

Bioavailability of soil pollutants and risk assessment

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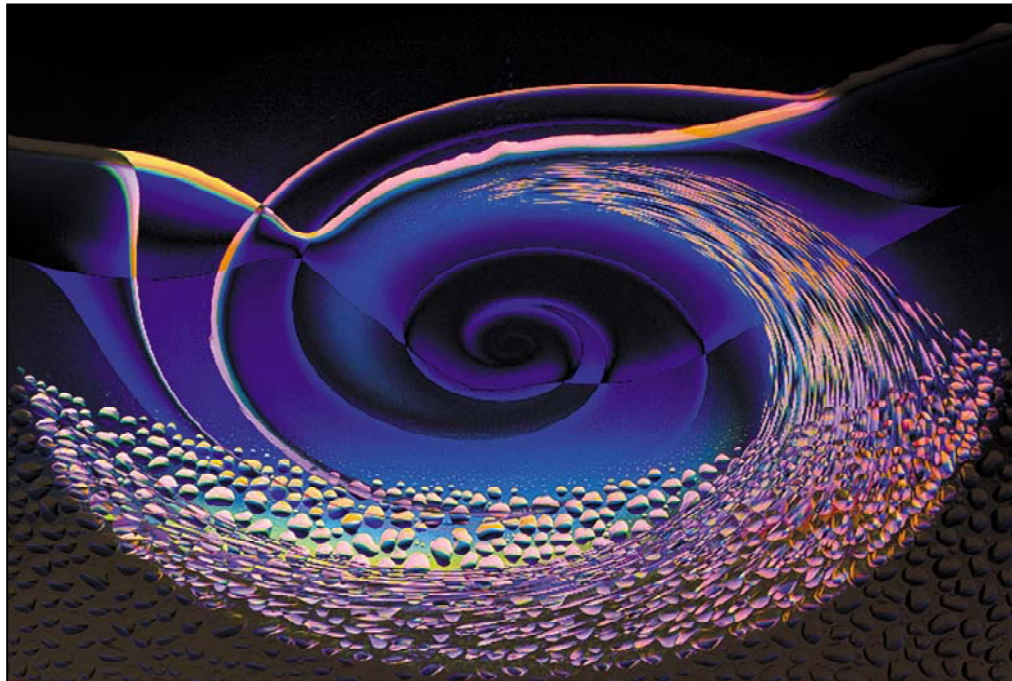
2nd International Workshop

February 2-7, 2003

Centro Stefano Franscini

Ascona, Switzerland

Bioavailability of Soil Pollutants and Risk Assessment



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2nd International Workshop on

Bioavailability of Soil Pollutant and Risk Assessment

February 2-7, 2003
Centro Stefano Franscini, Monte Verita
Ascona, Switzerland

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<http://www.ito.umnw.ethz.ch/bioavailability.monteverita>

Programme

Sunday, Februar 2nd

- 13.30 – 16.00 Registration
- 16.15 Welcome
- 16.30 – 17.10 Opening Lecture: **Wilfried Ernst**
Bioavailability of heavy metals to plants: restricted options
for generalization

Monday, February 3rd

- 8.30 – 9.10 **Domy Adriano** (keynote)
- 9.10 – 9.35 **Bernd Nowack** *Metal-ligand complexes: a coin with two sides*
- 9.35 – 10.00 **Piet Seuntjens** *Modelling leaching and root uptake of heavy metals
in the presence of organic ligands*
- 10.00 – 10.40 **Coffee break**
- 10.40 – 11.20 **Herman van Leeuwen** (keynote) *Speciation dynamics and
bioavailability of metals in aqueous systems*
- 11.20 – 11.45 **Jing Song** *Assessment of phytoavailability of soil zinc*
- 11.45 – 12.10 **Rai Kookana** *The role of black carbon in bioavailability of organic
contaminated soils*
- 12.10 – 13.30 **Lunch break**
- 14.00 – 14.30 **Ravi Naidu** (keynote/ launching discussion) *Bioavailability of soil
pollutants and risk assessment: Abiotic Factors*
- 14.30 – 16.00 **Discussion in working groups**
Ravi Naidu / Domy Adriano / Rai Kookana
- 16.00 – 16.30 **Plenary discussion** (Ravi Naidu)
- 16.30 – 17.00 **Coffee break**
- 17.00 – 18.30 Postersession 1: Abiotic Factors
- 19.00 **Dinner**

