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on
ENVIRONMENTAL BIOTECHNOLOGY

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C O N T E N T S

BACKGROUND

Environmental Information System (ENVIS) is established in the year 1984 as a network of Information Centre. It is planned by the Ministry of Environment and Forest. Aim of this centre is to provide descriptive data and environmental subject related numerical data. Now 35 centres are working under this network on various subjects area in the country. The focal point of this network is at the Ministry of Environmental and Forest, Government of India, New Delhi.

EMCB-ENVIS Centre is established for studies on Environmental Biotechnology as Pollutant Degradation at the University of Kalyani, Department of Environmental Science, Nadia-741235, West Bengal.

The objective of this centre is to collect data, related to the above mentioned subject, from different major libraries in Kolkata with different journals, Annual reviews, Internet and to generate a database and to create a website with this database. View point of this journal abstract is to help the interested research workers, scientist, administrator and the public.

This is the third publication of this ENVIS Centre. This contains the abstract of research papers collected in the area of Environmental Biotechnology from various journal published during June 2000 onwards. Here various topics like Bio-engineering, Bio-degradation, Bio-remediation, Bio-transformation etc. are covered. We are grateful to the various libraries and their staff for their extended cooperation in the collection of the articles.

Abstract Format

The format of the abstract is as follows:

Abstract : The abstracts were arranged in alphabetic orders different subheads.

Author: Name of the authors are given in the order in which they appear in the original document. These names are given in succession.

Address of Authors: Address of the author is given in parenthesis at the end of the author name. When the address of any other author is found, it is written after wards delimited by stop(.).

Locus: The name of the journal are followed by the volume number, the issue number, the year of publication and the page no.

GENERAL INFORMATION

Abstract have been taken directly from source document like research report, journals, Internet, seminars, proceedings, standards and patents. All the resources published within the year 2000-2003.

Abstract are broadly classified and arranged under the following heads:

Bioaccumulation: It studies address the buildup of bioaccumulative compounds through biomagnification and/or bioconcentration. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Understanding the dynamic process of bioaccumulation is very important in protecting human beings and other organisms from the adverse effects of chemical exposure, and it has become a critical consideration in the regulation of chemicals.

Bioremediation: It is a clean-up technology that uses naturally occurring microorganisms to degrade hazardous substances into less toxic or nontoxic compounds. These microorganisms may:

1. Ingest and degrade organic substances as their food and energy source,
2. Degrade organic substances, such as chlorinated solvents or petroleum products, that are hazardous to living organisms, including humans, anddegrade the organic contaminants into inert products.

Because the microorganisms already occur naturally in the environment they pose no contamination risk.

Bio-Transformation: This is a process of Biological changes of complex compound to simpler toxic to non-toxic or vice-versa. Several microorganism are capable of transforming a varity of compound founding nature but generally with respect to synthetic compound they are unable to show any appropriate action. Biotransfer appears to be one of the major detoxication method known so far.

Biomarker: It is a biological response to a chemical that gives a measure of exposure and, sometimes, of toxic effect.Biological markers found in crude oils and source rock extracts can provide molecular evidence of the correlation among oils and their sources.

Bioenergy: In recent decades, efforts were made for evolving were non-polluting bioenergy sources or energy generation from organic waste or biomass. These are all ecofreindly solution. Biomass energy supply demand balances have become a

component of energy sector analysis and planning and assumed greater importance in countries. These are variety of biological energy sources. Biomass, Biogas, Hydrogen are the example of Bioenergy.

Biofertilizer: To reduce the impact of excess chemical fertilizers in the field of agriculture the biofertilizer is a potential tool, biologically fixed nitrogen is such a source which can supply an adequate amount of Nitrogen to plants and other nutrients to some extent. Many free living and symbiotic bacteria which fix atmospheric Nitrogen were used as biofertiliser material as a substitute for Nitrogen fertilizer. In general two types of biofertiliser are used

1. Bacterial Biofertilizer
2. Algal Biofertilizer

Biocomposting: It involves combining organic materials under conditions that enables them to decompose more quickly than they would in nature. Think about logs and leaves on the ground in a forest. The leaves will break down and disappear within a year. Logs of course will take much longer to crumble away. Composting involves combining organic materials under conditions that enables them to decompose more quickly than they would in nature.

Biopesticide: pest control by biological antagonism appears to be very useful tool in recent years. Bacterial pesticides are being developed. Heliothis complex, which lives in close association with plant roots, consists of two major crop pests budworm and ball warm. Biological insecticides against both these insects are being prepared by transfer of a gene from *Bacillus thuringiensis*

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Biodegradation: It is nature's way of recycling wastes, breaking down organic matter into nutrients that can be used by other organisms. "Degradation" means decay, and the "bio-" prefix means that the decay is carried out by a huge assortment of bacteria, fungi, maggots, worms, and other organisms that eat dead material and recycle it into new forms.

In nature, there is no waste because everything gets recycled. The waste products from one organism become the food for others, providing nutrients and energy while breaking down the waste organic matter. Some organic materials will break down much faster than others, but all will eventually decay.

By harnessing these natural forces of biodegradation, people can reduce wastes and clean up some types of environmental contaminants. Through **composting**, we accelerate natural biodegradation and convert organic wastes to a valuable resource.

Biosensor: Biosensor represents biophysical devices which will detect the presence and measure the quantities of specific substances in a varity of environments. These specific substances may include sugars, proteins, or humas and varity of toxins in the industrial effluents. In designing a biosensor an enzyme or an antibody or even microbial cells are associated with microchip devices which are used for quantitative estimate of a substance.

Bioengineering: It is a developing speciality featuring a multidisciplinary approach to the solution of problems in medicine and biology, based on the application of advances in science, engineering and technology. A major focus for bioengineering is to improve the quality of life of people with medical conditions that restrict independent living and integration within the community.

Pollen-Biotechnology: This is a new field of science delaying with the pollen chemistry allerginicity of aerospora. This subject also covers genetic manipulation of pollen development of haploid culture. Such haploid plant have remains values in genetic research.

Biotechnology Policy Issue: Biotechnology appears to be a emerging science in present decades. Genetic manipulation and development of genetically modified organism in human welfare is now showed a potential prospect and risk. Thus researches and application of Biotechnology in diverse field is a major policy issue in the present decades.

Agricultural Biotechnology: Over the years tremendous success was made in diverse field of agriculture by applying Biotechnology. It includes development of genetically modified crops, genetically improvement in sericulture practices, improvement in Biofertilizer development and similar other aspects. Crop production against pest and disease stress resistance of crops also considered to be emerging area of Agricultural Biotechnology.

ABBREVIATIONS USED IN ADDRESSES AND CITED JOURNALS

Acad	Academy	Chem	Chemistry
Adm	Administration	Chemi	Chemical
Admn	Administrative	Clini	Clinical
Adv	Advance	Co	Company
Agric	Agriculture	Coil	College
Agrici	Agricultural	Comm	Committee
Amer,	American	Commn	Commission
An	Annual	Comp	Comparative
Analyt	Analytical	Conf	Conference
Anat	Anatomy	Conv	Convention
Anim	Animal	Conserv	Conservation
Ann	Annals	Conti	Control
Appt	Applied	Contam	Contamination
Arch	Archives	Corpn	Corporation
Archaeo	Archaeology	Coun	Council
Archaeol	Archaeological	Cult	Culture
Architect	Architecture	Cultl	Cultural
Assoc	Association	Curr	Current
Asst	Assistant	Dept	Department
Atom	Atomic	Dev	Development
Bacterio	Bacteriology	Develop	Developmental
Bacteriol	Bacteriological	Dig	Digest
Bd	Board	Div	Division
Bio	Biology	Divl	Divisional
Biochem	Biochemistry	Dte	Directorate
Biochemi	Biochemical	Dy	Deputy
Bioengng	Bioengineering	Eco	Ecology
Biol	Biological	Ecol	Ecological
BiometeO	Biometeorology	Econ	Economics
Biophys	Biophysics	Ecosys	Ecosystem
Biometeol	Biometeorological	Exotoxico	Ecotoxicology
Biotech	Biotechnique(s)	Endocrinol	Endocrinological
Biotechno	Biotechnology	Engng	Engineering
Biotechnol	Bitechnological	Engrs	Engineers
Bidg	Building	Env	Environment
Bot	Botany	Environ	Environmental
Boti	Botanical	Epidemic	Epidemiology
Br	Branch	Epidemiol	Epidemiological
Bull	Bulletin	Estb	Establishment
Cent	Centre	Ethnopharmaco	Ethnopharmacology
Centl	Central	Exot	Experiment

Expti	Experimental	Microbiol	Microbiological
Fac	Faculty	Min	Ministry
Fd	Food	Monit	Monitoring
Fedn	Federation	Myco	Mycology
Fert	Fertiliser	Mycol	Mycological
Fmg	Farming	Nat	Natural
Gaz	Gazette	Natl	National
Genet	Genetics	N-E	North Eastern
Geo	Geology	Nut	Nutrition
Geogr	Geography	No	Number
Geogri	Geographical	Occ	Occassional
Geol	Geological	Occupl	Occupational
Geosci	Geoscience	Oceanogr	Oceanogoraphy
Govt	Government	Org	Organic
Hist	History	Orgn	Organisation
Hlth	Health	Pharmaco	Pharmacology
Hort	Horticulture	Pharmacol	Pharmacological
Hosp	Hospital	Phyl	Physical
Hydro	Hydrology	Patho	Pathology
Hydrol	Hydrological	Pathol	Pathological
Immuno	Immunology	Petrochemi	Petrochemical
Immunol	Immunlogical	Petro	Petrology
Ind	Industry	PG	Post Graduate
Inf	Information	Phys	Physics
Inst	Institute	Physio	Physiology
Instn	Institution	Phytopath	Phytopathology
Int	International	Phytopathol	Phytopathological
Irrig	Irrigation	Plang	Planning
J	Journal	Polln	Pollution
Lab	Laboratory	Proc	Proceedings
Lett	Letter(s)	Prot	Protection
Ltd	Limited	Pub	Publication
Malario	Malariology	Pvt	Private
Malariol	Malariological	Qlty	Quality
Manag	Management	Qr	Quarter
Med	Medicine	Rad	Radiation
Medl	Medical	Radio	Radiology
Metab	Metabolism	Radiol	Radiological
Metall	Metallurgy	Rd	Road
Metallurg	Metallurgical	Recd	Received
Meteo	Meteorology	Reg	Region
Meteol	Meteorological	Regl	Regional
Microbio	Microbiology		

Rep	Report	Stud	Studies
Reptr	Reporter	Surv	Survey
Res	Research	Syst	System
Rev	Review	Tax	Taxonomy
Sch	School(s)	Techi	Technical
Sci	Sciences(s)	Techno	Technology
Scient	Scientific	Technol	Technological
S-E	South East	Toxico	Toxicology
Sec	Section	Toxicol	Toxicological
Sect	Sector	Trans	Transcations
Semin	Seminar	Trans	Transportation
Ser	Services	Tmg	Training
Soc	Society	Trop	Tropical
Sod	Social	Univ	University
Stat	Statistics	Util	Utilisation
Stati	Statistical	Vet	Veterinary
Stand	Standard(s)	Zoo	Zoology
Std	Study	Zool	Zoological

Bioaccumulation

A A Hamdy. (Microbial and Natural Products Chemistry Department, National Research Center, Dokki, Cairo, Egypt). **Biosorption of Heavy Metals by Marine Algae**. Current Microbiology, 41(4) (2000), 0232 – 0238.

The ability of four different algae (three brown and one red) that have not been previously studied to adsorb Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} ions was investigated. The metal uptake was dependent on the type of biosorbent, with different accumulation affinities towards the tested elements. The HCl-treated biomass decreased the metal biosorptive capacity particularly in the case of Cr^{3} adsorption with Laurencia obtusa. The extent of uptake of the different metals with the tested algae was assessed under different conditions such as pH, time of algal residence in solution with the metal, and concentration of algal biomass. The rate of uptake of the different metals was very fast in the first 2 h; thereafter the increase in metal uptake was insignificant. The amount of the metal uptake (5-15 mg range) increased steeply by increasing the weight of the biomass. An exception was L. obtusa, where a parallel increase of the uptake of different metals was observed on increasing the algal mass from 5 to 50 mg.

A. E. El-Enany, A. A. Issa. (Botany Department, Faculty of Science, Assiut University, Assiut, Egypt). **Cyanobacteria as a biosorbent of heavy metals in sewage water**. Environmental Toxicology and Pharmacology, 8(2) (2000), 95-101.

The effect of sewage water on some physiological activities of cyanobacteria was studied. Metal-tolerant cyanobacterium (*Nostoc linckia*) and metal-sensitive (*Nostoc rivularis*) were grown at three levels of sewage water (25, 50 and 75%). The growth rate showed significant stimulation in low and moderate levels (50% for *N. linckia* and 25% for *N. rivularis*). Not only the number of cells was elevated but also, the time required to reach the exponential and the stationary phases was reduced. Also, low levels of sewage water increased chl.a content, photosynthetic O_2-evolution, respiration and protein content. Similarly, heterocyst frequency as well as nitrogenase activity were increased in cyanobacteria grown at low and moderate levels (25 and 50% sewage). On the other hand, the high level of waste (75%) reduced growth and metabolic activities of the two species. *N. linckia* accumulated about 30-fold of Zn and ten-fold of Cd than those of growth medium (50% sewage water). Also, *N. rivularis* accumulated about ten-fold of Zn and two-fold of Cd. The distribution of Cd and Zn in cells were investigated. About 65–60% of Cd or Zn were found in pellets (sediment) as insoluble form in the two species. The soluble form (cytosolic fraction) after being fractionated on sephadex G-(75-100) revealed two peaks with molecular weights of 70–75 and 40–45 kDa. These peaks were in coincidence with Cd and Zn maxima. *Nostoc rivulais* showed more sensitivity to heavy metals than *N. linckia*, and accumulated less amount of metal-binding proteins. *Nostoc linckia* seems to be tolerant to heavy metals (Zn and Cd) and is able to accumulate this metal by adsorption on the pellets (cell surface) and/or through sequestration via metal-binding protein. Therefore it can be recommended it to be employed in the purification of waste contaminated with these heavy metals.

A. Heikens, W. J. G. M. Peijnenburg, A. J. Hendriks. (Institute for Inland Water Management and Waste Water Treatment, PO Box 17, 8200 AA Lelystad, The Netherlands. National Institute of Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands). **Bioaccumulation of heavy metals in terrestrial invertebrates.** Environmental Pollution, 113(3) (2001), 385-393.

In this literature study, accumulation data of metals in terrestrial invertebrates were collected and compared (Arthropoda and Lumbricidae). Based on total soil concentrations and body concentrations, regression equations were calculated for each metal (Cd, Cu, Pb and Zn) and each taxonomic group. We also tried to find out whether or not accumulation levels of metals in Lumbricidae are representative for all of the studied terrestrial invertebrates. Taxonomic groups

could be ordered according to the extent of metal accumulation. Significant differences in accumulation levels of a factor 2–12 were found between taxonomic groups. Overall, metal concentrations were high in Isopoda and low in Coleoptera. The concentrations in Lumbricidae were in between. It should be kept in mind that the data for Lumbricidae were mainly derived from laboratory experiments, while the data for other groups were derived from field studies. The internal Pb, Cd and Cu concentration increased with the soil concentration for most taxonomic groups in the order Pb>Cd>Cu. Body concentrations of Zn were quite constant over a range of soil concentrations. The differences in accumulation level between taxonomic groups show the relevance of including detailed information on feeding behaviour in risk assessment for invertebrate-eating animals.

A. L. Lawrence and R. P. Mason. (Chesapeake Biological Laboratory, Center for Environmental Science, The University of Maryland, PO Box 38, Solomons, MD 20688, USA). **Factors controlling the bioaccumulation of mercury and methylmercury by the estuarine amphipod *Leptocheirus plumulosus.*** Environmental Pollution, 111(2) (2001), 217-231.

The bioaccumulation of inorganic mercury (Hg_I) and monomethylmercury (MMHg) by benthic organisms and subsequent trophic transfer couples the benthic and pelagic realms of aquatic systems and provides a mechanism for transfer of sedimentary contaminants to aquatic food chains. Experiments were performed to investigate the bioavailability and bioaccumulation of particle-associated Hg_I and MMHg by the estuarine amphipod *Leptocheirus plumulosus* to further understand the controls on bioaccumulation by benthic organisms. Hg_I and MMHg are particle reactive and have a strong affinity for organic matter, a potential food source for amphipods. Microcosm laboratory experiments were performed to determine the effects of organic matter on Hg bioaccumulation and to determine the major route of Hg uptake (i.e. sediment ingestion, uptake from water/porewater, or uptake from `food'). Amphipods living in organic-rich sediment spiked with Hg accumulated less Hg than those living in sediments with a lower organic matter content. Feeding had a significant impact on the amount of Hg_I and MMHg accumulated. Similarly, amphipods living in water with little organic matter accumulated more Hg than those living in water with a greater percentage of organic matter. MMHg was more readily available for uptake than Hg_I. Experimental results, coupled with results from a bioaccumulation model, suggest that accumulation of Hg_I and MMHg from sediment cannot be accurately predicted based solely on the total Hg, or even the MMHg, concentration of the sediment, and sediment-based bioaccumulation factors. All routes of exposure need to be considered in determining the accumulation of Hg_I and MMHg from sediment to benthic invertebrates.

Antonia Concetta Elia, Roberta Galarini, Maria Illuminata Taticchi, Ambrosius Josef Martin Dörr, Luciana Mantilacci. (Department of Animal Biology and Ecology, University of Perugia, Via Elce di Sotto, I-06123, Perugia, Italy. Experimental Zooprophylactic Institute of Umbria-Marche, Via Salvemini, 1 I-06126, Perugia, Italy). **Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure.** Ecotoxicology and Environmental Safety, 55(2) (2003), 162-167.

Laboratory experiment was carried out to determine mercury accumulation in tissues (gills, kidneys, liver, and muscle) and biochemical responses in the liver of freshwater teleost *Ictalurus melas*. Catfish were subjected to different concentrations of Hg^{2+} (35, 70, and 140 μg/L) for 10 days. The chemical analyses showed higher mercury concentrations for all treatments in gills and kidneys followed by liver and muscle. At the lowest mercury concentration a decrease in glutathione (GSH) content and an increase of GSH peroxidase Se-dependent and glyoxalase II enzymes were observed. An increasing trend was observed also for GSH-*S*-transferase and

glyoxalase I, while GSH peroxidase Se-independent enzyme and GSH reductase showed no significant variation in activities. The increase in the enzymes activities of catfish, involved in the inactivation of reactive molecules formed during oxidative stress, could provide an additional protection against the oxidative damage induced by mercury.

Aran Incharoensakdi, Pissopa Kitjaharn. (Laboratory of Biochemical Products, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand). **Zinc Biosorption from Aqueous Solution by a Halotolerant Cyanobacterium Aphanothece halophytica**. Current Microbiology, 45(4) (2002), 261 – 264.

We have investigated the characteristics of zinc biosorption by Aphanothece halophytica. Zinc could be rapidly taken up from aqueous solution by the cells with an equilibrium being reached within 15 min of incubation with 100 mg Lm1 ZnCl2. The adsorbed zinc was desorbed by treatment with 10 mM EDTA. The presence of glucose, carbonyl cyanide m-chlorophenylhydrazone (CCCP), and N,N'-dicyclohexylcarbodiimide (DCCD) did not affect the uptake of zinc. The specific uptake of zinc increased at low cell concentration and decreased when cell concentration exceeded 0.2 g Lm1. The binding of zinc followed Langmuir isotherm kinetics with a maximum zinc binding capacity of 133 mg gm1 and an apparent zinc binding constant of 28 mg Lm1. The presence of an equimolar concentration of Mn2+, Mg2+, Co2+, K+, or Na+ had no effect on zinc biosorption, whereas Ca2+, Hg2+, and Pb2+ showed an inhibitory effect. The biosorption of zinc was low at a pH range from 4 to 6, but increased progressively at pH 6.5 and 7.

B. Clasona, W. J. Langstonb, G. -P. Zauke. (Carl von Ossietzky Universität Oldenburg, Institut für Chemie und Biologie des Meeres (ICBM), Postfach 2503, D-26111, Oldenburg, Germany. Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, UK). **Bioaccumulation of trace metals in the amphipod Chaetogammarus marinus (Leach, 1815) from the Avon and Tamar estuaries (UK): comparison of two-compartment and hyperbolic toxicokinetic models**. Water Research, 37(10) (2003), 2505-2511.

Bioaccumulation of Cd, Cr, Cu, Co, Pb, Ni and Zn was investigated in the eulittoral gammaridean amphipod Chaetogammarus marinus (Leach, 1815) from the Avon and Tamar estuaries (UK). The main goals were to provide information on accumulation strategies of the organisms tested and to verify toxicokinetic models as a predictive tool. The organisms accumulated metals upon exposure and it was possible to estimate significant model parameters of two-compartment and hyperbolic models, with the exception of Zn. Both types of models yielded significant model estimates in two independent toxicokinetic studies using gammarids from the Avon and Tamar estuaries, respectively. Kinetic BCFs at theoretical equilibrium were 58–102 for Cd, 95–215 for Cr, 400–963 for Cu, 38–59 for Co, 150–750 for Pb and 33–63 for Ni, and were in most cases distinctly lower than the range reported in the literature for other amphipods. To demonstrate the potential of toxicokinetic models as a predictive tool, attempts were made, further to verify time-dependent model data (Tamar) with independent experimental data (Avon). In this case only for Cd were both in good agreement, whilst for all other elements the models overestimated the measured values. The second approach was to verify time-dependent model data (Tamar) with measured values from an independent concentration-dependent uptake study (Tamar) or, alternatively, to estimate the model parameters simultaneously for both studies. A good agreement between observed and predicted values was obtained for all elements for the simultaneous two-compartment models. Only for Cd was a simultaneous estimation of the hyperbolic model more promising. A tentative estimation showed the following sequence of sensitivity of C. marinus to an increase of soluble metal exposure: 0.6 g Cd l-1, 1.7 g Cr l-1, 16 g Cu l-1, 3.5 g Co l-1, 0.8 g Pb l-1 and 7 g Ni l-1. Available

information can be used to quantify a measure of agreement or disagreement between bioaccumulation in different amphipods. This can be regarded as an important step in the calibration of biomonitors which is necessary to assess the potential for bioaccumulation on a large geographical scale.

Badri N. Badriyha, Varadarajan Ravindran, Walter Den, Massoud Pirbazari. (Department of Civil and Environmental Engineering, University of Southern California, Los Angeles, CA 90089-2531, USA). **Bioadsorber efficiency, design, and performance forecasting for alachlor removal**. Water Research, 37(17) (2003), 4051-4072.

This study discusses a mathematical modeling and design protocol for bioactive granular activated carbon (GAC) adsorbers employed for purification of drinking water contaminated by chlorinated pesticides, exemplified by alachlor. A thin biofilm model is discussed that incorporates the following phenomenological aspects: film transfer from the bulk fluid to the adsorbent particles, diffusion through the biofilm immobilized on adsorbent surface, adsorption of the contaminant into the adsorbent particle. The modeling approach involved independent laboratory-scale experiments to determine the model input parameters. These experiments included adsorption isotherm studies, adsorption rate studies, and biokinetic studies. Bioactive expanded-bed adsorber experiments were conducted to obtain realistic experimental data for determining the ability of the model for predicting adsorber dynamics under different operating conditions. The model equations were solved using a computationally efficient hybrid numerical technique combining orthogonal collocation and finite difference methods. The model provided accurate predictions of adsorber dynamics for bioactive and non-bioactive scenarios. Sensitivity analyses demonstrated the significance of various model parameters, and focussed on enhancement in certain key parameters to improve the overall process efficiency. Scale-up simulation studies for bioactive and non-bioactive adsorbers provided comparisons between their performances, and illustrated the advantages of bioregeneration for enhancing their effective service life spans. Isolation of microbial species revealed that fungal strains were more efficient than bacterial strains in metabolizing alachlor. Microbial degradation pathways for alachlor were proposed and confirmed by the detection of biotransformation metabolites and byproducts using gas chromatography/mass spectrometry. Kinetics of 4-nitrophenol biodegradation in a sequencing batch reactor

C. A. Oliveira Ribeiro, E. Pelletier, W. C. Pfeiffer, C. Rouleau. (Departamento de Biologia Celular, Universidade Federal do Paraná, Caixa Postal 19031, CEP, 81531-970, Curitiba, PR, Brazil. Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, 310 allée des Ursulines Rimouski, Québec, G5L 3A1, Canada. Laboratório de Radioisótopos, I.B.C.C.F. CCS BlocoG, Universidade Federal do Rio de Janeiro, CEP, 21949-900, Rio de Janeiro, RJ, Brazil. Institut Maurice Lamontagne, Pêches, et Océans, C.P. 1000, Mont-Joli, Québee, G5H 3Z4, Canada). **Comparative Uptake, Bioaccumulation, and Gill Damages of Inorganic Mercury in Tropical and Nordic Freshwater Fish.** Environmental Research, 83(3) (2000), 286-292.

This paper reports comparative results on the bioaccumulation of inorganic mercury and resulting gill damages in the tropical fish, *Trichomycterus zonatus*, and a nordic species, *Salvelinus alpinus*, using radioisotope ^{203}Hg techniques and scanning electron microscopy. Uptake of inorganic Hg from water was much more important in *T. zonatus* than in *S. alpinus* and the Hg concentration in *S. alpinus* increased at a slower rate in all tissues during the first 24 h of exposure. After 96 h, Hg concentration was 70 times higher in the kidney, 10 times higher in liver, intestine, skin, and brain, and 3 times higher in gills, muscle, and the rest of body of *T. zonatus* compared to *S. alpinus*. Gill damages in *T. zonatus* were more evident and occurred

much earlier than for *S. alpinus*. According to our data, the high differences observed in the inorganic mercury uptake, bioaccumulation, and gills damages strongly suggest that *T. zonatus* is more sensitive to inorganic mercury pollution than *S. alpinus*. Further studies are urgently needed to determine whether the high sensitivity observed for *T. zonatus* to inorganic Hg is also present in the majority of tropical species or whether this species presents an isolated case.
Cevdet Uguz, Mesude Iscan, Ayse Ergüven, Belgin Isgor and Inci Togan. (Afyon Kocatepe Universitesi, Veteriner Fakultesi, A.N. Sezer Kampusu, 03100, Afyon, Turkey. Department of Biological Sciences, Middle East Technical University, 06531, Ankara, Turkey). **The bioaccumulation of nonyphenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*)**. Environmental Research, 92(3) (2003), 262-270.

Alkylphenol polyethoxylates (APEs) are widely used as nonionic surfactants. Nonylphenol (NP), one of the derivatives of APEs, has been found in the aquatic environment in ranges from nanograms per liter to milligrams per liter. In this study, juvenile rainbow trout were exposed to 0 (control), 66, 220, or 660 μg NP/L for up to 28 days. Fish remained healthy under NP exposures of 0, 66, and 220 μg/L for the length of the experiment. All fish died after 4 days of exposure to 660 μg NP/L. Time-dependent NP bioaccumulation was detected in the tissues of fish exposed to 220 μg NP/L ($P<0.05$) and histopathological changes were observed in the livers of fish exposed to 220 μg NP/L. Furthermore, an increase in the activity of glutathione-*S*-transferase (GST) was found in the liver of fish exposed to 220 μg NP/L for 1 week ($P<0.05$). There was an increase in GST activity in the liver of fish exposed to 66 μg NP/L but it did not occur before 2 weeks of exposure to NP. The GST activity then decreased in a time-dependent manner in treatment groups, and this decrease was lower in the livers of fish treated with 66 and 220 μg NP/L than in control fish after 3 weeks of exposure ($P<0.05$). These results indicated that sublethal doses of NP were accumulating in the bodies of the fish and causing histopathological and biochemical changes in the livers of rainbow trout.
D. C. Su, J. W. C. Wong. (Department of Plant Nutrition, China Agricultural University, Beijing 100094, PR China. Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, PR China). **Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge**. Environment International, 29(7) (2004), 895-900.

A sequential extraction method was used to determine chemical forms of Cu, Zn, Ni and Cd in fly ash-stabilized sludge. A loamy acid soil amended with fly ash-stabilized sludge was used to grow corn under greenhouse conditions. Sewage sludge amended with coal fly ash can reduce the availability of Cu, Zn, Ni and Cd in the sludge. Increasing fly ash amendment rate significantly reduced DTPA-extractable Cu, Zn, Ni and Cd concentrations. Percentages of Cu, Zn and Ni in residual fraction increased with an increase in fly ash amendment rates. Majority of Cu was associated with organic form, but Zn and Ni were associated with Fe–Mn oxide and residual forms. Addition of ash-amended sludge to soil significantly increased dry mass of corn. With coal fly ash amendment rate increasing, concentrations of Zn and Cu in shoot tissues of corn decreased significantly, but concentrations of Cd and Ni did not change significantly. Significant correlations were found between concentrations of Cu and Zn in corn shoot and oxide and total Cu fractions, and all chemical fractions of Zn in fly ash-stabilized sludge, respectively. Hence, ash amendment significantly reduced the availability of heavy metals by chemical modification of their chemical speciation into less available forms.
D. Mackay, A. Fraser. (Canadian Environmental Modelling Centre, Trent University, Peterborough, Ontario, Canada K9J 7B8). **Bioaccumulation of persistent organic chemicals: mechanisms and models.** Environmental Pollution, 110(3) (2000), 375-391.

A review is presented of bioaccumulation of organic substances in organisms, especially fish, including the incentives for developing a tiered predictive approach for addressing the large number of chemicals of commerce. From a review of the existing estimation methods it is suggested that the simplest Tier 1 approach is an empirical correlation for bioconcentration factor as a function of the octanol–water partition coefficient. For more detailed Tier 2 evaluation, the bioaccumulation factor is best predicted using a mechanistic mass balance model applied to the organism at steady state in which relevant uptake and loss processes are quantified. The equivalence of rate constant and fugacity models is demonstrated and methods of obtaining parameter values are discussed. Such a model reveals the relative significance of gill ventilation, food uptake, egestion, and metabolism. The most detailed Tier 3 evaluation should involve prediction of the potential for biomagnification in a food chain involving both fish and air-breathing animals. Research needs are discussed with a view to understanding the mechanisms more fully and developing more accurate quantitative descriptions or models of bioaccumulation phenomena.

D. W. Berzins, K. J. Bundy. (Department of Biomedical Engineering and the Center for Bioenvironmental Research, Tulane University, 500 Lindy Boggs Building, New Orleans, LA 70118, USA). **Bioaccumulation of lead in *Xenopus laevis* tadpoles from water and sediment.** Environment International, 28(1-2) (2002), 69-77.

The overall objective of this research was to monitor the uptake kinetics of lead in an amphibian model and correlate metal content with embryo development. Based upon the concentration of lead found in the water and sediment of a Louisiana swamp adjacent to a Superfund site, a controlled laboratory experiment exploring lead uptake from water and sediment by *Xenopus laevis* tadpoles was conducted. For 5 weeks, tadpoles were exposed to water and a simulated sediment, kaolin, spiked with 1, 5, or 10 times the concentration of lead found in field water and sediment samples. Additionally, organisms were exposed to the 5× condition for 3 and 6 weeks. The experimental controls consisted of unexposed tadpoles and ones exposed to lead originating from water or sediment exclusively. At the end of the exposure periods, developmental data, i.e., body weight and developmental stage, were recorded, and the tadpoles were analyzed for whole body lead concentration. Lead extraction was accomplished by dry ashing, and its amount was quantified polarographically. Results showed that lead inhibited the normal development of these amphibians, in a manner that generally was more severe as exposure level increased. The hindrance of tadpole development also coincided with an increase in whole body lead concentration at higher exposures. Temporally, at the 5× exposure concentration, the mean lead level increased with time, but this difference was not statistically significant ($P<.05$). Additionally, control animals exposed to lead (either in water or in sediment) showed no statistical difference with regard to weight and lead uptake, indicating that lead originating from both water and sediment is incorporated into the tadpole. The controlled laboratory experimental protocol used here is thus capable of investigating the uptake of a single metal (Pb in this case) and determining its effect on the development of tadpoles while differentiating the significance of multiple sources of exposure.

F. B. Pyatt. (Department of Life Sciences, The Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, United Kingdom). **Copper and Lead Bioaccumulation by *Acacia retinoides* and *Eucalyptus torquata* in Sites Contaminated as a Consequence of Extensive Ancient Mining Activities in Cyprus**. Ecotoxicology and Environmental Safety, 50(1) (2001), 60-64.

Aspects of the industrial archaeology of the northwestern part of the island of Cyprus are outlined. Wastes resultant from copper mining activities of approximately two millennia ago continue to exert an important influence on organisms. Detailed chemical analysis of two tree

species growing on archaeologically important metalliferous spoil tips has indicated their ability to bioaccumulate heavy metals and sulfur primarily from the substratum; the bioaccumulation and biomagnification of lead and sulfur are particularly marked in both *Acacia* and *Eucalyptus*. The concentrations of elements in different parts of the two tree species are discussed and partitioning is noted together with the fact that while the pod of *Acacia* and the fruit capsule of *Eucalyptus* may have an enhanced metal loading, the values in the seeds are much reduced; the importance of this is discussed. The seeds of *Acacia* differ chemically from those of *Eucalyptus*. The importance of these plants as biomonitors of environmental quality is noted.

G. Gorbi, M. G. Corradi, M. Invidia, M. Bassi. (Department of Environmental Sciences, University of Parma, I-43100, Parma, Italy. Department of Evolutive and Functional Biology, University of Parma, I-43100, Parma, Italy). **Light Intensity Influences Chromium Bioaccumulation and Toxicity in Scenedesmus acutus (Chlorophyceae)**. Ecotoxicology and Environmental Safety, 48(1) (2001), 36-42.

The influence of light intensity on chromium uptake was studied in two strains of the freshwater unicellular alga *Scenedesmus acutus* (Chlorophyceae) having different sensitivity to Cr poisoning and light intensity. The two strains were subjected to different Cr treatments at 3000 and 80 lux. Cr toxicity was assessed by algal growth rate, recovery test, methylene blue staining, and determination of photosynthetic activity. After 2 and 4 days of treatment, bioaccumulated chromium, cell dry mass, and protein and carbohydrate contents were also assessed. When the algae were treated at 3000 lux, different bioaccumulation patterns were obtained when Cr content was related to dry mass, cell number, or protein content. A direct relationship between Cr content and cell mortality was observed only when the amount of Cr was related to protein content. In both strains Cr uptake was slower in subdued light, suggesting that it is linked to energy-dependent processes. The difference between the strains in sensitivity to Cr poisoning was also evident in subdued light.

G. R. MacFarlane, A. Pulkownik and M. D. Burchett. (School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, 2308, Australia. Centre for Ecotoxicology and Department of Environmental Sciences, University of Technology, Sydney, Gore Hill, NSW 2065, Australia). **Accumulation and distribution of heavy metals in the grey mangrove, Avicennia marina (Forsk.)Vierh.: biological indication potential.** Environmental Pollution, 123(1) (2003), 139-151.

The accumulative partitioning of the heavy metals Cu, Pb and Zn in the grey mangrove, *Avicennia marina*, were studied under field conditions. Copper and Pb were accumulated in root tissue to levels higher than surrounding sediment levels. Zinc was accumulated to levels reflecting sediment concentrations. Strong linear relationships existed for all metals in sediments with metals in root tissue. Accumulation of Cu in leaf tissue followed a linear relationship at lower sediment concentrations, with an exclusion or saturation mechanism at higher sediment concentrations. Lead showed little mobility to leaf tissue. Zn showed restricted accumulation in leaf tissue, which correlated with sediment concentrations. Decreases in sediment pH were found to increase Zn accumulation to root tissue. Increasing concentrations of Pb and Zn in sediments resulted in a greater accumulation of Pb to both root and leaf tissue. *A. marina* roots may be employed as a biological indicator of environmental exposure of Cu, Pb and Zn and leaves for Zn, with temporal monitoring.

Gregorio Fernandez-Leborans and Yolanda Olalla Herrero. (Departamento de Biologia Animal I (Zoologia), Pnta. 9^{a}, Universidad Complutense, 28040, Madrid, Spain). **Toxicity and Bioaccumulation of Lead and Cadmium in Marine Protozoan Communities.** Ecotoxicology and Environmental Safety, 47(3) (2000), 266-276.

The behavior of lead and cadmium in a protozoan community was studied in order to obtain new data regarding the toxicity and bioaccumulation of these heavy metals. For this purpose, microcosms with different concentrations of the pollutants (without metals, 500 μg Cd and $Pb \cdot L^{-1}$ 1000 μg Cd and $Pb \cdot L^{-1}$) were used. Protozoans bioaccumulated 7.03–207.00 μg $Pb \cdot g^{-1}$ dry weight (dw) and 0.05–332.75 μg Cd·g-1 dw, representing an accumulation capacity of up to 161.45 μg $Pb \cdot g^{-1}$ dw and 310.75 μg $Cd \cdot g^{-1}$ dw more than that of the bacteria. The addition of both metals caused a significant reduction in the density of protozoans. These data were compared with those obtained previously in treatments that used these metals separately.
H. M. Anawar, J. Akai, K. M. G. Mostofa, S. Safiullah, S. M. Tareq. (Graduate School of Science and Technology, Niigata University, Ikarashi 2-no-cho, Niigata 950-2181, Japan. Department of Geology, Niigata University, Ikarashi 2-no-cho, Niigata 950-2181, Japan. Department of Hydrospheric––Atmospheric Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan. Department of Environmental Science, Jahangirnagar University, Savar, Dhaka, Bangladesh). **Arsenic poisoning in groundwater: Health risk and geochemical sources in Bangladesh.** Environment International, 27(7) (2002), 597-604.

Of the 2508 water samples analyzed in 10 districts of Bangladesh, 51%, on an average, contained arsenic levels of 0.05 to 2.50 mg/l. 95% of nail, 96% of hair, and 94% of urine samples contained arsenic above the normal level. Approximately 3.58 million people out of a total of 17.92 million who are drinking water containing arsenic levels >0.20 mg/l are potentially exposed to high risk of health hazard. Eight thousand and five hundred arsenic patients are identified; they are suffering from various skin lesions, gangrene in leg, skin, lung, bladder, liver, and renal cancer. A big portion of the total population is highly vulnerable to various internal cancers. Lowest arsenic concentration in drinking water producing dermatological disease is found to be 0.103 mg/l. However, the exposure time to develop arsenicosis varies from case to case reflecting its dependence on arsenic level in drinking water and food, nutritional status, genetic variant of human being, and compounding factors. This study has determined the high intensity of fluorescent humic substances in drinking water containing elevated concentrations of arsenic and very low concentrations of heavy metals. The synergistic/antagonistic effect of fluorescent compounds present in drinking water may aggravate the toxicity of arsenic. Geochemical study suggests that arsenic may be released from both reductive dissolution of Fe and Mn (oxy)hydroxide and microbial oxidation of organic matter.
J. Calderón, M. E. Navarro, M. E. Jimenez-Capdeville, M. A. Santos-Diaz, A. Golden, I. Rodriguez-Leyva, V. Borja-Aburto, F. Díaz-Barriga. (Facultad de Medicina, Universidad Autónoma de San Luis Potosí, México. Escuela de Psicología, Universidad Autónoma de San Luis Potosí, México. Department of Community and Preventive Medicine, Mount Sinai School of Medicine, City University of New York, New York City, USA. Centro Nacional de Salud Ambiental, México, D.F., México). **Exposure to Arsenic and Lead and Neuropsychological Development in Mexican Children.** Environmental Research, 85(2) (2001), 69-76.

This cross-sectional study examined the effects of chronic exposure to lead (Pb), arsenic (AS) and undernutrition on the neuropsychological development of children. Two populations chronically exposed to either high (41 children) or low (39 children) levels of As and Pb were analyzed using the Wechsler Intelligence Scale for Children, Revised Version, for México (WISC-RM). Geometric means of urinary arsenic (AsU) and lead in blood (PbB) were 62.9±0.03 (μgAs/g creatinine) and 8.9±0.03 (μg/dl) for the exposed group and 40.2±0.03 (μgAs/g creatinine) and 9.7±0.02 (μg/dl) for the reference group. The height for age index (HAI) was used as an indicator of chronic malnutrition and sociodemographic information was obtained with a questionnaire. Lead and arsenic were measured by atomic absorption spectrophotometry.

Data on full, verbal, and performance intelligence quotients (IQ) scores, long-term memory, linguistic abstraction, attention span, and visuospatial organization were obtained through the WISC-RM. After controlling for significant potential confounders verbal IQ ($P<0.01$) decreased with increasing concentrations of AsU. The HAI correlated positively with full-scale and performance IQ ($P<0.01$). Higher levels of AsU were significantly related to poorer performance on WISC-RM factors examining long-term memory and linguistic abstraction, while lower scores in WISC-RM factors measuring attention were obtained at increasing values of PbB. Our results suggest that exposure to As and chronic malnutrition could have an influence on verbal abilities and long-term memory, while Pb exposure could affect the attention process even at low levels.

J. M. Roper, J. W. Simmers, D. S. Cherry. (AScI Corporation, Vicksburg, MS 39180, USA. Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, MS 39180, USA. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA). **Bioaccumulation of butyltins in *Dreissena polymorpha* at a confined placement facility in Buffalo, New York.** Environmental Pollution, 111(3) (2001), 447-452.

This study involves a site characterization followed by biomonitoring with the zebra mussel, *Dreissena polymorpha*, at the Times Beach confined placement facility (CPF), located in Buffalo, NY. The contaminant of interest was tributyltin (TBT) and the degradation products dibutyltin (DBT) and monobutyltin (MBT). At study initiation (Day 0) TBT levels in the baseline mussels were 5.86±0.43 ng Sn/g, DBT levels were 2.25±0.37 ng Sn/g. No MBT was detected in the Day 0 baseline samples. Caged reference mussels placed back in the Black Rock Channel Lock and retrieved on Days 19 and 34 had TBT, DBT, and MBT levels which did not differ significantly from the Day 0 baseline levels. Mussels placed at the Times Beach CPF had TBT concentrations that were significantly lower at both Days 19 (3.65±0.90 ng Sn/g) and 34 (3.50±1.03 ng Sn/g) than the Day 0 baseline analysis (5.86±0.43 ng Sn/g). The results of this study indicate that butyltins were detected at the CPF site in the sediment (7.33±5.70 ng Sn/g) but not the water column (not detected at 0.01 μg/l). In this study the zebra mussel was able to depurate TBT even in the presence of contaminated sediment. TBT may be bioaccumulated from the sediments. However, the initial levels in the mussels were so high, levels actually dropped as sediment–tissue equilibria levels were reached by the mussels.

J. Ruelas-Inzunza, F. Páez-Osuna. (Technological Institute of the Sea, P.O. Box 757, Mazatlán 82000, Sinaloa, Mexico. Graduate Program on Marine Sciences and Limnology, P.O. Box 811, Mazatlán 82000, Sinaloa, Mexico. Inst. Ciencias del Mar y Limnologia, Unidad Academica Mazatlan, Universidad Nacional Autonoma Mexico, Apdo Postal 811, Mazatlán 82000, Sinaloa, Mexico). **Distribution of Cd, Cu, Fe, Mn, Pb and Zn in selected tissues of juvenile whales stranded in the SE Gulf of California (Mexico)**. Environment International, 28(4) (2002), 325-329.

With the aim of knowing the concentration and distribution of essential and nonessential metals in selected tissues of whales, analysis of Cd, Cu, Fe, Mn, Pb and Zn were carried out in kidney, liver and muscle of the gray whale Eschrichtius robustus and the sperm whale Physeter catodon. Whales were found stranded in the southeast Gulf of California. Individuals were in a juvenile stage; mean length of whales was 9.3 m for E. robustus and 7 m for P. catodon. Sequence of metal concentrations was Fe>Zn>Cu>Mn>Cd>Pb in E. robustus, and Fe>Zn>Cu>Cd>Mn>Pb in P. catodon. In E. robustus, highest concentrations of Cu, Mn, Pb and Zn (17.2, 19.6, 0.9 and 388 μg g-1, respectively) were measured in liver, Cd (5.7 μg g-1) in kidney and Fe (1009 μg g-1) in muscle. In P. catodon, the highest levels of Cu, Fe and Pb (48.6, 5200 and 4.2 μg g-1, respectively) were found in liver, Cd and Zn (94 and 183 μg g-1) in kidney and Mn (8 μg g-1) in

muscle. Metal concentrations reported here were not considered to contribute to the stranding of specimens.

Jaqueline García-Hernández, Edward P. Glenn, Janick Artiola, Don J. Baumgartner. (The University of Arizona, Department of Soil, Water, and Environmental Science, College of Agriculture, 12601 E. Airport Drive, Tucson, Arizona, 85706-6895. Shantz Building, Tucson, Arizona, 85721). **Bioaccumulation of Selenium (Se) in the Cienega de Santa Clara Wetland, Sonora, Mexico**. Ecotoxicology and Environmental Safety, 46(3) (2000), 298-304.

The Cienega de Santa Clara, on the east side of the Colorado River delta, is a brackish wetland supported by agricultural drainage water from the United States that provides habitat for endangered fish and bird species. Bioaccumulation of selenium has created toxicity problems for wildlife in similar wetlands in the United States. This is the first selenium survey in the Cienega de Santa Clara. Ten sites were selected to collect water (dissolved), sediments (total), plants, invertebrates, and fish. Samples were collected from October 1996 to March 1997. Selenium was detected in all samples. Concentrations in water ranged from 5 to 19 μg/L and increased along a salinity gradient. Although water levels of selenium exceeded EPA criterion for protection of wildlife, levels in sediments (0.8–1.8 mg/kg), aquatic plants (0.03–0.17 mg/kg), and fish (2.5–5.1 mg/kg whole body, dry wt) did not exceed USFWS recommended levels. It is concluded from this study that the levels of selenium in water did not affect the overall health of the fish sampled. Therefore, it is important to maintain or improve the water quality entering this wetland to continue to have normal levels of Se in the food chain components.

Jerzy Falandysz, Krzysztof Lipka, Magdalena Gucia, Masahide Kawano, Katarzyna Strumnik, Kurunthachalam Kannan. (Department of Environmental Chemistry and Ecotoxicology, University of Gdańsk, 18 Sobieskiego Str., PL 80-952, Gdańsk, Poland. Department of Environmental Analytical Chemistry, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan. National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA). **Accumulation factors of mercury in mushrooms from Zaborski Landscape Park, Poland**. Environment International, 28(5) (2002), 421-427.

Total mercury concentrations were determined by cold-vapour atomic absorption spectroscopy (CV-AAS) in 117 samples of caps, 117 of stalks and 47 of whole fruiting bodies of 13 species of wild mushrooms and in 164 underlying soil substrate collected from Zaborski Landscape Park during 1997 and 1998. The study area is a background, forested site with rural landscape and no known local sources of mercury emission. Mean mercury concentrations in mushrooms varied widely (range: 50±20 to 3700±1700 ng/g, dry matter) depending on the site and mushroom species investigated. However, mercury concentrations in soil samples varied less (range: 3.0±3.0 to 43±17 ng/g dry matter). Fruiting bodies of Common Puffball (*Lycoperdon perlatum*) and King Bolete (*Boletus edulis*) contained the greatest concentrations of mercury of 3700±1700 and 2600±1200 ng/g dry matter, respectively. A positive correlation existed between mercury concentrations in the caps of Slippery Jack (*Suillus luteus*) and Fly Agaric (*Amanita muscaria*) ($p<0.01$) and mercury concentrations in corresponding soils. However, concentrations of mercury in The Sickener (*Russula emetica*) was negatively correlated with its soil substrate ($p<0.01$). Bioconcentration factors (BCFs: concentrations ratios of mercury in mushroom to soil) of total mercury in whole fruiting bodies or caps were greatest for Common Puffball (*L. perlatum*), Larch Bolete (*Suillus grevillei*) and King Bolete (*B. edulis*) and varied between 130±78 and 160±120, while for the other species BCFs were between 4.0±6.0 and 61±20 in caps, and 4.4±3.1 and 70±68 in stalks. The concentration ratios of Hg in cap to stalk were from 1.1±0.5 for Poison Pax (*Paxillus involutus*) to 2.7±1.7 in Larch Bolete (*S. grevillei*).

John E. Weinstein, Denise M. Sanger, A. Frederick Holland. (Department of Biology, The Citadel, Military College of South Carolina, Charleston, SC 29409, USA. South Carolina

Marine Resources Research Institute, 217 Fort Johnson Rd., Charleston, SC 29422, USA). **Bioaccumulation and toxicity of fluoranthene in the estuarine oligochaete *Monopylephorus rubroniveus*.** Ecotoxicology and Environmental Safety, 55(3) (2003), 278-286.

The tolerance of the estuarine oligochaete *Monopylephorus rubroniveus* to fluoranthene was characterized both in the presence and absence of ultraviolet (UV) radiation. Using waterborne exposures, the 72-h median lethal concentration (LC_{50}) and median lethal dose (LD_{50}) were 0.7 (95% CI, 0.4–0.8) μg/L and 8.0 (5.6–9.6) μg/g worm dry weight, respectively, in the presence of UV radiation [UV-A=64.7±1.0 $\mu W/cm^2$ (mean±standard deviation)]. In the absence of UV radiation, little mortality was observed, even at the water solubility limits of fluoranthene (120.4 μg/L). Mean bioconcentration factors across all treatments was 10,893±2828. Using sediment exposures, little mortality was observed following 10 days at concentrations as high as 3912 μg fluoranthene/g sediment dry weight in both the presence of UV radiation (UV-A=108.4±1.3 $\mu W/cm^2$) and its absence. Bioaccumulation of sediment-associated fluoranthene was comparatively high and varied little among the five sediment treatments. The results of the present study demonstrate that *M. rubroniveus* is (1) sensitive to waterborne fluoranthene in the presence of UV radiation and (2) highly tolerant of fluoranthene in the presence of sediment, despite the ability to bioaccumulate fluoranthene to comparatively high levels. These findings suggest that those environmental factors which could potentially increase their exposure to UV radiation need to be considered when assessing the overall risk of fluoranthene to *M. rubroniveus*.

K. Borgå, M. Poltermann, A. Polder, O. Pavlova, B. Gulliksen, G. W. Gabrielsen, J. U. Skaare. (Norwegian Polar Institute, N-9296 Tromsø, Norway. Norwegian College of Fishery Science, University of Tromsø, N-9037 Tromsø, Norway. Norwegian School of Veterinary Science, PO Box 8146, N-0033 Oslo, Norway. UNIS, PO Box 156, 9170 Longyearbyen, Norway. National Veterinary Institute, PO Box 8156 Dep., N-0033 Oslo, Norway). **Influence of diet and sea ice drift on organochlorine bioaccumulation in Arctic ice-associated amphipods**. Environmental Pollution, 117(1) (2002), 47-60.

The drifting sea ice has been suggested as important in the transport and concentration of organic matter and pollutants in the Arctic. We collected sea ice-associated amphipods in the marginal ice zone north of Svalbard and in the Fram Strait in September 1998 and 1999 to assess contaminant accumulation in ice-associated organisms. Organochlorine concentrations increased from the more herbivorous *Apherusa glacialis* to the more carnivorous *Gammarus wilkitzkii* and the more necrophagous *Onisimus* spp. The relative contribution of compound classes to the sum of organochlorines differed between the amphipod families, with a higher relative contribution of hexachlorocyclohexanes (HCHs) in *A. glacialis*. The composition of the compound classes HCHs, chlordanes and dichlorodiphenyltrichloroethanes (DDTs) was similar between the amphipod families, whereas the profiles of polychlorinated biphenyls (PCBs) differed. The occurrence of organochlorines differed spatially, with higher α-HCH concentrations in amphipods from the Fram Strait in comparison with amphipods collected north of Svalbard. This could be related to the sea ice drift route, since sea ice in the Fram Strait had a drift route across the central Arctic Ocean, while the sea ice north of Svalbard had a western drift route to the sampling stations. Even though marine invertebrates have direct uptake by passive diffusion of contaminants across their gills, our results imply that the species' ecology such as diet is important in the bioaccumulation process of organic pollutants. In addition, the results show that sea ice drift route influences the concentrations of organochlorine pollutants in ice-associated organisms.

K. Saeki, M. Nakajima, T. R. Loughlin, D. C. Calkins, N. Baba, M. Kiyota, R. Tatsukawa. (Department of Environmental Conservation, Ehime University, Tarumi 3-5-7, Matsuyama, Ehime, 790, Japan. National Marine Mammal Laboratory, 7600 Sand Point Way NE, Seattle, Washington 98115-0070, USA. Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99502, USA. National Research Institute of Far Seas Fisheries, Orido 5-7-1, Shimizu, Shizuoka 424-8633, Japan). **Accumulation of silver in the liver of three species of pinnipeds**. Environmental Pollution, 112(1) (2001), 19-25.

Silver in the three species of pinnipeds [northern fur seal (*Callorhinus ursinus*), Steller sea lion (*Eumetopias jubatus*), and harbor seal (*Phoca vitulina*)] caught in the North Pacific Ocean were analyzed using inductively coupled plasma-mass spectrometry, in order to understand accumulation and distribution of silver in pinnipeds. In northern fur seals, relatively high concentrations of silver were observed in the liver and body hair. Some 70% of the silver burden was concentrated in the liver. Hepatic silver concentrations were significantly correlated to age in northern fur seals (r=0.766, P<0.001, n= 49) and Steller sea lions (r=0.496, P<0.01, n=28). Levels of silver concentrations per wet weight ($\mu g\ g^{-1}$) in the three pinnipeds ranged from 0.04 to 0.55 for northern fur seals, from 0.1 to 1.04 for Steller sea lions and from 0.03 to 0.83 for harbor seals. Silver concentrations in liver for all pinnipeds were significantly correlated with mercury, and selenium (P<0.001). Molar ratios between silver to selenium approximated 1:180 in northern fur seals, 1:120 in Steller sea lions, and 1:60 in harbor seals. The silver–mercury molar ratios were approximately 1:170 in northern fur seals, and 1:80 both in the other species. Increase in silver accumulation in the liver was caused by the retention in nuclei and mitochondria fraction together with mercury and selenium in the cells of northern fur seals.

M. Barwick, W. Maher. (Ecochemistry Laboratory, Applied Ecology Group, University of Canberra, Bruce, ACT 2601, Australia). **Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia**. Marine Environmental Research, 57(3) (2004), 171-195.

In this study the biotransference of selenium copper, cadmium, zinc, arsenic and lead was measured in a contaminated seagrass ecosystem in Lake Macquarie, NSW, Australia, to determine if biomagnification of these trace metals is occurring and if they reach concentrations that pose a threat to the resident organisms or human consumers. Selenium was found to biomagnify, exceeding maximum permitted concentrations for human consumption within carnivorous fish tissue, the highest trophic level examined. Selenium concentrations measured within carnivorous fish were also above those shown to elicit sub-lethal effects in freshwater fish. As comparisons are made to selenium concentrations known to effect freshwater fish, inferences must be made with caution. There was no evidence of copper, cadmium, zinc or lead biomagnification within the food web examined. Copper, cadmium, zinc and lead concentrations were below concentrations shown to elicit adverse responses in biota. Copper concentrations within crustaceans M. bennettae and P. palagicus were found to exceed maximum permitted concentrations for human consumption. It is likely that copper concentrations within these species were accumulated due to the essential nature of this trace metal for many species of molluscs and crustaceans. Arsenic showed some evidence of biomagnification. Total arsenic concentrations are similar to those found in other uncontaminated marine ecosystems, thus arsenic concentrations are unlikely to cause adverse effects to aquatic organisms. Inorganic arsenic concentrations are below maximum permitted concentrations for human consumption.

M. Concepción Contreras López. (University of the Basque Country, Faculty of Sciences, Leioa, Bizkaia, Spain). **Determination of potentially bioaccumulating complex mixtures of organochlorine compounds in wastewater: a review.** Environment International, 28(8) (2003), 751-759.

Organic chlorine compounds can be persistent environmental contaminants and may be accumulated through the food chain to the aquatic organisms, to fish and humans, depending basically on their hydrophobic properties. Consequently, there is an interest to measure these organic compounds from both the scientific and regulatory communities. The analytical essays have been improved for measuring specific organic chlorine compounds that present the most toxicological potential (polychlorinated biphenyls [PCBs], certain pesticides and dioxins), although they are tedious and time-consuming procedures. The existing tests to measure adsorbable organic halogens (AOX) or extractable organic halogens (EOX) do not distinguish the more hydrophobic organic chlorine matter. The intention of this paper is to make a review of the existing methods to measure the potentially bioaccumulating organochlorine compounds (OCs) from wastewater and propose a methodology to a standardisation procedure for complex mixtures of OCs in wastewater, such as pulp mill effluents. A new method has been proposed for determining the most hydrophobic part of the extractable organic halogens (EOX_{fob}), the lowest reported value is 0.6 µg/l, expressed as chloride, and the relative standard deviation at 20 µg/l is 7% on laboratory samples and 30% on real effluents. This new procedure could be a valuable tool to complement environmental risk assessment studies of wastewater discharges.
M. Kantola, R. Purkunen, P. Kröger, A. Tooming, J. Juravskaja, M. Pasanen, S. Saarikoski, T. Vartiainen. (Department of Chemistry, University of Kuopio, FIN-70211, Kuopio, Finland. Department of Environmental Sciences, University of Kuopio, FIN-70211, Kuopio, Finland. Department of Chemistry, National Public Health Institute, 95, FIN-70701, Kuopio, Finland. Central Hospital of Rakvere, Rakvere, Estonia. Institute for Advanced Training of Physicians, St. Petersburg, Russia. Department of Pharmacology and Toxicology, University of Oulu and National Agency for Medicines, Oulu, Finland. Clinic of Gynaecology and Obstetrics, University Hospital of Kuopio, Kuopio, Finland). **Accumulation of Cadmium, Zinc, and Copper in Maternal Blood and Developmental Placental Tissue: Differences between Finland, Estonia, and St. Petersburg.** Environmental Research, 83(1) (2000), 54-66.

Cadmium, zinc, and copper from placental tissue and blood samples at the first trimester (*n*=64) and at term (*n*=152) were analyzed; the welfare of newborns and placental 7-eothoxycoumarin *O*-deethylase (ECOD) activities *in vitro* were determined. The study material was collected from Finland, Estonia, and Russia. The results demonstrate that Cd starts to accumulate in the placenta during the first trimester and that Zn and Cu contents were significantly higher at the first trimester than at term. Among nonsmokers a negative correlation was found between placental Cu content and birth weight of neonates. Among smokers a positive correlation between placental Zn content and birth weight and ECOD activity was found. The birth weights correlated inversely with the length of time the mothers smoked. The highest Cd concentrations were detected in the samples collected from St. Petersburg. The data demonstrate an inverse accumulation of Zn and Cd throughout the pregnancy in the placenta and maternal blood samples. Zn may act as a positive marker or even an enzymatic enhancement for the human placental vital functions. Smoking, parity, age, and especially the place of residence affect the Cd, Zn, and Cu contents and ratios in placenta and mother's blood.
M. -L. Bouché, F. Habets, S. Biagianti-Risbourg, G. Vernet. (Laboratoire d'Éco-toxicologie, Faculté des Sciences, Université de Reims Champagne-Ardenne, Bâtiment Europol'Agro, 1039, F-51687, Reims cedex 2, France. Laboratoire de Toxicologie des Métaux, Faculté de Pharmacie, Université de Reims Champagne-Ardenne, 51 rue Cognacq Jay, 51096, Reims cedex, France). **Toxic Effects and Bioaccumulation of Cadmium in the Aquatic Oligochaete *Tubifex tubifex***. Ecotoxicology and Environmental Safety, 46(3) (2000), 246-251.

Although *Tubifex tubifex* (Oligochaeta, Tubificida) has been proposed as a test organism for ecotoxocological studies, very few data concerning sublethal toxicity and bioaccumulation are available on this worm. The aims of this work were to assess the toxicity of cadmium, one of the most toxic metals frequently encountered in polluted areas, on *T. tubifex* and the ability of the worm to accumulate this metal. Acute toxicity was analyzed by measurement of the 96-h LC_{50} and daily survival rates. Results indicated that *T. tubifex* undergoes an adaptation period to Cd, the duration of which decreases with increasing Cd concentration. The various parameters affecting toxicity are discussed. Sublethal toxicity was studied by scanning electron microscopy. Observations revealed that Cd induced autotomy of the caudal region and mucus production. Autotomy is proposed as a criterion for sublethal toxicity. The results of bioaccumulation studies revealed that Cd is highly and rapidly taken up by the worm, suggesting involvement of efficient detoxification mechanisms. Consequently, the ability to accumulate large amounts of Cd may represent a potential toxicological risk to predators of the worm if Cd is accumulated in bioavailable forms.

M. Oudeh, M. Khan, J. Scullion. (Faculty of Agriculture, Al-Baath University, PO Box 77, Homs, Syria. World Wide Fund for Nature Pakistan, PO Box 5180, Ferozepur Road Lahore-54600, Pakistan. Soil Science Unit, University of Wales, Penglais, Aberystwyth SY23 3DE, UK). **Plant accumulation of potentially toxic elements in sewage sludge as affected by soil organic matter level and mycorrhizal fungi.** Environmental Pollution, 116(2) (2002), 293-300.

Leek (*Allium ameloprasum*) was grown in pot trials in two clay loams of contrasting organic contents, with and without indigenous mycorrhizal propagules. Sewage sludges containing varying levels of Cd, Cu and Zn were added. Extractable soil metals, plant growth, major nutrient content and accumulation of metals, and soil microbial indices were investigated. The aim was to establish whether soil organic content and mycorrhizal status affected plant and microbial exposure to these metals. Extractable metals were higher and responses to inputs more pronounced in the arable, lower organic matter soil, although only Cd showed a soil difference in the $CaCl_2$ fraction. There were no metal toxic effects on plants and some evidence to suggest that they promoted growth. Uptake of each metal was higher in the larger plants of the grassland, higher organic matter soil. Inoculation with arbuscular mycorrhizal fungi increased root Cd and Zn concentrations. With the exception of Cd (roots) and Zn (shoots), higher inputs of sludge metals did not increase plant metals. Zn and Cu, but not Cd, concentrations were higher in roots than in shoots.

M. Soto-Jiménez, F. Páez-Osuna, F. Morales-Hernández. (Programa de Posgrado en Ciencias del Mar y Limnología, Unidad Académica Mazatlán, UNAM, Mexico. Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, UNAM, Apdo Postal 811, Mazatlán 82000, Sinaloa, Mexico). **Selected trace metals in oysters (*Crassostrea iridescens*) and sediments from the discharge zone of the submarine sewage outfall in Mazatlán Bay (southeast Gulf of California): chemical fractions and bioaccumulation factors**. Environmental Pollution, 114(3) (2001), 357-370.

Concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the soft tissue of *Crassostrea iridescens* and the associated surface sediments (bulk and bioavailable metal concentrations) from an area influenced by a sewage outfall in Mazatlán Bay (southeast Gulf of California), were determined by atomic absorption spectrophotometry. Significant spatial differences in metal concentrations in both the bulk and bioavailable forms in the sediments were identified. An enrichment of Cu, Ni, Pb and Zn in sites located on a south–north transect was detected indicating a dominant influence of the sewage outfall toward the north. *C. iridescens* accumulated more Zn, Cu, Ni, Fe, Cd; and less Mn, Cr and Pb than were bioavailable in the sediments, as measured using

conventional extraction analysis. The degree of enrichment and the bioavailable metal concentrations in the sediments of the south portion of Mazatlán Bay is discussed. The potential ability of *C. iridescens* as a biomonitor of metallic pollutants is postulated.

M. Topashka- Ancheva, R. Metcheva, S. Teodorova. (Institute of Zoology, Bulgarian Academy of Sciences, Bd.Tzar Osvoboditel 1, Sofia 1000, Bulgaria. Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko shaussee, Sofia 1784, Bulgaria). **Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice *Mus musculus alba* II. Genetic, cell, and metabolic disturbances.** Environmental Research, 92(2) (2003), 152-160.

An ecologo–toxicological experiment was carried out with laboratory mice *Mus musculus alba* of the inbred line BALB/c. The experimental animals (male and female) were exposed for 120 days to polymetal industrial dust containing zinc, copper, lead, and cadmium, which was mixed with conventional animal food. Chromosome aberration frequency and pathological changes in hematological indices, oxygen consumption, body temperature, and body weight were studied in the context of heavy metal bioaccumulation and interactions. Samples for analyses were taken on days 15, 40, 60, and 90. An increased frequency of chromosome aberrations (up to 22%), lead-induced anemia, and significant decreases in body temperature were observed. A strong correlation between hemoglobin content and oxygen consumption (O_2/ g h) was established. An increase in hematocrit, accompanied by a loss of body weight after day 60, suggests dehydration resulting from lead and cadmium poisoning.

Md. Zahangir Alam, A. Fakhru'l-Razia, Abul H. Mollaa. (Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia. Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100, Kuala Lumpur, Malaysia). **Biosolids accumulation and biodegradation of domestic wastewater treatment plant sludge by developed liquid state bioconversion process using a batch fermenter**. Water Research, 37(16) (2003), 3967-3977.

The biosolids accumulation and biodegradation of domestic wastewater treatment plant (DWTP) sludge by filamentous fungi have been investigated in a batch fermenter. The filamentous fungi Aspergillus niger and Penicillium corylophilum isolated from wastewater and DWTP sludge was used to evaluate the treatment performance. The optimized mixed inoculum (A. niger and P. corylophilum) and developed process conditions (co-substrate and its concentration, temperature, initial pH, inoculum size, and aeration and agitaion rate) were incorporated to accelerate the DWTP sludge treatment process. The results showed that microbial treatment of higher strength of DWTP sludge (4% w/w of TSS) was highly influenced by the liquid state bioconversion (LSB) process. In developed bioconversion processes, 93.8 g/kg of biosolids was enriched with fungal biomass protein of 30 g/kg. Enrichment of nutrients such as nitrogen (N), phosphorous (P), potassium (K) in biosolids was recorded in 6.2% (w/w), 3.1% (w/w) and 0.15% (w/w) from its initial values of 4.8% (w/w), 2.0% (w/w) and 0.08% (w/w) respectively after 10 days of fungal treatment. The biodegradation results revealed that 98.8% of TSS, 98.2% of TDS, 97.3% of turbidity, 80.2% of soluble protein, 98.8% of reducing sugar and 92.7% of COD in treated DWTP sludge supernatant were removed after 8 days of microbial treatment. The specific resistance to filtration (SRF) in treated sludge (1.4×1012 m/kg) was decreased tremendously by the microbial treatment of DWTP sludge after 6 days of fermentation compared to untreated sample (85×1012 m/kg).

Michaël Cœurdassier, Annette Gomot-de Vaufleury, Christiane Lovy, Pierre-Marie Badot. (Laboratoire de Biologie et Ecophysiologie (EA 3184 MRT/INRA), Institut des Sciences et Techniques de l'Environnement, Université de Franche-Comté, Place Leclerc, 25030, Besançon cedex, France). **Is the cadmium uptake from soil important in**

bioaccumulation and toxic effects for snails? Ecotoxicology and Environmental Safety, 53(3) (2002), 425-431.

To evaluate the contribution of digestive and epithelial transfer of Cd from soil to snail, *Helix aspersa* was exposed to an artificial substrate contaminated with 0,100,500, and 1000 $\mu gCd \cdot g^{-1}$ for 4 weeks under laboratory conditions. Two modes of exposure were used: (1) the snails were in direct contact with the substrate (DC) or (2) were separated from substrate with a perforated plate (no contact (NC)) which allowed ingestion of substrate but avoided epithelial contact. Cd concentrations in DC snails were twice as high as in NC snails. The bioaccumulation factors were 0.51±0.13 in DC snails and 0.26±0.04 in NC snails. Dose-dependent growth inhibition was noted in DC snails ($500<EC_{50}<1000$ $\mu g \cdot g^{-1}$ and EC_{10} around 100 $\mu g \cdot g^{-1}$) while only slight effects were noted in NC snails. Internal Cd concentrations in DC and NC snails were linearly correlated with growth effect whatever the contamination route.
N. R. Verrengia Guerrero, M. G. Taylor, E. A. Wider, K. Simkiss. (Biomarkers Lab., Dept. of Biological Chemistry, Faculty of Exact and Natural Sciences, University of Buenos Aires, 4 piso, Pab. II, 1428, Buenos Aires, Argentina. School of Animal and Microbial Sciences, The University of Reading, PO Box 228, Reading RG6 6AJ, UK). **Influence of particle characteristics and organic matter content on the bioavailability and bioaccumulation of pyrene by clams.** Environmental Pollution, 121(1) (2003), 115-122.

Hydrophobic chemicals are known to associate with sediment particles including those from both suspended particulate matter and bottom deposits. The complex and variable composition of natural particles makes it very difficult therefore, to predict the bioavailability of sediment-bound contaminants. To overcome these problems we have previously devised a test system using artificial particles, with or without humic acids, for use as an experimental model of natural sediments. In the present work we have applied this experimental technique to investigate the bioavailability and bioaccumulation of pyrene by the freshwater fingernail clam *Sphaerium corneum*. The uptake and accumulation of pyrene in clams exposed to the chemical in the presence of a sample of natural sediment was also investigated. According to the results obtained, particle surface properties and organic matter content are the key factors for assessing the bioavailability and bioaccumulation of pyrene by clams.
Naim Sezgin, H. Kurtulus Ozcan, Goksel Demir, Semih Nemlioglu, Cuma Bayat. (Faculty of Engineering, Department of Environmental Engineering, Istanbul University, 34850 Avcilar, Istanbul, Turkey). **Determination of heavy metal concentrations in street dusts in Istanbul E-5 highway**. Environment International, 29(7) (2004), 979-985.

Components and quantity of street dust are environmental pollution indicators especially in big cities. Street dust is generally composed of car exhaust gas originated particles and wind-transported particles. Heavy metals, which are found in street dust, such as Pb, Cu, Mn, Zn, Cd and Ni are significant for environmental pollution. According to the kind of vehicle in traffic, quantity and type of heavy metals vary in street dust. The use of leaded gasoline gives a boost to the importance of lead level especially in street dust even at the start of 21st century. These metals possess bioaccumulation property, and the possibility of the amount of these metals reaching a critical value and threatening human health increases the importance of this issue. In this study, street dusts have been collected from E-5 Highway from Topkapi to Avcilar regions that spans about 18 km in Istanbul, Turkey, and Pb, Cu, Mn, Zn, Cd and Ni concentrations have been detected in street dust. Twenty-two street dust samples were taken from a total of 22 different points at previously decided 14 main areas. Analyses were conducted using Leeds Public Analyst method. According to the results of this study, Pb, Cu and Zn concentrations in E-5 Highway between Topkapi and Avcilar region in Istanbul were higher than maximum

concentration levels of these heavy metals in normal soil. This situation indicates that there is heavy metal pollution in the inspected area in E-5 Highway in Istanbul.

Nakajima Akira. (Department of Chemistry, Faculty of Medicine, Miyazaki Medical College, Kiyotake, Miyazaki 889□16, Japan). ***Accumulation of gold by microorganisms*** . Journal of Microbiology and Biotechnology, 19(4) (2003), 369-374.

Thirty species of microorganisms (8 bacteria, 9 actinomycetes, 8 fungi and 5 yeasts) were screened for maximal gold accumulation. Extremely high abilities to accumulate gold from a solution containing hydrogen tetrachloroaurate(III) were found in bacterial strains, such as *Escherichia coli* and *Pseudomonas maltophilia*. Most of the actinomycetes, fungi and yeasts had lower ability to accumulate gold than bacteria. Some microorganisms could accumulate similar amounts of gold from a solution containing sodium gold(I) thiomalate as those from gold(III) solution. However, most microorganisms tested accumulated far lesser amounts of gold from a solution containing sodium dicyanoaurate(I) than from the other two gold solutions. The accumulation of gold from the solution containing hydrogen tetrachloroaurate(III) by *Pseudomonas maltophilia* was very rapid, was affected by the pH of the solution, and obeyed the Langmuir adsorption isotherm. *Pseudomonas maltophilia* cells immobilized in polyacrylamide gel adsorbed gold effectively from the solution containing hydrogen tetrachloroaurate(III). The gold adsorbed on the cells was easily desorbed with 0.1 M thiourea solution. The immobilized *Pseudomonas* cells could be used repeatedly in the adsorption–desorption cycle using 0.1 M thiourea solution as desorbent.

O. S. Okay, P. Donkin, L. D Peters, D. R Livingstone. (TÜBİTAK, MRC, Energy Systems and Environmental Research Institute, PO Box 21, 41470, Gebze, Kocaeli, Turkey. CCMS, Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK. CCMS, Plymouth Marine Laboratory, Citadel Hill, Plymouth PL1 2PB, UK). **The role of algae (*Isochrysis galbana*) enrichment on the bioaccumulation of benzo[a]pyrene and its effects on the blue mussel *Mytilus edulis.*** Environmental Pollution, 110(1) (2000), 103-113.

The role of algal concentration in the transfer of organic contaminants in a food chain has been studied using the ubiquitous model polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP) as the contaminant, *Isochrysis galbana* as the phytoplankton food source, and the common mussel (*Mytilus edulis*) as the primary consumer. The effect of algal concentration on BaP uptake by *M. edulis* was determined by feeding *M. edulis* daily with *I. galbana* which had previously been kept in the presence of BaP for 24 h. Four combinations of concentrations of algae and BaP were used to give final exposure concentrations of 30,000 or 150,000 algal cells ml^{-1} in combination with either 2 or 50 μg BaP l^{-1}. BaP concentrations were determined fluorometrically in rest tissues (excluding digestive glands) and digestive gland microsomal fractions of *M. edulis* after 1, 7 and 15 days exposure, and also in isolated algae. Potentially toxic effects of BaP on *M. edulis* were examined in terms of blood cell lysosomal membrane damage (neutral red dye retention assay) and induction of digestive gland microsomal mixed-function oxygenase (MFO) parameters [BaP hydroxylase (BPH) and NADPH-cytochrome c (P450) reductase activities]. BaP bioaccumulation in rest tissues (and to a lesser extent in digestive gland microsomes) of *M. edulis* increased with both increasing BaP and algal exposure concentrations, and over time, producing maximal bioconcentration factors in rest tissues after 15 days exposure to 150,000 algal cells ml^{-1} and 50 μg BaP l^{-1} of 250,000. The five-fold higher concentration of algae increased BaP bioaccumulation by a factor of approximately 2 for 50 μg BaP l^{-1} at day 15. Blood cell neutral red dye retention time decreased linearly with increasing log_{10} tissue BaP body burden, indicating an increased biological impact on *M. edulis* with increasing BaP exposure possibly due to a direct effect of BaP on blood cell lysosomal membrane integrity. An increase

was seen in NADPH-cytochrome c reductase activity, and indicated in BPH activity, with 1 but not 7 or 15 days exposure to BaP, indicating a transient response of the digestive gland microsomal MFO system to BaP exposure.

P. Adamo, S. Giordano, S. Vingiani, R. Castaldo Cobianchi and P. Violante. (Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli Federico II, Via Università, 100 80055, Portici (NA), Italy. Dipartimento di Biologia Vegetale, Università di Napoli Federico II, Via Foria, 223 80139, Napoli, Italy). **Trace element accumulation by moss and lichen exposed in bags in the city of Naples (Italy).** Environmental Pollution, 122(1) (2003), 91-103.

This paper presents the results of a bioaccumulation study of trace elements in the Naples urban area based on the use of the moss *Sphagnum capillifolium* (Ehrh.) Hedw. and the lichen *Pseudevernia furfuracea* (L.) Zopf exposed in bags in 23 sites. Moss and lichen bags were exposed for 4 months starting from the beginning of July 1999. Bags gathering was carried out after 10 weeks of exposure, at the end of the dry season, and after 17 weeks, during the wet season. The elements Al, As, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Mo, Ni, Pb, Ti, V and Zn were analysed by inductively coupled plasma–mass spectrometry ICP–MS in both biomonitors. For the majority of the elements the total amounts found in *S. capillifolium* were higher than in *P. furfuracea* whether considering the whole period of exposure or the weekly uptake. It was observed that there was a much greater difference in metal accumulation by *P. furfuracea* between the dry and wet seasons compared with *S. capillifolium.* In the wet period, the lichen seems to accumulate a larger quantity of metals. With the exception of Mn, trace element concentrations did not appear to be significantly affected by the washing away of rainfall. K loss during exposure suggested cell membrane damage in both organisms. For *P. furfuracea* the K leakage was limited to the dry period of exposure. A clear distinction between "lithophilic" and "anthropogenic" elements was achieved by cluster analysis. Significant correlations were found among Fe–Cu–Cr–Ni, Pb–Cd–Co, V–Cr–Ni, Zn–Ni–Pb, suggesting a common source for each group of elements.

P. E. Karlsson, E. L. Medin, G. Selldén, G. Wallin, S. Ottosson, H. Pleijel, L. Skärby. (Swedish Environmental Research Institute (IVL), PO Box 47086, S-402 58 Göteborg, Sweden. Botanical Institute, Göteborg University, Box 461, S-405 30, Göteborg, Sweden. Institute of Environmental Science and Conservation, Göteborg University, Box 464, SE-405 30 Göteborg, Sweden). **Impact of ozone and reduced water supply on the biomass accumulation of Norway spruce saplings.** Environmental Pollution, 119(2) (2002), 237-244.

Norway spruce saplings [*Picea abies* (L.) Karst.] were exposed during four growing seasons to two different ozone treatments in open-top chambers: charcoal filtered air (CF), and non-filtered air with extra ozone (NF+, 1.4×ambient concentrations). Within each ozone treatment the saplings were either kept well watered or treated with a 7–8 week period with reduced water supply each growing season. The total biomass of the trees was measured in April and September during each of the last three growing seasons. NF+ significantly reduced the total biomass accumulation of Norway spruce saplings during the fourth growing season. No interaction between ozone and reduced water supply could be detected. The magnitude of the ozone impact after 4 years of exposure was an 8% reduction of the total plant biomass and a 1.5% reduction of the RGR. The reduced water supply reduced the total biomass 29% and the RGR 12%.

P. F. Chaton, P. Ravanel, M. Tissut, J. C. Meyran. (Laboratoire Ecosystèmes et Changements Environnementaux, Centre de Biologie Alpine, Université Joseph Fourier, 53, F-38041, Grenoble Cedex 9, France). **Toxicity and Bioaccumulation of Fipronil in the**

Nontarget Arthropodan Fauna Associated with Subalpine Mosquito Breeding Sites. Ecotoxicology and Environmental Safety, 52(1) (2002), 8-12.