Tuesday, February 4th

8.30 – 9.10	Walter Wenzel (keynote) <i>Biotic factors controlling pollutant bioavailability: Rhizosphere processes</i>
9.10 – 9.35	Ann Kripka <i>Arbuscular mycorrhizal fungi can affect radionuclides transport into herbaceous plants</i>
9.35 – 10.00	Hauke Harms <i>About the impracticability of generalizations concerning pollutant bioavailability</i>
10.00 – 10.40	Coffee break
10.40 – 11.05	Lukas Wick <i>Bacteria as bioavailability enhancing agents</i>
11.05 – 11.30	José-Julio Ortega-Calvo <i>Physiological promotion by bacteria of bioavailability of polycyclic aromatic hydrocarbons</i>
11.30 – 11.55	Matthias Kästner <i>Formation and stability of residues derived from microbial degradation of organic pollutants: bound residues versus refractory soil organic matter</i>
12.10 – 13.30	Lunch break
14.00 – 14.30	Ken Killham (keynote/ launching discussion) <i>Bioavailability of contaminants – relation to risk and bioremediation potential</i>
14.30 – 16.00	Discussion in working groups Ken Killham / Walter Wenzel
16.00 – 16.30	Plenary discussion (Ken Killham)
16.30 – 17.00	Coffee break
17.00 – 18.30	Postersession 2 & 3: Biotic Factors & Toxicity and Ecotoxicity Risks
19.00	Dinner

Wednesday, February 5th

8.30 – 9.10	Nico van Straalen (keynote) <i>Indicators of bioavailability: uptake fluxes or residues</i>
9.10 – 9.35	Kristin Becker-van Slooten <i>The use of biomarkers for the assessment of the bioavailability of soil pollutants – an approach with the collembolan <i>Folsomia candida</i></i>
9.35 – 10.00	Petra Gulz <i>The toxic effects of arsenic on growth of sunflower</i>
10.00 – 10.40	Coffee break
10.40 – 11.20	Rufus Chaney <i>Evidence of Effective in situ Inactivation of Soil Bp Using Phosphate or Composted Biosolids in the IINERT Field Test at Joplin, Missouri</i>
11.20 – 11.45	Madelaine Goerg-Günthardt <i>Model forest ecosystem to test heavy metal effects in an interdisciplinary approach “From Cell to Tree”</i>
12.00 – 13.30	Lunch break
13.30 – 14.00	Jörg Römbke (keynote/ launching discussion) <i>Bioavailability in soil: the role of invertebrate behaviour</i>
14.00 – 15.30	Discussion in working groups Jörg Römbke / Nico van Straalen / Kristin Becker
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16.15	Excursion, Gardada
19.00	Workshop Dinner

Thursday, February 5th

8.30 – 9.10	Sjoerd von der Zee (keynote) <i>Contamination, bioavailability, and risk assessment</i>
9.10 – 9.35	Jan Japenga / Paul Römken <i>Phytoremediation: using bioavailability to improve estimates of uptake rates by crops</i>
9.35 – 10.00	Nicholas Jarvis <i>Modelling carbon turnover and cadmium bioavailability and leaching in sludge-amended soil</i>
10.00 – 10.40	Coffee break
10.40 – 11.05	Sokrat Sinaj <i>Assessment of exchangeable cadmium in soils using isotope exchange kinetics</i>
11.05 – 11.30	Beat Frey <i>the use of microbial bioassays and activity measurements for the evaluation of metal-contaminated soil quality</i>
11.30 – 11.55	Joëlle Fismes <i>Forecast methods to assess the contamination of edible vegetable growing on soils polluted by organic chemicals</i>
12.10 – 13.30	Lunch break
14.00 – 14.30	Jaco Vangronsveld (keynote/ launching discussion) <i>Measures to control and reduce pollutant bioavailability and risk assessment</i>
14.30 – 16.00	Discussion in working groups Jaco Vangronsveld / Sjoerd van der Zee
16.00 – 16.30	Plenary discussion (Jaco Vangronsveld)
16.30 – 17.00	Coffee break
17.00 – 18.30	Postersession 4 & 5: Risk Assessment & Risk Management
19.00	Dinner

Friday, February 6th

8.30 – 9.30	Peter Schröder <i>Workshop Summary – Conclusions</i>
9.30 – 10.00	<i>Final discussion</i>
10.00 – 10.30	Coffee break
10.30 – 12.00	<i>Final discussion – Statements / Poster Awards / Closing Remarks</i>
12.00 – 13.30	Lunch break
	End of Workshop

Poster Session 1, Monday February 3rd

Abiotic Factors

Majid Afyuni	<i>Sequential Extraction of Cd, Pb, and Ni in a calcareous soil amended with sewage sludge</i>
Marta Camps Arbestain	<i>Reductive dechlorination of a, b, and g-hexachlorocyclohexane isomers by hydroxocobalamin in the presence of dithiotreitol as reducing agent</i>
Marta Camps Arbestain	<i>Sorption of hexachlorocyclohexane isomers on soils which differ in the type of organic matter and clay fraction</i>
Karaj Dhillon	<i>Distribution and plant availability of selenium fractions in seleniferous soils of northwest India</i>
Catarina Joaquim	<i>Bioavailability of chemical elements in soils and sediments contaminated by the sulphide mine exploitation</i>
Agnes Piquet-Pissaloux	<i>Bioavailability of Ni and Cr in grassland from volcanic geochemical deposit</i>
Jürgen Ruoss	<i>New methods to measure and estimate the bioavailability of ¹⁴C-organic chemicals in soils</i>
Susan Tandy	<i>The role of NTA in Copper and Zinc Uptake by Sunflowers</i>
Patrizia Zaccheo	<i>Effect of different N fertilizers on heavy metal bioavailability in soil</i>
Kathrin Wenger	<i>Phytoextraction of atrazine and metabolites from spiked nutrient solutions</i>
Sascha Oswald	<i>Modelling kinetic phenol biodegradation processes and the associated hydrogen and acetate concentrations in a batch microcosm</i>

Poster Session 2 & 3, Tuesday February 4th

Biotic Factors & Toxicity and Ecotoxicity Risks

Marta Camps Arbestain	<i>Impact of vegetation on the bioavailability of HCH isomers in contaminated soils</i>
Catherine Daoust	<i>Integrating bioavailability of metals into life-cycle impact assessment of terrestrial ecotoxicity</i>
Sandra Hermle	<i>Which influence have heavy metal pollution, soil type and acidic irrigation on physiological activities and biomass production on a young forest ecosystem?</i>
Anna Lazzaro	<i>Approaches to study effects of heavy metals on soil microbial communities: a conceptual work</i>
Manoj Menon	<i>Plant water uptake in heavy metal contaminated soil</i>
Shiv Pandey	<i>Residues of atrazine in rhizosphere and non-rhizosphere soils</i>
Daniel Ragnvaldsson / Rune Berglind / Per Leffler	<i>Ecotoxicological risk analysis of soil polluted from pyrotechnical activities</i>
Lu Zhao	<i>Influence of root exudates on transport of heavy metals in soils: assessment by combining unsaturated column experiments with spatially resolved soil solution monitoring factories based on wind direction</i>