In order to examine ecological impact of fipronil use for larval culicine control in natural hydrosystems, toxicity and bioaccumulation of this new insecticide were analyzed on aquatic species representative of the nontarget arthropodan fauna (nonculicine larval Diptera: Chaoboridae, Chironomidae; planktonic Crustacea: Cladocera, Copepoda, Ostracoda) associated with target larval mosquito populations in the subalpine breeding sites. Standard toxicological bioassays using fipronil aqueous solutions from 1 to 2000 nM indicated different sensitivity levels among species. Insecticide bioaccumulation analyses, using [^{14}C]fipronil solutions in simplified laboratory ecosystem, also indicated large differences among species. These differences may come from biological parameters characteristic of each species. Taking into account these nontarget effects of fipronil, a possible strategy of use of this insecticide for integrated mosquito control management was proposed, which is based upon selective dietary absorption of the insecticide by larval Culicidae.

P. Waranusantigul, P. Pokethitiyook, M. Kruatrachue, E. S. Upatham. (Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand. Mahidol University International College, Mahidol University, Nakhonpathom 73170, Thailand. Faculty of Science, Burapha University, Chonburi 20131, Thailand). **Kinetics of basic dye (methylene blue) biosorption by giant duckweed (*Spirodela polyrrhiza*).** Environmental Pollution, 125(3) (2003), 385-392.

Wastewater containing pigments and/or dyes can cause serious water pollution problems in the form of reduced light penetration and photosynthesis, and the toxicity from heavy metals associated with pigments and/or dyes. Laboratory investigations, of the potential use of dried *Spirodela polyrrhiza* biomass as an adsorbent for the removal of the basic dye methylene blue from aqueous solution were conducted. A series of experiments were undertaken in an agitated batch adsorber to assess the effect of the system variables, i.e. sorbent dosage, pH, and contact time. The results showed that as the amount of the dried *S. polyrrhiza* increased, the percentage of dye sorption increased accordingly. At pH 2.0 the sorption of dye was not favorable, while the sorption at other pHs (3.0–11.0) was remarkable. There was no significant difference in the dye concentration remaining when the pH was increased from 3.0 to 11.0. The dye removal time was influenced by the initial dye concentration, and the process followed the first-order rate kinetics. The rate constants for intraparticle diffusion were 1.00 and 3.27 mg/g/min$^{1/2}$ for 300 and 500 mg/l of dye, respectively.

Piyush Kant Pandey, Sushma Yadav, Sumita Nair, Ashish Bhui. (Department of Engineering Chemistry, Bhilai Institute of Technology, Bhilai House Durg, CG, Bhilai, MP 491002, India). **Arsenic contamination of the environment- A new perspective from central-east India**. Environment International, 28(4) (2002), 235-245.

This paper reports a regional contamination of the environment in central-east India that does not share geology or boundary with the Bengal Delta Plain. About 30,000 people residing in 30 villages and towns are directly exposed to arsenic and more than 200,000 people are "at risk." Complete geographical extent of this contamination is being established, and this newly reported contaminated area could be quite large. This paper further reports that the mechanisms involved in arsenic mobilisation are complex and the two theories of arsenic mobilisation, i.e., pyrite oxidation and oxyhydroxides reduction, do not fully explain the high levels of arsenic contamination. This paper also proposes the "oxidation–reduction theory" for arsenic mobilisation where the arsenic originates from the arsenopyrite oxidation and the arsenic thus mobilised forms the minerals and gets reduced underground in favourable Eh conditions. The stoppage of water withdrawal from the contaminated sources did not result in lowering of arsenic

levels as expected according to the heavy groundwater extraction theory (pyrite oxidation theory). Cases of arsenicosis in the region are on the rise and the switchover to less contaminated water has not reversed the arsenicosis progression in the affected persons even after 2 years. Surface water of the rivers is also being contaminated because of the probable dislocation of contaminated groundwater due to the heavy rains in monsoon season, which indicates that the river water could be a major carrier of arsenic in dissolved or adsorbed forms that may be a cause of contamination of the delta plains.

R. Kubota, T. Kunito, S. Tanabe. (Center for Marine Environmental Studies (CMES), Ehime University, Tarumi 3-5-7, Matsuyama 790-8566, Japan). **Arsenic accumulation in the liver tissue of marine mammals.** Environmental Pollution, 115(2) (2001), 303-312.

Arsenic concentrations were determined in livers of 226 individuals representing 16 different marine mammal species to elucidate its accumulation with age, sex, and feeding habits. Arsenic concentrations varied widely among species and individuals, and ranged from <0.10 to 7.68 g g^{-1} dry weight. Marine mammals feeding on cephalopods and crustaceans contained higher arsenic concentrations than those feeding on fishes. No significant gender difference in arsenic concentration was found for almost all the species. Also, no apparent trend with age (or body length) in arsenic accumulation was found for most of the species. It was noted that two seal species, Baikal seal and Caspian seal, from landlocked water environments, contained lower arsenic concentrations than the marine species. To our knowledge, this is the first comprehensive study of arsenic accumulation in a wide range of marine mammal species.

R. Metcheva, S. Teodorova, M. Topashka-Ancheva. (Institute of Zoology, Bulgarian Academy of Sciences, Bd. Tzar Osvoboditel 1, 1000, Sofia, Bulgaria. Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko shausse, 1784, Sofia, Bulgaria). **A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria I: monitoring points and bioaccumulation features.** Ecotoxicology and Environmental Safety, 54(2) (2003), 176-187.

Data on liver and body copper, zinc, lead, and cadmium content of small mammals (rodents and insectivorous) were collected and analyzed. Data comparisons were performed in two aspects: (1) points and years of monitoring; (2) monitor species bioaccumulations. Specific bioaccumulation features were observed in some of the monitor species. A method for comparative evaluation of heavy metal loads in the different species is proposed using data for liver and body contamination. The loads of *Clethrionomys glareolus* and *Apodemus flavicollis* were compared, and the data are in agreement with data from other authors in Central Europe. A correlation between heavy metal content in the food and liver of snow vole was established. The data demonstrate that two of the regions investigated in Rila Mountain National Park could be assumed to be background locations. Some possible reasons for the heavy metal contamination of the low-altitude region in Rila are discussed. Not very significant pollution was observed around industrial facilities. Correlations between heavy metal levels in zoomonitors and meteorological factors were established.

R. Scheifler, A. Gomot-de Vaufleury, P. -M. Badot. (Université de Franche-Comté, Institut des Sciences et Techniques de l'Environnement, Laboratoire de Biologie et Ecophysiologie, EA 3184 MRT USC INRA, Place Leclerc, F-25030, Besançon Cedex, France). **Transfer of Cadmium from Plant Leaves and Vegetable Flour to the Snail *Helix aspersa:* Bioaccumulation and Effects.** Ecotoxicology and Environmental Safety, 53(1) (2002), 148-153.

Juvenile *Helix aspersa* snails were exposed for 4 weeks to fresh rape leaves (Brassica napus), contaminated by simulating superficial deposits of increasing concentrations of cadmium (Cd).

The Cd concentration in leaves was 0.1 in control and 38.4, 93.1, and 177.2 $\mu g.g^{-1}$ (dry mass) in Cd-treated food. The concentration in snail tissues increased with increasing Cd concentrations in the food. The bioaccumulation factors ranged from 4.8 (control) to 2.4 (highest exposed group), indicating a biomagnification of Cd in this food chain. The growth of the snails treated with Cd was reduced by 17, 24, and 43% respectively, compared to the control group. Comparison of these results with those obtained with snails exposed to similar Cd concentrations in a vegetable flour revealed that accumulation and effects were relatively consistent, demonstrating a comparable bioavailability of Cd in the two diets. Tests using growing H. aspersa snails exposed to metals in flour or fresh leaves can be useful for risk assessment purposes.

Ron van der Oost, Jonny Beyer, Nico P. E. Vermeulen. (Department of Environmental Toxicology, OMEGAM Environmental Research Institute, PO Box 94685, 1090 GR, Amsterdam, The Netherlands. Department of Marine Environment, RF-Rogaland Research, Stavanger, Norway. Department of Molecular Toxicology, Vrije Universiteit, Amsterdam, The Netherlands). **Fish bioaccumulation and biomarkers in environmental risk assessment: a review**. Environmental Toxicology and Pharmacology, 13(2) (2003), 57-149.

In this review, a wide array of bioaccumulation markers and biomarkers, used to demonstrate exposure to and effects of environmental contaminants, has been discussed in relation to their feasibility in environmental risk assessment (ERA). *Fish bioaccumulation markers* may be applied in order to elucidate the aquatic behavior of environmental contaminants, as bioconcentrators to identify certain substances with low water levels and to assess exposure of aquatic organisms. Since it is virtually impossible to predict the fate of xenobiotic substances with simple partitioning models, the complexity of bioaccumulation should be considered, including toxicokinetics, metabolism, biota-sediment accumulation factors (BSAFs), organ-specific bioaccumulation and bound residues. Since it remains hard to accurately predict bioaccumulation in fish, even with highly sophisticated models, analyses of tissue levels are required. The most promising fish bioaccumulation markers are body burdens of persistent organic pollutants, like PCBs and DDTs. Since PCDD and PCDF levels in fish tissues are very low as compared with the sediment levels, their value as bioaccumulation markers remains questionable. Easily biodegradable compounds, such as PAHs and chlorinated phenols, do not tend to accumulate in fish tissues in quantities that reflect the exposure. Semipermeable membrane devices (SPMDs) have been successfully used to mimic bioaccumulation of hydrophobic organic substances in aquatic organisms. In order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, the following suite of *fish biomarkers* may be examined: biotransformation enzymes (phase I and II), oxidative stress parameters, biotransformation products, stress proteins, metallothioneins (MTs), MXR proteins, hematological parameters, immunological parameters, reproductive and endocrine parameters, genotoxic parameters, neuromuscular parameters, physiological, histological and morphological parameters. All fish biomarkers are evaluated for their potential use in ERA programs, based upon six criteria that have been proposed in the present paper. This evaluation demonstrates that phase I enzymes (e.g. hepatic EROD and CYP1A), biotransformation products (e.g. biliary PAH metabolites), reproductive parameters (e.g. plasma VTG) and genotoxic parameters (e.g. hepatic DNA adducts) are currently the most valuable fish biomarkers for ERA. The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring. Many of the biological measurements form the only way of integrating effects on a large number of individual and interactive processes in aquatic organisms. Moreover, biological and biochemical effects may link the bioavailability of the

compounds of interest with their concentration at target organs and intrinsic toxicity. The limitations of biomonitoring, such as confounding factors that are not related to pollution, should be carefully considered when interpreting biomarker data. Based upon this overview there is little doubt that measurements of bioaccumulation and biomarker responses in fish from contaminated sites offer great promises for providing information that can contribute to environmental monitoring programs designed for various aspects of ERA.

Roshan T. Ramessur, Toolseeram Ramjeawon. (Faculty of Science, University of Mauritius, Reduit, Mauritius). **Determination of lead, chromium and zinc in sediments from an urbanized river in Mauritius**. Environment International, 28(4) (2002), 315-324.

The mean concentration of Cr (105±30 mg kg-1), Zn (167±30 mg kg-1) and Pb (14±7 mg kg-1) in the sediments along St. Louis River situated in an urbanized and industrialized area in Mauritius were well below the limits of 600, 2500 and 700 mg kg-1 quoted for contaminated sediments adopted from the draft standards (24% clay and 10% organic matter by weight) from the Netherlands [Van Veen RJ, Stortelder PBM. Research on contaminated sediments in the Netherlands. In: Wolf K, Van de Brink WJ, Colon FJ, editors. Contaminated soil. Academic Publisher, 1998. p. 1263–1275.]. Industrial contamination appeared to undergo rapid dilution in the estuary as Cr had high levels near point sources from industries, but decreased rapidly in amount in the estuary possibly because of dilution by other sediments. The significant levels of Zn in sediments from upstream to the estuary suggest that the potential sources could be from the adjacent motorway and road runoff causing significant quantities to be trapped within the St. Louis River. Pb was two folds higher in the sediments in the estuary of St. Louis River compared to upstream and downstream indicating accumulation of Pb in estuarine sediments, which could be released continuously into the lagoon. The potential sources of Pb in sediments from upstream to the estuary were from the adjacent motorway and road runoff causing significant quantities to be trapped within the St. Louis River. Pb and Zn were significantly positively correlated in the sediments along St. Louis River indicating a common source for Pb and Zn. Significant negative correlations were also found for both Pb and Zn with dissolved oxygen in summer along St. Louis River which indicated that the presence of anoxic waters influenced the trapping of Zn and Pb in the sediment phase. This study has also highlighted that a phasedown of Pb in petrol is necessary and with the introduction of unleaded petrol and vehicles equipped with catalytic converters, studies on levels of Pd and Pt to provide baseline data need to be done in the near future and integrated in environmental development schemes and effective coastal zone management of small island states.

S. Hadjispyrou, A. Kungolos, A. Anagnostopoulos. (Laboratory of Inorganic Chemistry, Department of Chemical Engineering, Aristotle University of Thessaloniki, 540 06, Thessaloniki, Greece. Department of Planning and Regional Development, University of Thessaly, 38334, Volos, Greece). **Toxicity, Bioaccumulation, and Interactive Effects of Organotin, Cadmium, and Chromium on *Artemia franciscana.*** Ecotoxicology and Environmental Safety, 49(2) (2001), 179-186.

The effects of three organotin compounds—trimethyltin chloride, dimethyltin dichloride, and dibutyltin diacetate—and two heavy metals—cadmium and hexavalent chromium—on *Artemia franciscana* mortality are investigated in this study. Of all the compounds tested in this work, trimethyltin chloride was, by far, the most toxic. The toxicity order for the five compounds was trimethyltin chloride>potassium dichromate>dimethyltin dichloride>dibutyltin diacetate>cadmium chloride. The big difference in toxicity between dialkyltin and trialkyltin was not accompanied by an equally big difference in bioaccumulation. At a Sn concentration in water of 10 mg/L, the bioconcentration factor was 50 for dimethyltin dichloride and 75 for trimethyltin chloride. At a Sn concentration in water of 100 mg/L, the bioconcentration factor for 6 for

dimethyltin dichloride and 9 for trimethyltin chloride. The interactive effect of trimethyltin chloride and cadmium, as well as that of trimethyltin chloride and chromium, was found to be synergistic. Also found to be synergistic was the interactive effect of trimethyltin chloride with cadmium and chromium applied together.

S. Hadjispyrou, A. Kungolos, A. Anagnostopoulos. (Laboratory of Inorganic Chemistry, Department of Chemical Engineering, Aristotle University of Thessaloniki, 540 06, Thessaloniki, Greece. Department of Planning and Regional Development, University of Thessaly, 38334, Volos, Greece). **Toxicity, Bioaccumulation, and Interactive Effects of Organotin, Cadmium, and Chromium on *Artemia franciscana*.** Ecotoxicology and Environmental Safety, 49(2) (2001), 179-186.

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S. Teodorova, R. Metcheva, M. Topashka-Ancheva. (Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko chaussee, 1784, Sofia, Bulgaria. Institute of Zoology, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel boul. 1000, Sofia, Bulgaria). **Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice *Mus musculus alba*. I. Analysis of Zn, Cu, Pb, and Cd disposition and mathematical model for Zn and Cd bioaccumulations**. Environmental Research, 91(2) (2003), 85-94.

The concentrations of Zn, Cu, Pb, and Cd in the liver, kidneys, spleen, bones, and carcass of laboratory mice BALB/cy were observed in toxicological experiments. Polymetal industrial dust containing these metals was given to experimental animals at 1% concentration mixed with conventional animal food. Samples for analyses were taken on Days 15, 40, 60, 90, and 120 posttreatment. The experimental data clearly support the established antagonistic interactions among cadmium, zinc, copper, and lead. A mathematical model was proposed to study the main tendencies of heavy metal bioaccumulation under conditions of metal interaction and excessive exposure. The experimental results were assessed on the basis of the model. A rate constant of renal excretion greater than that of hepatic excretion was obtained, which agrees with the observed inversion of cadmium kidney/liver ratio in the conditions of very high exposure.

S. Teodorova, R. Metcheva, M. Topashka-Ancheva. (Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko chaussee, 1784, Sofia, Bulgaria. Institute of Zoology, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel boul. 1000, Sofia, Bulgaria). **Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice *Mus musculus albaI.*** Environmental Research, 91(2) (2003), 85-94.

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Sheryl A. Tittlemier, Aaron T. Fisk, Keith A. Hobson, Ross J. Norstrom. (Centre for Analytical and Environmental Chemistry, Carleton University, Ottawa, Ontario, Canada, K1S 5B6. Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5E2. Prairie and Northern Wildlife Research Centre, Canadian Wildlife Service, Saskatoon, Saskatchewan, Canada, S7N 0X4. National Wildlife Research Centre, Environment Canada, Hull, Quebec, Canada, J8Y 4V8). **Examination of the bioaccumulation of halogenated dimethyl bipyrroles in an Arctic marine food web using stable nitrogen isotope analysis.** Environmental Pollution, 116(1) (2002), 85-93.

Concentrations of four possibly naturally produced organohalogens — 1,1'-dimethyl-3,3',4-tribromo-4,5,5'-trichloro-2,2'-bipyrrole (DBP-Br_3Cl_3), 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrole (DBP-Br_4Cl_2), 1,1'-dimethyl-3,3',4,4',5-pentabromo-5'-chloro-2,2'-bipyrrole (DBP-Br_5Cl) and 1,1'-dimethyl-3,3',4,4',5,5'-hexabromo-2,2'-bipyrrole (DBP-Br_6) — were quantitated and the extent of their magnification through an entire Arctic marine food web [measured as integrated trophic magnification factors (TMFs)] were calculated. The food web consisted of three zooplankton species (*Calanus hyperboreus*, *Mysis oculata*, and *Sagitta* sp.), one fish species [Arctic cod (*Boreogadus saida*)], four seabird species [dovekie (*Alle alle*), black guillemot (*Cepphus grylle*), black-legged kittiwake (*Rissa tridactyla*), and glaucous gull (*Larus hyperboreus*)], and one marine mammal species [ringed seal (*Phoca hispida*)]. Trophic levels in the food web were calculated from ratios of stable isotopes of nitrogen ($^{15}N/^{14}N$). All halogenated dimethyl bipyrrole (HDBP) congeners were found to significantly ($P<0.02$) biomagnify, or increase in concentration with trophic level in the invertebrate — fish — seabird food web. DBP-Br_4Cl_2 (TMF=14.6) was found to biomagnify to a greater extent than DBP-Br_3Cl_3 (TMF=5.2), DBP-Br_5Cl (TMF=6.9), or DBP-Br_6 (TMF=7.0), even though the K_{ow} of DBP-Br_4Cl_2 was predicted to be lower than those of DBP-Br_5Cl and DBP-Br_6. None of the four HDBP congeners in ringed seals followed the general trend of increasing concentration with trophic level, which was possibly due to an ability of the seals to metabolize HDBPs.

Thomas A. Davis, Bohumil Volesky, Alfonso Mucci. (Department of Chemical Engineering, McGill University, 3610 University Street, Montreal, Que., Canada H3A 2B2. Department of Earth and Planetary Sciences, McGill University, 3450 University Street, Montreal, Que., Canada H3A 2A7). **Bioaccumulation**. Water Research, 37(20) (2003), 4843-4854.

The passive removal of toxic heavy metals such as Cd2+, Cu2+, Zn2+, Pb2+, Cr3+, and Hg2+ by inexpensive biomaterials, termed biosorption, requires that the substrate displays high metal uptake and selectivity, as well as suitable mechanical properties for applied remediation scenarios. In recent years, many low-cost sorbents have been investigated, but the brown algae have since proven to be the most effective and promising substrates. It is their basic biochemical constitution that is responsible for this enhanced performance among biomaterials. More specifically, it is the properties of cell wall constituents, such as alginate and fucoidan, which are chiefly responsible for heavy metal chelation. In this comprehensive review, the emphasis is on outlining the biochemical properties of the brown algae that set them apart from other algal biosorbents. A detailed description of the macromolecular conformation of the alginate biopolymer is offered in order to explain the heavy metal selectivity displayed by the brown algae. The role of cellular structure, storage polysaccharides, cell wall and extracellular polysaccharides is evaluated in terms of their potential for metal sequestration. Binding mechanisms are discussed, including the key functional groups involved and the ion-exchange process. Quantification of metal–biomass interactions is fundamental to the evaluation of potential implementation strategies, hence sorption isotherms, ion-exchange constants, as well as models used to characterize algal biosorption are reviewed. The sorption behavior (i.e., capacity, affinity) of brown algae with various heavy metals is summarized and their relative performance is evaluated.

Uguz, Mesude Iscan, Ayse Ergüven, Belgin Isgor, Inci Togan. (Afyon Kocatepe Universitesi, Veteriner Fakultesi, A.N. Sezer Kampusu, 03100, Afyon, Turkey. Department of Biological Sciences, Middle East Technical University, 06531, Ankara, Turkey). **The bioaccumulation of nonyphenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*).** Environmental Research, 92(3) (2003), 262-270.

Alkylphenol polyethoxylates (APEs) are widely used as nonionic surfactants. Nonylphenol (NP), one of the derivatives of APEs, has been found in the aquatic environment in ranges from nanograms per liter to milligrams per liter. In this study, juvenile rainbow trout were exposed to 0 (control), 66, 220, or 660 μg NP/L for up to 28 days. Fish remained healthy under NP exposures of 0, 66, and 220 μg/L for the length of the experiment. All fish died after 4 days of exposure to 660 μg NP/L. Time-dependent NP bioaccumulation was detected in the tissues of fish exposed to 220 μg NP/L ($P<0.05$) and histopathological changes were observed in the livers of fish exposed to 220 μg NP/L. Furthermore, an increase in the activity of glutathione-*S*-transferase (GST) was found in the liver of fish exposed to 220 μg NP/L for 1 week ($P<0.05$). There was an increase in GST activity in the liver of fish exposed to 66 μg NP/L but it did not occur before 2 weeks of exposure to NP. The GST activity then decreased in a time-dependent manner in treatment groups, and this decrease was lower in the livers of fish treated with 66 and 220 μg NP/L than in control fish after 3 weeks of exposure ($P<0.05$). These results indicated that sublethal doses of NP were accumulating in the bodies of the fish and causing histopathological and biochemical changes in the livers of rainbow trout.

V. O. Sipiä, H. T. Kankaanpää, S. Pflugmacher, J. Flinkman, A. Furey, K. J. James. (Finnish Institute of Marine Research, PO Box 33, FIN-00931, Helsinki, Finland. University of Western Sydney, Campbelltown Campus, Campbelltown, Locbed Bag 1797, Penrith Sout, 1797, New South Wales, Australia. Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12561, Berlin, Germany. Ecotoxicology Research Unit, Cork Institute

of Technology, Chemistry Department, Bishopstown, Cork, Ireland). **Bioaccumulation and Detoxication of Nodularin in Tissues of Flounder (*Platichthys flesus*), Mussels (*Mytilus edulis, Dreissena polymorpha*), and Clams (*Macoma balthica*) from the Northern Baltic Sea.** Ecotoxicology and Environmental Safety, 53(2) (2002), 305-311.

Cyanobacterial hepatotoxin accumulation in mussels (*Mytilus edulis, Dreissena polymorpha*), clam (*Macoma balthica*), and flounder (*Platichthys flesus*) tissues was measured. Flounder were caught with gillnets from the western Gulf of Finland on 21 August 1999, 25 July 2000, and 25 August 2000. Blue mussels were collected from: (1) a steel cage at a depth of 3 m on 20 August 1999, (2) an enclosure at depths of 3–5 m, and (3) an artificial reef (wreck at 25–30 m) in the western Gulf of Finland between June and September 2000. Furthermore, blue mussels were collected from two sites between August and October 2000: south of the town of Hanko at depths of 5 and 20 m in the western Gulf of Finland and south of the city of Helsinki at a depth of 7 m in the central Gulf of Finland. *M. balthica* and *D. polymorpha* were collected at a depth of 12 m from Russian waters in the eastern Gulf of Finland on 1–4 August 2000. The samples were analyzed for the cyanobacterial hepatotoxins nodularin (NODLN) and microcystins (MCs) using enzyme-linked immunosorbent assay (ELISA), liquid chromatography–mass spectrometry (LC-MS), and matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF-MS). ELISA indicated a time-dependent accumulation of hepatotoxins in flounder liver up to 400±10 (SD) µg/kg on 25 August 2000. No hepatotoxins were detected in flounder muscle samples. In blue mussels, collected from an enclosure 3–5 m deep in the western Gulf of Finland on 23 August 2000, ELISA indicated cyanobacterial hepatotoxins up to 1490±60 µg/kg dry wt. Blue mussels collected from the other sites contained less cyanobacterial hepatotoxins (40–130 µg/kg dry wt). Clams and mussels from Russian waters contained cyanobacterial hepatotoxin at about 100–130 µg/kg dry wt. Total hepatotoxin levels in mussels from enclosures decreased from August to September, indicating at least partial detoxication/depuration of the toxins. LC-MS verified the presence of NODLN in mussels and flounder. Typical detoxication conjugates were observed by MALDI-TOF-MS in mussel samples collected during August 2000. In deeper-living wreck mussels cyanobacterial hepatotoxin levels continued to increase, from August to September, indicating that portions of cyanobacterial hepatotoxins reach the sea floor. NODLN bioaccumulation is a constant phenomenon in the area.

Wei Maa, J. M. Tobin. (Department of Chemistry, Dalian University of Technology, 116023, China. School of Biotechnology, Dublin City University, Dublin 9, Ireland). **Development of multimetal binding model and application to binary metal biosorption onto peat biomass**. Water Research, 37(16) (2003), 3803.

Biosorption of Cr3+, Cu2+ and Cd2+ from binary metal solutions onto peat in the batch systems was investigated at pH 4. The order of maximum uptake was CrCu>Cd and maximum uptake levels of ca. 0.4 mmol/g were observed for chromium and copper while cadmium was taken up to a maximum of ca. 0.2 mmol/g. Co-ion competition resulted in up to 70 percent decrease of primary metal uptake. A novel approach to multicomponent sorption modelling involving regression to the total metal taken up was adopted. Two extended Langmuir-type models were found to exhibit good fit to the experimental data. Using the simpler model of these, three-dimensional sorption surfaces were generated which describe the metal uptake as a function of equilibrium concentrations of both metals. These methods allow prediction of metal uptakes over a continuum of concentrations of both metals in binary systems.

Weon Bae, Cindy H. Wu, Jan Kostal, Ashok Mulchandani, and Wilfred Chen. (Department of Chemical and Environmental Engineering, Environmental Toxicology Program, University of California, Riverside, California 92521). **Enhanced Mercury Biosorption by Bacterial**

Cells with Surface-Displayed MerR. Applied and Environmental Microbiology, 69(6) (2003), 3176-3180.

The metalloregulatory protein MerR, which exhibits high affinity and selectivity toward mercury, was exploited for the construction of microbial biosorbents specific for mercury removal. Whole-cell sorbents were constructed with MerR genetically engineered onto the surface of Escherichia coli cells by using an ice nucleation protein anchor. The presence of surface-exposed MerR on the engineered strains enabled sixfold-higher Hg2+ biosorption than that found in the wild-type JM109 cells. Hg2+ binding via MerR was very specific, with no observable decline even in the presence of 100-fold excess Cd2+ and Zn2+. The Hg2+ binding property of the whole-cell sorbents was also insensitive to different ionic strengths, pHs, and the presence of metal chelators. Since metalloregulatory proteins are currently available for a wide variety of toxic heavy metals, our results suggest that microbial biosorbents overexpressing metalloregulatory proteins may be used similarly for the cleanup of other important heavy metals.

X. Deng, Q. B. Lia, Y. H. Lua, D. H. Suna, Y. L. Huanga, X. R. Chenb. (Department of Chemical Engineering, Xiamen University, Xiamen 361005, People's Republic of China. Department of Biology, Xiamen University, Xiamen 361005, People's Republic of China). **Bioaccumulation of nickel from aqueous solutions by genetically engineered Escherichia coli**. Water Research, 37(11) (2003), 2748-2756.

This study constructed a genetically engineered Escherichia coli JM109 which simultaneously expressed nickel transport system and metallothionein to remove and recover Ni2+ from aqueous solution. Bioaccumulation process was rapid and followed linearized Langmuir isotherm. A more than six-fold increase of Ni2+ binding capacity was obtained by genetically engineered E. coli cells compared with original host E. coli cells. A pH assay showed genetically engineered E. coli cells accumulated Ni2+ effectively over a broad range of pH (4–10). The presence of 1000 mg/L Na+ and Ca2+, or 50 mg/L Cd2+ or Pb2+ did not have a significant effect on Ni2+ bioaccumulation, while Mg2+, Hg2+ and Cu2+ posed a severe adverse influence on Ni2+ uptake by genetically engineered E. coli. Furthermore, genetically engineered E. coli cells did not require extra nutrients for Ni2+ bioaccumulation.

Xinde Cao, Lena Q. Ma, Aziz Shiralipour. (Department of Soil and Water Science, University of Florida, Gainesville, FL 32611, USA. Center for Natural Resources, University of Florida, Gainesville, FL 32611, USA). **Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris vittata* L.** Environmental Pollution, 126(2) (2003), 157-167.

Chinese brake fern (*Pteris vittata* L.), an arsenic (As) hyperaccumulator, has shown the potential to remediate As-contaminated soils. This study investigated the effects of soil amendments on the leachability of As from soils and As uptake by Chinese brake fern. The ferns were grown for 12 weeks in a chromated–copper–arsenate (CCA) contaminated soil or in As spiked contaminated (ASC) soil. Soils were treated with phosphate rock, municipal solid waste, or biosolid compost. Phosphate amendments significantly enhanced plant As uptake from the two tested soils with frond As concentrations increasing up to 265% relative to the control. After 12 weeks, plants grown in phosphate-amended soil removed >8% of soil As. Replacement of As by P from the soil binding sites was responsible for the enhanced mobility of As and subsequent increased plant uptake. Compost additions facilitated As uptake from the CCA soil, but decreased As uptake from the ASC soil. Elevated As uptake in the compost-treated CCA soil was related to the increase of soil water-soluble As and As(V) transformation into As(III). Reduced As uptake in the ASC soil may be attributed to As adsorption to the compost. Chinese brake fern took up As mainly from the iron-bound fraction in the CCA soil and from the water-

soluble/exchangeable As in the ASC soil. Without ferns for As adsorption, compost and phosphate amendments increased As leaching from the CCA soil, but had decreased leaching with ferns when compared to the control. For the ASC soil, treatments reduced As leaching regardless of fern presence. This study suggest that growing Chinese brake fern in conjunction with phosphate amendments increases the effectiveness of remediating As-contaminated soils, by increasing As uptake and decreasing As leaching.

Zakaria A. Mohamed. (Department of Botany, Faculty of Science (Sohag), South Valley University, Sohag, 82524, Egypt). **Accumulation of Cyanobacterial Hepatotoxins by *Daphnia* in Some Egyptian Irrigation Canals**. Ecotoxicology and Environmental Safety, 50(1) (2001), 4-8.

In this study, microcosm experiments were run in the laboratory to test the possibility of feeding of *Daphnia parvula* on toxic *Microcystis aeruginosa* in some Egyptian irrigation canal at Sohag city. The results demonstrated that *Daphnia* has a priority of feeding on green algae and the diatom *Melosira granulata* over toxic *M. aeruginosa* during the first 10 days. Thereafter, when the green algae and diatom were depleted from the water, *Daphnia* started to feed on toxic *Microcystis*. This presumably indicates that *Daphnia* feeds facultatively on toxic cyanobacteria under the conditions of depletion of edible food. Additionally, the results indicated that *Daphnia* accumulates the *Microcystis* toxins "microcystins" in its body with a level of 1.78 μg toxin/25 daphnids. No release of toxin into the water was detected during the experimental period. This emphasizes that the disappearance of toxic *Microcystis* was due to the feeding by *Daphnia*, not to death or cell lysis. Such an accumulation of cyanobacterial hepatotoxins in the primary consumers (*Daphnia*) should be taken into consideration when zooplankton are used in the biomanipulation of toxic phytoplankton.

Biocomposting

B. Kluczek-Turpeinen, M. Tuomela, A. Hatakka, M. Hofrichter. (Department of Applied Chemistry and Microbiology, Biocenter 1, University of Helsinki, Viikinkaari 9, P.O. Box 56, 00014, Helsinki, Finland. International Graduate School Zittau, Markt 23, 02763, Zittau, Germany). **Lignin degradation in a compost environment by the deuteromycete Paecilomyces inflatus**. Applied Microbiology and Biotechnology, 61(4) (2003), 374 – 379.

Two strains of the deuteromycete Paecilomyces inflatus were isolated from compost samples consisting of municipal wastes, paper and wood chips. Lignin degradation by P. inflatus was studied following the mineralization of a synthetic 14Cg-labeled lignin (side-chain labeled dehydrogenation polymer, DHP). Approximately 6.5% of the synthetic lignin was mineralized during solid-state cultivation of the fungus in autoclaved compost; and 15.5% was converted into water-soluble fragments. Laccase was the only ligninolytic enzyme detectable when the isolates were grown in autoclaved compost. Production of the enzyme was growth-associated and dependent on the culture conditions. The optimal pH for laccase production was between 4.5 and 5.5 and the optimal temperature was around 30 °C. Activity levels of laccase increased in the presence of low-molecular-mass aromatic compounds, such as veratryl alcohol, veratric acid, vanillin and vanillic acid.

M. Ohkuma.(Molecular Microbial Ecology Division, Bioscience Technology Center, RIKEN and ICORP, Japan Science and Technology Corporation, 351-0198, Wako, Saitama, Japan). **Termite symbiotic systems: efficient bio-recycling of lignocellulose**. Applied Microbiology and Biotechnology, 61(1) (2003), 1 – 9.

Termites thrive in great abundance in terrestrial ecosystems and play important roles in biorecycling of lignocellulose. Together with their microbial symbionts, they efficiently decompose lignocellulose. In so-called lower termites, a dual decomposing system, consisting of

the termite's own cellulases and those of its gut protists, was elucidated at the molecular level. Higher termites degrade cellulose apparently using only their own enzymes, because of the absence of symbiotic protists. Termite gut prokaryotes efficiently support lignocellulose degradation. However, culture-independent molecular studies have revealed that the majority of these gut symbionts have not yet been cultivated, and that the gut symbiotic community shows a highly structured spatial organization. In situ localization of individual populations and their functional interactions are important to understand the nature of symbioses in the gut. In contrast to cellulose, lignin degradation does not appear to be important in the gut of wood-feeding termites. Soil-feeding termites decompose humic substances in soil at least partly, but little is known about the decomposition. Fungus-growing termites are successful in the almost complete decomposition of lignocellulose in a sophisticated cooperation with basidiomycete fungi cultivated in their nest. A detailed understanding of efficient biorecycling systems, such as that for lignocellulose, and the symbioses that provide this efficiency will benefit applied microbiology and biotechnology.

Biodegradation

A. A. M. Langenhoff, J. J. M. Staps, C. Pijls, A. Alphenaar, G. Zwiep, H. H. M. Rijnaarts. (TNO Environmental, Energy and Process Innovation, Department of Environmental Biotechnology, Apeldoorn, The Netherlands. Tauw Milieu Consultancy, Deventer, The Netherlands. Akzo Nobel Chemicals, Hengelo, The Netherlands). **Intrinsic and Stimulated *In Situ* Biodegradation of Hexachlorocyclohexane (HCH).** Water, Air and Soil Pollution: Focus, 2(3) (2002) 171-181.

The feasibility of the biodegradation of HCH and its intermediates has been investigated. A recent characterisation of two sites in The Netherlands has shown intrinsic biodegradation of HCH. At one site, breakdown products (monochlorobenzene, benzene and chlorophenol) were found in the core of the HCH-plume, whereas the HCH-concentration decreased over time and space. Characterisation of a second, industrial site indicated less intrinsic biodegradation and the need to stimulate biodegradation. In the laboratory, enhanced HCH degradation was tested with soil and groundwater material from both sites, and the required conversion to the intermediates benzene and monochlorobenzene was demonstrated. Furthermore, the biodegradation of these intermediates could be initiated by adding low amounts of oxygen (<5%). Adding nitrate enhanced this degradation. We hypothesise that this occurs through anaerobic nitrate reducing conversion of oxidised intermediates. At the non-industrial other site, intrinsic degradation took place, as shown in the laboratory experiments. Interpretation of the field data with computer codes Modflow and RT3D was performed. As a result of the modelling study, it has been proposed to monitor natural attenuation for several years before designing the final approach. At the industrial site, the results of the batch experiments are applied. Anaerobic HCH degradation to monochlorobenzene and benzene is stimulated via the addition of an electron donor. Infiltration facilities have been installed at the site to create an anaerobic infiltration zone in which HCH will be degraded, and these facilities are combined with the redevelopment of the site.

A. D. Satroutdinov, E. G. Dedyukhina, T. I. Chistyakova, I. G. Minkevich, V. K. Eroshin, T. Egli. (Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia, Department of Microbiology, EAWAG CH-8600 Dubendorf, Switzerland). **Bacterial Degradation of EDTA**. Microbiology, 72(1) (2003), 8-11.

Degradation of EDTA (ethylenediaminetetraacetic acid) or metal–EDTA complexes by cell suspensions of the bacterial strain DSM 9103 was studied. The activity of EDTA degradation

was the highest in the phase of active cell growth and decreased considerably in the stationary phase, after substrate depletion in the medium. Exponential-phase cells were incubated in HEPES buffer (pH 7.0) with 1 mM of uncomplexed EDTA or EDTA complexes with Mg2+, Ca2+, Mn2+, Pb2+, Co2+, Cd2+, Zn2+, Cu2+, or Fe3+. The metal–EDTA complexes (Me–EDTA) studied could be divided into three groups according to their degradability. EDTA complexes with stability constants *K* below 1016 (log *K* < 16), such as Mg–EDTA, Ca–EDTA, and Mn–EDTA, as well as uncomplexed EDTA, were degraded by the cell suspensions at a constant rate to completion within 5–10 h of incubation. Me–EDTA complexes with log *K* above 16 (Zn–EDTA, Co–EDTA, Pb–EDTA, and Cu–EDTA) were not completely degraded during a 24-h incubation, which was possibly due to the toxic effect of the metal ions released. No degradation of Cd–EDTA or Fe(III)–EDTA by cell suspensions of strain DSM 9103 was observed under the conditions studied.

A. I. Okoh. (Department of Microbiology, Obafemi Awolowo University, Ile – Ife, Nigeria). **Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria**. African Journal of Biotechnology, 2(5) (2003), 104-108.

In an effort at developing an active indigenous bacterial consortium that could be of relevance in bioremediation of petroleum contaminated systems in Nigeria, four hydrocarbon degrading bacteria strains were isolated. Partial sequencing of the 16S rDNA of the isolates suggests that they are all strains of *Pseudomonas aeruginosa*. Axenic cultures of the isolates biodegraded Bonny light crude oil in soil microcosm. Amount of crude oil biodegraded in 15 days ranged significantly ($P < 0.05$) from 4.9% to 29.6%. Degradation rates and specific growth rates varied significantly ($P < 0.05$) between 0.049 and 0.351 day^{-1} and 0.017 and 0.028 $hour^{-1}$ respectively. Major peak components of the oil were reduced by between 6.5% and 70.6%. It would appear that oil degradation capability of axenic cultures of at least three of these isolates was not different from that of their consortium. Also, the multiple antibiotic resistance observed in the isolates is an important factor to consider in their eventual use in bioremediation exercises.

Albert D. Venosa, Xueqing Zhu. (National Risk Management Research Laboratory, US Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA. Department of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, OH 45221-0071, USA). **Biodegradation of Crude Oil Contaminating Marine Shorelines and Freshwater Wetlands**. Spill Science & Technology Bulletin, 8(2) (2003), 163-178.

This paper is a summary of the various factors influencing weathering of oil after it has been released into the environment from a spill incident. Special emphasis has been placed on biodegradation processes. Results from two field studies conducted in 1994 and 1999 involving bioremediation of an experimental oil spill on a marine sandy shoreline in Delaware and a freshwater wetland on the St. Lawrence River in Quebec, Canada have been presented in the paper.

Ana Soares, Benoit Guieysse, Bo Mattiasson. (Biotechnology Department, Lund University, P.O. Box 124, S-221 00 Lund, Sweden). **Biodegradation of nonylphenol in a continuous packed-bed bioreactor**. Biotechnology Letters, 25(12) (2003)), 927-933.

A packed bed bioreactor, with 170 ml glass bead carriers and 130 ml medium, was tested for the removal of the endocrine disrupter, nonylphenol, with a *Sphingomonas* sp. The bioreactor was first continuously fed with medium saturated with nonylphenol in an attempt to simulate groundwater pollution. At best, nonylphenol was degraded by 99.5% at a feeding rate of 69 ml h-1 and a removal rate of 4.3 mg nonylphenol day-1, resulting in a 7.5-fold decrease in effluent toxicity according to the Microtox. The bioreactor was then fed with soil leachates at 69 ml h-1 from artificially contaminated soil (1 g nonylphenol kg-1 soil) and a real contaminated soil

(0.19 g nonylphenol kg-1 soil). Nonylphenol was always completely removed from the leachates of the two soils. It was removed by 99% from the artificial soil but only 62% from real contaminated soil after 18 and 20 d of treatment, respectively, showing limitation due to nonylphenol adsorption.

Ana Soares, Benoit Guieysse, Osvaldo Delgado, Bo Mattiasson. (Biotechnology Department, Lund University, P.O. Box 124, S-221 00 Lund, Sweden). **Aerobic biodegradation of nonylphenol by cold-adapted bacteria**. Biotechnology Letters, 25(9) (2003), 731-738.

Three strains capable of mineralizing nonylphenol as sole carbon source were isolated from a sample of contaminated soil and characterized as two *Pseudomonas* spp. and a *Stenotrophomonas* sp. The two *Pseudomonas* spp. expressed characteristics typical of psychrophiles growing optimally of 10 °C and capable of growing at 0 °C. The *Stenotrophomonas* sp. was more likely psychrotrophic because it had an optimal temperature between 14 and 22 °C although it was not capable of growing at 4 °C. At 14 °C, one of the *Pseudomonas* spp. exhibited the highest rate of degradation of nonylphenol (4.4 mg l^{-1} d^{-1}), when compared with axenic or mixed cultures of the isolates. This study represents, to the best of our knowledge, the first reported case of cold-adapted microorganisms capable of mineralizing nonylphenol.

Ana Soares, Benoit Guieysse, Osvaldo Delgado, Bo Mattiasson. (Biotechnology Department, Lund University, P.O. Box 124, S-221 00 Lund, Sweden). **Aerobic biodegradation of nonylphenol by cold-adapted bacteria.** Biotechnology Letters, 25(9) (2003), 731-738.

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Andrew J. Daugulis, Colleen M. McCracken. (Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6). **Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp**. Biotechnology Letters, **25**(17) (2003), 1441-1444.

A mixture of six polyaromatic hydrocarbons (naphthalene, phenanthrene, fluoranthene, pyrene, chyrysene and benzo[a]pyrene), varying in size from 2 to 5 rings, was dissolved in dodecane, and used as the delivery phase of a partitioning bioreactor. Two species of *Sphingomonas* were then used individually, and as a consortium, to determine which of the PAHs were degraded. Only low molecular weight PAHs (naphthalene, phenanthrene and fluoranthene) were degraded by the individual strains, but the consortium degraded all substrates either to completion or near completion.

B Chardin, A Dolla, F Chaspoul, M Fardeau, P Gallice, M Bruschi. (Laboratoire de Bioénergétique et Ingénierie des Protéines, Institut de Biologie Structurale et Microbiologie - CNRS, 31 ch. Joseph Aiguier, 13009 Marseille, France. Laboratoire de Chimie Générale et Prevention des Risques et Nuisances Technologiques, Faculté de Pharmacie, 27 bd. Jean Moulin, 13385 Marseille cedex 05, France. Laboratoire de Microbiologie IRD, Université de Provence, CESB-ESIL, Case 925, 163 Avenue de Luminy, 13288 Marseille Cedex 9, France).

Bioremediation of chromate: thermodynamic analysis of the effects of Cr(VI) on sulfate-reducing bacteria. Applied Microbiology and Biotechnology, 60(3) (2002), 352 – 360.

Developing new bioremediation processes for soils and effluents polluted by Cr(VI) requires the selection of the most efficient and the most heavy-metal-resistant bacteria. The effects of Cr(VI) on bioenergetic metabolism in two sulfate-reducing bacteria (SRB), Desulfovibrio vulgaris Hildenborough and Desulfomicrobium norvegicum, were monitored using isothermal microcalorimetry. The complete reduction of Cr(VI) to Cr(III) was studied by spectrophotometry and by speciation using a combination of high-performance liquid chromatography and inductively coupled plasma-mass spectrometry. Results revealed that Cr(VI) induces an inhibition of growth with concomitant production of energy, which can be compared to the reaction of the bacteria to a stress such as oxidative stress. Moreover, the sensitivity of bacteria towards this metal is as a characteristic of the strain, which leads to differences in the kinetics of Cr(VI) reduction. The study by microcalorimetry of heavy metal effects on SRB bioenergetic metabolism thus appears an appropriate tool to identify better strains to be used for industrial bioremediation process development.

Benedict C. Okeke, William T. Frankenberger Jr. **Biodegradation of methyl tertiary butyl ether (MTBE) by a bacterial enrichment consortia and its monoculture isolates**. Microbiological Research, 158(2), 99-106.

Methyl tertiary butyl ether (MTBE), an important gasoline additive, is a recalcitrant compound posing serious environmental health problems. In this study, MTBE-degrading bacteria were enriched from five environmental samples. Enrichments from Stewart Lake sediments and an MTBE contaminated soil displayed the highest rate of MTBE removal; 29.6 and 27.8% respectively, in 28 days. A total of 12 bacterial monocultures isolated from enrichment cultures were screened for MTBE degradation in liquid cultures. In a nutrient-limited medium containing MTBE as the sole source of carbon and energy, the highest rate of MTBE elimination was achieved with IsoSL1, which degraded 30.6 and 50.2% in 14 and 28 days, respectively. In a nutrient-rich medium containing ethanol and yeast extract, the bacterium (Iso2A) substantially removed MTBE (20.3 and 28.1% removal in 14 and 28 days, respectively). Based upon analysis of the 16s rRNA gene sequence and data base comparison, IsoSL1 and Iso2A were identified as a Streptomyces sp. and Sphingomonas sp., respectively. The Streptomyces sp. is a new genera of bacteria degrading MTBE and could be useful for MTBE bioremediation.

Brian J. Reid, Terry R. Fermor, Kirk T. Semple. (Department of Environmental Science, Institute of Environmental and Natural Sciences, Lancaster University, Lancaster LA1 4YQ, UK. Department of Plant Pathology and Microbiology, Horticulture Research International, Wellesbourne CV35 9EF, UK). **Induction of PAH-catabolism in mushroom compost and its use in the biodegradation of soil-associated phenanthrene.** Environmental Pollution, 118(1) (2002), 65-73.

This paper describes the induction of phenanthrene-catabolism within Phase II mushroom compost resulting from its incubation with (1) phenanthrene, and (2) PAH-contaminated soil. Respirometers measuring mineralization of freshly added ^{14}C-9-phenanthere were used to evaluate induction of phenanthrene-catabolism. Where pure phenanthrene (spiked at a concentration of 400 mg $kg^{-1}_{wet\ wt.}$) was used to induce phenanthrene-catabolism in compost, induction was measurable, with maximal mineralization observed after 7 weeks phenanthrene-compost contact time. Where PAH-contaminated soil was used to induce phenanthrene-catabolism in un-induced compost, induction was observed after 5 weeks soil–compost contact time. Microcosm-scale amelioration of soil contaminated with ^{14}C-phenanthrene (aged in soil for 516 days prior to incubation with compost) indicated that both induced (using pure

phenanthrene) and uninduced Phase II mushroom composts were equally able to promote degradation of this soil-associated contaminant. After 111 days incubation time, 42.7±6.3% loss of soil-associated phenanthrene was observed in the induced-compost soil mixture, while 36.7±2.9% loss of soil-associated phenanthrene was observed in the uninduced-compost soil mixture. These results are notable as they indicate that while pre-induction of phenanthrene-catabolism within compost is possible, it does not significantly increase the extent of degradation when the compost is used to ameliorate phenanthrene-contaminated soil. Thus, compost could be used directly in the amelioration of contaminated land i.e. without pre-induction of catabolism.
C. D. Johnston, A. Desvignes. (CSIRO Land and Water, Private Bag 5, PO Wembley, W.A., 6913, Australia. Hydrogeology Centre of the University of Neuchâtel, Neuchâtel, Switzerland). **Evidence for biodegradation and volatilisation of dissolved petroleum hydrocarbons during *in situ* air sparging in large laboratory columns**. Water, Air and Soil Pollution: Focus, 3(3) (2003), 25-33.

Laboratory column experiments run for up to 13 days compared air sparging of groundwater contaminated by dissolved petroleum hydrocarbons in sterile and non-sterile aquifer sediments as well as uncontaminated sediments and groundwater. Loss of dissolved BTEX compounds in the contaminated columns was very rapid, occurring through volatilisation. The majority of the dissolved total organic carbon (TOC) persisted for much longer periods however. A direct comparison between losses from sterile and non-sterile columns suggested a negligible contribution of biodegradation to the removal of TOC. This was difficult to confirm through examination of O_2 utilisation because oxidation of a small amount of reduced sulphur in the aquifer materials was the dominant sink for O_2. Despite this, it was possible to conclude that less than 22% of the removal of TOC was through biodegradation during the first three days of air sparging.
D. Y. Kim, Y. H. Rhee. (Department of Microbiology, Chungnam National University, 305-764, Daejeon, Korea). **Biodegradation of microbial and synthetic polyesters by fungi**. Applied Microbiology and Biotechnology, 61(4) (2003), 300 – 308.

A variety of biodegradable polyesters have been developed in order to obtain useful biomaterials and to reduce the impact of environmental pollution caused by the large-scale accumulation of non-degradable waste plastics. Polyhydroxyalkanoates, poly(k-caprolactone), poly(l-lactide), and both aliphatic and aromatic polyalkylene dicarboxylic acids are examples of biodegradable polyesters. In general, most aliphatic polyesters are readily mineralized by a number of aerobic and anaerobic microorganisms that are widely distributed in nature. However, aromatic polyesters are more resistant to microbial attack than aliphatic polyesters. The fungal biomass in soils generally exceeds the bacterial biomass and thus it is likely that fungi may play a considerable role in degrading polyesters, just as they predominantly perform the decomposition of organic matter in the soil ecosystem. However, in contrast to bacterial polyester degradation, which has been extensively investigated, the microbiological and environmental aspects of fungal degradation of polyesters are unclear. This review reports recent advances in our knowledge of the fungal degradation of microbial and synthetic polyesters and discusses the ecological importance and contribution of fungi in the biological recycling of waste polymeric materials in the biosphere.
David Schleheck, Melanie Lechner, René Schönenberger, Marc J.-F. Suter, Alasdair M. Cook. (Department of Biology, The University of Konstanz, D-78457 Konstanz, Germany, Swiss Federal Institute for Environmental Science and Technology, CH-8600 Dübendorf, Switzerland). **Desulfonation and Degradation of the Disulfodiphenylethercarboxylates from Linear Alkyldiphenyletherdisulfonate Surfactants**.Applied and Environmental Microbiology, February, 69(2) (2003), 938-944.

Earlier work showed that the biodegradation of a commercial linear monoalkyldiphenyletherdisulfonate surfactant as a carbon source for microbial growth leads to the quantitative formation of corresponding disulfodiphenylether carboxylates (DSDPECs), which were not degraded. α-Proteobacterium strain DS-1 (DSM 13023) catalyzes these reactions. These DSDPECs have now been characterized by high-pressure liquid chromatography coupled via an electrospray interface to a mass spectrometer. DSDPECs were a complex mixture of compounds which indicated catabolism via ω-oxygenation and ß-oxidation. DSDPECs were subject to quantitative desulfonation in bacterial cultures in which they served as sole sulfur sources for bacterial growth. On average, one sulfonate group per DSDPEC species was removed, and the organism responsible for this desulfonation was isolated and identified as Rhodococcus opacus ISO-5. The products were largely monosulfodiphenylether carboxylate-phenols (MSDPEC-phenols). MSDPEC-phenols were subject to extensive dissimilation by bacteria from activated sludge.

E S Gilbert, A W Walker, J D Keasling. (Department of Biology, Georgia State University, 24 Peachtree Center Avenue, Atlanta, GA 30303, USA. Sandia Laboratories, Livermore, Calif., USA. Department of Chemical Engineering, University of California, Berkeley, CA 94720, USA). **A constructed microbial consortium for biodegradation of the organophosphorus insecticide parathion**. Applied Microbiology and Biotechnology, 61(1) (2003), 77 – 81.

A consortium comprised of two engineered microorganisms was assembled for biodegradation of the organophosphate insecticide parathion. Escherichia coli SD2 harbored two plasmids, one encoding a gene for parathion hydrolase and a second carrying a green fluorescent protein marker. Pseudomonas putida KT2440 pSB337 contained a p-nitrophenol-inducible plasmid-borne operon encoding the genes for p-nitrophenol mineralization. The co-culture effectively hydrolyzed 500 wM parathion (146 mg lm1) and prevented the accumulation of p-nitrophenol in suspended culture. Kinetic analyses were conducted to characterize the growth and substrate utilization of the consortium members. Parathion hydrolysis by E. coli SD2 followed Michaelis-Menten kinetics. p-Nitrophenol mineralization by P. putida KT2440 pSB337 exhibited substrate-inhibition kinetics. The growth of both strains was inhibited by increasing concentrations of p-nitrophenol, with E. coli SD2 completely inhibited by 600 wM p-nitrophenol (83 mg lm1) and P. putida KT2440 pSB337 inhibited by 1,000 wM p-nitrophenol (139 mg lm1). Cultivation of the consortium as a biofilm indicated that the two species could cohabit as a population of attached cells. Analysis by confocal microscopy showed that the biofilm was predominantly comprised of P. putida KT2440 pSB337 and that the distribution of E. coli SD2 within the biofilm was heterogeneous. The use of biofilms for the construction of degradative consortia may prove beneficial.

Elbieta Grabiska-Sota and Joanna Kalka. (Department of Environmental Biotechnology, Faculty of Environmental Engineering and Energy, Silesian University of Technology, Akademicka 2, PL-44-101, Gliwice, Poland). **An assessment of the toxicity of pyridinium chlorides and their biodegradation intermediates**. Environment International, 28(8) (2003), 711-717.

Toxicity investigations were conducted for four pyridinium chlorides belonging to cationic surface-active substances (CSAS), which differed from each other in the numbers of methyl groups (CH3) in pyridinium ring. The crustacean Daphnia magna, the fish Lebistes reticulatus and the alga Scenedesmus quadricauda were chosen as biotests. Toxicity of examined preparations appeared to be very high but did not depend on their chemical structure. S. quadricauda was the most sensitive organism. Toxicity of intermediate products obtained in biological oxidation process was also examined. Biodegradation was conducted according to the

"river water test". It was found that only partial degradation took place while pyridinium chlorides constituted main energy and carbon source. Presence of biodegradation intermediate products was shown on the basis of 1H NMR analysis. Intermediates were not toxic to any biotests.

Ewa Liwarska-Bizukojc, Stanislaw Ledakowicz. (Department of Environmental Engineering, Technical University of Lodz, Al. Politechniki 6, 93-590 Lodz, Poland Author for correspondence. Department of Bioprocess Engineering, Technical University of Lodz, Wolczanska 213/215, 93-005 Lodz, Poland). **Stoichiometry of the Aerobic Biodegradation of the Organic Fraction of Municipal Solid Waste (MSW)**. Biodegradation, 14(1) (2003), 51-56.

An elemental analysis was applied to describe the composition of the organic fraction of municipal solid waste (MSW). The initial elemental composition was constant at5H8.5O4N0.2. The changes of the composition during the biodegradation process and the final waste composition were strictly dependent on the process conditions. The decrease in carbon content due to biodegradation increased with temperature at which the experiments were conducted, from 20% at 20 °C to about 40% at 37–42 °C after 96 hours. It was correlated with the amount of oxygen that was utilised in the investigated processes of aerobic biodegradation of the waste suspension. The amount of oxygen required for biodegradation of organic fraction of MSW was estimated on the basis of stoichiometric equations and increased from 0.92 moles per 1 mole of waste at 20 °C to 1.6 moles at 42 °C within 96 hours of the experiments.

F Solano-Serena, R Marchal, T Huet, J -M Lebeault, J -P Vandecasteele. (Institut Français du Pétrole, Département de Microbiologie, 1 & 4 avenue de Bois Préau, 92852 Rueil-Malmaison Cedex, France. Université de Technologie de Compiègne, Centre de Recherches de Royallieu, BP 60319, 60203 Compiègne Cedex, France). **Biodegradability of volatile hydrocarbons of gasoline**. Applied Microbiology and Biotechnology, 54(1) (2000), 121 – 125.

The biodegradability under aerobic conditions of volatile hydrocarbons (4-6 carbons) contained in gasoline and consisting of n-alkanes, iso-alkanes, cycloalkanes and alkenes, was investigated. Activated sludge was used as the reference microflora. The biodegradation test involved the degradation of the volatile fraction of gasoline in closed flasks under optimal conditions. The kinetics of biodegradation was monitored by CO_2 production. Final degradation was determined by gas chromatographic analysis of all measurable hydrocarbons (12 compounds) in the mixture after sampling the headspace of the flasks. The degradation of individual hydrocarbons was also studied with the same methodology. When incubated individually, all hydrocarbons used as carbon sources, except 2,2-dimethylbutane and 2,3-dimethylbutane, were completely consumed in 30 days or less with different velocities and initial lag periods. When incubated together as constituents of the light gasoline fraction, all hydrocarbons were metabolised, often with higher velocities than for individual compounds. Cometabolism was involved in the degradation of dimethyl isoalkanes.

Flemming Ingerslev, Bent Halling-Sørensen. (Institute of Pharmaceutical and Analytical Chemistry, Section of Environmental Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, Copenhagen, DK-2100, Denmark). **Biodegradability of Metronidazole, Olaquindox, and Tylosin and Formation of Tylosin Degradation Products in Aerobic Soil–Manure Slurries**. Ecotoxicology and Environmental Safety, 48(3) (2001), 311-320.

The use of veterinary drugs (primarily antibiotics) in animal husbandry harbors the risk that these compounds end up in the farmland when manure is used as fertilizer. The biodegradability of three compounds, olaquindox (OLA), metronidazole (MET), and tylosin (TYL), was

simulated in soil–manure slurries with 50 g of soil per liter. Supplemental batch sorption tests revealed that insignificant amounts of OLA and MET were located in the soil phase, whereas only 0.1 to 10% of the added amounts of TYL remained in the liquid phase. This may reduce the bioavailability and thus biodegradation rates of TYL. Unidentified metabolites of OLA and TYL and four known TYL metabolites were detected using HPLC. However, none of these substances were seen to persist in the biodegradation experiments, indicating that OLA and TYL most likely were mineralized in the experiments. Neither the use of sandy or clayey soil nor the use of 0, 1, or 10% (V/V) of manure added to these soils had a significant effect on the degradation rates. Degradation half-lives for the primary degradation were 3.3–8.1 days for TYL, 5.8–8.8 days for OLA, and 13.1–26.9 days for MET. Based on comparisons of results obtained with the benchmark chemical aniline and degradation half-lives of this compound in nature, it was assessed that results obtained with the current test method slightly overestimate real-world biodegradation rates.

Flemming Ingerslev, Niels Nyholm.(Department of Environmental Science and Engineering, Building 115, Technical University of Denmark, DK-2800, Lyngby, Denmark). **Shake-Flask Test for Determination of Biodegradation Rates of ^{14}C- Labeled Chemicals at Low Concentrations in Surface Water Systems.** Ecotoxicology and Environmental Safety, 45(3) (2000), 274-283.

A simple shake-flask surface water biodegradability die away test with ^{14}C-labeled chemicals added to microgram per liter concentrations (usually 1–100 µg/L) is described and evaluated. The aim was to provide information on biodegradation behavior and kinetic rates at environmental (low) concentrations in surface water systems. The basic principle of measurement was to determine evolved CO_2 indirectly from measurements of total organic activity in subsamples after stripping off their content of CO_2. Used with surface water alone the test simulates a pelagic environment and amended with sediments (0.1–1 dry weight/L) the test is intended to simulate a water environment with suspended solids (e.g., resuspended sediments). A protocol of the test used with the ^{14}C technique or with specific chemical analysis was recently developed by the International Organization for Standardization. Practical experience with the method is presented for a set of reference substances. These substances could be ranked in five groups of decreasing biodegradability: aniline>*p*-nitrophenol, 2,4-dichlorophenoxyacetic acid>4-chloroaniline>maleic hydrazide, pentachlorophenol>atrazine. It was found that degradation rates and lag periods varied considerably among sampling sites and sometimes also among samples from the same site. No significant correlation could be established between degradation rates and microbial biomass estimates. Even small portions of added sediments greatly enhanced biodegradation of the absorbable compound pentachlorophenol, probably by providing sites for microbial attachment. Repeated tests indicated consistent degradation behavior for the readily degradable substances, whereas degradation sometimes stopped or failed with the more recalcitrant substances. A preadaptation step involving regular reinoculation with freshly collected surface water could, however, overcome the problems of false-negative results.

Francesco Pomati, Gianluca Manarolla, Olivia Rossi, Davide Vigetti and Carlo Rossetti. (DBSF, University of Insubria, via J.H. Dunant 3, 21100 Varese, Italy). **Possible role in paralytic shellfish toxin metabolism in the cyanobacterium Planktothrix sp. FP1. The purine degradation pathway**. Environment International, 27(6) (2001), 463-470.

The paralytic shellfish toxins (PSTs) are potent neurotoxic alkaloids and their major biological effect is due to the blockage of voltage-gated sodium channels in excitable cells. They have been recognised as an important health risk for humans, animals, and ecosystems worldwide. The metabolic pathways that lead to the production and the degradation of these toxic metabolites are still unknown. In this study, we investigated the possible link between PST accumulation and the

activation of the metabolism that leads to purine degradation in the filamentous freshwater cyanobacterium Planktothrix sp. FP1. The purine catabolic pathway is related to the nitrogen microcycle in water environments, in which cyanobacteria use traces of purines and ureides as a nitrogen source for growth. Thus, the activity of allantoicase, a key inducible enzyme of this metabolism, was used as tool for assaying the activation of the purine degradation pathway. The enzyme and the pathway were induced by allantoic acid, the direct substrate of allantoicase, as well as by adenine and, to a lower degree, by urea, one of the main products of purine catabolism. Crude cell extract of Escherichia coli was also employed and showed the best induction of allantoicase activity. In culture, Planktothrix sp. FP1 showed a differential accumulation of PST in consequence of the induction with different substrates. The cyanobacterial culture induced with allantoic acid accumulated 61.7% more toxins in comparison with the control. On the other hand, the cultures induced with adenine, urea, and the E. coli extract showed low PST accumulation, respectively, 1%, 38%, and 5% of the total toxins content detected in the noninduced culture. A degradation pathway for the PSTs can be hypothesised: as suggested for purine alkaloids in higher plants, saxitoxin (STX) and derivatives may also be converted into xanthine, urea, and further to CO2 and NH4+ or recycled in the primary metabolism through the purine degradation pathway.

Friedrich Widdel and Ralf Rabuszx. (Max-Planck-Institut für Marine Mikrobiologie, Celsiusstrasse 1, D-28359 Bremen, Germany). **Anaerobic biodegradation of saturated and aromatic hydrocarbons**. Current Opinion in Biotechnology, 12(3) (2001), 259-276.

Saturated and aromatic hydrocarbons are wide-spread in our environment. These compounds exhibit low chemical reactivity and for many decades were thought to undergo biodegradation only in the presence of free oxygen. During the past decade, however, an increasing number of microorganisms have been detected that degrade hydrocarbons under strictly anoxic conditions.

G. M. Walker, L. R. Weatherley. (School of Chemical Engineering, The Queen's University of Belfast, Belfast BT9 5AG, Northern Ireland, UK. Department of Chemical and Process Engineering, University of Canterbury, Christchurch, New Zealand). **Biodegradation and biosorption of acid anthraquinone dye.** Environmental Pollution, 108(2) (2000), 219-223.

The acid anthraquinone dye Tectilon Blue (TB4R) is a major coloured component from the aqueous effluent of a carpet printing plant in Northern Ireland. The aerobic biodegradation of TB4R has been investigated experimentally in batch systems, using three strains of bacteria, namely, *Bacillus gordonae* (NCIMB 12553), *Bacillus benzeovorans* (NCIMB 12555) and *Pseudomonas putida* (NCIMB 9776). All three strains successfully decolourised the dye, and results were correlated using Michaelis–Menten kinetic theory. A recalculation of the reaction rate constants, to account for biosorption, gave an accurate simulation of the colour removal over a 24-h period. Up to 19% of the decolorisation was found to be caused by biosorption of the dye onto the biomass, with the majority of the decolorisation caused by utilisation of the dye by the bacteria. The reaction rate was found to be intermediate between zero and first order at dye concentrations of 200–1000 mg/l.

Hanumanthanaik P Doddamani, Harichandra Z Ninnekar. (Department of Biochemistry, Karnatak University, Dharwad-580 003, India). **Biodegradation of Carbaryl by a Micrococcus Species.** Current Microbiology, 43(1) (2001), 0069 – 0073.

A bacterium capable of utilizing carbaryl as sole source of carbon was isolated from garden soil and identified as a Micrococcus species. The organism also utilized carbofuran, naphthalene, 1-naphthol, and several other aromatic compounds as growth substrates. The organism degraded carbaryl by hydrolysis to yield 1-naphthol and methylamine. 1-Naphthol was further metabolized via salicylate by a gentisate pathway, as evidenced by oxygen uptake and enzymatic studies.

Hanumanthanaik P Doddamani, Harichandra Z Ninnekar. (Department of Biochemistry, Karnatak University, Dharwad--580 003, India). **Biodegradation of Phenanthrene by a Bacillus Species**. Current Microbiology, 41(1) (2000), 0011 – 0014.

A bacterial strain capable of utilizing phenanthrene as sole source of carbon was isolated from soil and identified as a Bacillus sp. The organism also utilized naphthalene, biphenyl, anthracene, and other aromatic compounds as growth substrates. The organism degraded phenanthrene through the intermediate formation of 1-hydroxy-2-naphthoic acid, which was further metabolized via o-phthalate by a protocatechuate pathway, as evidenced by oxygen uptake and enzymatic studies.

Harald J Ruijssenaars, Francesca Stingele, Sybe Hartmans. (Division of Industrial Microbiology, Department of Food Technology and Nutritional Sciences, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Bioscience Department, Nestlé Research Center, P.O. Box 44, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland). **Biodegradability of Food-Associated Extracellular Polysaccharides.** Current Microbiology, 40(3) (2000), 0194 – 0199.

Exopolysaccharides (EPSs) produced by lactic acid bacteria, which are common in fermented foods, are claimed to have various beneficial physiological effects on humans. Although the biodegradability of EPSs is important in relation to the bioactive properties, knowledge on this topic is limited. Therefore, the biodegradability of eight EPSs, six of which were produced by lactic acid bacteria, was compared with microorganisms from human feces or soil. EPS-degradation was determined from the decrease in polysaccharide-sugar concentration and by high-performance size exclusion chromatography (HPSEC). Xanthan, clavan, and the EPSs produced by Streptococcus thermophilus SFi 39 and SFi 12 were readily degraded, in contrast to the EPSs produced by Lactococcus lactis ssp. cremoris B40, Lactobacillus sakei 0-1, S. thermophilus SFi20, and Lactobacillus helveticus Lh59. Clearly, the susceptibility of exopolysaccharides to biological breakdown can differ greatly, implying that the physiological effects of these compounds may also vary a lot.

Heiko Feitkenhauer, Rudolf Müller, Herbert MAuml;rkl. (Laboratory of Chemical Engineering and Industrial Chemistry, ETH Zürich, Hönggerberg, Building HCI F106, CH-8093 Zürich, Switzerland. Institute of Technical Biochemistry, Technical University of Hamburg-Harburg, Denickestr. 15, 21071 Hamburg, Germany. Institute of Bioprocess and Biochemical Engineering, Technical University of Hamburg-Harburg, Denickestr. 15, D-21071 Hamburg, Germany). **Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 6070 °C by Thermus and Bacillus spp**. Biodegradation, 14(6) (2003), 367-372.

Although polycyclic aromatic hydrocarbons (PAH) and alkanes are biodegradable at ambient temperature, in some cases low bioavailabilities are the reason for slow biodegradation. Considerably higher mass transfer rates and PAH solubilities and hence bioavailabilities can be obtained at higher temperatures. Mixed and pure cultures of aerobic, extreme thermophilic microorganisms (Bacillus spp., Thermus sp.) were used to degrade PAH compounds and PAH/alkane mixtures at 65 °C. The microorganisms used grew on hydrocarbons as sole carbon and energy source. Optimal growth temperatures were in the range of 60–70 °C at pH values of 6–7. The conversion of PAH with 3–5 rings (acenaphthene, fluoranthene, pyrene, benzo[e]pyrene) was demonstrated. Efficient PAH biodegradation required a second, degradable liquid phase. Thermus brockii Hamburg metabolized up to 40 mg (l h)-1 pyrene and 1000 mg (1 h)-1 hexadecane at 70 °C. Specific growth rates of 0.43 h-1 were measured for this strain with hexadecane/pyrene mixtures as the sole carbon and energy source in a 2-liter stirred bioreactor.

About 0.7 g cell dry weight were formed from 1 g hydrocarbon. The experiments demonstrate the feasibility and efficiency of extreme thermophilic PAH and alkane biodegradation.
Helia Radianingtyas, Phillip C. Wright. (Biochemical Engineering and Environmental Technologies Group, Department of Chemical and Process Engineering, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK. Biological and Environmental Systems Group, Department of Chemical and Process Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK). **2-Propanol degradation by *Sulfolobus solfataricus*.** Biotechnology Letters, 25(7) (2003), 579-583.

Sulfolobus solfataricus used 2-propanol and 2-propanone (acetone) when grown in static cultures at 78 °C with or without glucose at 10 g l-1. The presence of 3.92 g 2-propanol l-1 in both cases inhibited growth. However, acetone accumulation following 2-propanol depletion suggested that 2-propanol was co-metabolized via the acetone metabolic pathway. Glucose at 10 g l-1 increased 2-propanol and acetone utilization from 0.93 g l-1 to 1.77 g l-1 and from 0.11 g l-1 to 1.62 g l-1, respectively. Without glucose, immobilized *S. solfataricus* cells increased the 2-propanol removal rate to 0.035 g l-1 h-1, compared to 0.0012 g l-1 h-1 by its suspended counterpart. The results suggest the establishment of an immobilized reactor configuration is preferential for the treatment of high temperature solvent waste streams by this acidothermophilic Crenarchaeon.
Hojae Shima, EungBai Shina, Shang-Tian Yangb. (Department of Civil and Environmental Engineering, Hanyang University, 1271 Sa-1-Dong, Ansan, Kyungkido 425-791, South Korea. Department of Chemical Engineering, The Ohio State University, 140 West 19th Avenue, Columbus, OH 43210, USA). **A continuous fibrous-bed bioreactor for BTEX biodegradation by a co-culture of Pseudomonas putida and Pseudomonas fluorescens**. Advances in Environmental Research,7(4)(2003),889-900.

A co-culture of Pseudomonas putida and P. fluorescens immobilized in a fibrous-bed bioreactor was used to degrade benzene, toluene, ethylbenzene and xylenes (collectively known as BTEX), present as sole carbon sources in contaminated water. The kinetics of BTEX biodegradation in the fibrous-bed bioreactor operated under the liquid-continuous condition was studied. Biodegradation rates of BTEX increased with increasing BTEX concentration and reactor loading rate. For benzene, the maximum biodegradation rate was 38 mg/l/h at a loading rate of 265 mg/l/h. For toluene, the rate was 45 mg/l/h at a 100 mg/l/h loading rate. Aeration was not used in the process and the addition of hydrogen peroxide (H2O2) as an additional oxygen source improved benzene and toluene biodegradation for the high strength synthetic wastewater feeds. When benzene, toluene, ethylbenzene and para-xylene were present as a mixture in the feed, they were concurrently and completely biodegraded under hypoxic conditions (no addition of air or H2O2). The total BTEX biodegradation rate was as high as 600 mg/l/h at the highest BTEX loading rate, 1000 mg/l/h, studied. Individual BTEX compounds were efficiently and concurrently degraded at a retention time of less than 15 h. Immobilized cells adapted in the bioreactor showed no preferential degradation of BTEX present as mixtures. The bioreactor also had a stable long-term performance, maintaining its ability for efficient BTEX degradation without requiring additional nutrients (e.g. glucose) for more than 1 year. The good performance of the fibrous-bed bioreactor was attributed to the high cell density and unique cell immobilization process provided by the fibrous matrix, which allowed use of the reactor for continued regeneration, adaptation and selection of efficient BTEX degraders in the bioreactor environment.
Hong-Gyu Song. (Division of Biological Sciences, Kangwon National University Hyoja-dong 192-1, Chuncheon 200-701, South Korea). **Degradation of humus-bound metabolites generated from toluene and o-xylene in soil**. International Biodeterioration & Biodegradation, 51(2) (2003), 129-132.

We investigated the fate of 14C-labeled toluene and o-xylene in soil. Some of the metabolites of toluene and o-xylene were bound to the humic matrix rather than mineralized or incorporated into biomass. The distribution of the bound radioactive metabolites from toluene and o-xylene were respectively, 33.4% and 32.1% of original radioactivity. Most of the humus-bound metabolites (83.5–85.4%) from toluene and o-xylene were found in the humin fraction and less than 10% were incorporated to fulvic and humic acid, respectively. The bound metabolites from radioactive toluene and o-xylene were not extracted with various solvents, and they showed slow biodegradation. The mineralization rates of bound metabolites generated from toluene and o-xylene did not differ significantly, and the turnover times ranged between 3.9 and 4.6 years.
Horacio Bach, Yevgeny Berdichevsky, and David Gutnick. (Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Science, Tel Aviv University, Tel Aviv 69978, Israel). **An Exocellular Protein from the Oil-Degrading Microbe Acinetobacter venetianus RAG-1 Enhances the Emulsifying Activity of the Polymeric Bioemulsifier Emulsan**. Applied and Environmental Microbiology, 69(5) (2003), 2608-2615.

The oil-degrading microorganism Acinetobacter venetianus RAG-1 produces an extracellular polyanionic, heteropolysaccharide bioemulsifier termed emulsan. Emulsan forms and stabilizes oil-water emulsions with a variety of hydrophobic substrates. Removal of the protein fraction yields a product, apoemulsan, which exhibits much lower emulsifying activity on hydrophobic substrates such as n-hexadecane. One of the key proteins associated with the emulsan complex is a cell surface esterase. The esterase (molecular mass, 34.5 kDa) was cloned and overexpressed in Escherichia coli BL21(DE3) behind the phage T7 promoter with the His tag system. After overexpression, about 80 to 90% of the protein was found in inclusion bodies. The overexpressed esterase was recovered from the inclusion bodies by solubilization with deoxycholate and, after slow dialysis, was purified by metal chelation affinity chromatography. Mixtures containing apoemulsan and either the catalytically active soluble form of the recombinant esterase isolated from cell extracts or the solubilized inactive form of the enzyme recovered from the inclusion bodies formed stable oil-water emulsions with very hydrophobic substrates such as hexadecane under conditions in which emulsan itself was ineffective. Similarly, a series of esterase-defective mutants were generated by site-directed mutagenesis, cloned, and overexpressed in E. coli. Mutant proteins defective in catalytic activity as well as others apparently affected in protein conformation were also active in enhancing the apoemulsan-mediated emulsifying activity. Other proteins, including a His-tagged overexpressed esterase from the related organism Acinetobacter calcoaceticus BD4, showed no enhancement.
J. Blok. (Haskoning Consulting Engineers and Architects, 151, 6500 AD, Nijmegen, The Netherlands). **Probability of Biodegradation, a Novel Concept for Improving Chemical Classification and Risk Assessment.** Ecotoxicology and Environmental Safety, 47(3) (2000), 221-230.

In this article biodegradability is considered as a combination of an inherent substance property, defined as the maximum specific growth rate, μ_{max} and a condition of the environment defined as a specific fraction (f_s) of the total viable biomass. By proper analysis of test results it is possible to quantify both parameters by one single standard test. Calculations with literature data indicate that for the majority of the degradable substances, μ_{max} may vary between 0.5 and 10 per day. The specific fractions, however, may vary 5 or 6 orders of magnitude and can be as low as 10^{-8}. This concept gives a valuable tool in environmental risk assessment. As the results will be less influenced by test conditions, data will be more reproducible and can be more predictive for a specific environment. The results allow predicting the time needed to achieve adaptation in a treatment plant and, in particular, the behavior under conditions with discontinuous discharge.

By using threshold criteria for μ_{max}, f_s, and percentage mineralization, a new classification scheme with eight different classes for biodegradability is proposed. Compared to the currently used system with two classes, the prediction of biodegradation will be more sophisticated. The proposed system differentiates for six types of inherently degradable substances and identifies those substances with an abnormal growth curve, due to inhibition or toxicity, poor water solubility, or incomplete mineralization. For these classes the proposed equations are not directly applicable and more research will be required to predict their behavior.
J. Blok. (HASKONING Consulting Engineers and Architects, 151, 6500AD, Nijmegen, The Netherlands). **A Single Model for Mass Transfer and Growth for Biodegradation Rates in Activated Sludge**. Ecotoxicology and Environmental Safety, 48(2) (2001), 148-160.

In the scope of environmental risk assessment for new and existing chemicals a generic model is developed to predict the possible concentration of pollutants after passage of sewage treatment plants. The model combines Monod kinetics with mass transfer rates through an imaginary boundary layer between mixed liquor and active biomass. For each specific substance a combined rate constant can be calculated. This combined rate constant is dependent on the influent concentration of the substance and the maximum growth rate of the substance as the main inherent substance property. All other parameters are inherent for the activated sludge process and can be quantified on the basis of generic default values. Monod kinetics for bacterial growth are modified for a concentration gradient between the mixed liquor and the suspended solids. This gradient causes a difference between an apparent half-saturation value ($K_{S\ app}$) and the real half-saturation value ($K_{S\ real}$). Real half-saturation values are estimated by linear extrapolation of a plot of the apparent values against mass transfer rates, whereas the slope of this plot gives the specific diffusion limitated mass transfer rate constant (K_{diff}). From the available data it is concluded that values for $K_{S\ real}$ for water-soluble substances are below 100 µg COD/L, whereas the value for K_{diff} varies between 100 and 500/day. Compared to the currently applied default values, which are based on a classification between ready and inherent biodegradability, the proposed method is more sophisticated.
J. P. Del'Arco, F. P. de França. (Departamento de Engenharia Bioquímica, Escola de Química, Cento de Tecnologia, Bloco E, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, CEP 21.949-900, Brazil). **Influence of oil contamination levels on hydrocarbon biodegradation in sandy sediment.** Environmental Pollution, 112(3) (2001), 515-519.