Poster Session 4 & 5, Thursday February 6th

Risk Assessment and Risk Management

Majid Afyuni	<i>Spatial variability of Pb, Zn and Ni around two industrial factories based on wind direction</i>
Amparo Cortés	<i>Changes on lead bioavailability after induced phytoextraction</i>
Satish Kumar Gupta	<i>Role of bioavailable metals in contaminated soils to formulate ordinance on risk assessment and to develop risk management techniques</i>
Nicholas Jarvis	<i>Long-term effects of sewage sludge applications on cadmium bioavailability, distribution and leaching in an arable field soil</i>
Cathérine Keller	<i>Mobility and bioavailability of Cd, Cu and Zn in soils after repeated croppings of the hyperaccumulator <i>Thlaspi caerulescens</i></i>
Carmen Lobo	<i>Effects of organic and inorganic amendments on cadmium, zinc and lead fractionation in contaminated soils</i>
David Rais	<i>Risk assessment of heavy metal leaching by soil solution monitoring: chemical behaviour of different soil water samplers</i>
Paul Romkens / Jan Japenga	<i>Bioavailability: from scientific concept to applicable approach?</i>
Walter Rosselli	<i>Phytoextraction capacity of trees growing on a metal contaminated soil</i>
Boris Soroichinsky	<i>The use of plant technologies for the clean-up of soils and aquatic systems contaminated with radionuclides is limited with pollutants' bioavailability</i>
Stefan Zimmermann	<i>Vulnerability of a calcareous soil to further Cd-input compared to its calculated critical load for Cd</i>

Participants

Name, affiliation, e-mail	Title: <i>oral</i>, poster	Page
Abreu, Maria Manuela Instituto Superior de Agronomia Dept.de Ciências do Ambiente Tapada da Ajuda, 1349-017 Lisboa, Portugal manuelaabreu@isa.utl.pt	<i>Bioavailability of chemical elements in soils and sediments contaminated by the sulphide mine exploitation</i>	1
Adriano, Domi The University of Georgia, Savannah Environ. Soil Science River Ecology Lab, Drawer E, 29802 Aiken SC, United States Adriano@srel.edu	<i>Biogeochemical Processes and Abiotic Factors Controlling Bioavailability</i>	5
Afyuni, Majid Isfahan University of Technology Department of Soil Science College of Agriculture 84154 Isfahan, Iran afyuni@cc.iut.ac.ir	<i>1 Sequential Extraction of Cd, Pb, and Ni in a calcareous soil amended with sewage sludge</i>	7
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Chaney, Rufus US Department Agr.-ARS Animal Manure & By-Products Lab Bldg. 007, BARC-West 20705-2350 Beltsville, MD, United States chaneyr@ba.ars.usda.gov	<i>Evidence of Effective in situ inactivation of soil by using phosphate or composted biosolids in the INERT field test at Joplin, Missouri</i>	17

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Ernst, Wilfried Vrije Universiteit Ecology and Physiology of Plants De Boelelaan 1085, 1081 HV Amsterdam, Netherlands wernst@bio.vu.nl	<i>Bioavailability of heavy metals to plants: Restricted options for generalization</i>	31
Fismes, Joëlle ENSAIA-INPL/INRA Laboratoire Sols et Environnement 2, av. de la Forêt de Haye, BP172 54505 Vandoeuvre lès Nancy, France Joelle.Fismes@ensaia.inpl-nancy.fr	<i>Forecast methods to assess the contamination of edible vegetables growing on soils polluted by organic chemicals</i>	32
Frey, Beat Swiss Federal Research Institute WSL Soil Ecology Zuercherstr.111, 8903 Birmensdorf, Switzerland beat.frey@wsl.ch	<i>The use of microbial bioassays and activity measurements for the evaluation of metal-contaminated soil quality</i>	36
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Goerg-Günthardt, Madeleine Swiss Federal Research Institute WSL WUS; Bioindications Zürcherstr. 111, 8903 Birmensdorf, Switzerland madeleine.goerg@wsl.ch	<i>Model forest ecosystems to test heavy metal effects in an interdisciplinary approach "From Cell to Tree"</i>	41