The influence of oil concentration on hydrocarbon biodegradation in a sandy sediment was studied in polyvinyl chloride reactors (0.45×0.28×0.31 m) containing 76.8 kg of beach sand in natura, where the upper layer was artificially contaminated with petroleum. The oil-degrading microorganisms used consisted of a mixed culture named N_D, obtained from landfarming and associated with indigenous microorganisms. On the 28th day of the process, the degradation in reactors containing sandy sediment contaminated with light Arabian oil and presenting an initial oil content of 14, 21 or 28 g kg^{-1} reached the following levels (%): 33.7, 32.9 and 28.9 for oil and grease; up to 88.3, 35.3 and 13.0 for C_{14}–C_{26} *n*-alkanes; and 100, 61.3 and 59.4 for pristane, respectively. Phytane removal (37.1%) was only detected in the reactor contaminated with the lowest oil concentration studied. These results, together with the expressive bacterial growth observed (from 10^6 to 10^{11} cfu g^{-1}) give strong support to the argument that biodegradation was the dominant component of the remediation process. Susceptibility to biodegradation was inversely proportional to increasing oil contamination. The degradation of branched alkane: pristane was not repressed by the presence of *n*-alkanes.

Jerold Scott Teeter, Roger D. Meyerhoff. (Lilly Research Laboratories, A Division of Eli Lilly and Company, 2001 West Main Street, Drop Code GL45, Greenfield, IN 46140, USA). **Aerobic degradation of tylosin in cattle, chicken, and swine excreta.** Environmental Research, 93(1) (2003), 45-51.

Tylosin, a fermentation-derived macrolide antibiotic, was tested to determine its aerobic degradation rate in cattle, chicken, and swine excreta. For chicken, excreta from a hen administered ^{14}C-tylosin as part of a metabolism study were used. For cattle and swine, ^{14}C-tylosin was added to control excreta. The formation of ^{14}C volatile breakdown products and $^{14}CO_2$ was not observed throughout the study. Material balance for the cabon-14 label ranged between 94% and 104%. Initial, day-0, concentrations of tylosin-A averaged 119.52±4.39, 35.01±1.34, and 62.82±2.11 μg/g (dry weight basis) for cattle, chicken, and swine excreta samples, respectively. After 30 days, samples averaged 4.16±0.69 and 4.11±0.69 μg/g tylosin-A in cattle and swine excreta, respectively. No residues of tylosin-A or its factors were apparent in the chicken excreta samples after 30 days of incubation. In each case, tylosin declined to less than 6.5% of the initial level after 30 days. Calculated first-order half-lives under the test conditions were 6.2 days, <7.6 days, and 7.6 days for cattle, chicken, and swine excreta, respectively. The results indicate that tylosin residues degrade rapidly in animal excreta. Therefore, tylosin residues should not persist in the environment.

Ji-Dong Gu. (Laboratory of Environmental Toxicology, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, People's Republic of China. The Swire Institute of Marine Science, The University of Hong Kong, Shek O, Cape d'Aguilar, Hong Kong SAR, People's Republic of China). **Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances**. International Biodeterioration & Biodegradation, 52(2) (2003), 69-91.

Biodeterioration of polymeric materials affect a wide range of industries. Degradability of polymeric materials is a function of the structures of polymeric materials, the presence of degradative microbial population and the environmental conditions that encourage microbial growth. Our understanding of polymer degradation has been advanced in recent years, but the subject is still inadequately addressed. This is clearly indicated by the lack of information available on biodeterioration of polymeric materials, particularly the mechanisms involved and the microorganisms participated. In this review, polymers are treated according to their origin and biodegradability, and grouped as biopolymer, chemically modified natural polymers and recalcitrant polymers. Selective examples are used to illustrate the mechanisms and microorganisms involved in degradation of specific polymeric materials, and detection methods used for degradation and deterioration tests are discussed. In addition, new detection techniques and preventive measures are also presented.

José L. Sanz, Elayne Culubret, Juan de Ferrer, Alfonso Moreno, José L. Berna. (Unit of Applied Microbiology, Centre of Molecular Biology, Autónoma University of Madrid, 28049 Madrid, Spain. Petroquímica Española (Petresa). Avenida Partenón 12, 28042 Madrid, Spain). **Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors**. Biodegradation, 14(1) (2003), 57-64.

The anaerobic biodegradation of Linear Alkylbenzene Sulfonate (LAS) was studied in Upflow Anaerobic Sludge Blanket Reactors (UASB). One reactor was fed with easily degradable substrates and commercial LAS solution during a period of 3 months (Reactor 1), meanwhile a second reactor was fed with a commercial LAS solution without co-substrate (Reactor 2) during 4 months. Both reactors were operated with an organic loading rate of 4–5 mg-LAS/l*day and a hydraulic retention time of one day. The LAS biodegradation was determined by full mass balance. LAS was analysed by HPLC in the liquid phase (influent and effluent streams of the

reactors) as well as in the solid phase (granular sludge used as biomass). The results indicate a high level of removal (primary biodegradation: 64–85%). Biodegradation was higher in the absence of external co-substrates than in the presence of additional sources of carbon. This indicates that the surfactant can be partially used as carbon and energy source by anaerobic bacteria. Under the operating conditions used, inhibition of the methanogenic activity or any other negative effects on the biomass due to the presence of LAS were not observed. The methanogenic activity remained high and stable throughout the experiment.

Joseph G. Leahy, Karen D. Tracy, Michael H. Eley. (Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL 35899, USA). **Degradation of volatile hydrocarbons from steam-classified solid waste by a mixture of aromatic hydrocarbon-degrading bacteria**. Biotechnology Letters, 25(6) (2003), 479-483.

Steam classification is a process for treatment of solid waste that allows recovery of volatile organic compounds from the waste via steam condensate and off-gases. A mixed culture of aromatic hydrocarbon-degrading bacteria was used to degrade the contaminants in the condensate, which contained approx. 60 hydrocarbons, of which 38 were degraded within 4 d. Many of the hydrocarbons, including styrene, 1,2,4-trimethylbenzene, naphthalene, ethylbenzene, *m*-/*p*-xylene, chloroform, 1,3-dichloropropene, were completely or nearly completely degraded within one day, while trichloroethylene and 1,2,3-trichloropropane were degraded more slowly.

Junko Hata, Kazuhiro Takamizawa, Naoyuki Miyata, Keisuke Iwahori. (Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan. Department of Bioprocessing, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan). **Biodegradation of *cis*-1,2-Dichloroethylene and Vinyl Chloride in Anaerobic Cultures Enriched from Landfill Leachate Sediment Under Fe(III)-Reducing Conditions**. Biodegradation, 14(4) (2003), 275-283.

An anaerobic, Fe(III)-reducing enrichment culture, which originated from a sediment sample collected at a landfill in Nanji-do, Seoul, Korea, was capable of degrading *cis*-1,2-dichloroethylene (*cis*-DCE) and vinyl chloride (VC). Although it exhibited the ability under Fe(III)-reducing conditions, the chlorinated ethenes degradation was not linked to the Fe(III) reduction. During *cis*-DCE degradation, no VC, ethene, or ethane was detected through the experimental period. Also, this culture did not accumulate ethene and ethane during the VC degradation. It was unlikely that *cis*-DCE was reductively dechlorinated to VC and then the VC formed was dechlorinated fast enough. Because the kinetic data showed that the rate of *cis*-DCE degradation was 3.5 times higher than that of VC. Whereas glucose supported the culture growth and the degradation, formate, acetate, butyrate, propionate, lactate, pyruvate, and yeast extract did not. The results appeared consistent with the involvement of oxidative degradation mechanism rather than reductive dechlorination mechanism. The traits of the culture described here are unusual in the anaerobic degradation of chlorinated ethenes and may be useful for searching an effective organism and mechanism regarding anaerobic *cis*-DCE and VC degradation.

K. C. Das, Matt C. Smith, David K. Gattie, Dorothy D. Hale Boothe. (Department of Biological and Agricultural Engineering, Driftmier Engineering Center, University of Georgia, Athens, GA 30602-4435, USA). **Stability and quality of municipal solid waste compost from a landfill aerobic bioreduction process**. Advances in Environmental Research, 6(4) (2002), 411-418.

Solid waste compost product from an aerobic bioreduction process at a full-scale landfill was characterized. The landfill was sampled after 5 months of aerobic bioreduction for spatial variations in biological stability. The product after 14 months of bioreduction was excavated and

screened in three different ways to improve product quality. After 5 months of bioreduction, the stability index (SI) of solid waste in the landfill ranged from low activity (0.15–0.67 mg g-1 h-1) in the 6.1–7.6-m depth layer to high activity (1.42–2.14 mg g-1 h-1) at the 4.6–6.1-m depth layer. After 14 months of bioreduction, the 9.5-mm trommel screen provided a superior product among those tested and resulted in total inert content of 3.5% compared to 9.0% (dry basis) when using a 19.1-mm screen. Product SI ranged from 0.39 to 0.55 mg g-1 h-1, indicating stability. Regulated heavy metals were below EPA exceptional quality compost levels. Lead, nickel, chromium and zinc were at relatively higher levels than other metals.
K. Fadil, A. Chahlaoui, A. Ouahbi, A. Zaid, R. Borja. (Laboratoire de Biochimie et Pharmacognosie, Département de Biologie, Faculté des Sciences de Meknès, BP 4010, Beni Mhamed, Meknès, Maroc. Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012-, Sevilla, Spain). **Aerobic biodegradation and detoxification of wastewaters from the olive oil industry**. International Biodeterioration & Biodegradation, 51(1) (2003), 37-41.

Growth and polyphenol biodegradation by three microorganisms namely Geotrichum sp., Aspergillus sp. and Candida tropicalis were studied on olive mill wastewater (OMW). These three microorganisms were selected for their tolerance to the polyphenols. The biodegradation process of OMW was investigated in batch regime by conducting experiments where the initial concentration of chemical oxygen demand (COD) was varied. Furthermore, some tests were performed to determine the most important nutrients necessary for aerobic degradation of OMW. Average COD removals were 55.0%, 52.5% and 62.8% in wastewaters fermented with Geotrichum sp., Aspergillus sp. and C. tropicalis, respectively. The maximum removal of polyphenols was 46.6% (Geotrichum sp.), 44.3% (Aspergillus sp.) and 51.7% (C. tropicalis). In addition, significant decolorization was evident.
K. Nam, J. Y. Kim and D. I. Oh. (School of Civil, Urban & Geosystem Engineering, Seoul National University, Seoul 151-742, The, Republic of Korea). **Effect of soil aggregation on the biodegradation of phenanthrene aged in soil.** Environmental Pollution, 121(1) (2003), 147-151.

A study was conducted to determine the possible role of soil aggregates in the sequestration of phenanthrene and thus in the declined biodegradation of the hydrocarbon. Phenanthrene aged in Lima loam (2-mm aggregates) showed declined biodegradation with time of aging to the test bacterium P5-2 capable of using sorbed phenanthrene. In contrast, the compound aged in a soil reconstructed with 68% clay–silt and 32% sand that had been separated from the Lima loam was readily mineralized. The percentages of each fraction used were the same as those of the original soil. Biodegradation of aged phenanthrene was not affected significantly by varying the ratios of each fraction in reconstructed mixtures. In experiments with Lima loam, its clay–silt fraction, and its sand fraction, mineralization extent was much lower in soil aggregates compared with the other samples while all had similar organic carbon content of ca. 1.51%. This suggests that aggregation may be another important determinant in the reduced biodegradation of aged phenanthrene.
Kananbala Sarangthem, Th. Nabakumar Singh. (Plant Physiology Research Laboratory. Department of Life Sciences, Manipur University, Manipur 795 003. India). **Microbial bioconversion of metabolites from fermented succulent bamboo shoots into phytosterols.** Current Science, 84(12) (2003), 1544.

Fermented succulent shoots of bamboo (Bambusa balcooa and Dendrocalamus strictus) are an enriched source of phytosterol. Microorganisms from the 'soibum exudate' involved in microbial bioconversion of phytosterol during fermentation of succulent bamboo shoots were isolated and identified as Bacillus subtilis, B. licheniformis, B. coagulans and Micrococcus

tuteus. Crude phytosterol was purified to isolate β-sitosterol by thin layer chromatography and identified by IR and mass spectral data. The isolated β-sitosterol was then subjected to microbial bioconversion using B. subtilis yielding a considerable amount of androstadienedione in the presence of metal chelate inhibitor (0.1% α, α'-dipyridyl).

Kazuya Watanabe, Natsuko Hamamura. (Laboratory of Applied Microbiology, Marine Biotechnology Institute, 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan). **Molecular and physiological approaches to understanding the ecology of pollutant degradation**. Current Opinion in Biotechnology, 14(3) (2003), 289-295.

Pollutant biodegradation in the environment occurs in the context of various interactions among microorganisms. To understand this ecological process, identification of functionally important populations is considered to be the primary step, which can be followed by isolation and laboratory pure-culture studies of the important organisms. Laboratory studies can then proceed to the analysis of *in situ* activity and interactions with other organisms. Such studies will shape a deeper understanding of the ecology of pollutant degradation and facilitate the development of new bioremediation strategies.

Kensuke Furukawa. (Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan). **Engineering dioxygenases for efficient degradation of environmental pollutants**. Current Opinion in Biotechnology, 11(3) (2000), 244-249.

Dioxygenases have recently been engineered to improve their capabilities for environmental pollutant degradation. The techniques used to achieve this include in vitro DNA shuffling and subunit or domain exchanges between dioxygenases of different bacterial origins. Such evolved enzymes acquire novel and enhanced degradation capabilities of xenobiotic compounds, such as polychlorinated biphenyls, trichloroethylene and a variety of aromatic compounds. Hybrid strains in which the evolved genes are integrated into the chromosomal operons exhibit efficient degradation of xenobiotic chlorinated compounds.

Kirsi-Maarit Lehto, Helge Lemmetyinen. (Institute of Materials Chemistry Author for correspondence Jaakko A. Puhakka. Institute of Environmental Engineering and Biotechnology, Tampere University of Technology, PO Box 541, FIN-33101 Tampere, Finland. 1Institute of Materials Chemistry). **Biodegradation of selected UV-irradiated and non-irradiated polycyclic aromatic hydrocarbons (PAHs)**. Biodegradation, 14(4) (2003), 249-263.

Biodegradation of UV-irradiated anthracene, pyrene, benz[a]anthracene, and dibenz[a, h]anthracene was compared to that of the non-irradiated samples, individually and in synthetic mixtures with enrichment cultures. Combined treatment was repeated for individual anthracene and for the PAH mixture with Sphingomonas sp. strain EPA 505 and Sphingomonas yanoikuyae. Enrichment culture studies were performed on the PAH mixtures in the presence of the main photoproduct of anthracene, pure 9,10-anthracenedione. Photochemically pretreated creosote solutions were also subjected to biodegradation and the results were compared to those of the non-irradiated solutions. The primary interest was on 16 polycyclic aromatic hydrocarbons (PAHs) listed as priority pollutants by European Union (EU) and the United States Environmental Protection Agency (USEPA). Irradiation accelerated the biodegradation onset for anthracene, pyrene, and benz[a]anthracene when they were treated individually. The biodegradation of irradiated pyrene started with no lag phase andwas complete by 122 h whereas biodegradation of the non-irradiated sample had a lag of 280 h and resulted in complete degradation by 720 h. Biodegradation of PAHs was accelerated in synthetic mixtures, especially in the presence of pure 9,10-anthracenedione. In general, irradiation had no effect on the biodegradation of PAHs incubated in synthetic mixtures or with pure cultures. Under current

experimental conditions, the UV-irradiation invariably reduced the biodegradation of PAHs in creosote. Based on the results of the present and previous photochemical-biological studies of PAHs, the influence of the photochemical pretreatment on the biodegradation is highly dependent on the compounds being treated and other process parameters.

L. J. Forney, W. -T. Liu, J. B. Guckert, Y. Kumagai, E. Namkung, T. Nishihara, R. J. Larson. (Center for Microbial Ecology, Michigan State University, East Lansing, Michigan, 48824-1325. Environmental Science Department, The Procter & Gamble Co. Ross, Ohio, 45061. Professional and Regulatory Services, Procter & Gamble Asia, 1-17 Koyoh-cho, Naka, Higashinda-ku, Kobe, 658, Japan. Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Shuita-shi, Osaka, 565-0871, Japan. Corporate Microbiology, The Procter & Gamble Co. Ross, Ohio, 45061). **Structure of Microbial Communities in Activated Sludge: Potential Implications for Assessing the Biodegradability of Chemicals**. Ecotoxicology and Environmental Safety, 49(1) (2001), 40-53.

Various methods used to assess the biodegradability of chemicals often employ activated sludge as an inoculum since chemicals that ultimately enter the environment are often discharged through wastewater. Differences in the structure and function of activated sludge microbial communities that may complicate interpretation of biodegradation tests could arise from differences in wastewater composition, wastewater treatment plant (WWTP) operation, or manipulations done after collection of the activated sludge. In this study, various methods were used to characterize the structure of microbial communities found in freshly collected activated sludge from WWTPs in Japan, Europe, and the United States, as well as sludge that had been continuously fed either sewage or a glucose-peptone mixture for several weeks after collection. Comparisons of biomass levels, whole-community substrate utilization (determined using Biolog GN and GP plates), and phospholipid fatty acid (PLFA) profiles indicated there were both geographical and temporal differences among freshly collected activated sludge samples. Moreover, marked shifts in the structure of activated sludge microbial communities occurred upon continuous cultivation in the laboratory for 5 weeks using a glucose-peptone feed. These shifts were evident from whole-community substrate utilization and PLFA profiles as well as differences in the profiles of 16S rDNA genes from numerically dominant populations obtained by denaturing gradient gel electrophoresis and terminal restriction fragment analyses. Further studies are needed to better define the variability within and between activated sludge from wastewater treatment plants and laboratory reactors and to assess the impact of such differences on the outcome of biodegradability tests.

L. Pitzurra, B. Moroni, A. Nocentini, G. Sbaraglia, G. Poli, F. Bistoni. (Department of Experimental Medicine and Biochemical Sciences, Microbiology Section, University of Perugia, Val del giochetto, Perugia 0616, Italy. Department of Earth Sciences, University of Perugia, Perugia, Italy). **Microbial growth and air pollution in carbonate rock weathering**. International Biodeterioration & Biodegradation, 52(2) (2003), 63-68.

Preliminary results on limestone weathering caused by air pollution and microbial colonization are presented in this study. Outdoor exposure experimental assays were performed on Scaglia limestone samples. Samples were exposed in two areas in Perugia (Italy) that differ in degree of urban air pollution. At different times of exposure, ranging from 1 to 12 months, microbial contamination of sampled surfaces was evaluated by microbiological techniques, genotyping and scanning electron microscopy. After 1 year of exposure, a significant fungal colonization and the presence of weathering products (i.e., gypsum) were detected on sampled surfaces.

Laleh Yerushalmi, Sylvie Rocheleau, Ruxandra Cimpoia, Manon Sarrazin, Geoffrey Sunahara, Adriana Peisajovich, Gervais Leclair, Serge R. Guiot. (Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Canada, H4P 2R2. Transport Canada, 700 Leigh Caperol, Dorval, Quebec, Canada H4Y 1G7.

Environment Canada, 105 McGill Street, Montreal, Quebec, Canada H2Y 2E7. Present address: Atara Corporation, 390 Guy Street, Montreal, Quebec, Canada H3J 1S6). **Enhanced Biodegradation of Petroleum Hydrocarbons in Contaminated Soil**. Bioremediation Journal, 7(1) (2003), 37-51.

Soil samples taken from a contaminated site in Northern Quebec, Canada, exhibited a low capacity for biodegradation of total petroleum hydrocarbons (TPH), despite a high capacity for the mineralization of aromatic hydrocarbons and a low toxicity of soil leachates as measured by Microtox assay. Toxicity assays directly performed on surface soil, including earthworm mortality and barley seedling emergence, indicated moderate to high levels of toxicity. Soil biostimulation did not improve the removal of petroleum hydrocarbons, while bioaugmentation of soil with a developed enrichment culture increased the efficiency of hydrocarbon removal from 20.4% to 49.2%. A considerable increase in the removal of TPH was obtained in a bioslurry process, enhancing the mass transfer of hydrocarbons from soil to the aqueous phase and increasing the efficiency of hydrocarbon removal to over 70% after 45 days of incubation. The addition of ionic or nonionic surfactants did not have a significant impact on biodegradation of hydrocarbons. The extent of hydrocarbon mineralization during the bioslurry process after 45 days of incubation ranged from 41.3% to 58.9%, indicating that 62.7% to 83.1% of the eliminated TPH were transformed into CO2 and water.

Lilian Schoefer, Ruchika Mohan, Andreas Schwiertz, Annett Braune, Michael Blaut. (Deutsches Institut für Ernährungsforschung, 14558 Bergholz-Rehbrücke, Germany). **Anaerobic Degradation of Flavonoids by Clostridium orbiscindens**. Applied and Environmental Microbiology, 69(10) (2003), 5849-5854.

An anaerobic, quercetin-degrading bacterium was isolated from human feces and identified as Clostridium orbiscindens by comparative 16S rRNA gene sequence analysis. The organism was tested for its ability to transform several flavonoids. The isolated C. orbiscindens strain converted quercetin and taxifolin to 3,4-dihydroxyphenylacetic acid; luteolin and eriodictyol to 3-(3,4-dihydroxyphenyl)propionic acid; and apigenin, naringenin, and phloretin to 3-(4-hydroxyphenyl)propionic acid, respectively. Genistein and daidzein were not utilized. The glycosidic bonds of luteolin-3-glucoside, luteolin-5-glucoside, naringenin-7-neohesperidoside (naringin), quercetin-3-glucoside, quercetin-3-rutinoside (rutin), and phloretin-2'-glucoside were not cleaved. Based on the intermediates and products detected, pathways for the degradation of the flavonol quercetin and the flavones apigenin and luteolin are proposed. To investigate the numerical importance of C. orbiscindens in the human intestinal tract, a species-specific oligonucleotide probe was designed and tested for its specificity. Application of the probe to fecal samples from 10 human subjects proved the presence of C. orbiscindens in 8 out of the 10 samples tested. The numbers ranged from 1.87 x 108 to 2.50 x 109 cells g of fecal dry mass-1, corresponding to a mean count of 4.40 x 108 cells g of dry feces-1.

Lonnie G. Kennedy, Jess W. Everettb. (Earth Science Services, 5815 Hickory Bend, Norman, OK 73026, USA. Department of Civil Engineering, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, USA). **Microbial degradation of simulated landfill leachate: solid iron/sulfur interactions**. Advances in Environmental Research, 6(1) (2001), 17-27.

Microcosms were prepared to test if added mineral Fe3+ and SO42- could treat landfill leachate and to examine intrinsic microbial/mineral interactions related to natural attenuation. Two oxidized native sediments were used from central Oklahoma. Three types of anoxic microcosms were prepared which included the addition of: (1) mineral ferrihydrite (Fe(OH)3); (2) mineral gypsum (CaSO42H2O); and (3) no mineral amendments. Each received a synthetic leachate consisting of 2000 mg/l non-purgable organic carbon (NPOC). Measurements of substrate consumption, dissolved ions, mineral utilization/precipitation, and biological gases were made

over 12 weeks. The added CaSO42- and Fe(OH)3 were used as electron acceptors: CaSO42- by first order kinetics (k0.12 week-1) and Fe3+ by zero order kinetics (k0.16 mM week-1). The addition of either CaSO42- or Fe(OH)3 did not increase organic carbon degradation rates over methanogenesis, which was predominate in the non-amended microcosm set. Adding solid electron acceptors promoted carbonate and sulfide mineral formation and controlled greenhouse gases including CH4 and CO2. It is suggested that reduced Fe and S minerals could be used to assess organic contaminant degradation occurring due to Fe3+ and SO42- microbial reduction processes for natural attenuation studies.

Luis A. Rios-Hernandez, Lisa M. Gieg, and Joseph M. Suflita. (Institute for Energy and the Environment and Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019). **Biodegradation of an Alicyclic Hydrocarbon by a Sulfate-Reducing Enrichment from a Gas Condensate-Contaminated Aquifer**. Applied and Environmental Microbiology, 69(1) (2003), 434-443.

We used ethylcyclopentane (ECP) as a model alicyclic hydrocarbon and investigated its metabolism by a sulfate-reducing bacterial enrichment obtained from a gas condensate-contaminated aquifer. The enrichment coupled the consumption of ECP with the stoichiometrically expected amount of sulfate reduced. During ECP biodegradation, we observed the transient accumulation of metabolite peaks by gas chromatography-mass spectrometry, three of which had identical mass spectrometry profiles. Mass-spectral similarities to analogous authentic standards allowed us to identify these metabolites as ethylcyclopentylsuccinic acids, ethylcyclopentylpropionic acid, ethylcyclopentylcarboxylic acid, and ethylsuccinic acid. Based on these findings, we propose a pathway for the degradation of this alicyclic hydrocarbon. Furthermore, a putative metabolite similar to ethylcyclopentylsuccinic acid was also found in samples of contaminated groundwater from the aquifer. However, no such finding was evident for samples collected from wells located upgradient of the gas condensate spill. Microbial community analysis of the ECP-degrading enrichment by denaturing gradient gel electrophoresis revealed the presence of at least three different organisms using universal eubacterial primers targeting 550 bp of the 16S rRNA gene. Based on sequence analysis, these organisms are phylogenetically related to the genera Syntrophobacter and Desulfotomaculum as well as a member of the Cytophaga-Flexibacter-Bacteroides group. The evidence suggests that alicyclic hydrocarbons such as ECP can be anaerobically activated by the addition to the double bond of fumarate to form alkylsuccinate derivatives under sulfate-reducing conditions and that the reaction occurs in the laboratory and in hydrocarbon-impacted environments.

M. Veselý, M. Pátek, J. Newvera, A. *ejková, J. Masák, V. JirkŒ. (Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeeská 1083, 14220, Prague 4, Czech Republic. Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, 16628, Prague 6, Czech Republic). **Host-vector system for phenol-degrading Rhodococcus erythropolis based on Corynebacterium plasmids**. Applied Microbiology and Biotechnology, 61(5-6) (2003), 523 – 527.

The strain Rhodococcus erythropolis CCM2595, which was shown to degrade phenol, was chosen for genetic studies. To facilitate strain improvement using the methods of gene manipulation, the technique of genetic transfer was introduced and cloning vectors were constructed. Using the plasmid pFAJ2574, an electrotransformation procedure yielding up to 7×104 transformants/μg DNA was optimized. Escherichia coli-R. erythropolis shuttle vectors were constructed using the replicons pSR1 and pGA1 from Corynebacterium glutamicum. The small vector pSRK21 (5.8 kb) provides six unique cloning sites and selection of recombinant clones using α-complementation of β-galactosidase in E. coli. This vector, exhibiting high

segregational stability under non-selective conditions in R. erythropolis CCM2595, was applied to cloning and efficient expression of the gene coding for green fluorescent protein (gfpuv).

M.E. Acuña-Argüelles, P. Olguin-Lora, E. Razo-Flores. (Programa de Biotecnología, Instituto Mexicano del Petróleo, Eje Central Lázaro Cárdenas 152 Nte, C.P. 07730, México D.F., México). **Toxicity and kinetic parameters of the aerobic biodegradation of the phenol and alkylphenols by a mixed culture**. Biotechnology Letters, 25(7) (2003), 559-564.

A mixed culture aerobically metabolized phenol, cresol isomers (*o*-,*m*-,*p*-), 2-ethylphenol and xylenol isomers (2,5-DMP and 3,4-DMP) as the sole carbon and energy source. This culture had a high tolerance towards phenol with values of maximum degradation rate (V\\max) of 47 µM phenol mg-1 protein h-1 and inhibition substrate constant (Ki) of 10 mM. These kinetic parameters were considerably diminished and the toxicity increased with the alkylphenols. For example with 2,5-xylenol, V\\max and Ki values of 0.8 µM 2,5-xylenol mg-1 protein h-1 and 1.3 mM, respectively, were obtained. The cresols were 5-fold more toxic than phenol, whereas 2-ethylphenol and 3,4-xylenol were 11-fold more toxic, and 2,5-xylenol was 34-fold more toxic than phenol.

Mark R. Bruins, Sanjay Kapil, Frederick W. Oehme. (Department of Diagnostic Medicine–Pathobiology, College of Veterinary Medicine, Manhattan, Kansas, 66506). ***Pseudomonas pickettii:* A Common Soil and Groundwater Aerobic Bacteria with Pathogenic and Biodegradation Properties**. Ecotoxicology and Environmental Safety, 47(2) (2000), 105-111.

Pseudomonas pickettii is an aerobic, nonfermentative, Gram-negative rod-shaped, bacterium that has been isolated from soil, water, humans, and recently the bovine intestinal tract. It belongs to the rRNA group II of the genus *Pseudomonas* and has three biovars: Va-1, Va-2, and biovar 3/thomasii. *P. pickettii* can cause pneumonia, meningitis, endocarditis, and osteomyelitis in humans. It frequently is associated with nosocomial infections that often are linked to contaminated injectable solutions. *P. pickettii* exhibits remarkable ability to degrade a variety of toxic compounds such as chlorophenols, aromatic hydrocarbons, 2,4-dichlorophenoxyacetic acid, and pentacyclic triterpeniod compounds. The genes that encode for these properties are chromosome- and plasmid-associated. Strains of the organism also have demonstrated resistance to heavy metals, such as cadmium, copper, and zinc. This species can survive in a nutrient-poor environment and use a variety of toxic compounds as carbon and energy sources, making it an ideal candidate for study in the biodegradation of toxic compounds found in wastewater and soils.

Masayuki Shimao. (Department of Biotechnology, Faculty of Engineering, Tottori University, Tottori 680-8552, Japan). **Biodegradation of plastics**. Current Opinion in Biotechnology, 12(3) (2001), 242-247.

Widespread studies on the biodegradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste. Recent work has included studies of the distribution of synthetic polymer-degrading microorganisms in the environment, the isolation of new microorganisms for biodegradation, the discovery of new degradation enzymes, and the cloning of genes for synthetic polymer-degrading enzymes.

Mayra A. Laraa, Antonio J. Rodríguez-Malaverb, Orlando J. Rojas, Otón Holmquistc, Aura M. Gonzáleza, Johnny Bullóna, Nancy Peñalozab, Elisa Araujoa. (Lab. FIRP, Escuela de Ingeniería Química, Facultad de Ingeniería, Universidad de Los Andes, Mérida 5101, Venezuela. Lab. Bioquímica Adaptativa, Facultad de Medicina, Universidad de Los Andes, Mérida 5101, Venezuela. Lab. Patología Forestal, Facultad de Ciencias Forestales y Ambientales, Universidad de Los Andes, Mérida 5101, Venezuela). **Black liquor lignin**

biodegradation by Trametes elegans. International Biodeterioration & Biodegradation, 52(4) (2003), 261-267.

The white rot fungus Trametes elegans was used for direct treatment of spent black liquor from pulping processes with the aim to degrade solubilized lignin which is the primary organic by-product from the chemical digestion of lignocellulosic raw materials. The enzymatic activity of T. elegans and the resulting degradation was confirmed by UV/VIS spectrometric and size-exclusion chromatography (SEC) measurements on incubated and control samples of both industrial and synthetic black liquors. Opposing polymerization and depolymerization reactions occurred after treatment of the black liquor with T. elegans in liquid media. It is noteworthy that these effects, which originate from ligninolytic enzyme systems, occur after the direct treatment of the black liquor even if no nutrients are added. The effect of the suspension pH and incubation time on the lignolytic action of T. elegans is also presented.

Md. Zahangir Alam, A. Fakhru'l-Razia, Abul H. Mollaa. (Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia. Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100, Kuala Lumpur, Malaysia). **Biosolids accumulation and biodegradation of domestic wastewater treatment plant sludge by developed liquid state bioconversion process using a batch fermenter**. Water Research, 37(16) (2003), 3967-3977.

The biosolids accumulation and biodegradation of domestic wastewater treatment plant (DWTP) sludge by filamentous fungi have been investigated in a batch fermenter. The filamentous fungi Aspergillus niger and Penicillium corylophilum isolated from wastewater and DWTP sludge was used to evaluate the treatment performance. The optimized mixed inoculum (A. niger and P. corylophilum) and developed process conditions (co-substrate and its concentration, temperature, initial pH, inoculum size, and aeration and agitaion rate) were incorporated to accelerate the DWTP sludge treatment process. The results showed that microbial treatment of higher strength of DWTP sludge (4% w/w of TSS) was highly influenced by the liquid state bioconversion (LSB) process. In developed bioconversion processes, 93.8 g/kg of biosolids was enriched with fungal biomass protein of 30 g/kg. Enrichment of nutrients such as nitrogen (N), phosphorous (P), potassium (K) in biosolids was recorded in 6.2% (w/w), 3.1% (w/w) and 0.15% (w/w) from its initial values of 4.8% (w/w), 2.0% (w/w) and 0.08% (w/w) respectively after 10 days of fungal treatment. The biodegradation results revealed that 98.8% of TSS, 98.2% of TDS, 97.3% of turbidity, 80.2% of soluble protein, 98.8% of reducing sugar and 92.7% of COD in treated DWTP sludge supernatant were removed after 8 days of microbial treatment. The specific resistance to filtration (SRF) in treated sludge (1.4×1012 m/kg) was decreased tremendously by the microbial treatment of DWTP sludge after 6 days of fermentation compared to untreated sample (85×1012 m/kg).

Meltem Urgun-Demirtas, Krishna R. Pagilla, Benjamin C. Stark, Dale Webster. (Department of Chemical and Environmental Engineering, Illinois Institute of Technology Chicago, IL 60616, USA. Department of Biological, Chemical and Physical Sciences, Illinois Institute of Technology Chicago, IL 60616, USA). **Biodegradation of 2-Chlorobenzoate by Recombinant Burkholderia Cepacia Expressing Vitreoscilla Hemoglobin Under Variable Levels of Oxygen Availability**. Biodegradation, 14(5) (2003), 357-365.

The influence of bacterial hemoglobin, VHb, on dechlorination and degradation of 2-chlorobenzoate (2-CBA) by recombinant Burkholderia sp. under variable oxygen availability with an initial dissolved oxygen concentration of 0.27 mM-0.72 mM was investigated in batch and continuous culture. Ability to express VHb was provided to recombinant Burkholderia by transformation with the VHb gene, vgb, on plasmid pSC160. 100% of 0.5 mM CBA was

degraded in cultures with 85% and 70% of total volume as headspace air in closed reactors by both wild type and recombinant Burkholderia. The recombinant cultures were able to dechlorinate and degrade 100% of the 2-CBA in less than 48 hours at 30 °C compared to more than 120 hours for wild type cultures. The rate and extent of CBA degradation by recombinant cultures with 40% of total volume as headspace air was higher than those achieved by wild type cells at the end of the 168 hours of incubation period, 98 and 73%, respectively. The chloride released: CBA degraded molar ratio for cultures with 40% of total volume headspace air was nearly stoichiometric (molar ratio = 1.0) for recombinant strains, whereas it was non-stoichiometric (molar ratio = 0.24) for wild type cells. The results suggest a suicidal meta-pathway for wild type cells and a complete dechlorination and degradation pathway for recombinant cells under hypoxic conditions. The degradation and dechlorination ability of both types of cells was also investigated in continuous reactor studies by varying the dilution rate under hypoxic conditions. Regarding potential of the recombinant strain for 2-CBA degradation in either open ecosystems or closed bioreactor bioremediation systems, the stability of the plasmid containing vgb in the recombinant cells was also studied; the plasmid was 100% stable at 0.025 h-1 dilution rate (~1.7 d hydraulic retention time), even after one month.

Mikael Eriksson, Erik Sodersten, Zhongtang Yu, Gunnel Dalhammar, William W. Mohn. (Departmentof Microbiology and Immunology, University of British Columbia,Vancouver, British Columbia V6T 1Z3,Canada. Department of Biotechnology,Royal Institute of Technology, KTH, SE-100 44 Stockholm,Sweden). **Degradation of Polycyclic Aromatic Hydrocarbons at Low Temperature under Aerobic and Nitrate-Reducing Conditions in Enrichment Cultures from Northern Soils**. Applied and Environmental Microbiology, 69(1) (2003), 275-284.

Thepotential for biodegradation of polycyclic aromatic hydrocarbons (PAHs)at low temperature and under anaerobic conditions is not wellunderstood, but such biodegradation would be very useful forremediation of polluted sites. Biodegradation of a mixture of 11different PAHs with two to five aromatic rings, each at a concentrationof 10 µg/ml, was studied in enrichment cultures inoculated withsamples of four northern soils. Under aerobic conditions, lowtemperature severely limited PAH biodegradation. After 90 days, aerobiccultures at 20°C removed 52 to 88% of the PAHs. The mostextensive PAH degradation under aerobic conditions at 7°C,53% removal, occurred in a culture from creosote-contaminatedsoil. Low temperature did not substantially limit PAH biodegradationunder nitrate-reducing conditions. Under nitrate-reducing conditions,naphthalene, 2-methylnaphthalene, fluorene, and phenanthrene weredegraded. The most extensive PAH degradation under nitrate-reducingconditions at 7°C, 39% removal, occurred in a culturefrom fuel-contaminated Arctic soil. In separate transfer cultures fromthe above Arctic soil, incubated anaerobically at 7°C, removalof 2-methylnaphthalene and fluorene was stoichiometrically coupled tonitrate removal. Ribosomal intergenic spacer analysis suggested thatenrichment resulted in a few predominant bacterial populations,including members of the genera Acidovorax,Bordetella, Pseudomonas, Sphingomonas, andVariovorax. Predominant populations from different soils oftenincluded phylotypes with nearly identical partial 16S rRNA genesequences (i.e., same genus) but never included phylotypes withidentical ribosomal intergenic spacers (i.e., different species orsubspecies). The composition of the enriched communities appeared to bemore affected by presence of oxygen, than by temperature or source oftheinoculum.

N. Awasthi, A. K. Singh, R. K. Jain, B. S. Khangarot, A. Kumar. (Industrial Toxicology Research Center Mahatma Gandhi Marg Post Box No 80 226 001 Lucknow India. Institute of Microbial Technology Chandigarh India. Department of Biochemistry and Molecular Biology, New Jersey Medical School University of Medicine and Dentistry of New Jersey Newark NJ 07103 USA). **Degradation and detoxification of endosulfan isomers by a defined co-**

culture of two Bacillus strains. Applied Microbiology and Biotechnology, 62(2-3) (2003), 279 – 283.

The degradation of a and ß isomers of endosulfan by a two-member bacterial co-culture was studied. Results were similar whether the two isomers were present individually or together, as in technical endosulfan. The degradation of both isomers was accompanied by the formation of endosulfan diol and endosulfan lactone. Accumulation of the metabolite, endosulfan sulfate was, however, not observed during the reaction with either of the isomers. The microbial degradation of endosulfan isomers was also accompanied by a decrease in its toxicity to the test organism Tubifex tubifex Müller.

Nico Boon, Eva M. Top, Willy Verstraete, and Steven D. Siciliano. (Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, B-9000 Ghent, Belgium). **Bioaugmentation as a Tool To Protect the Structure and Function of an Activated-Sludge Microbial Community against a 3-Chloroaniline Shock Load**. Applied and Environmental Microbiology, 69(3) (2003), 1511-1520.

Bioaugmentation of bioreactors focuses on the removal of xenobiotics, with little attention typically paid to the recovery of disrupted reactor functions such as ammonium-nitrogen removal. Chloroanilines are widely used in industry as a precursor to a variety of products and are occasionally released into wastewater streams. This work evaluated the effects on activated-sludge reactor functions of a 3-chloroaniline (3-CA) pulse and bioaugmentation by inoculation with the 3-CA-degrading strain Comamonas testosteroni I2 gfp. Changes in functions such as nitrification, carbon removal, and sludge compaction were studied in relation to the sludge community structure, in particular the nitrifying populations. Denaturing gradient gel electrophoresis (DGGE), real-time PCR, and fluorescent in situ hybridization (FISH) were used to characterize and enumerate the ammonia-oxidizing microbial community immediately after a 3-CA shock load. Two days after the 3-CA shock, ammonium accumulated, and the nitrification activity did not recover over a 12-day period in the nonbioaugmented reactors. In contrast, nitrification in the bioaugmented reactor started to recover on day 4. The DGGE patterns and the FISH and real-time PCR data showed that the ammonia-oxidizing microbial community of the bioaugmented reactor recovered in structure, activity, and abundance, while the number of ribosomes of the ammonia oxidizers in the nonbioaugmented reactor decreased drastically and the community composition changed and did not recover. The settleability of the activated sludge was negatively influenced by the 3-CA addition, with the sludge volume index increasing by a factor of 2.3. Two days after the 3-CA shock in the nonbioaugmented reactor, chemical oxygen demand (COD) removal efficiency decreased by 36% but recovered fully by day 4. In contrast, in the bioaugmented reactor, no decrease of the COD removal efficiency was observed. This study demonstrates that bioaugmentation of wastewater reactors to accelerate the degradation of toxic chlorinated organics such as 3-CA protected the nitrifying bacterial community, thereby allowing faster recovery from toxic shocks.

O. A. Gorelova, S. Yu. Kleimenov. (Faculty of Biology, Moscow State University, Vorob'evy gory, Moscow, 119992 Russia. Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences, ul. Vavilova 26, Moscow, 117808 Russia). **The Accumulation and Degradation Dynamics of Cyanophycin in Cyanobacterial Cells Grown in Symbiotic Associations with Plant Tissues and Cells.** Microbiology, 72(3) (2003), 318-326.

Five different artificial associations of cyanobacterial cells with the cells or tissues of nightshade and rauwolfia were studied. The associations grown on nitrogen-containing media produced heterocysts. Cyanobacterial cells in the associations retained their ability to take up combined nitrogen from the medium, to store it in the form of cyanophycin granules, and to use them in the process of symbiotic growth. The synthesis and degradation of cyanophycin granules in

cyanobacterial cells were more active in the associations than in monocultures. In the symbiotic associations of *Chlorogloeopsis fritschii* ATCC 27193 with *Solanum laciniatum* cells and of *Nostoc muscorum* CALU 304 with the *Rauwolfia serpentina* callus, heterocysts were produced with a 3- to 30-fold higher cyanophycin content than in pure cyanobacterial cultures. In contrast, in the association of *N. muscorum* CALU 304 with the *Solanum dulcamara* callus, heterocysts were produced with a lower cyanophycin content than in the *N. muscorum* CALU 304 pure culture. The degradation of cyanophycin granules in *N. muscorum* CALU 304 cells grown in associations with plant tissues or cells was subjected to mathematical analysis. The activation of cyanophycin degradation and heterocyst differentiation in the associations *N. muscorum* CALU 304–*R. serpentina* and *C. fritschii–S. laciniatum* was accompanied by an enhanced synthesis of the nitrogen-containing alkaloids in plant cells. The data obtained suggest that an integrated system of nitrogen homeostasis can be formed in symbiotic associations. Depending on the growth stage of an association, its plant member can either stimulate the accumulation of combined nitrogen in vegetative cyanobacterial cells in the form of cyanophycin granules, activate their degradation, or initiate the formation of heterocysts independently of the cyanobacterial combined nitrogen deprivation sensing-signaling pathway.

Olaf Kniemeyer, Thomas Fischer, Heinz Wilkes, Frank Oliver Glöckner, Friedrich Widdel. (Max-Planck-Institut für Marine Mikrobiologie, D-28359 Bremen, Institut für Erdöl und Organische Geochemie, Forschungszentrum Jülich GmbH, D-52425 Jülich, GeoForschungsZentrum Potsdam, D-14473 Potsdam, Germany). **Anaerobic Degradation of Ethylbenzene by a New Type of Marine Sulfate-Reducing Bacterium**. Applied and Environmental Microbiology, 69(2) (2003), 760-768.

Anaerobic degradation of the aromatic hydrocarbon ethylbenzene was studied with sulfate as the electron acceptor. Enrichment cultures prepared with marine sediment samples from different locations showed ethylbenzene-dependent reduction of sulfate to sulfide and always contained a characteristic cell type that formed gas vesicles towards the end of growth. A pure culture of this cell type, strain EbS7, was isolated from sediment from Guaymas Basin (Gulf of California). Complete mineralization of ethylbenzene coupled to sulfate reduction was demonstrated in growth experiments with strain EbS7. Sequence analysis of the 16S rRNA gene revealed a close relationship between strain EbS7 and the previously described marine sulfate-reducing strains NaphS2 and mXyS1 (similarity values, 97.6 and 96.2%, respectively), which grow anaerobically with naphthalene and m-xylene, respectively. However, strain EbS7 did not oxidize naphthalene, m-xylene, or toluene. Other compounds utilized by strain EbS7 were phenylacetate, 3-phenylpropionate, formate, n-hexanoate, lactate, and pyruvate. 1-Phenylethanol and acetophenone, the characteristic intermediates in anaerobic ethylbenzene degradation by denitrifying bacteria, neither served as growth substrates nor were detectable as metabolites by gas chromatography-mass spectrometry in ethylbenzene-grown cultures of strain EbS7. Rather, (1-phenylethyl)succinate and 4-phenylpentanoate were detected as specific metabolites in such cultures. Formation of these intermediates can be explained by a reaction sequence involving addition of the benzyl carbon atom of ethylbenzene to fumarate, carbon skeleton rearrangement of the succinate moiety (as a thioester), and loss of one carboxyl group. Such reactions are analogous to those suggested for anaerobic n-alkane degradation and thus differ from the initial reactions in anaerobic ethylbenzene degradation by denitrifying bacteria which employ dehydrogenations.

Patrick A. W. Van Hees, David L. Jones, Douglas L. Godbold. (School of Agricultural and Forest Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, UK and Department of Environmental Sciences, Mid Sweden University, 851 70 Sundsvall, Sweden. School of Agricultural and Forest Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, UK).

Biodegradation of Low Molecular Weight Organic Acids in a Limed Forest Soil. Water, Air and Soil Pollution: Focus, 3(4) (2003), 121-144.

The effect of liming (3.45 and 8.75 t ha^{-1} dolomite; 16 yr after application) on the biodegradation of three low molecular weight organic acids (citrate, oxalate and propionate) in forest soils was investigated. The concentration of organic acids in the soil solution followed the series propionate > citrate > oxalate with liming having no significant impact on soil solution concentrations (mean organic acid concentration = 8.7 ± 2.3 μM). Organic acid mineralization by the soil microbial community was rapid in surface organic horizons (mean half-life for citrate = 2–6 h), with biodegradation rate gradually declining with soil depth. Concentration-dependent biodegradation studies (0 to 350 μM) showed that the mineralization kinetics generally conformed well to a single Michaelis–Menten equation with V_{max} values following the series oxalate > citrate > propionate (mean = 9.8 ± 1.0 nmol g^{-1} h^{-1}) and K_M values following the series oxalate = citrate > propionate (mean 168 ± 25 μM). The V_{max} values declined with soil depth, which was consistent with a general reduction in microbial activity down the soil profile. Liming induced a significant increase in V_{max} for citrate with no change for propionate and reduction in V_{max} for oxalate. The latter was probably due to adsorption and precipitation of Ca-oxalate making it unavailable for microbial uptake. The higher adsorption/precipitation capacity for oxalate in the limed soils was confirmed by adsorption isotherms. Generally, liming increased soil microbial activity by approximately 10 to 35% with calculations based on soil solution concentrations indicating that organic acid mineralization constituted approximately 3 to 15% of the total soil respiration.

R Muñoz, B Guieysse, B Mattiasson. (Department of Biotechnology, Lund University, P.O. Box 124, 22100, Lund, Sweden). **Phenanthrene biodegradation by an algal-bacterial consortium in two-phase partitioning bioreactors**. Applied Microbiology and Biotechnology, 61(3) (2003), 261 – 267.

An algal-bacterial consortium formed by Chlorella sorokiniana and a phenanthrene-degrading Pseudomonas migulae strain was able to biodegrade 200-500 mg/l of phenanthrene dissolved in silicone oil or tetradecane under photosynthetic conditions and without any external supply of oxygen. Phenanthrene was only removed when provided in organic solvent, which confirms the potential of two-phase systems for toxicity reduction. Phenanthrene was degraded at highest rates when provided in silicone oil rather than in tetradecane since this solvent probably sequestered the PAH, reducing its mass transfer to the aqueous phase. The influence of phenanthrene concentration, amount of inoculum and light intensity on pollutant removal was also investigated and, under the best conditions, phenanthrene was degraded at 24.2 g $m^{m3}\cdot h^{m1}$. In addition to being cost-effective and mitigating the release of greenhouse gases into the atmosphere, photosynthetic oxygenation was especially beneficial to the use of two-phase partitioning bioreactors since it prevented solvent emulsification and/or volatilization and evidence was found that the microalgae release biosurfactants that could further enhance phenanthrene degradation

R. Jayasekara, S. Sheridan, E. Lourbakos, H. Beh, G. B. Y. Christie, M. Jenkins, P. B. Halley, S. McGlashan, G. T. Lonergan. (Centre for Applied Colloid and Biocolloid Science, School of Engineering and Science, Swinburne University of Technology, John Street, Hawthorn, 3122, Melbourne, Australia. CSIRO Division of Manufacturing Science and Technology, Cooperative Research Centre for International Food Manufacture and Packing Science, Clayton, Victoria 31169, Australia. Materials Characterisation and Processing Centre, The University of Queensland, Queensland 4072, Australia). **Biodegradation and ecotoxicity evaluation of a bionolle and starch blend and its degradation products in compost**. International Biodeterioration & Biodegradation, 51(1) (2003), 77-81.

A polymer based on a blend of starch and "BionolleTM" has been prepared and tested for biodegradation in compost. The polymer was completely mineralised to carbon dioxide in 45 days. The potential toxicity of the polymer was tested against the earthworm Eisenia fetida using a modification of the American Standard for Testing Materials E1976-97. The earthworms were exposed to 30 g of the polymer for 28 days and changes in weight recorded. In addition, the polymer was firstly degraded by the compost and the worms exposed to the breakdown products for 28 days. Differences in weight were also recorded. In each case the production of juveniles was noted and all earthworms were examined for pathology. The results obtained were processed statistically using a t-test. The number of juveniles, produced from the breakdown products, was highly significant ($P<0.001$) when compared to the earthworms added to the intact polymer. There was a definitely significant difference ($P<0.01, t=3.25$) in change in weight between the earthworms that were exposed to the polymer directly and those that were exposed to the breakdown products. There was no indication of any pathology of any earthworms. The polymer is considered safe for this species.

R. Margesin, D. Labbé, F. Schinner, C. W. Greer, L. G. Whyte. (Institute of Microbiology, University of Innsbruck, A-6020 Innsbruck, Austria,1 NRC—Biotechnology Research Institute, Montreal, Quebec, Canada H4P 2R2,2 Department of Natural Resource Sciences, McGill University, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9). **Characterization of Hydrocarbon-Degrading Microbial Populations in Contaminated and Pristine Alpine Soils**. Applied and Environmental Microbiology, 69(6) (2003), 3085-3092.

Biodegradation of petroleum hydrocarbons in cold environments, including Alpine soils, is a result of indigenous cold-adapted microorganisms able to degrade these contaminants. In the present study, the prevalence of seven genotypes involved in the degradation of n-alkanes (Pseudomonas putida GPo1 alkB; Acinetobacter spp. alkM; Rhodococcus spp. alkB1, and Rhodococcus spp. alkB2), aromatic hydrocarbons (P. putida xylE), and polycyclic aromatic hydrocarbons (P. putida ndoB and Mycobacterium sp. strain PYR-1 nidA) was determined in 12 oil-contaminated (428 to 30,644 mg of total petroleum hydrocarbons [TPH]/kg of soil) and 8 pristine Alpine soils from Tyrol (Austria) by PCR hybridization analyses of total soil community DNA, using oligonucleotide primers and DNA probes specific for each genotype. The soils investigated were also analyzed for various physical, chemical, and microbiological parameters, and statistical correlations between all parameters were determined. Genotypes containing genes from gram-negative bacteria (P. putida alkB, xylE, and ndoB and Acinetobacter alkM) were detected to a significantly higher percentage in the contaminated (50 to 75%) than in the pristine (0 to 12.5%) soils, indicating that these organisms had been enriched in soils following contamination. There was a highly significant positive correlation ($P < 0.001$) between the level of contamination and the number of genotypes containing genes from P. putida and Acinetobacter sp. but no significant correlation between the TPH content and the number of genotypes containing genes from gram-positive bacteria (Rhodococcus alkB1 and alkB2 and Mycobacterium nidA). These genotypes were detected at a high frequency in both contaminated (41.7 to 75%) and pristine (37.5 to 50%) soils, indicating that they are already present in substantial numbers before a contamination event. No correlation was found between the prevalence of hydrocarbon-degradative genotypes and biological activities (respiration, fluorescein diacetate hydrolysis, lipase activity) or numbers of culturable hydrocarbon-degrading soil microorganisms; there also was no correlation between the numbers of hydrocarbon degraders and the contamination level. The measured biological activities showed significant positive correlation with each other, with the organic matter content, and partially with the TPH content and a significant negative correlation with the soil dry-mass content ($P < 0.05$ to 0.001).

R. Muñoz, B. Guieysse, B. Mattiasson. (Department of Biotechnology, Lund University, P.O. Box 124, 22100, Lund, Sweden). **Phenanthrene biodegradation by an algal-bacterial consortium in two-phase partitioning bioreactors**. Applied Microbiology and Biotechnology, 61(3) (2003), 261 – 267.

An algal-bacterial consortium formed by Chlorella sorokiniana and a phenanthrene-degrading Pseudomonas migulae strain was able to biodegrade 200-500 mg/l of phenanthrene dissolved in silicone oil or tetradecane under photosynthetic conditions and without any external supply of oxygen. Phenanthrene was only removed when provided in organic solvent, which confirms the potential of two-phase systems for toxicity reduction. Phenanthrene was degraded at highest rates when provided in silicone oil rather than in tetradecane since this solvent probably sequestered the PAH, reducing its mass transfer to the aqueous phase. The influence of phenanthrene concentration, amount of inoculum and light intensity on pollutant removal was also investigated and, under the best conditions, phenanthrene was degraded at 24.2 g mm3·hm1. In addition to being cost-effective and mitigating the release of greenhouse gases into the atmosphere, photosynthetic oxygenation was especially beneficial to the use of two-phase partitioning bioreactors since it prevented solvent emulsification and/or volatilization and evidence was found that the microalgae release biosurfactants that could further enhance phenanthrene degradation.

Rainer U. Meckenstock, Barbara Morasch, Matthias Kästner, Andrea Vieth, Hans Hermann Richnow. (Eberhard-Karls University of Tübingen, Center for Applied Geoscience, Tübingen, Germany. University of Konstanz, Faculty of Biology, Konstanz, Germany. Center for Environmental Research, Department of Remediation Research, Leipzig, Germany). **Assessment of Bacterial Degradation of Aromatic Hydrocarbons in the Environment by Analysis of Stable Carbon Isotope Fractionation**. Water, Air and Soil Pollution: Focus, 2(3) (2002), 141-152.

13C/12C stable carbon isotope fractionation was used to assess biodegradation in contaminated aquifers with toluene as a model compound. Different strains of anaerobic bacteria (*Thauera aromatica*, *Geobacter metallireducens*, and the sulfate-reducing strain TRM1) showed consistent 13C/12C carbon isotope fractionation with fractionation factors between $\alpha C = 1.0017$ and 1.0018. In contrast, three cultures of aerobic organisms, using different mono- and dioxygenase enzyme systems to initiate toluene degradation, showed variable isotope fractionation factors of $\alpha C = 1.0027$ (*Pseudomonas putida* strain mt-2), $\alpha C = 1.0011$ (*Ralstonia picketii*), and $\alpha C = 1.0004$ (*Pseudomonas putida* strain F1). The great variability of isotope fractionation between different aerobic bacterial strains suggests that interpretation of isotope data in oxic habitats can only be qualitative. A soil column was run as a model system for contaminated aquifers with toluene as the carbon source and sulfate as the electron acceptor and samples were taken at different ports along the column. Microbial toluene degradation was calculated based on the 13C/12C isotope fractionation factors of the batch culture experiments together with the observed 13C/12C isotope shifts of the residual toluene fractions. The calculated percentage of biodegradation, *B*, correlated well with the decreasing toluene concentrations at the sampling ports and indicated the increasing extent of biodegradation along the column. The theoretical toluene concentrations as calculated based on the isotope values matched the measured concentrations at the different sampling ports indicating that the Rayleigh equation can be used to calculate biodegradation in quasi closed systems based on measured isotope shifts. A similar attempt was performed to assess toluene degradation in a contaminated, anoxic aquifer. A transect of groundwater wells was monitored along the main direction of the groundwater flow and revealed decreasing concentrations accompanied with an increase in the 13C/12C stable

carbon isotope ratio of the residual toluene. Calculation of the extent of biodegradation based on the isotope values and laboratory derived isotope fractionation factors showed that the residual toluene was degraded to more than 99% by microbial activity. Calculation of the theoretical residual toluene concentrations based on the measured isotope values described the strongly decreasing concentrations along the plume. Other aromatic hydrocarbons like benzene and naphthalene which were analysed in the same course also showed decreasing concentrations along the groundwater flow path accompanied by increasing δ13C values indicating biodegradation.

René van Herwijnen, Dirk Springael, Pieter Slot, Harrie A. J. Govers, John R. Parsons. (Department of Environmental and Toxicological Chemistry (IBED/MTC), University of Amsterdam, 1018WV Amsterdam, The Netherlands, Vlaamse Instelling voor Technologisch Onderzoek (Vito), B-2400 Mol, Belgium). **Degradation of Anthracene by Mycobacterium sp. Strain LB501T Proceeds via a Novel Pathway, through o-Phthalic Acid**. Applied and Environmental Microbiology, 69(1) (2003), 186-190.

Mycobacterium sp. strain LB501T utilizes anthracene as a sole carbon and energy source. We analyzed cultures of the wild-type strain and of UV-generated mutants impaired in anthracene utilization for metabolites to determine the anthracene degradation pathway. Identification of metabolites by comparison with authentic standards and transient accumulation of o-phthalic acid by the wild-type strain during growth on anthracene suggest a pathway through o-phthalic acid and protocatechuic acid. As the only productive degradation pathway known so far for anthracene proceeds through 2,3-dihydroxynaphthalene and the naphthalene degradation pathway to form salicylate, this indicates the existence of a novel anthracene catabolic pathway in Mycobacterium sp. LB501T.

Robert M. Garrett, Stephen J. Rothenburger and Roger C. Prince. (ExxonMobil Research and Engineering Co., Annandale, NJ 08801, USA). **Biodegradation of Fuel Oil Under Laboratory and Arctic Marine Conditions**. Spill Science & Technology Bulletin, 8(5-6) (2003), 503-507.

The aerobic biodegradation of the components of a fuel oil under Arctic summer conditions follows a pattern that is indistinguishable from that exhibited under temperate conditions. Straight chain alkanes and small aromatics are degraded first, followed by branched alkanes and larger and alkylated aromatics. We present data on the biodegradation of heptadecane as a representative n-alkane, pristane as a representative iso-alkane, and naphthalene, phenanthrene, and chrysene and their alkylated forms as representative two-, three- and four-ring aromatic hydrocarbons. In particular, the pattern of degradation of the alkylated aromatics allows the identification of biodegradation in samples collected from the field and the estimation of the extent of biodegradation that occurred in the In-Situ Treatment of Oiled Sediment Shorelines Field Trials.

S Lotfabad, M Gray. (Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta, T6G 2G6 Canada). **Kinetics of biodegradation of mixtures of polycyclic aromatic hydrocarbons**. Applied Microbiology and Biotechnology, 60(3) (2002), 361 – 366.

The kinetics of biodegradation of polycyclic aromatic hydrocarbons (PAHs) by a mixed culture were determined in a creosote-contaminated soil and in a pristine soil. A competitive-inhibition model was able to represent the kinetics of degradation of PAHs from the creosote-contaminated soil, from the lag phase through to active degradation, but not data from pristine soil with the same PAHs alone and in mixtures. The presence of phenanthrene introduced a lag phase of 4.5 days in the degradation of fluoranthene and 5 days for chrysene. Rapid degradation of pyrene followed a lag phase of circa 5 days, regardless of the presence of other PAHs. These results

show that even when kinetics of PAH degradation by mixed cultures appear to follow competitive-inhibition kinetics, the underlying mechanisms may be more complex.

S. Rodríguez Couto, R. Rodríguez , P. P. Gallego, A. Sanromán. (University of Vigo, Department of Chemical Engineering, 36200 Vigo, Spain. University of Vigo, Faculty of Science, Laboratory of Plant Physiology and Biotechnology, 36200 Vigo, Spain). **Biodegradation of Grape Cluster Stems and Ligninolytic Enzyme Production by *Phanerochaete chrysosporium* during Semi-Solid-State Cultivation.** Acta Biotechnologica, 23(1) (2003), 65-74.

The degradation undergone by grape cluster stems (woody component of vine bagasse), an agroindustrial waste, was investigated during the semi-solid-state cultivation of *Phanerochaete chrysosporium* BKM-F-1767 (ATCC 24725). For this, the content of lignin, cellulose and hemicellulose in grape cluster stems was determined before and after the enzymatic process. It was found that about 20% of Klason lignin, 48% of hemicellulose and 5% of cellulose were degraded during the process, being the ligninolytic enzymes (manganese-dependent peroxidase and lignin peroxidase) produced by such cultures responsible for the degradation of grape cluster stems. In parallel, semi-solid-state cultures of *P. chrysosporium* grown on an inert support (cubes of nylon sponge), which is not susceptible to undergoing degradation during the enzymatic process, were used as reference cultures.In addition, the *in vivo* decolourisation of a model dye, the polymeric dye Poly R-478, by both grape cluster stem and nylon cultures was studied in order to assess their degradative ability. A percentage of biological decolourisation higher than 90% after four days of dye addition was obtained using nylon sponge cultures, whereas grape cluster stem cultures led to a decolourisation of around 70% after eight days of dye incubation. The lower percentage of dye degradation achieved by the cultures grown on grape cluster stems was due to the enzymes produced, which were not only employed in the decolourisation of the dye but also in the degradation of the support, as indicated by the data mentioned above.

Sandra Trott, Shirley F. Nishino, Jalal Hawari, and Jim C. Spain. (Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403, Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada). **Biodegradation of the Nitramine Explosive CL-20**. Applied and Environmental Microbiology, 69(30) (2003), 1871-1874.

The cyclic nitramine explosive CL-20 (2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane) was examined in soil microcosms to determine whether it is biodegradable. CL-20 was incubated with a variety of soils. The explosive disappeared in all microcosms except the controls in which microbial activity had been inhibited. CL-20 was degraded most rapidly in garden soil. After 2 days of incubation, about 80% of the initial CL-20 had disappeared. A CL-20-degrading bacterial strain, *Agrobacterium* sp. strain JS71, was isolated from enrichment cultures containing garden soil as an inoculum, succinate as a carbon source, and CL-20 as a nitrogen source. Growth experiments revealed that strain JS71 used 3 mol of nitrogen per mol of CL-20.

Sangchul Hwang, Teresa J. Cutright. (Department of Geography and Environmental Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA. Department of Civil Engineering, The University of Akron, Akron, OH 44325, USA). **Preliminary exploration of the relationships between soil characteristics and PAH desorption and biodegradation**. Environment International, 29(7) (2004), 887-894.

Desorption and biodegradation of pyrene (PYR) were investigated and their relationships to soil characteristics were addressed. The results indicated that maximum achievable desorption was 30.2, 10.4, and 1.0 mg/kg for soils that had 1.7, 2.2, and 4.4 wt.% of expandable clays (smectite and vermiculite), respectively. Neither dissolved organic matter (DOM) nor total clay amounts

made a good prediction of the desorption trend. Subsequently, the ease of desorption facilitated a faster aqueous biodegradation rate. The slowest aqueous biodegradation rate, 0.02 l/h, was achieved for the soil system that had the greatest amount of expandable clays, whereas the soil containing 1.7% expandable clays only achieved 0.73 l/h. The soil with 2.2% expandable clays depicted 0.41 l/h of aqueous biodegradation rate. A good linear correlation was obtained between maximum achievable desorption and aqueous biodegradation rate (R2=0.92). Soil analysis revealed that the total (soil+water) biodegradation reached was 65%, 78.3%, and 81.8% of the initial concentration (100 mg/kg) for the sandy clay loam (Colombian), sandy loam (Ohio), and silty loam (New Mexico) soils, respectively. This biodegradation extent was also in good agreement of expandable clay amount. Although aqueous PYR bioavailability was limited due to the strong association with the expandable clays, microbial movement and adhesion to those clays seemed to result in a great extent of the soil-phase biodegradation.

Sato Akira, Watanabe Tsuneo, Watanabe Yoshio, Kurane Ryuichiro. (Bioconsortia Program Laboratory, National Institute of Advanced Industrial Science and Technology, Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki Pref. 3058566, Japan; Bioresource Laboratories, Mercian Corporation, 4-9-1 Johnan, Fujisawa, Kanagawa Pref. 251□0057, Japan; Biological Research Center, National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba Pref. 292-0818, Japan. Bioconsortia Program Laboratory, National Institute of Advanced Industrial Science and Technology, Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki Pref. 305-8566, Japan. Bioresource Laboratories, Mercian Corporation, 4-9 Johnan, Fujisawa, Kanagawa Pref. 251-0057, Japan). **Enhancement of biodegradation of 2,7-dichlorodibenzo-p-dioxin by addition of fungal culture filtrate.** Journal of Microbiology and Biotechnology, 19(4) (2003), 439-441.

The hydrophilicity of 2,7-dichlorodibenzo-*p*-dioxin (2,7-DCDD), a model dioxin compound, increased when incubated with the culture filtrates of several strains of fungi. The possibility that the addition of these filtrates could enhance the biodegradation of 2,7-DCDD by the white□rot basidiomycetous fungus *Phanerochaete sordida* YK-624 was examined. The decrease of 2,7-DCDD after 3 weeks incubation in a YK-624 culture containing these filtrates was greater (30%) than that in the culture of YK-624 alone (15%). This is the first report describing the enhancement of dioxin decrease by the addition of a fungal filtrate.

Silvia A. Mancini, Ania C. Ulrich, Georges Lacrampe-Couloume, Brent Sleep, Elizabeth A. Edwards, and Barbara Sherwood Lollar. (Stable Isotope Laboratory, Department of Geology, Department of Chemical Engineering and Applied Chemistry, Department of Civil Engineering, University of Toronto, Toronto, Canada). **Carbon and Hydrogen Isotopic Fractionation during Anaerobic Biodegradation of Benzene**. Applied and Environmental Microbiology, 69(1) (2003), 191-198.

Compound-specific isotope analysis has the potential to distinguish physical from biological attenuation processes in the subsurface. In this study, carbon and hydrogen isotopic fractionation effects during biodegradation of benzene under anaerobic conditions with different terminal-electron-accepting processes are reported for the first time. Different enrichment factors (ε) for carbon (range of -1.9 to -3.6‰) and hydrogen (range of -29 to -79‰) fractionation were observed during biodegradation of benzene under nitrate-reducing, sulfate-reducing, and methanogenic conditions. These differences are not related to differences in initial biomass or in rates of biodegradation. Carbon isotopic enrichment factors for anaerobic benzene biodegradation in this study are comparable to those previously published for aerobic benzene biodegradation. In contrast, hydrogen enrichment factors determined for anaerobic benzene biodegradation are significantly larger than those previously published for benzene biodegradation under aerobic conditions. A fundamental difference in the previously proposed initial step of aerobic versus proposed anaerobic biodegradation pathways may account for these

differences in hydrogen isotopic fractionation. Potentially, C-H bond breakage in the initial step of the anaerobic benzene biodegradation pathway may account for the large fractionation observed compared to that in aerobic benzene biodegradation. Despite some differences in reported enrichment factors between cultures with different terminal-electron-accepting processes, carbon and hydrogen isotope analysis has the potential to provide direct evidence of anaerobic biodegradation of benzene in the field.

Silvia R. Peressutti, Héctor M. Alvarez, Oscar H. Pucci. (Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo N0 1, Mar del Plata, 7600, Buenos Aires, Argentina. Departamento de Bioquímica, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, Km 4, 9000, Comodoro Rivadavia, Argentina). **Dynamics of hydrocarbon-degrading bacteriocenosis of an experimental oil pollution in Patagonian soil**. International Biodeterioration & Biodegradation, 52(1) (2003), 21-30.

Changes of the bacteriocenosis in oil polluted Patagonian soil were studied during a bioremediation process performed in a controlled laboratory experiment. A selective enrichment of hydrocarbon-degrading bacteria was observed, from 0.028% at the beginning of the assay to almost 100% 2 months after spillage. During the experiment 45.48% of oil was degraded. Significant correlation between crude oil, aliphatic, aromatic and asphaltic fractions, and the bacteria able to utilize such hydrocarbons was observed. Both the asphaltenes fraction and asphaltene-degrading bacteria increased throughout the assay, suggesting a biological transformation of hydrocarbons in compounds of higher complexity. In addition, important variations in the physiological and taxonomic diversity of the bacteriocenosis were detected. After oil pollution, representative strains of the bacterial community exhibited the ability to metabolize a broad spectrum of hydrocarbons. During the first 5 months of the experiment a predominance of Gram negative bacteria was found and, subsequently, Gram positive microorganisms increased. Some of the isolated bacteria, such as members of *Rhodococcus* genus, displayed a wide potential for hydrocarbon biodegradation and persistence in the studied environment. These results indicate the importance of autochthonous bacteria for hydrocarbon-polluted soil bioremediation.

Stefaan De Wildemana, Hendrik Nolleta, Herman Van Langenhoveb and Willy Verstraete. (Laboratory for Microbial Ecology and Technology (LabMET1), Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium. Laboratory of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium). **Reductive biodegradation of 1,2-dichloroethane by methanogenic granular sludge in lab-scale UASB reactors**. Advances in Environmental Research, 6(4) (2002), 401-409.

Dechlorination of 1,2-dichloroethane (1,2-DCA) dosed to a model wastewater in lab-scale upflow anaerobic sludge blanket (UASB) reactors was examined. Anaerobic granular sludge was used as a biocatalyst. Ethanol served as the main methanogenic substrate. For 3 months, two types of UASB reactors were studied, the first type consisting of a sludge blanket and the second type containing an additional layer of activated carbon. When subjected to 1,2-DCA at an average volumetric loading rate of 87.6 mg l-1 day-1, the latter type obtained an average removal efficiency of 82%. Increasing the volumetric loading rate of ethanol from 5 to 15 g COD l-1 day-1 resulted in higher 1,2-DCA conversion rates. No chlorinated intermediates or residues were found. 1,2-DCA was converted mainly to ethene (65–80%) and ethane (<1%). Both autoclaved sludge and cell extracts were not able to degrade 1,2-DCA, which indicates the need for metabolic activity. The reactor effluents were less toxic relative to the influent when analyzed by Nitrox tests, indicating that such UASB treatments can protect a subsequent aerobic nitrifying system. The 1,2-DCA removal rates achieved, and the safe nature of the endproducts, warrant the

combination of granular sludge and UASB technology for practical decontamination of waters containing such types of organochlorines.

Stephanie Fiorenza and Hanadi S. Rifai. **Review of MTBE Biodegradation and Bioremediation**. Bioremediation Journal, 7(1) (2003), 1-35.

Conclusive evidence of methyl tert-butyl ether (MTBE) biotransformation and complete mineralization under aerobic conditions in environmental samples and enrichment cultures is reviewed, in addition to increasing evidence of MTBE biotransformation under anaerobic conditions. The metabolic pathway of MTBE appears to have two key intermediates, tert-butyl alcohol (TBA) and 2-hydroxy isobutyric acid (HIBA). The first enzyme in MTBE biodegradation has been identified as either a cytochrome P450 or a nonhemic monooxygenase in different isolates. Mixed and pure cultures of microorganisms have utilized MTBE as a sole carbon and energy source. Cometabolism of MTBE with n-alkanes at rates of 3.9 to 52 nmol/min/mg protein has been documented. The presence of co-contaminants such as BTEX has either not affected or seemed to limit MTBE biodegradation. Some studies of MTBE natural attenuation have attributed mass loss to biodegradation, while others have attributed mass loss to dilution and dispersion. Recent advances in the assessment of MTBE biodegradation have indicated the potential for natural anaerobic transformation of MTBE. In situ bioremediation of MTBE has been enhanced by adding air or oxygen, or by adding microorganisms and air or oxygen. Bioreactors have attained significant removal of MTBE from MTBE-contaminated influent. Despite historical concerns about the biodegradability of MTBE, several biological methods can now be used for MTBE remediation.

T Mori, S Kitano, R Kondo. (Laboratory of Systematic Forest and Forest Products Sciences, Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, 812-8581, Fukuoka, Japan). **Biodegradation of chloronaphthalenes and polycyclic aromatic hydrocarbons by the white-rot fungus Phlebia lindtneri.** Applied Microbiology and Biotechnology, 61(4) (2003), 380 – 383.

The biodegradation of chloronaphthalene (CN) and polycyclic aromatic hydrocarbons by the white-rot fungus Phlebia lindtneri, which can degrade dichlorinated dioxins and non-chlorinated dioxin-like compounds, was investigated. Naphthalene, phenanthrene, 1-chloronaphthalene (1-CN) and 2-chloronaphthalene (2-CN) were metabolized by the fungus to form several oxidized products. Naphthalene and phenanthrene were metabolized to the corresponding hydroxylated and dihydrodihydroxylated metabolites. 2-CN was metabolized to 3-chloro-2-naphtol, 6-chloro-1-naphtol and two other chloronaphtols, CN-dihydrodiols and CN-diols. Significant inhibition of the degradation of these substrates was observed when they were incubated with the cytochrome P-450 monooxygenase inhibitors 1-aminobenzotriazole and piperonyl butoxide. These results suggest that P. lindtneri initially oxidizes these substrates by a cytochrome P-450 monooxygenase.

T Yoshida, T Nagasawa. (Department of Biomolecular Science, Gifu University, Yanagido 1-1, 501-1193, Gifu, Japan). **k-Poly-*l*-lysine: microbial production, biodegradation and application potential**. Applied Microbiology and Biotechnology, 62(1) (2003), 21–26.

k-Poly-*l*-lysine (k-PL) is a homo-poly-amino acid characterized by the peptide bond between the carboxyl and k-amino groups of *l*-lysine. k-PL shows a wide range of antimicrobial activity and is stable at high temperatures and under both acidic and alkaline conditions. The mechanism of the inhibitory effect of k-PL on microbial growth is the electrostatic adsorption to the cell surface of microorganisms on the basis of its poly-cationic property. Due to this antimicrobial activity, k-PL is now industrially produced in Japan as a food additive by a fermentation process using Streptomyces albulus. In spite of the practical application of k-PL, the biosynthetic mechanisms

of k-PL have not been clarified at all. k-PL producers commonly possess membrane-bound k-PL-degrading aminopeptidase, which might play a role in self-protection.

T. Teeraphatpornchai, T. Nakajima-Kambe, Y. Shigeno-Akutsu, M. Nakayama, N. Nomura, T. Nakahara, H. Uchiyama. (Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan PRESTO, Japan Science and Technology Corporation (JST), Kawaguchi Center Building 1-8, Honcho 4, Kawaguchi, Saitama 332-0012, Japan. PRESTO, Japan Science and Technology Corporation (JST), Kawaguchi Center Building 1-8, Honcho 4, Kawaguchi, Saitama 332-0012, Japan. Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan). **Isolation and characterization of a bacterium that degrades various polyester-based biodegradable plastics**. Biotechnology Letters, 25(1) (2003), 23-28.

Microorganisms isolated from soil samples were screened for their ability to degrade various biodegradable polyester-based plastics. The most active strain, designated as strain TB-13, was selected as the best strain for degrading these plastics. From its phenotypic and genetic characteristics, strain TB-13 was closely related to *Paenibacillus amylolyticus*. It could degrade poly(lactic acid), poly(butylene succinate), poly(butylene succinate-*co*-adipate), poly(caprolactone) and poly(ethylene succinate) but not poly(hydroxybutylate-*co*-valerate). However, it could not utilize these plastics as sole carbon sources. Both protease and esterase activities, which may be involved in the degradation of plastic, were constitutively detected in the culture broth.

Y Prabhu, P S Phale. (Biotechnology group, BJM School of Biosciences and Bioengineering, Indian Institute of Technology Bombay, 400 076, Powai, Mumbai, India). **Biodegradation of phenanthrene by Pseudomonas sp. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation**. Applied Microbiology and Biotechnology, 61(4) (2003), 342 – 351.

Pseudomonas sp. strain PP2 isolated in our laboratory efficiently metabolizes phenanthrene at 0.3% concentration as the sole source of carbon and energy. The metabolic pathways for the degradation of phenanthrene, benzoate and p-hydroxybenzoate were elucidated by identifying metabolites, biotransformation studies, oxygen uptake by whole cells on probable metabolic intermediates, and monitoring enzyme activities in cell-free extracts. The results obtained suggest that phenanthrene degradation is initiated by double hydroxylation resulting in the formation of 3,4-dihydroxyphenanthrene. The diol was finally oxidized to 2-hydroxymuconic semialdehyde. Detection of 1-hydroxy-2-naphthoic acid, f-naphthol, 1,2-dihydroxy naphthalene, and salicylate in the spent medium by thin layer chromatography; the presence of 1,2-dihydroxynaphthalene dioxygenase, salicylaldehyde dehydrogenase and catechol-2,3-dioxygenase activity in the extract; O_2 uptake by cells on f-naphthol, 1,2-dihydroxynaphthalene, salicylaldehyde, salicylate and catechol; and no O_2 uptake on o-phthalate and 3,4-dihydroxybenzoate supports the novel route of metabolism of phenanthrene via 1-hydroxy-2-naphthoic acid M [f-naphthol] M 1,2-dihydroxy naphthalene M salicylate M catechol. The strain degrades benzoate via catechol and cis,cis-muconic acid, and p-hydroxybenzoate via 3,4-dihydroxybenzoate and 3-carboxy-cis,cis-muconic acid. Interestingly, the culture failed to grow on naphthalene. When grown on either hydrocarbon or dextrose, the culture showed good extracellular biosurfactant production. Growth-dependent changes in the cell surface hydrophobicity, and emulsification activity experiments suggest that: (1) production of biosurfactant was constitutive and growth-associated, (2) production was higher when cells were grown on phenanthrene as compared to dextrose and benzoate, (3) hydrocarbon-grown cells were more hydrophobic and showed higher affinity towards both aromatic and aliphatic hydrocarbons compared to dextrose-grown cells, and (4) mid-log-phase cells were significantly (2-fold) more hydrophobic than stationary phase cells. Based on these results, we hypothesize

that growth-associated extracellular biosurfactant production and modulation of cell surface hydrophobicity plays an important role in hydrocarbon assimilation/uptake in Pseudomonas sp. strain PP2.

Yang Hongwei, Jiang Zhanpeng, Shi Shaoqi, W. Z. Tang. (Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, China. Department of Civil and Environmental Engineering, Florida International University, Miami, FL 33199, USA). **INT–dehydrogenase activity test for assessing anaerobic biodegradability of organic compounds.** Ecotoxicology and Environmental Safety, 53(3) (2002), 416-421.

This study assessed anaerobic biodegradability of organic compounds from microorganism activity. Dehydrogenase activity can be a good parameter characterizing the microorganism activity. A modified method of 2-(*p*-iodophenyl-3-(*p*-nitrophenyl)-5-pheny tetrazolium chloride-dehydrogenase activity determination was proposed in anaerobic biodegradability assessment. Cubic spline curves were adopted to link the data points. This curve was integrated twice to calculate areas. The microorganism activity index in anaerobic biodegradability assessment was calculated by standardizing the integral. According to the results of the activity index, 14 kinds of organic compounds were classified into readily, partially, and poorly biodegradable under anaerobic conditions, respectively. As a result, some conclusions for anaerobic biodegradability of organic compounds were reached, based on the activity index value.

Zita Snellinx, Safieh Taghavi, Jaco Vangronsveld, Daniël van der Lelie.(Environmental Technology Expertise Centre, Vlaamse Instelling voor Technologisch Onderzoek, Boeretang 200, 2400 Mol, Belgium Environmental Biology, Limburgs Universitair Centrum, Universitaire Campus, 3590 Diepenbeek, Belgium. Environmental Biology, Limburgs Universitair Centrum, Universitaire Campus, 3590 Diepenbeek, Belgium). **Microbial consortia that degrade 2,4-DNT by interspecies metabolism: isolation and characterization**. Biodegradation, 14 (1) (2003), 19-29.

Two consortia, isolated by selective enrichment from a soil sample of a nitroaromatic-contaminated site, degraded 2,4-DNT as their sole nitrogen source without accumulating one or more detectable intermediates. Though originating from the same sample, the optimised consortia had no common members, indicating that selective enrichment resulted in different end points. Consortium 1 and consortium 2 contained four and six bacterial species respectively, but both had two members that were able to collectively degrade 2,4-DNT. Variovorax paradoxus VM685 (consortium 1) and Pseudomonas sp. VM908 (consortium 2) initiate the catabolism of 2,4-DNT by an oxidation step, thereby releasing nitrite and forming 4-methyl-5-nitrocatechol (4M5NC). Both strains contained a gene similar to the *dntAa* gene encoding 2,4-DNT dioxygenase. They subsequently metabolised 4M5NC to 2-hydroxy-5-methylquinone (2H5MQ) and nitrite, indicative of DntB or 4M5NC monooxygenase activity. A second consortium member, *Pseudomonas marginalis* VM683 (consortium 1) and *P. aeruginosa* VM903, *Sphingomonas* sp. VM904, *Stenotrophomonas maltophilia* VM905 or *P. viridiflava* VM907 (consortium 2), was found to be indispensable for efficient growth of the consortia on 2,4-DNT and for efficient metabolisation of the intermediates 4M5NC and 2H5MQ. Knowledge about the interactions in this step of the degradation pathway is rather limited. In addition, both consortia can use 2,4-DNT as sole nitrogen and carbon source. A gene similar to the *dntD* gene of *Burkholderia* sp. strain DNT that catalyses ring fission was demonstrated by DNA hybridisation in the second member strains. To our knowledge, this is the first time that consortia are shown to be necessary for 2,4-DNT degradation.

Bioenergy

Guido Dietrich, Hans-Joachim Mollenkopf, Heinz Weber, Bernhard Knapp, Klaus-Dieter Diehl, Jürgen Hess, Friedrich Blackkolb, Michael Bröker, Stefan H. E. Kaufmann, Erika Hundt. (Chiron Behring GmbH & Co., D-35006 Marburg, Germany. Max-Planck-Institute for Infection Biology, D-10117 Berlin, Germany). **Cultivation of Mycobacterium bovis BCG in bioreactors**. Journal of Biotechnology, 96(3) (2002), 259-270.

The Mycobacterium bovis BCG vaccine for commercial use is classically produced as surface pellicles by culture on synthetic medium. Under these conditions, reproducibility of the cultures and quality assessment are hampered by slow growth of the bacilli, the formation of bacterial aggregates and a high proportion of dead bacilli after processing and final formulation of the vaccine. Here, we established dispersed cultures of M. bovis BCG in synthetic media in small-scale bioreactors. These cultures allow recording and adjusting of culture parameters and give rise to single bacilli with a high degree of live bacteria. In the murine model, bioreactor-grown M. bovis BCG exhibited slightly stronger replication and persistence than the vaccine produced under the classical conditions. The protective efficacy against challenge with M. tuberculosis was identical for both vaccine preparations.

Ilana S Aldor and Jay D Keasling. (Department of Chemical Engineering, 201 Gilman Hall, University of California, Berkeley, 94720-1462, USA). **Process design for microbial plastic factories: metabolic engineering of polyhydroxyalkanoates**. Current Opinion in Biotechnology, 14(5) (2003), 475-483.

Implementing several metabolic engineering strategies, either individually or in combination, it is possible to construct microbial plastic factories to produce a variety of polyhydroxyalkanoate (PHA) biopolymers with desirable structures and material properties. Approaches include external substrate manipulation, inhibitor addition, recombinant gene expression, host cell genome manipulation and, most recently, protein engineering of PHA biosynthetic enzymes. In addition, mathematical models and molecular methods can be used to elucidate metabolically engineered systems and to identify targets for performance improvement.

Julie C. Brodeur, Finn Økland, Bengt Finstad, D. George Dixon and R. Scott McKinley. (Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada. Norwegian Institute for Nature Research, Tungasletta 2, N-7005, Trondheim, Norway). **Effects of Subchronic Exposure to Aluminium in Acidic Water on Bioenergetics of Atlantic Salmon (*Salmo salar*).** Ecotoxicology and Environmental Safety, 49(3) (2001), 226-234.

Atlantic salmon (*Salmo salar*) were exposed for 36 days to water from the acidified (pH 5.2) Fossbekk River (Norway) with 50 $\mu g \cdot L^{-1}$ of aluminium (Al) added as $AlCl_3$, or to circumneutral water (pH 6.6) from Ims River (Norway). Food consumption, cardiac output, swimming activity, and growth rate were measured to determine the effects of sublethal levels of Al in acidic water on the bioenergetics of the fish. Food consumption was reduced in acidic water and Al during the first 12 days, but was not significantly different from the control group during the rest of the exposure. A significant decrease in body weight was concomitant with the reduction in food consumption. The fish also kept losing weight during the rest of the exposure despite the return of their appetite to normal. Cardiac output of the fish exposed to acidic water and Al was not significantly different from the control group after 36 days of exposure. Swimming activity remained significantly elevated throughout the exposure to acidic water and Al. These results indicate that sublethal levels of Al can alter the energy budget of Atlantic salmon living in acidified surface waters.

Kenneth Möllersten, Jinyue Yan, Jose R. Moreira. (Department of Chemical Engineering and Technology/Energy Processes, TR 50, Royal Institute of Technology, SE-100 44, Stockholm,

Sweden. National Center of Biomass, Av. Luciano Gualberto 1289, Cid. Universitaria, 05508-900, São Paulo, Brazil). **Potential market niches for biomass energy with CO2 capture and storage––Opportunities for energy supply with negative CO2 emissions**. Biomass and Bioenergy, 25(3) (2003), 273-285.

The paper presents an analysis of biomass energy with CO2 capture and storage (BECS) in industrial applications. Sugar cane-based ethanol mills and chemical pulp mills are identified as market niches with promising prospects for BECS. Calculations of CO2 balances of BECS in these applications show that the introduction of CO2 capture and storage in biomass energy systems can significantly increase the systems' CO2 abatement potentials. CO2 emissions of the total systems are negative. The CO2 reduction potentials of these technologies are discussed in regional and global contexts. An economic assessment of each system is carried out and opportunities for cost-effective technologies for CO2 capture, transportation and storage are identified. Furthermore, potentials for system improvements that could substantially decrease the CO2 abatement cost are addressed.

M. Senthil Kumar, A. Ramesh, B. Nagalingam. (Department of Mechanical Engineering, Indian Institute of Technology Madras, Chennai 600 036, India). **An experimental comparison of methods to use methanol and Jatropha oil in a compression ignition engine**. Biomass and Bioenergy, 25(3) (2003), 309-318.

In this work various methods of using vegetable oil (Jatropha oil) and methanol such as blending, transesterification and dual fuel operation were studied experimentally. A single cylinder direct injection diesel engine was used for this work. Tests were done at constant speed of 1500 revmin-1 at varying power outputs. In dual fuel operation the methanol to Jatropha oil ratio was maintained at 3:7 on the volume basis. This is close to the fraction of methanol used to prepare the ester with Jatropha oil. Brake thermal efficiency was better in the dual fuel operation and with the methyl ester of Jatropha oil as compared to the blend. It increased form 27.4% with neat Jatropha oil to a maximum of 29% with the methyl ester and 28.7% in the dual fuel operation. Smoke was reduced with all methods compared to neat vegetable oil operation. The values of smoke emission are 4.4 Bosch Smoke Units (BSU) with neat Jatropha oil, 4.1 BSU with the blend, 4 BSU with methyl ester of Jatropha oil and 3.5 BSU in the dual fuel operation. The Nitric Oxide (NO) level was lower with Jatropha oil compared to diesel. It was further reduced in dual fuel operation and the blend with methanol. Dual fuel operation showed higher hydrocarbon (HC) and carbon monoxide (CO) emissions than the ester and the blend. Ignition delay was higher with neat Jatropha oil. It increased further with the blend and in dual fuel operation. It was reduced with the ester. Peak pressure and rate of pressure rise were higher with all the methods compared to neat Jatropha oil operation. Jatropha oil and methyl ester showed higher diffusion combustion compared to standard diesel operation. However, dual fuel operation resulted in higher premixed combustion. On the whole it is concluded that transesterification of vegetable oils and methanol induction can significantly enhance the performance of a vegetable oil fuelled diesel engine.

Monique Hoogwijk, André Faaij, Richard van den Broek, Göran Berndes, Dolf Gielen, Wim Turkenburg. (Utrecht University, Department of Science, Technology and Society, Padualaan 14, 3584 CH, Utrecht, The Netherlands. Chalmers University, Department of Physical Resource Theory, Göteborg, Sweden. Netherlands Energy Research Foundation (ECN), Petten, The Netherlands. National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands). **Exploration of the ranges of the global potential of biomass for energy**. Biomass and Bioenergy, 25(2) (2003), 119-133.

This study explores the range of future world potential of biomass for energy. The focus has been put on the factors that influence the potential biomass availability for energy purposes

rather than give exact numbers. Six biomass resource categories for energy are identified: energy crops on surplus cropland, energy crops on degraded land, agricultural residues, forest residues, animal manure and organic wastes. Furthermore, specific attention is paid to the competing biomass use for material. The analysis makes use of a wide variety of existing studies on all separate categories. The main conclusion of the study is that the range of the global potential of primary biomass (in about 50 years) is very broad quantified at 33-1135 EJy-1. Energy crops from surplus agricultural land have the largest potential contribution (0–988 EJy-1). Crucial factors determining biomass availability for energy are: (1) The future demand for food, determined by the population growth and the future diet; (2) The type of food production systems that can be adopted world-wide over the next 50 years; (3) Productivity of forest and energy crops; (4) The (increased) use of bio-materials; (5) Availability of degraded land; (6) Competing land use types, e.g. surplus agricultural land used for reforestation. It is therefore not "a given" that biomass for energy can become available at a large-scale. Furthermore, it is shown that policies aiming for the energy supply from biomass should take the factors like food production system developments into account in comprehensive development schemes.

P.N.L. Lens, R. Gastesi, G. Lettinga. (Author for correspondence Sub-Department of Environmental Technology, Agricultural University of Wageningen, ``Biotechnion'' – Bomenweg 2, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Sub-Department of Environmental Technology, Agricultural University of Wageningen, ``Biotechnion'' – Bomenweg 2, P.O. Box 8129, 6700 EV Wageningen, The Netherlands). **Use of Sulfate Reducing Cell Suspension Bioreactors for the Treatment of SO2 Rich Flue Gases**. Biodegradation, 14(3) (2003), 229-240.

This paper describes a novel bioscrubber concept for biological flue gas desulfurization, based on the recycling of a cell suspension of sulfite/sulfate reducing bacteria between a scrubber and a sulfite/sulfate reducing hydrogen fed bioreactor. Hydrogen metabolism in sulfite/sulfate reducing cell suspensions was investigated using batch activity tests and by operating a completely stirred tank reactor (CSTR). The maximum specific hydrogenotrophic sulfite/sulfate reduction rate increased with 10% and 300%, respectively, by crushing granular inoculum sludge and by cultivation of this sludge as cell suspension in a CSTR. Operation of a sulfite fed CSTR (hydraulic retention time 4 days; pH 7.0; sulfite loading rate 0.5–1.5 g SO32- l-1 d-1) with hydrogen as electron donor showed that high (up to 1.6 g l-1) H2}S concentrations can be obtained within 10 days of operation. H2S inhibition, however, limited the sulfite reducing capacity of the CSTR. Methane production by the cell suspension disappeared within 20 days reactor operation. The outcompetition of methanogens in excess of H2 can be attributed to CO2 limitation and/or to sulfite or sulfide toxicity. The use of cell suspensions opens perspectives for monolith or packed bed reactor configurations, which have a much lower pressure drop compared to air lift reactors, to supply H2 to sulfite/sulfate reducing bioreactors.

Peter M. Deak, Sabine Lutz-Wahl, Harald Bothe, Lutz Fischer. (Lehrstuhl für Biotechnologie, Institut für Lebensmitteltechnologie, Universität Hohenheim, Emil-Wolff-Straße 14, 70599 Stuttgart, Germany). **Bioreactor cultivation of *Escherichia coli* for production of recombinant penicillin G amidase from *Alcaligenes faecalis*.** Biotechnology Letters, 25(5) (2003), 397-400.

The penicillin G amidase (PGA) from *Alcaligenes faecalis*, which has interesting properties for use in combinatorial biochemistry, was produced by recombinant expression in *Escherichia coli*. The corresponding gene was cloned into a multicopy vector under the strict regulatory control of the rhamnose inducible promoter. Cells were grown in a synthetic minimal medium in a bioreactor (5 l working vol.), and production of PGA was induced by repeated addition of the

inducer rhamnose, that served also as a carbon source. The fermentation yield was about 4500 units PGA activity per liter of culture medium.

Wahidul K. Biswas, Paul Bryce, Mark Diesendorf. (Institute for Sustainable Futures, University of Technology, Sydney, PO Box 123, Broadway NSW 2007, Australia. Faculty of Engineering, University of Technology, Sydney, PO Box 123, Broadway NSW 2007, Australia). **Model for empowering rural poor through renewable energy technologies in Bangladesh.** Environmental Science & Policy, 4(6) (2001), 333-344.

This paper proposes an integrated ecological, economic and social model to assist sustainable rural development in villages in Bangladesh. In the model, renewable energy technologies (RETs) create income-generating activities for male landless and marginal farmers and for women from such households, while reducing environmental problems, like deforestation and indoor air pollution from cooking with poor-quality fuels. Because of the high capital costs of RETs, the model proposes an extension of the well-known micro-credit approach developed by such NGOs as the Grameen Bank and BRAC. With the assistance of an External Agency composed of NGO, business, government and university representatives, such groups of villagers would form Village Organizations, comprising cooperatives or other forms of business, borrow money from a bank or large NGO, and purchase a RET based on biogas, solar or wind, depending upon location. By selling energy to wealthier members of the village, the Village Organizations would repay their loans, thus gaining direct ownership and control over the technology and its applications.

Zhongming Zheng and Jeffrey Philip Obbard. (Department of Chemical & Environmental Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore). **Removal of surfactant solubilized polycyclic aromatic hydrocarbons by Phanerochaete chrysosporium in a rotating biological contactor reactor**. Journal of Biotechnology, 96(3) (2002), 241-249.

White rot fungi can oxidize surfactant solubilized polycyclic aromatic hydrocarbons (PAH). The objective of this study was to evaluate the performance of immobilized white rot fungus, Phanerochaete chrysosporium, to remove surfactant Tween 80 solubilized PAH i.e. phenanthrene, pyrene and benzo(α)pyrene in a rotating biological contactor (RBC) reactor. Results indicated that the immobilized P. chrysosporium in the RBC reactor system in continuous operation could effectively remove the three tested PAH at specific hydraulic loading rates and concentrations tested for each individual PAH. Batch operation of RBC reactor showed that the immobilized P. chrysosporium was stable and effective for the eight successive batch treatments of PAH in solution medium i.e. PAH removal was greater than 90% after 60 h, although only low levels of ligninolytic enzyme activity were detected. The removal of phenanthrene and pyrene in solution medium has been found to be a first order reaction in batch operation. A mass balance calculation indicated that biological oxidation was the main factor for removal of benzo(α)pyrene i.e. 95.7% in the RBC reactor. However, for phenanthrene and pyrene, both biological oxidation (i.e. 49 and 56%, respectively) and RBC disc foam adsorption (i.e. 44 and 34%, respectively) made a significant contribution to the removal of PAH.

Bioengineering

A Johri, W Blank, D Kaplan. (Department of Chemical and Biological Engineering, Bioengineering and Biotechnology Center, Tufts University, 4 Colby Street, Medford, MA 02155 USA). **Bioengineered emulsans from Acinetobacter calcoaceticus RAG-1 transposon mutants.** Applied Microbiology and Biotechnology, 59(2-3), 2002, 217 – 223.

Transposon mutants of Acinetobacter calcoaceticus strain RAG-1 were studied in an effort to control fatty acid (FA) substitution patterns of emulsan, a bioemulsifier secreted by the

organism. The disrupted genes, involved in the biosynthetic pathways of biotin, histidine, cysteine or purines, influenced the level and types of FAs incorporated into emulsan. The structural variants of emulsan generated by the transposon mutants were characterized for yield, FA content, molecular weight, and emulsification behavior when grown on a series of FAs of different chain lengths from C11 to C18. Yields of emulsan from the transposon mutants were found to be lower than the parent strain and depended on the type of FA used to supplement the growth medium. Mutants 13D (His-) and 52D (Cys-) grown on LB plus C16 or C14, respectively, exhibited enhanced emulsifying activity compared to A. calcoaceticus RAG-1. The presence and composition of long chain FAs on the polysaccharide backbone influenced emulsification behavior: particularly a high mole percentage of C16 (48%) and C18 (42%). The results provide important insight into the bioengineering of bioemulsifier-producing microorganisms and provide a path towards highly tailored novel amphipathic structures to utilize as biodegradable in environmental, biomedical, and personal care applications.

Anna de Raadt and Herfried Griengl. (Institute of Organic Chemistry, Technical University Graz, Stremayrgasse 16, A-8010, Graz, Austria). **The use of substrate engineering in biohydroxylation**. Current Opinion in Biotechnology, 13(6) (2002), 537-542.

In the biohydroxylation of nonactivated carbon atoms, substrate engineering has been found to be a very useful and simple means to influence substrate acceptance and the regioselectivity and stereoselectivity of this transformation. Recently, this methodology has been applied to the hydroxylation of a large number of compounds including cycloalkane carboxylic acids, ketones, amines, amides and alcohols.

E. Record, M. Asther, C. Sigoillot, S. Pagès, P. J. Punt, M. Delattre, M. Haon, C. A. M. J. J. van den Hondel , J.-C. Sigoillot, L. Lesage-Meessen, Marcel Asther. (UMR 1163 INRA/Université de Provence de Biotechnologie des Champignons Filamenteux, IFR-BAIM Universités de Provence et de la Méditerranée ESIL, 163 avenue de Luminy Case Postale 925 13288 Marseille Cedex 09 France. Laboratoire de Bioénergétique et Ingénierie des Protéines CNRS UPR9036 31 chemin Joseph Aiguier 13402 Marseille Cedex 09 France. Université de Provence 3 place Victor Hugo 13331 Marseille France. Department of Applied Microbiology and Gene Technology TNO Nutrition and Food Research Institute Utrechtseweg 48 P.O. Box 360 3700 AJ Zeist The Netherlands). **Overproduction of the Aspergillus niger feruloyl esterase for pulp bleaching application**. Applied Microbiology and Biotechnology, 62(4) (2003), 349 – 355.

A well-known industrial fungus for enzyme production, Aspergillus niger, was selected to produce the feruloyl esterase FAEA by homologous overexpression for pulp bleaching application. The gpd gene promoter was used to drive FAEA expression. Changing the nature and concentration of the carbon source nature (maltose to glucose; from 2.5 to 60 g l -1), improved FAEA activity 24.5-fold and a yield of 1 g l -1 of the corresponding protein in the culture medium was achieved. The secreted FAEA was purified 3.5-fold to homogeneity in a two-step purification procedure with a recovery of 69%. The overproduced protein was characterised and presented properties in good agreement with those of native FAEA. The recombinant FAEA was tested for wheat straw pulp bleaching, with or without a laccase mediator system and xylanase. Best results were obtained using a bi-sequential process with a sequence including xylanase, FAEA and laccase, and yielded very efficient delignification—close to 75%—and a kappa number of 3.9. This is the first report on the potential application of recombinant FAEA in the pulp and paper sector.

Glynis Giddings. (Institute of Biological Sciences, Cledwyn Building, University of Wales Aberystwyth, Aberystwyth, Ceredigion SY23 3DD, UK). **Transgenic plants as protein factories**. Current Opinion in Biotechnology, 12(5) (2001), 450-454.

Transgenic plants are showing considerable potential for the economic production of proteins, with a few already being marketed. Recent clinical trials of pharmaceuticals produced from transgenic plants are encouraging, with plant glycans showing reassuringly poor immunogenicity. Our increasing understanding of protein targeting and accumulation should further improve the potential of this new technology.

Hassan Brim, Amudhan Venkateswaran, Heather M. Kostandarithes, James K. Fredrickson, Michael J. Daly. (Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814,1 Pacific Northwest National Laboratory, Richland, Washington 99352). **Engineering Deinococcus geothermalis for Bioremediation of High-Temperature Radioactive Waste Environments**. Applied and Environmental Microbiology, 69(8) (2003), 4575-4582.

Deinococcus geothermalis is an extremely radiation-resistant thermophilic bacterium closely related to the mesophile Deinococcus radiodurans, which is being engineered for in situ bioremediation of radioactive wastes. We report that D. geothermalis is transformable with plasmids designed for D. radiodurans and have generated a Hg(II)-resistant D. geothermalis strain capable of reducing Hg(II) at elevated temperatures and in the presence of 50 Gy/h. Additionally, D. geothermalis is capable of reducing Fe(III)-nitrilotriacetic acid, U(VI), and Cr(VI). These characteristics support the prospective development of this thermophilic radiophile for bioremediation of radioactive mixed waste environments with temperatures as high as 55°C.

José M Luengo, Belén García, Angel Sandoval, Germán Naharro, Elías R Olivera. (Departamento de Bioquímica y Biología Molecular, Facultades de Biología y de Veterinaria, Universidad de León, 24007, León, Spain. Departamento de Patología Animal (Sanidad Animal), Facultad de Veterinaria, Universidad de León, 24007, León, Spain). **Bioplastics from microorganisms**. Current Opinion in Microbiology, 6(3) (2003), 251-260.

The term `biomaterials' includes chemically unrelated products that are synthesised by microorganisms (or part of them) under different environmental conditions. One important family of biomaterials is bioplastics. These are polyesters that are widely distributed in nature and accumulate intracellularly in microorganisms in the form of storage granules, with physico-chemical properties resembling petrochemical plastics. These polymers are usually built from hydroxy-acyl–CoA derivatives via different metabolic pathways. Depending on their microbial origin, bioplastics differ in their monomer composition, macromolecular structure and physical properties. Most of them are biodegradable and biocompatible, which makes them extremely interesting from the biotechnological point of view.

Ken Oofusa, Osamu Tooi, Akihiko Kashiwagi, Keiko Kashiwagi, Yasuyuki Kondo, Masanobu Obara, Katsutoshi Yoshizato. (Tissue Regeneration Project, Hiroshima Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, Japan Science and Technology Corporation, Hiroshima Prefecture Institute of Industrial Science and Technology, Higashihiroshima, Hiroshima 739-0046, Japan. Laboratory of Developmental Biology, Department of Biological Science, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashihiroshima, Hiroshima 739-8526, Japan. ProPhoenix Company Ltd., Higashihiroshima, Hiroshima 739-0046, Japan. Towa-Kagaku Corporation Ltd., Hiroshima, Hiroshima 730-0841, Japan. Institution of Amphibians, Graduate School of Science, Hiroshima University, Higashihiroshima, Hiroshima 739-8526, Japan). **Metal ion-responsive transgenic *Xenopus laevis* as an environmental**

monitoring animal. Environmental Toxicology and Pharmacology, 13(3) (2003), 153-159.

We generated germ line-transgenic *Xenopus laevis* that monitors environmental heavy metal ions. Sperm nuclei were transduced with cDNA of enhanced green fluorescent protein (EGFP) driven by murine metallothionein-1 gene promoters and were microinjected into unfertilized eggs. The eggs developed to sexually matured adults. The transgenic tadpoles at the premetamorphic stage were reared in water containing Zn^{2+} and Cd^{2+} separately at the concentrations of 0.38–1.52 and 0.09–0.44 μM, respectively. These animals responded to Zn^{2+} at as low as 0.38 μM and Cd^{2+} at as low as 0.44 μM. The level of EGFP fluorescence emitted by tadpoles increased as the concentration increased up to 1.52 μM and the exposure time prolonged up to 120 h. The fluorescent response was much more sensitive to Cd^{2+} than to Zn^{2+}. We concluded that these transgenic tadpoles are useful as an animal indicator of environmental heavy metal ions.

Kimberly L Cook, Gary S Sayler. (Department of Microbiology, Center for Environmental Biotechnology, 676 Dabney Hall, University of Tennessee, Knoxville, TN 37996, USA). **Environmental application of array technology: promise, problems and practicalities**. Current Opinion in Biotechnology, 14(3) (2003), 311-318.

Array technology has been applied in environmental research using innovative approaches in gene expression, comparative genomics and mixed community analysis. Greater fundamental understanding of sources of experimental and analytical error in array experiments should facilitate the future application of array technology to environmental analysis.

Michael J Daly. (Department of Pathology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA). **Engineering radiation-resistant bacteria for environmental biotechnology**. Current Opinion in Biotechnology, 11(3) (2000), 280-285.

Seventy million cubic meters of ground and three trillion liters of groundwater have been contaminated by leaking radioactive waste generated in the United States during the Cold War. A cleanup technology is being developed based on the radiation-resistant bacterium *Deinococcus radiodurans*, which is being engineered to express bioremediating functions.

Roseanne M. Hofmann and Tom W. Muir. (Laboratory of Synthetic Protein Chemistry, The Rockefeller University, New York, NY 10021, USA). **Recent advances in the application of expressed protein ligation to protein engineering**. Current Opinion in Biotechnology, 13(4) (2002), 297-303.

Expressed protein ligation is a technique for joining recombinantly expressed proteins to polypeptides containing biophysical probes, post-translational modifications or unnatural amino acids. Recent advances have expanded the scope of expressed protein ligation and have allowed the approach to be applied to the study of basic biological questions.

Biofertilizer

Hongxia Yu, Huihua Shang, Tielian Xu, Yuxia Cui, Ling Yang, Hongjun Jin, Liansheng Wang. (State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, China). **Application of toxicity identification evaluation procedures to an effluent from a nitrogenous fertilizer plant in China.** Ecotoxicology and Environmental Safety, 55(2) (2003), 223-226.

The integrated method combining chemistry and toxicology, toxicity identification evaluation (TIE), was conducted to identify key toxicants in an effluent from a nitrogen fertilizer plant in China. Toxicity characterization, phase I of TIE, revealed that the suspected toxicant in the

effluent was an anion that could be changed into a volatile acid. The results of toxicity identification and confirmation procedures indicated potassium cyanide to be the primary toxicant in the effluent.

J. Derome. (Rovaniemi Research Station, Finnish Forest Research Institute, PO Box 16, FIN-96301 Rovaniemi, Finland). **Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilization.** Environmental Pollution, 107(1) (2000), 79-88.

Four experiments were established in 1992 in Scots pine stands (*Pinus sylvestris* L.) on relatively infertile sites at distances of 0.5, 2, 4 and 8 km to the south-east of the Cu–Ni smelter at Harjavalta, south-west Finland, in order to investigate the effects of liming, correction fertiliser and site-specific fertiliser treatments on heavy metal (Cu, Ni) and macronutrient (Ca, Mg, K) availability in the organic layer. The organic layer samples were analysed for total, plant-available ($BaCl_2$+EDTA) and water-extractable Ca, Mg, K, Cu and Ni. A high proportion of the Cu and Ni at 0.5 km was in a non-toxic, immobilised form. Liming had only a relatively small reducing effect on free and exchangeable Cu and Ni concentrations at 0.5 km. The lack of pH increase following liming may be due to the precipitation of Fe, present in very high concentrations close to the smelter, as $Fe(OH)_3$, resulting in the loss of neutralising bicarbonate and hydroxyl ions, but the release of Ca and Mg. Liming strongly increased Ca and Mg availability. The correction fertiliser had no effect on Ca or K availability at any of the sites.

Morihiro Maeda, Bingzi Zhao, Yasuo Ozaki, Tadakatsu Yoneyama. (Department of Soils and Fertilizers, National Agricultural Research Center, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan. Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan). **Nitrate leaching in an Andisol treated with different types of fertilizers.** Environmental Pollution, 121(3) (2003), 477-487.

Nitrate (NO_3) leaching was studied in an Andisol treated with four N fertilizers (SC: swine compost, CU: coated urea, AN: ammonium N, or NF: no fertilizer) for 7 years. Sweet corn (*Zea mays* L.) was grown in summer, followed by Chinese cabbage (*Brassica rapa* L. var. *amplexicaulis*) or cabbage (*Brassica oleracea* L. var. *capitata*) in autumn each year. In chemical fertilizer plots treated with AN or CU, NO_3–N concentrations in soil water at 1-m depth increased markedly in the summer of the second year and fluctuated between 30 and 60 mg l^{-1}. In the SC plot, NO_3–N concentration started increasing in the fourth year, reaching the same level as in the AN and CU plots in the late period of the experiment. In the NF plot, NO_3–N concentration was about 10 mg l^{-1} for the first 4 years and decreased to 5 mg l^{-1}. The potential NO_3–N concentrations by an N and water balance equation satisfactorily predicted NO_3–N concentration in the AN and CU plots, but substantially overestimated that in the SC plot, presumably because a large portion of N from SC first accumulated in soil in the organic form. Our results indicate that, under the Japanese climate (Asian monsoon), excessive N from chemical fertilizers applied to Andisols can cause substantial NO_3 leaching, while compost application is promising to establish high yields and low N leaching during a few years but would cause the same level of NO_3 leaching as in chemically fertilized plots over longer periods.

N. Vassilev, M. Vassileva. (Estación Experimental del Zaidin, CSIC, Prof. Albareda 1, 18008, Granada, Spain). **Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes**. Applied Microbiology and Biotechnology, 61(5-6) (2003), 435 – 440.

Rock phosphate (RP) is an important natural material traditionally used for the production of phosphorus (P) fertilizers. Compared with chemical treatment, microbial solubilization of RP is an alternative environmentally mild approach. An overview of biotechnological techniques,

mainly based on solubilization processes involving agro-industrial residues, is presented. Potential advantages of composting, solid-state fermentation, and liquid submerged fermentation employing free and immobilized microorganisms that produce organic acids and simultaneously solubilize RP are discussed. Subsequent introduction of the final fermented products into soil-plant systems promotes plant growth and P acquisition.

Biomarkers

A D Correia, G Lima , M H Costa , D R Livingstone. (IMAR - Institute of Marine Research, Department of Environmental Sciences and Engineering, College of Sciences and Technology, Lisbon New University, 2829-516 Caparica, Portugal). **Studies on biomarkers of copper exposure and toxicity in the marine amphipod *Gammarus locusta* (Crustacea): I. Induction of metallothionein and lipid peroxidation**. Biomarkers, 7(5) (2002), 422 – 437.

Sublethal exposures of the marine amphipod *Gammarus locusta* to a concentration range of copper (Cu) in water (4 days' exposure; 3, 5 and 10 µg Cu l^{-1}) or spiked sediments (28 days' exposure; 1, 3 and 6 mg Cu kg^{-1} dry weight) were performed, and the resulting bioaccumulation of Cu and effects on putative metallothionein (MT) and lipid peroxidation (LP) were investigated. A time-course exposure study (over 10 days) to a single water-borne concentration of Cu (4 µg l^{-1}) was also carried out. MT and LP were quantified, respectively, by differential pulse polarography and as thiobarbituric acid-reactive malondialdehyde equivalents. The increasing levels of Cu in water and sediment exposures resulted in enhanced uptake of the metal by *G. locusta*. Synthesis of putative MT occurred in response to exposure to water-borne Cu, the levels being higher ($p < 0.05$) over the dose range of Cu compared with controls. A positive correlation was observed between putative MT levels and the Cu body-burden concentration ($p < 0.001$). However, no increase in LP was observed in these animals. In contrast, in the time-course experiment, LP levels increased within 1 day of exposure, subsequently peaking at 4 days (68% greater than control, $p < 0.001$), before returning to control values by day 6. Higher levels of MT were also observed in this exposure, but at days 6 and 10 (55% and 38%, respectively), paralleling the decrease in LP. No increase in MT levels was recorded with exposure to Cu-contaminated sediments, whereas higher levels of LP were seen in comparison with controls ($p < 0.001$). Overall, the inverse relationship between putative MT induction and the occurrence of LP indicates that MT may protect against the prooxidant effects of Cu. It is concluded that MT and LP offer potential for application as biomarkers in *G. locusta*.

A. Callaghan, G. Hirthe, T. Fisher, M. Crane. (School of Animal & Microbial Sciences, The University of Reading, Whiteknights, 228, Reading, RG6 6AJ, United Kingdom. School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, United Kingdom). **Effect of Short-Term Exposure to Chlorpyrifos on Developmental Parameters and Biochemical Biomarkers in *Chironomus riparius* Meigen.** Ecotoxicology and Environmental Safety, 50(1) (2001), 19-24.

Chironomus riparius Meigen were exposed to sediment spiked with 0, 0.01, 0.05, and 0.10 mg chlorpyrifos/kg dry sediment for 48 h as late third-instar larvae. Acetylcholinesterase activity, glutathione-*S*-transferase activity, burrowing behavior, emergence time, and adult dry weight were measured to determine which were the most sensitive biomarkers of exposure and effect for short-term sublethal exposures. There was a significant drop in acetylcholinesterase activity at the two highest concentrations, but no effect on glutathione-*S*-transferase activity. Behavioral and life history biomarkers were sensitive only to the highest concentration of chlorpyrifos. The association of life history changes (female weight and male emergence times) with a significant

inhibition of acetylcholinesterase activity suggests that this can be used as a biomarker of exposure and effect.

A. R. Linde, S. Sánchez-Galán, P. Vallés-Mota, E. García-Vázquez. (Departamento de Biología Funcional, Area de Genética, Facultad de Medicina, Facultad de Química, C/ Julián Clavería, s/n, 33006, Oviedo, Spain. Area de Genética, Facultad de Medicina, Departamento de Química Física y Analítica, Facultad de Química, C/ Julián Clavería, s/n, 33006, Oviedo, Spain). **Metallothionein as Bioindicator of Freshwater Metal Pollution: European Eel and Brown Trout.** Ecotoxicology and Environmental Safety, 49(1) (2001), 60-63.

The aim of this work was to evaluate the potential of metallothionein (MT) as a bioindicator of heavy metal pollution in brown trout and European eel in the field situation. River Ferrerias (North Spain) provided a good gradient of metal contamination: concentrations of heavy metals were elevated both in water and in sediments at the downstream (polluted) site and were low at the upstream (unpolluted) site. MT levels of brown trout exhibited statistically significant differences between sites. Although European eel at the polluted site had a higher MT content, differences were not significant. It is concluded that MT is a good bioindicator of heavy metal pollution in brown trout.

Amanda Callaghan, Thomas C. Fisher, Albania Grosso, Graham J. Holloway, Mark Crane. (Division of Zoology, School of Animal and Microbial Sciences, The University of Reading, Whiteknights, P.O. Box 228, Reading, Berkshire, RG6 6AJ, United Kingdom. School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, TW20 OEX, United Kingdom). **Effect of Temperature and Pirimiphos Methyl on Biochemical Biomarkers in *Chironomus riparius* Meigen**. Ecotoxicology and Environmental Safety. 52(2) (2002), 128-133.

Fourth-instar *Chironomus riparius* Meigen larvae were exposed to the organophosphate (OP) insecticide pirimiphos methyl (0, 0.1, 1.0, and 10 μg/L) for 48, 72, or 96 h at three temperatures (3, 12, or 22°C). Two biochemical biomarkers, acetylcholinesterase (AChE) and glutathione *S*-transferase (GST), were measured in individual larvae from each treatment. AChE activity was inhibited by the OP in a dose-responsive fashion. This response remained similar at all three temperatures, demonstrating that AChE is a robust and specific biomarker. Exposure duration had little effect on AChE activity. In contrast, GST activity was induced at the highest OP insecticide concentration, but induction was also evident at 3°C. There was a significant effect of exposure duration, with an overall decline in GST activity over time. This result agrees with previous work suggesting that GSTs are not particularly suitable for use as a biomarker of pesticide exposure or effect in *Chironomus*.

B. Bhattacharya, S. K. Sarkar, R. Das. (Department of Metallurgical Engineering, Jadavpur University, Kolkata 700032, India. Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700019, India). **Seasonal variations and inherent variability of selenium in marine biota of a tropical wetland ecosystem: implications for bioindicator species.** Ecological Indicators, 2(4) (2003), 367-375.

The present study was designed to study the seasonal variations of selenium in marine biota of different trophic levels collected from Sunderban deltaic complex, northeast India. The primary objective of this work is to provide baseline data for a future environmental quality programme. In general, the sequence of Se accumulation observed in biota is as follows: Bivalve>Zooplankton>Macroalgae>Pisces>Seagrass. An elevated level of Se was recorded during monsoon season. The Se level in zooplankton (~4 μg/g) was three to four times higher than seagrass. The content of various soft tissues and shell in bivalves molluscs showed an organ-specific pattern in the following order: gill > visceral MASS = mantle > adductor muscle

> podium > shell. Fish contain less Se in comparison with macroalgae, zooplankton and bivalves revealing poor or insignificant trophic transfer of Se in the marine food chain. *Pelecyora trigona* (Bivalve) seems to be reliable indicator of Se contamination because of its high accumulation capacity. An in-depth monitoring program is recommended in order to clarify the present trend and to establish the studied biota as indicator species.

C. Abdennour, K. Khelili, M. S. Boulakoud, A. Nezzal, S. Boubsil, S. Slimani. (Department of Biology, Faculty of Sciences, University of Annaba, Annaba, 23000, Algeria. Occupational Medicine Centre, C.H.U. Annaba, 23000, Algeria). **Urinary Markers of Workers Chronically Exposed to Mercury Vapor.** Environmental Research, 89(3) (2002), 245-249.

The effects of chronic mercury intoxication on urinary markers in workers from northeast Algeria were investigated. Workmen were chosen from highly and moderately mercury-exposed factories, while controls were selected from a nonexposed site. The number of proteinuria cases were higher in the highly exposed subjects, although the nature (glomerular or tubular) of proteinuria remains unclear. However, it appears difficult to assess the degree of renal disturbance among the different exposure levels, such as the amount of excreted proteins, which have not clearly reflected the kidney lesion severity. The results also reveal that urine acidity increased progressively with increased levels of exposure, while a remarkable inverse relationship between urinary pH and urinary Hg in the highly exposed workers has been recorded. Furthermore, the significant differences in blood and urinary mercury concentrations of the three sites reflect the dose–response relationships.

C. Gravato, M. A. Santos. (Animal Physiology/Ecotoxicology Sector, Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal). **Genotoxicity biomarkers' association with B(*a*)P biotransformation in *Dicentrarchus labrax* L.** Ecotoxicology and Environmental Safety, 55(3) (2003), 352-358.

Dicentrarchus labrax (sea bass) were exposed during 0, 4, 8, 16, 24, 48, and 96 h to 0 and 0.1 μM benzo(*a*)pyrene (B(*a*)P), an environmental pollutant, and the following biomarkers were measured: (1) liver cytochrome P450 (P450) content and ethoxyresorufin-*O*-deethylase (EROD) activity as phase I biotransformation parameters, (2) liver gluthathione-*S*-transferase (GST) activity as a phase II biotransformation conjugation enzyme, (3) biliary and liver cytosolic B(*a*)P-type metabolites by fixed wavelength fluorescence detection (FF), and (4) erythrocytic micronuclei (EMN) and erythrocytic nuclear abnormalities (ENA) as genotoxicity biomarkers. Liver EROD activity (4 h), P450 content (24 h), GST activity (4, 8, and 96 h), bile (4–96 h), and liver cytosolic (4–24 h) B(*a*)P-type metabolites increased significantly in sea bass exposed to B(*a*)P as well as EMN (8–96 h) and ENA (4–96 h) frequencies. B(*a*)P genotoxicity is associated with increase in B(*a*)P-type metabolites in liver cytosol due to an impaired phase II conjugation. This increase seems to be responsible for the decrease in liver EROD and GST activities.

C.Cossu, A. Doyotte, M. Babut, A. Exinger, P. Vasseur. (EBSE Centre des Sciences de l'Environnement, Université de Metz, 94025, 57040, Metz Cédex, France. Agence de l'Eau Rhin Meuse, Rozérieulle, 19, 57061, Moulins les Metz, France. Centre d'Analyses et de Recherche, Université L. Pasteur de Strasbourg, 24, Illkirch Cédex, France). **Antioxidant Biomarkers in Freshwater Bivalves, *Unio tumidus*, in Response to Different Contamination Profiles of Aquatic Sediments.** Ecotoxicology and Environmental Safety, 45(2) (2000), 106-121.

Antioxidant systems were studied in the freshwater bivalve *Unio tumidus* transplanted from a control site to four different contaminated areas, in order to study the biological response according to the contamination characteristics. Reduced and oxidized glutathione (GSH, GSSG), the activities of antioxidant enzymes such as selenium-dependent and non-selenium-dependent

glutathione peroxidases (SeGPx and non-SeGPx), and glutathione reductase (GRd) were measured in the gills and the digestive gland of the mussels after 15 days of exposure at different sites. Lipid peroxidation (LPO) was evaluated by means of malondialdehyde measurements (MDA). The four sites investigated were located in the valleys of Fensch (F), Moselle (M), Lot et Garonne (LG), and Sarthe (S). At each site, the bivalves were placed upstream (Up) from an identified pollution source (a cokery, a laundry, or a foundry) and downstream (Do), close to the effluent outfall (Do_1) or farther (Do_2). The goal was to study the antioxidant response in relation to the pollution gradient. Metals and congeners of PAHs, PCBs, and organochlorinated pesticides were analyzed in the river sediments of each station. The exposure of the bivalves at the most highly polluted sites or close to the pollution source led to a sharp depletion in some antioxidant parameters, namely GRd, SeGPx, and GSH. The decrease in enzyme activities could reach 80% for GRd and 70% for SeGPx, while GSH depletion could yield 70%, leading then in an induction of lipid peroxidation, either in the digestive gland or in the gills. The higher the MDA concentrations, the lower the activity of these three antioxidant parameters, suggesting that they could be biomarkers for toxicity. Yet, a depletion in these parameters was sometimes insufficient for cytotoxicity to be induced, since lipid peroxidation failed to appear in some cases where antioxidant depletion was clear, although not so severe. The response of the gills and the digestive gland was not always paralleled, which can be explained by differences in the bioavailability of pollutants. In some cases, a relationship was not found between the antioxidant response and the degree and the type of contamination in sediments, suggesting that the effects could result from nonidentified pollutants or/and be indicators of bio-availability.

Chuanlun L. Zhang. (Department of Geological Sciences, University of Missouri, Columbia, MO 65211, USA). **Stable carbon isotopes of lipid biomarkers: analysis of metabolites and metabolic fates of environmental microorganisms**. Current Opinion in Biotechnology, 13(1) (2002), 25-30.

Lipid biomarkers are specific compounds that are characteristic of certain groups or species of microorganisms. The use of natural or labeled carbon isotopes of lipid biomarkers has enabled a better understanding of carbon flow pathways at the molecular level. Recent advances include, but are not limited to, the elucidation of mechanisms of anaerobic methane oxidation mediated by syntrophic sulfate-reducing bacteria and Archaea, linking microbial populations with specific microbial processes or bacterial transport mechanisms in natural or contaminated environments, and elucidation of the biosynthetic pathways of cellular material.

D. J. Reid and G. R. MacFarlane. (School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia). **Potential biomarkers of crude oil exposure in the gastropod mollusc, *Austrocochlea porcata*: laboratory and manipulative field studies.** Environmental Pollution, 126(2) (2003), 147-155.

Surveys conducted after a crude oil spill indicated that the intertidal gastropod mollusc *Austrocochlea porcata* may be highly sensitive to the pollutant, and therefore also valuable as a biomonitoring organism. Toxicity tests conducted in the laboratory and field established cause-effect for *A. porcata* mortalities on exposure to environmentally relevant concentrations of crude oil constituents. Glutathione antioxidant system components (glutathione and glutathione peroxidase, GPx) and oxidative damage (lipid peroxidation) in *A. porcata* were measured to determine whether any of these biochemical parameters showed potential as biomarkers of sublethal oil exposure. GPx was the most promising candidate for field-based biomarker studies after showing a dose-dependent induction to a crude oil water accommodated fraction (WAF) in laboratory assays. However, subsequent manipulative field experimentation indicated that the GPx response was not sufficiently sensitive and not necessarily predictive of population level effects when measured in situ.

D. S. Maycock, M. M. Prennera, R. Kheira, S. Morrisb, A. Callaghanc, P. Whitehoused, D. Morritta, M. Cranea. (School of Biological Sciences, Royal Holloway, University of London, Egham Hill, Egham, Surrey TW20 0EX, UK. CEFAS, Remembrance Avenue, Burnham-on-Crouch, Essex CM0 8HA, UK. School of Animal and Microbial Sciences, The University of Reading, Whiteknights, P.O. Box 228, Reading, Berkshire RG6 6AJ, UK. WRc-NSF, Medmenham, Marlow, Buckinghamshire SL7 2HD, UK). **Incorporation of in situ and biomarker assays in higher-tier assessment of the aquatic toxicity of insecticides**. Water Research, 37(18) (2003), 4311-4330.

This study was designed to test the feasibility of integrating in situ, single species exposures and biomarker analysis into microcosm studies. Experimental ponds were dosed with pirimiphos methyl (PM) and lindane. C. riparius fourth instar larvae were deployed for 48 h on nine separate occasions during the study period before and after treatment. Surviving larvae were analysed for acetylcholinesterase activity (AChE). Survival and biomarker data were compared to chironomid assemblage analysis by monitoring insects emerging from the microcosms. Survival of chironomids within the in situ systems commenced on day +16 after treatment with 31.6% and 53.3% survival in the lindane and PM treated ponds, respectively. In contrast, the first emergence from the microcosms occurred on days +27, in respect to lindane, and +59 for the PM treated ponds. Thus the in situ bioassay was able to demonstrate gradual reduction in toxicity within the sediment before this was evident from macroinvertebrate monitoring. Significant AChE inhibition was only detected on exposure to PM. Levels decreased from 75% on day +16 to 26% by day +29. The biomarker analysis confirmed that, by the end of the study, the insecticide was no longer exerting an effect. We discuss how the use of in situ bioassays could also aid comparison of microcosm studies by adding a standardized dimension.

Daria Pereg, Jean Lagueux, Éric Dewailly, Guy G Poirier, Pierre Ayotte. (Unité de recherche en santé et environnement, Centre de Recherche du Pavillon CHUL, Centre Hospitalier Universitaire de Québec, Ste-Foy, Québec G1V 4G2, Canada. Unité de recherche en santé publique, Centre de Recherche du Pavillon CHUL, Centre Hospitalier Universitaire de Québec, Beauport, Québec G1E 7G9, Canada). **Cigarette smoking during pregnancy: comparison of biomarkers for inclusion in epidemiological studies.** Biomarkers, 6(2) (2001), 161 – 173.

Prenatal exposure to tobacco smoke represents an important confounding factor in epidemiological studies addressing developmental effects and requires careful controlling by the use of biomarkers. We compared the following biomarkers of exposure to tobacco smoke during pregnancy and related biological effects in 23 smokers and 17 non-smokers: placental concentrations of heavy metals (cadmium, chrome, lead and zinc), cotinine concentration in meconium, placental CYP1A1 activity (EROD) and bulky DNA adducts. Cadmium was detected in all samples and found in higher concentration in placentas of smokers compared with non-smokers (geometric mean - GSD: 56.1 - 1.8 vs 27.4-1.6 µg kg--1 dry weight; p <0.001). Cotinine was not detected in meconium samples from the non-smoker group, while samples from the smoker group contained a mean concentration of 114.1-2.9 µg kg--1. Correlation analysis of biomarkers among smokers revealed that daily cigarette consumption was strongly correlated to placental cadmium (Pearson's r =0.83, p <0.001) and to cotinine (r =0.73, p <0.001). EROD activity was also higher in smokers than in non-smokers (9.4-3.4 vs 2.5-1.8 pmol resorufin min-1 mg-1 protein; p <0.001) and values were correlated to cotinine concentration in meconium (r =0.80, p <0.001) and placental cadmium level (r =0.66, p <0.001). The amount of bulky DNA adducts in placenta was highly variable and poorly associated with smoking status. Because of their high sensitivity and specificity to detect women who smoke during pregnancy, cotinine concentrations in meconium and placental EROD activity should be incorporated in

epidemiological studies that investigate adverse developmental effects induced by in utero exposure to environmental contaminants.

Denise Fernandes, Joanna Potrykus, Cinzia Morsiani, Demetrio Raldua, Ramón Lavado and Cinta Porte. (Environmental Chemistry Department, IIQAB-CSIC, C/Jordi Girona, 18, 08034, Barcelona, Spain). **The Combined Use of Chemical and Biochemical Markers to Assess Water Quality in Two Low-Stream Rivers (NE Spain)**. Environmental Research, 90(2) (2002), 169-178.

Carps (*Cyprinus carpio*) and red swamp crayfish (*Procambarus clarkii*) were sampled from two low- stream Mediterranean rivers (Anoia and Cardener) receiving extensive urban and industrial waste water discharges. Tissue residues of selected pollutants (organochlorinated compounds, polycyclic aromatic hydrocarbons (PAHs)) were determined in conjunction with different biochemical responses (cytochrome P450, phase II enzymes) with the aim of investigating whether resident organisms were responsive to changes in water quality. Biota inhabiting those rivers were highly exposed to complex mixtures of polychlorobiphenyls and dichlorodiphenyltrichloroethanes (up to 19 ng/g w.w.) and PAHs (up to 6097 ng/g of hydroxylated PAHs in bile), the highest residues being observed in carps from Cardener. This has a reflection on 7-ethoxyresorufin *O*-deethylase (EROD) activity; that in carps from Cardener ranged between 350 and 550 pmol/min/mg protein, whereas in carps from Anoia ranged between was 90 and 250 pmol/min/mg protein. The highest activity recorded was downstream of the sewage treatment plants in both rivers. Phase II enzymes were less sensitive to pollutant exposure than EROD; nonetheless, both glutathione *S*-transferase and UDP–glucuronyl transferase were higher in carps from Cardener. The results support the usefulness of the combined use of chemical and biochemical responses to assess and diagnose pollution in highly stressed ecosystems.

E Gibney, J Gault, J Williams. **The use of stress proteins as a biomarker of sub-lethal toxicity: induction of heat shock protein 70 by 2-isobutyl piperidine and transition metals at sub-lethal concentrations**. Biomarkers, 6(3) (2001), 204 – 217.

The stress response is a highly conserved reaction to various physical, chemical and biological stimuli. The ubiquity of the response occurring across taxonomic classes has identified heat shock proteins as potential biomarkers. In this study using the neutral red assay, silver stained one-dimensional SDS-PAGE, Western blotting and ELISA, the use of heat shock proteins as biomarkers of sub-lethal toxicity was examined. Hsp70 was induced in the mouse connective tissue cell line (L929) at sub-lethal concentrations for three transition metals (cadmium, mercury and copper) and for 2-isobutyl piperidine, a novel compound whose chemical structure is similar to a toxin found in the Colorado potato beetle (*Leptinotarsa decemlineata*). Hsp70 induction was found to increase in a dose-dependent fashion. Expression of other potentially interfering proteins was found to decrease with increasing toxin concentration. The induction of hsp70 at sub-lethal concentrations by the transition metals and 2-isobutyl piperidine demonstrates the potential of hsp70 as a biomarker of sub-lethal toxicity.

E M Alvarez Leite , A Leroyer , C Nisse , J M Haguenoer , C Y De Burbure , J P Buchet , A Bernard. **Urinary homovanillic acid and serum prolactin levels in children with low environmental exposure to lead**. Biomarkers, 7(1) (2002), 49 – 57.

Current evidence suggests that the neurotoxic effects of lead may partially be mediated through interference with the dopaminergic system. The aim of this study was to assess the levels of two peripheral dopaminergic markers- serum prolactin (Pro-S) and urinary homovanillic acid (HVA-U) - in children living around two lead smelters, who are presumed to be exposed to high environmental lead pollution (n = 200), and compare their results with 200 age- and sex-matched controls living in an area unpolluted by heavy metals, giving a total of 400 children (200 boys

and 200 girls). The influence of lead exposure on HVA-U and Pro-S was assessed by stepwise multiple regression, testing lead concentrations in blood (Pb-B), age, sex and area of residence as predictors. Though lead levels were significantly higher in boys and in the lead-polluted environment, mean Pb-B values were relatively low, indicating a low uptake of lead in the contaminated environment (39.5 µg l-1, range 4.6-165µgl-1, n = 200), and no significant correlation could be found with either Pro-S or HVA-U. However, when the subgroup of 121 children with Pb-B levels above 50 µg l-1 were considered, a weak positive correlation was found between Pb-B and HVA-U (r2 = 0.04, p = 0.03), whilst in the even smaller subgroup of 15 children with Pb-B levels above 100 µg l-1, Pro-S appeared to be positively correlated with Pb-B, though the numbers of children were too small for the correlation to reach statistical significance (p = 0.095). These weak associations, probably not important in biological terms, indicate that Pro-S and HVA-U are not useful biomarkers at present exposure levels to lead in the environment. Nevertheless, the finding of subtle biochemical alterations in the dopaminergic system at Pb-B levels of around 100µgl-1 supports the recommended setting of the action level at this value.

Eric Lindesjöö, Margaretha Adolfsson-Erici, Gunilla Ericson, Lars Förlin. (Institute of Applied Environmental Research, Stockholm University, SE-106 91, Stockholm, Sweden. Department of Zoophysiology, Göteborg University, 463, SE-405 30, Göteborg, Sweden). **Biomarker Responses and Resin Acids in Fish Chronically Exposed to Effluents from a Total Chlorine-Free Pulp Mill during Regular Production**. Ecotoxicology and Environmental Safety, 53(2) (2002), 238-247.

This study used rainbow trout (*Oncorhynchus mykiss*) to investigate the biological effects of long-term exposure to a total chlorine-free (TCF) pulp mill effluent. Fish tanks were set up on the premises of the pulp mill, and fresh effluent water was led through a pipe directly from the pulp mill to the tanks. The fish were exposed to effluent for up to 50 days and kept for up to 70 days afterward to study the recovery process. Two independent experiments were carried out. Ethoxyresorufin-*O*-deethylase (EROD), glutathione-*S*-transferase (GST), and glutathione reductase (GR) activity were elevated in fish exposed to a 2% concentration of pulp mill effluent, as were levels of DNA adducts. These effects could be detected during exposure and also after a period of recovery. The results demonstrate that the pulp mill effluent contains substances that affect the detoxification process and also have genotoxic potential. The continued occurrence of effects after a 70-day recovery period was demonstrated in both experiments, and may indicate that the effluent contained compounds with persistent properties The content of free and conjugated resin acids in the bile of the fish was found to be a useful indicator of exposure to pulp mill effluent.

F. Monaci, F. Moni, E. Lanciotti, D. Grechi, R. Bargagli. (Dipartimento di Biologia Ambientale, Università degli Studi di Siena, Via delle Cerchia 3, 53100 Siena, Italy. Dipartimento di Sanità Pubblica, Sezione di Igiene, Università di Firenze, Viale Morgagni 48, 50100 Florence, Italy. Agenzia Regionale per la Protezione dell'Ambiente Toscana, Dipartimento di Firenze, Via Ponte alle Mosse 211, 50100 Florence, Italy). **Biomonitoring of airborne metals in urban environments: new tracers of vehicle emission, in place of lead**. Environmental Pollution, 107(3) (2000), 321-327.

Samples of *Quercus ilex* leaves and of the inhalable fraction of atmospheric particulate (PM_{10}) were collected along a busy road and in a park in Florence (Italy). Quantitative comparisons and correlations of element concentrations in PM_{10} collected by air samplers at two sites showed that Ba, Cu, Fe, Mn, Pb and Zn were the main metal pollutants emitted by vehicles in Florence. Very similar results were obtained by the analysis of *Q. ilex* leaves which were found to accumulate airborne metals as a function of the exposure time (i.e. their age). One-year-old leaves showed

the highest rate of metal accumulation. Our results show that the progressive phasing-out of leaded petrol in Italy has resulted in a decrease of about 20% per year in the Pb concentrations in PM_{10}. Both PM_{10} and *Q. ilex* analysis singled out Ba and Zn as valid tracers of automotive traffic instead of Pb.

F. R. de la Torre, A. Salibián, L. Ferrari. (Applied Ecophysiology Program, National University of Lujan, Casilla de Correo 221, 6700-, Luján, Argentina. CONICET, Buenos Aires, Argentina. CIC-, Buenos Aires, 1900-La Plata, Argentina). **Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium.** Environmental Pollution, 109(2) (2000), 277-282.

The impact of long-term exposure to waterborne cadmium (Cd) on *Cyprinus carpio* was evaluated through changes of selected parameters considered as biomarkers of toxicity. Fish were exposed to 1.6 mg l^{-1} Cd for 14 days and then transferred to Cd-free water for 19 days. The measured parameters were gill ATPases, brain acetylcholinesterase (AchE), liver glutamate oxaloacetate (GOT) and glutamate pyruvate (GPT) transaminases, muscle water content, and protein content of liver, gills and brain. Condition factor and liver somatic index were also calculated. Branchial ATPase activities were impaired in a dissimilar way: the (Na^+,K^+)-ATPases were inhibited by approximately 30%, while the Mg^{2+}-ATPase was significantly activated by 70%. Brain AchE showed no changes after Cd exposure. Both liver GOT and GPT activities were increased by the metal by 63 and 98%. Water content of the skeletal muscle showed no significant alterations. After the 19-day recovery phase, changes in the Mg^{2+}-ATPase and GPT were reversed to values similar to controls, but the Cd exposure resulted in an irreversible alteration in GOT activity. Results indicate that the sublethal Cd concentrations are stressful to carp, particularly with reference to branchial enzymes which may disrupt the osmotic and ionic balance of the animals.

Francesco Nonnis Marzano, Pier Giovanni Bracchi, Paola Pizzetti. (Dipartimento di Biologia Evolutiva e Funzionale, Faculty of Sciences, Faculty of Veterinary Medicine, University of Parma, 43100, Parma, Italy. Faculty of Sciences, Instituto di Scienza e Tecnologia degli Alimenti, Faculty of Veterinary Medicine, University of Parma, 43100, Parma, Italy). **Radioactive and Conventional Pollutants Accumulated by Edible Mushrooms (*Boletus* sp.) Are Useful Indicators of Species Origin.** Environmental Research, 85(3) (2001), 260-264.

Concentrations of artificial radionuclides and trace elements in *Boletus* samples collected in different areas of the world were detected, respectively, by gamma spectrometry and neutron activation analysis. The particular commercial value of *Boletus edulis*, *B. aestivalis*, and *B. pinophilus* from the Taro Valley (Parma, Italy) has often stimulated local factories to trade edible *Boletus* imported from several areas of the world as the real Taro Valley mushroom. Starting from this evidence, the calculation of the Chernobyl radioactive contamination in the mushrooms coupled with the presence of particular stable elements and their concentration factors has been demonstrated to be a potential useful tool for identifying the real origin of the samples. In fact, major differences in the radiocesium activity levels and trace element presence were observed even in mushrooms collected in nearby valleys. The radiometric data are supported by the statistical analysis. In particular, both the principal component analysis and the concentration distribution along a regression line support the idea of two different clusters: one referred to the "king boletus" of the Taro Valley and another one to the other conspecific samples from different ecosystems.

Freek Ariese, Wilfried H. O. Ernst, Dick T. H. M. Sijm. (Department of Analytical Chemistry and Applied Spectroscopy, Vrije Universiteit Amsterdam, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands. Department of Ecology and Ecotoxicology of Plants, Vrije Universiteit Amsterdam, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. Centre

for Substances and Risk Assessment (CSR), National Institute of Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands). **Natural and synthetic organic compounds in the environment––a symposium report**. Environmental Toxicology and Pharmacology, 10(3) (2001), 65-80.

In March 2000, an international two-day symposium was organized in Noordwijkerhout, The Netherlands, on `Natural and synthetic organic compounds in the environment'. The emphasis of the symposium was on the following classes of compounds: polycyclic aromatic hydrocarbons, xeno-estrogens, phyto-estrogens, and veterinary drugs. Sources, environmental distribution, uptake, biotransformation and toxic effects from the molecular to the population level were discussed. Other important aspects were the development of biomarkers, analytical methods, bioassays, molecular modelling and other research tools. Finally, the implications of the findings for government policies were discussed. In this paper, a summary is given of the most important facts and views presented at the symposium.

H. Kinnunen, T. Holopainen, L. Kärenlampi. (Department of Ecology and Environmental Science, University of Kuopio, P.O. Box 1627, FIN-70211, Kuopio, Finland. Department of Biology/Botany, University of Oulu, P.O. Box 3000, FIN-90014, Oulu, Finland). **Sources of error in epiphytic lichen variables mapped as bioindicators: needs to modify the Finnish standard.** Ecological Indicators, 3(1) (2003), 1-11.

A standardized lichen mapping method, to assess the environmental quality in urban and industrial areas, has been used in Finland since 1990. Comparisons of the data collected by different field workers showed large variation in both numbers of species recorded and their observed frequencies. It is recommended that a reduced number of species, which are easily and reliably identified, should be used when assessing environmental quality, and correspondingly, the standard method should be revised.

H. SandermannJr. (GSF––Forschungszentrum für Umwelt und Gesundheit GmbH, Institut für Biochemische Pflanzenpathologie, D-85758 Oberschleißheim, Germany). **Ozone/biotic disease interactions: molecular biomarkers as a new experimental tool.** Environmental Pollution, 108(3) (2000), 327-332.

Current climate change scenarios predict a further increase of tropospheric ozone which is well known to inhibit plant photosynthesis and growth processes. Ozone can also predispose plants to enhanced biotic attack, as proposed in particular for necrotrophic fungi, root-rot fungi and bark beetles. However, at present it does not seem possible to predict whether increased ambient ozone will lead to a higher or lower disease likelihood in particular plant–pathogen systems. It has been stated repeatedly in the literature that periods of high ambient ozone are essentially non-coincident with infection periods of most fungal pathogens. This implies minimal interactive risks. However, it now appears that the various ozone-induced metabolic changes can persist in plants over days or months. Visible ozone symptoms also may be greatly delayed. Certain stress transcripts, proteins and metabolites have been developed as ozone biomarkers in controlled exposure experiments, but these biomarkers remain to be examined on field sites. A simple epidemiological scenario based on `memory' time spans of ozone effects is proposed as a tool to make ozone–plant disease interactions more predictable.

Henri Teisseire, Guy Vernet. (Laboratoire d'Eco-Toxicologie, Unité de Recherche Vigne et Vins de Champagne E. A. 2069, Bâtiment Europol'Agro, Faculté des Sciences, Université de Reims Champagne-Ardenne, BP 1039, F-51687 Reims-02, France). **Ascorbate and glutathione contents in duckweed, *Lemna minor*, as biomarkers of the stress generated by copper, folpet and diuron.** Biomarkers, 5(4) (2000), 263 – 273.

Glutathione and ascorbate are essential components of the general antioxidative strategy to overcome oxidative stress due to environmental constraints such as pollution. The variation of

glutathione and ascorbate contents in duckweed (*Lemna minor*) was investigated after a 48 h exposure to copper, diuron and folpet under laboratory conditions in order to determine whether changes in their level could serve as suitable and early biomarkers of pollution. One could observe that diuron and folpet caused the glutathione level to increase, its redox status remaining unchanged, while copper led to a depletion of this antioxidant and to an increase in its oxidation rate. When duckweed was contaminated by folpet and the metal, an increase of the ascorbate pool size occurred from concentrations as low as 1 mg l^{-1} and 50 µg l^{-1} respectively. While the ascorbate pool became more oxidized because of exposure to copper concentrations h 200 µg l^{-1}, folpet caused an increase in its reduction rate. Diuron was responsible for depletion of ascorbate, the redox status of which remained unchanged. Because it is an adaptation to stress and a defence process, the increase in the antioxidant pool size was proposed as a biomarker of exposure to an unsafe environment. Since depletion of antioxidant and an increase in its oxidation rate weakened cellular defences and indicated a precarious state, they could constitute early indicators of toxicity. So they were proposed as potential biomarkers of toxicity. It was concluded that the antioxidant content in duckweed might serve as a useful biomarker for monitoring water quality.

Indranil Mukhopadhyay, Aamir Nazir, D K Saxena, D Kar Chowdhuri. (Embryotoxicology Section, Industrial Toxicology Research Centre, PO Box 80, M. G. Marg, Lucknow 226 001, Uttar Pradesh, India). **Toxicity of cypermethrin: hsp70 as a biomarker of response in transgenic *Drosophila***. Biomarkers, 7(6) (2002), 501 – 510.

Heat shock protein induction is often associated with a cellular response to a harmful environment or to adverse life conditions. The main aims of our study were (1) to evaluate the cytotoxic potential of cypermethrin; and (2) to investigate the suitability of stress-induced heat shock protein Hsp70 as a biomarker for environmental pollutants in transgenic *Drosophila melanogaster* (*Hsp70-lac*Z)*Bg*[9]. Different concentrations of cypermethrin (0.002, 0.2, 0.5 and 50.0 p.p.m.) were mixed with food. Third instar larvae of transgenic *Drosophila melanogaster* were allowed to feed on these mixtures for different time intervals (2, 4, 6, 12, 24 and 48h). Following feeding, hsp70 induction and tissue damage were evaluated. In the highest concentration treatment group (50 p.p.m.), 100% larval mortality was recorded after 12 h exposure. Hsp70 was found to be induced even at the lowest concentration (0.002 p.p.m.) of the insecticide, while tissue damage was observed in the larvae exposed for 48 h. While an insignificant decline in hsp70 expression was observed in the larvae exposed to cypermethrin at a dietary concentration of 0.002 p.p.m. after 48 h compared with those exposed for 24 h, in the next two higher concentrations of the toxicant, a similar but significant decline in hsp70 expression was evident in the exposed larvae after 48 h. The present study reveals the cytotoxic potential of cypermethrin and further proposes that hsp70 induction in transgenic *Drosophila melanogaster* could be used as a sensitive biomarker in risk assessment.

Iqbal Sayeed, Suhel Parvez, Suwarna Pandey, Bilal Bin-Hafeez, Rizwanul Haque and Sheikh Raisuddin. (Ecotoxicology Laboratory, Department of Medical Elementology and Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110 062, India). **Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, Channa punctatus Bloch**. Ecotoxicology and Environmental Safety, 56(2) (2003), 295-301.

The pyrethroid class of insecticides, including deltamethrin, are being used as substitutes for organochlorines and organophosphates in pest-control programs because of their low environmental persistence and toxicity. Ecotoxicological consequences of deltamethrin, particularly its effects on antioxidants in fish and other aquatic organisms, are not well understood. We investigated the effect of deltamethrin (0.75 µg/L) on antioxidants in a freshwater fish, Channa punctatus Bloch, using standard laboratory conditions. A single

exposure for 48 h caused induction of various antioxidant enzymes and nonenzymatic antioxidants in kidney and liver. The induction of these antioxidants was not very prominent in gills. In fact, certain antioxidants were found to be depleted in gills. Catalase activity was decreased in all the tissues. Deltamethrin also induced lipid peroxidation in all the tissues, gills showing the highest levels. Glutathione, which is an established nonenzymatic antioxidant in fish, was significantly ($P<0.001$) increased in all the tissues. Ascorbic acid content increased in kidney and liver while it decreased in gills. The findings of the present investigation show that deltamethrin has oxidative-stress-inducing potential in fish, and gills are the most sensitive organs. It is also interesting to note that gills are the primary sites of deltamethrin absorption and their antioxidant potential is also very poor. The various parameters studied in this investigation can also be used as biomarkers of exposure to deltamethrin. It is suggested that appropriate ecotoxicological risk assessment should be made in the areas where deltamethrin is proposed to be used in pest control activities.

J Ruíz-Laguna, C García-Alfonso, J Peinado, S Moreno, L A Ieradi, M Cristaldi, J López-Barea. (Department of Biochemistry and Molecular Biology,University of Córdoba, Spain. Department of Applied Biology,Estación Biológica de Doñana, CSIC, Seville, Spain. Center for Nucleic Acid Studies, CNR, Department of Genetics and Molecular Biology,University of 'La Sapienza', Rome, Italy. Department of Animal and Human Biology, University of 'La Sapienza', Rome, Italy). **Biochemical bio markers of pollution in Algerian mouse (Mus spretus) to assess the effects of the AznalcÓllar disaster on Doñana Park (Spain)**. Biomarkers, 6(2) (2001), 146 – 160.

On April 25, 1998, a tailings dam of the Aznalcóllar pyrite mine partially collapsed and released to the Guadiamar river acidic water (pH < 3) and mud containing toxic metals (Fe, Zn, Pb, As, Cu, Sb, Co, Tl, Bi, Cd, Ag, Hg, Sr), threatening the Doñana National Park, a Spanish wildlife reserve. To assess possible biological effects in terrestrial ecosystems, biochemical biomarkers have been assayed for the first time in Algerian mice (Mus spretus), a non-protected and free-living species, from several areas of Doñana and Guadiamar. Biomarkers assayed responded to different types of contaminants: I-metals and oxidant compounds (Se-glutathione peroxidase (SeGSHPx) and antioxidant activities, malondialdehyde (MDA), and glutathione redox status); II-Aromatic chemicals (ethoxyresorufin-O-deethylase (EROD) activity); III-Compounds of both types (glutathione-S-transferase (GST) activities). Before the Aznalcóllar spill (October 1997), mice from the 'Brazo de la Torre' had SeGSHPx and EROD activities close to animals from the Huelva Industrial Park, suggesting similar levels of oxidant and aromatic contaminants at both sites. Six months after the spill (October 1998), mice from the lower Guadiamar areas ('Cangrejo Grande' and 'Brazo de la Torre') also showed significant increase of soluble and microsomal GST activities, and altered levels of several antioxidant enzymes. Thus, the spilled chemicals could have induced further biological effects in mice from the exposed areas. Although no significant responses to contamination were found after the spill at Doñana core, further investigations should be carried out to monitor the situation.

J. A. Almeida, Y. S. Diniz, S. F. G. Marques, L. A. Faine, B. O. Ribas, R. C. Burneiko, E. L. B. Novelli. (Zoology, Instituto de Biociencias, Universidade Estadual Paulista––UNESP, Botucatu, São Paulo, Brazil. Departamento de Química e Bioquímica, Instituto de Biociências, IB, Universidade Estadual Paulista, UNESP, 18618-000 Botucatu, São Paulo, Brazil. Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Madrid, Spain). **The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination.** Environment International, 27(8) (2002), 673-679.

Water contaminants have a high potential risk for the health of populations. Protection from toxic effects of environmental water pollutants primarily involves considering the mechanism of

low level toxicity and likely biological effects in organisms who live in these polluted waters. The biomarkers assessment of oxidative stress and metabolic alterations to cadmium exposure were evaluated in Nile tilapia, *Oreochromis niloticus*. The fish were exposed to 0.35, 0.75, 1.5, and 3.0 mg/l concentrations of Cd^{2+} ($CdCl_2$) in water for 60 days. Fish that survived cadmium exposure showed a metabolic shift and a compensatory development for maintenance of the body weight gain. We observed a decreased glycogen content and decreased glucose uptake in white muscle. Lactate dehydrogenase (LDH) and creatine phosphokinase (CK) activities were also decreased, indicating that the glycolytic capacity was decreased in this tissue. No alterations were observed in total protein content in white muscle due to cadmium exposure suggesting a metabolic shift of carbohydrate metabolism to maintenance of the muscle protein reserve. There was an increase in glucose uptake, CK increased activity, and a clear increase of LDH activity in red muscle of fish with cadmium exposure. Since no alterations were observed in lipoperoxide concentration, while antioxidant enzymes glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were changed in the liver and the red and white muscle of fish with cadmium exposure, we can conclude that oxygen free radicals are produced as a mediator of cadmium toxicity. Resistance development is related with increased activities of antioxidant enzymes, which were important in the protection against cadmium damage, inhibiting lipoperoxide formation.

J. K. Jansson, K. Björklöf, A. M. Elvang, K. S. Jørgensen. (Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, S-10691 Stockholm, Sweden. Research Laboratory, Finnish Environment Institute, Hakuninmaantie 4-6, FIN-00430 Helsinki, Finland). **Biomarkers for monitoring efficacy of bioremediation by microbial inoculants.** Environmental Pollution, 107(2) (2000), 217-223.

Bioaugmentation of contaminated sites with microbes that are adapted or genetically engineered for degradation of specific toxic compounds is an area that is currently being expored as a clean-up option. Biomarkers have been developed to track the survival and efficacy of specific bacteria that are used as inocula for bioremediation of contaminated soil. Examples of biomarkers include the *luc* gene, encoding firefly luciferase and the *gfp* gene, encoding the green fluorescent protein (GFP). The *luc* gene was used to tag different bacteria used for bioremediation of gasoline or chlorophenols. The bacteria were monitored on the basis of luciferase activity in cell extracts from soil. The *gfp* gene was also used to monitor bacteria during degradation of chlorophenol in soil, based on fluorescence of the GFP protein. Other biomarkers can also be used for monitoring of microbial inocula used for bioaugmentation of contaminated sites. The choice of biomarker and monitoring system depends on the particular site, bacterial strain and sensitivity and specificity of detection required.

J. P. Odendaal, A. J. Reinecke. (Department of Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa). **Quantifying histopathological alterations in the hepatopancreas of the woodlouse *Porcellio laevis* (Isopoda) as a biomarker of cadmium exposure.** Ecotoxicology and Environmental Safety, 56(2) (2003), 319-325.

The aims of this study were to determine and quantify the effects of cadmium sulfate on the histological structure of the hepatopancreas of *Porcellio laevis*, to determine the extent of the changes due to cadmium exposure, and to assess whether these effects could serve as biomarkers of cadmium exposure. For this purpose, different batches of *P. laevis* were exposed for 6 weeks to shredded leaves contaminated with a range of different concentrations of cadmium sulfate. Histological sections of different parts of the hepatopancreases revealed that the exposure changed the structure of the hepatopancreas. Quantification of this change in hepatopancreas structure (expressed as the percentage cellular area or PCA) showed that there was a dose-related change in certain areas of the hepatopancreas of woodlice exposed to cadmium sulfate in

comparison to those areas of unexposed controls. This change was evident despite the fact that PCA also varied over the length of the hepatopancreas in the control specimens. This study showed that PCA can be a useful measure of change in the hepatopancreas resulting from toxic stress. Although it may not be practical for regular monitoring, it could serve as a useful research tool and general biomarker in studies of toxic stress in woodlice.

Jacob Garty, Sharon Tomer, Tal Levin, Haya Lehr. (Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel. Institute for Nature Conservation Research, Tel Aviv University, Tel Aviv 69978, Israel). **Lichens as biomonitors around a coal-fired power station in Israel**. Environmental Research, 91(3) (2003), 186-198.

In the present study epiphytic lichens were applied as biomonitors of air pollution to determine the environmental impact of a coal-fired power station. Thalli of the lichen *Ramalina lacera* (With.) J.R. Laund. growing on carob twigs (*Ceratonia siliqua* L.) were collected with their substrate in July 2000 in a relatively unpolluted forest near HaZorea, Ramoth Menashe, Northeast Israel, and transplanted to 10 biomonitoring sites in the vicinity of the coal-fired power station Oroth Rabin near the town of Hadera. The lichens were retrieved in January 2001. We examined the following parameters of lichen vitality: (a) potential quantum yield of photosynthesis expressed as fluorescence ratio F_v/F_m, (b) stress-ethylene production, and (c) electric conductivity expressing integrity of cell membranes. Following an exposure of 7 months, the lichens were retrieved and physiological parameters and data of elemental content were analyzed comparatively. Electric conductivity values correlated positively with B, Fe, Mg, Mn, Na, Pb, S, Sn, and Ti content. Concentrations of stress-ethylene correlated positively with Al, Ba, Pb, S, and V content and negatively with Cu and Sn. F_v/F_m ratios correlated negatively with S content. Some of the heavy metals reached lower levels than those reported in the relevant literature despite a wind regime that should have blown pollutants toward the biomonitoring sites.

Jean-Jacques Legrand , Cecile Fisch, Pierre-Olivier Guillaumat, Jean-Marc Pavard, Mahmoud Attia, Stephane De Jouffrey, Jean-Roger Claude. (Beaufour-Ipsen Pharma, Paris, France. CIT, Evreux, France. Faculty of Pharmacy, Paris, France). **Use of biochemical markers to monitor changes in bone turnover in cynomolgus monkeys**. Biomarkers, 8(1) (2003), 63 – 77.