Gupta, Satish Kumar Swiss Federal Research Station for Agroecology and Agriculture Reckenholzstrasse 191 8046 Zurich, Switzerland gupta@ito.umnw.ethz.ch	<i>Role of bioavailable metals in contaminated soils to formulate ordinance on risk assessment and to develop risk management techniques</i>	45
Harms, Hauke Swiss Federal Institute of Technology, ENAC-ISTE-LPE, Bâtiment GR 1015 Lausanne, Switzerland hauke.harms@epfl.ch	About the impracticability of generalizations concerning pollutant bioavailability	
Hermle, Sandra Swiss Federal Research Institute WSL WUS; Bioindications, Zuercherstr.111 8903 Birmensdorf, Switzerland sandra.hemle@wsl.ch	<i>Which influence have heavy metal pollution, soil type and acidic irrigation on physiological activities and biomass production of a young forest ecosystem</i>	49
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Lobo, Carmen Instituto Madrileño de Investigación Agraria y Alimentaria. IMIA Finca "El Encín", Apartado 127 28800 Madrid, Spain mcarmen.lobo@imia.comadrid.es	<i>Effects of organic and inorganic amendments on Cd, Zn and Pb fractionation in contaminated soils</i>	66
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Ragnvaldsson, Daniel FOI NBC Defence Dept. of Threat Assessment Cementvägen 20, 901 82 Umeå, Sweden daniel.ragnvaldsson@foi.se	<i>Ecotoxicological risk analysis of soil polluted from pyrotechnical activities</i>	78
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Schweigert, Nina Swiss Federal Institute for Environmental Science and Technology, Umwelt-Mikrobiologie und Molekulare Ökotoxikologie Ueberlandstrasse 133 8600 Dübendorf, Switzerland schweigert@eawag.ch	Guest	
Seuntjens, Piet Integral Environmental studies Vito, Flemish Institute for Technological Research Boeretang 200, 2400 Mol, Belgium piet.seuntjens@vito.be	Modelling leaching and root uptake of heavy metals in the presence of organic ligands	91
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Bioavailability of chemical elements in soils and sediments contaminated by the sulphide mine exploitation

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INTRODUCTION

The S. Domingos sulphide mine in south Portugal was exploited as underground and open cast mining. Exploitation started in pre-roman and roman times for Au, Cu and Ag. Modern exploitation began in XIX century for Cu and Zn, and occurred until 1960's. Several facilities were developed to support mining works and ore transportation, including a village, water reservoirs, cementation tanks, sulphur factory, network channels for acid water evaporation, a railway and a harbour for ore transportation. Several environmental problems are clearly visible in an area of 50 km² due to mine waste material, acid waters and landscape disruption. Soils and sediments in this area are contaminated with several pollutants, such as As, Pb, Cu, Hg, Sb, and Zn. In this scenery, an endemic species of heather (*Erica andevalensis*) occurs on sediments and soils located near the acid mine drainage stream, being the single plant species found in these sediments. *Erica andevalensis* is in community with *Erica australis*, growing on soils in the same area. This plant community was identified only in two sites of the Iberian Peninsula, associated to sulphide mines (Rio Tinto – Spain, and S. Domingos – Portugal).

The objectives of this work are to evaluate the element availability to heather plants growing on soils contaminated and non-contaminated by hazardous chemical elements, and to compare the behaviour of two heather species (*Erica australis* and *Erica andevalensis*).

MATERIALS AND METHODS

Soil and plant samples were collected from S. Domingos mining area (20 samples) and on two non-contaminated sites: Moreanes (4 samples), near S. Domingos, but not contaminated by mining activity, and Cabo Espichel (3 samples) on a different geological environment. The objective of this sampling was to evaluate heather absorption ability for soil elements in three different environments. Soils from Moreanes were developed on schists and greywackes, away from mining contamination. Soils from S. Domingos were developed on a similar lithology which is sometimes mixed with waste mining materials. Soils from Cabo Espichel were developed on calcareous sandstone and marls. S. Domingos and Moreanes are submitted to dry climatic conditions whereas Cabo Espichel, located on a littoral environment receive the influence of the moist sea winds.