The ovariectomized old cynomolgus monkey is a recognized model of human osteoporosis, and the same species can be used for the assessment of the efficacy and potential toxicity of agents intended to prevent or treat osteoporosis. Several assays have been developed that can measure the same biochemical markers of bone turnover as are used in human patients for the diagnosis and treatment follow-up of bone-related diseases, including osteoporosis. The aim of the present study was to describe the results obtained with these assays in normal control monkeys, their variations with age and sex, and their sensitivity in monitoring the bone turnover induced by ovariectomy in old skeletally mature cynomolgus monkeys. Seven old cynomolgus monkeys were bilaterally ovariectomized and 13 age-matched monkeys were sham-operated. Bone mineral density and biochemical markers were measured before and at regular intervals after surgery for up to 20 months. Total alkaline phosphatase (total ALP), bone-specific alkaline phosphatase isoenzyme (bone ALP) and osteocalcin (OC) were highly correlated to the decrease in bone mineral density (BMD) induced by ovariectomy. Deoxypyridinoline (DPD) measured by enzyme-linked immunoassay was insensitive to the bone resorption induced by ovariectomy, but cross-linked N-telopeptide (NTX-I) was higher in ovariectomized monkeys than in control monkeys. These results demonstrate that reliable biochemical parameters are available to adequately monitor and provide insight into osteoclastic bone resorption and osteoblastic bone

formation, the two components of bone turnover in this animal model, and can thus be used to assess the efficacy and toxicity of potential therapeutic agents.
Jonathan D Tugwood, Laura E Hollins, Mark J Cockerill. (Molecular Toxicology Group Safety Assessment Department, AstraZeneca Pharmaceuticals Alderley Park Macclesfield. University of Manchester Manchester). **Genomics and the search for novel biomarkers in toxicology**. Biomarkers, 8(2) (2003), 79 – 92.

The advent of 'genomics' technology, in particular transcript profiling, has already had a measurable impact on the drug discovery process in the areas of target identification and validation. This review is concerned with the potential application of this technology to toxicology and drug safety assessment, with particular emphasis on biomarker discovery and characterization. An advantage (or possibly a drawback!) of transcript profiling is that candidate biomarkers of toxicity can be speedily identified, with the caveat that a significant amount of subsequent experimental and bioinformatic effort needs to be expended in order to evaluate and validate them. Attention is also drawn to the critical need for robust experimental design with studies of this type and to issues associated with the analysis of large data sets. In summary, while genomics technology undoubtedly offers much that can assist drug safety assessment, its potential has yet to be realized fully in this area. However, a large amount of resource continues to be applied to 'toxicogenomics'. Tangible benefits, in terms of new biomarkers of toxicity and reduced numbers of adverse drug effects, remain realistic objectives.
Jonathon E. Ericson, Amber Rinderknecht, Elisabeth J. Gonzalez, Francis M. Crinella, Michael T. Kleinman. (Department of Environmental Analysis and Design, School of Social Ecology, College of Medicine, University of California, Irvine, California, 92697-7070. School of Social Ecology, Department of Pediatrics, College of Medicine, University of California, Irvine, California, 92697-7070. School of Social Ecology, Department of Community and Environmental Medicine, College of Medicine, University of California, Irvine, California, 92697-7070). **Measurements of Manganese with Respect to Calcium in Histological Enamel Cross Sections: Toward a New Manganese Biomarker.** Environmental Research, 86(1) (2001), 46-50.

Airborne Mn may become an important route of exposure if the use of Mn-containing gasoline additives becomes more widespread. We report on the measurement of manganese and calcium in histological cross sections of shed deciduous tooth enamel of three human subjects. The goal of this research was to measure Mn in tooth enamel for use as a biomarker in assessing manganese exposure in cross-sectional and longitudinal studies. The histological locations can be time-specific (analogous to examining growth rings in trees). This technique, which may identify critical windows of exposure, can be important for evaluating potential vulnerability of the fetus and neonate to inhaled or ingested Mn.
Judith S. Weisa, Jennifer Samsona, Tong Zhoua, Joan Skurnickb, Peddrick Weis. (Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA. Department of Preventive Medicine and Community Health, UMDNJ–NJ Medical School, Newark, NJ 07101-1709, USA. Department of Radiology (G-621), UMDNJ- NJ Medical School, P.O. Box 1709, Newark, NJ 07103-1709, USA). **Evaluating prey capture by larval mummichogs (Fundulus heteroclitus) as a potential biomarker for contaminants**. Marine Environmental Research, 55(2) (2003), 137-159.

We evaluated larval prey capture as a "behavioral biomarker" of contamination by examining feeding behavior of larval mummichogs (Fundulus heteroclitus) from many different sites, including a severely contaminated "Superfund" site, moderately contaminated sites, and reference areas. Prey capture ability was related to sediment contaminant levels. The levels of contaminants at a site were highly correlated with each other, so that the impact of individual contaminants was confounded. The number of captures of brine shrimp by mummichog larvae

from all sites was highly variable, but significant negative correlations of prey capture were seen with mercury, lead, zinc, cadmium, and PCBs. As observed previously with adults, polyaromatic hydrocarbons (PAHs) did not appear to impair prey capture ability. The only site in which prey capture rates of 8-day old larvae were severely affected was the most highly contaminated Superfund site, Berry's Creek, NJ. This implies that larval prey capture is not as sensitive a behavioral biomarker for contamination as adult behavior studied previously.

Luigi Manzo, Anna F. Castoldi, Teresa Coccini, Leon D. Prockop. (Toxicology Division, University of Pavia, "Salvatore Maugeri" Foundation IRCCS, Institute of Pavia, Pavia, 27100, Italy. Department of Neurology, College of Medicine, University of South Florida, Tampa, Florida). **Assessing Effects of Neurotoxic Pollutants by Biochemical Markers.** Environmental Research, 85(1) (2001), 31-36.

Neurotoxins cause biochemical and molecular events which indicate early stage effects in exposed persons well before or well below the induction of overt disease. Monitoring these early events may represent a valid approach to developing markers of neurotoxicity in individuals exposed to environmental chemicals. In neurotoxicology, the use of biochemical markers is more problematic compared to other fields due to the complexity of central nervous system function, the multistage nature of neurotoxic events, and the inaccessibility of target tissue. Nevertheless, new biochemical assays have been developed in recent years to assess exposure, subclinical effects, and susceptibility to neurotoxic disorders. This paper reviews novel biomarkers of neurotoxicity and discusses perspectives and limitations of their use in occupational and environmental medicine.

Lynn H Booth, Vanessa J Heppelthwaite, Ray Webster, Kathryn O'Halloran. (CENTOX (Centre for Environmental Toxicology), Landcare Research, PO Box 69, Lincoln, New Zealand). **Lysosomal neutral red retention time as a biomarker of organophosphate exposure in the earthworm Aporrectodea caliginosa: laboratory and semi-field experiments**. Biomarkers, 6(1) (2001), 77 – 82.

Aporrectodea caliginosa is the most common endogeic (topsoil) earthworm in New Zealand and, because of its habitat, is potentially vulnerable to surface-applied pesticides. Lysosomal damage to earthworms, which can be visualized by the use of the neutral red retention assay (NRRA) has been evaluated in this species as a biomarker of organophosphate exposure. Earthworms were exposed in the laboratory to sub-lethal concentrations of chlorpyrifos and diazinon. In a semi-field experiment, earthworms were placed in mesocosms in a field sprayed with these pesticides at the rate recommended for a vegetable crop. In the laboratory, the neutral red retention time (NRRT) was significantly reduced following exposure to both pesticides compared with controls. In the semi-field experiment, earthworm NRRT was significantly reduced by both pesticides. These experiments have shown that the NRRA is very sensitive to exposure to chlorpyrifos and diazinon even at field rates. It therefore shows promise as a potential biomarker of contamination of soil by organophosphates.

M. S. Maboeta, S. A. Reinecke and A. J. Reinecke. (Department of Zoology, Stellenbosch University, X1, Matieland, 7602, South Africa). **The Relation Between Lysosomal Biomarker and Population Responses in a Field Population of *Microchaetus* sp. (Oligochaeta) Exposedto the Fungicide Copper Oxychloride.** Ecotoxicology and Environmental Safety, 52(3) (2002), 280-287.

The ecological relevance of the neutral red retention assay as a biomarker in an indigenous earthworm population (*Microchaetus* sp.) exposed to the fungicide copper oxychloride was investigated. Changes in earthworm biomass and numbers were monitored and related to changes in neutral red retention times of coelomocytes as well as changes in copper concentrations in the soil and earthworm body tissues. Results indicated that *Microchaetus* sp.

responded sensitively to the copper oxychloride by showing an initial decrease in biomass 2 months after spraying started. This was followed by a significant decrease in worm numbers after 3 months. The neutral red retention times of earthworm coelomocytes decreased significantly within the first month of treatment and correspond to a significant increase in soil copper concentrations, but not with an immediate increase in body burdens of copper. More than a year after spraying had stopped the worm biomass and numbers were still significantly lower in the treated plots compared with the control plots. Since the reduction in neutral red retention times could be attributed to the presence of copper oxychloride and preceded the decline in population density and biomass, we conclude that this biomarker has a useful role to play in environmental risk assessment and could provide a warning of impending ecological damage.
Madeleine Nyman, Magnus Bergknutb, Marie Louise Fanta, Hannu Raunioc, Marika Jestoid, Charlotta Bengsa, Albertinka Murke, Jaana Koistinenf, Christina Bäckmand, Olavi Pelkoneng, Mats Tysklindb, Timo Hirvid, Eero Hellea. (Finnish Game and Fisheries Research Institute, Box 6, 00721, Helsinki, Finland. Department of Chemistry, Umeå University, SE-90187, Umeå, Sweden. Department of Pharmacology and Toxicology, University of Kuopio, Box 1627, 70211, Kuopio, Finland. National Veterinary and Food Research Institute, Box 368, 00231, Helsinki, Finland. Wageningen University, Division of Toxicology, Box 8000, 6400 EA, Wageningen, The, Netherlands. Division of Environmental Health, National Public Health Institute, Box 95, 70701, Kuopio, Finland. Department of Pharmacology and Toxicology, University of Oulu, Box 5000, 90401, Oulu, Finland). **Contaminant exposure and effects in Baltic ringed and grey seals as assessed by biomarkers**. Marine Environmental Research, 55(1) (2003), 59-71.

The Baltic Sea ecosystem has suffered from a heavy pollutant load for more than three decades. Persistent organic pollutants (POPs) and heavy metals have been of most concern due to their persistence and toxic properties. Ringed seals (Phoca hispida baltica) and grey seals (Halichoerus grypus) living in the Baltic Sea have been suffering from pathological impairments, including reproductive disturbances, which have resulted in a depressed reproductive capacity. We investigated several biochemical parameters as potential biomarkers for exposure to and effects of the contaminant load in the Baltic seals. Seals from less polluted areas were used as reference material in terms of the pollution load. In both Baltic seal populations, the levels of some biochemical parameters diverged from those in the reference seals, and some of these showed a clear correlation with the individual contaminant load. Of the potential bioindicators, we propose cytochrome P4501A activity and vitamin E levels, in blubber or plasma, as exposure biomarkers for polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) in both species. The arylhydrocarbon receptor-mediated chemical-activated luciferase gene expression (CALUX) response reflects the whole PCB and DDT burden in ringed seals. Retinyl palmitate (vitamin A) levels showed a negative correlation with the individual POP load, and is proposed as potential effect biomarkers for the depletion of the vitamin A stores. As the nutritional levels of both vitamin A and E have an impact on the vitamin levels in the seals, more information on the dietary vitamin levels is needed before any conclusions can be drawn. As the relationship between biochemical parameters and contaminants varied between the two species, species-specific characteristics has to be considered when monitoring the health status and possible toxic effects of the contaminant load in ringed and grey seals.
Mark Crane, Wanwisu Sildanchandra, Rania Kheir, Amanda Callaghan. (School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 OEX, UK. Division of Zoology, School of Animal and Microbial Sciences, The University of Reading, Whiteknights, P.O. Box 228, Reading, Berkshire RG6 6AJ, UK). **Relationship between biomarker activity and developmental endpoints in *Chironomus riparius* Meigen exposed to an organophosphate insecticide.** Ecotoxicology and Environmental Safety, 53(3) (2002), 361-369.

The biomarkers acetylcholinesterase (AChE) and glutathione *S*-transferase (GST) were measured in fourth-instar *Chironomus riparius* Meigen larvae exposed to the organophosphate insecticide pirimiphos methyl (0, 5, 10, and 50 ng/g) for 48 or 96 h, and at high or low food ration. Larvae exposed to 50 ng/g pirimiphos methyl died within 48 h. The weight of larvae exposed to 10 ng/g pirimiphos methyl was significantly lower than those exposed to 0 and 5 ng/g. AChE activity was significantly reduced in larvae exposed to 10 ng/g, but GST activity remained unaffected. Lower food ration reduced larval weights across all treatments but did not affect biomarker measurements. Insecticide exposure was associated with a longer time to adult emergence and oviposition, fewer egg masses, a greater proportion of deformed egg masses, and fewer eggs.
Mary-Laure Vidal, Anne Bassères, Jean-François Narbonne. (Laboratoire de Physico-Toxicochimie des Systèmes Naturels (LPTC), UPRES-A 5472 CNRS, Département de Toxicologie Biochimique, Université Bordeaux 1, Avenue des Facultés, 33405 Talence, France. ELF, Groupement de Recherches de Lacq, Service Environnement, BP 34, 64170 Lacq, France). **Potential biomarkers of trichloroethylene and toluene exposure in *Corbicula fluminea***. Environmental Toxicology and Pharmacology, 9(3) (2001), 87-97.

Freshwater clams *Corbicula fluminea* were exposed in aquariums to four doses of trichloroethylene-TCE-(1.56 up to 100 mg/1) or toluene-TOL-(7.5 up to 60 mg/1) for 5 days. At the end of exposure, components of (de)toxification metabolism of phases I and II, parameters related to oxidative stress and propionylcholinesterase activity were assayed. Determination of TCE and TOL concentrations in water revealed an important evaporative loss during the experiment, characteristic of acute and occasional contaminations by such products occurring in the environment. Appropriate statistical methods such as ANOVA, Tukey test and discriminant analysis underlined the relevance of cytochromes *P*450 and *P*418, NADH-cytochrome *c* reductase, catalase, peroxided and peroxidizable lipids and net peroxidation as biomarkers of exposure to these solvents in *C. fluminea*. This experiment emphasised the importance of a multi-biomarker approach in environmental surveys and will be completed further by mesocosm studies.
Michael Meger, Irmtrud Meger-Kossien, Kirsten Riedel, Gerhard Scherer. **Biomonitoring of environmental tobacco smoke (ETS)-related exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)**. Biomarkers, 5(1) (2000), 33 – 45.

The exposure of non-smokers to the tobacco-specific N-nitrosamine 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a rodent lung carcinogen, was determined in the air of various indoor environments as well as by biomonitoring of non-smokers exposed to environmental tobacco smoke (ETS) under real-life conditions using the urinary NNK metabolites 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and [4-(N-methylnitrosamino)-1-(3-pyridyl)but-1-yl]-beta-O-D-glucosiduronic acid (NNAL-Gluc). NNK was not detectable (<0.5 ng m^{-3}) in 11 rooms in which smoking did not occur. The mean NNK concentration in 19 rooms in which smoking took place was 17.5 (2.4-50.0) ng m^{-3}. The NNK levels significantly correlated with the nicotine levels (r=0.856; p< 0.0001). Of the 29 non-smokers investigated, 12 exhibited no detectable NNAL and NNAL-Gluc excretion (<3 pmol day) in their urine. The mean urinary excretion of NNAL and NNAL-Gluc of the 17 remaining non-smokers was 20.3 (<3-63.2) and 22.9 (<3-90.0) pmol day^{-1}, respectively. Total NNAL excretion (NNAL+NNAL-Gluc) in all non-smokers investigated significantly correlated with the amount of nicotine on personal samplers worn during the week prior to urine collection (r=0.88; <0.0001) and with the urinary cotinine levels (r=0.40; p=0.038). No correlation was found between NNAL excretion and the reported extent of ETS exposure. Average total NNAL excretion in the non-smokers with detectable NNAL levels was 74 times less than in 20 smokers who were also investigated. The cotinine/total NNAL ratios in urine of smokers (9900) and non-smokers (9300) were

similar. This appears to be at variance with the ratios of the corresponding precursors (nicotine/NNK) in mainstream smoke (16400) and ETS (1000). Possible reasons for this discrepancy are discussed. The possible role of NNK as a lung carcinogen in non-smokers is unclear, especially since NNK exposure in non-smokers is several orders of magnitude lower than the ordinary exposure to exogenous and endogenous N-nitrosamines and the role of NNK as a human lung carcinogen is not fully understood.

Michael Neumann, Joachim Baumeister, Matthias Liess, Ralf Schulz. (Department of Limnology, Zoological Institute, Technical University Braunschweig, Fasanenstrasse 3, D-38092, Braunschweig, Germany. Department of Artificial Intelligence and Applied Computer Science, University of Würzburg, Am Hubland, D-97074, Würzburg, Germany. Department of Chemical Ecotoxicology, UFZ Center for Environmental Research, Permoserstr. 15, D-04318, Leipzig, Germany). **An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators II. The knowledge base of LIMPACT.** Ecological Indicators, 2(4) (2003), 391-401.

The development and the evaluation of a biological indicator system for pesticide pollution in streams are presented. For small headwater streams with an agricultural catchment area, the expert system LIMPACT estimates the pesticide contamination according to the four classes: Not Detected (ND), Low (L), Moderate (M) and High (H) contamination without any specification of the chemical agents. The input parameters are the abundance data of benthic macroinvertebrate taxa within four time frames in a year (March/April, May/June, July/August, September/October) and nine basic water-quality and morphological parameters. The heuristic knowledge base was developed with the shell-kit D3 and contains 921 diagnostic rules with scores either to establish or to de-establish a diagnosis. The 418 rules had less than three symptoms, and only 47 rules had more than four symptoms in their rule condition. We differentiate between positive indicator (PI) taxa, which indicate contamination by high abundance values and positive abundance dynamics, and negative indicator (NI) taxa, a high abundance of which rules out contamination and indicates an uncontaminated site. We analysed 39 taxa and found 13 positive and 24 negative indicators. The database comprises 157 investigations per stream and year with rainfall event-controlled pesticide sampling and repeated benthic sampling as described in Part 1 [Ecol. Indicators, this issue]. For the evaluation of LIMPACT, we used the same cases. The correct class for the 157 investigations per stream and year is established by LIMPACT in 66.7–85.5% of the cases, with better results for uncontaminated sites. The overall alpha error probability (false positive) is 9.6% while the beta error probability (false negative) varied between 0 and 8% depending on the contamination class. If each stream is considered only once in the system (n=104), the correct diagnosis is established by LIMPACT in 51.9–88.6% of the cases. In most of the remaining cases no diagnosis is established instead of a wrong one.

Michel Couderchet, Guy Vernet. (Laboratoire d'Eco-Toxicologie, Unité de Recherches Vigne et Vin de Champagne (URVVC), UPRES EA 2069, Université de Reims Champagne-Ardenne, BP 1039, F-51687, Reims Cedex 02, France). **Pigments as biomarkers of exposure to the vineyard herbicide flazasulfuron in freshwater algae**. Ecotoxicology and Environmental Safety, 55(3) (2003), 271-277.

Weed control in Champagne vineyards has long relied on the use of diuron and substituted triazines; these compounds are now being replaced by flazasulfuron, a sulfonylurea that is used at a much lower dosage. The vineyards of Champagne are planted on steep slopes and runoff is important, and even though low doses of these herbicides are used, they may present some potential risk for freshwater ecosystems. Therefore, the effects of the sulfonylurea herbicide, flazasulfuron (formulated as Katana) was investigated on the unicellular green alga *Scenedesmus*

obliquus. The pigment content of the algal suspensions was followed as a biomarker of exposure to the herbicide. The results demonstrate that flazasulfuron induced a reduction in chlorophyll content at concentrations of 10 μg/L, while the increase of pigment content in the culture was reduced with the lowest concentration tested (0.1 μg/L). Among the three pigments tested, chlorophyll *a* appeared to be the most sensitive biomarker. In the algal medium, flazasulfuron was slowly degraded (DT_{50} approximately 8 days) in a compound that was tentatively identified. The toxicity of this herbicide for the algae was comparable to that of older herbicides which are used at a much higher rate. Therefore, we may speculate that even if flazasulfuron comes into contact with freshwater ecosystems, its effects on algae will be less deleterious than that of traditional herbicides.

Michèle Roméo, Pascal Hoarau, Ginette Garello, Mauricette Gnassia-Barelli, Jean Pierre Girard. (UMR INRA-UNSA 1112, ROSE––Réponse des organismes aux Stress Environnementaux, Faculté des Sciences, BP 71, 06108, Nice Cedex 2, France). **Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean.** Environmental Pollution, 122(3) (2003), 369-378.

Mussels, coming from an aquaculture farm located in a clean open bay, were transplanted to several stations of the bays of Nice and Cannes (NW Mediterranean) including a reference site for one month at three periods. Several biomarkers: activities of glutathione *S*-transferase (GST; exposure to organics), of catalase (exposure to oxidative stress) and of acetylcholinesterase (inhibited by some pesticides) and the lipid peroxidation (thiobarbituric acid reactive substances: TBARS) were measured in transplanted mussels. Cd, Cu and Zn concentrations were also measured as well as their condition index. The results demonstrated some seasonal variations in GST and catalase activities with higher levels in June compared to October. The condition index was also higher in June than in October. Principal component analyses performed with the whole set of data allowed to separate stations or groups of stations according to their responses. The mussels from the harbour of Nice were characterized by high TBARS levels and catalase activity in October 1999 whereas in the harbour of Cannes, animals presented very high copper concentrations and GST activities in June 2000. At the reference site, mussels generally presented low enzymatic activities (except AChE activity) and peroxidation levels and low heavy metal concentrations.

P. Flammarion, A. Devaux, S. Nehls, B. Migeon, P. Noury, J. Garric. (Cemagref, Division Biologie des Ecosystèmes Aquatiques, 3 bis Quai Chauveau CP 220, 69336, Lyon cedex 9, France. INRA-SCRIBE, Equipe d'Ecotoxicologie Aquatique, Campus de Beaulieu, 35042, Rennes cedex, France. ENTPE, Laboratoire des Sciences de l'Environnement, rue Maurice Audin, 69518, Vaulx en Velin cedex, France). **Multibiomarker Responses in Fish from the Moselle River (France)**. Ecotoxicology and Environmental Safety, 51(2) (2002), 145-153.

The response of wild fish to pollutants was studied using two biomakers in chub (*Leuciscus cephalus*) at five stations in the Moselle River (France) in 1998 and in 1999. The induction of cytochrome P450 1A was quantified by the ethoxyresorufin *O*-deethylase (EROD) activity in the liver and the level of DNA single-strand breaks was determined in erythrocytes using the comet assay. EROD activity was observed to be up to 10-fold induced in both males and females from the downstream stations in comparison to the fish from the upstream station. Levels of DNA damage did not parallel EROD induction. Chemical analyses did not clearly explain the responses of the studied biomarkers, confirming the great difficulty in relating chemical and biological information in the field. This study confirms the difficulty in assessing the biological effects of mixtures of pollutants and points out the usefulness of a large array of biomarkers.

Pål A Olsvik, Kjetil Hindar, Karl E Zachariassen, Rolf A Andersen. **Brown trout (*Salmo trutta*) metallothioneins as biomarkers for metal exposure in two Norwegian rivers**. Biomarkers, 6(4) (2001), 274 – 288.

The potential use of the metal binding protein metallothionein (MT) as a biomarker for trace metal exposure has been evaluated in brown trout (Salmo trutta) from the Cucontaminated Rugla and the Cd/Zn-contaminated Naustebekken Norwegian rivers, as well as in hatchery control trout. Metal concentrations were measured in gills, liver and kidney as well as in ambient water, and compared with Cd/Zn MT and Cu MT levels, measured by two Cd-saturation techniques. In addition haematocrit, plasma chloride and condition factors (weight 2 100/length3) were measured, and genetic diversity determined. A negative correlation was found between the Cd/Zn MT content and the condition factor in the different trout populations, and Naustebekken trout, having the lowest condition factor, were less heterozygous than Rugla trout, indicating that Naustebekken trout have adapted to the metal in their environment. Significant positive correlations were found between accumulated levels of Cd and Cd/Zn MT in liver and kidney, suggesting that liver and kidney, but not gill, Cd/Zn MT can be used as a biomarker for chronic Cd exposure. Cu MT seems less suitable as a biomarker for Cu exposure. These data together further emphasize that MTs must be applied with caution, when assessing these proteins in biomonitoring of the natural environment.

Paolo Vineis. (Dipartimento di Scienze Biomediche e Oncologia Umana, via Santena 7, University of Torino, Torino, Italy). **The randomized controlled trial in studies using biomarkers**. Biomarkers, 8(1) (2003), 13 – 32.

The randomized controlled trial (RCT) is a scientific experiment during which observations on the effects of therapy or a preventive action are conducted by the researcher under rigorous control. The purpose of the experiment is to clear the uncertainties surrounding a clinical/research issue and involves isolating the 'treatment' and 'end result' variables from external influences. RCTs therefore make use of scientific method standards: measuring, which includes the possibility of reproducing observations; controlling factors unconnected to the cause-effect relationship of interest; and the external verification or 'falsification' of the cause-effect relationship. Many RCTs are now including biomarkers to answer scientific questions in a more accurate way. In the present methodological paper, the main aspects involved in the design and conduction of a trial are discussed, with special emphasis on the use of biomarkers. Aspects that are often overlooked by scientists involved in the design of trials include multiple comparisons, subgroup analysis, the duration of the observations, the use of surrogate endpoints, and ethical issues. This review summarizes the main issues that should be addressed in a protocol, and illustrates these with an example.

Paule Vasseur, and Carole Cossu-Leguille. (E.B.S.E Faculty of Sciences, rue Delestraint, 57070, Metz, France). **Biomarkers and community indices as complementary tools for environmental safety**. Environment International, 28(8) (2003), 751-759.

Research on biomarkers as early bioindicators of perturbation in populations and individuals has been gaining ground over the last decade. This ecotoxicological approach relies on the fact that changes occur at low levels of organization before the community is affected and thus they can be monitored to assess environmental safety. Changes may concern behavior, physiology, biochemistry, or genomic structure and functioning, and may impair population dynamics in the long-term. Ecotoxicity studies based on biomarkers allow us to measure the impact of environmental stressors and to easily follow the evolution of the systems towards degradation or restoration. Over and above their use as simple indices of exposure to specific pollutants, biomarkers can give an insight into ecosystem health. The results of our experience in field

studies involving ecotoxicologists and ecologists will be presented in order to illustrate the relevance of such an integrating strategy for environmental quality assessment.

Pete Kolsky, David Butler. (Water and Sanitation Program, World Bank, BP 1850 Abidjan 01, Côte d'Ivoire. Urban Water Research Group, Department of Civil and Environmental Engineering, Imperial College, Imperial College Road, SW7 2BU London, UK). **Performance indicators for urban storm drainage in developing countries**. Urban Water, 4(4) (2002), 415-421.

This paper describes conceptual and practical aspects of urban storm drainage performance indicators, based on the authors' experience in developing countries, particularly India. The paper begins by presenting a general framework of objectives and performance indicators as logical intermediate steps between values and the decisions taken to reflect them. The paper then considers practical approaches to performance and indicator measurement, based on field experience in India. General conclusions about drainage performance indicators are then presented, stressing the challenge of finding indicators which are (1) valid indicators of performance, (2) relatively easy to measure, and (3) helpful to the decision-maker.

Pierre Yves Robidoux, Claus Svendsen, Manon Sarrazin, Jalal Hawari, Sonia Thiboutot, Guy Ampleman, Jason M Weeks, Geoffrey I Sunahara. (Applied Ecotoxicology Group, Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec, H4P 2R2, Canada). **Evaluation of tissue and cellular biomarkers to assess 2,4,6-trinitrotoluene (TNT) exposure in earthworms: effects-based assessment in laboratory studies using *Eisenia andrei***. Biomarkers, 7(4) (2002), 306 – 321.

The lysosomal neutral red retention time (NRRT) assay, a biomarker for lysosomal membrane stability, and the total immune activity (TIA) assay, a measure of non-specific immune system activity, were used in laboratory studies to assess the toxic effects of 2,4,6-trinitrotoluene (TNT) on earthworms (*Eisenia andrei*) *in vivo*. The results were compared with the concentration of TNT and its metabolites in earthworm tissue, as well as standard sublethal toxicity endpoints including growth (i.e. weight change) and reproduction effects from previously published studies. Filter paper experiments indicated a significant decrease in NRRT at S1.8 µg TNT cm^{-2}, whereas sublethal (weight loss) and lethal effects to earthworms were detected at S3.5 and 7.1 µg TNT cm^{-2}, respectively. Experiments in artificial soil showed that NRRT effects could be detected at lower TNT concentrations (S55 mg TNT kg^{-1} soil dry weight) compared with other sublethal endpoints (effects on growth and reproduction). The TIA biomarker did not significantly respond to TNT. Copper (as $CuSO_4$, filter paper contact tests) and 2-chloroacetamide (soil tests), which were used as reference toxicants, also decreased the NRRT. The use of the NRRT assay linked with tissue concentrations of TNT metabolites in earthworms was identified as a potentially appropriate biomarker approach for TNT exposure assessment under laboratory conditions and a novel tool for effects-based risk assessment.

R. Figueira, C. Sérgio, A. J. Sousa. (Museu, Laboratório e Jardim Botânico, R. Escola Politécnica 58, 1250-102 Lisboa, Portugal. CVRM, Geo-Systems Centre, IST, Av. Rovisco Pais, 1049-001 Lisboa, Portugal). **Distribution of trace metals in moss biomonitors and assessment of contamination sources in Portugal.** Environmental Pollution, 118(1) (2002), 153-163.

A biomonitoring survey using the moss species [*Hypnum cupressiforme* Hedw. and *Scelopodium touretii* (Brid.) L. Kock] was performed in the whole territory of Portugal, in order to evaluate the atmospheric deposition of the following elements: Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The concentrations of the same elements were also obtained in two types of soil samples, collected

under the moss and in nearby plots without any plant coverage, and relationships between moss and soil concentrations was investigated using the multivariate statistical method of Co-inertia Analysis. Also, relationships between concentrations in moss and several anthropogenic, geologic, pedologic and environmental parameters were screened using the same method of Co-inertia Analysis. Higher concentrations of Cu, Pb and Zn were found in areas of higher population density, with higher gasoline consumption, while higher values of Fe and Cr occur in the driest region, with lower plant coverage, indicating strong contamination by resuspended soil particles. Results also show good agreement between moss and soil contents, even for elements with high contribution of anthropogenic sources. The spatial pattern in Portugal of element contents in mosses were also detected and discussed in relation to local contamination sources.

Roli Budhwar, Vipin Bihari, Neeraj Mathur, Ak Srivastava, Sushil Kumar. (Industrial Toxicology Research Center MG Marg Lucknow). **DNA-protein crosslinks as a biomarker of exposure to solar radiation: a preliminary study in brick-kiln workers**. Biomarkers, 8(2) (2003), 162 – 166.

In India, fired clay bricks are produced in small-scale factories. There are 60t000 active brick kilns, providing employment to nearly 12 million people in different suboccupations. This industry is largely non-mechanized and operates from November to June. Almost all the workers are exposed to direct sunlight for 8-10 h a day. Cellular DNA-protein crosslinks (DPCs) are the biologically active nucleoprotein complexes formed between DNA and proteins. Ultraviolet light and n-rays, and other suspected carcinogens in humans, induce DPC formation in blood cells. DPCs have therefore been identified as a biomarker for monitoring exposure to these hazardous agents. Here we report steady-state levels of DPCs in human peripheral lymphocytes from 46 brick-kiln workers exposed occupationally for 8-10 h a day to solar radiation in brickfields and 25 unexposed controls. A significant increase ($p<0.05$) in DPC content and DPC coefficients in peripheral lymphocytes was observed in the brick-kiln workers compared with the controls. The data suggest that the DPC content of lymphocytes could be a possible biomarker of exposure to solar radiation. However, further work is necessary to confirm this.

Ross V. Hyne, William A. Maher. (A Cooperative Research Centre for Freshwater Ecology and Ecotoxicology Section, Environment Protection Authority, New South Wales, located at the EPA/UTS Centre for Ecotoxicology, Westbourne Street, Gore Hill, New South Wales 2065, Australia. Cooperative Research Centre for Freshwater Ecology & Science and Design, University of Canberra, ACT 2601, Australia). **Invertebrate biomarkers: links to toxicosis that predict population decline**. Ecotoxicology and Environmental Safety, 54(3) (2003), 366-374.

The application of biochemical measurements that can be used as individual biomarkers of impaired biological function in invertebrates is reviewed to evaluate whether biochemical biomarkers of aquatic invertebrates can predict changes in natural populations. Biomarkers that measure toxic effects at the molecular level (e.g., the inhibition of brain acetylcholinesterase activity by organophosphorus pesticides) have been shown to provide rapid quantitative predictions of a toxic effect upon individuals in laboratory studies. Such biomarkers should not be used as a replacement for conventional aquatic monitoring techniques, but should be applied as supplementary approaches for demonstrating links between sublethal biochemical and adverse effects in natural populations in field studies. The research challenge for using biomarker measurements in aquatic invertebrates is to predict effects at the population level from effects at the individual level measured upon individuals collected in the field.

S Marshall Adams. (Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA). **Biomarker/bioindicator response profiles of organisms can help differentiate between sources of anthropogenic stressors in aquatic ecosystems**. Biomarkers, 6(1) (2001), 33 – 44.

Aquatic ecosystems can be chronically stressed by multiple environmental factors which originate from a variety of point and non-point sources. In addition, these stressors may vary both spatially and temporally, and, combined with synergestic and cumulative interactions of these stressors, complicate the interpretation and evaluation of stress responses in organisms. To help identify and differentiate between sources of anthropogenic stressors in aquatic systems, a diagnostic approach based on exposure-response profiles in sentinel organisms was developed from the known effects of various anthropogenic activities on biological systems. To generate these exposure-effects profiles, biomarkers of exposure were plotted against bioindicators of corresponding effects for several major anthropogenic activities including petrochemical, pulp and paper, domestic sewage, mining operations, land-development, and agricultural activities. Biomarkers of exposure to environmental stressors varied widely depending on the type of anthropogenic activity involved. Bioindicator effects, however, including histopathological lesions, bioenergetic status, growth, reproductive impairment, and community-level endpoints were similar among several of the major anthropogenic activities because responses at these higher levels are less specific to stressors than are biomarkers. This approach appears useful for helping to identify and diagnose sources of stress in environments impacted by multiple stressors. By identifying the types and sources of environmental stressors impacting key components of biological systems, aquatic ecosystems can be more effectively protected, regulated, and managed to help improve and maintain environmental quality and ecosystem fitness.

Sandy Kennedy. (Oxford GlycoSciences (UK) Ltd, The Forum, 86 Milton Park, Abingdon, Oxfordshire OX14 4RY, UK; sandy). **The role of proteomics in toxicology: identification of biomarkers of toxicity by protein expression analysis.** Biomarkers, 7(4) (2002), 269 – 290.

Proteomics, i.e. the high throughput separation, display and identification of proteins, has the potential to be a powerful tool in drug development. It could increase the predictability of early drug development and identify non-invasive biomarkers of toxicity or efficacy. This review provides an introduction to modern proteomics, with particular reference to applications in toxicology. A literature search was carried out to identify studies in two broad classes: screening/predictive toxicology, and mechanistic toxicology. The strengths and limitations of current methods and the likely impact of techniques in drug development are also considered. Proteomics can increase the speed and sensitivity of toxicological screening by identifying protein markers of toxicity. Proteomics studies have already provided insights into the mechanisms of action of a wide range of substances, from metals to peroxisome proliferators. Current limitations involving speed of throughput are being overcome by increasing automation and the development of new techniques. The isotope-coded affinity tag (ICAT) method appears particularly promising. The application of proteomics to drug development has given rise to the new field of pharmacoproteomics. New associations between proteins and toxicopathological effects are constantly being identified, and major progress is on the horizon as we move into the post-genomic era.

Sara M. Long, Kelly J. Ryder, Douglas A. Holdway. (Department of Biotechnology and Environmental Biology, RMIT-University, Bundoora Campus, GPO Box 71, Victoria 3083, Australia. NERC Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire, PE28 2LS, UK). **The use of respiratory enzymes as biomarkers of petroleum hydrocarbon exposure in *Mytilus edulis planulatus*.** Ecotoxicology and Environmental Safety, 55(3) (2003), 261-270.

The effect of exposure to petroleum hydrocarbons via the water column and through contaminated sediment upon changes in respiratory enzymes in the common mussel (*Mytilus*

edulis planulatus) was investigated. Mussels were exposed to three concentrations of the water-accommodated fraction (WAF) of Bass Strait crude oil, for 24, 48, and 96 h. In a second study mussels were exposed to three concentrations of crude oil-contaminated sediment for 2 weeks and 1, 2, 4 and 6 months. Activities of citrate synthase (CS) and lactate dehydrogenase (LDH) were measured in the gills. In mussels exposed to WAF, a significant decrease in CS activity was observed over time ($P<0.05$), whereas treatment did not cause a significant change in CS activity ($P>0.05$); neither treatment nor time had an effect on LDH activity. Exposure to contaminated sediment did not have a significant effect on CS activity, however, time had a significant effect on CS activity ($P<0.05$). Both time and treatment had an effect on LDH activity ($P<0.05$). Results demonstrated that changes in gill CS and LDH are not sensitive biomarkers of petroleum hydrocarbon exposure in *M. edulis planulatus*

Scott M. Bartell, Rafael A. Ponce, Ravi N. Sanga and Elaine M. Faustman. (Department of Environmental Health and Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, Washington, 98105-6099). **Human Variability in Mercury Toxicokinetics and Steady State Biomarker Ratios.** Environmental Research, 84(2) (2000), 127-132.

Regulatory guidelines regarding methylmercury exposure depend on dose–response models relating observed mercury concentrations in maternal blood, cord blood, and maternal hair to developmental neurobehavioral endpoints. Generalized estimates of the maternal blood-to-hair, blood-to-intake, or hair-to-intake ratios are necessary for linking exposure to biomarker-based dose–response models. Most assessments have used point estimates for these ratios; however, significant inter-individual and interstudy variability has been reported. For example, a maternal ratio of 250 ppm in hair per mg/L in blood is commonly used in models, but a 1990 WHO review reports mean ratios ranging from 140 to 370 ppm per mg/L. To account for interindividual and interstudy variation in applying these ratios to risk and safety assessment, some researchers have proposed representing the ratios with probability distributions and conducting probabilistic assessments. Such assessments would allow regulators to consider the range and likelihood of mercury exposures in a population, rather than limiting the evaluation to an estimate of the average exposure or a single conservative exposure estimate. However, no consensus exists on the most appropriate distributions for representing these parameters. We discuss published reviews of blood-to-hair and blood-to-intake steady state ratios for mercury and suggest statistical approaches for combining existing datasets to form generalized probability distributions for mercury distribution ratios. Although generalized distributions may not be applicable to all populations, they allow a more informative assessment than point estimates where individual biokinetic information is unavailable. Whereas development and use of these distributions will improve existing exposure and risk models, additional efforts in data generation and model development are required.

Sergei A Kharitonov, Peter J Barnes. **Biomarkers of some pulmonary diseases in exhaled breath**. Biomarkers, 7(1) (2002), 1 – 32.

Analysis of various biomarkers in exhaled breath allows completely non-invasive monitoring of inflammation and oxidative stress in the respiratory tract in inflammatory lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), bronchiectasis and interstitial lung diseases. The technique is simple to perform, may be repeated frequently, and can be applied to children, including neonates, and patients with severe disease in whom more invasive procedures are not possible. Several volatile chemicals can be measured in the breath (nitric oxide, carbon monoxide, ammonia), and many non-volatile molecules (mediators, oxidation and nitration products, proteins) may be measured in exhaled breath condensate. Exhaled breath analysis may be used to quantify inflammation and oxidative stress

in the respiratory tract, in differential diagnosis of airway disease and in the monitoring of therapy. Most progress has been made with exhaled nitric oxide (NO), which is increased in atopic asthma, is correlated with other inflammatory indices and is reduced by treatment with corticosteroids and antileukotrienes, but not (g2-agonists. In contrast, exhaled NO is normal in COPD, reduced in CF and diagnostically low in primary ciliary dyskinesia. Exhaled carbon monoxide (CO) is increased in asthma, COPD and CF. Increased concentrations of 8-isoprostane, hydrogen peroxide, nitrite and 3-nitrotyrosine are found in exhaled breath condensate in inflammatory lung diseases. Furthermore, increased levels of lipid mediators are found in these diseases, with a differential pattern depending on the nature of the disease process. In the future it is likely that smaller and more sensitive analysers will extend the discriminatory value of exhaled breath analysis and that these techniques may be available to diagnose and monitor respiratory diseases in the general practice and home setting.

Shelley S Sehnert , Long Jiang , James F Burdick , Terence H Risby. **Breath biomarkers for detection of human liver diseases: preliminary study**. Biomarkers, 7(2) (2002), 174 – 187.

Chronic liver disease is initially occult, has multiple aetiologies, involves complex diagnostic questions, and requires follow-up because progression is likely. Blood tests and biopsies are generally used, but have disadvantages. We have developed a new test for liver disease based on abnormal concentrations of metabolic products detected in exhaled breath. This test can be used, in conjunction with other clinically accepted diagnostic protocols, to detect and classify chronic liver diseases. Samples of breath collected from spontaneously breathing human subjects (86 patients presenting with 13 liver diseases and 109 subjects with normal liver function) were concentrated cryogenically and analysed by wide-bore capillary gas chromatography using various detectors. The concentrations of various molecules in exhaled breath were examined for potential use as biomarkers of liver function. Subjects with chronic liver diseases could be differentiated from those with normal liver function by comparing levels of breath carbonyl sulphide, carbon disulphide and isoprene; these differences were confirmed and correlated by comparing the levels with standard clinical blood markers of liver damage. The presence of chronic liver failure can thus be detected with sensitivity and specificity by quantifying sulphur-containing compounds arising from the abnormal metabolism associated with liver disease. The breath test we have developed appears to distinguish between hepatocellular and biliary tract aetiologies, and allows staging for severity. This approach may provide the clinician with a simple, non-invasive technique for use in the screening of large populations and follow-up for patients with chronic liver disease.

Shoichi Fujita, Issei Chiba, Mayumi Ishizuka, Hidenobu Hoshi, Hisato Iwata, Akihito Sakakibara, Shinsuke Tanabe, Akio Kazusaka, Makihiko Masuda, Yasushi Masuda, Hajime Nakagawa. (Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University,Sapporo, Japan. Department of Agriculture, Ehime University,Ehime, Japan. Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University,Sapporo, Japan. Shiretoko Museum, Shari, Japan). **P450 in wild animals as a biomarker of environmental impact**. Biomarkers, 6(1) (2001), 19 – 25.

The impact of environmental pollution on selected animals was tested by monitoring the hepatic content of cytochromes P450 and their enzyme activities or by calculating TEQ values from the concentration of pollutants in the body. Fish-eating Stellars Sea Eagles, Haliaeetus pelagicus, found dead in the northern part of Hokkaido island accumulated high levels of PCBs and DDT and metabolites. The TEQ values calculated from the PCB concentration in the eagles were high enough to cause a significant toxic effect in other birds living in the same environment. Some of

these birds were also contaminated with high concentrations of lead. Spotted seals, Phoca largha, captured along the coast-line of Hokkaido accumulated PCBs in their fat at about 100 million times the concentrations in the surface sea water. The levels of expressions of hepatic microsomal CYP 1A1and related enzyme activities in these seals showed good correlation to the levels of PCBs accumulated in the fat. The fresh water crabs, Eriocheir japonicus, were captured from three different rivers with various degrees of pollution. The P450 content and the related enzyme activities showed good correlation to TEQ values obtained from the concentrations of PCBs and PCDDs in the crabs from the rivers. The wild rodents, Clethrionomys rufocanus, were captured from urban, agricultural, and forest areas in Hokkaido. Those from the forest area had the lowest CYP content and related enzyme activities, comparable to those in laboratory-raised animals. Those from the urban areas, presumably contaminated with PAHs from fuel combustion, showed increased CYP 1A1 content and related enzyme activities. Those from the agricultural areas showed increased levels of CYP 1A1, 2B, 2E1. Rats treated with some of the agrochemicals used in the area resulted in a similar pattern of induction. It is concluded that P450 can be a useful biomarker for assessing the environmental impact of chemical pollutants on wild animals.

Silvano Monarca, Donatella Feretti, Ilaria Zerbini, Adriana Alberti, Claudia Zani, Sergio Resola, Umberto Gelatti, Giuseppe Nardi. (Department of Experimental and Applied Medicine, Hygiene Section, University of Brescia, I-25123, Brescia, Italy. Local Health Unit of Brescia, Brescia, Italy). **Soil Contamination Detected Using Bacterial and Plant Mutagenicity Tests and Chemical Analyses**. Environmental Research, 88(1) (2002), 64-69.

Soil contaminants are common in industrialized countries, causing widespread contamination directly of soil and indirectly of ground water and food. Among these pollutants particular attention should be paid to soil mutagens and carcinogens due to their potentially hazardous effects on animal populations and human health. The aim of this research was to evaluate the genotoxicity of contaminated soils by means of an integrated chemical/biological approach, using a short-term bacterial mutagenicity test (Ames test), a plant genotoxicity test (Tradescantia/micronucleus test), and chemical analyses. Soil samples were collected in a highly industrialized area in the Lombardy region, in Northern Italy. Soil samples were extracted with water or with organic solvents. Water extracts of soil samples were tested using the Tradescantia genotoxicity test and organic solvent extracts were analyzed for their polycyclic aromatic hydrocarbon (PAH) concentrations and for their mutagenicity with the Ames test. Heavy metal concentrations were also studied. Some soil samples showed mutagenic activity with the Ames test and clastogenicity with the Tradescantia/micronucleus test. The same soils showed high concentrations of genotoxic PAH and heavy metals.

Susanne P. Baden, Douglas M. Neilb. (Department of Marine Ecology, Göteborg University, Kristineberg Marine Research Station, 450 34, Fiskebäckskil, Sweden. Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK). **Manganese accumulation by the antennule of the Norway lobster Nephrops norvegicus (L.) as a biomarker of hypoxic events**. Marine Environmental Research, 55(1) (2003), 27-38.

In laboratory tests, manganese accumulation by the appendages of the sediment burrowing Norway lobster, Nephrops norvegicus (L.) (including the lateral antennules) was approximately three times greater [600 g Mn g-1 (dry weight) after 5 days in 20 mg Mn l-1] than that by the carapace. The accumulation was linearly dose-dependent (10–40 mg Mn l-1) and duration-dependent (2–30 days), and showed no decrease after 3 weeks in undosed seawater. A high manganese uptake to the lateral antennules during hypoxia in the field was verified from the SE

Kattegat, Sweden. These results indicate that accumulation of Mn on the mobile appendages of the Norway lobster fulfils most of the criteria for a biomarker of exposure to hypoxia. Using these measurements in conjunction with Mn concentrations in the internal tissues, it may be possible to resolve both the timing and the extent of the Mn exposure and the underlying hypoxic event.

Tamara Do, Angela Gambelunghe, Habibul Ahsan, Joseph Graziano, Mary Perrin, Vesna Slavkovich, Faruque Parvez, Abul Hasnat Milton, Paul Brandt-Rauf. (The Mailman School of Public Health of Columbia University, New York, NY 10032, USA. NGO Forum, Dhaka, Bangladesh. The Mailman School of Public Health of Columbia University, New York, NY 10032, USA). **Urinary transforming growth factor-alpha in individuals exposed to arsenic in drinking water in Bangladesh**. Biomarkers, 6(2) (2001), 127 – 132.

Recent evidence suggests that the development of skin lesions from arsenic exposure may be mediated by increases in the expression of various growth factors, including transforming growth factor-alpha (TGFf). To investigate this association in humans, levels of total urinary arsenic and urinary TGFf were determined in 41 individuals with and without arsenic-associated skin lesions from Bangladesh who have chronic exposure to arsenic in their drinking water. After adjusting for age and sex, total urinary arsenic was found to be correlated with urinary TGFf ($R 2 = 0.37$; $p < 0.0001$), particularly in those individuals with arsenic-associated skin lesions ($R 2 = 0.70$; $p < 0.0001$). Stratification of the cohort into quartiles based on urinary TGFf levels demonstrated a trend of increasing odds ratios for the presence of arsenic-associated skin lesions with increasing urinary TGFf, although this was not significant ($p = 0.15$). These results suggest that urinary TGFf may be a useful biomarker for the epidermal effects of arsenic exposure.

Tom Kosatsky, Jean-Philippe Weber. (Montreal Public Health Department, 1301 Sherbrooke East, Montreal, Québec, Canada, H2L1M3. Departments of Epidemiology and Biostatistics and Occupational Health, McGill University, Montreal, Québec, Canada. Direction de la toxicologie humaine, Institut national de santé publique du Québec, 2705 Boulevard Laurier, Sainte-Foy, Québec, Canada, G1V 4G2). **Using biomarkers to characterise contaminant exposure among eaters of Great Lakes and St. Lawrence River fish**. Environmental Toxicology and Pharmacology, 12(2) (2002), 69-74.

Analyses of xenobiotics in human tissues are used to screen individuals whose habits or environments put them at high risk of overexposure, for the surveillance of exposure patterns over time and between places, and as part of research to identify the sources and pathways of exposure in a population. Both study goals and technical considerations should guide the choice of which xenobiotics to sample and in which tissues. Understanding the absorption and distribution of the specific compound to be measured is essential to the choice of when to sample and how to interpret results. Laboratory quality is of major concern. A particular issue in the statistical treatment of results are those near to the laboratory's detection limit. Theoretical concepts are discussed in the context of studies of xenobiotic exposure among eaters of Great Lakes and St. Lawrence River sport fish.

W. M. De Coen, C. R. Janssen, H. Segner. (Laboratory for Biological Research in Aquatic Pollution, University of Ghent, J. Plateaustraat 22, B-9000, Ghent, Belgium. Department of Chemical Ecotoxicology, UFZ-Centre for Environmental Research, Permoserstrasse 15, D-04318, Leipzig, Germany). **The Use of Biomarkers in *Daphnia magna* Toxicity Testing V. *In Vivo* Alterations in the Carbohydrate Metabolism of *Daphnia magna* Exposed to Sublethal Concentrations of Mercury and Lindane**. Ecotoxicology and Environmental Safety, 48(3) (2001), 223-234.

Aspects of the carbohydrate metabolism of *Daphnia magna* exposed for 48 and 96 h to sublethal concentrations of mercury and lindane were investigated. General as well as toxicant-specific perturbations in the intermediary metabolism were observed. Both model toxicants caused an

increase in glycolytic and hexose-monophosphate shunt activity. Mercury exposure increased lactate dehydrogenase and isocitrate activity (only after 96 h), while lindane exposure, on the contrary, inhibited the cellular lactate formation and increased the Krebs' cycle activity (only after 48 h). Daphnids exposed to sublethal mercury concentrations clearly exhibited increased glycogenolytic activity, while in lindane-exposed organisms mainly glycogen phosphorylase inhibition was detected. The short-term enzyme-based effect levels (48–96 h LOEC and EC_{10} values) were compared with the effects on the population dynamics. This evaluation for both model toxicants suggests that threshold levels (LOEC or EC_{10} values) based on pyruvate kinase activity after 48 and 96 h of exposure could be potential early warning signals for long-term effects. A set of enzymatic endpoints, based on the intermediary metabolism, is suggested to characterize the metabolic state of the daphnids.

Yohan Mourgaud, Éric Martinez, Alain Geffard, Bruno Andral, Jean-Yves Stanisiere, Jean-Claude Amiard. (Service d'Écotoxicologie, CNRS GDR 1117, ISOMer, SMAB, 2 Rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France. Laboratoire de Toulon, IFREMER DEL/TL, Zone portuaire de Brégaillon, BP 303, 83507 La Seyne-sur-Mer, France). **Metallothionein concentration in the mussel *Mytilus galloprovincialis* as a biomarker of response to metal contamination: validation in the field**. Biomarkers, 7(6) (2002), 479 – 490.

Mussels were translocated from a shell-fish breeding area (Sète, on the French Mediterranean coast) to sites exposed to trace element inputs in April 2000. They were recovered 3 months later. Whole soft tissues from all of the sites (n = 97) were analysed for arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc. Metallothioneins (MTs) were also measured in the digestive gland and in the remaining tissues (allowing calculation of whole soft tissue concentrations) at 22 of the 97 sites. MT concentrations in the digestive gland and the whole soft tissues were strongly correlated. The condition index varied with food availability at different sites. This did not influenced MT concentrations in the whole soft tissues, whereas the condition index was negatively correlated to trace element concentrations. A model is proposed to minimize this influence of condition. Metal concentrations adjusted using this model showed significant correlations with MT levels for those metals (cadmium, copper, nickel and zinc) that are known to bind to this protein, with the exception of mercury. Even in moderately contaminated sites, measurement of the MT level in the soft tissues of mussels was generally able to discriminate between different levels of contamination, allowing the use of a simplified procedure compared with dissection of the digestive gland. It is recommended to avoid translocation and sampling during the reproductive period, which is well documented for commercial species such as *Mytilus* sp.

Zhou Qun-fang, Li Zhong-yang, Jiang Gui-bin, Yang Rui-qiang. (Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PO Box 2871, Beijing, 100085, China). **Preliminary investigation of a sensitive biomarker of organotin pollution in Chinese coastal aquatic environment and marine organisms.** Environmental Pollution, 125(3) (2003), 301-304.

In nine batches of sea bivalves collected from Chinese coastal cities during the year of 2000 to 2002, a special sample named *Mya arenaria* was found to have strong ability of butyltin accumulation compared with the other sampled bivalves in the corresponding batches. Tributyltin compound was the predominant pollutant with the detection rate high up to 100%. Special high levels of μg Sn/g were detected in some *Mya arenaria* samples. The results obtained showed that *Mya arenaria* was potentially a biomarker to indicate organotin pollution in coastal aquatic environment.

Biopesticide

Badal Bhattacharya, Santosh Kumar Sarkar, Nilanjana Mukherjee. (Department of Metallurgical Engineering, Jadavpur University, Calcutta 700032, India. Department of Marine Science, University of Calcutta, Calcutta 700019, India). **Organochlorine pesticide residues in sediments of a tropical mangrove estuary, India: implications for monitoring**. Environment International, 29(5) (2003), 587-592.

The paper examines the concentrations of isomers of hexachlorocyclohexane (HCHs), dichlorodiphenyl trichloroethane and its metabolites (DDTs), -endosulfan and endosulfan sulfate in surface sediment samples collected from the mouth of Hugli estuary in the vicinity of Sundarban mangrove environment, eastern part of India. An overall pattern of accumulation of these pesticides was in the order of: HCH>endosulfan sulfate>DDT>-endosulfan. The concentration of these compounds was quite low. An elevated level of HCH, DDT and endosulfan sulfate were marked during premonsoon months, a period characterized by high salinity and pH values. Among the isomers and metabolites of HCH and DDT, HCH, pp'-DDT and pp'-DDE were found to be dominant. The sources of contamination are closely related to human activities, such as domestic and industrial discharges, agricultural chemical applications and soil erosion due to deforestation. The study is compared to other estuarine environment in India and abroad. The present data will serve as a baseline against which future anthropogenic effects may be assessed.

Jörg Degenhardt, Jonathan Gershenzon, Ian T Baldwin, André Kessler. (Department of Biochemistry, Max Planck Institute for Chemical Ecology, Beutenberg Campus, Winzerlaer Strasse 10, D-07745, Jena, Germany. Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Beutenberg Campus, Winzerlaer Strasse 10, D-07745, Jena, Germany). **Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies.** Current Opinion in Biotechnology, 14(2) (2003), 169-176.

When attacked by herbivorous insects or mites, some plant species call on other arthropods for help. They emit mixtures of volatile compounds, dominated by terpenes, to attract carnivorous arthropods that prey on or parasitise herbivores and so reduce further damage. This fascinating defence strategy offers a new, environmentally friendly approach to crop protection. Using recent advances in the biochemistry and molecular genetics of terpene biosynthesis, it should now be possible to engineer crop plants that release terpenes for attracting herbivore enemies. By introducing or selectively altering the existing rate of terpene emission and composition, plant breeders could enable attacked plants to attract enemies and reduce additional herbivory, without compromising the effectiveness of other modes of defence.

Matthias P. Lutz, Georg Feichtinger, Geneviève Défago, Brion Duffy. (Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Swiss Federal Research Station for Fruit Production, Viticulture and Horticulture, CH-8820 Wädenswil, Switzerland). **Mycotoxigenic *Fusarium* and Deoxynivalenol Production Repress Chitinase Gene Expression in the Biocontrol Agent *Trichoderma atroviride* P1**. Applied and Environmental Microbiology, 69(6) (2003), 3077-3084.

Mycotoxin contamination associated with head blight of wheat and other grains caused by *Fusarium culmorum* and *F. graminearum* is a chronic threat to crop, human, and animal health throughout the world. One of the most important toxins in terms of human exposure is deoxynivalenol (DON) (formerly called vomitoxin), an inhibitor of protein synthesis with a broad spectrum of toxigenicity against animals. Certain *Fusarium* toxins have additional antimicrobial activity, and the phytotoxin fusaric acid has recently been shown to modulate fungus-bacterium interactions that affect plant health (Duffy and Défago, Phytopathology

87**:**1250-1257, 1997). The potential impact of DON on *Fusarium* competition with other microorganisms has not been described previously. Any competitive advantage conferred by DON would complicate efforts to control *Fusarium* during its saprophytic growth on crop residues that are left after harvest and constitute the primary inoculum reservoir for outbreaks in subsequent plantings. We examined the effect of the DON mycotoxin on ecological interactions between pathogenic *Fusarium* and *Trichoderma atroviride* strain P1, a competitor fungus with biocontrol activity against a wide range of plant diseases. Expression of the *Trichoderma* chitinase genes, *ech42* and *nag1*, which contribute to biocontrol activity, was monitored in vitro and on crop residues of two maize cultivars by using *goxA* reporter gene fusions. We found that DON-producing *F. culmorum* and *F. graminearum* strains repressed expression of *nag1-gox*. DON-negative wild-type *Fusarium* strains and a DON-negative mutant with an insertional disruption in the tricothecene biosynthetic gene, *tri5*, had no effect on antagonist gene expression. The role of DON as the principal repressor above other pathogen factors was confirmed. Exposure of *Trichoderma* to synthetic DON or to a non-DON-producing *Fusarium* mutant resulted in the same level of *nag1-gox* repression as the level observed with DON-producing *Fusarium*. DON repression was specific for *nag1-gox* and had no effect, either positive or negative, on expression of another key chitinase gene, *ech42*. This is the first demonstration that a target pathogen down-regulates genes in a fungal biocontrol agent, and our results provide evidence that mycotoxins have a novel ecological function as factors in *Fusarium* competitiveness.

Sarah A. Keim and Michael C. R. Alavanja. (Occupational Epidemiology Branch, National Cancer Institute, Bethesda, Maryland, 20892). **Pesticide Use by Persons Who Reported a High Pesticide Exposure Event in the Agricultural Health Study.** Environmental Research, 85(3) (2001), 256-259.

Almost 16% of the pesticide applicators in the Agricultural Health Study (AHS) cohort (a cohort that includes 52,629 private applicators) reported having a high pesticide exposure event (i.e., an incident or experience while using a pesticide that caused an unusually high personal exposure). Pesticides involved in these events were compared to the frequency with which specific pesticides were ever used by the AHS cohort. Generally, pesticides with greater acute toxicity were more frequently involved with the high pesticide exposure event than were other pesticides. Whereas it is clear that the use of acutely toxic pesticides may be related to more frequent visits to health care facilities, the reason that the spills and immersions of the high pesticide exposure events are associated with the acute toxicity of the pesticide is not intuitively clear. This analysis suggests that current practices directed at minimizing pesticide exposures may not be sufficient for acutely toxic or irritating chemicals.

Siegfried Keller, Philip Kessler, Christian Schweizer. (Swiss Federal Research Station for Agroecology and Agriculture, Reckenholzstrasse 191, CH-8046 Zurich, Switzerland). **Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metharhizium anisopliae*.** BioControl, 48(3) (2003), 307-319.

Between 1998 and 2000 soil samples from 82 fields in north, east, central and south west of Switzerland were analysed for presence of insect pathogenic soil fungi using a selective medium and the *Galleria* bait method. The detection rates with either method were very similar. 96% of the fields distributed over all examined regions contained *Metarhizium anisopliae*. The presence of *Beauveria brongniartii* was limited to sites colonised by its host, *Melolontha melolontha*. It was also present at a site where *M. melolontha* disappeared about 40 years ago. On the other hand, *B. brongniartii* was not found in four fields containing *M. melolontha* populations. *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Conidiobolus* sp. were other

entomopathogenic fungi isolated during this survey. Differences in the presence of *M. anisopliae* between arable fields and adjacent meadows as well as between orchards and meadows are discussed.

Sushil K. Shahi, Mamta Patra, A.C. Shukla, Anupam Dikshit. (Biological Product Laboratory, Botany Department, University of Allahabad, Allahabad -211002, India). **Use of essential oil as botanical-pesticide against post harvest spoilage in *Malus pumilo* fruit**. BioControl, 48(2) (2003), 223-232.

During antifungal screening of the essential oils of some angiospermic plants, oil of *Cymbopogon flexuosus* showed potent bioactivity against dominant post harvest fungal pathogens. The minimum bioactive concentrations with fungicidal action of the oil was found to be 0.2 µl ml-1 for *Alternaria alternata*, 0.4 µl ml-1 for *Aspergillus flavus, A. fumigatus, A. niger, A. parasiticus, Cladosporium cladosporioides, Colletotrichum capsici, C. falcatum, Curvularia lunata, Fusarium cerealis, F. culmorum, F. oxysporum, F. udum, Gloeosporium fructigenum, Penicillium expansum, P. italicum, P. implicatum, P. digitatum, P. minio-luteum, P. variable*, and 0.5 µl ml-1 for *Botrytis cinerea, Helminthosporium oryzae, H. maydis, Phoma violacea, Rhizopus nigricans*. The oil exhibited potency against heavy doses (30 mycelial disc, each of 5 mm in diameter) of inoculum at 1.0 µl ml-1 concentrations. The bioactivity of the oil was thermostable up to 100°C and lasted up to 48 months. The oil preparation did not exhibit any phytotoxic effect on the fruit skins of *Malus pumilo* up to 50 µl ml-1 concentrations. In vivo trials of the oil as a fungicidal spray on Malus pumilo for checking the rotting of fruits, it showed that 20 µl ml-1 concentration controls 100% infection by pre-inoculation treatment, while in post-inoculation treatment, 30 µl ml-1 concentration of fungicidal spray was required for the 100% control of rotting. The fungicidal spray was found to be cost effective (INR 15/l), has long shelf life (48 month) and was devoid of any adverse effects. Therefore, it can be used as a potential source of sustainable eco-friendly botanical pesticide, after successful completion of wide range trials.

Young Ah Kim, Hye Sung Lee, Yong Chae Park, Yong Tae Lee. (Department of Food Science and Nutrition, Kyungpook National University, Taegu, 702-701, Republic of Korea. Department of Biochemistry, Yeungnam University, Kyongsan, 712-749, Republic of Korea). **A Convenient Method for Oxidation of Organophosphorus Pesticides in Organic Solvents.** Environmental Research, 84(3) (2000), 303-309.

Since organophosphorus pesticides can be oxidized to oxons *in vivo* and in the environment and their determination based on inhibition of cholinesterases can be more sensitive after their oxidation to oxons, development of an efficient method for their *in vitro* oxidation is important for their toxicological and analytical studies. This study demonstrated that treatment of organophosphorus pesticides with 10 molar excess bromine in acetonitrile is a rapid and efficient method for their oxidation. For the nine organophosphorus pesticides tested, the reaction was complete within a few seconds. All reactions gave the respective oxons as single major product, except that of fenthion, which gave two major products, the respective oxon and another product from further oxidation of the oxon. The yields of the oxons were 82–100%. The inhibitory power of the pesticides on acetylcholinesterase before and after oxidation was measured and, for all pesticides tested, the power after oxidation was much higher than that before oxidation. Inhibition calibration curves for both unoxidized and oxidized forms of fenitrothion and parathion were obtained. The sensitivity of the detection of these pesticides was much higher after oxidation.

Bioremediation

Anna Muratova, Thorsten Hübner, Neeru Narula, Helmut Wand, Olga Turkovskaya, Peter Kuschk, Richard Jahn, Wolfgang Merbach. **Rhizosphere microflora of plants used for**

the phytoremediation of bitumen-contaminated soil. Microbiological Research, 158(2), 151-161.

The microbial communities and their degradative potential in rhizospheres of alfalfa (Medicago sativa) and reed (Phragmites australis) and in unplanted soil in response to bitumen contamination of soil were studied in pot experiments. According to the results of fluorescence microscopy, over a period of 27 months, bitumen contamination of soil reduced the total number of microorganisms more significantly (by 75%) in unplanted than in rhizosphere soil (by 42% and 7% for reed and alfalfa, respectively) and had various effects on some important physiological groups of microorganisms such as actinomycetes as well as nitrogen-fixing, nitrifying, denitrifying, ammonifying, phosphate-solubilizing, sulphur-oxidizing, cellulolytic and hydrocarbon-degrading microorganisms. The changes in the physiological structure of the microbial community under bitumen contamination were found to hinge on not merely the presence of plants but also their type. It was noted that the rhizosphere microflora of alfalfa was less inhibited by hydrocarbon pollution and had a higher degradative potential than the rhizosphere microflora of reed.

Bharat Bhushan, Sandra Trott, Jim C. Spain, Annamaria Halasz, Louise Paquet, Jalal Hawari. (Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada,1 U.S. Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403). **Biotransformation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) by a Rabbit Liver Cytochrome P450: Insight into the Mechanism of RDX Biodegradation by Rhodococcus sp. Strain DN_{22}**. Applied and Environmental Microbiology, 69(3) (2003), 1347-1351.

A unique metabolite with a molecular mass of 119 Da (C2H5N3O3) accumulated during biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Rhodococcus sp. strain DN22 (D. Fournier, A. Halasz, J. C. Spain, P. Fiurasek, and J. Hawari, Appl. Environ. Microbiol. 68:166-172, 2002). The structure of the molecule and the reactions that led to its synthesis were not known. In the present study, we produced and purified the unknown metabolite by biotransformation of RDX with Rhodococcus sp. strain DN22 and identified the molecule as 4-nitro-2,4-diazabutanal using nuclear magnetic resonance and elemental analyses. Furthermore, we tested the hypothesis that a cytochrome P450 enzyme was responsible for RDX biotransformation by strain DN22. A cytochrome P450 2B4 from rabbit liver catalyzed a very similar biotransformation of RDX to 4-nitro-2,4-diazabutanal. Both the cytochrome P450 2B4 and intact cells of Rhodococcus sp. strain DN22 catalyzed the release of two nitrite ions from each reacted RDX molecule. A comparative study of cytochrome P450 2B4 and Rhodococcus sp. strain DN22 revealed substantial similarities in the product distribution and inhibition by cytochrome P450 inhibitors. The experimental evidence led us to propose that cytochrome P450 2B4 can catalyze two single electron transfers to RDX, thereby causing double denitration, which leads to spontaneous hydrolytic ring cleavage and decomposition to produce 4-nitro-2,4-diazabutanal. Our results provide strong evidence that a cytochrome P450 enzyme is the key enzyme responsible for RDX biotransformation by Rhodococcus sp. strain DN22.

Bharati J Bhadbhade, Seema S Sarnaik, Pradnya P Kanekar. (Microbial Sciences Division, Agharkar Research Institute, G. G. Agarkar Road, Pune 411 004, Maharashtra, India). **Bioremediation of an Industrial Effluent Containing Monocrotophos.** Current Microbiology, 45(5) (2002), 346 – 349.

Almost 30% of the precious agricultural output of India is lost owing to pest infestation. In India, pesticide consumption for protecting crops is about 3% of the total world consumption. Monocrotophos (MCP), an organophosphorus insecticide, is widely used to control insects on crops. Being readily water soluble and highly toxic, its removal from wastewater generated

during manufacture becomes inevitable. Bioremediation of wastewater containing MCP by Arthrobacter atrocyaneus, Bacillus megaterium, and Pseudomonas mendocina was highest at pH 8.0, but maximum reduction in Chemical Oxygen Demand (COD) was at pH 7.0. Removal of MCP and reduction in COD by B. megaterium and Ps. mendocina were highest at 35°C, while with A. atrocyaneus, it was maximum at 30°C, under aerated culture condition and inoculum density of 10^8 cells/ml. Use of pure cultures for bioremediation of effluent containing MCP appears to be the first such attempt.
C. Lobo, M. Sanchez, C. Garbi, E. Ferrer, M. J. Martinez-Iñigo, J. L. Allende, C. Martín, L. Casasús, R. Alonso-Sanz, A. Gibello, M. Martin. (Inst. Madrileño de Investigacion Agraria y Alimentaria, Apt. 127, 28800 Madrid, Spain. Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spai. ETSIA y ETSII, Universidad Politécnica, 28040 Madrid, Spain. Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain). **Three Different Topics for Bioremediation Application on Soils and Waters Contaminated with Aromatic Compounds, by Using Immobilized Bacteria.** Water, Air and Soil Pollution: Focus, 3(3) (2003), 35-46.

Molecular techniques and modelling are presented as powerful tools required in the performance of efficient soil and water bioremediation systems. An *Escherichia coli* CC118-D strain was constructed by inserting the *Klebsiella pneumoniae hpa* B gene, coding for the unstable 3,4-dihydroxyphenylacetate 2,3-dioxygenase, into its chromosome. When the constructed strain was immobilized, both enzyme stability and viability increased along the studied period, in absence of antibiotic. We proposed this strategy as an approach to overcoming plasmid instability and to enhance enzyme activity and stability, avoiding antibiotic utilization. A model was developed to understand and predict the behaviour of bacteria and pollutants in a bioreactor system, considering: fluid dynamics, molecular/cellular scale processes and biofilm formation.
C. Wittmann, K. P. Suominen, M. S. Salkinoja-Salonen. (Department of Applied Chemistry and Microbiology, Biocenter, POB 56 Helsinki University, FIN 00014, Finland). **Evaluation of ecological disturbance and intrinsic bioremediation potential of pulp mill-contaminated lake sediment using key enzymes as probes.** Environmental Pollution, 107(2) (2000), 255-261.

A rapid protocol was developed to measure 10 different enzymic activities from a large number of 1-cm-sliced freshly collected lake sediments. Layers heavily polluted by organic halogens (4900 mg Cl kg^{-1}) revealed severe depression of phosphatase, sulfatase, leucine-aminopeptidase, chitinase, acetate esterase and butyrate esterase activities as compared to layers above and below the most polluted zone. α-Glucosidase, β-glucosidase, β-xylosidase and palmitate esterase were less affected. Methane oxidation potential was dramatically depressed in the polluted strata whereas tetrachloromethane dehalogenating activity was observed in the polluted sediment only. The sediment layers formed after the chlorine discharges into the lake had diminished to 1/10, and showed restoration of the activities close to those observed in non-recipient sediment, in spite of the persisting presence of >1000 mg of organic chlorine (kg dry wt)$^{-1}$. We conclude that certain enzymic activities involved in breakdown or oxidation of organic matter in the sediments are useful probes for assessing the degree of ecological damage and its potential for restoration in recipient lakes of industrial discharges.
Carsten Vogt, Albin Alfreider, Helmut Lorbeer, Joerg Ahlheim, Bernd Feist, Olaf Boehme, Holger Weiss, Wolfgang Babel, Lothar Wuensche. (UFZ Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany. University of Technology Dresden, Institute of Waste Management and Contaminated Site Treatment, Pirna, Germany. GFE GmbH Halle, Halle/Saale, Germany). **Two Pilot Plant Reactors Designed for the *In Situ* Bioremediation of Chlorobenzene-contaminated Ground Water:**

Hydrogeological and Chemical Characteristics and Bacterial Consortia. Water, Air and Soil Pollution: Focus, 2(3) (2002), 161-170.

The SAFIRA *in situ* pilot plant in Bitterfeld, Saxonia-Anhalt, Germany, currently serves as the test site for eight different *in situ* approaches to remediate anoxic chlorobenzene (CB)-contaminated ground water. Two reactors, both filled with original lignite-containing aquifer material, are designed for the microbiological *in situ* remediation of the ground water by the indigenous microbial consortia. In this study, the hydrogeological, chemical and microbiological conditions of the inflowing ground water and reactor filling material are presented, in order to establish the scientific basis for the start of the bioremediation process itself. The reactors were put into operation in June 1999. In the following, inflow CB concentrations in the ground water varied between 22 and 33 mg L-1; a chemical *steady state* for CB in both reactors was reached after 210 till 260 days operation time. The sediments were colonized by high numbers of aerobic, iron-reducing and denitrifying bacteria, as determined after 244 and 285 days of operation time. Furthermore, aerobic CB-degrading bacteria were detected in all reactor zones. Comparative sequence analysis of 16S rDNA gene clone libraries suggest the dominance of *Proteobacteria* (*Comamonadaceae*, *Alcaligenaceae*, *Gallionella} group*, *Acidithiobacillus*) and members of the class of low G+C gram-positive bacteria in the reactor sediments. In the inflowing ground water, sequences with phylogenetic affiliation to sulfate-reducing bacteria and sequences not affiliated with the known phyla of Bacteria, were found.

Chen-Ko Kwok, Kai-Chee Loh. (Department of Chemical and Environmental Engineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore). **Effects of Singapore soil type on bioavailability of nutrients in soil bioremediation**, Advances in Environmental Research, 7(4) (2003), 881-887.

The biodegradation of phenanthrene by Pseudomonas putida (ATCC 17484) was studied in six different synthetic soils representing the chemical compositions of six Singapore soil types. The nutrients used were ammonium nitrate to provide nitrogen, potassium dihydrogen phosphate (KH2PO4) and phenyl phosphate (PP) disodium salt to provide phosphorus. Sorption isotherms of the nutrients in each soil type were determined. Bioavailability of nutrients is measured by the ratio of the rate of biodegradation in the presence of nutrients to that in the absence of nutrients. The bioavailability was found to correspond closely to the KH2PO4 sorption capacity of a soil. Increasing KH2PO4 increased the biodegradation rate of phenanthrene across all soil types, indicating that phosphorus was limiting. The organic phosphorus compound, PP, has greater sorption ability than KH2PO4. It was tested as a substitute for KH2PO4 in the biodegradation of phenanthrene and was found to increase the bioavailability of phosphorus. It is proposed that bacteria attach themselves on the external surfaces of soil particles and utilize the nutrients sorbed there. The bioavailability of phosphorus from both KH2PO4 and PP was well correlated with the effective concentration of phosphorus—defined by the product of the equilibrium concentration of phosphorus sorbed in a soil and the fraction of external surface area over total surface area of soil particles. The Monod model fits the above correlation reasonably well. This study can be applied to the bioremediation of local soils in determining the amount of nutrients to use.

Dal-Heui Lee, Robert D. Cody, Dong-Ju Kim, Sangil Choi. (Department of Earth and Environmental Sciences, Korea University, Anam-dong, Sungbuk-ku, Seoul 136-701, South Korea. Department of Geological and Atmospheric Sciences, 253 Science I, Iowa State University, Ames, IA 50011, USA. Department of Environmental Engineering, Kwangwoon University, Wolgye-dong, Nowon-ku, Seoul 39-701, South Korea). **Effect of soil texture on surfactant-based remediation of hydrophobic organic-contaminated soil**. Environment International, 27(8) (2002), 681-688.

Surfactants may be used in remediation of subsoil and aquifer contaminated with hydrophobic compounds. The objectives of this study were to examine the effect of soil texture on hydrophobic organic contaminant (HOC; toluene, or 1,2,4-trichlorobenzene [TCB]) removal from six soils and to evaluate the optimal composition of soil texture for maximum HOC removal using aqueous surfactant solution. Selected surfactants were 4% (vol/vol) sodium diphenyl oxide disulfonate (DOSL) and 4% (wt/vol) sodium lauryl sulfate (LS). Toluene and TCB were selected as the lighter-than-water nonaqueous phase liquid (LNAPL) and denser-than-water nonaqueous phase liquid (DNAPL) model substances, respectively. Soil types used for this study were Ottawa sand and five Iowa soils (Fruitfield, Keomah, Crippin, Webster, and Galvar). The greatest recovery of toluene and TCB in batch tests was 73% and 84%, respectively, which was obtained with DOSL surfactant in Ottawa sand. The toluene removal of 95% in column tests has been achieved in the Ottawa sand and three Iowa soils (Fruitfield, Keomah, Crippin) with DOSL after effluent volume of 3750 ml (about 32 pore volume) passed. TCB removal of 98% in column tests has been achieved in Ottawa sand and three Iowa soils (Fruitfield, Keomah, Crippin) with DOSL after effluent volume of 2500 ml (about 21 pore volume) passed. These results were related with soil texture (clay content 30%), clay mineralogy (kaolinite and smectite), as a function of transported pore volume.

David Oppong, Vanja M. King, Judith A. Bowen. (Buckman Laboratories International Inc., 1256 North Mclean Blvd., Memphis, TN 38108, USA). **Isolation and characterization of filamentous bacteria from paper mill slimes**. International Biodeterioration & Biodegradation, 52(2) (2003), 53-62.

The composition of filamentous bacteria in paper machine systems has received little scientific scrutiny, even though these organisms have been associated with many problems that affect machine efficiency and paper quality. The objective of the study was to isolate and characterize filamentous bacteria in paper mill slimes using conventional microbiological techniques, fatty acid methyl ester (FAME) analysis, and 16S rRNA gene sequencing. Filamentous or long, thread-like bacteria from different genera were observed. Pink or red-pigmented filamentous bacteria identified as Flectobacillus sp. were commonly isolated from pink slime deposits, but they were also obtained from deposits that did not appear pink or red. Two organisms had certain characteristics that were different from similar organisms previously described. One was a Gram-negative, filamentous bacterium with golden yellow colonies. This organism was esculin positive, and hydrolyzed starch but did not produce hydrogen sulfide. A BLAST search of GenBank database produced an identity of 92% with Cytophaga sp. or Flavobacterium columnare. A Gram-positive bacterium that produced very long or filamentous structures was also observed. On tryptic soy agar, this organism produced yellow colonies, but on plate count agar, the colonies were white. The plate count agar-grown cells were atypical with many of them having bulbs either in the middle or at the ends of the cell. In indole nitrite broth, the organism produced a very extensive filamentous structure. The FAME and 16S rRNA gene analyses of this organism showed that the organism was a Bacillus sp., but no spores were produced in any of the media studied, including a sporulating medium. Various spore-formers, identified as Bacillus psychrophilus and Paenibacillus sp., also had long continuous chains of cells. Two species of Chryseobacterium produced long filaments. Actinomycetes with branched mycelia and identified as Norcardiopsis alba and Streptomyces albidoflavus were isolated from wet lap pulp samples. These actinomycetes produced a very strong earthy odor both in culture and in field samples. Information, such as growth requirements and presence or absence of endospores, gained about these organisms will help in their control.

Dietmar H Pieper, Walter Reineke. (Department of Environmental Biotechnology, Gesellschaft für Biotechnologische Forschung mbH (GBF), Mascheroder Weg 1, D-38124

Braunschweig, Germany. Chemical Microbiology, Bergische Universität-Gesamthochschule Wuppertal, Gausstrasse 20, D-42097 Wuppertal, Germany). **Engineering bacteria for bioremediation**. Current Opinion in Biotechnology, 11(3) (2000), 262-270.

The treatment of environmental pollution by microorganisms is a promising technology. Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation. New information on the metabolic routes and bottlenecks of degradation is still accumulating, enlarging the available toolbox. With molecular methods allowing the characterization of microbial community structure and activities, the performance of microorganisms under *in situ* conditions and in concert with the indigenous microflora will become predictable.

E. Bozau, G. Strauch. (Department of Hydrogeology, UFZ-Centre for Environmental Research Leipzig-Halle, Halle/Saale, Germany). **Hydrogeological Basis for Biotechnological Remediation of the Acidic Mining Lake `RL 111', Lusatia (Germany)**. Water, Air and Soil Pollution: Focus, 2(3) (2002) 15-25.

The drainage basin of the acidic mining lake `RL 111' was characterized by hydrogeological and geochemical models to assess its influence on a planned biotechnological remediation of the lake water and lake sediment. Ground, seepage and lake water, as well as the surrounding sediments, were examined to model the hydrodynamic processes and the geochemical development of the lake. The geochemical conditions seem to behave in a stable manner (steady state conditions). A reduction of the high sulphate and acid input has not been observed since the beginning of our investigations. The biotechnological remediation of the whole lake should consider a treatment of the dump sediments to improve the quality of the inflowing groundwater, as well as to reduce erosion.

Eliora Z. Ron, Eugene Rosenberg. (Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel 69978). **Biosurfactants and oil bioremediation**. Current Opinion in Biotechnology, 13(3) (2002), 249-252.

Oil pollution is an environmental problem of increasing importance. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in oil-containing environments, have an important role in the biological treatment of this pollution. One of the limiting factors in this process is the bioavailability of many fractions of the oil. The hydrocarbon-degrading microorganisms produce biosurfactants of diverse chemical nature and molecular size. These surface-active materials increase the surface area of hydrophobic water-insoluble substrates and increase their bioavailability, thereby enhancing the growth of bacteria and the rate of bioremediation.

Gary S Sayler, Steven Ripp. (Center for Environmental Biotechnology, University of Tennessee, Knoxville, 676 Dabney Hall, Knoxville, TN 37996, USA). **Field applications of genetically engineered microorganisms for bioremediation processes**. Current Opinion in Biotechnology, 11(3) (2000), 286-289.

Genetically engineered microorganisms (GEMs) have shown potential for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities encompassing a wide range of chemical contaminants. However, the vast majority of studies pertaining to genetically engineered microbial bioremediation are supported by laboratory-based experimental data. In general, relatively few examples of GEM applications in environmental ecosystems exist. Unfortunately, the only manner in which to fully address the competence of GEMs in bioremediation efforts is through long-term field release studies. It is therefore essential that field studies be performed to acquire the requisite information for determining the overall effectiveness and risks associated with GEM introduction into natural ecosystems.

Geoffrey Michael Gadd. (Department of Biological Sciences, University of Dundee, Dundee, DD1 4HN, UK). **Bioremedial potential of microbial mechanisms of metal mobilization and immobilization**. Current Opinion in Biotechnology, 11(3) (2000), 271-279.

Microorganisms play important roles in the environmental fate of toxic metals and radionuclides with a multiplicity of mechanisms effecting transformations between soluble and insoluble forms. These mechanisms are integral components of natural biogeochemical cycles and are of potential for both *in situ* and *ex situ* bioremedial treatment processes for solid and liquid wastes.
H. Ksheminska, A. Jaglarz, D. Fedorovych, L. Babyak, D. Yanovych, P. Kaszycki, H. Koloczek. **Bioremediation of chromium by the yeast Pichia guilliermondii: toxicity and accumulation of Cr (III) and Cr (VI) and the influence of riboflavin on Cr tolerance**. Microbiological Research, 158(1), 59- 67.

A comparative study has been made on the sensitivity of the yeast Pichia guilliermondii to Cr (III) and Cr (VI) as well as on the Cr uptake potential at growth-inhibitory concentrations of chromium. The strains used in the study were either isolated from natural sources or obtained from a laboratory strain collection. The results show that most of the natural strains were more tolerant to chromium and were able to grow in the presence of 5 mM Cr (III) or 0.5 mM Cr (VI), that is at concentrations which substantially inhibited the growth of laboratory strains. The cellular Cr content after treatment was similar for both strain types and ranged from 1.2-4.0 mg/g d.w. and 0.4-0.9 mg/g d.w., for Cr (III) and Cr (VI) forms, respectively; however, in one case of a natural strain it reached the value of 10 mg Cr (III)/g dry mass. Natural-source strains were grouped into four groups based on the yeasts' differential response to Cr (III) and Cr (VI). Hexavalent Cr-resistant mutants of a P. guilliermondii laboratory strain, which revealed markedly changed capabilities of chromium accumulation, were obtained by means of UV-induced mutagenesis. Cr (VI) treatment triggered oversynthesis of riboflavin and the addition of exogenous riboflavin increased P. guilliermondii resistance to both Cr (III) and Cr (VI). Electrophoretic protein profiles revealed the induction and/or suppression of several proteins in response to toxic Cr (VI) levels.
H. Suhara, C. Daikoku, H. Takata, S. Suzuki, Y. Matsufuji, K. Sakai, R. Kondo. (Laboratory of Systematic Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, 6–10–1 Hakozaki, Higashi-ku, 812–8581 Fukuoka, Japan. Department of Geriatric Research, National Institute for Longevity Sciences, 36–3 Gengo, 474–8522 Morioka Obu, Aichi, Japan. Department of Civil Engineering, Faculty of Engineering, Fukuoka University, 8–19–1 Nakamura, Jonan-ku, 814–0180 Fukuoka, Japan. Laboratory of Forest Chemistry and Biochemistry, Faculty of Agriculture, Kyushu University, 6–10–1 Hakozaki, Higashi-ku, 812–8581 Fukuoka, Japan). **Monitoring of white-rot fungus during bioremediation of polychlorinated dioxin-contaminated fly ash**. Applied Microbiology and Biotechnology, 62(5-6) (2003), 601 – 607.

Bioremediation is a low-cost treatment alternative for the cleanup of polychlorinated-dioxin-contaminated soils and fly ash when pollution spread is wide-ranging. An interesting fungus, Ceriporia sp. MZ-340, with a high ability to degrade dioxin, was isolated from white rotten wood of a broadleaf tree from Kyushu Island in Japan. We have attempted to use the fungus for bioremediation of polychlorinated-dioxin-contaminated soil on site. However, we have to consider that this trial has the potential problem of introducing a biohazard to a natural ecosystem if this organism is naturalized. We have therefore developed a monitoring system for the introduced fungus as a part of the examination and evaluation of bioremediation in our laboratory. We have also developed a PCR-based assay to reliably detect the fungus at the bioremediation site. DNA isolated from the site was amplified by PCR using a specific primer

derived from internal transcribed spacer region (ITS: ITS1, 5.8S rDNA and ITS2) sequences of Ceriporia sp. MZ-340. We successfully monitored Ceriporia sp. MZ-340 down to 100 fg/µl DNA and down to 2 mg/g mycelium. We also successfully monitored the fungus specifically at the bioremediation site. The polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran content was observed to decrease in response to treatment with the fungus. The species-specific PCR technique developed in the present work is useful in evaluating the possibility of on-site bioremediation using the fungus Ceriporia sp. MZ-340.

Helmut Lorbeer, Sophie Starke, Misri Gozan, Andreas Tiehm, Peter Werner. (Dresden University of Technology, Institute of Waste Management and Contaminated Site Treatment, Pirna, Germany. Water Technology Centre Karlsruhe, Karlsruhe, Germany). **Bioremediation of Chlorobenzene-Contaminated Groundwater on Granular Activated Carbon Barriers**. Water, Air and Soil Pollution: Focus, 2(3) (2002), 183-193.

During the past decade, various promising technologies have been developed for the decontamination of groundwater *in situ* which do not require long-term pumping or high energy consumption. One approach is to use funnel and gate technology. In the case described here, the combination of adsorption of contaminants on granular activated carbon (GAC) and its biodegradation is applied to considerably extend the operating time of the filling material in the barrier system. Monochlorobenzene (MCB), a recalcitrant groundwater contaminant under anaerobic conditions, undergoes high-capacity adsorption on GAC up to about 450 mg per gram. Aerobic enrichment cultures, obtained from a contaminated aquifer, were able to mineralize initially adsorbed MCB. In respirometer experiments the rate of carbon dioxide formation was dependent on the equilibrium concentration of MCB. The oxygen consumption of activated carbon by means of autoxidative reactions may delay aerobic biodegradation in GAC filters. The oxygen uptake of pristine activated carbon amounted to 5.6 mg per gram GAC in laboratory column experiments. When GAC was pre-loaded with MCB, autoxidation rates were considerably reduced. Hence, it is advisable not to stimulate the biodegradation of MCB by oxygen supply in GAC biobarriers until after an initial period of solely sorptive MCB removal from the groundwater flow.

I. D. Pulford, C. Watson. (Environmental, Agricultural and Analytical Chemistry Section, Chemistry Department, University of Glasgow, Glasgow G12 8QQ, UK). **Phytoremediation of heavy metal-contaminated land by trees.3–a review.** Environment International, 29(4) (2003), 529-540.

This paper reviews the potential for using trees for the phytoremediation of heavy metal-contaminated land. It considers the following aspects: metal tolerance in trees, heavy metal uptake by trees grown on contaminated substrates, heavy metal compartmentalisation within trees, phytoremediation using trees and the phytoremediation potential of willow (*Salix* spp.).

I. Wagner-Döbler . (National Research Institute for Biotechnology Mascheroder Weg 1 38124 Braunschweig Germany). **Pilot plant for bioremediation of mercury-containing industrial wastewater**. Applied Microbiology and Biotechnology, 62(2-3) (2003), 124 – 133.

Mercury is an extremely toxic pollutant that is currently being emitted mainly by low level industrial sources. It is distributed globally through the atmosphere, from where it precipitates onto the surface of the Earth, enters aquatic organisms, accumulates in fish and finally affects the health of human populations. Microbes have evolved a mechanism for mercury detoxification [mercury resistance operon (mer)] based on intracellular reduction of Hg 2+ to non-toxic Hg 0 by the mercuric reductase enzyme and subsequent diffusional loss of Hg 0 from the cell. It was shown that Hg 0 produced by microbial detoxification can be retained quantitatively in packed bed bioreactors, in which biofilms of mercury-resistant bacteria are grown on porous carrier

material. This review describes operation of this system on a technical, fully automated, scale, and its operation at a chloralkali electrolysis factory. It was shown to work with high efficiency under fluctuating mercury concentrations and to be robust against transiently toxic conditions. The gradient of mercury concentration in the technical scale system exerted a strong selective pressure on the microbial community, which resulted in a succession of mercury-resistant strains at high mercury concentrations and an increase in phylogenetic and functional diversity at low mercury concentrations. Clean-up of mercury-containing wastewater by mercury-resistant microbes is a simple, environmentally friendly and cost-effective alternative to current treatment technologies.

Ines P**ö**hler, Dirk F. Wenderoth, Katrin Wendt-Potthoff, Manfred G. H**ö**fle. (*GBF-German Research Centre for Biotechnology, Dept. of Environmental Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany).* **Bacterioplankton Community Structure and Dynamics in Enclosures During Bioremediation Experiments in an Acid Mining Lake.** Water, Air and Soil Pollution: Focus, 2(3) (2002), 111-121.

In an acid mining lake (pH 2.6) enclosure experiments were performed with the addition of different concentrations of organic carbon, nitrogen and phosphorus. SSCP-community fingerprints, based on 16S rRNA gene amplicons, were performed to monitor changes in the structure of the total bacterial community and the sulfate reducing bacteria (SRB) in the mesocosms. Total bacterial cell counts, as assessed by epifluorescence microscopy, were increased in the mesocosms amended with organic carbon. The addition of carbon also increased the number of abundant bacterial taxa substantially along depth. Sulfate reducing bacteria (SRB) could be detected in all enclosures and all parts of the water column. These SRB belonged to genus *Desulfobacter* as indicated by coroborating molecular data.

J Widada, H Nojiri, T Omori. (Biotechnology Research Center, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Laboratory of Soil and Environmental Microbiology, Department of Soil Science, Faculty of Agriculture, Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia). **Recent developments in molecular techniques for identification and monitoring of xenobiotic-degrading bacteria and their catabolic genes in bioremediation.** Applied Microbiology and Biotechnology, 60(1-2) (2002), 45 – 59.

The pollution of soil and water with xenobiotics is widespread in the environment and is creating major health problems. The utilization of microorganisms to clean up xenobiotics from a polluted environment represents a potential solution to such environmental problems. Recent developments in molecular-biology-based techniques have led to rapid and accurate strategies for monitoring, discovery and identification of novel bacteria and their catabolic genes involved in the degradation of xenobiotics. Application of these techniques to bioremediation has also improved our understanding of the composition, phylogeny, and physiology of metabolically active members of the microbial community in the environment. This review provides an overview of recent developments in molecular-biology-based techniques and their application in bioremediation of xenobiotics.

J. F. Ferguson, J. M. H. Pietari. (Department of Civil and Environmental Engineering, University of Washington, Seattle, WA 98195, USA). **Anaerobic transformations and bioremediation of chlorinated solvents.** Environmental Pollution, 107(2) (2000), 209-215.

Chlorinated aliphatic compounds, notably the chlorinated solvents, are common contaminants in soil and groundwater at hazardous waste sites. While these compounds are often recalcitrant, under favorable conditions they can be transformed and degraded through microbially mediated processes. There is great interest in understanding the transformations that are observed at

contaminated sites and in manipulating these systems to achieve remediation. An important class of transformations occurs in anaerobic environments. Many of the transformations are reductive, and many yield useful energy to specific anaerobic bacteria. They include reductive dechlorination, dehydrochlorination and dichloroelemination. Of these, reductive dechlorination is often a growth-supporting reaction, while the others may be abiological or catalyzed by biological molecules. The reactions may result in chlorinated products, but there are often reaction sequences leading to completely dechlorinated products. The behavior of carbon tetrachloride (CT), 1,1,2,2-tetrachloroethane (TeCA) and the chloroethenes, perchloroethylene (PCE) and trichloroethylene (TCE), illustrate the range of anaerobic transformations that are possible, as well as the limited transformation that often is seen in the environment. CT undergoes reductive and substitutive reactions that are catalyzed by biological molecules but do not support bacterial growth. The anaerobic degradation of TeCA, which is a major contaminant at a site near Tacoma, WA, USA, provides examples of each type of transformation, and the products formed are consistent with the chlorinated compounds that are found in groundwater extraction wells. A laboratory study, using anaerobic sludge that had been fed chlorinated compounds, a cell-free extract from the sludge, and killed controls, showed that TeCA was transformed to four products and that these were further transformed, suggesting that it might be possible to degrade TeCA to innocuous products. Reductive dechlorination of PCE and TCE has been studied in many laboratories. Studies with mixed anaerobic consortia and with several dehalogenating bacteria, including strain 195 (Maymó-Gatell, X., Chien, Y-T., Gosset, J.M., Zinder, S.M, 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethane to ethane. Science 276, 1568–1571) that transforms PCE to ethene, have demonstrated that reductive dechlorination supports growth of the novel bacteria that carry out the reactions. Hydrogen has been shown to be an electron donor for the bacterial dehalogenation reactions, and kinetic and thermodynamic considerations indicate that dehalogenators can compete in some, but not all, anaerobic environments, pointing to applications of in situ bioremediation and to its limitations. Selected field studies of anaerobic transformations help delineate the applications of this type of bioremediation.

J. H. Langwaldt and J. A. Puhakka. (Institute of Water and Environmental Engineering, Tampere University of Technology, PO Box 541, FIN-33101 Tampere, Finland). **On-site biological remediation of contaminated groundwater: a review.** Environmental Pollution, 107(2) (2000), 187-197.

On-site biological treatment has been used for groundwater cleanup from industrial and agricultural chemicals. The pump-and-treat efficiency is controlled by retardation of contaminants by sorption onto the saturated subsurface solids and by the presence of non-aqueous-phase liquids in the aquifer. On-site bioreactors have been widely used for treatment of contaminants such as petroleum hydrocarbons, monoaromatic hydrocarbons, chlorinated aliphatics and aromatics. The most commonly used reactor types for groundwater include the following: trickling filter, upflow fixed-film reactor and fluidized bed reactor. Bioreactor processes have limitations mainly because of their design to operate at elevated temperatures and thereby by high operational costs.

J. Haimi. (Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, FIN-40351 Jyväskylä, Finland). **Decomposer animals and bioremediation of soils.** Environmental Pollution, 107(2) (2000), 233-238.

Although microorganisms are degrading the contaminants in bioremediation processes, soil animals can also have important — while usually an indirect — role in these processes. Soil animals are useful indicators of soil contamination, both before and after the bioremediation. Many toxicity and bioavailability assessment methods utilizing soil animals have been developed

for hazard and risk-assessment procedures. Not only the survival of the animals, but also more sensitive parameters like growth, reproduction and community structure have often been taken into account in the assessment. The use of bioassays together with chemical analyses gives the most reliable results for risk analyses. This is because physical, chemical and biological properties of the remediated soil may be changed during the process, and it is possible that transformation rather than mineralization of the contaminants has taken place. In addition, the soil may contain other harmful substances than those searched in chemical analyses. Finally, because the ultimate goal of the bioremediation should be — together with mineralization of the harmful substances — the ecological recovery of the soil, development of diverse decomposer community as a basis of the functioning ecosystem should be ensured. Soil animals, especially the large ones, can also actively take part in the ecological recovery processes through their own activity. The potential risk of transfer of contaminants accumulated in soil animals to the above-ground food webs should be borne in mind.

Jessica Leighton, Susan Klitzman, Slavenka Sedlar, Thomas Matte, Neal L. Cohen. (Lead Poisoning Prevention Program, New York City Department of Health, 253 Broadway, 12th floor, Box CN58, New York, NY 10007, USA. Hunter College, City University of New York, New York, NY 10010, USA. New York City Department of Health, New York, NY 10007, USA. Columbia University, New York, NY 10027, USA. New York Academy of Medicine/Center for Urban Epidemiologic Studies, New York, NY, USA. US Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. New York City Department of Health, New York, NY 10013, USA. Academic Medicine Development Corporation (AMDeC), New York, NY 10020, USA). **The effect of lead-based paint hazard remediation on blood lead levels of lead poisoned children in New York City**. Environmental Research, 92(3) (2003), 182-190.

Despite the widespread use of lead paint hazard control for children with lead poisoning, few controlled studies that estimate the effect of such control on children's blood lead levels have been published. This retrospective follow-up study examined the effects of lead hazard remediation and its timing on the blood lead levels of lead-poisoned children. From the New York City child blood lead registry, 221 children were selected who had an initial blood lead level of 20–44 μg/dL between 1 July 1994 and 31 December 1996; were 6 months to 6 years of age; had a report of a follow-up blood lead test between 10 and 14 months after the initial test; had a lead-based paint hazard identified in the primary dwelling unit prior to the 10- to 14-month follow-up blood lead test; had resided or spent time at only one address with an identified lead-based paint hazard; and were not chelated. The decline in geometric mean blood lead levels from baseline to 10–14 months later was compared for children whose homes were remediated and whose homes were not remediated during the follow-up period. Regardless of remediation, geometric mean blood lead levels declined significantly from 24.3 μg/dL at the initial diagnosis to 12.3 μg/dL at the 10- to 14-month follow-up blood lead test ($P<0.01$). Among the 146 children whose homes were remediated the geometric mean blood lead levels declined 53% compared to 41% among the 75 children whose homes were not remediated by the follow-up blood lead test, a remediation effect of approximately 20% ($P<0.01$). After adjusting for potential confounders, the remediation effect was 11%, although it was no longer significant. Race was the only factor that appeared to confound the relationship: Black children had higher follow-up blood lead levels even after controlling for other factors, including the natural logarithm of the initial blood lead level. The effect of remediation appeared to be stronger for younger (10 to <36 months old) than for older (36 to 72 months old) children ($P=0.06$). While children in homes with earlier remediation (within less than 3 months) appeared to have greater declines in blood lead levels at the follow-up test than children in homes with later remediation (after 3 or more months), this trend was not significant when controlling for confounding factors.

The findings of this study suggest that early identification of lead-poisoned children and timely investigation and abatement of hazards contribute to reducing blood lead levels. However, the apparent effect is modest and further research is needed to systematically test and improve the effectiveness of lead hazard controls.

K. Knöller, G. Strauch. (*UFZ-Center for Environmental Research Leipzig-Halle, Department of Hydrogeology, Halle/Saale, Germany).* **The Application of Stable Isotopes for Assessing the Hydrological, Sulfur, and Iron Balances of Acidic Mining Lake ML 111 (Lusatia, Germany) as a Basis for Biotechnological Remediation.** Water, Air and Soil Pollution: Focus, 2(3) (2002), 3-14.

Stable isotope ($\delta^{18}O–H_2O$, $\delta^{2}H–H_2O$ $\delta^{34}S–SO_4^{2-}$) and hydrochemical data (SO_4^{2-}, Fe-concentrations) have been used to estimate the annual groundwater inflow and outflow of mining lake ML 111 and to calculate the total amount of dissolved sulfate and iron that is carried into the lake by groundwater. The hydrological balance suggests an annual groundwater inflow of 23 700 m^3 and an annual groundwater outflow of 15 700 m^3. The calculation of the sulfur and iron balances yielded an annual sulfate input of 37 800 kg and an annual iron input of 7000 kg with the groundwater inflow. Furthermore it was shown that significant fluxes of these elements go into the lake sediments, which results in continuous release of acidity in the lake water.

K. S. Jørgensen, J. Puustinen, A. -M. Suortti. (Finnish Environment Institute, Research Laboratory, Hakuninmaantie 4-6, FIN-00430 Helsinki, Finland). **Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles.** Environmental Pollution, 107(2) (2000), 245-254.

Composting of contaminated soil in biopiles is an ex situ technology, where organic matter such as bark chips are added to contaminated soil as a bulking agent. Composting of lubricating oil-contaminated soil was performed in field scale (5×40 m^3) using bark chips as the bulking agent, and two commercially available mixed microbial inocula as well as the effect of the level of added nutrients (N,P,K) were tested. Composting of diesel oil-contaminated soil was also performed at one level of nutrient addition and with no inoculum. The mineral oil degradation rate was most rapid during the first months, and it followed a typical first order degradation curve. During 5 months, composting of the mineral oil decreased in all piles with lubrication oil from approximately 2400 to 700 mg (kg dry w)$^{-1}$, which was about 70% of the mineral oil content. Correspondingly, the mineral oil content in the pile with diesel oil-contaminated soil decreased with 71% from 700 to 200 mg (kg dry w)$^{-1}$. In this type of treatment with addition of a large amount of organic matter, the general microbial activity as measured by soil respiration was enhanced and no particular effect of added inocula was observed.

Kazuya Watanabe. (Marine Biotechnology Institute, Kamaishi Laboratories, 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan) **Microorganisms relevant to bioremediation**. Current Opinion in Biotechnology, 12(3) (2001), 237-241.

Naturally occurring microbial consortia have been utilized in a variety of bioremediation processes. Recent developments in molecular microbial ecology offer new tools that facilitate molecular analyses of microbial populations at contaminated and bioremediated sites. Information provided by such analyses aids in the evaluation of the effectiveness of bioremediation and the formulation of strategies that might accelerate bioremediation.

Kelly P. Nevin, Kevin T. Finneran, and Derek R. Lovley. (Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003). **Microorganisms Associated with Uranium Bioremediation in a High-Salinity Subsurface Sediment.** Applied and Environmental Microbiology, 69(6) (2003), 3672-3675.

Although stimulation of dissimilatory metal reduction to promote the reductive precipitation of uranium has been shown to successfully remove uranium from some aquifer sediments, the

organisms in the family *Geobacteraceae* that have been found to be associated with metal reduction in previous studies are not known to grow at the high salinities found in some uranium-contaminated groundwaters. Studies with a highly saline uranium-contaminated aquifer sediment demonstrated that the addition of acetate could stimulate the removal of U(VI) from the groundwater. This removal was associated with an enrichment in microorganisms most closely related to *Pseudomonas* and *Desulfosporosinus* species.
Kensuke Furukawa. (Department of Bioscience and Biotechnology, Kyushu University, Fukuoka 812-8581, Japan). **`Super bugs' for bioremediation.** Trends in Biotechnology, 21(5) (2003), 187-190.

Chlorinated organic compounds are among the most significant pollutants in the world. Sequential use of anaerobic halorespiring bacteria, which are the key players in biological dehalogenation processes, and aerobic bacteria whose oxygenases are modified by directed evolution could lead to efficient and total degradation of highly chlorinated organic pollutants. Recently three interesting papers on halorespiration and polychlorinated biphenyl biodegradation were published.
Kristin Van Gestel, Joris Mergaert, Jean Swings, Jozef Coosemans, Jaak Ryckeboer. (Laboratory of Phytopathology and Plant Protection, Faculty of Agricultural and Applied Biological Sciences, Katholieke Universiteit Leuven, W. de Croylaan 42, B-3001, Leuven, Belgium. Laboratorium voor Microbiologie, Vakgroep Biochemie, Fysiologie, en Microbiologie, Faculteit Wetenschappen, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000, Gent, Belgium). **Bioremediation of diesel oil-contaminated soil by composting with biowaste.** Environmental Pollution, 125(3) (2003), 361-368.

Soil spiked with diesel oil was mixed with biowaste (vegetable, fruit and garden waste) at a 1:10 ratio (fresh weight) and composted in a monitored composting bin system for 12 weeks. Pure biowaste was composted in parallel. In order to discern the temperature effect from the additional biowaste effect on diesel degradation, one recipient with contaminated soil was hold at room temperature, while another was kept at the actual composting temperature. Measurements of composting parameters together with enumerations and identifications of microorganisms demonstrate that the addition of the contaminated soil had a minor impact on the composting process. The first-order rate constant of diesel degradation in the biowaste mixture was four times higher than in the soil at room temperature, and 1.2 times higher than in the soil at composting temperature.
L. Suominen, M. M. Jussila, K. Mäkeläinen, M. Romantschuk, K. Lindström. (Department of Applied Chemistry and Microbiology, PO Box 56, Viikki Biocenter, 00014 University of Helsinki, Helsinki, Finland. Department of Biosciences, PO Box 56, Viikki Biocenter, 00014 University of Helsinki, Helsinki, Finland). **Evaluation of the *Galega–Rhizobium galegae* system for the bioremediation of oil-contaminated soil.** Environmental Pollution, 107(2) (2000), 239-244.

The bioremediation potential of a nitrogen-fixing leguminous plant, *Galega orientalis*, and its microsymbiont *Rhizobium galegae* was evaluated in BTX (benzene, toluene, xylene)-contaminated soils in microcosm and mesocosm scale. To measure the intrinsic tolerance of the organisms to *m*-toluate, a model compound representing BTX, *G. orientalis* and *R. galegae* were cultivated under increasing concentrations of *m*-toluate alone and in association with *Pseudomonas putida* pWWO, a bacterial strain able to degrade toluene-derived compounds. The test plants and rhizobia remained viable in *m*-toluate concentrations as high as 3000 ppm. Plant growth was inhibited in concentrations higher than 500 ppm, but restituted when plants were transferred into *m*-toluate-free medium. Nodulation was blocked under the influence of *m*-toluate, but was restored after the plants were transferred into the non-contaminated media. In the

mesocosm assay the *Galega* plants showed good growth, nodulation and nitrogen fixation, and developed a strong rhizosphere in soils contaminated with oil or spiked with 2000 ppm *m*-toluate. Thus, this legume system has good potential for use on oil-contaminated sites.
Laura Bardi, Roberto Ricci, Mario Marzona. (Istituto Sperimentale per la Nutrizione della Piante, Via Pianezza, 115 – 10151 Torino, Italy. Biosearch Ambiente S.r.l., Via Livorno, 60 – 10100 Torino, Italy.Dipartimento di Chimica Generale ed Organica Applicata, Corso Massimo D'Azeglio, 48 – 10125 Torino, Italy). ***In situ* bioremediation of a hydrocarbon polluted site with cyclodextrin as a coadjuvant to increase bioavailability**. Water, Air and Soil Pollution: Focus, 3(3) (2003), 15-23.

Bioavailability is one main factor that influences the extent of biodegradation of hydrocarbons. They are very poorly soluble in water and easily adsorbed to clay or humus fractions, so they pass very slowly to the aqueous phase where they are metabolised by microorganisms. Cyclodextrins are natural compounds that form soluble inclusion complexes with hydrophobic molecules and increase degradation rate of hydrocarbons *in vitro*. In the perspective of an *in situ* application, we previously checked that β-cyclodextrin does not increase eluviation of hydrocarbons through the soil and consequently does not increase the risk of groundwater pollution. The results of an *in situ* application of β-cyclodextrin for bioremediation of a hydrocarbon polluted site are presented. We stated that the combination of bioaugmentation and enhanced bioavailability due to β-cyclodextrin was effective for a full degradation.
Lucas Ruberto, Susana C. Vazquez and Walter P. Mac Cormack. (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina. Instituto Antártico Argentino, Cerrito 1248, Buenos Aires (1010), Argentina). **Effectiveness of the natural bacterial flora, biostimulation and bioaugmentation on the bioremediation of a hydrocarbon contaminated Antarctic soil**. International Biodeterioration & Biodegradation, 52(2) (2003), 115-125.

Microcosms systems (250 g soil in 1 l flasks) were performed in Jubany Station (King George Island, South Shetland Islands) to analyse biodegradation of gas-oil in Antarctic soils under natural conditions. Abiotic loss of hydrocarbons, biodegradation activity of indigenous microflora and biostimulation with N and P were studied. In addition, biaugmentation with a previously isolated psychrotolerant strain (B-2-2) was analysed. Hydrocarbon concentration, heterotrophic and hydrocarbon-degrading bacterial counts and predominant bacterial groups were evaluated during 51 days. A significant loss of hydrocarbons was observed in abiotic control. Indigenous microflora showed increased heterotrophic counts and hydrocarbon-degrading/heterotrophic ratio. This fact was associated with a significant degrading activity (35% higher than the control). Bioaugmentation with B-2-2 strain improved the bioremediation efficiency (75% of the hydrocarbon was removed). High levels of N and P produced an initial inhibition of bacterial growth. Finally, bacterial diversity was reduced in contaminated soil. Our results showed that autochthonous bacterial flora from Antarctic soils is able to degrade an important fraction of the gas-oil and that bioaugmentation represents a valuable alternative tool to improve bioremediation.
M Dua, A Singh, N Sethunathan, A Johri. (Department of Zoology, University of Delhi, Delhi 110007, India. Department of Biology, University of Waterloo, Waterloo, N2L 3G1, Ontario, Canada. CSIRO Land and Water, PMB 2, Glen Osmond, SA 5064, Australia, Present address: Channing Laboratory, Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA, Present address: Department of Biomedical Research, Division of Hematology/Oncology, St. Elizabeth Medical Center, School of Medicine, Tufts University, Boston, MA 02135, USA). **Biotechnology and bioremediation: successes and limitations.** Applied Microbiology and Biotechnology, 59(2-3) (2002), 143 – 152.

With advances in biotechnology, bioremediation has become one of the most rapidly developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants, such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters, nitroaromatic compounds, industrial solvents, pesticides and metals. A number of bioremediation strategies have been developed to treat contaminated wastes and sites. Selecting the most appropriate strategy to treat a specific site can be guided by considering three basic principles: the amenability of the pollutant to biological transformation to less toxic products (biochemistry), the accessibility of the contaminant to microorganisms (bioavailability) and the opportunity for optimization of biological activity (bioactivity). Recent advances in the molecular genetics of biodegradation and studies on enzyme-tailoring and DNA-shuffling are discussed in this paper.

M. Romantschuk, I. Sarand, T. Petänen, R. Peltola, M. Jonsson-Vihanne, T. Koivula, K. Yrjälä and K. Haahtela. (Helsinki Biocenter, Department of Biosciences, PO Box 56 (Viikinkarig), **FIN-00014 University of Helsinki, Finland). Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches.** Environmental Pollution, 107(2) (2000), 179-185.

Different aspects of bacterial degradation of organic contaminants in soil, and how to improve the efficiency and reproducibility is discussed in this review. Although bioremediation in principle includes the use of any type of organism in improving the condition of a contaminated site, most commonly bacteria are the degraders and other organisms, such as soil animals or plant roots, play a role in dissemination of bacteria and, indirectly, plasmids between bacteria, and in providing nutrients and co-substrates for the bacteria active in the degradation process. There are a number of different procedures that have been tested more-or-less successfully in attempts to improve reliability, cost efficiency and speed of bioremediation. The methods range from minimal intervention, such as mere monitoring of intrinsic bioremediation, through in situ introduction of nutrients and/or bacterial inocula or improvement of physico-chemical conditions, all the way to excavation followed by on site or ex situ composting in its different varieties. In the past the rule has been that more intervention (leading to higher costs) has been more reliable, but novel ideas are continuously tried out, both as a means to come up with new truly functional applications and also as a line of studies in basic soil microbial ecology. Both approaches generate valuable information needed when predicting outcome of remediation activities, evaluating environmental risks, deciding on cleaning-up approaches, etc. The emphasis of this review is to discuss some of the novel methods for which the value has not been clearly shown, but that in our view merit continued studies and efforts to make them work, separately or in combination.

Matthew T. Hardin, Tony Howes, David A. Mitchell. (Chemical and Materials Engineering Department, University of Auckland, Auckland, New Zealand. Department of Chemical Engineering, University of Queensland, St Lucia, Qld 4072, Australia. Departamento de Bioquímica, Universidade Federal do Paraná, Cx. P. 19046, 81531-990 Curitiba, Parana, Brazil). **Mass transfer correlations for rotating drum bioreactors**. Journal of Biotechnology, 97(1) (2002), 89-101.

Evaporative cooling is extremely important for large-scale operation of rotating drum bioreactors (RDBs). Outlet water vapour concentrations were measured for a RDB containing wet wheat bran with the aim of determining the mass transfer coefficient for evaporation from the bran bed to the headspace. Mass transfer was expressed as the mass transfer coefficient times the area for transfer per unit volume of void space in the drum. Values of ka' were determined under combinations of aeration superficial velocities ranging from 0.006 to 0.017 ms-1 and rotation rates ranging from 0 to 9 rpm. Mass transfer coefficients were evaluated using a variety of

residence time distributions (RTDs) for flow in the gas phase including plug flow and well-mixed and a Central Jet RTD based on RTD studies. If plug flow is assumed, the degree of holdup at low effective Peclet (Peeff) numbers gives an apparent under-estimate of ka' compared with empirical correlations. Values of ka' calculated using the Central Jet RTD agree well with values of ka' from literature correlations. There was a linear relationship between ka' and effective Peclet number: ka'=2.32×10-3Peeff.

Matthias Koschorreck, René Frömmichen, Peter Herzsprung, Jörg Tittel, Katrin Wendt-Potthoff. (UFZ-Centre for Environmental Research Leipzig-Halle GmbH, Department of Inland Water Research, Magdeburg, Germany). **Functions of Straw for *In Situ* Remediation of Acidic Mining Lakes.** Water, Air and Soil Pollution: Focus, 2(3) (2002), 97-109.

The addition of straw in combination with `Carbokalk', a by-product from the sugar-industry, was successfully used to stimulate microbial alkalinity generation in an acidic mining lake. To get detailed information about functions of straw, an enclosure experiment was carried out. Straw bundles were placed at the sediment surface of an acidic mining lake (ML 111) and the physiochemical conditions and the microbiology of the sediment-water contact zone were studied. Straw was degraded by anaerobic microorganisms and dissolved organic carbon (DOC) leached from straw bundles. Pigmented flagellates responded to the DOC supply in the water column and a considerable amount of algal carbon was transported to the sediment. Straw addition led to microbial reduction of iron and sulfate in the sediment. Sulfate reduction was observed at a pH of 5.5. The pH, however, was not high enough to precipitate H_2S completely. Thus, some H_2S diffused into the water column, where it was reoxidized. Straw did not create or stabilize an anoxic water body above the sediment. Microbial sulfate reduction and pyrite formation only took place in the sediment, whereas iron reduction also took place in the straw. Straw, however, altered the flow conditions above the sediment surface and prevented complete mixing of the profundal water. Straw did not serve as a substratum for a reactive biofilm. We conclude that the most important function of straw for mining lake remediation is to be a long-term nutrient source for microbial alkalinity generation in the sediment.

N. Papassiopi K. Vaxevanidou, I. Paspaliaris. (School of Mining and Metallurgical Engineering, National Technical University of Athens, GR 157 80 Zografos, Greece). **Investigating the Use of Iron Reducing Bacteria for the Removal of Arsenic from Contaminated Soils**. Water, Air and Soil Pollution: Focus, 3(3) (2003), 81-90.

Clean-up techniques, which were developed for removing cationic heavy metals from contaminated soils, are inappropriate for the metalloid As, which is a common and highly toxic pollutant. Because arsenic is mainly found associated with the hydrous ferric oxides of the soil, a possible mechanism for the mobilisation of this element is the reductive dissolution of Fe(III) oxyhydroxides. In this paper we investigate the possibility to mobilise arsenic, using the Fe(III)-reducing bacterium *Desulfuromonas Palmitatis*. The initial experiments were carried out using a crystalline ferric arsenate as model compound, i.e. scorodite (FeAsO4.2H2O). *D. palmitatis* was found able to reduce the trivalent iron of scorodite at a percentage of 80% within 16 days, but arsenic remained in the pentavalent state, and reprecipitated with Fe(II) in the form of low solubility ferrous arsenates. To avoid the precipitation of ferrous arsenates the subsequent experiments with soil were conducted by combining the reducing ability of *D. palmitatis* with the chelating strength of EDTA (ethylenediamine tetracetic acid), which can form strong aqueous complexes with Fe(II). Approximately 60% of Fe and 75% of As were recovered in the aqueous solution in the presence of EDTA, while in the simple biological treatment no Fe was dissolved and only a 3% of As was mobilised.

N. Rangsayatorn, E. S. Upatham, M. Kruatrachue, P. Pokethitiyook, G. R. Lanza. (Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok, 10400 Thailand. Faculty of Science, Burapha University, Chonburi, Thailand. Environmental Sciences Program and Department of Microbiology, University of Massachusetts, Amherst, MA 01003-7240, USA). **Phytoremediation potential of *Spirulina* (*Arthrospira*) *platensis*: biosorption and toxicity studies of cadmium.** Environmental Pollution, 119(1) (2002), 45-53.

This study examines the possibility of using *Spirulina (Arthrospira) platensis* TISTR 8217 to remove low concentrations of cadmium (less than 100 mg/l) from wastewater. The cyanobacteria were exposed to six different cadmium concentrations for 96 h, and the growth rate was determined using an optical density at 560 nm. The inhibiting concentration (IC_{50}) was estimated using probit analysis. The IC_{50} at 24, 48, 72, and 96 h were 13.15, 16.68, 17.28, and 18.35 mg/l Cd, respectively. Cellular damage was studied under a light microscope and a transmission electron microscope. Swollen cells and fragmented filaments were observed. Cell injury increased with increasing concentrations of cadmium. Ultrastructural changes were observed in the algae exposed to cadmium concentrations both close to IC_{50} (14.68 mg/l) and at IC_{50} (18.35 mg/l). The alterations induced by cadmium were disintegration and disorganization of thylakoid membranes, presence of large intrathylakoidal space, increase of polyphosphate bodies, and cell lysis. In addition, the cadmium adsorption by algal cells was studied. Environmental factors were found to have an effect on biosorption. The uptake of cadmium was not affected by the temperature of the solution, but the sorption was pH dependent. The optimum pH for biosorption of algal cells was 7. The cadmium uptake process was rapid, with 78% of metal sorption completed within 5 min. The sorption data fit well to the Langmuir isotherm. The maximum adsorption capacity for *S. platensis* was 98.04 mg Cd per g biomass.

N. Vasudevan, P. Rajaram. (Centre for Environmental Studies, Anna University, Chennai 600 025, India). **Bioremediation of oil sludge-contaminated soil**. Environment International, 26(5-6) (2001), 409-411.

Bioremediation has become an important method for the restoration of oil-polluted environments by the use of indigenous or selected microbial flora. Several factors such as aeration, use of inorganic nutrients or fertilizers and the type of microbial species play a major role in the remediation of oil-contaminated sites. Experiments were undertaken for bioremediation of oil sludge-contaminated soil in the presence of a bacterial consortium, inorganic nutrients, compost and a bulking agent (wheat bran). Experiments were conducted in glass troughs for the 90-day period. Bulked soil showed more rapid degradation of oil compared to all other amendments. During the experimental period, wheat bran-amended soil showed 76% hydrocarbon removal compared to 66% in the case of inorganic nutrients-amended soil. A corresponding increase in the number of bacterial populations was also noticed. Addition of the bacterial consortium in different amendments significantly enhanced the removal of oil from the petroleum sludge from different treatment units.

O V Singh, S Labana, G Pandey, R Budhiraja, R K Jain. (Institute of Microbial Technology, Sector-39A, 160036, Chandigarh, India). **Phytoremediation: an overview of metallic ion decontamination from soil**. Applied Microbiology and Biotechnology, 61(5-6) (2003), 405 – 412.

In recent years, phytoremediation has emerged as a promising ecoremediation technology, particularly for soil and water cleanup of large volumes of contaminated sites. The exploitation of plants to remediate soils contaminated with trace elements could provide a cheap and sustainable technology for bioremediation. Many modern tools and analytical devices have provided insight into the selection and optimization of the remediation process by plant species.

This review describes certain factors for the phytoremediation of metal ion decontamination and various aspects of plant metabolism during metallic decontamination. Metal-hyperaccumulating plants, desirable for heavily polluted environments, can be developed by the introduction of novel traits into high biomass plants in a transgenic approach, which is a promising strategy for the development of effective phytoremediation technology. The genetic manipulation of a phytoremediator plant needs a number of optimization processes, including mobilization of trace elements/metal ions, their uptake into the root, stem and other viable parts of the plant and their detoxification and allocation within the plant. This upcoming science is expanding as technology continues to offer new, low-cost remediation options.

Omry Koren, Vishnia Knezevic, Eliora Z. Ron, and Eugene Rosenberg. (Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel 69978). **Petroleum Pollution Bioremediation Using Water-Insoluble Uric Acid as the Nitrogen Source**. Applied and Environmental Microbiology, 69(10) (2003), 6337-6339.

The biodegradation of hydrocarbon pollutants in open systems is limited by the availability of a utilizable nitrogen source. This limitation can be overcome by using uric acid. Enrichment cultures grown on crude oil-uric acid media yielded mixed and pure cultures that degraded petroleum. In a simulated open system, uric acid bound to crude oil and was available for bacterial growth and petroleum biodegradation.

P. Vervaeke, S. Luyssaert, J. Mertens, E. Meers, F. M. G. Tack, N. Lust. (Laboratory of Forestry, Department of Forest and Water Management, Ghent University, Geraardsbergse steenweg 267, 9090, Gontrode, Belgium. Laboratory of Analytical Chemistry and Applied Ecochemistry, Department of Applied Analytical and Physical Chemistry, Ghent University, Coupure Links 265, 9000, Ghent, Belgium). **Phytoremediation prospects of willow stands on contaminated sediment: a field trial.** Environmental Pollution, 126(2) (2003), 275-282.

Establishing fast growing willow stands on land disposed contaminated dredged sediment can result in the revaluation of this material and opens possibilities for phytoremediation. A field trial was designed to assess the impact of planting a willow stand *(Salix viminalis* L. `Orm') on the dissipation of organic contaminants (mineral oil and PAHs) in dredged sediment. In addition, the accumulation of heavy metals (Cd, Cu, Pb and Zn) in the biomass was determined. After 1.5 years, a significant decrease of 57% in the mineral oil concentration in the sediment planted with willow was observed. Degradation of mineral oil in sediment which was left fallow, was only 15%. The mineral oil degradation under willow was most pronounced (79%) in the root zone of the stand. In the sediment which was left fallow there was a significant reduction of the total PAH content by 32% compared with a 23% reduction in the planted sediment. The moderate and selective metal uptake, measured in this study, limits the prospects for phytoextraction of metals from dredged sediment.

P. Visoottiviseth, K. Francesconi, W. Sridokchan. (Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand. Institute of Biology, University of Southern Denmark, DK 5230, Odense M, Denmark). **The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land.** Environmental Pollution, 118(3) (2002), 453-461.

To assess the potential of the native plant species for phytoremediation, plant and soil samples were collected from two areas in Thailand that have histories of arsenic pollution from mine tailings. The areas were the Ron Phibun District (Nakorn Si Thammarat province) and Bannang Sata District (Yala province), and samples were taken in 1998 and 1999 and analysed for total arsenic by atomic absorption spectrophotometry. Arsenic concentrations in soil ranged from 21 to 14,000 $\mu g\ g^{-1}$ in Ron Phibun, and from 540 to 16,000 $\mu g\ g^{-1}$ in Bannang Sata. The criteria

used for selecting plants for phytoremediation were: high As tolerance, high bioaccumulation factor, short life cycle, high propagation rate, wide distribution and large shoot biomass. Of 36 plant species, only two species of ferns (*Pityrogramma calomelanos* and *Pteris vittata*), a herb (*Mimosa pudica*), and a shrub (*Melastoma malabrathricum*), seemed suitable for phytoremediation. The ferns were by far the most proficient plants at accumulating arsenic from soil, attaining concentrations of up to 8350 $\mu g\ g^{-1}$ (dry mass) in the frond.

Paul R. Adler, Steven T. Summerfelt, D. Michael Glenn, Fumiomi Takeda. (United States Department of Agriculture, Agricultural Research Service, National Center for Cool and Cold Water Aquaculture, 11876 Leetown Road, Kearneysville, WV 25430, USA. The Conservation Fund's Freshwater Institute, PO Box 1889, Shepherdstown, WV 25443, USA. United States Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430, USA). **Mechanistic approach to phytoremediation of water.** Ecological Engineering, 20(3) (2003), 251-264.

Conventional thinking regarding the use of food crops to clean aquaculture effluents has been that plants cannot remove nutrients in water to low levels without a reduction in productivity and quality. Because greenhouse space is expensive, productivity is critical for a profitable operation. A production strategy, called the conveyor production system (CPS), was developed using thin-film technology for plant production in dilute aquaculture effluents. With the CPS, young plants were positioned near the solution inlet in a gutter receiving the effluent and moved progressively, like along a conveyor belt, towards the outlet as they grew. Luxury consumption by lettuce plants (*Lactuca sativa* L. cv. Ostinata) enabled them to store P in their tissues early in their growth cycle for use later as water P levels decreased and influx could no longer meet current demands. If water is distributed in a horizontal plug-flow pattern, without the CPS, all nutrients will be luxury consumed at the inlet, making nutrients limiting at the outlet and significant greenhouse space will be dedicated to growing plants that have no market value. The object of this study was to construct and operate a pilot-scale CPS, collect data demonstrating its potential to clean effluent and produce a marketable product, and develop a mechanistic model describing the process. Greenhouse studies demonstrated that by using the CPS, phosphorus could be reduced from 0.52 to <0.01 mg l^{-1} by lettuce without an apparent reduction in production or quality. The mechanistic model described in this paper simulated experimental data collected during the operation of the CPS growing lettuce and defines critical data necessary for the general comparison of effluents for treatment.

Paul Römkens, Lucas Bouwman, Jan Japenga, Cathrina Draaisma. (ALTERRA –– Green World Research, Department of Water and the Environment, Wageningen University and Research Center, PO Box 47, NL-6700 AA Wageningen, The Netherlands). **Potentials and drawbacks of chelate-enhanced phytoremediation of soils.** Environmental Pollution, 116(1) (2002), 109-121.

Chelate-enhanced phytoremediation has been proposed as an effective tool for the extraction of heavy metals from soils by plants. However, side-effects related to the addition of chelates, e.g. metal leaching and effects on soil micro-organisms, were usually neglected. Therefore, greenhouse and lysimeter studies were conducted to study the phytoremedation potential of EDGA and citric acid and to evaluate its effects on microbial activity and leaching of Cd, Zn Cu and Pb. Grass, lupine and yellow mustard were grown on a moderately polluted acid (pH 4.5) sandy soil that contained 2 mg kg^{-1} Cd and 200 mg kg^{-1} Zn. Citric acid appeared to be degraded microbially within a few days after addition which limited its potential for long-lasting remediation studies. EDGA enhanced metal solubility but plant uptake did not increase accordingly. The metal shoot:root ratio increased upon addition of EDGA but it also reduced the net shoot and root biomass production of both lupine and yellow mustard. Bacterial biomass was

higher in both the citric and EDGA treated pots but bacterial activity remained unaffected. The number of microbivorous nematodes was greatly reduced upon addition of EDGA which was most likely related to the reduced biomass production and, to a smaller extent, to the changes in the composition of the available food. Furthermore, EDGA enhanced metal leaching in the lysimeter study which could lead to groundwater pollution. To prevent these unwanted side-effects, careful management of phytoremediation methods, therefore, seems necessary.

R. Margesin , G. Walder, F. Schinner. (Universität Innsbruck, Institut für Mikrobiologie, Technikerstrasse 25, 6020 Innsbruck, Austria). **Bioremediation Assessment of a BTEX-Contaminated Soil.** Acta Biotechnologica, 23(1) (2003), 29-36.

The elimination of BTEX (benzene, toluene, ethylbenzene, *o*-xylene) compounds from soil was studied. After 18 days at 20 °C, 21% of the initial total BTEX contamination (400 mg/kg soil) was lost due to sorption onto soil. Biodegradation decreased in the order ethylbenzene > toluene > benzene > *o*-xylene. NPK fertilisation stimulated biodegradation, particularly that of benzene and toluene, significantly, and oleophilic fertilisation inhibited biodegradation. After 18 days, the residual contamination in the NPK-fertilised, unfertilised and with oleophilic nutrients amended soil was 96, 166 and 196 mg total BTEX/kg soil, respectively. The presence of BTEX initially inhibited the biological activity of the soil (fluorescein diacetate hydrolysis) considerably. This short-term, reversible inhibition was significantly higher in the unfertilised soil than in the fertilised soil.

Robert M. Garrett, Stephen J. Rothenburger, Roger C. Prince. (ExxonMobil Research and Engineering Co., Annandale, NJ 08801, USA). **In-Situ Treatment and Fate of Oil Stranded on Coarse-Sediment Shorelines**. Spill Science & Technology Bulletin, 8(3) (2003), 297-302.

The aerobic biodegradation of the components of a fuel oil under Arctic summer conditions follows a pattern that is indistinguishable from that exhibited under temperate conditions. Straight chain alkanes and small aromatics are degraded first, followed by branched alkanes and larger and alkylated aromatics. We present data on the biodegradation of heptadecane as a representative n-alkane, pristane as a representative iso-alkane, and naphthalene, phenanthrene, and chrysene and their alkylated forms as representative two-, three- and four-ring aromatic hydrocarbons. In particular, the pattern of degradation of the alkylated aromatics allows the identification of biodegradation in samples collected from the field and the estimation of the extent of biodegradation that occurred in the In-Situ Treatment of Oiled Sediment Shorelines Field Trials.

Rutchadaporn Sriprang, Makoto Hayashi, Mitsuo Yamashita, Hisayo Ono, Kazuhiko Saeki, Yoshikatsu Murooka. (Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita-shi, Osaka 565-0871, Japan. Department of Biology, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka-shi, Osaka 560-0043, Japan). **A novel bioremediation system for heavy metals using the symbiosis between leguminous plant and genetically engineered rhizobia**. Journal of Biotechnology, 99(3) (2002), 279-293.

A novel plant–bacterial remediation system for heavy metals (HM) was developed by expression of tetrameric human metallothionein (MTL4) in Mesorhizobium huakuii subsp. rengei B3, a strain which infects and forms nodules on a green manure, Astragalus sinicus. The MTL4 gene was fused to the nifH and nolB promoters, which generated nodule- specific expression of the MTL4 gene. The expression analysis of the MTL4 gene was demonstrated in free-living cells in the presence of Cd2+ and Cu2+, under the low oxygen condition. The MTL4 under the nifH and nolB promoters was expressed and increased the accumulation of Cd2+, but not Cu2+ in free-living cells. The expression of the integrated nifH-MTL4 gene in the chromosome of strain B3

was also expressed stably and accumulated Cd2+ in the bacterial cells. The MTL4 transcripts were detected by in situ hybridization in bacteroids of mature nodules of A. sinicus containing nifH-MTL4 and nolB-MTL4 fusion gene. Moreover the MTL4 protein was detected by immunostaining. By infection of the recombinant B3, A. sinicus established symbiosis with the recombinant B3 that was grown in Cd2+ and Cu2+-polluted soils. The symbionts increased Cd2+ accumulation in nodules 1.7–2.0-fold, whereas, no significantly increase in Cu2+ accumulation was noted.

S. Kärenlampi, H. Schat, J. Vangronsveld, J. A. C. Verkleij, D. van der Lelie, M. Mergeay, A. I. Tervahauta. (Department of Biochemistry and Biotechnology, University of Kuopio, PO Box 1627, FIN-70211, Finland. Department of Ecology and Ecotoxicology, Faculty of Biology, Free University of Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, Netherlands. Limburgs Universitaire Centrum, Universitaire Campus, B-3590 Diepenbeek, Belgium. Vlaamse Instelling voor Technologisch Onderzoek (VITO), Boeretang 200, B-2400 Mol, Belgium). **Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils.** Environmental Pollution, 107(2) (2000), 225-231.

Metal concentrations in soils are locally quite high, and are still increasing due to many human activities, leading to elevated risk for health and the environment. Phytoremediation may offer a viable solution to this problem, and the approach is gaining increasing interest. Improvement of plants by genetic engineering, i.e. by modifying characteristics like metal uptake, transport and accumulation as well as metal tolerance, opens up new possibilities for phytoremediation. So far, only a few cases have been reported where one or more of these characteristics have been successfully altered; e.g. mercuric ion reduction causing improved resistance and phytoextraction, and metallothionein causing enhanced cadmium tolerance. These, together with other approaches and potentially promising genes for transformation of target plants are discussed.

S. Teodorova, a, R. Metchevab, M. Topashka-Anchevab. (Institute for Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko chaussee, 1784, Sofia, Bulgaria. Institute of Zoology, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel boul. 1000, Sofia, Bulgaria). **Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice Mus musculus alba**. Environmental Research, 91(2) (2003), 85-94.

The concentrations of Zn, Cu, Pb, and Cd in the liver, kidneys, spleen, bones, and carcass of laboratory mice BALB/cy were observed in toxicological experiments. Polymetal industrial dust containing these metals was given to experimental animals at 1% concentration mixed with conventional animal food. Samples for analyses were taken on Days 15, 40, 60, 90, and 120 posttreatment. The experimental data clearly support the established antagonistic interactions among cadmium, zinc, copper, and lead. A mathematical model was proposed to study the main tendencies of heavy metal bioaccumulation under conditions of metal interaction and excessive exposure. The experimental results were assessed on the basis of the model. A rate constant of renal excretion greater than that of hepatic excretion was obtained, which agrees with the observed inversion of cadmium kidney/liver ratio in the conditions of very high exposure.

Sanjeet Mishra, Jeevan Jyot, Ramesh Chander Kuhad, Banwari Lal. (Tata Energy Research Institute, New Delhi 110 003, India. Department of Microbiology, University of Delhi South Campus, New Delhi, India). **In Situ Bioremediation Potential of an Oily Sludge-Degrading Bacterial Consortium**. Current Microbiology, 43(5) (2001), 0328 – 0335.

A field-scale study was conducted in a 4000 m2 plot of land contaminated with an oily sludge by use of a carrier-based hydrocarbon-degrading bacterial consortium for bioremediation. The land belonged to an oil refinery. Prior to this study, a feasibility study was conducted to assess the

capacity of the bacterial consortium to degrade oily sludge. The site selected for bioremediation contained approximately 300 tons of oily sludge. The plot was divided into four blocks, based on the extent of contamination. Blocks A, B, and C were treated with the bacterial consortium, whereas Block D was maintained as an untreated control. In Block A, at time zero, i.e., at the beginning of the experiment, the soil contained as much as 99.2 g/kg of total petroleum hydrocarbon (TPH). The application of a bacterial consortium (1 kg carrier-based bacterial consortium/10 m2 area) and nutrients degraded 90.2% of the TPH in 120 days, whereas in block D only 16.8% of the TPH was degraded. This study validates the large-scale use of a carrier-based bacterial consortium and nutrients for the treatment of land contaminated with oily sludge, a hazardous hydrocarbon waste generated by petroleum industry.

Steve P McGrath, Fang-Jie Zhao. (Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK). **Phytoextraction of metals and metalloids from contaminated soils**. Current Opinion in Biotechnology, 14(3) (2003), 277-282.

The removal of inorganic contaminants by plants is termed phytoextraction. Recent studies have looked at the feasibility of phytoextraction, and demonstrate that both good biomass yields and metal hyperaccumulation are required to make the process efficient. Adding chelating agents to soil to increase the bioavailability of contaminants can sometimes induce hyperaccumulation in normal plants, but may produce undesirable environmental risks. Thus, it is necessary to investigate the mechanisms responsible for hyperaccumulation, using natural hyperaccumulators as model plant species. Recent advances have been made in understanding the mechanisms responsible for hyperaccumulation of Zn, Cd, Ni and As by plants. Attempts to engineer metal tolerance and accumulation have so far been limited to Hg, As and Cd, and although promising results have been obtained they may be some way from practical application. More fundamental understanding of the traits and mechanisms involved in hyperaccumulation are needed so that phytoextraction can be optimised.

Susan Eapen, K. N. Suseelan, Suchita Tivarekar, S. A. Kotwal, R. Mitra. (Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India). **Potential for rhizofiltration of uranium using hairy root cultures of Brassica juncea and Chenopodium amaranticolor**. Environmental Research, 91(2) (2003), 127-133.

Hairy root cultures of Brassica juncea and Chenopodium amaranticolor were developed by genetic transformation using Agrobacterium rhizogenes. The stable, transformed root systems demonstrated a high growth rate of 1.5–3.0 g/g dry weight/day in Murashige and Skoog medium. In the present study, hairy root system was used for removal of uranium from the solution of concentration up to 5000 μM. The results indicated that the hairy roots could remove uranium from the aqueous solution within a short period of incubation. B. juncea could take up 20–23% of uranium from the solution containing up to 5000 μM, when calculated on g/g dry weight basis. C. amaranticolor showed a slow and steady trend in taking up uranium, with 13% uptake from the solution of 5000 μM concentration. Root growth was not affected up to 500 μM of uranium nitrate over a period of 10 days.

Tobias Frische. (Centre for Environmental Research and Technology (UFT), University of Bremen, Leobener Straße, 28359, Bremen, Germany). **Ecotoxicological evaluation of in situ bioremediation of soils contaminated by the explosive 2,4,6-trinitrotoluene (TNT).** Environmental Pollution, 121(1) (2003), 103-113.

To evaluate the environmental relevance of in situ bioremediation of contaminated soils, effective and reliable monitoring approaches are of special importance. The presented study was conducted as part of a research project investigating in situ bioremediation of topsoils contaminated by the explosive 2,4,6-trinitrotoluene (TNT). Changes in soil toxicity within

different experimental fields at a former ordnance factory were evaluated using a battery of five bioassays (plant growth, Collembola reproduction, soil respiration, luminescent bacteria acute toxicity and mutagenicity test) in combination to chemical contaminant analysis. Resulting data reveal clear differences in sensitivities between methods with the luminescent bacteria assay performed with soil leachates as most sensitive toxicity indicator. Complete test battery results are presented in so-called soil toxicity profiles to visualise and facilitate the interpretation of data. Both biological and chemical monitoring results indicate a reduction of soil toxicity within 17 months of remediation.

Walter J. Fitz, Walter W. Wenzel. (Institute of Soil Science, University of Agricultural Sciences Vienna-BOKU, Gregor Mendel Strasse 33, A-1180, Vienna, Austria). **Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation**. Journal of Biotechnology, 99(3) (2002), 259-278.

This paper reviews major processes that potentially affect the fate of arsenic in the rhizosphere of plants. Rhizosphere interactions are deemed to play a key role in controlling bioavailability to crop plants and for a better understanding and improvement of phytoremediation technologies. Substantial progress has been made towards an understanding of As transformation processes in soils. However, virtually no information is available that directly addresses the fate of As in the rhizosphere. We are proposing a conceptual model of the fate of As in the soil–rhizosphere–plant system by integrating the state-of-the art knowledge available in the contributing disciplines. Using this model and recent studies on hyperaccumulation of As, we discuss research needs and the potential application of rhizosphere processes to the development of phytoremediation technologies for As-polluted soils.

Wan Namkoong, Eui-Young Hwang, Joon-Seok Park, Jung-Young Choi. (Department of Environmental Engineering, College of Engineering, Konkuk University, Seoul 143-701, South Korea. Institute of Technology, Kyong-Ho Engineering Co. Ltd., Kuri, 471-722, South Korea. Department of Environmental Industry, Shinsung College, Dangjin, 343-861, South Korea). **Bioremediation of diesel-contaminated soil with composting.** Environmental Pollution, 119(1) (2002), 23-31.

The major objective of this research was to find the appropriate mix ratio of organic amendments for enhancing diesel oil degradation during contaminated soil composting. Sewage sludge or compost was added as an amendment for supplementing organic matter for composting of contaminated soil. The ratios of contaminated soil to organic amendments were 1:0.1, 1:0.3, 1:0.5, and 1:1 as wet weight basis. Target contaminant of this research was diesel oil, which was spiked at 10,000 mg/kg sample on a dry weight basis. The degradation of diesel oil was significantly enhanced by the addition of these organic amendments relative to straight soil. Degradation rates of total petroleum hydrocarbons (TPH) and n-alkanes were the greatest at the ratio of 1:0.5 of contaminated soil to organic amendments on wet weight basis. Preferential degradation of n-alkanes over TPH was observed regardless of the kind and the amount of organic amendments. The first order degradation constant of n-alkanes was about twice TPH degradation constant. Normal alkanes could be divided in two groups (C10–C15 versus C16–C20) based on the first order kinetic constant. Volatilization loss of TPH was only about 2% of initial TPH. Normal alkanes lost by volatilization were mainly by the compounds of C10 to C16. High correlations (r=0.80–0.86) were found among TPH degradation rate, amount of CO_2 evolved, and dehydrogenase activity.

Weon Bae, Cindy H. Wu, Jan Kostal, Ashok Mulchandani, Wilfred Chen. (Department of Chemical and Environmental Engineering,1 Environmental Toxicology Program, University of California, Riverside, California 92521). **Enhanced Mercury Biosorption by Bacterial Cells with Surface-Displayed MerR**. Applied and Environmental Microbiology, 69(6) (2003), 3176-3180.

The metalloregulatory protein MerR, which exhibits high affinity and selectivity toward mercury, was exploited for the construction of microbial biosorbents specific for mercury removal. Whole-cell sorbents were constructed with MerR genetically engineered onto the surface of Escherichia coli cells by using an ice nucleation protein anchor. The presence of surface-exposed MerR on the engineered strains enabled sixfold-higher Hg2+ biosorption than that found in the wild-type JM109 cells. Hg2+ binding via MerR was very specific, with no observable decline even in the presence of 100-fold excess Cd2+ and Zn2+. The Hg2+ binding property of the whole-cell sorbents was also insensitive to different ionic strengths, pHs, and the presence of metal chelators. Since metalloregulatory proteins are currently available for a wide variety of toxic heavy metals, our results suggest that microbial biosorbents overexpressing metalloregulatory proteins may be used similarly for the cleanup of other important heavy metals.

William J Hunter. (USDA-ARS, P. O. Box E, Fort Collins, CO 80522, USA). **Bioremediation of Chlorate or Perchlorate Contaminated Water Using Permeable Barriers Containing Vegetable Oil.** Current Microbiology, 45(4) (2002), 287 – 292.

A scale model of an in situ permeable barrier, formed by injecting vegetable oil onto laboratory soil columns, was used to remove chlorate and perchlorate from flowing groundwater. The hypothesis that trapped oil would serve as a substrate enabling native microorganisms to reduce chlorate or perchlorate to chloride as water flowed through the oil-rich zone had merit. Approximately 96% of the 0.2 mM chlorate and 99% of the 0.2 mM perchlorate present in the water was removed as water was pumped through columns containing vegetable oil barriers. The product formed was chloride. When nitrate at 1.4 mM was added to the water, both nitrate and chlorate were removed. High concentrations of chlorate or perchlorate can be treated; 24 mM chlorate and 6 mM perchlorate were completely reduced to chloride during microcosm incubations. Microorganisms capable of reducing perchlorate are plentiful in the environment.

Y. Meriah Arias, Bradley M. Tebo. (Marine Biology Research Division and Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0202). **Cr(VI) Reduction by Sulfidogenic and Nonsulfidogenic Microbial Consortia**. Applied and Environmental Microbiology, 69(3) (2003), 1847-1853.

In time course experiments, bacterial community compositions were compared between a sulfidogenic and two nonsulfidogenic Cr(VI)-reducing consortia enriched from metal-contaminated sediments. The consortia were subjected to 0 and 0.85 mM or 1.35 mM Cr(VI), and Cr(VI) reduction, growth, and denaturing gradient gel electrophoresis profiles of PCR products of small-subunit (16S) ribosomal genes were compared. Results showed that although Cr(VI) was completely reduced by the three consortia, Cr(VI) inhibited cell growth, with sulfate-reducing bacteria being particularly sensitive to Cr(VI) toxicity relative to other bacteria in the consortia.

Biosensor

A. Guzmán-Vázquez de Prada, N. Peña, M. L. Mena, A. J. Reviejo and J. M. Pingarrón. (Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040, Madrid, Spain). **Graphite–Teflon composite bienzyme amperometric biosensors for monitoring of alcohols**. Biosensors and Bioelectronics, 18(10) (2003), 1279-1288.

Composite graphite–Teflon electrodes, in which the enzymes alcohol oxidase (AOD) and horseradish peroxidase (HRP), as well as the mediator ferrocene, are incorporated into the electrode matrix, are reported for the reliable monitoring of alcohols in food and beverages. The bienzyme electrodes are constructed by simple physical inclusion of the enzymes and the

mediator in the bulk of graphite–70% Teflon rigid cylindrical pellets. The composite biosensors are robust and reusable because of the renewability of the electrode surface by polishing. Reproducible amperometric responses at 0.00 V were obtained with different electrodes constructed from the same pellet and from different pellets. No significant loss of the enzymes activities was found after at least 3 months of storage at 0 °C. The detection limits obtained by amperometry in stirred solutions can be advantageously compared with those achieved with commercial sensors for alcohols. The bienzyme electrodes are suitable to be used under flow-injection conditions, as well as for amperometric detection in HPLC. The bioelectrodes were employed for the determination of ethanol in beers, wines and liquors, using both batch- and flow-injection modes, and for the determination of methanol in wines and liquors by HPLC with amperometric detection. Only a dilution of the beverages was needed as sample treatment in all cases.

A. R. Linde, S. Sánchez-Galán, P. Vallés-Mota, E. García-Vázquez. (Departamento de Biología Funcional, Area de Genética, Facultad de Medicina, Facultad de Química, C/ Julián Clavería, s/n, 33006, Oviedo, Spain. Area de Genética, Facultad de Medicina, Departamento de Química Física y Analítica, Facultad de Química, C/ Julián Clavería, s/n, 33006, Oviedo, Spain). **Metallothionein as Bioindicator of Freshwater Metal Pollution: European Eel and Brown Trout.** Ecotoxicology and Environmental Safety, 49(1) (2001), 60-63.

The aim of this work was to evaluate the potential of metallothionein (MT) as a bioindicator of heavy metal pollution in brown trout and European eel in the field situation. River Ferrerias (North Spain) provided a good gradient of metal contamination: concentrations of heavy metals were elevated both in water and in sediments at the downstream (polluted) site and were low at the upstream (unpolluted) site. MT levels of brown trout exhibited statistically significant differences between sites. Although European eel at the polluted site had a higher MT content, differences were not significant. It is concluded that MT is a good bioindicator of heavy metal pollution in brown trout.

Ajit Sadana. (Chemical Engineering Department, University of Mississippi, Post Office Box 1848, University, MS 38677-1848, USA). **A fractal analysis of protein to DNA binding kinetics using biosensors.** Biosensors and Bioelectronics, 18(8) (2003), 985-997.

A fractal analysis of a confirmative nature only is presented for the binding of estrogen receptor (ER) in solution to its corresponding DNA (estrogen response element, ERE) immobilized on a sensor chip surface [J. Biol. Chem. 272 (1997) 11384], and for the cooperative binding of human 1,25-dihydroxyvitamin D_3 receptor (VDR) to DNA with the 9-*cis*-retinoic acid receptor (RXR) [Biochemistry 35 (1996) 3309]. Ligands were also used to modulate the first reaction. Data taken from the literature may be modeled by using a single- or a dual-fractal analysis. Relationships are presented for the binding rate coefficient as a function of either the analyte concentration in solution or the fractal dimension that exists on the biosensor surface. The binding rate expressions developed exhibit a wide range of dependence on the degree of heterogeneity that exists on the surface, ranging from sensitive (order of dependence equal to 1.202) to very sensitive (order of dependence equal to 12.239). In general, the binding rate coefficient increases as the degree of heterogeneity or the fractal dimension of the surface increases. The predictive relationships presented provide further physical insights into the reactions occurring on the biosensor surface. Even though these reactions are occurring on the biosensor surface, the relationships presented should assist in understanding and in possibly manipulating the reactions occurring on cellular surfaces.

Alfred Fürst, Stefan Smidt, Friedl Herman. (Federal Office and Research Centre for Forests, A-1130 Vienna, Seckendorff-Gudent-Weg 8, Austria). **Native Plants as Bioindicators of**

Air Pollutants: Contributed Papers to a Symposium held in conjunction with the 34th Air Pollution Workshop. Environmental Pollution, 125(1) (2003), 13-19.

In Austria, the impact of sulphur has been assessed since 1985 with the help of the Austrian Bioindicator Grid on 760 sample plots with *Picea abies* as the main tree species (90%). The annual sampling allows a precise evaluation of the temporal and regional development of the impact of sulphur on the basis of legal standards. Despite the reduction of SO_2 emissions in Austria, the legal standard is still exceeded on 8% of the plots. These plots are mainly located near large Austrian emittors, but also in areas affected by transboundary sulphur emissions from neighbouring countries. The present paper describes how the Bioindicator Grid can be applied for the control of legal requirements to enact effective clean air measures in Austria and take supportive measures that reduce the impact of sulphur from emittors in neighbouring countries.
Andreas Klumpp, Therese Hintemann, Josanídia Santana Lima, Ellen Kandeler. (Institute for Landscape and Plant Ecology (320), University of Hohenheim, D-70593, Stuttgart, Germany. Institute for Soil Science (310), University of Hohenheim, D-70593, Stuttgart, Germany. Institute for Biology, Federal University of Bahia (UFBa), 40170-290, Salvador, BA, Brazil). **Bioindication of air pollution effects near a copper smelter in Brazil using mango trees and soil microbiological properties.** Environmental Pollution, 126(3) (2003), 313-321.

A field study near the copper smelter of a large industrial complex examined air pollution effects on vegetation and soil parameters in Camaçari (northeast Brazil). Close to the smelter, soil pH-value was lower and total acidity as well as organic carbon contents were higher compared with a site far from the source and two reference sites. The acidification of top soil particularly and the drastically enhanced plant-available copper concentrations were caused by atmospheric deposition. High sulphur and copper deposition significantly reduced microbial biomass and altered functional diversity of soil microorganisms (arylsulphatase and xylanase). Large accumulations of sulphur, arsenic and copper were detected in mango leaves (*Mangifera indica*) growing downwind from the smelter suggesting potential food chain-mediated risk.
B. Bhattacharya, S. K. Sarkar, R. Das. (Department of Metallurgical Engineering, Jadavpur University, Kolkata 700032, India. Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700019, India). **Seasonal variations and inherent variability of selenium in marine biota of a tropical wetland ecosystem: implications for bioindicator species**. Ecological Indicators, 2(4) (2003), 367-375.

The present study was designed to study the seasonal variations of selenium in marine biota of different trophic levels collected from Sunderban deltaic complex, northeast India. The primary objective of this work is to provide baseline data for a future environmental quality programme. In general, the sequence of Se accumulation observed in biota is as follows: Bivalve>Zooplankton>Macroalgae>Pisces>Seagrass. An elevated level of Se was recorded during monsoon season. The Se level in zooplankton (~4 μg/g) was three to four times higher than seagrass. The content of various soft tissues and shell in bivalves molluscs showed an organ-specific pattern in the following order: gill > visceral MASS = mantle > adductor muscle > podium > shell. Fish contain less Se in comparison with macroalgae, zooplankton and bivalves revealing poor or insignificant trophic transfer of Se in the marine food chain. *Pelecyora trigona* (Bivalve) seems to be reliable indicator of Se contamination because of its high accumulation capacity. An in-depth monitoring program is recommended in order to clarify the present trend and to establish the studied biota as indicator species.
Byung Hong Kim, In Seop Chang, Geun Cheol Gil, Hyung Soo Park, Hyung Joo Kim. (Water Environment and Remediation Research Centre, Korea Institute of Science and Technology, 39-1, Hawolgok-dong, Sungpook-ku, Seoul 136-791, Korea Fax: +82-2-958-5839). **Novel**

BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. Biotechnology Letters, 25(7) (2003), 541-545.

A microbial fuel cell type of biosensor was used to determine the biochemical oxygen demand (BOD) of wastewater. The biosensor gave a good correlation between the BOD value and the coulomb produced. The BOD sensor has been operated for over 5 years in a stable manner without any servicing. This is much longer that that of previously reported BOD biosensors.
C. Brandon Davis, Lisa M. Shamansky, Steven Rosenwald, Joan K. Stuart, Werner G. Kuhr and Sara A. Brazill. (Department of Chemistry, University of California, Riverside, CA 92521, USA). **Independently-addressable micron-sized biosensor elements**. Biosensors and Bioelectronics, 18(10) (2003), 1299-1307.

With the continuing development of micro-total analysis systems and sensitive biosensing technologies, it is often desirable to immobilize biomolecules onto a surface in a small well-defined area. A novel method was developed to electrochemically attach DNA probes to micron-sized regions of a gold surface using biotin-LC-hydrazide (BH). Previously, we have found that the radical produced during the oxidation of BH will attach to a wide variety of electroactive surfaces. An array of micron-sized gold band electrodes (75 μm wide) was fabricated onto glass microscope slides and BH was deposited onto each electrode through the application of an oxidizing potential. Subsequent attachment of avidin to the biotinylated surface created the `molecular sandwich' architecture necessary for further immobilization of biotinylated biomolecules to the surface. In this work, we utilized biotinylated DNA probes of varying sequence to illustrate the specificity of the attachment scheme. The immobilization of avidin, DNA probe, and hybridization of DNA target is visualized with fluorescence tags and the spatially selective attachment and hybridization of unique DNA sequences is demonstrated.
C. Tibazarwa, P. Corbisier, M. Mench, A. Bossus, P. Solda, M. Mergeay, L. Wyns and D. van der Lelie. (Environmental Technology Expertise Centre, Flemish Institute of Technological Research (Vito), Boeretang 200, B-2400 Mol, Belgium. Department of Ultrastructure, Free University of Brussels, Flemish Interuniversity Institute of Biotechnology (VIB), Paardenstraat 65,B-1640 St-Genesius-Rode, Belgium. Agronomy Unit, Inra Bordeaux Aquitaine Research Center, BP 81, 33883 Villenave d'Ornon, France). **A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants.** Environmental Pollution, 113(1) (2001), 19-26.

Ralstonia eutropha strain AE2515 was constructed and optimised to serve as a whole-cell biosensor for the detection of bioavailable concentrations of Ni^{2+} and Co^{2+} in soil samples. Strain AE2515 is a *Ralstonia eutropha* CH34 derivative containing pMOL1550, in which the *cnr*YXH regulatory genes are transcriptionally fused to the bioluminescent *lux*CDABE reporter system. Strain AE2515 was standardised for its specific responses to Co^{2+} and Ni^{2+}. The detection limits for AE2515 were 0.1 μM Ni^{2+} and 9 μM Co^{2+}, respectively. The signal to noise (S/N) bioluminescence response and the metal cation concentration could be linearly correlated: for Ni^{2+} this was applicable within the range 0.1–60 μM, and between 9 and 400 μM for Co^{2+}. The AE2515 biosensor strain was found to be highly selective for nickel and cobalt: no induction was observed with Zn(II), Cd(II), Mn(II), Cu(III) and Cr(VI). In mixed metal solutions, the bioluminescent response always corresponded to the nickel concentrations. Only in the presence of high concentrations of Co^{2+} (2 mM), the sensitivity to nickel was reduced due to metal toxicity. AE2515 was used to quantify the metal bioavailability in various nickel-enriched soils, which had been treated with additives for in situ metal immobilisation. The data obtained with strain AE2515 confirmed that the bioavailability of nickel was greatly reduced following the treatment of the soils with the additives beringite and steel shots. Furthermore, the data were found to correlate linearly with those on the biological accumulation of Ni^{2+} in specific parts of

important agricultural crops, such as maize and potato. Therefore, the test can be used to assess the potential transfer of nickel to organisms of higher trophic levels, in this case maize and potato plants grown on nickel-enriched soils, and the potential risk of transfer of these elements to the food chain.