Soil samples were stored in polyethylene recipients and were kept moist under refrigeration. Available fraction of chemical elements was extracted with 0.5 M

$\text{Ca}(\text{NO}_3)_2$ adjusted to soil pH in a 1:40 (w:v) relation for 16 h, on the < 2mm moist soil fraction. Soil samples were also air dried and used for soil characterization: pH(H_2O) in a 1:20 (w:v) soil suspension, organic carbon by Walkley-Black method, free Fe and Al oxides extracted by DCD and measured by atomic absorption spectrometry. Dry fraction < 2 mm was analysed for total chemical content by ICP and INAA. Plants were water washed twice and dried under 30°C. Dried shoots and flowers were ground and analysed for total chemical content by ICP and INAA.

RESULTS AND DISCUSSION

S. Domingos soils present pH < 4.5 (median 3.7; min. 3; max. 4.5). Moreanes soil pH lies between 5.0 and 4.6 (median 5.0) and the other non-contaminated site (C. Espichel) present the highest pH values (between 8.0 and 6.6), with a 7.5 median. Organic carbon content presents a high variability in the contaminated soils (3.0-137.4 mg kg⁻¹; median 10 mg kg⁻¹) whereas for Moreanes and C. Espichel the variability is much lower although the single values are higher (median 39.9 and 21.3 mg kg⁻¹ respectively). As expected, free Fe oxides present higher values in S. Domingos (median 32 mg kg⁻¹) than in Moreanes (median 11.7 mg kg⁻¹) and even lower in C. Espichel (median 4.1 mg kg⁻¹). Free Al oxides content is similar for S. Domingos and Moreanes (median 1.1 mg kg⁻¹) and zero for C. Espichel.

The basic statistic results for some elements in soils and plants (total content) are shown in tables 1 and 2. Both plant species showed high levels of chemical elements, frequently greater than the total soil content: As (9-808 mg kg⁻¹), Pb (17-4940 mg kg⁻¹), Cu (113-720 mg kg⁻¹), Mn (604-28600), Cr (14-70 mg kg⁻¹), V (9-53 mg kg⁻¹), and B (580-2530 mg kg⁻¹). However, Sb presented smaller levels in plants than in soils.

Table 1: Basic statistic results of total element soil content (mg kg⁻¹).

	Site	n	average value	Median value	Min. value	Max. value	coefficient of variation
As	S. Domingos.	20	1803.6	1330.0	175.0	11600.0	1.376
	Moreanes	4	15.0	14.8	12.6	18.9	0.182
	C. Espichel	3	5.9	5.7	4.1	7.9	0.323
Cu	S. Domingos.	20	243.8	224.5	25.2	988.8	0.939
	Moreanes	4	21.5	19.7	15.1	31.5	0.327
	C. Espichel	3	14.1	14.1	13.2	15.0	0.064
Hg	S. Domingos.	20	6.1	5.0	0.5	32.0	1.241
	Moreanes	4	-	-	-	-	-
	C. Espichel	3	-	-	-	-	-
Mn	S. Domingos.	20	205.9	175.4	18.7	655.2	0.768
	Moreanes	4	280.6	137.6	64.8	853.6	1.367
	C. Espichel	3	459.9	394.2	392.1	593.3	0.251
Pb	S. Domingos.	20	3434.3	1859.4	217.8	24930.0	1.585
	Moreanes	4	23.7	26.2	18.4	26.8	0.161
	C. Espichel	3	23.0	23.0	22.2	23.8	0.035
Sb	S. Domingos.	20	317.4	185.0	19.2	2150.0	1.442
	Moreanes	4	3.3	1.9	1.5	8.2	0.991
	C. Espichel	3	0.7	0.8	0.6	0.8	0.158
Zn	S. Domingos.	20	187.3	106.9	36.4	1368.7	1.571
	Moreanes	4	26.4	24.9	19.5	38.6	0.319
	C. Espichel	3	26.0	25.9	24.1	28.1	0.077

Table 2: Basic statistic results of plant elemental content (shoots and flowers).