Christopher Rensing, Raina M. Maier. (Department of Soil, Water, and Environmental Science, University of Arizona, Room 429, Shantz Boulevard # 38, Tucson, AZ 85721, USA). **Issues underlying use of biosensors to measure metal bioavailability.** Ecotoxicology and Environmental Safety, 56(1) (2003), 140-147.

Heavy metal-mediated toxicity in the environment is dependent on bioavailable metal concentrations both internal and external to microbial cells. Both internal and external metal bioavailability are influenced by multiple factors in the soil environment. External factors include pH, redox potential, ionic strength, organic matter and clay content. The internal bioavailable metal concentration is dependent on both the aforementioned external factors, as well as metal uptake and efflux activities that are specific for each microorganism. The metal-specific biosensors discussed in this article can be used to measure internal metal bioavailability.

Claude Durrieu, Canh Tran-Minh. (Laboratoire des Sciences de l'Environnement, Ecole Nationale des Travaux Publiques de l'Etat, 3 Rue Maurice Audin, 69518, Vaulx en Velin cedex, France. Centre SPIN/Génie Enzymatique, Ecole Nationale Supérieure des Mines, 158 Cours Fauriel, 42023, Saint Etienne cedex 2, France). **Optical Algal Biosensor using Alkaline Phosphatase for Determination of Heavy Metals.** Ecotoxicology and Environmental Safety, 51(3) (2002), 206-209.

A biosensor is constructed to detect heavy metals from inhibition of alkaline phosphatase (AP) present on the external membrane of *Chlorella vulgaris* microalgae. The microalgal cells are immobilized on removable membranes placed in front of the tip of an optical fiber bundle inside a homemade microcell. *C. vulgaris* was cultivated in the laboratory and its alkaline phosphatase activity is strongly inhibited in the presence of heavy metals. This property has been used for the determination of those toxic compounds.

D. E. Yuska, J. M. Skelly, J. A. Ferdinand, R. E. Stevenson, J. E. Savage, J. D. Mulik, A. Hines. (Environmental Pollution Control, Graduate Program, The Pennsylvania State University, University Park, PA 16802, USA. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA. Environmental Resources Research Institute, The Pennsylvania State University, University Park, PA 16802, USA. Atmospheric Research and Assessment Lab, Exposure Methods and Monitoring Branch USEPA, Research Triangle Park, NC 27711, USA). **Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania**. Environmental Pollution, 125(1) (2003), 71-80.

Ambient concentrations of tropospheric ozone and ozone-induced injury to black cherry (*Prunus serotina*) and common milkweed (*Asclepias syriaca*) were determined in north central Pennsylvania from 29 May to 5 September 2000 and from 28 May to 18 September 2001. Ogawa passive ozone samplers were utilized within openings at 15 forested sites of which six were co-located with TECO model 49 continuous ozone monitors. A significant positive correlation was observed between the Ogawa passive samplers and the TECO model 49 continuous ozone monitors for the 2000 (r=0.959) and 2001 (r=0.979) seasons. In addition, a significant positive correlation existed in 2000 and 2001 between ozone concentration and elevation (r=0.720) and (r=0.802), respectively. Classic ozone-induced symptoms were observed on black cherry and common milkweed. In 2000, initial injury was observed in early June, whereas for the 2001 season, initial injury was initially observed in late June. During both seasons, injury was noted at most sites by mid- to late-July. Soil moisture potential was measured for the 2001 season and a significant positive relationship (P<0.001) showed that

injury to black cherry was a function of cumulative ozone concentrations and available soil moisture.

E. Rizzio, L. Bergamaschi, M. G. Valcuvia, A. Profumo, M. Gallorini. (CNR Centro di Radiochimica e Analisi per Attivazione, V.le Taramelli 12, 27100 Pavia, Italy. Dip. di Ecologia del Territorio, Università di Pavia, 27100 Pavia, Italy). **Trace elements determination in lichens and in the airborne particulate matter for the evaluation of the atmospheric pollution in a region of northern Italy**. Environment International, 26(7-8) (2001), 543-549.

Lichens as biomonitors and neutron activation analysis as analytical technique have been employed to study the distribution of trace elements (TE) in a mountain region of north Italy (Biella) characterized by settlements of wool industry. Samples of airborne particulate matter collected onto filters, different species of lichens and samples of soils have been analyzed for the calculation of the enrichment factors (EFs) of more than 25 TE. By comparison of the corresponding EFs, the most suitable lichen species (*Parmelia caperata*) was selected as specific TE biomonitor of the area investigated. Samples of this lichen were collected and analyzed for the evaluation of the TE distribution in four different locations. The results obtained from the analysis of the lichens gave information about the predominant direction of pollutants transportation, while those related to the concentrations found in the air particulate allowed the evaluation of the degree of the local TE atmospheric pollution.

Feng Hong, Nils-Olof Nilvebrant, Leif J. Jönsson. (Department of Applied Microbiology, Lund University/Lund Institute of Technology, P.O. Box 124, SE-22100, Lund, Sweden. Biochemistry, Division for Chemistry, Karlstad University, SE-65188, Karlstad, Sweden. STFI, Swedish Pulp and Paper Research Institute, P.O. Box 5604, SE-11486, Stockholm, Sweden). **Rapid and convenient determination of oxalic acid employing a novel oxalate biosensor based on oxalate oxidase and SIRE technology**. Biosensors and Bioelectronics, 18(9) (2003), 1173-1181.

A new method for rapid determination of oxalic acid was developed using oxalate oxidase and a biosensor based on SIRE (sensors based on injection of the recognition element) technology. The method was selective, simple, fast, and cheap compared with other present detection systems for oxalate. The total analysis time for each assay was 2–9 min. A linear range was observed between 0 and 5 mM when the reaction conditions were 30 °C and 60 s. The linear range and upper limit for concentration determination could be increased to 25 mM by shortening the reaction time. The lower limit of detection in standard solutions, 20 μM, could be achieved by means of modification of the reaction conditions, namely increasing the temperature and the reaction time. The biosensor method was compared with a conventional commercially available colorimetric method with respect to the determination of oxalic acid in urine samples. The urine oxalic acid concentrations determined with the biosensor method correlated well (R=0.952) with the colorimetric method.

Francesco S. Violante, Giovanni Sanguinetti, Anna Barbieri, Antonio Accorsi, Stefano Mattioli, Rossano Cesari, Carmela Fimognari, Patrizia Hrelia. (Occupational Health Unit, Policlinico Sant' Orsola-Malpighi Hospital, Via Palagi 9, Bologna 40138, Italy. Department of Pharmacology, University of Bologna, Bologna, Italy). **Lack of correlation between environmental or biological indicators of benzene exposure at parts per billion levels and micronuclei induction.** Environmental Research, 91(3) (2003), 135-142.

Despite growing concern for possible carcinogenic effects associated with environmental benzene exposure in the general population, few studies exist at parts per billion (ppb) levels. We investigated the existence of a relationship between airborne/biological measurements of benzene exposure (i.e., personal/area sampling and unmodified urinary benzene/*trans,trans*-muconic acid; t,t-MA) and micronuclei induction (cytochalasin B technique) among exposed

chemical laboratory workers (n=47) and traffic wardens (n=15). Although urinary t,t-MA (106.9±123.17 μg/L_{urine}) correlated (R^2=0.37) with urinary benzene (0.66±0.99 μg/L_{urine}), neither biological measurement correlated with environmental benzene exposure (14.04±9.71 μg/m^3; 4.39±3.03 ppb), suggesting that, at ppb level (1 ppb=3.2 μg/m^3), airborne benzene constitutes a fraction of the total intake. Traffic wardens and laboratory workers had comparable numbers of micronuclei (4.70±2.63 versus 5.76±3.11; n.s.), similar to levels recorded in the general population. With univariate/multivariate analysis, no association was found between micronuclei induction and air/urinary benzene exposure variables. Notably, among the personal characteristics examined (including age, gender, smoking, drinking, etc.), high body mass index correlated with micronuclei induction while, among females, use of hormonal medication was associated with less micronuclei. Thus the present study provides no evidence that ppb levels of environmental benzene exposure appreciably affect micronuclei incidence (against the background of other relevant factors). However, this should not be taken as an argument against efforts aiming to reduce environmental benzene pollution.

Frieder W Scheller, Ulla Wollenberger, Axel Warsinke, Fred Lisdat. (University of Potsdam, Institute of Biochemistry and Biology, Karl-Liebknecht-Strasse 24-25, 14476 Golm, Germany). **Research and development in biosensors**. Current Opinion in Biotechnology, 12(1) (2001), 35-40.

Progress in biosensors has mainly been made by the improvement of the biological components and the implementation of microsystem technologies. Enzymes are still the most appropriate recognition elements because they combine high chemical specificity and inherent biocatalytic signal amplification. A breakthrough has been achieved in the application of membrane-integrated receptor systems for analyte recognition and signal transduction in biosensors. Sensor integration of RNA aptamers has been initiated, and the performance of fully synthetic molecularly imprinted polymers has been improved.

G. Kirchner, O. Daillant. (University of Bremen, FB 1, Postfach 330440, D-28334 Bremen, Germany. Observatoire Mycologique, F-71250 Mazille, France). **The potential of lichens as long-term biomonitors of natural and artificial radionuclides.** Environmental Pollution, 120(1) (2002), 145-150.

Lichens were used as biomonitors of Chernobyl fallout ^{137}Cs, of cosmogenic ^{7}Be and of radioactive members of the natural uranium and thorium decay chains. Samples were taken from two locations in France, including lichens sampled at different distances of a coal fired power plant and close to a uranium ore processing waste disposal site. All samples were analyzed gamma-spectrometrically after equilibrium concentrations of short-lived isotopes had been attained. Activity concentrations of the members of the uranium and thorium decay chains in *Parmelia sulcata* sampled 1994 decrease with distance from the plant, whereas in lichens taken at the waste disposal site a decrease with time was observed. Comparison of ^{7}Be activity concentrations measured in lichens with atmospheric deposition rates confirms that *P. sulcata* can be used as a quantitative biomonitor of radioactive trace substances. Retention half-lives calculated with a simple one-compartment model are 2.6±1.2 years for cesium, which was detected in all samples even more than a decade after the Chernobyl accident, and of 0.7 (±0.1) to 3.3 (±0.7) years for lead. Consequences of our results for model identifiability and parameter estimation of a two-compartment model are discussed.

G. Thouand, H. Horry, M. J. Durand, P. Picart, L. Bendriaa, P. Daniel, M. S. DuBow. (Laboratoire de Capteur Bactérien pour l'Analyse et le Contrôle, département Génie Biologique IUT de la Roche-sur-Yon Cedex, Université de Nantes 18 Bd G. Defferre 85035 La Roche-sur-Yon Cedex France. École Nationale Supérieure d'Ingénieurs du Mans rue Aristote 72085 Le Mans Cedex 9 France. Laboratoire d'Acoustique de l'Université du Maine UMR

CNRS 6613 Avenue Olivier Messiaen 72085 Le Mans Cedex 9 France. Laboratoire de Physique de l'État Condensé UMR CNRS 6087 Avenue Olivier Messiaen 72085 Le Mans Cedex 9 France. Institut de Génétique et de Microbiologie Université Paris-Sud Bâtiment 409 91405 Orsay Cedex France). **Development of a biosensor for on-line detection of tributyltin with a recombinant bioluminescent Escherichia coli strain**. Applied Microbiology and Biotechnology, 62(2-3) (2003), 218 – 225.

A biosensor was developed for the detection of tributyltin (TBT), using a bioluminescent recombinant Escherichia coli:: luxAB strain. Dedicated devices allowed the on-line measurement of bioluminescence, pH and dissolved oxygen values and the feed-back regulation of temperature. Bacterial physiology was monitored by the measurement of the cellular density, respiratory activity and the intracellular level of ATP, glucose and acetate levels. Our results showed that a synthetic glucose medium gave a better TBT detection limit than LB medium (respectively 0.02 µM and 1.5 µM TBT). High growth and dilution rates (D=0.9 h -1) allowed maximum light emission from the bacterium. Moreover, simple atmospheric air bubbling was sufficient to provide oxygen for growth and the bioluminescence reaction. Real-time monitoring of bioluminescence after TBT induction occurred with continuous addition of decanal up to 300 µM, which was not toxic throughout a 7-day experiment. The design of our biosensor and the optimization of the main parameters that influence microbial activity led to the capacity for the detection of TBT.

Glenn W. Suter, II. (United States Environmental Protection Agency, National Center for Environmental Assessment, 26 West Martin Luther King Drive, Cincinnati, OH 45268, USA). **Applicability of indicator monitoring to ecological risk assessment**. Ecological Indicators, 1(2) (2001), 101-112.

Although ecological risk assessment (ERA) and environmental monitoring would seem to be potentially complimentary activities, they have been disjunct in practice. This is because of differences in goals and products. Environmental monitoring determines status and trends in indicators to determine whether the environment is improving. ERA estimates effects of stressors on endpoint attributes to support decision making. Indicators are, by definition, indicative of some unmeasured condition. Assessment endpoints are valued properties of the environment that are susceptible to stressors of concern. Indicators are justified by the logic of the monitoring program, which may be self-referential. Assessment endpoints are justified by their potential susceptibility and by environmental policies and public values. Indicators are often expressed in terms of indices or scores that obscure the actual condition of the environment. Because assessment endpoints must be clear to decision makers and the public, they require real units of actual environmental properties. Monitoring programs are peripherally concerned about causal relationships, while risk assessment is devoted to elucidating causal relationships. As a result, risk assessments may use the results of monitoring studies, but only after disaggregating the indicators to their components and choosing those that are appropriate. Monitoring programs could be more useful if they used a risk-based approach to address important problems rather than simply tracking indicators.

H. Kinnunen, T. Holopainen, L. Kärenlampi. (Department of Ecology and Environmental Science, University of Kuopio, P.O. Box 1627, FIN-70211, Kuopio, Finland. Department of Biology/Botany, University of Oulu, P.O. Box 3000, FIN-90014, Oulu, Finland). **Sources of error in epiphytic lichen variables mapped as bioindicators: needs to modify the Finnish standard**. Ecological Indicators, 3(1) (2003) 1-11.

A standardized lichen mapping method, to assess the environmental quality in urban and industrial areas, has been used in Finland since 1990. Comparisons of the data collected by different field workers showed large variation in both numbers of species recorded and their

observed frequencies. It is recommended that a reduced number of species, which are easily and reliably identified, should be used when assessing environmental quality, and correspondingly, the standard method should be revised.

Hiroaki Suzuki, Ayumi Kumagai. (Institute of Materials Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan). **A disposable biosensor employing a glucose-sensitive biochemomechanical gel.** Biosensors and Bioelectronics, 18(10) (2003), 1289-1297.

A novel approach to construct a disposable biosensor is proposed. By immobilizing glucose oxidase in a pH-sensitive copolymerized poly(*N*-isopropylacrylamide)/acrylic acid gel, a glucose-sensitive gel can be obtained. When the gel makes contact with a sample solution containing glucose, the pH of the gel decreases as a result of the enzymatic reaction, inducing the collapse of the gel. The response time to the shrinkage depended upon the activity of the enzyme and the concentration of glucose, although the final ratio of shrinkage settled to approximately the same value irrespective of the concentration of glucose. The transient stage can be used for sensing. The volume change of the gel was transduced into the movement of a colored solution in a flow channel with the help of a silicone rubber diaphragm. The mechanism was used to construct a disposable glucose sensor. A clear dependence of the column length of the colored solution on the concentration of glucose was observed. By measuring the change at a predetermined time, the glucose concentration was obtained. This sensor can be considered the ultimate style in disposable sensors, as detection does not require any electrical measurement or spectroscopic procedures.

Hussein S. Hussein, Norman Terry. (Department of Environmental Sciences, Faculty of Science, Alexandria University, Alexandria, Egypt. Department of Plant and Microbial Biology, University of California, Berkeley, CA USA). **Phytomonitoring the unique colonization of oil-contaminated saline environment by *Limoniastrum monopetalum* (L.) Boiss in Egypt.** Environment International, 28(1-2) (2002), 127-135.

A site that covers over 20 acres of coastal saline depression in the western Mediterranean coastal desert of Egypt (El-Hammra station, the main crude oil pipeline terminal in Al-Alamein) is contaminated with crude oil spill as a result of activities from refineries, oilfield blowouts, tanker and pipeline break-ups. This area, prior to contamination, was dominated by different common halophytes. However, *Limoniastrum monopetalum* is now the only species found growing in the oil-contaminated soil. A specific question addressed in the present study was: what are the biochemical changes occurring in a desert plant growing in oil-contaminated soils? Major metabolites such as proline, betaine, free amino acids, fatty acid esters and mineral elements were studied. The plant samples were collected from the oil-contaminated, as well as noncontaminated, sites. The higher concentration in the selected organic metabolites in the plants growing in the contaminated site compared to those in noncontaminated site may be due to differences in a number of receptors. The sensitivity of such receptors for the environmental signal that cause differences in genetic expression leads to differences in physiological processes. The change in the landscape of the contaminated area and the elimination of the natural vegetation, except *L. monopetalum*, may explain the competitive balance toward the oil-resistant species.

J. S. Lima, E. B. Fernandes, W. N. Fawcett. (Universidade Federal da Bahia, Instituto de Biologia, 40170, 290 Salvador-Ba, Brasil. Kings College, London). ***Mangifera indica* and *Phaseolus vulgaris* in the Bioindication of Air Pollution in Bahia, Brazil**. Ecotoxicology and Environmental Safety, 46(3) (2000), 275-278.

In this article are reported the results of a study on the concentration of ascorbic acid (AA) in *Mangifera indica* as passive monitor and in *Phaseolus vulgaris* as active monitor with the

intention to study the effects of industrial emissions from the Petrochemical Complex of Camaçari (PCC), Bahia, Brazil, on the vegetation. Leaves from *M. indica* were collected in two sites in the region under direct influence of industrial emissions and in one presumed nonpolluted reference (background) site. Pots with *P. vulgaris* were exposed in the same sites. The AA increase in the leaves of *M. indica* from PCC sites indicates a stress situation. The small AA increase in the *P. vulgaris* exposed in the more polluted site indicates that the active monitor is a sensitive one. The decrease of its leaf area indicates the inability of this specie to activate physiological protection mechanisms like an increase in AA production.

J. W. Ferry Slik, Paul J. A. Keßler, Peter C. van Welzen. (Nationaal Herbarium Nederland, Universiteit Leiden Branch, P.O. Box 9514, 2300, RA Leiden, The Netherlands). ***Macaranga* and *Mallotus* species (Euphorbiaceae) as indicators for disturbance in the mixed lowland dipterocarp forest of East Kalimantan (Indonesia)**. Ecological Indicators, 2(4) (2003), 311-324.

The indicator value (IV) of *Macaranga* and *Mallotus* species (Euphorbiaceae) for different types of disturbance in lowland dipterocarp forest was assessed by counting and identifying all individuals of species of these genera taller than 30 cm in 45 (10 m×300 m) plots at nine locations. Twelve *Macaranga* and nine *Mallotus* species were found. The main forest disturbance types (primary forest, secondary forest, selectively logged forest, forest burned once, and repeatedly burned forest used for shifting-cultivation) each had their own set of indicator species. The level of disturbance in the forest types was assessed by measuring nine forest structural parameters. The occurrence of *Macaranga* and *Mallotus* species was closely related to the level of disturbance in a forest. Most *Macaranga* species were characteristic of high disturbance levels, while most *Mallotus* species preferred intermediate to low levels of disturbance. However, both genera had species at both disturbance extremes. Using multiple regression analysis, combinations of *Macaranga* and *Mallotus* species were formed and used to predict the separate forest structural parameters and the general level of disturbance of a forest. The *Macaranga* and *Mallotus* species could be grouped into (1) primary forest `remnant' species; (2) generalist pioneer species; and (3) high disturbance pioneer species.

Jacqueline Muñoz Cifuentes, Peter H. Becker, Ute Sommer, Patricia Pacheco, Roberto Schlatter. (Institut für Vogelforschung "Vogelwarte Helgoland", An der Vogelwarte 21, D-26386, Wilhelmshaven, Germany. Aquatic Systems Research Unit, EULA-Chile Centre, University of Concepción, Post Office Box 160-C, Concepción, Chile. Instituto de Zoología, Universidad Austral de Chile, Post office Box 567, Valdivia, Chile). **Seabird eggs as bioindicators of chemical contamination in Chile.** Environmental Pollution, 126(1) (2003), 123-137.

Seabird eggs were used as bioindicators of chemical contamination in Chile. Brown-hooded Gull (*Larus maculipennis*), Kelp Gull (*Larus dominicanus*), Trudeau's Tern (*Sterna trudeaui*), Neotropic Cormorant (*Phalacrocorax brasilianus*), and Pink-footed Shearwater (*Puffinus creatopus*) eggs were sampled at different breeding sites during the 1990s. Mercury and organochlorines (PCBs, DDT, HCB, HCH, and PCP) were quantified to reveal the interspecific differences, spatial and temporal trends in contamination levels. Trudeau's Tern displayed the highest levels of mercury (486 ng g^{-1} wet weight). The highest ΣDDT concentrations were measured in Brown-hooded Gulls (726 ng g^{-1}). PCB levels were similar among the species (102–236 ng g^{-1}), but the composition of the PCB mixture was different in Pink-footed Shearwaters. With the exception of the Brown-hooded Gull, all species studied presented similar and low levels of organochlorines (ΣOHa). Residues of PCB and related compounds were not detected in any of the seabird eggs analyzed in Chile. Geographical variation was low, although levels of industrial chemicals were slightly higher in eggs from Concepción Bay, and agricultural

chemicals in eggs from Valdivia. Also interannual variation was low, but some evidence was found of decreasing levels in gull eggs throughout the time of the study. The causes of the low levels and small variability in space and time of environmental chemicals in Chilean seabirds are discussed. We propose the use of seabirds in future monitoring of the development of chemical contamination in Chile.

Jan Tkac, Igor Vostiar, Lo Gorton, Peter Gemeiner, Ernest Sturdik. (Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, SK-842 38, Bratislava, Slovak Republic. Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinskeho 9, SK-812 37, Bratislava, Slovak Republic. Department of Analytical Chemistry, Lund University, PO Box 124, SE-22 100, Lund, Sweden). **Improved selectivity of microbial biosensor using membrane coating. Application to the analysis of ethanol during fermentation**. Biosensors and Bioelectronics, 18(9) (2003), 1125-1134.

A ferricyanide mediated microbial biosensor for ethanol detection was prepared by surface modification of a glassy carbon electrode. The selectivity of the whole *Gluconobacter oxydans* cell biosensor for ethanol determination was greatly enhanced by the size exclusion effect of a cellulose acetate (CA) membrane. The use of a CA membrane increased the ethanol to glucose sensitivity ratio by a factor of 58.2 and even the ethanol to glycerol sensitivity ratio by a factor of 7.5 compared with the use of a dialysis membrane. The biosensor provides rapid and sensitive detection of ethanol with a limit of detection of 0.85 μM (S/N=3). The selectivity of the biosensor toward alcohols was better compared to previously published enzyme biosensors based on alcohol oxidase or alcohol dehydrogenases. The biosensor was successfully used in an off-line monitoring of ethanol during batch fermentation by immobilized *Saccharomyces cerevisiae* cells with an initial glucose concentration of 200 g l^{-1}.

Katharine Kierek, Paula I. Watnick. (Division of Geographic Medicine and Infectious Diseases, New England Medical Center, Boston, Massachusetts 02111). **Environmental Determinants of Vibrio cholerae Biofilm Development**. Applied and Environmental Microbiology, 69(9) (2003), 5079-5088.

Vibrio cholerae is a versatile bacterium that flourishes in diverse environments, including the human intestine, rivers, lakes, estuaries, and the ocean. Surface attachment is believed to be essential for colonization of all of these natural environments. Previous studies have demonstrated that the vps genes, which encode proteins required for exopolysaccharide synthesis and transport, are required for V. cholerae biofilm development in Luria-Bertani broth. In this work, we showed that V. cholerae forms vps-dependent biofilms and vps-independent biofilms. The vps-dependent and -independent biofilms differ in their environmental activators and in architecture. Our results suggest that environmental activators of vps-dependent biofilm development are present in freshwater, while environmental activators of vps-independent biofilm development are present in seawater. The distinct environmental requirements for the two modes of biofilm development suggest that vps-dependent biofilm development and vps-independent biofilm development may play distinct roles in the natural environment.

Ken-ichiro Kamei, Tetsuya Haruyama, Masayasu Mie, Yasuko Yanagida, Eiry Kobatake, Masuo Aizawa. (Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259, Nagatsuta, Midori-ku, Yokohama 226-8501, Japan). **Cellular biosensing system for assessing immunomodulating effects on the inducible nitric oxide synthase (iNOS) cascade**. Biotechnology Letters, 25(4) (2003), 321-325.

A cellular biosensing system has been constructed to assess the biological safety/toxicity of chemicals. Detection of nitric oxide (NO) by the cellular biosensing system was used as a

readout for assessing the immunomodulating effects of various chemicals, because some are known to induce NO synthase (iNOS) activity thereby increasing NO production. The macrophage-like cell line, RAW264.7, was cultured on the electrode coated with a polyion complex layer. The potent immune activating abilities of lipopolysaccharide could be verified by the cellular biosensing system: NO release from cells was detected within 600 ms by double potential step chronoamperometry.

Kevin A Gray, Gregory T Mrachko, Charles H Squires. (Diversa Corporation, 4955 Director's Place, San Diego, CA 92121, USA. The Dow Chemical Company, 5501 Oberlin Dr, San Diego, CA 92121, USA). **Biodesulfurization of fossil fuels**. Current Opinion in Microbiology, 6(3) (2003), 229-235.

Biotechnological techniques enabling the specific removal of sulfur from fossil fuels have been developed. In the past three years there have been important advances in the elucidation of the mechanisms of biodesulfurization; some of the most significant relate to the role of a flavin reductase, DszD, in the enzymology of desulfurization, and to the use of new tools that enable enzyme enhancement via DNA manipulation to influence both the rate and the substrate range of Dsz. Also, a clearer understanding of the unique desulfinase step in the pathway has begun to emerge.

Leonard H. Weinstein, Alan W. Davison. (Boyce Thompson Institute, Tower Road, Ithaca, NY 14850, USA. School of Biology, Ridley Building, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK). **Native plant species suitable as bioindicators and biomonitors for airborne fluoride.** Environmental Pollution, 125(1) (2003), 3-11.

For 30–40 years airborne fluoride, usually in the form of HF or SiF_4, was one of the most important and damaging air pollutants affecting forests, crops and natural vegetation. It is much more toxic than most other air pollutants such as O_3 or SO_2 because injury to the most sensitive species begins when they are exposed to a concentration below 1 ppb (ca. 0.8 $\mu g\ m^{-3}$) for a 1- to 3-day period. The long-term threshold concentration is around 0.25–0.30 $\mu g\ m^{-3}$. Higher concentrations and longer durations of exposure induce much more rapid and extensive injury. However, there is a difference in sensitivity between the most and least sensitive species of around 2–3 orders of magnitude and most species possess a degree of resistance. Dramatic improvements in engineering technology have greatly reduced emissions but because of the high toxicity, cases of vegetation injury are still common, even in developed countries, and cases involving litigation still occur. Therefore there is a continuing need for bioindicators and biomonitoring of fluorides, so this paper reviews the subject, drawing attention to the strengths and limitations of the techniques. Visible symptoms are described and illustrated and tables of relative sensitivity are given and their limitations discussed. Finally, examples of biomonitoring in Europe and the USA are presented.

Li Kim Lee, Charles M Roth. (Department of Chemical and Biochemical Engineering, Rutgers University, 98 Brett Road, Piscataway, NJ 08854, USA. Department of Biomedical Engineering, Rutgers University, 98 Brett Road, Piscataway, NJ 08854, USA). **Antisense technology in molecular and cellular bioengineering**. Current Opinion in Biotechnology, 14(5) (2003), 505-511.

Antisense technology is finding increasing application not only in clinical development, but also for cellular engineering. Several types of antisense methods (e.g. antisense oligonucleotides, antisense RNA and small interfering RNA) can be used to inhibit the expression of a target gene. These antisense methods are being used as part of metabolic engineering strategies to downregulate enzymes controlling undesired pathways with regard to product formation. In addition, they are beginning to be utilized to control cell phenotype in tissue engineering

constructs. As improved methods for antisense effects that can be externally regulated emerge, these approaches are likely to find increased application in cellular engineering applications.
M. A. Alonso Lomillo, J. M. Kauffmann and M. J. Arcos Martinez. (Dpto. de Química, Facultad de Ciencias, Área de Química Analítica, Universidad de Burgos, Plaza Misael Bañuelos, s/n, E-09001, Burgos, Spain. Institut de Pharmacie, Université Libre de Bruxelles, Campus Plaine, CP 205/6, B-1050, Brussels, Belgium). **HRP-based biosensor for monitoring rifampicin.** Biosensors and Bioelectronics, 18(9) (2003), 1165-1171.

Pyrrole was electropolymerized onto a Pt electrode in the presence of $LiClO_4$ and horseradish peroxidase (HRP). This HRP-based biosensor has been used for the amperometric detection of rifampicin (RIF) in the presence of a constant concentration of H_2O_2. The C_{H2O2} as well as the applied potential (E_{ap}) and the pH of the phosphate buffer have simultaneously been optimized through a central composite design. Under these conditions, repeatability, reproducibility, and stability of the modified electrode have been analyzed. The detection limit for RIF has been calculated taking into account the probability of false-positive (α) and -negative (β), reaching a value of 5.06×10^{-6} mol dm^{-3}. The biosensor was applied to the determination of RIF in pharmaceutical preparations and biological samples.
M. E. Conti, G. Cecchetti. (Dipartimento di Controllo e Gestione delle Merci e del loro Impatto sull'Ambiente, Facoltà di Economia, Università "La Sapienza", Via Del Castro Laurenziano 9, 00161 Rome, Italy. Facoltà di Scienze Ambientali, Università degli Studi di Urbino, ex Sogesta, Località Crocicchia, 61029, Urbino, Italy). **Biological monitoring: lichens as bioindicators of air pollution assessment –– a review.** Environmental Pollution, 114(3) (2001), 471-492.

Often as part of environmental impact studies and, above all, to obtain authorisations in accordance with prescriptions from the Ministry for the Environment (Italy), surveys and controls that use biological indicators are required. This is because such indicators are valid instruments for evaluating the quality of the air ensuing from the subject (often an industrial plant) of the Environmental Impact Assessment (EIA). In this context, this paper aims to analyse some of the theoretical aspects of biological monitoring and to provide a progress report on the use of lichens as bioindicators of air quality, with a particular eye to the situation in Italy. The object of this paper is that of pointing out the most important lines in the current state of knowledge in this field, evaluating the methodological applications and their advantages/disadvantages with respect to traditional surveying methods.
Marc A. Breimer, Yevgeny Gelfand and Omowunmi A. Sadik. (Department of Chemistry, State University of New York at Binghamton, PO Box 6016, Binghamton, NY 13902-6016, USA). **Integrated capillary fluorescence DNA biosensor**. Biosensors and Bioelectronics, 18(9) (2003), 1135-1147.

Covalent attachment of dsDNA molecules inside a glass capillary without the need for hybridization is described. It is shown that the glass capillary has a surface density of 2.5×10^{13} molecules/cm^2 with specific binding capacity of 62.5%. The resulting substrate was used to develop a biosensor for determining fluorescent organic analytes and metal binding with DNA. The biosensor combines highly specific immobilization chemistry with a capillary-geometry flow cell arrangement. The results show that fluorescent dyes are retained in the dsDNA-modified surface and that exposure to concentrations of nickel and lead ions resulted in a recoverable, highly reproducible diminishment of the fluorescence intensity.
Marcelo Enrique Conti, Gaetano Cecchetti. (Dipartimento di Controllo e Gestione delle Merci e del loro Impatto sull'Ambiente, Università di Roma "La Sapienza", Via del Castro Laurenziano 9, 00161, Rome, Italy. Centro per le Valutazioni Ambientali delle Attività Industriali, Facoltà di Scienze Ambientali, Università degli Studi di Urbino, Campus Scientifico Sogesta, 61029 Urbino (Pu), Italy). **A biomonitoring study: trace metals in**

algae and molluscs from Tyrrhenian coastal areas. Environmental Research, 93(1) (2003), 99-112.

Marine organisms were evaluated as possible biomonitors of heavy metal contamination in marine coastal areas. Concentrations of Cd, Cr, Cu, Pb, and Zn were measured in the green algae *Ulva lactuca* L., the brown algae *Padina pavonica* (L.) Thivy, the bivalve mollusc *Mytilus galloprovincialis* Lamarck, and the two gastropod molluscs *Monodonta turbinata* Born and *Patella cerulea* L. collected at six coastal stations in the area of the Gulf of Gaeta (Tyrrhenian Sea, central Italy). The coastal area of the Regional Park of Gianola and Monte di Scauri (a "Protected Sea Park" area) was chosen as a control site. Seawater samples were also collected in each site to assess soluble and total metal concentrations and to gain additional information on both the environmental conditions of the area and possible bioaccumulation patterns. Metal concentrations detected in algae and molluscs did not show significant differences among all stations studied. Moreover, statistical analyses (ANOVA, multiple comparison tests, cluster analysis) showed that the Sea Park station was not significantly different from the others. The hypothesis that the Protected Sea Park would be cleaner than the others must therefore be reconsidered. Data from this study were also compared with those previously obtained from uncontaminated sites in the Sicilian Sea, Italy. The results show clearly differences between these two marine ecosystems. The species examined showed great accumulations of metals, with concentration factors (CFs) higher than 10,000 with respect to the concentrations (soluble fractions) in marine waters. Metal concentrations recorded in this area may be used for background levels for intraspecific comparison within the Tyrrhenian area, a body of water about which information is still very scarce.

Martine Naessens, Jean Claude Leclerc, Canh Tran-Minh. (Ecole Nationale Supérieure des Mines, Centre SPIN/Génie Enzymatique, 158 Cours Fauriel, 42023, Saint Etienne cedex 2, France. Faculté des Sciences, Equipe d'Ecophysiologie, 23 Rue Dr Paul Michelon, 42023, Saint Etienne cedex 2, France). **Fiber Optic Biosensor Using *Chlorella vulgaris* for Determination of Toxic Compounds.** Ecotoxicology and Environmental Safety, 46(2) (2000), 181-185.

A new biosensor is constructed for the detection of some herbicides based on kinetic measurements of chlorophyll-a fluorescence in *Chlorella vulgaris* cells. The microalgae are immobilized on removable membranes placed in front of the tip of an optical fiber bundle inside a homemade microcell. *C. vulgaris* was easily cultivated in laboratory and very sensitive to herbicides that effect the photosynthesis process. The response of the algal biosensor is studied in terms of detection limits, reversibility, and long-term activity. The effects of temperature and pH are also reported. The biosensor can be used to measure the concentration of a toxic chemical in the form of a single drop or dissolved in a continuous flow. The detection of 0.1 $\mu g \cdot L^{-1}$ of a single herbicide as is required by European Community legislation for drinking water is possible with this algal biosensor especially for atrazine, simazine, and diuron.

Michael Neumann, Joachim Baumeister, Matthias Liess, Ralf Schulz. (Department of Limnology, Zoological Institute, Technical University Braunschweig, Fasanenstrasse 3, D-38092, Braunschweig, Germany. Department of Artificial Intelligence and Applied Computer Science, University of Würzburg, Am Hubland, D-97074, Wurzburg, Germany. Department of Chemical Ecotoxicology, UFZ Center for Environmental Research, Permoserstr. 15, D-04318, Leipzig, Germany). **An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators II. The knowledge base LIMPACT.** Ecological Indicators, 2(4) (2003), 391-401.

The development and the evaluation of a biological indicator system for pesticide pollution in streams are presented. For small headwater streams with an agricultural catchment area, the

expert system LIMPACT estimates the pesticide contamination according to the four classes: Not Detected (ND); Low (L); Moderate (M) and High (H) contamination without any specification of the chemical agents. The input parameters are the abundance data of benthic macroinvertebrate taxa within four time frames in a year (March/April; May/June; July/August; September/October) and nine basic water-quality and morphological parameters. The heuristic knowledge base was developed with the shell-kit D3 and contains 921 diagnostic rules with scores either to establish or to de-establish a diagnosis. The 418 rules had less than 3 symptoms, and only 47 rules had more than 4 symptoms in their rule condition. We differentiate between positive indicator (PI) taxa, which indicate contamination by high abundance values and positive abundance dynamics, and negative indicator (NI) taxa, a high abundance of which rules out contamination and indicates an uncontaminated site. We analysed 39 taxa and found 13 positive and 24 negative indicators. The database comprises 157 investigations per stream and year with rainfall event-controlled pesticide sampling and repeated benthic sampling as described in Part 1 (Neumann et al., this issue). For the evaluation of LIMPACT, we used the same cases. The correct class for the 157 investigations per stream and year is established by LIMPACT in 66.7–85.5% of the cases, with better results for uncontaminated sites. The overall alpha error probability (false positive) is 9.6% while the beta error probability (false negative) varied between 0 and 8% depending on the contamination class. If each stream is considered only once in the system (n=104), the correct diagnosis is established by LIMPACT in 51.9–88.6% of the cases. In most of the remaining cases no diagnosis is established instead of a wrong one.

Michael Neumann, Matthias Liess, Ralf Schulz. (Department of Limnology, Zoological Institute, Technical University Braunschweig, Fasanenstrasse 3, D-38092, Braunschweig, Germany. Department of Chemical Ecotoxicology, UFZ Center for Environmental Research, Permoserstr. 15, D-04318, Leipzig, Germany). **An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrates as bioindicators**. Ecological Indicators, 2(4) (2003), 379-389.

We developed an expert system (LIMPACT) to estimate the pesticide contamination of streams using macroinvertebrate indicators. Here, we present the database consisting of 157 data sets obtained from 1992 to 2000 through investigation of 104 small headwater streams with an agricultural catchment area. The contamination by pesticides (insecticides, fungicides and herbicides) during rainfall events varied greatly in both water and suspended-particle samples, occasionally reaching ecotoxicologically relevant levels. On the basis of standardised toxicities, the data sets were grouped into Not Detected (n=55), Low (34), Moderate (42) and High (26) contamination with pesticides. Additionally, nine water-quality and morphological parameters were evaluated with regard to their influence on the fauna and are used to exclude unsuitable streams from LIMPACT. The benthic macroinvertebrate fauna data were divided into four time frames (March/April; May/June; July/August; September/October) and analysed regarding the abundance and the abundance dynamics of the 39 most common taxa. In contaminated streams, lower abundance for negative and higher for positive indicator taxa were observed. The number of taxa was significantly lower (unpaired t-test P<0.015) in the most severely contaminated streams. Information abstracted from this empirical approach was used to create rules indicating or not indicating contamination and to build up the heuristic knowledge base of LIMPACT as shown in the Part 2 paper (M. Neumann, J. Baumeister, M. Liess, R. Schulz, An expert system to estimate the pesticide contamination of small streams using benthic macroinvertebrate as bioindicators. Part 2. The knowledge base of LIMPACT, Ecological Indicators, this issue).

Michelle A. Brusatori and Paul R. Van Tassel. (Department of Chemical Engineering and Materials Science, Wayne State University, 5050 Anthony Wayne Drive, Detroit, MI 48202, USA). **Biosensing under an applied voltage using optical waveguide lightmode spectroscopy**. Biosensors and Bioelectronics, 18(10) (2003), 1269-1277.

An applied dc voltage offers a means of controlling immobilization during biosensor fabrication and detection during biosensing application. We present a method to directly and continuously measure the adsorption of biomacromolecules or other polyelectrolytes, under an applied potential difference, based on optical waveguide lightmode spectroscopy (OWLS). An indium tin oxide (ITO) film of thickness ca. 10 nm coated onto a silicon titanium oxide (STO) waveguiding film serves as the working (sensing) electrode. We observe the effective refractive index of the 0th transverse electric guided mode to increase significantly in the presence of an applied potential due to charging of the interfacial double layer and, possibly, modest electrochemical oxidation. Adsorption from solution onto the ITO electrode is detected by a further increase in the effective refractive index. We achieve accurate detection by employing an optical model in which the STO and ITO layers are combined into a single waveguiding film. No improvement is found using models treating the ITO as a separate layer, either dielectric or conducting. Using this method, we find the adsorption of human serum albumin and horse heart cytochrome *c* to be considerably enhanced in the presence of an applied potential exceeding 1 V. We attribute this behavior to adsorption at positions on the protein molecules of complementary charge.

Neelam Verma, Minni Singh. (Department of Biotechnology, Punjabi University, Patiala 147002, PB, India). **A disposable microbial based biosensor for quality control in milk**. Biosensors and Bioelectronics, 18(10) (2003), 1219-1224.

The food industry needs suitable analytical methods for quality control, that is, methods that are rapid, reliable, specific and cost-effective as current wet chemistries and analytical practices are time consuming and may require highly skilled labor and expensive equipment. The need arises from heightened consumer concern about food composition and safety. The present study was carried out keeping in view the recently emerging concern of the presence of urea in milk, called "synthetic milk". The biocomponent part of the urea biosensor is an immobilized urease yielding bacterial cell biomass isolated from soil and is coupled to the ammonium ion selective electrode of a potentiometric transducer. The membrane potential of all types of potentiometric cell based probes is related to the activity of electrochemically detected product, and thus to the activity of the substrate by a form of the Nernst equation. Samples of milk were collected and analyzed for the presence of urea by the developed biosensor with a response time as low as 2 min. The results were in good correlation with the pure enzyme system.

P.V. Preejith, C.S. Lim, A. Kishen, M.S. John, A. Asundi. (School of Mechanical and Production Engineering, c/o School of Mechanical and Production Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798. Biomedical Engineering Research Centre, c/o School of Mechanical and Production Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 Fax: 65-6791-18590). **Total protein measurement using a fiber-optic evanescent wave-based biosensor**. Biotechnology Letters, 25(2) (2003), 105-110.

A novel method and instrumental system to determine the total protein concentration in a liquid sample is described. It uses a fiber optic total protein sensor (FOPS) based on the principles of fiber optic evanescent wave spectroscopy. The FOPS applies a dye-immobilized porous glass coating on a multi-mode optical fiber. The evanescent waves at the fiber optic core-cladding interface are used to monitor the protein-induced changes in the sensor element. The FOPS offers a single-step method for quantifying protein concentrations without destroying the sample. The response time and reusability of the FOPS are evaluated. This unique sensing method presents a sensitive and accurate platform for the quantification of protein.

Rebecca L Rich, David G Myszka. (Huntsman Cancer Institute, University of Utah, 50 North Medical Drive, #4A417, Salt Lake City, UT 84132, USA). **Advances in surface plasmon resonance biosensor analysis**. Current Opinion in Biotechnology, 11(1) (2000), 54-61.

The number and diversity of surface plasmon resonance (SPR) biosensor applications continue to increase. Evolutions in instrument and sensor chip technology, experimental methodology, and data analysis are making it possible to examine a wider variety of biomolecular interactions in greater mechanistic detail. SPR biosensors are poised to make a significant impact in basic research and pharmaceutical discovery.

Ronald R. Breaker. (Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA). **Engineered allosteric ribozymes as biosensor components**. Current Opinion in Biotechnology, 13(1) (2002), 31-39.

RNA and DNA molecules can be engineered to function as molecular switches that trigger catalytic events when a specific target molecule becomes bound. Recent studies on the underlying biochemical properties of these constructs indicate that a significant untapped potential exists for the practical application of allosteric nucleic acids. Engineered molecular switches can be used to report the presence of specific analytes in complex mixtures, making possible the creation of new types of biosensor devices and genetic control elements.

S. Loppi, F. Riccobono, Z. H. Zhang, S. Savic, D. Ivanov, S. A. Pirintsos. (Department of Environmental Science, University of Siena, Italy. Institute of High Energy Physics, Chinese Academy of Sciences of Beijing, China. Natural History Museum of Belgrade, Yugoslavia. Botanic Garden, University of Varna, Bulgaria. Department of Biology, University of Crete, Greece). **Lichens as biomonitors of uranium in the Balkan area.** Environmental Pollution, 125(2) (2003), 277-280.

The contribution of the conflict of 1999 to the environmental levels of uranium in the Balkan area was evaluated by means of lichens used as biomonitors. The average U concentration found in lichens in the present study was in line with the values reported for lichens from other countries and well below the levels found in lichens collected in areas with natural or anthropogenic sources of U. Measurement of isotopic ratios $^{235}U/^{238}U$ allowed to exclude the presence of depleted uranium. According to these results, we could not detect widespread environmental contamination by depleted uranium in the Balkan area.

Shimshon Belkin. (Division of Environmental Sciences, The Fredy and Nadin Herrmann Graduate School of Applied Science, Hebrew University, Jerusalem 91904, Israel). **Microbial whole-cell sensing systems of environmental pollutants**. Current Opinion in Microbiology, 6(3) (2003), 206-212.

The past decade has witnessed the development of a novel class of tools for environmental monitoring: genetically engineered microorganisms `tailored' to respond in a dose-dependent manner to changes in environmental conditions. Recent advances in the field include the expansion of available reporter functions with multicolored fluorescent proteins, a broadening of the detected chemical effects such as the availability of nutrients and enhancement of the spectrum of reporter microorganisms to include cyanobacteria, yeast and fungi. Most importantly, the stage has been set for the incorporation of such cells into various whole-cell array formats on silicon chips, optic fibres and other configurations. The future of such multiplex detection and analysis systems seems bright.

Simon J. Grove. (Rainforest Cooperative Research Centre, School of Tropical Environment Studies and Geography, James Cook University, P.O. Box 6811, Cairns, Qld 4870, Australia). **Tree basal area and dead wood as surrogate indicators of saproxylic insect faunal integrity: a case study from the Australian lowland tropic**. Ecological Indicators, 1(3) (2002), 171-188.

Saproxylic (dead wood associated) insects are well-known in Europe for their associations with the mature timber habitat (old trees and dead wood)—features which have a bearing on their response to forest management and which have encouraged their use as indicators of ecological continuity. In the tropics and elsewhere, their relationships with the mature timber habitat have yet to be characterised, preventing their consideration in determining the sustainability of forest management. Furthermore, the practical difficulties of adequately sampling a tropical saproxylic insect fauna may well preclude their consideration as indicators in their own right, in which case surrogate structural indicators will need to be sought. To investigate this, the saproxylic beetle fauna of lowland rainforest was studied in a series of sites in the Daintree region of northeast Queensland, Australia. Study sites were chosen to represent a range of intensities of past management from old-growth forest, through selectively logged forest to re-growth forest that had arisen following previous clearance. Beetle abundance, incidence, species richness and assemblage composition were considered in relation to various mature timber habitat attributes, comprising living tree basal area, coarse woody debris and standing dead trees. Volume of coarse woody debris proved the strongest positive correlate of species richness, while the basal area of larger-diameter trees proved a more robust indicator of abundance, incidence and assemblage composition, and was also correlated with species richness. Given its ease of measurement compared to recording dead wood or saproxylic beetles, the basal area of larger-diameter trees is considered an appropriate surrogate measure of saproxylic beetle faunal integrity in tropical rainforest. Given the wider ecological role of old trees in forest ecosystems, the development of locally-defined indicators based on larger-diameter trees for monitoring forest management is strongly encouraged.

Tanyarut Boontheekul, David J Mooney. (University of Michigan, 1011 North University Avenue, 5213 Dental Building, Ann Arbor, MI 48109-1078, USA). **Protein-based signaling systems in tissue engineering. Current Opinion in Biotechnology,** 14(5) (2003), 559-565.

Tissue engineering aims to replace damaged tissues or organs using either transplanted cells or host cells recruited to the target site. Protein signaling is crucial to regulate cell phenotype and thus engineered tissue structure and function. Biomaterial vehicles are being designed to incorporate and locally deliver various molecules involved in this signaling, including both growth factors and peptides that mimick whole proteins. Controlling the concentration, local duration and spatial distribution of these factors is key to their utility and efficacy. Recent advances have been made in the development of polymeric delivery systems intended to achieve this control.

Thomas Ruckstuhl, Michael Rankl and Stefan Seeger. (Physikalisch-Chemisches Institut, Universität Zürich, Winterthurerstr. 190, CH-8057, Zürich, Switzerland). **Highly sensitive biosensing using a supercritical angle fluorescence (SAF) instrument**. Biosensors and Bioelectronics, 18(9) (2003), 1193-1199.

We present a new optical biosensor for probing molecular binding to a water/glass interface. The system is designed to measure the kinetics of surface reactions down to low analyte concentrations straightforwardly. The selective detection of surface bound fluorescence is achieved by collecting supercritical angle fluorescence (SAF) emission of surface bound molecules into the glass. Thereby the expansion of the detection volume into the aqueous probe is reduced to about one sixth of the fluorescence wavelength, consequently bulk fluorescence from the solution is rejected successfully. The SAF-signal is captured by a parabolic glass lens, which leads to high spatial collection efficiency and detection sensitivity. The sensor has an inverted optical design and is compatible with common glass cover slips, which strongly facilitates operation for the user working in the biological and biochemical fields. The

performance of the system is demonstrated by real time measurements of antibody-antigen reactions. Rate constants of the reaction were extracted. Antigen concentrations were detected down to 10^{-13} mol/l.

Valentyna N. Arkhypova, Sergei V. Dzyadevych, Alexey P. Soldatkin, Anna V. El'skaya, Claude Martelet, Nicole Jaffrezic-Renault. (Laboratory of Biomolecular Electronics, Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, 150 Zabolotnogo Street, Kiev 03143, Ukraine. Ecole Centrale de Lyon, IFoS UMR 5621, BP 163, 69131, Ecully Cedex, France). **Development and optimisation of biosensors based on pH-sensitive field effect transistors and cholinesterases for sensitive detection of solanaceous glycoalkaloids.** Biosensors and Bioelectronics, 18(8) (2003), 1047-1053.

Highly sensitive biosensors based on pH-sensitive field effect transistors and cholinesterases for detection of solanaceous glycoalkaloids have been developed, characterised and optimised. The main analytical characteristics of the biosensors developed have been studied under different conditions and an optimal experimental protocol for glycoalkaloids determination in model solution has been proposed. Using such a biosensor and an enzyme reversible inhibition effect, the total potato glycoalkaloids content can be determined within the range of 0.2–100 μM depending on the type of alkaloid, with lowest detection limits of 0.2 μM for α-chaconine, 0.5 μM for α-solanine and 1 μM for solanidine. The dynamic ranges for the compounds examined show that such biosensors are suitable for a quantitative detection of glycoalkaloids in real potato samples. High reproducibility, operational and storage stability of the biosensor developed have been shown.

W. J. Manning, B. Godzik, R. Musselman. (Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA. Institute of Botany, Polish Academy of Sciences, Krakow, Poland. USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO 80526-2098, USA). **Potential bioindicator plant species for ambient ozone in forested mountain areas of central Europe.** Environmental Pollution, 119(3) (2002), 283-290.

From 1993 to 2000, trees, shrubs, forbs and vines were evaluated for symptoms of probable ozone injury in the vicinity of passive ozone samplers or active ozone monitors in forest condition assessment networks in mostly mountainous regions, principally the Carpathian Mountain Range, in the central European countries Czech Republic, Poland, Romania, Slovakia and Ukraine. Each country was visited at least twice during the time period. Over the course of eight seasons, 29 species of native plants were identified as potential bioindicators of ozone. This is the first report of probable ozone injury on native plants in central Europe. Forbs and shrubs made up the bulk of the species (21 of 29). Potential bioindicators that are widely distributed include the forbs *Centaurea nigra*, and *Impatiens parviflora* and the shrubs *Alnus incana*, *Corylus avellana*, and *Sambucus racemosa*. Ozone concentrations in forested areas of central Europe appear to be high enough and of sufficient duration to cause foliar injury on a wide variety of native plants.

Xu Chen and Shaojun Dong. (State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China). **Sol-gel-derived titanium oxide/copolymer composite based glucose biosensor**. Biosensors and Bioelectronics, 18(8) (2003), 999-1004.

A new type of sol-gel-derived titanium oxide/copolymer composite material was developed and used for the construction of glucose biosensor. The composite material merged the best properties of the inorganic species, titanium oxide and the organic copolymer, poly(vinyl alcohol) grafting 4-vinylpyridine (PVA-g-PVP). The glucose oxidase entrapped in the composite

matrix retained its bioactivity. Morphologies of the composite-modified electrode and the enzyme electrode were characterized with a scanning electron microscope. The dependence of the current responses on enzyme-loading and pH was studied. The response time of the biosensor was <20 s and the linear range was up to 9 mM with a sensitivity of 405 nA/mM. The biosensor was stable for at least 1 month. In addition, the tetrathiafulvalene-mediated enzyme electrode was constructed for the decrease of detection potential and the effect of three common physiological sources that might interfere was also investigated.

Z. Jeran, R. Jačimović, F. Batič , R. Mavsar. (Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia. Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia. Slovenian Forestry Institute, Večna pot 2, SI-1000 Ljubljana, Slovenia). **Lichens as integrating air pollution monitors.** Environmental Pollution, 120(1) (2002), 107-113.

In this work an attempt to combine the results of lichen mapping with the quantitative levels of certain trace elements in *Hypogymnia physodes* (L.) Nyl. collected on a national scale is presented. An Index of Atmospheric Purity (IAP) was calculated using a simple method of mapping lichens based on the assessment of the cover and frequency of crustose, foliose and fruticose lichens on different tree species. For determination of trace elements in lichens k_0-instrumental neutron activation analysis was used. From the IAP results it can be concluded that the epiphytic lichen flora look quite poor with more than 70% of the territory in the fourth and third classes, which represent highly polluted and moderately polluted air. By comparing IAP results with elemental levels in *H. physodes* using multivariate statistical methods it was found that the elemental levels do not have a direct negative effect on the diversity of lichens but can help in identification of the type of possible pollution sources and their origin.

Zh. H. Zhang, Z. F. Chai, X. Y. Mao, J. B. Chen. (Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, PO Box 918, Beijing 100039, China. Systematical Mycology and Lichenology Lab, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China). **Biomonitoring trace element atmospheric deposition using lichens in China.** Environmental Pollution, 120(1) (2002), 157-161.

Concentrations of 32 elements, Ag, As, Au, Ba, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, U, W, Yb, and Zn were determined by instrumental neutron activation analysis (INAA) in the early preserved epiphytic lichens (*Parmotrema recticulatum*) from the remote southwestern China area in the 1960s, 1980s and 1990s. The concentrations of Ag, As, Ce, Cr, Cs, Eu, Fe, Hf, La, Nd, Ni, Sc, Se, Sm, Tb, U, Yb and Zn were dropped down by the temporal prolongation. The elemental concentration levels obtained from the organisms indicate that the extent of heavy metal atmospheric deposition among the sampling sites has been declining during the past decades.

Biotechnology-Agricultural Issue

Chensheng Lu, Richard A. Fenske, Nancy J. Simcox and David Kalman. (Department of Environmental Health, 357234, School of Public Health and Community Medicine, University of Washington, Seattle, Washington, 98195-7234). **Pesticide Exposure of Children in an Agricultural Community: Evidence of Household Proximity to Farmland and Take Home Exposure Pathways**. Environmental Research, 84(3) (2000), 290-302.

Children's exposure to organophosphorus (OP) pesticides in an agricultural community in central Washington State was determined. Spot urine and hand wipe samples were collected from 109 children 9 months to 6 years of age, as were house dust samples, and wipe samples from various surfaces. Children were categorized based on parental occupation (agricultural vs

nonagricultural) and on household proximity to pesticide-treated orchards. Median house dust concentrations of dimethyl OP pesticides in homes of agricultural families were seven times higher than those of reference families (1.92 vs 0.27 μg/g; $P<0.001$). Median pesticide metabolite concentrations in agricultural children were five times higher than those in reference children (0.05 vs 0.01 μg/ml; $P=0.09$). Median pesticide concentrations in housedust ($P=0.01$) and metabolite concentrations in urine ($P=0.01$) from agricultural families were significantly higher in the children living near treated orchards (within 200 ft or 60 m) than those living more distant. Ten of 61 agricultural children had detectable OP pesticide levles on their hands, whereas none of the reference children had detectable levels. These findings indicate that children living with parents who work with agricultural pesticides, or who live in proximity to pesticide-treated farmland, have higher exposures than do other children living in the same community.

David T. Mage, Michael C. R. Alavanja, Dale P. Sandler, Cheryl J. McDonnell, Burt Kross, Andrew Rowland, Aaron Blair. (U.S. Environmental Protection Agency, National Center for Environmental Assessment, MD-52, Research Triangle Park, North Carolina, 27711. Epidemiology and Statistics Program, National Cancer Institute, Bethesda, Maryland. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. SRA Technologies, Inc. Falls Church, Virginia. University of Iowa, Iowa City, Iowa). **A Model for Predicting the Frequency of High Pesticide Exposure Events in the Agricultural Health Study**. Environmental Research, 83(1) (2000), 67-71.

The frequency of self-reported high pesticide exposure events (HPEE) has been recorded in the NCI/EPA/NIEHS Agricultural Health Study (AHS). Fourteen percent (14%) of the enrolled applicators responding reported "an incident or experience while using *any* pesticide which caused an unusually high exposure." These data show, as expected, that the probability of a report of an HPEE increases with the cumulative number of days of pesticide application reported by the applicator. We have developed a three-parameter model that predicts the risk odds ratio (OR) of an HPEE as a function of the number of days that pesticides are applied. These events are costly in terms of resulting health-care visits, lost time from work, and potential risk for cancer and other chronic diseases. We propose that failure to carefully follow all the pesticide manufacturer's label requirements, inexperience, and random events (i.e., breaking hose) are the three factors responsible for the events observed. Special precautions for new or infrequent users of pesticides are indicated.

Diane S. Rohlman, Steffani R. Bailey, W. Kent Anger and Linda McCauley. (Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, Oregon, 97201). **Assessment of Neurobehavioral Function with Computerized Tests in a Population of Hispanic Adolescents Working in Agriculture**. Environmental Research, 85(1) (2001), 14-24.

In recent years there has been heightened concern over the potential of occupational or environmental exposures to affect neurological function in children and adolescents. The current study was designed to develop computerized tests to effectively assess neurobehavioral function in Hispanic adolescents working in agriculture and to evaluate those tests in Hispanic youths working in agriculture and in a non-agricultural group. After exclusions, 96 adolescents currently working in agriculture (AG) and 51 adolescents currently non-migratory and not working in agriculture (Non-AG) were tested. Neurobehavioral tests were selected from the computerized Behavioral Assessment and Research System. AG test performance was significantly below Non-AG performance on the cognitive tests. However, educational and cultural differences between the AG and Non-AG groups may explain this difference. Repeat testing of the AG group revealed substantially improved performance, further supporting educational or cultural

differences as an explanation for the group differences. Together, these results expose the limitations in case-control or cross-sectional designs for testing migrant worker populations in the United States. Longitudinal or cross-sectional designs with repeat testing offer more promise and may be essential for drawing accurate conclusions in migrant worker groups where there are no truly equivalent comparison or control groups.

Helge E. Lundekvam, Eirik Romstad, Lillian Øygarden. (Department of Soil and Water Sciences, Agricultural University of Norway, P.O. Box 5028, NO-1432, As, Norway. Department of Economics and Social Sciences, Agricultural University of Norway, P.O. Box 5033, NO-1432, As, Norway. Øygarden, Centre for Soil and Environmental Research, Frederik A. Dahls vei 20, NO-1432, As, Norway). **Agricultural policies in Norway and effects on soil erosion.** Environmental Science & Policy, 6(1) (2003), 57-67.

Norwegian agriculture depends heavily on governmental subsidies, due to small farming units and high costs. Due to a limited home market, many agricultural productions are also quantum regulated. Milk and grain production was regulated starting in the 1950 using region specific prices. At the level of three counties in south–eastern Norway, this policy resulted in an increase in the grain producing area from 30 to 80% of total agricultural area causing a similar reduction in grassland area over a 30 year period. The change in land use caused by this policy more than doubled the estimated soil losses by water erosion. During the late seventies extensive land levelling in the same region stimulated by subsidies lead to an estimated two–three fold increase in soil erosion. The increase was especially high when former ravine landscapes used for pasture were levelled and turned into arable land that was ploughed in autumn. Very visible erosion and increasing negative offsite effects on water quality together with overproduction put an end to the subsidies for land levelling. Erosion research was started around 1980 and the results from this research lead to the introduction of several kinds of payments in the early 1990 to encourage more sustainable agricultural production. Since the policy changed there has been changes in cultivating systems and a reduction in soil erosion has been estimated. Thus, farmers' behaviour and soil erosion in Norway is strongly influenced by agricultural and environmental policy.

J. Bouma, B. J. van Alphen, J. J. Stoorvogel. (Laboratory of Soil Science and Geology, Wageningen University, P.O. Box 37, 6700 AA Wageningen, The Netherlands). **Fine tuning water quality regulations in agriculture to soil differences.** Environmental Science & Policy, 5(2) (2002), 113-120.

Groundwater quality has been defined in terms of threshold values for nitrate (50 mg l^{-1}) and pesticides (0.1 μg l^{-1} active substance). Variability in space and time, and cost and safety considerations have made it unattractive to verify water quality by repeated measurements. Proxy values have, therefore, been defined to characterise water quality. For nitrate, maximum allowable fertilisation rates have been specified and farmers have to apply the MINAS book-keeping system to keep track of their N-flows. For pesticides, listing of allowed pesticides functions as another proxy quality measure. Field tests and simulations on a Dutch farm demonstrated that water quality assessment using these proxy values does not correspond with direct assessment based on measurements and a comparison with the threshold values, which represent the true standard. A second problem is the generic character of the proxy methods, which do not reflect quite different nitrate and pesticide dynamics in different types of soil. These problems make the proxy approach quite problematic. We, therefore, propose the systematic introduction of information technology to be used for deriving soil-specific management practices that do not lead to an increase of the thresholds. Existing techniques for precision agriculture can be used, and the current registration of all parcels in The Netherlands in a geographical information system, including occurrence of different soil types, will be quite

helpful. Such an information system on internet will allow better control than the current generic proxy systems and is likely to be quite motivating to farmers.

J. Ikea, I. Ingelbrecht, A. Uwaifo, G. Thottappilly. **Stable gene transformation in cowpea (Vigna unguiculata L. walp.) using particle gun method**. African Journal of Biotechnology, 2(8) (2003), 211-218.

We investigated the possibility of transforming and obtaining transgenic cowpea (Vigna unguiculata L Walp) plants using the particle bombardment process. Meristematic explants that could give rise to whole fertile plants were used in transformation experiments with reporter and selectable marker genes driven by a 35S CaMV promoter. Conditions for optimal delivery of DNA to explants were established based on transient gus expression assays two days after bombardment. The size of microcarriers, microflight distance and helium pressure significantly affected transient expression of reporter genes. A total of 1692 explants were bombarded with DNA-coated particles and placed on 3 mg/l bialaphos selective medium. Only 12 regenerated shoots produced seeds eventually, and all were Gus negative even though 7 gave positive PCR signals with the bar primer. Eight out of 1400 seeds from To plants were GUS positive. DNA from eight of the GUS positive seedlings were amplified with both the gus and bar primers in PCR analysis but only two gave a positive Southern signal. Only two of the 3557 T2 seedlings obtained were GUS positive. However, 3 seedlings survived Basta spray. The two GUS positive and 3 Basta surviving seedlings gave positive Southern hybridisation signals. Twelve T3 seedlings from these were GUS positive and also gave positive Southern hybridisation signals. The positive reaction of T1, T2 and T3 seedlings under Southern analysis confirms the stable integration of introduced genes and the transfer of such genes to progenies. However, the level of expression of introduced genes in cowpea cells is very low and this accounted for the high mortality rate of progenies under Basta spray.

Jürg Fuhrer, Fitzgerald Booker. (Swiss Federal Research Station for Agroecology and Agriculture (FAL), Air Pollution/Climate Group, Reckenholzstrasse 191, CH-8046, Zurich, Switzerland. USDA/ARS, Air Quality–Plant Growth and Development Research Unit, North Carolina State University, 3908 Inwood Road, Raleigh, NC 27603, USA. Department of Crop Science, North Carolina State University, 3908 Inwood Road, Raleigh, NC 27603, USA). **Ecological issues related to ozone: agricultural issues.** Environment International, 29(2-3) (2003), 141-154.

Research on the effects of ozone on agricultural crops and agro-ecosystems is needed for the development of regional emission reduction strategies, to underpin practical recommendations aiming to increase the sustainability of agricultural land management in a changing environment, and to secure food supply in regions with rapidly growing populations. Major limitations in current knowledge exist in several areas: (1) Modelling of ozone transfer and specifically stomatal ozone uptake under variable environmental conditions, using robust and well-validated dynamic models that can be linked to large-scale photochemical models lack coverage. (2) Processes involved in the initial reactions of ozone with extracellular and cellular components after entry through the stomata, and identification of key chemical species and their role in detoxification require additional study. (3) Scaling the effects from the level of individual cells to the whole-plant requires, for instance, a better understanding of the effects of ozone on carbon transport within the plant. (4) Implications of long-term ozone effects on community and whole-ecosystem level processes, with an emphasis on crop quality, element cycling and carbon sequestration, and biodiversity of pastures and rangelands require renewed efforts. The UNECE Convention on Long Range Trans-boundary Air Pollution shows, for example, that policy decisions may require the use of integrated assessment models. These models depend on quantitative exposure–response information to link quantitative effects at each level of

organization to an effective ozone dose (i.e., the balance between the rate of ozone uptake by the foliage and the rate of ozone detoxification). In order to be effective in a policy, or technological context, results from future research must be funnelled into an appropriate knowledge transfer scheme. This requires data synthesis, up-scaling, and spatial aggregation. At the research level, interactions must be considered between the effects of ozone and factors that are either directly manipulated by man through crop management, or indirectly changed. The latter include elevated atmospheric CO_2, particulate matter, other pollutants such as nitrogen oxides, UV-B radiation, climate and associated soil moisture conditions.

M. Agrawal, B. Singh, M. Rajput, F. Marshall, J. N. B. Bell. (Department of Botany, Banaras Hindu University, Varanasi- 221 005, India. Department of Environment Science and Technology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK). **Effect of air pollution on peri-urban agriculture: a case study.** Environmental Pollution, 126(3) (2003), 323-329.

Peri-urban agriculture is vital for the urban populations of many developing countries. Increases in both industrialization and urbanization, and associated air pollution threaten urban food production and its quality. Six hour mean concentrations were monitored for SO_2, NO_2 and O_3 and plant responses were measured in terms of physiological characteristics, pigment, biomass and yield. Parameter reductions in mung bean (*Vigna radiata*), palak (*Beta vulgaris*), wheat (*Triticum aestivum*) and mustard (*Brassica compestris*) grown within the urban fringes of Varanasi, India correlated directly with the gaseous pollutants levels. The magnitude of response involved all three gaseous pollutants at peri-urban sites; O_3 had more influence at a rural site. The study concluded that air pollution in Varanasi could negatively influence crop yield.

N Vassilev, M Vassileva. (Estación Experimental del Zaidin, CSIC, Prof. Albareda 1, 18008, Granada, Spain). **Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes**. Applied Microbiology and Biotechnology, 61(5-6) (2003), 435 – 440.

Rock phosphate (RP) is an important natural material traditionally used for the production of phosphorus (P) fertilizers. Compared with chemical treatment, microbial solubilization of RP is an alternative environmentally mild approach. An overview of biotechnological techniques, mainly based on solubilization processes involving agro-industrial residues, is presented. Potential advantages of composting, solid-state fermentation, and liquid submerged fermentation employing free and immobilized microorganisms that produce organic acids and simultaneously solubilize RP are discussed. Subsequent introduction of the final fermented products into soil-plant systems promotes plant growth and P acquisition.

Sagar Krupa. (Department of Plant Pathology, 495 Borlaug Hall, 1991 Upper Buford Circle, University of Minnesota, St. Paul, MN 55108, USA). **Atmosphere and agriculture in the new millennium.** Environmental Pollution, 126(3) (2003), 293-300.

Effects of changing climate (CO_2, O_3, aerosols, UV-B radiation, temperature and precipitation) on crops are predominantly based on univariate studies. Limited bivariate studies suggest rising CO_2 levels would be beneficial to crops but may be offset by adverse O_3 effects. Elevated UV-B and ambient crop yields are difficult to project due also to limited research. Climate warming concerns, using average daily temperatures may be less important than the effects of rising nocturnal temperatures on crop growth. Traditional approaches of examining air pollutant-induced visible foliar injury or the effects of single air pollutants on crop productivity need to be redirected to the analysis of integrated holistic systems. In that context, present and future agriculture in India and the USA are compared.

T. Robinson, B. Chandran, P. Nigam. (School of Biomedical Sciences, University of Ulster, Coleraine, Londonberry BT52 1SA, Northern Ireland, UK). **Removal of dyes from an**

artificial textile dye effluent by two agricultural waste residues, corncob and barley husk. Environment International, 28(1-2) (2002), 29-33.

The use of a previously untried biosorbent, barley husk, for dye removal is compared to corncob. The effectiveness of adsorption as a means of dye removal has made it an ideal alternative to other more costly treatments. This paper deals with two low-cost, renewable biosorbents, which are agroindustrial by-products, for textile dye removal. Experiments at total dye concentrations of 10, 20, 30, 40, 50, 100, 150, and 200 mg l^{-1} were carried out with an artificial effluent consisting of an equal mixture of five textile dyes. The effects of initial dye concentration, biosorbent particle size, dose of biosorbent, effective adsorbance, and dye removal kinetics were examined. One gram (per 100 ml) of <600 μm corncob was found to be effective in removing a high percentage of dyes at a rapid rate (92% in 48 h). One gram of 1×4 mm barley husk was found to be the most effective weight and particle size combination for the removal of dyes (92% in 48 h). The results illustrate how barley husk and corncob are effective biosorbents concerning the removal of textile dyes from effluent.

Biotechnology Policy Issue

David P. Robertson, R. Bruce Hull. (College of Natural Resources, Virginia Tech and International Honors Program (IHP), Boston University, 108 Evergreen Ridge, Lynchburg, Virginia 24503, USA. College of Natural Resources, Virginia Tech 0324, Blacksburg, VA 24061, USA). **Public ecology: an environmental science and policy for global society.** Environmental Science & Policy, 6(5) (2003), 399-410.

Public ecology exists at the interface of science and policy. Public ecology is an approach to environmental inquiry and decision making that does not expect scientific knowledge to be perfect or complete. Rather, public ecology requires that science be produced in collaboration with a wide variety of stakeholders in order to construct a body of knowledge that will reflect the pluralist and pragmatic context of its use (decision context), while continuing to maintain the rigor and accountability that earns scientific knowledge its privileged status in contemporary society. As such, public ecology entails both *process* and *content*. The process is that of a post-modern scientific method: a process that values the participation of extended peer communities composed of a diversity of research specialists, professional policy-makers, concerned citizens and a variety of other stakeholders. The content of public ecology is a biocultural knowledge of dynamic human ecosystems that directly relates to and results from the participatory, democratic processes that distinguish public ecology as a *citizen science*. The primary goal of public ecology is to build common ground among competing beliefs and values for the environment. The purpose of this paper is to help unify and establish public ecology as a distinctive approach to environmental science and policy in global society.

Joanna Burger, Donny E. Roush, Robert Ramos, Michael Gochfeld. (Nelson Biological Laboratory, Department of Ecology, Evolution, and Natural Resources, Rutgers University, Piscataway, New Jersey, 08854-8082. Environmental and Occupational Health Sciences Institute, Piscataway, New Jersey, 08855. Environmental Science and Research Foundation, 101 South Park Avenue, Suite 2, Idaho Falls, Idaho, 83402. Natural Heritage Project, Idaho Museum of Natural History, Idaho State University, 8044, Pocatello, Idaho, 83209. Environmental and Community Medicine, UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey, 08854). **Risk Concerns, Land Use, Stewardship, and the Idaho National Engineering and Environmental Laboratory: Attitudes of the Shoshone–Bannock and Other American Indians.** Environmental Research, 83(3) (2000), 298-310.

This paper examines the attitudes and perceptions of 277 American Indians about hunting and fishing, risk, and future land use of the Idaho National Engineering and Environmental

Laboratory (INEEL) in southeastern Idaho. Nearly half of our sample were Shoshone–Bannock tribal members living on the nearby Fort Hall Reservation, and half were American Indians from elsewhere in the western United States. We also interviewed an additional 44 White people. We examine the hypothesis that there are differences in environmental concerns and attitudes toward future land use at INEEL as a function of tribal affiliation (ethnicity), educational level, gender, and age. Such perceptions are important because of the existence of tribal treaties that govern the legal and cultural rights of the Shoshone–Bannock. Returning INEEL to the Shoshone–Bannock Tribes, hunting, fishing, camping, hiking, and a National Environmental Research Park ranked as the highest preferred future land uses, whereas continuing nuclear materials reprocessing and increasing the storage of nuclear wastes ranked as the lowest. There were tribal differences in land use preferences, with those of the Fort Hall Indians being more similar to those of the local Whites than to other American Indians. All groups ranked storage of nuclear material, storage of additional nuclear material, and spills and accidents as the most serious of a list of concerns provided about the site. Fort Hall Indians answered an open-ended question with concerns for population levels and migration routes of game animals and other wildlife, more than hunting and human health. The Shoshone–Bannock from Fort Hall showed an environmental sensitivity for the well-being of wildlife and the health of the ecosystem and were interested in long-term stewardship, in addition to concern for human health.

Jos C. S. Kleinjans, Frederik-Jan van Schooten. (Department of Health Risk Analysis and Toxicology, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands). **Ecogenotoxicology: the evolving field**. Environmental Toxicology and Pharmacology, 11(3-4) (2002), 173-179.

The occurrence of chemical contaminants with DNA-damaging capacity in the environment represents a threat to human health as well as to the health of the ecosystem. This mini-review describes studies that were aimed to monitor at field conditions, the presence of such environmental toxicants and their DNA-damaging effects in aquatic and terrestrial species, as well as in birds. It is obvious that these studies, in particular are abundantly performed in fish and aquatic invertebrates, have brought forward new information on the levels and genotoxic effects of these compounds which complements data coming from monitoring the abiotic fractions of the ecosystem, thereby demonstrating that the ecogenotoxicological approach is fruitful. However, in order to assess the genotoxic impact on the health of the ecosystem, a second generation type of field studies is required focusing on adverse effects on biodiversity and on survival potency. For this, the application of DNA microarray-based technologies provides new opportunities.

Lynda BM Ellis. (Center for Biodegradation Research and Informatics and Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455, USA). **Environmental biotechnology informatics.** Current Opinion in Biotechnology, 11(3) (2000), 232-235.

Environmental biotechnology informatics is in its infancy and is growing fast. Computer and information science can assist environmental biotechnology by developing biological databases and building mathematical models of biological systems. Funding and training limitations in this field may, however, hinder its future growth.

M Arroyo, I de la Mata, C Acebal, M Pilar Castillón. (Departamento de Bioquímica y Biología Molecular I, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, 28040 Madrid, Spain). **Biotechnological applications of penicillin acylases: state-of-the-art.** Applied Microbiology and Biotechnology, 60(5) (2003), 507 – 514.

This review describes the most recent developments in the biotechnological applications of penicillin acylases. This group of enzymes is involved mainly in the industrial production of 6-

aminopenicillanic acid and the synthesis of semisynthetic #-lactam antibiotics. In addition, penicillin acylases can also be employed in other useful biotransformations, such as peptide synthesis and the resolution of racemic mixtures of chiral compounds. Particular emphasis is placed on advances in detection of new enzyme specificities towards other natural penicillins, enzyme immobilization, and optimization of enzyme-catalyzed hydrolysis and synthesis in the presence of organic solvents.

M. A. V. Borrero, J. T. V. Pereira, E. E. Miranda. (Federal University of Rondonia, 789000-500, Porto Velho, RO, Brazil. State University of Campinas, P.O. Box 6122, 13083-970, Campinas, SP, Brazil. EMBRAPA - Environmental Monitoring Center, Campinas, Brazil). **An environmental management method for sugar cane alcohol production in Brazil**. Biomass and Bioenergy, 25(3) (2003), 287-299.

This paper presents an environmental management method, focusing on environmental efficiency for agro-industry. The main idea is to perform a joint analysis of the ecological, economical and social aspects related to agro-industrial activities. The result of the analysis is a measurement of environmental efficiency, on a numerical scale. The lower values, encompassing 70% of the scale range, classify the low environmental efficiency activities. The values taking the upper 10% reveal the high efficiency ones. A case study focusing on the Brazilian alcohol production, including the agricultural and industrial phases, is presented. The study emphasizes the impact on the soil, water and air. Moreover, it also deals with the social and economic aspects related to the level of employment and productivity. According to the assumptions adopted, none of the three agro-industries analyzed achieved the highest environmental efficiency level established.

Patrick D Schloss and Jo Handelsman. (Department of Plant Pathology, 1630 Linden Drive, Russell Laboratories, University of Wisconsin, Madison, WI 53706, USA). **Biotechnological prospects from metagenomics**. Current Opinion in Biotechnology, 14(3) (2003), 303-310.

The recognition that most microorganisms in the environment cannot be cultured by standard methods stimulated the development of metagenomics, which is the genomic analysis of uncultured microorganisms. Two types of analysis have been used to obtain information from metagenomic libraries: a function-driven approach, in which metagenomic libraries are initially screened for an expressed trait, and a sequence-driven approach, in which libraries are initially screened for particular DNA sequences. New antibiotics and enzymes are among the early discoveries from metagenomics. Future refinement of methods that enrich for genes of particular function will accelerate the rate of discovery of useful molecules.

Paul Whitehouse. (National Centre for Environmental Toxicology, WRc-NSF, Medmenham, Marlow, United Kingdom). **Measures for Protecting Water Quality: Current Approaches and Future Developments.** Ecotoxicology and Environmental Safety, 50(2) (2001), 115-126.

An important component in protecting the ecological quality of watercourses is the regulation of point source discharges. These are usually expressed in terms of "end of pipe" limits on certain physical parameters or on the concentrations of specific chemicals. Sometimes discharge licences take the form of Emission Limit Values (ELVs) which are based on Best Available Technology with respect to emissions and apply to all discharges (usually for a specified industry sector) irrespective of the dilution capacity of the receiving watercourse. Alternatively, they may be based on water quality standards (e.g., EQSs) for specific chemicals which describe thresholds below which no adverse impact on the receiving water is predicted and which take explicit account of available dilution at different discharge locations. The strengths and limitations of these approaches are reviewed, along with a consideration of new approaches that

address some of the limitations associated with control measures based on ELVs or EQSs. These include approaches for controlling well-defined mixtures by a "Toxic Equivalent" approach, exemplified here for discharges containing alkylphenol ethoxylate surfactants, and a fundamentally different approach for regulating complex effluents based on Direct Toxicity Assessment of whole effluents. Circumstances under which these different approaches to controlling point source emissions to surface waters might be appropriate are discussed.

Rafia Afroz, Mohd Nasir Hassan, Noor Akma Ibrahim. (Department of Environmental Sciences, Faculty of Science and Environmental Studies, University Putra Malaysia 43400 UPM, Serdang Selangor D.E., Malaysia. Institute for Mathematical Research, University Putra Malaysia, Serdang Selangor D.E., Malaysia). **Review of air pollution and health impacts in Malaysia**. Environmental Research, 92(2) (2003), 71-77.

In the early days of abundant resources and minimal development pressures, little attention was paid to growing environmental concerns in Malaysia. The haze episodes in Southeast Asia in 1983, 1984, 1991, 1994, and 1997 imposed threats to the environmental management of Malaysia and increased awareness of the environment. As a consequence, the government established Malaysian Air Quality Guidelines, the Air Pollution Index, and the Haze Action Plan to improve air quality. Air quality monitoring is part of the initial strategy in the pollution prevention program in Malaysia. Review of air pollution in Malaysia is based on the reports of the air quality monitoring in several large cities in Malaysia, which cover air pollutants such as Carbon monoxide (CO), Sulphur Dioxide (SO_2), Nitrogen Dioxide (NO_2), Ozone (O_3), and Suspended Particulate Matter (SPM). The results of the monitoring indicate that Suspended Particulate Matter (SPM) and Nitrogen Dioxide (NO_2) are the predominant pollutants. Other pollutants such as CO, O_x, SO_2, and Pb are also observed in several big cities in Malaysia. The air pollution comes mainly from land transportation, industrial emissions, and open burning sources. Among them, land transportation contributes the most to air pollution. This paper reviews the results of the ambient air quality monitoring and studies related to air pollution and health impacts.

Timothy L. McDaniels. (Eco-Risk Research Unit, Institute for Resources and Environment, School of Community and Regional Planning, University of British Columbia, Vancouver BC, Canada V6T 1Z2). **Creating and using objectives for ecological risk assessment and management.** Environmental Science & Policy, 3(6) (2000), 299-304.

This paper provides guidance for ecological risk assessors and managers about how to structure objectives for these efforts. It first outlines why objectives are crucial for ecological risk assessment and management. Then, it draws on the writing within applied decision analysis to outline a process for structuring objectives for ecological risk-management efforts involving one or several decision-makers. The characteristics of a ood set of objectives are outlined, and the various uses for a set of objectives within ecological risk-management efforts are summarized. The paper concludes with practical advice for managers and facilitators charged with developing objectives to guide these complex processes.

Wu-Yuan Chen, Hsing-I Tseng, Ming-Tsang Wu, Hsin-Chia Hung, Hui-Tsu Wu, Hsiu-Lin Chen, Chu-Chong Lu. (Department of Pediatrics, Medical College, Kaohsiung Medical University, 100 Shu-Chuan 1st Road, Kaohsiung 100, Taiwan. Graduate Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung, Taiwan. School of Dental Hygiene, Kaohsiung Medical University, Kaohsiung, Taiwan). **Synergistic effect of multiple indoor allergen sources on atopic symptoms in primary school children**. Environmental Research, 93(1) (2003), 1-8.

Accumulating data show that the complex modern indoor environment contributes to increasing prevalence of atopic diseases. However, the dose–response relationship between allergic

symptoms and complexity of indoor environmental allergen sources (IEAS) has not been clearly evaluated before. Therefore, we designed this study to investigate the overall effect of multiple IEAS on appearance of asthma (AS), allergic rhinitis (AR), and eczema (EC) symptoms in 1472 primary school children. Among various IEAS analyzed, only stuffed toys, cockroaches, and mold patches fit the model of `more IEAS, higher odds ratio (OR) of association'. The association of IEAS and AR increased stepwise as more IEAS appeared in the environment (1.71, 2.47, to 2.86). In AS and EC, the association was significant only when all three IEAS were present (1.42, 1.98, to 4.11 in AS; 1.40, 1.76, to 2.95 in EC). These results showed that different IEAS had a synergistic effect on their association with atopic symptoms and also suggest that there is a dose–response relationship between kinds of IEAS and risk of appearance of atopic diseases.

Biotranformation

A. E. Glenn, F. I. Meredith, W. H. Morrison III, C. W. Bacon. (Toxicology and Mycotoxin Research Unit,[1] Quality Assessment Research Unit, Russell Research Center, USDA Agricultural Research Service, Athens, Georgia 30604). **Identification of Intermediate and Branch Metabolites Resulting from Biotransformation of 2-Benzoxazolinone by *Fusarium verticillioides*.** Applied and Environmental Microbiology, 69(6) (2003), 3165-3169.

Detoxification of the maize (*Zea mays*) antimicrobial compound 2-benzoxazolinone by the fungal endophyte *Fusarium verticillioides* involves two genetic loci, *FDB1* and *FDB2*, and results in the formation of *N*-(2-hydroxyphenyl)malonamic acid. Intermediate and branch metabolites were previously suggested to be part of the biotransformation pathway. Evidence is presented here in support of 2-aminophenol as the intermediate metabolite and 2-acetamidophenol as the branch metabolite, which was previously designated as BOA-X. Overall, 2-benzoxazolinone metabolism involves hydrolysis (*FDB1*) to produce 2-aminophenol, which is then modified (*FDB2*) by addition of a malonyl group to produce *N*-(2-hydroxyphenyl)malonamic acid. If the modification is prevented due to genetic mutation (*fbd2*), then 2-acetamidophenol may accumulate as a result of addition of an acetyl group to 2-aminophenol. This study resolves the overall chemistry of the 2-benzoxazolinone detoxification pathway, and we hypothesize that biotransformation of the related antimicrobial 6-methoxy-2-benzoxazolinone to produce *N*-(2-hydroxy-4-methoxyphenyl)malonamic acid also occurs via the same enzymatic modifications. Detoxification of these antimicrobials by *F. verticillioides* apparently is not a major virulence factor but may enhance the ecological fitness of the fungus during colonization of maize stubble and field debris.

Adrie J. J. Straathof, Sven Panke, Andreas Schmid. (Delft University of Technology, Kluyver Laboratory for Biotechnology, Julianalaan 67, NL-2628 BC, Delft, The Netherlands. Institute of Process Engineering, Swiss Federal Institute of Technology (ETH), Zürich, Sonnegstrasse 3, CH-8092, Zürich, Switzerland. Institute of Biotechnology, Swiss Federal Institute of Technology (ETH), Zürich, Hoenggerberg, CH-8093, Zurich, Switzerland). **The production of fine chemicals by biotransformations**. Current Opinion in Biotechnology, 13(6) (2002), 548-556.

Today, biocatalysis is a standard technology for the production of chemicals. An analysis of 134 industrial biotransformations reveals that hydrolases (44%) and redox biocatalysts (30%) are the most prominent categories. Most products are chiral (89%) and are used as fine chemicals. In the chemical industry, successful product developments involve on average a yield of 78%, a volumetric productivity of 15.5 g/(L.h) and a final product concentration of 108 g/L. By contrast,

the pharmaceutical industry focuses on time-to-market. The implications of this for future research and development on biocatalysis are discussed.

Andreas Steinreiber and Kurt Faber. (Department of Chemistry, Organic and Bio-organic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria). **Microbial epoxide hydrolases for preparative biotransformations**. Current Opinion in Biotechnology, 12(6) (2001), 552-558.

Epoxide hydrolases from microbial sources are highly versatile biocatalysts for the asymmetric hydrolysis of epoxides on a preparative scale. Besides kinetic resolution, which furnishes the corresponding vicinal diol and remaining non-hydrolysed epoxide in nonracemic form, enantioconvergent processes are possible: these are highly attractive as they lead to the formation of a single enantiomeric diol from a racemic oxirane. The data accumulated over recent years reveal a common picture of the substrate structure selectivity pattern of microbial epoxide hydrolases and indicate that substrates of various structural types can be selectively hydrolysed with enzymes from certain microbial sources.

Ata Akcil, Terry Mudder. (BIOMIN Group, Suleyman Demirel University, TR 32260 Isparta, Turkey). **Microbial destruction of cyanide wastes in gold mining: process review**. Biotechnology Letters, 25(6) (2003), 445-450.

Microbial destruction of cyanide and its related compounds is one of the most important biotechnologies to emerge in the last two decades for treating process and tailings solutions at precious metals mining operations. Hundreds of plant and microbial species (bacteria, fungi and algae) can detoxify cyanide quickly to environmentally acceptable levels and into less harmful by-products. Full-scale bacterial processes have been used effectively for many years in commercial applications in North America. Several species of bacteria can convert cyanide under both aerobic and anaerobic conditions using it as a primary source of nitrogen and carbon. Other organisms are capable of oxidizing the cyanide related compounds of thiocyanate and ammonia under varying conditions of pH, temperature, nutrient levels, oxygen, and metal concentrations. This paper presents an overview of the destruction of cyanide in mining related solutions by microbial processes.

Bharat Bhushan, Louise Paquet, Jim C. Spain, Jalal Hawari. (Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada, U.S. Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403). **Biotransformation of 2,4,6,8,10,12-Hexanitro-2,4,6,8,10,12-Hexaazaisowurtzitane (CL-20) by Denitrifying Pseudomonas sp. Strain FA1**. Applied and Environmental Microbiology, 69(9) (2003), 5216-5221.

The microbial and enzymatic degradation of a new energetic compound, 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20), is not well understood. Fundamental knowledge about the mechanism of microbial degradation of CL-20 is essential to allow the prediction of its fate in the environment. In the present study, a CL-20-degrading denitrifying strain capable of utilizing CL-20 as the sole nitrogen source, Pseudomonas sp. strain FA1, was isolated from a garden soil. Studies with intact cells showed that aerobic conditions were required for bacterial growth and that anaerobic conditions enhanced CL-20 biotransformation. An enzyme(s) involved in the initial biotransformation of CL-20 was shown to be membrane associated and NADH dependent, and its expression was up-regulated about 2.2-fold in CL-20-induced cells. The rates of CL-20 biotransformation by the resting cells and the membrane-enzyme preparation were 3.2 ± 0.1 nmol h-1 mg of cell biomass-1 and 11.5 ± 0.4 nmol h-1 mg of protein-1, respectively, under anaerobic conditions. In the membrane-enzyme-catalyzed reactions, 2.3 nitrite ions (NO2-), 1.5 molecules of nitrous oxide (N2O), and 1.7 molecules of formic acid (HCOOH) were produced per reacted CL-20 molecule. The membrane-enzyme preparation

reduced nitrite to nitrous oxide under anaerobic conditions. A comparative study of native enzymes, deflavoenzymes, and a reconstituted enzyme(s) and their subsequent inhibition by diphenyliodonium revealed that biotransformation of CL-20 is catalyzed by a membrane-associated flavoenzyme. The latter catalyzed an oxygen-sensitive one-electron transfer reaction that caused initial N denitration of CL-20.

Bharat Bhushan, Sandra Trott, Jim C. Spain, Annamaria Halasz, Louise Paquet, and Jalal Hawari. (Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada, U.S. Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403). **Biotransformation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) by a Rabbit Liver Cytochrome P450: Insight into the Mechanism of RDX Biodegradation by *Rhodococcus* sp. Strain DN22**. Applied and Environmental Microbiology, 69(3) (2003), 1347-1351.

A unique metabolite with a molecular mass of 119 Da ($C_2H_5N_3O_3$) accumulated during biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by *Rhodococcus* sp. strain DN22 (D. Fournier, A. Halasz, J. C. Spain, P. Fiurasek, and J. Hawari, Appl. Environ. Microbiol. 68:166-172, 2002). The structure of the molecule and the reactions that led to its synthesis were not known. In the present study, we produced and purified the unknown metabolite by biotransformation of RDX with *Rhodococcus* sp. strain DN22 and identified the molecule as 4-nitro-2,4-diazabutanal using nuclear magnetic resonance and elemental analyses. Furthermore, we tested the hypothesis that a cytochrome P450 enzyme was responsible for RDX biotransformation by strain DN22. A cytochrome P450 2B4 from rabbit liver catalyzed a very similar biotransformation of RDX to 4-nitro-2,4-diazabutanal. Both the cytochrome P450 2B4 and intact cells of *Rhodococcus* sp. strain DN22 catalyzed the release of two nitrite ions from each reacted RDX molecule. A comparative study of cytochrome P450 2B4 and *Rhodococcus* sp. strain DN22 revealed substantial similarities in the product distribution and inhibition by cytochrome P450 inhibitors. The experimental evidence led us to propose that cytochrome P450 2B4 can catalyze two single electron transfers to RDX, thereby causing double denitration, which leads to spontaneous hydrolytic ring cleavage and decomposition to produce 4-nitro-2,4-diazabutanal. Our results provide strong evidence that a cytochrome P450 enzyme is the key enzyme responsible for RDX biotransformation by *Rhodococcus* sp. strain DN22.

Burkhard Schmidt, Hildegard Patti, Claudia Niewersch, Ingolf Schuphan. (Department of Biology V (Ecology, Ecotoxicology, Ecochemistry), RWTH Aachen University, Worringerweg 1, 52056 Aachen, Germany). **Biotransformation of [*ring*-U-14C]4-*n*-nonylphenol by *Agrostemma githago* cell culture in a two-liquid-phase system**. Biotechnology Letters, 25(16) (2003), 1375-1381.

The biotransformation of [14C]4-*n*-nonylphenol (5 mg l-1; 10 mg l-1) by *Agrostemma githago* cell suspensions was studied using a batch two-liquid-phase system (medium/*n*-hexadecane 200:1, v/v). The highly lipophilic 4-*n*-nonylphenol was applied *via n*-hexadecane phase. After 7 d of incubation, more than 85% of applied 4-*n*-nonylphenol was absorbed by the cells, and 40% was transformed to 10 side-chain monohydroxylated metabolites (two with additional double bond at side-chain). The primary metabolites were analyzed by GC-EIMS. In the cells, the monohydroxylated products and residual 4-*n*-nonylphenol were present as glycosides. The method proved to be suitable for the production of primary metabolites of 4-*n*-nonylphenol on a larger scale for identification purposes and for metabolic profiling of the compound.

Byung-Taek Oh, Patrick J. Shea, Rhae A. Drijber, Galina K. Vasilyeva, Gautam Sarath. (School of Chemical Engineering, BK21, Seoul National University, San 56-1, Shinlim-dong, Gwanak-gu, Seoul 151-744, Korea. School of Natural Resources, BK21, Seoul National University, San 56-1, Shinlim-dong, Gwanak-gu, Seoul 151-744, Korea. Department of

Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583-0915, USA. Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, Pushchino, Russia 142292. USDA-ARS & Department of Biochemistry, University of Nebraska, Lincoln, NE 68583-0664, USA). **TNT Biotransformation and Detoxification by a Pseudomonas Aeruginosa Strain**. Biodegradation, 14(5) (2003) 309-319.

Successful microbial-mediated remediation requires transformation pathways that maximize metabolism and minimize the accumulation of toxic products. Pseudomonas aeruginosa strain MX, isolated from munitions-contaminated soil, degraded 100 mg TNT L-1 in culture medium within 10 h under aerobic conditions. The major TNT products were 2-amino-4,6-dinitrotoluene (2ADNT, primarily in the supernatant) and 2,2'-azoxytoluene (2,2'AZT, primarily in the cell fraction), which accumulated as major products via the intermediate 2-hydroxylamino-4,6-dinitrotoluene (2HADNT). The 2HADNT and 2,2'AZT were relatively less toxic to the strain than TNT and 2ADNT. Aminodinitrotoluene (ADNT) production increased when yeast extract was added to the medium. While TNT transformation rate was not affected by pH, more HADNTs accumulated at pH 5.0 than at pH 8.0 and AZTs did not accumulate at the lower pH. The appearance of 2,6-diamino-4-nitrotoluene (2,6DANT) and 2,4-diamino-6-nitrotoluene (2,4DANT); dinitrotoluene (DNT) and nitrotoluene (NT); and 3,5-dinitroaniline (3,5DNA) indicated various routes of TNT metabolism and detoxification by P. aeruginosa strain MX.
C. Gravato and M. A. Santos. (Animal Physiology/Ecotoxicology Sector, Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal). **Juvenile Sea Bass Liver Biotransformation and Erythrocytic Genotoxic Responses to Pulp Mill Contaminants.** Ecotoxicology and Environmental Safety, 53(1) (2002), 104-112.

This research represents the first study concerning liver phase I biotransformation induction, measured as cytochrome *P*-450 (P450) and ethoxyresorufin-*O*-deethylase (EROD), and genotoxic responses, measured as erythrocytic micronuclei (EMN) and nuclear abnormalities (ENA), in *Dicentrarchus labrax* L. (sea bass) exposed to pulp and paper mill contaminants. Juvenile sea bass were exposed for 6 h to 0 (control) and 0.0125 μM concentrations of the resin acids (RAs) abietic acid (AA) and dehydroabietic acid (DHAA) or 7-isopropyl-1-methylphenanthrene (retene) (Experiment 1). Sea bass were exposed for 6 h to 0 (control), 0.78, 1.56, 3.12, 6.25, and 12.5% bleached kraft pulp mill environmental contaminated water (BKPMECW) collected at the old sewage outlet of a pulp and paper industry (Experiment 2). The time-dependent response was studied in sea bass at 0, 8, 16, 24, 48, and 72 h exposure to 0.78 and 12.5% BKPMECW (Experiment 3). The experimental results demonstrated the presence of genotoxic compounds in BKPMECW. AA, DHAA, and retene may be the constituents responsible for high BKPMECW genotoxicity, since they induced similar sea bass EMN and ENA frequency increases. The BKPMECW, the RAs, and retene failed to significantly increase liver EROD activity and P450 content at 6 h. Furthermore, 3.12% BKPMECW, 0.0125 μM AA, and 0.0125 μM retene significantly decreased liver EROD activity. However, P450 was significantly increased from 8 up to 72 h exposure to BKPMECW. Therefore, the low or inhibited EROD levels could be a consequence of a general membrane disturbance by BKPMECW, RAs, and retene. However, liver ALT results indicate significant liver damage or enzyme inhibition only at 8, 16, 48, and 72 h exposure to BKPMECW.
C. Gravato, M. A. Santos. (Animal Physiology/Ecotoxicology Sector, Department of Biology, University of Aveiro, 3810-193, Aveiro, Protugal). **Juvenile Sea Bass Liver Biotransformation Induction and Erythrocytic Genotoxic Responses to Resin Acids**. Ecotoxicology and Environmental Safety, 52(3) (2002), 238-247.

Juvenile *Dicentrarchus labrax* L. (sea bass) were exposed for 0, 2, 4, 6, and 8 h to abietic acid (AA) and dehydroabietic acid (DHAA) in concentration ranges 0, 0.0125, 0.025, 0.05, 0.1, 0.3,

0.9, and 2.7 μM. Liver cytochrome *P*-450 content (P450) and liver ethoxyresorufin-*O*-deethylase activity (EROD) were determined as phase I biotransformation biomarkers. Genotoxicity was measured as erythrocytic micronuclei (EMN) and nuclear abnormalities (ENA). Liver damage was assessed as liver alanine aminotransferase activity (ALT) and liver somatic index (LSI) was used as a general health indicator. AA inhibited EROD (at 2 h exposure to 0.05 μM) or failed to induce it, whereas a significant P450 increase was observed at 2 h exposure to 0.05 (2.3-fold) and 2.7 μM (6.3-fold). A significant P450 increase was also observed at 6 and 8 h exposure, respectively, to 0.0125 (3.4-fold) and 0.025 μM (4.9-fold) AA. EMN and ENA frequency were significantly increased at 2 h exposure to 0.9 μM AA. A significant EMN and ENA time-related increase was observed with an increased exposure length up to 8 h. Therefore, all the AA concentrations tested promoted an EMN and ENA increase at 8 h exposure. DHAA induced a significant EROD increase (3.2-fold) at 2 h exposure to the lowest concentration tested. Liver P450 was significantly increased at 2 h exposure to 0.025 (1.8-fold) and 0.3 μM (2.5-fold), at 4 h exposure to 0.1 μM (3.6-fold), and at 6 h exposure to 0.1 (3.2-fold) and 0.3 μM (2.8-fold), whereas it was significantly decreased (30% of control value) at 4 h exposure to 0.9 μM. EMN and ENA frequency were significantly increased from 0 to 2 h exposure to all DHAA concentrations tested and remained high from 2 to 8 h. EMN frequency was increased 20 times over control at 2 h exposure to 0.0125 μM DHAA, whereas it increased 9-fold at 2 h exposure to 0.9 μM AA. Furthermore, ENA frequency was increased 3 times over control at 2 h exposure to 0.0125 μM DHAA, and 2-fold after exposure to 0.9 μM AA. Maximal EMN and ENA induction was observed at 2 h exposure to 0.0125 μM DHAA and only at 4 h exposure to 2.7 μM AA. Therefore, the comparative analysis of the two resin acids genotoxic effects, measured as EMN and ENA, indicated that DHAA is more genotoxic than AA.

C. Gravato, M. A. Santos. (Department of Biology, Animal Physiology/Ecotoxicology Sector, University of Aveiro, Aveiro 3810-193, Portugal). ***Dicentrarchus labrax* biotransformation and genotoxicity responses after exposure to a secondary treated industrial/urban effluent**. Ecotoxicology and Environmental Safety, 55(3) (2003), 300-306.

The present research work was designed to study *Dicentrarchus labrax* (sea bass) biotransformation and genotoxicity responses to the soluble fraction of a secondary treated industrial/urban effluent (SF-STIUE) discharged through a submarine pipe outlet into the Aveiro coastal area. Sea bass was exposed for 4, 8, 16, 24, 48, and 96 h to 0%, 0.1%, and 1% SF-STIUE and the following biological responses were measured: (1) liver cytochrome P450 (P450) content and ethoxyresorufin-*O*-deethylase (EROD) activity, as phase I biotransformation parameters; (2) liver gluthathione *S*-transferase (GST) activity as a phase II conjugation enzyme; (3) biliary and liver cytosol naphthalene (Naph)- and benzo(*a*)pyrene (B(*a*)P)-type metabolites, by fixed wavelength fluorescence detection (FF); (4) liver DNA strand breaks, erythrocytic micronuclei (EMN), and erythrocytic nuclear abnormalities (ENA) as genotoxicity parameters. Both SF-STIUE dilutions (0.1% and 1%) failed to significantly increase liver EROD activity, despite a significant increase of liver P450 at 16 and 48 h exposure to 0.1%. Liver GST activity increased significantly at 4 h of sea bass exposure to 1% SF-STIUE, being inhibited at 96 h of exposure to this SF-STIUE dilution. Naph- and B(*a*)P-type metabolite contents were not significantly increased in bile. However, Naph-type metabolite contents increased significantly in liver cytosol at 4 h exposure to 1% SF-STIUE, and at 24 h exposure to 0.1% and 1% SF-STIUE. Furthermore, B(*a*)P-type metabolites increased significantly in liver cytosol at 4 h exposure to 1% SF-STIUE, and 16 h exposure to 0.1% and 1% SF-STIUE. EMN and ENA frequencies increased significantly at 4, 8, 16, 24, 48, and 96 h exposure to 0.1% and 1% SF-STIUE. Liver DNA integrity decreased significantly at 96 h of sea bass exposure to 1% SF-STIUE. The STIUE

discharged into Aveiro coastal area is of great ecotoxicological concern due to its genotoxic potential.

Charles E Nakamura and Gregory M Whited. (DuPont Central Research & Development Experimental Station, 328/245A, Wilmington, DE 19880-0328, USA. Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304, USA). **Metabolic engineering for the microbial production of 1,3-propanediol. Current Opinion in Biotechnology,** 14(5) (2003), 454-459.

Improvements in the biological production of 1,3-propanediol, a key component of an emerging polymer business, have been realized. Utilizing genes from natural strains that produce 1,3-propanediol from glycerol, metabolic engineering has enabled the development of a recombinant strain that utilizes the lower cost feedstock D-glucose. This accomplishment bodes well for future metabolic engineering efforts and, ultimately, for increased societal benefit obtained through the production of chemicals from renewable resources.

Chi Ming So, Craig D. Phelps, L. Y. Young. (Biotechnology Center for Agriculture and the Environment,1 Department of Environmental Sciences, Cook College, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08901-8520). **Anaerobic Transformation of Alkanes to Fatty Acids by a Sulfate-Reducing Bacterium, Strain Hxd3**. Applied and Environmental Microbiology, 69(7) (2003), 3892-3900.

Strain Hxd3, an alkane-degrading sulfate reducer previously isolated and described by Aeckersberg et al. (F. Aeckersberg, F. Bak, and F. Widdel, Arch. Microbiol. 156:5-14, 1991), was studied for its alkane degradation mechanism by using deuterium and 13C-labeled compounds. Deuterated fatty acids with even numbers of C atoms (C-even) and 13C-labeled fatty acids with odd numbers of C atoms (C-odd) were recovered from cultures of Hxd3 grown on perdeuterated pentadecane and [1,2-13C2]hexadecane, respectively, underscoring evidence that C-odd alkanes are transformed to C-even fatty acids and vice versa. When Hxd3 was grown on unlabeled hexadecane in the presence of [13C]bicarbonate, the resulting 15:0 fatty acid, which was one carbon shorter than the alkane, incorporated a 13C label to form its carboxyl group. The same results were observed when tetradecane, pentadecane, and perdeuterated pentadecane were used as the substrates. These observations indicate that the initial attack of alkanes includes both carboxylation with inorganic bicarbonate and the removal of two carbon atoms from the alkane chain terminus, resulting in a fatty acid one carbon shorter than the original alkane. The removal of two terminal carbon atoms is further evidenced by the observation that the [1,2-13C2]hexadecane-derived fatty acids contained either two 13C labels located exclusively at their acyl chain termini or none at all. Furthermore, when perdeuterated pentadecane was used as the substrate, the 14:0 and 16:0 fatty acids formed both carried the same numbers of deuterium labels, while the latter was not deuterated at its carboxyl end. These observations provide further evidence that the 14:0 fatty acid was initially formed from perdeuterated pentadecane, while the 16:0 fatty acid was produced after chain elongation of the former fatty acid with nondeuterated carbon atoms. We propose that strain Hxd3 anaerobically transforms an alkane to a fatty acid through a mechanism which includes subterminal carboxylation at the C-3 position of the alkane and elimination of the two adjacent terminal carbon atoms.

David J. Hopper, Lisa Cottrell. (Institute of Biological Sciences, University of Wales, Aberystwyth, Ceredigion SY23 3DD, United Kingdom). **Alkylphenol Biotransformations Catalyzed by 4-Ethylphenol Methylenehydroxylase**. Applied and Environmental Microbiology, 69(6) (2003), 3650-3652.

4-Ethylphenol methylenehydroxylase from *Pseudomonas putida* JD1 acts by dehydrogenation of its substrate to give a quinone methide, which is then hydrated to an alcohol. It was shown to be

active with a range of 4-alkylphenols as substrates. 4-*n*-Propylphenol, 4-*n*-butylphenol, chavicol, and 4-hydroxydiphenylmethane were hydroxylated on the methylene group next to the benzene ring and produced the corresponding chiral alcohol as the major product. The alcohols 1-(4'-hydroxyphenyl)propanol and 1-(4'-hydroxyphenyl)-2-propen-1-ol, produced by the biotransformation of 4-*n*-propylphenol and chavicol, respectively, were shown to be *R*(+) enantiomers. 5-Indanol, 6-hydroxytetralin, 4-isopropylphenol, and cyclohexylphenol, with cyclic or branched alkyl groups, gave the corresponding vinyl compounds as their major products.
David R. Nicholas, Srividhya Ramamoorthy, Vince Palace, Stefan Spring, Johnnie N. Moore, R. Frank Rosenzweig. (Division of Biological Sciences, Program in Microbial Ecology, University of Montana, Missoula, MT 59812-4824, USA. Habitat Impacts Research, Department of Fisheries and Oceans, 501 University Crescent, Winnipeg, Manitoba, Canada. DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Zellkurturen GmbH, Mascheroden Weg 1b, D-38124 Braunschweig, Germany. Department of Geology, University of Montana, Missoula, MT 59812-4824, USA). **Biogeochemical transformations of arsenic in circumneutral freshwater sediments.** Biodegradation, 14(2) (2003), 123-137.

Arsenic is a wide-spread contaminant of soils and sediments, and many watersheds worldwide regularly experience severe arsenic loading. While the toxicity of arsenic to plants and animals is well recognized, the geochemical and biological transformations that alter its bioavailability in the environment are multifaceted and remain poorly understood. This communication provides a brief overview of our current understanding of the biogeochemistry of arsenic in circumneutral freshwater sediments, placing special emphasis on microbial transformations. Arsenic can reside in a number of oxidation states and complex ions. The common inorganic aqueous species at circumneutral pH are the negatively charged arsenates ($H_2As^VO_4^-$ and $Has^VO_4^{2-}$) and zero-charged arsenite ($H_3As^{III}O_3^0$). Arsenic undergoes diagenesis in response to both physical and biogeochemical processes. It accumulates in oxic sediments by adsorption on and/or co-precipitation with hydrous iron and manganese oxides. Burial of such sediments in anoxic/suboxic environments favors their reduction, releasing Fe(II), Mn(II) and associated adsorbed/coprecipitated As. Upward advection can translocate these cations and As into the overlying oxic zone where they may reprecipitate. Alternatively, As may be repartitioned to the sulfidic phase, forming precipitates such as arsenopyrite and orpiment. Soluble and adsorbed As species undergo biotic transformations. As(V) can serve as the terminal electron acceptor in the biological oxidation of organic matter, and the limited number of microbes capable of this transformations are diverse in their phylogeny and physiology. Fe(III)-respiring bacteria can mobilize both As(V) and As(III) bound to ferric oxides by the reductive dissolution of iron-arsenate minerals. SO_4^{2-}-reducing bacteria can promote deposition of As(III) as sulfide minerals via their production of sulfide. A limited number of As(III)-oxidizing bacteria have been identified, some of which couple this reaction to growth. Lastly, prokaryotic and eukaryotic microbes can alter arsenic toxicity either by coupling cellular export to its reduction or by converting inorganic As to organo-arsenical compounds. The degree to which each of these metabolic transformations influences As mobilization or sequestration in different sedimentary matrices remains to be established.
Dick B. Janssen, Jantien E. Oppentocht, Gerrit J. Poelarends. (Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, NL-9747 AG Groningen, The Netherlands). **Microbial dehalogenation**. Current Opinion in Biotechnology, 12(3) (2001), 254-258.

Novel dehalogenases have been identified recently in various bacteria that utilise halogenated substrates. X-ray studies and sequence analysis have revealed insight into the molecular

mechanisms of hydrolytic dehalogenases. Furthermore, genetic and biochemical studies have indicated that reductive dehalogenases are extra-cytoplasmic corrinoid-containing iron–sulphur proteins. Sequence analysis and mutagenesis studies indicate that several dehalogenases are homologous to enzymes that carry out transformations on non-halogenated substrates.
Erik J de Vries, Dick B Janssen. (Department of Biochemistry, Groningen Biomolecular Sciences & Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands). **Biocatalytic conversion of epoxides**. Current Opinion in Biotechnology, 14(4) (2003), 414-420.

Epoxides are attractive intermediates for producing chiral compounds. Important biocatalytic reactions involving epoxides include epoxide hydrolase mediated kinetic resolution, leading to the formation of diols and enantiopure remaining substrates, and enantioconvergent enzymatic hydrolysis, which gives high yields of a single enantiomer from racemic mixtures. Epoxides can also be converted by non-hydrolytic enantioselective ring opening, using alternative anionic nucleophiles; these reactions can be catalysed by haloalcohol dehalogenases. The differences in scope of these enzymatic conversions is related to their different catalytic mechanisms, which involve, respectively, covalent catalysis with an aspartate carboxylate as the nucleophile and non-covalent catalysis with a tyrosine that acts as a general acid-base. The emerging new possibilities for enantioselective biocatalytic conversion of epoxides suggests that their importance in green chemistry will grow.
Eva H. Hansen, Line Albertsen, Thomas Schäfer, Charlotte Johansen, Jens C. Frisvad, Søren Molin, Lone Gram. (Novozymes A/S, DK-2880 Bagsværd, Department of Seafood Research, Danish Institute for Fisheries Research at the Technical University of Denmark, Section of Food Biotechnology and Mycology, Section of Molecular Microbiology, BioCentrum, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark). **Curvularia Haloperoxidase: Antimicrobial Activity and Potential Application as a Surface Disinfectant**. Applied and Environmental Microbiology, 69(8) (2003), 4611-4617.

A presumed antimicrobial enzyme system, the Curvularia haloperoxidase system, was examined with the aim of evaluating its potential as a sanitizing agent. In the presence of hydrogen peroxide, Curvularia haloperoxidase facilitates the oxidation of halides, such as chloride, bromide, and iodide, to antimicrobial compounds. The Curvularia haloperoxidase system caused several-log-unit reductions in counts of bacteria (Pseudomonas spp., Escherichia coli, Serratia marcescens, Aeromonas salmonicida, Shewanella putrefaciens, Staphylococcus epidermidis, and Listeria monocytogenes), yeasts (Candida sp. and Rhodotorula sp.), and filamentous fungi (Aspergillus niger, Aspergillus tubigensis, Aspergillus versicolor, Fusarium oxysporum, Penicillium chrysogenum, and Penicillium paxilli) cultured in suspension. Also, bacteria adhering to the surfaces of contact lenses were killed. The numbers of S. marcescens and S. epidermidis cells adhering to contact lenses were reduced from 4.0 and 4.9 log CFU to 1.2 and 2.7 log CFU, respectively, after treatment with the Curvularia haloperoxidase system. The killing effect of the Curvularia haloperoxidase system was rapid, and 106 CFU of E. coli cells/ml were eliminated within 10 min of treatment. Furthermore, the antimicrobial effect was short lived, causing no antibacterial effect against E. coli 10 min after the system was mixed. Bovine serum albumin (1%) and alginate (1%) inhibited the antimicrobial activity of the Curvularia haloperoxidase system, whereas glucose and Tween 20 did not affect its activity. In conclusion, the Curvularia haloperoxidase system is an effective sanitizing system and has the potential for a vast range of applications, for instance, for disinfection of contact lenses or medical devices.
H S Vieira, J A Takahashi, M A D Boaventura. (Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil). **Biotransformation of methyl ent-17-hydroxy-16g-kauran-19-**

oate by Rhizopus stolonifer. Applied Microbiology and Biotechnology, 53(5) (2000), 0601 – 0604.

Methyl ent-17-hydroxy-16#-kauran-19-oate was fed to a 2-day-old culture of the fungus Rhizopus stolonifer, fermenting at room temperature (25 °C) in an orbital shaker (2 l). After 11 days, both broth and mycelia were extracted with ethyl acetate. Two novel compounds were isolated from this experiment: methyl ent-9!,17-dihydroxy-16#-kauran-19-oate and methyl ent-7!,17-dihydroxy-16#-kauran-19-oate. Their structures were fully confirmed by spectroscopic methods.

Henry H. Tabak, Rakesh Govind. (US Environmental Protection Agency, ORD, NRMRL, Cincinnati, OH 45268, USA Author for correspondence. Department of Chemical Engineering, University of Cincinnati, Cincinnati, OH 45221, USA). **Advances In Biotreatment of Acid Mine Drainage and Biorecovery of Metals: 2. Membrane Bioreactor System for Sulfate Reduction**. Biodegradation, 14(6) (2003), 437-452.

Several biotreatmemt techniques for sulfate conversion by the sulfate reducing bacteria (SRB) have been proposed in the past, however few of them have been practically applied to treat sulfate containing acid mine drainage (AMD). This research deals with development of an innovative polypropylene hollow fiber membrane bioreactor system for the treatment of acid mine water from the Berkeley Pit, Butte, MT, using hydrogen consuming SRB biofilms. The advantages of using the membrane bioreactor over the conventional tall liquid phase sparged gas bioreactor systems are: large microporous membrane surface to the liquid phase; formation of hydrogen sulfide outside the membrane, preventing the mixing with the pressurized hydrogen gas inside the membrane; no requirement of gas recycle compressor; membrane surface is suitable for immobilization of active SRB, resulting in the formation of biofilms, thus preventing washout problems associated with suspended culture reactors; and lower operating costs in membrane bioreactors, eliminating gas recompression and gas recycle costs. Information is provided on sulfate reduction rate studies and on biokinetic tests with suspended SRB in anaerobic digester sludge and sediment master culture reactors and with SRB biofilms in bench-scale SRB membrane bioreactors. Biokinetic parameters have been determined using biokinetic models for the master culture and membrane bioreactor systems. Data are presented on the effect of acid mine water sulfate loading at 25, 50, 75 and 100 ml/min in scale-up SRB membrane units, under varied temperatures (25, 35 and 40 °C) to determine and optimize sulfate conversions for an effective AMD biotreatment. Pilot-scale studies have generated data on the effect of flow rates of acid mine water (MGD) and varied inlet sulfate concentrations in the influents on the resultant outlet sulfate concentration in the effluents and on the number of SRB membrane modules needed for the desired sulfate conversion in those systems. The pilot-scale data indicate that the SRB membrane bioreactors systems can be applied toward field-scale biotreatment of AMD and for recovery of high purity metals and an agriculturally usable water.

Henry H. Tabak, Richard Scharp, John Burckle, Fred K. Kawahara, Rakesh Govind. (Author for correspondence U.S. Environmental Protection Agency, ORD, NRMRL, Cincinnati, OH 45268, USA. U.S. Environmental Protection Agency, ORD, NRMRL, Cincinnati, OH 45268, USA. University of Cincinnati, Department of Chemical Engineering, Cincinnati, OH 45221, USA). **Advances in biotreatment of acid mine drainage and biorecovery of metals: 1. Metal precipitation for recovery and recycle**. Biodegradation, 14(6) (2003), 423-436.

Acid mine drainage (AMD), an acidic metal-bearing wastewater, poses a severe pollution problem attributed to post mining activities. The metals usually encountered in AMD and considered of concern for risk assessment are arsenic, cadmium, iron, lead, manganese, zinc, copper and sulfate. The pollution generated by abandoned mining activities in the area of Butte,

Montana has resulted in the designation of the Silver Bow Creek–Butte Area as the largest Superfund (National Priorities List) site in the U.S. This paper reports the results of bench-scale studies conducted to develop a resource recovery based remediation process for the clean up of the Berkeley Pit. The process utilizes selective, sequential precipitation (SSP) of metals as hydroxides and sulfides, such as copper, zinc, aluminum, iron and manganese, from the Berkeley Pit AMD for their removal from the water in a form suitable for additional processing into marketable precipitates and pigments. The metal biorecovery and recycle process is based on complete separation of the biological sulfate reduction step and the metal precipitation step. Hydrogen sulfide produced in the SRB bioreactor systems is used in the precipitation step to form insoluble metal sulfides. The average metal recoveries using the SSP process were as follows: aluminum (as hydroxide) 99.8%, cadmium (as sulfide) 99.7%, cobalt (as sulfide) 99.1% copper (as sulfide) 99.8%, ferrous iron (sulfide) 97.1%, manganese (as sulfide) 87.4%, nickel (as sulfide) 47.8%, and zinc (as sulfide) 100%. The average precipitate purity for metals, copper sulfide, ferric hydroxide, zinc sulfide, aluminum hydroxide and manganese sulfide were: 92.4, 81.5, 97.8, 95.6 , 92.1 and 75.0%, respectively. The final produced water contained only calcium and magnesium and both sulfate and sulfide concentrations were below usable water limits. Water quality of this agriculturally usable water met the EPA's gold standard criterion.

Horacio Bach, Yevgeny Berdichevsky, David Gutnick. (Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Science, Tel Aviv University, Tel Aviv 69978, Israel). **An Exocellular Protein from the Oil-Degrading Microbe *Acinetobacter venetianus* RAG-1 Enhances the Emulsifying Activity of the Polymeric Bioemulsifier Emulsan**. Applied and Environmental Microbiology, 69(5) (2003), 2608-2615.

The oil-degrading microorganism *Acinetobacter venetianus* RAG-1 produces an extracellular polyanionic, heteropolysaccharide bioemulsifier termed emulsan. Emulsan forms and stabilizes oil-water emulsions with a variety of hydrophobic substrates. Removal of the protein fraction yields a product, apoemulsan, which exhibits much lower emulsifying activity on hydrophobic substrates such as *n*-hexadecane. One of the key proteins associated with the emulsan complex is a cell surface esterase. The esterase (molecular mass, 34.5 kDa) was cloned and overexpressed in *Escherichia coli* BL21(DE3) behind the phage T7 promoter with the His tag system. After overexpression, about 80 to 90% of the protein was found in inclusion bodies. The overexpressed esterase was recovered from the inclusion bodies by solubilization with deoxycholate and, after slow dialysis, was purified by metal chelation affinity chromatography. Mixtures containing apoemulsan and either the catalytically active soluble form of the recombinant esterase isolated from cell extracts or the solubilized inactive form of the enzyme recovered from the inclusion bodies formed stable oil-water emulsions with very hydrophobic substrates such as hexadecane under conditions in which emulsan itself was ineffective. Similarly, a series of esterase-defective mutants were generated by site-directed mutagenesis, cloned, and overexpressed in *E. coli*. Mutant proteins defective in catalytic activity as well as others apparently affected in protein conformation were also active in enhancing the apoemulsan-mediated emulsifying activity. Other proteins, including a His-tagged overexpressed esterase from the related organism *Acinetobacter calcoaceticus* BD4, showed no enhancement.

Jörg Overhage, Alexander Steinbüchel, and Horst Priefert. (Institut für Molekulare Mikrobiologie und Biotechnologie der Westfälischen Wilhelms-Universität Münster, D-48149 Münster, Germany). **Highly Efficient Biotransformation of Eugenol to Ferulic Acid and Further Conversion to Vanillin in Recombinant Strains of Escherichia coli**. Applied and Environmental Microbiology, 69(11) (2003), 6569-6576.

The vaoA gene from Penicillium simplicissimum CBS 170.90, encoding vanillyl alcohol oxidase, which also catalyzes the conversion of eugenol to coniferyl alcohol, was expressed in Escherichia coli XL1-Blue under the control of the lac promoter, together with the genes calA and calB, encoding coniferyl alcohol dehydrogenase and coniferyl aldehyde dehydrogenase of Pseudomonas sp. strain HR199, respectively. Resting cells of the corresponding recombinant strain E. coli XL1-Blue(pSKvaomPcalAmcalB) converted eugenol to ferulic acid with a molar yield of 91% within 15 h on a 50-ml scale, reaching a ferulic acid concentration of 8.6 g liter-1. This biotransformation was scaled up to a 30-liter fermentation volume. The maximum production rate for ferulic acid at that scale was 14.4 mmol per h per liter of culture. The maximum concentration of ferulic acid obtained was 14.7 g liter-1 after a total fermentation time of 30 h, which corresponded to a molar yield of 93.3% with respect to the added amount of eugenol. In a two-step biotransformation, E. coli XL1-Blue(pSKvaomPcalAmcalB) was used to produce ferulic acid from eugenol and, subsequently, E. coli(pSKechE/Hfcs) was used to convert ferulic acid to vanillin (J. Overhage, H. Priefert, and A. Steinbüchel, Appl. Environ. Microbiol. 65:4837-4847, 1999). This process led to 0.3 g of vanillin liter-1, besides 0.1 g of vanillyl alcohol and 4.6 g of ferulic acid liter-1. The genes ehyAB, encoding eugenol hydroxylase of Pseudomonas sp. strain HR199, and azu, encoding the potential physiological electron acceptor of this enzyme, were shown to be unsuitable for establishing eugenol bioconversion in E. coli XL1-Blue.

Juan Jauregui, Brenda Valderrama, Arnulfo Albores, Rafael Vazquez-Duhalt. (Instituto de Biotecnologia, UNAM Apartado Postal 510-3, Cuernavaca, Morelos. 62250 Mexico. CINVESTAV-IPN, México D.F, Mexico Author for correspondence). **Microsomal Transformation of Organophosphorus Pesticides by White Rot Fungi**. Biodegradation, 14(6) (2003), 397-406.

The enzymatic mechanism for the transformation of organophosphorus pesticides (OPPs) by different white-rot fungi strains was studied. With the exception of *Ganoderma* applanatum 8168, all strains from a collection of 17 different fungi cultures were able to deplete parathion. Three strains showing the highest activities were selected for further studies: Bjerkandera adusta 8258, Pleurotus ostreatus 7989 and Phanerochaete chrysosporium 3641. These strains depleted 50 to 96% of terbufos, azinphos-methyl, phosmet and tribufos after four-days exposure to the pesticides. In order to identify the cellular localization of the transformation activity, the extracellular and microsomal fractions of *Pleurotus ostreatus* 7989 were evaluated *in vitro*. While the activities of ligninolytic enzymes (lignin peroxidase, manganese peroxidase and laccase) were detected in the extracellular fraction, no enzymatic modification of any of the five pesticides tested could be found, suggesting the intracellular origin of the transformation activity. In accordance with this observation the microsomal fraction was found able to transform three OPPs with the following rates: 10 µmol mg prot-1 h-1 for phosmet, 5.7 µmol mg prot-1 h-1 for terbufos, and 2.2 µmol mg prot-1 h-1 for azinphos-methyl. The products from these reactions and from the transformation of trichlorfon and malathion, were identified by mass-spectrometry. These results, supported by specific inhibition experiments and the stringent requirement for NADPH during the *in vitro* assays suggest the involvement of a cytochrome P450.

Khursheed Karim, S. K. Gupta. (Centre for Environmental Science and Engineering, Indian Institute of Technology, Powai, Mumbai 400 076, India). **Continuous biotransformation and removal of nitrophenols under denitrifying conditions**. Water Research, 37(15) (2003), 3569-3578.

The effect of COD/NO3--N ratio on the biotransformation and removal of 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 2,4-dinitrophenol (2,4-DNP) was studied in bench scale upflow anaerobic sludge blanket (UASB) reactors. Sodium acetate and sodium nitrate were used as

electron donor (substrate) and electron acceptor, respectively. Nitrate nitrogen loading was increased from 0.098 to 0.6 kg/m3 d in order to keep COD/NO3--N ratio as 20.8, 14.3, 9.8, 5.0, 4.0 and 3.33. Throughout the study, input nitrophenolic concentration and hydraulic retention time (HRT) were kept constant as 30 mg/l and 24 h, respectively. 2-Aminophenol (2-AP), 4-aminophenol (4-AP) and 2-amino,4-nitrophenol (2-A,4-NP) were found as the major intermediate metabolite of 2-NP, 4-NP and 2,4-DNP, respectively. Removal of all the three nitrophenols increased with lowering of COD/NO3--N ratio. However, nitrophenols removal got adversely affected when COD/NO3--N ratio was reduced below 5. Maximum removal achieved were 91.63%, 90.17% and 86.10% for 2-NP, 4-NP and 2,4-DNP, respectively at a COD/NO3--N ratio of 5. Simultaneous denitrification and methanogenesis was observed in all the reactors throughout the study.

Kwotsair Chang, Chungsying Lu. (Department of Environmental Engineering, National Chung Hsing Universiφty, Taichung 402, Taiwan. Author for correspondence Department of Environmental Engineering, National Chung Hsing Universiφty, Taichung 402, Taiwan). **Biofiltration of Isopropyl Alcohol by a Trickle-Bed Air Biofilter**. Biodegradation, 14(1) (2003) 9-18.

The performance of trickle-bed air biofilter (TBAB) for the removal of isopropyl alcohol (IPA) was evaluated in concentrations varying from 100 to 500 ppmv and at empty-bed residence time (EBRT) varying from 20 to 90 s. Nearly complete IPA removal could be achieved for influent carbon loading between 6 and 88 $g/m^{3\cdots}h$. The TBAB appears efficient for controlling IPA emission under low-to-high carbon loading conditions. Carbon recoveries of 95-99% were achieved demonstrating the accuracy of results. Applicable operating conditions of TBAB for controlling IPA emission were suggested.

Lyudmila V Kuzina, Ernie D Miller, Baoxue Ge, Thomas A Miller. (Department of Entomology, University of California, Riverside, Riverside, CA 92521, USA. USDA-APHIS-PPQ-PPPC, Phoenix Plant Protection Center, 3645 E Wier, Phoenix, AZ 85040, USA). **Transformation of Enterobacter gergoviae Isolated from Pink Bollworm (Lepidoptera: Gelechiidae) Gut with Bacillus thuringiensis Toxin**. Current Microbiology, 44(1) (2002), 0001 – 0004.

Production of molecules with toxic activity by genetically transformed symbiotic bacteria of pest insects may serve as a powerful approach to biological control. The symbiont, Enterobacter gergoviae, isolated from the gut of the pink bollworm (PBW), has been transformed to express Cyt1A, a cytolytic protein toxin lethal to mosquito and black fly larvae, as a model system. These transgenic bacteria might be used to spread genes encoding insecticidal proteins to populations of agricultural insects or as replacement for chemical insecticides such as malathion used in bait formulation to control specific insect pests, because of extreme public pressure against organophosphate pesticide spraying.

M Bramucci, M Singh, V Nagarajan. (Central Research and Development, DuPont Company, P.O. Box 80328, Wilmington, DE 19880-0328, USA). **Biotransformation of p-xylene and 2,6-dimethylnaphthalene by xylene monooxygenase cloned from a Sphingomonas isolate**. Applied Microbiology and Biotechnology, 59(6) (2002), 679 – 684.

Sphingomonas strain ASU1 was isolated from an industrial wastewater bioreactor and grew on 2,6-dimethylnaphthalene (2,6-DMN) as the sole carbon/energy source. The genes for a xylene monooxygenase were cloned from strain ASU1. Expression of the ASU1 xylene monooxygenase was compared to expression of the pWWO xylene monooxygenase in Escherichia coli. Both monooxygenases transformed p-xylene and 2,6-DMN by initially hydroxylating one methyl group. In addition, the ASU1 monooxygenase also hydroxylated the second methyl group on p-xylene and 2,6-DMN whereas the pWWO monooxygenase hydroxylated the second methyl

group only on p-xylene. Endogenous E. coli enzymes contributed to further oxidation of the resulting aromatic alcohols to form aromatic carboxylates.

Mário Pacheco and Maria Ana Santos. (Animal Physiology/Ecotoxicology Sector, Biology Department, University of Aveiro, 3810-193, Aveiro, Portugal). **Biotransformation, Endocrine, and Genetic Responses of *Anguilla anguilla* L. to Petroleum Distillate Products and Environmentally Contaminated Waters.** Ecotoxicology and Environmental Safety, 49(1) (2001), 4-75.

The European eel (*Anguilla anguilla* L.) was exposed to diesel oil water-soluble fraction (DWSF) and gasoline water-soluble fraction (GWSF). The potential of these fractions to induce endocrine disruption, carbohydrate, and xenobiotic metabolism effects, as well as genotoxic responses, was investigated in a time-course laboratory study (3 h to 6 days). Both water-soluble fractions induced a time-related increase in liver ethoxyresorufin-*O*-deethylase (EROD) activity, as well as the appearance of erythrocytic nuclear abnormalities (ENA) after a 6-day exposure, revealing its genotoxic properties. Initially, DWSF exposure revealed an inhibition of the typical stress responses demonstrated by plasma cortisol and lactate decrease. Nevertheless, this effect progressively disappeared, allowing a plasma glucose and lactate increase after 6 days of exposure. Fish exposed to GWSF exhibited a liver alanine transaminase (ALT) activity increase after a short exposure while the longest exposure revealed liver damage expressed as an ALT activity decrease. A field caging experiment, carried out in a harbor area (Aveiro Lagoon, Portugal), and a complementary laboratory experiment were designed to assess the influence of the daily tide dynamic on polyaromatic hydrocarbon water distribution and effects on liver EROD and ALT activities, as well as ENA frequency. Eels exposed to low- and high-tide harbor waters, in the laboratory, exhibited a similar degree of genotoxicity, whereas clear differences were observed as EROD induction. In the field experiment, caged eels did not display significant responses enhancing the relevance of natural environmental factors on toxicity mechanisms as well as on the apparent lack of toxicity in harbor waters.

Mário Pacheco, Maria Ana Santos. (Animal Physiology/Ecotoxicology Sector, Biology Department, University of Aveiro, Aveiro 3810-193, Portugal). **Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.)**. Ecotoxicology and Environmental Safety, 53(3) (2002), 331-347.

A prolonged toxicity study was carried out in young European eel (*Anguilla anguilla* L.) to evaluate the effects of environmental contaminants, namely, two individual standard compounds, benzo[*a*]pyrene (BaP) and dehydroabietic acid (DHAA), and a complex mixture, bleached kraft pulp mill effluent (BKPME). Fish were exposed to BaP (0.22, 0.45, and 0.9 μM) and BKPME (3.12%, 6.25%, and 12.5% (v/v)) for 3, 7, and 30 days and to DHAA (0.07, 0.15, and 0.30 μM) for 3, 7, 30, 90, and 180 days. The biomarkers include biotransformation and genotoxicity indicators, such as total ethoxyresorufin *O*-deethylase (EROD) activity and frequency of erythrocytic nuclear abnormalities (ENAs), respectively. Hematological dynamics was assessed as frequency of immature erythrocytes (IEs). Histopathological examinations were carried out for the highest concentrations and for 30 days and longer exposures. Total EROD increases significantly only after 180 days of DHAA exposure. However, significant ENA induction was generally observed during exposure to all contaminants tested. Nevertheless, some of the ENA results suggest an altered genotoxic response, which may arise either from short-term exposures to the highest contaminant levels or long-term exposures to the lowest contaminant levels. IE frequency decreased significantly after 30 days of exposure to 0.45 μM BaP and 180 days of exposure to the entire DHAA concentration range. Increased density of pigmented macrophage aggregates in 30-day BaP- and BKPME-exposed fish as well as in 90- and 180-day DHAA-

exposed fish confirmed histopathological liver alterations. Bile accumulation in hepatocytes after BaP treatment, cytoplasmic vacuolization and cell atrophy following DHAA exposure, as well as liver loss of parenchymal cells in BKPME-exposed fish, were also detected. Dispersed necrosis and focal inflammation were observed in the livers of all treated groups. Fish exposed to DHAA and BKPME showed skin and gill disruption as well as kidney Malpighian corpuscle alterations. All 30-day-treated groups revealed intense spleen hemosiderosis, indicating increased erythrophagia. This splenic effect may be strongly correlated with the observed disappearance of ENAs. Neoplastic lesions were not found. A multibiomarker strategy, which includes EROD, ENA, and IE assays as well as histopathological studies, contributed to a better understanding of the global toxic process.

Michael Seeger, Myriam González, Beatriz Cámara, Liliana Muñoz, Emilio Ponce, Lorenzo Mejías, Carolina Mascayano, Yesseny Vásquez, Silvia Sepúlveda-Boza. (Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química, Universidad Técnica Federico Santa María, Valparaíso, Laboratorio de Química Médica y Ensayos Biológicos, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile). **Biotransformation of Natural and Synthetic Isoflavonoids by Two Recombinant Microbial Enzymes**. Applied and Environmental Microbiology, 69(9) (2003), 5045-5050.

Isolation and synthesis of isoflavonoids has become a frequent endeavor, due to their interesting biological activities. The introduction of hydroxyl groups into isoflavonoids by the use of enzymes represents an attractive alternative to conventional chemical synthesis. In this study, the capabilities of biphenyl-2,3-dioxygenase (BphA) and biphenyl-2,3-dihydrodiol 2,3-dehydrogenase (BphB) of Burkholderia sp. strain LB400 to biotransform 14 isoflavonoids synthesized in the laboratory were investigated by using recombinant Escherichia coli strains containing plasmid vectors expressing the bphA1A2A3A4 or bphA1A2A3A4B genes of strain LB400. The use of BphA and BphB allowed us to biotransform 7-hydroxy-8-methylisoflavone and 7-hydroxyisoflavone into 7,2',3'-trihydroxy-8-methylisoflavone and 7,3',4'-trihydroxyisoflavone, respectively. The compound 2'-fluoro-7-hydroxy-8-methylisoflavone was dihydroxylated by BphA at ortho-fluorinated and meta positions of ring B, with concomitant dehalogenation leading to 7,2',3',-trihydroxy-8-methylisoflavone. Daidzein (7,4'-dihydroxyisoflavone) was biotransformed by BphA, generating 7,2',4'-trihydroxyisoflavone after dehydration. Biotransformation products were analyzed by gas chromatography-mass spectrometry and nuclear magnetic resonance techniques.

N. R. Verrengia Guerrero, M. G. Taylor, N. A. Davies, M. A. M. Lawrence, P. A. Edwards, K. Simkiss, E. A. Wider. (Biomarkers Laboratory, Department of Biological Chemistry, Faculty of Exact and Natural Sciences, University of Buenos Aires, 4 piso, Pab. II, 1428, Buenos Aires, Argentina. School of Animal and Microbial Sciences, The University of Reading, PO Box 228, Reading RG6 6AJ, UK). **Evidence of differences in the biotransformation of organic contaminants in three species of freshwater invertebrates.** Environmental Pollution, 117(3) (2002), 523-530.

Acute static bioassays were performed using three freshwater invertebrate species (the oligochaete *Lumbriculus variegatus*, the fingernail clam *Sphaerium corneum* and the larvae *Chironomus riparius*) exposed separately to a variety of ^{14}C radiolabelled contaminants. The aim of this work was to investigate if the chemicals remained as parent compounds after the treatments. Chemicals used were 2,4-dichlorophenol; 2,4,5-trichlorophenol; pentachlorophenol; pyrene; Fenpropidin, and Trifluralin. Homogenates of the whole body tissue of each organism were prepared and total radioactivity was measured. Contaminants were then extracted into organic solvents and analysed by high-pressure liquid chromatography techniques. Chromatograms showed that most of the substances extracted were present as parent compounds

in *S. corneum* and in *L. variegatus*. In contrast, for *C. riparius* a low proportion of the chemicals was recovered as parent compounds. These results suggest that different metabolic processes could take place in the different species.

Rafael Clemente, David J. Walker, Asunción Roig, M. Pilar Bernal. (Author for Correspondence Department of Soil and Water Conservation and Organic Waste Management. Centro de Edafología y Biología Aplicada del Segura, CSIC. Apartado 4195, 30080 Murcia, Spain. Department of Soil and Water Conservation and Organic Waste Management. Centro de Edafología y Biología Aplicada del Segura, CSIC. Apartado 4195, 30080 Murcia, Spain**). Heavy metal bioavailability in a soil affected by mineral sulphides contamination following the mine spillage at Aznalcóllar (Spain).** Biodegradation, 14(3) (2003), 199-205.

A field experiment, lasting 14 months, was carried out in order to assess the effect of organic amendment and lime addition on the bioavailability of heavy metals in contaminated soils. The experiment took place in a soil affected by acid, highly toxic pyritic waste from the Aznalcóllar mine (Seville, Spain) in April 1998. The following treatments were applied (3 plots per treatment): cow manure, a mature compost, lime (to plots having pH < 4), and control without amendment. During the study two crops of *Brassica juncea* were grown, with two additions of each organic amendment. Throughout the study, the evolution of soil pH, total and available (DTPA-extractable) heavy metals content (Zn, Cu, Mn, Fe, Pb and Cd), electrical conductivity (EC), soluble sulphates and plant growth and heavy metal uptake were followed. The study indicates that: (1) soil acidification, due to the oxidation of metallic sulphides in the soil, increased heavy metal bioavailability; (2) liming succeeded in controlling the soil acidification; and (3) the organic materials generally promoted fixation of heavy metals in non-available soil fractions, with Cu bioavailability being particularly affected by the organic treatments.

S.J. Zhang, M. Yang, Q.X. Yang, Y. Zhang, B.P. Xin, F. Pan. (State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P.R. China). **Biosorption of reactive dyes by the mycelium pellets of a new isolate of *Penicillium oxalicum*.** Biotechnology Letters, 25(17) (2003), 1479-1482.

Three reactive dyes were rapidly adsorbed by the mycelium pellets of *Penicillium oxalicum*. Dye removal of Reactive Blue 19 was up to 60% in 10 min and 91% in 80 min. Dye adsorption isotherms fitted Langmuir model well and the maximum adsorption capacities at 20 °C were calculated to be 160 mg g-1 for Reactive Blue 19, 122 mg g-1 for Reactive Red 241 and 137 mg g-1 for Reactive Yellow 145, respectively. The pellets exhibited a high dye adsorption capacity (80–180 mg g-1) for all of the 3 dyes over a wide pH range (pH 2–10), and the maximum adsorption was obtained at pH 2. The adsorption capacity was mildly increased by increasing salinity.

Sandra Trott, Shirley F. Nishino, Jalal Hawari, Jim C. Spain. (Air Force Research Laboratory, Tyndall Air Force Base, Florida 32403,1 Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada). **Biodegradation of the Nitramine Explosive CL-20.** Applied and Environmental Microbiology, 69(3) (2003), 1871-1874.

The cyclic nitramine explosive CL-20 (2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane) was examined in soil microcosms to determine whether it is biodegradable. CL-20 was incubated with a variety of soils. The explosive disappeared in all microcosms except the controls in which microbial activity had been inhibited. CL-20 was degraded most rapidly in garden soil. After 2 days of incubation, about 80% of the initial CL-20 had disappeared. A CL-20-degrading bacterial strain, Agrobacterium sp. strain JS71, was

isolated from enrichment cultures containing garden soil as an inoculum, succinate as a carbon source, and CL-20 as a nitrogen source. Growth experiments revealed that strain JS71 used 3 mol of nitrogen per mol of CL-20.

Steinar Øvrebø, Aage Haugen, Vidar Skaug. (Department of Toxicology, National Institute of Occupational Health, 8149 DEP, N-0033, Oslo, Norway). **Biotransformation of Formaldehyde in Cultured Human Bronchus**. Environmental Research, 89(1) (2002), 38-42.

Biotransformation of formaldehyde to formic acid was studied to investigate the capacity of human bronchial epithelial cells to detoxicate formaldehyde. Normal human bronchial explants and normal primary bronchial epithelial cells were grown in medium containing 0.5 to 5 mM formaldehyde for up to 48 h. Formic acid was quantitated by analytical isotachophoresis. Explants were cultured with up to 5 mM formaldehyde for 48 h with approximately linear turnover, but at 5 mM the cells showed reduced biotransformation relative to the lower concentrations. The mean K_m values for explants were 1.4 and 5.1 mM for cells and the mean V_{max} values were 3.3 nmol/mg protein·min for the explants and 6.1 nmol/mg protein·min for the cells. By using the same technique with hepatocytes we found K_m 1.25 mM and V_{max} 4.2 nmol/mg protein·min, indicating that human bronchial epithelium cells have formaldehyde biotransforming activity similar to that of hepatocytes. Our results indicate that human bronchial epithelial cells oxidize formaldehyde at a relatively fast rate at concentrations up to 3 mM formaldehyde in the medium over a period up to 48 h.

V. L. Maria, A. C. Correia, M. A. Santos. (Department of Biology and Animal Physiology/Ecotoxicology Sector, University of Aveiro, 3810-193, Aveiro, Portugal. Department of Biology, Microbiology/Genetic Sector, University of Aveiro, 3810-193, Aveiro, Portugal). **Genotoxic and hepatic biotransformation responses induced by the overflow of pulp mill and secondary-treated effluents on *Anguilla anguilla* L**. Ecotoxicology and Environmental Safety, 55(1) (2003), 126-137.

Pulp and paper mill effluent compounds pollute the aquatic environment and are responsible for increased biochemical alterations and genotoxicity in aquatic organisms such as fish. Adult eels (*Anguilla anguilla* L) were exposed during 8, 16, 24, and 72 h to the following conditions: (1) aerated, filtered, and dechlorinated tap water (**C**); (2) 2.5% (v/v) sewage water previously treated with activated sludge (**T**); (3) bleached kraft pulp and paper mill effluent collected at the river Vouga, close to an ancient sewage outlet (Portucel), diluted in tap water [25% ($\mathbf{E_{25}}$) and 50% ($\mathbf{E_{50}}$)]; and (4) bleached kraft pulp and paper mill effluent sediment [water-soluble fraction (**S**)]. Liver biotransformation induced by the above conditions was measured as ethoxyresorufin-*O*-deethylase (EROD), cytochrome P450 (P450) (Phase I), and glutathione-*S*-transferase (GST) (Phase II). Genotoxicity was also determined as blood/liver DNA strand breaks and erythrocytic nuclear abnormalities (ENA) induced on European eel (*A. anguilla* L). Liver EROD activity was significantly increased in eels at 8 and 16 h exposure to $\mathbf{E_{25}}$, as well as at 16, 24, and 72 h exposure to $\mathbf{E_{50}}$. **S** exposure induced liver EROD activity only at 24 h. A significant decrease in liver P450 was observed at 72 h exposure to **T**, whereas a significant P450 increase at 16 h was followed by a significant decrease at 24 h exposure to $\mathbf{E_{25}}$. Another P450 significant increase was noticed at 72 h exposure to **S**. Liver GST activity (Phase II) demonstrated a significant increase at 72 h exposure to $\mathbf{E_{50}}$ and to **S**. A significant decrease in blood DNA integrity was observed at 72 h exposure to **T** and at 24 and 72 h to **S**. Blood DNA integrity significantly decreased at 16 and 24 h exposure to $\mathbf{E_{25}}$, as well as at 8, 16, and 24 h exposure to $\mathbf{E_{50}}$. Liver DNA integrity significantly decreased at 72 h exposure to **T** and at 16 h exposure to **S**. Moreover**,** liver DNA integrity was significantly decreased at 24 h exposure to $\mathbf{E_{25}}$ and $\mathbf{E_{50}}$, and 72 h to $\mathbf{E_{50}}$. *A. anguilla* L. increased ENA frequency was detected in **T** at 16, 24, and 72 h,

whereas in $\mathbf{E_{25}}$ and **S** it was observed at 8, 16, and 24 h. Furthermore, $\mathbf{E_{50}}$ ENA frequency increased at 24 h exposure.

Vlada Urlacher, Rolf D. Schmid. (Institute for Technical Biochemistry, University of Stuttgart, Allmandring 31, D-70569, Stuttgart, Germany). **Biotransformations using prokaryotic P450 monooxygenases**. Current Opinion in Biotechnology, 13(6) (2002), 557-564.

Recent studies on microbial cytochrome P450 enzymes have covered several new areas. Advances have been made in structure–function analysis and new non-enzymatic/electrochemical systems for the replacement of NAD(P)H in biocatalysis have been developed. Furthermore, the properties of some enzymes have been re-engineered by site-directed mutagenesis or by methods of directed evolution and new P450s have been functionally expressed and characterized. It is thought that a combination of these approaches will facilitate the use of isolated P450 monooxygenases in biocatalysis.

W A Duetz, H Bouwmeester, J B van Beilen, B Witholt. (Institute of Biotechnology, ETH Hönggerberg, HPT, 8093, Zürich, Switzerland. Plant Research International, P.O. Box 16, 6700 AA, Wageningen, The Netherlands. Enzyscreen B.V., Wassenaarseweg 72, 2333 AL, Leiden, The Netherlands). **Biotransformation of limonene by bacteria, fungi, yeasts, and plants.** Applied Microbiology and Biotechnology, 61(4) (2003), 269 – 277.

The past 5 years have seen significant progress in the field of limonene biotransformation, especially with regard to the regiospecificity of microbial biocatalysts. Whereas earlier only regiospecific biocatalysts for the 1,2 position (limonene-1,2-diol) and the 8-position (f-terpineol) were available, recent reports describe microbial biocatalysts specifically hydroxylating the 3-position (isopiperitenol), 6-position (carveol and carvone), and 7-position (perillyl alcohol, perillylaaldehyde, and perillic acid). The present review also includes the considerable progress made in the characterization of plant P-450 limonene hydroxylases and the cloning of the encoding genes.

Wang Jing-Yuan, Stabnikova Olena, Tay Stephen Tiong-Lee, Ivanov Volodymyr, Tay Joo-Hwa. (Environmental Engineering Research Centre, School of Civil and Environmental Engineering, Nanyang Technological University, Blk N1, 50 Nanyang Avenue, Singapore 639798, Singapore. Environmental Engineering Research Centre, School of Civil and Environmental Engineering, Nanyang Technological University, Blk N1, 50 Nanyang Avenue, Singapore 639798, Singapore). **Intensive bioconversion of sewage sludge and food waste by *Bacillus thermoamylovorans.*** Journal of Microbiology and Biotechnology, 19(4) (2003), 427-432.

The main aim of this work was to intensify conventional composting of a mixture of sewage sludge and solid food wastes by a one-stage thermophilic bioconversion of these wastes into an organic fertilizer. An intensive process was carried out in a closed system, with or without addition of a starter culture of *Bacillus thermoamylovorans*. The most effective thermophilic bioconversion of the mixture of food waste and sewage sludge, with addition of starter culture, was when the pH was buffered with calcium carbonate, or the pH drop in the material was prevented by preliminary removal of sulphides from sewage sludge by hydrogen peroxide.

Y. Shen, C. West and S. R. Hutchins. (National Risk Management Research Laboratory, Subsurface Protection and Remediation Division, U.S. Environmental Protection Agency, Ada, Oklahoma, 74820). **In Vitro Cytotoxicity of Aromatic Aerobic Biotransformation Products in Bluegill Sunfish BF-2 Cells.** Ecotoxicology and Environmental Safety, 45(1) (2000), 27-32.

Toluene (methylbenzene) is a common environmental pollutant that is found in many hazardous waste sites and it is an aquifer contaminant. A concern is the potential risk to human and

ecosystem health due to exposure to toluene and its major biotransformation products. The cytotoxicity of eight aromatic products of toluene aerobic biotransformation was investigated in bluegill sunfish BF-2 cells. The cytotoxicity was determined using several *in vitro* assay endpoints. BF-2 cells were propagated at 32°C in an atmosphere of 5% CO_2–95% air. The concentrations of these products causing 50% inhibition in cell replication, protein content, uptake of natural red, and colony formation were evaluated and compared. The results of the study indicate a direct relationship between the exposure concentration of these products and observed cytotoxic effects. In descending order of cytotoxicity, the compounds were 3-methylcatechol, 4-methylcatechol, catechol, *o*-cresol, *p*-cresol, *m*-cresol, benzaldehyde, and methyl benzoate.

Pollen Biotechnology

Constance Helen Katelaris, Therese Valerie Burke. (Department of Clinical Immunology and Allergy, ICPMR Westmead Hospital, Westmead 2145 Sydney, NSW Australia). **A 7 year pollen profile of major Olympic Games venues in Sydney, Australia**. Aerobiologia, 19(2) (2003), 121-124.

The year 2000 Olympic and Paralympic Games held in Sydney, Australia were unique in the history of the Games because they were staged in the early to mid spring. This led to the concern that pollen-sensitive athletes may have significant problems with allergic symptoms triggered by pollen exposure and that this may have compromised their ability to attain their best performance. Unfortunately, there was no systematic pollen count data available for the city of Sydney up until this time so the purpose of this study was to obtain a profile of the pattern and type of pollens in the region so that Olympic team managers and medical staff could be adequately advised and able to prepare allergic athletes for any exposures encountered while training and competing. We performed pollen monitoring of three major Olympic venues over the six years before the Games to provide a profile of the most prevalent species appearing over the spring. The pollen counts obtained at the major sites were extremely high over the periods of training and competition. Tree pollens appeared from late July, peaking in August and September, whilst grass pollens appeared from September and peaked in mid October. A relatively small number of pollen varieties comprise the majority of the pollen count.

Huey-Jen Su, Pei-Chih Wu, Hsiu-Ling Chen, Fang-Chun Lee and Li-Ling Lin. (Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan, Republic of China). **Exposure Assessment of Indoor Allergens, Endotoxin, and Airborne Fungi for Homes in Southern Taiwan.** Environmental Research, 85(2) (2001), 135-144.

This study was undertaken to examine the seasonal variations of domestic *Der p 1*, *Der p 2*, and endotoxin on mattress and airborne fungal concentrations in homes of asthmatic and nonasthmatic children in southern Taiwan, where temperature and relative humidity are usually high throughout the year. A group of asthmatic children (10–12 years old) were selected randomly based on a citywide questionnaire survey. The nonasthmatic children were chosen to be in the comparison group by matching in age, gender, and proximity of residence. Environmental sampling of domestic microbes was conducted once a month for a year. Twelve calendar months were grouped into spring, summer, fall, and winter according to weather data (mainly average temperature and humidity) from the Central Weather Bureau. Dust samples from a child's mattress and airborne samples from a child's bedroom were collected and analyzed for allergens of *Der p 1* and *Der p 2*, endotoxin, and fungi respectively. Results show that about 65% of children's mattresses in our region have *Der p 1* levels greater than 2 μg/g. It is also apparent that most airborne fungal concentrations found in homes of either asthmatic or

nonasthmatic children are higher than the recommended levels of concern. The predominant genera are *Cladosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, and yeast. In addition, seasonal effects seem to be a critical factor for the concentrations and distributions of domestic endotoxin in these study homes. The implication of long-term exposure to these high levels of environmental microbes and how their effects vary with seasons remain to be further characterized.

M.B. Joshi, S.K. Aher, B.N. Pande. (Stats and Demo (PPC) Dept ofOBGY, Govt. Medical College, Aurangabad 431 001 (M.S.). Department of Botony, New Arts Commerce and Science College, Pame" Dist. Ahmednagar414 302 (M.S). Department of Environmental Science, Dr. B.A. Marathwada University, Aurangabad 431004 (M.S.)). **Circadian Periodicity Curves of Some Air Borne Allergenic Components.** Poll Res., 21(4) (2002), 507-509.

Aerobiological survey was performed at Aurangabad in order to detect airborne inicrobial population in general and identification of the population of fungi constituting the aerial mycoflora. However, during the period of present investigation continuous volumetric Tilak air sampler was employed to obtain a continuous data on the incidence of air borne allergenic spores producing species of fungi in this area. The airborne microbial components were analyzed quantitatively. From allergy point of view spore types such as Alternaria, Aspergillus, Cladosporium, Fusarium, Periconia, insect parts, pollen grains, hyphal fragments etc. were found to be important. Fungal spore types showed variable peaks i.e. from early morning 02.00 hn tFusarium) to afternoon 16.00 hrs (Periconia). The circadian rhythm of miscellaneous bioparticles such as insect parts and hyphal fragments showed their highest peak in morning hrs and subsidiary peak in afternoon. Pollen grains also showed their diurnal peaks at 14.00 hrs.

Mona Mansour, Bruce P. Lanphear, Richard Hornung, Jane Khoury, David I. Bernstein, William Menrath, Joji Decolongon. (For the Cincinnati Lead and Allergen Sampling Study (CLASS) with Children's Hospital Medical Center, The Department of Pediatrics, Cincinnati, Ohio, 45229. For the Cincinnati Lead and Allergen Sampling Study (CLASS) with Children's Hospital Medical Center, Institute for Health Policy & Health Services Research, Cincinnati, Ohio, 45229. For the Cincinnati Lead and Allergen Sampling Study (CLASS) with Children's Hospital Medical Center, University of Cincinnati Departments of Environmental Health, Cincinnati, Ohio, 45229. For the Cincinnati Lead and Allergen Sampling Study (CLASS) with Children's Hospital Medical Center, Internal Medicine, Cincinnati, Ohio, 45229). **A Side-by-Side Comparison of Sampling Methods for Settled, Indoor Allergens**. Environmental Research, 87(1) (2001), 37-46.

Exposure to indoor allergens is associated with asthma, but there is no standardized sampling method for measuring allergens. We compared the association of measured allergen exposure and serum-specific IgE levels and the precision of three sampling methods (Cyclone, Mighty Mite, and Readivac II) to identify a standardized sampling method for indoor allergens. A random sample of 72 children, 5 to 17 years old, with doctor-diagnosed asthma who lived in the same residence >2 years were enrolled. Composite, side by side floor samples were obtained with all three methods. Dust allergen concentrations and serum-specific IgE levels were measured for *Der f I*, *Fel d I*, and *Bla g I*. Mean allergen concentration did not differ significantly by sampling method. Cat allergen was significantly correlated with serum-specific IgE for Cyclone (P=0.003) and Mighty Mite (P=0.008), but only marginally for Readivac II (P=0.07). Dust mite allergen was significantly correlated with serum-specific IgE for Readivac II (P=0.02) and Cyclone (P=0.038), but not for Mighty Mite (P=0.12). Cockroach allergen was not correlated with serum-specific IgE for any sampling method. In multiple linear regression, cat allergen was associated with serum-specific IgE for Cyclone (P=0.007) and Mighty Mite (P=0.02), but not for Readivac II (P=0.06). In contrast, dust mite allergen was marginally

associated with serum-specific IgE for Readivac II (*P*=0.07), but not for Mighty Mite (*P*=0.64) or Cyclone (*P*=0.27). The Cyclone and Mighty Mite were more precise than Readivac II for cat allergen, but there was no difference for dust mite allergen (*P*>0.05). No single method is superior for measurement of indoor allergens. In general, cat allergen collected with the Cyclone was a better predictor of serum-specific IgE levels to *Fel d I*, whereas dust mite allergen collected with the Readivac II was a better predictor of serum-specific IgE levels to *Der f I*.

Wu-Yuan Chen, Hsing-I Tseng, Ming-Tsang Wu, Hsin-Chia Hung, Hui-Tsu Wu, Hsiu-Lin Chen, Chu-Chong Lu. (Department of Pediatrics, Medical College, Kaohsiung Medical University, 100 Shu-Chuan 1st Road, Kaohsiung 100, Taiwan. Graduate Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung, Taiwan. School of Dental Hygiene, Kaohsiung Medical University, Kaohsiung, Taiwan). **Synergistic effect of multiple indoor allergen sources on atopic symptoms in primary school children.** Environmental Research, 93(1) (2003), 1-8.

Accumulating data show that the complex modern indoor environment contributes to increasing prevalence of atopic diseases. However, the dose–response relationship between allergic symptoms and complexity of indoor environmental allergen sources (IEAS) has not been clearly evaluated before. Therefore, we designed this study to investigate the overall effect of multiple IEAS on appearance of asthma (AS), allergic rhinitis (AR), and eczema (EC) symptoms in 1472 primary school children. Among various IEAS analyzed, only stuffed toys, cockroaches, and mold patches fit the model of `more IEAS, higher odds ratio (OR) of association'. The association of IEAS and AR increased stepwise as more IEAS appeared in the environment (1.71, 2.47, to 2.86). In AS and EC, the association was significant only when all three IEAS were present (1.42, 1.98, to 4.11 in AS; 1.40, 1.76, to 2.95 in EC). These results showed that different IEAS had a synergistic effect on their association with atopic symptoms and also suggest that there is a dose–response relationship between kinds of IEAS and risk of appearance of atopic diseases.

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