	Site	Plant species	n	average	median	min.	max.	coeff. of var.
Al	S. Domingos	E. australis	8	14 191.3	16 200.0	6 730.0	17 100.0	0.243
	S. Domingos		12	13 164.2	12 300.0	5 090.0	43 500.0	0.760
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	19 600.0	19 000.0	18 900.0	21 600.0	0.068
	C. Espichel	E. australis	3	19 633.3	15 300.0	13 900.0	29 700.0	0.445
As	S. Domingos	E. australis	8	193.4	155.0	74.3	462.0	0.746
	S. Domingos		12	334.1	232.0	84.0	808.0	0.756
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	45.8	48.0	33.0	56.0	0.208
	C. Espichel	E. australis	3	12.2	10.0	8.5	18.1	0.425
Cr	S. Domingos	E. australis	8	36.6	35.8	26.3	61.2	0.292
	S. Domingos		12	34.4	33.6	14.2	70.5	0.433
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	58.7	57.3	52.5	68.2	0.114
	C. Espichel	E. australis	3	31.8	29.0	27.9	38.6	0.186
Cu	S. Domingos	E. australis	8	269.8	244.9	143.6	473.2	0.478
	S. Domingos		12	260.8	236.0	131.6	720.4	0.619
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	258.2	274.3	207.2	294.2	0.144
	C. Espichel	E. australis	3	180.5	161.2	114.1	266.2	0.432
Hg	S. Domingos	E. australis	8	171.9	139.1	60.6	485.3	0.816
	S. Domingos		12	344.6	212.6	93.1	1 317.1	0.972
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	28.7	29.5	23.1	34.9	0.171
	C. Espichel	E. australis	3	29.7	27.7	22.0	39.4	0.298
Mn	S. Domingos	E. australis	8	10 890.0	9 830.0	4 000.0	21 300.0	0.514
	S. Domingos		12	16 021.7	17 400.0	8 250.0	28 600.0	0.398
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	18 050.0	17 100.0	11 700.0	27 400.0	0.369
	C. Espichel	E. australis	3	745.0	654.0	613.0	968.0	0.261
Pb	S. Domingos	E. australis	8	520.2	310.1	114.8	2 190.0	1.325
	S. Domingos		12	783.7	419.6	85.1	4 940.0	1.714
		<i>E. andevalensis</i>						
	Moreanes	E. australis	4	54.5	54.9	40.0	68.6	0.214
	C. Espichel	E. australis	3	25.7	18.2	17.2	41.6	0.537

All elements in mg kg⁻¹ except Hg in µg kg⁻¹.

Data were also assessed by principal component analysis (PCA). This methodology separates Moreanes and C. Espichel soils from those from S. Domingos, through the association pH and Mn *versus* Cu, Pb, Sb, Zn, Hg, As, Fe (DCB) and Al (DCB), respectively.

Concerning plant elemental content, the association Hg and As *versus* Al, Cr, V and B separates respectively *E. andevalensis* from *E. australis*. Considering both plant species

separately, data analysis from soils and plants shows that *E. australis* is associated to the high soil pH (3.5-8.0), and have higher levels of Al and V. Also, a correlation ($r=0.75$) was obtained between total soil Hg and plant Hg, however, available Hg was below the detection limit ($4 \mu\text{g kg}^{-1}$). No correlation was obtained between available Pb and plant content besides the specific methodology for Pb (Berti et al., 1997). Nevertheless, a high correlation ($r=0.83$) between plant and total soil Pb was obtained. Considering the available fraction of the other elements, only Mn showed correlation with plant Mn content ($r=0.75$).

Erica australis has a different behaviour in contaminated and non-contaminated soils. Plants growing in the non-contaminated soils contain levels of As, Hg, Cu, Mn, and Zn, higher than those present in the soils, showing the capacity to accumulate these elements in shoots and flowers. In the contaminated soils, plants only accumulate Mn and Zn, having a Cu content identical to the soils.

E. andevalensis is found only in contaminated soils and sediments with pH 3.0-4.1. This plant species seems to absorb more Pb and As than *E. australis* independently As and Pb soil content. Regarding available fraction, no correlations were found with plant element contents. However, there is correlation between total soil and plant Pb ($r=0.65$) and Cu ($r=0.57$).

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Biogeochemical Processes and Abiotic Factors Controlling Bioavailability

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The term bioavailability can mean differently to different disciplines. It has emerged as an important paradigm in ecological as well as human health risk assessment (Natl. Res. Council, *in press*; Adriano, 2001), replacing an old belief that biological response by receptor organisms could be predicted by the total concentrations of the chemicals of concern. A rather generic definition of bioavailability offered by the National Research Council states that it refers to a fraction of the total contaminant mass in soil and sediment actually available to receptor organisms, including human and ecological organisms. In short, it refers to the uptake by living organisms.

In the natural environment, chemicals deposited in soils are subject to natural attenuation, otherwise known as natural remediation. While organic residues can be naturally remediated in soils, mediated by natural indigenous processes, inorganic residues including metals and metalloids generally can merely undergo transformation of their labile fractions while maintaining its total mass. The most labile fraction, usually represented by the soluble metal component occurring as either free ions or soluble complexed ions (i.e., as ion pair or complexed with humic ligands), is considered as the immediately bioavailable fraction to receptor organisms. Ions that are weakly adsorbed on exchange surfaces in soils are also fairly labile and can also become immediately available. The combined soluble and adsorbed fractions (i.e., the two most labile fractions), is collectively known in plant nutrition as the bioavailable fraction.

The different natural remediation-bioavailability processes are depicted in Figure 1. In the initial phase (A), the dynamics of metals between the aqueous phase and solid phase are regulated by various physico-biogeochemical processes, the extent of which determines metal partitioning.

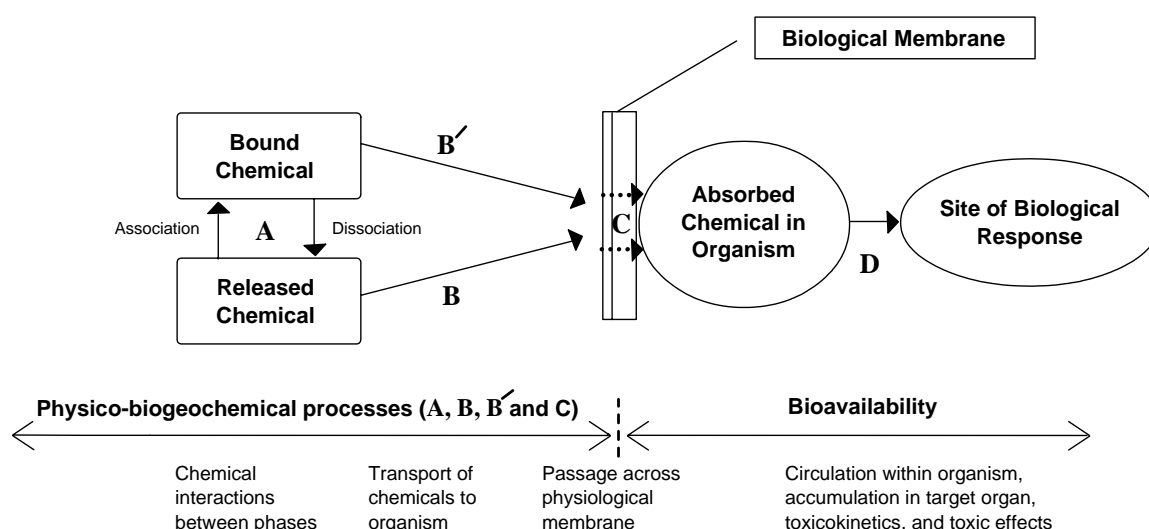


Fig. 1 Bioavailability processes in soils. Note the delineation of the whole scheme into "Natural remediation" (left) and "Bioavailability" (right). Modified from the NRC (*in press*).

Some of these processes include basic ones like desorption/adsorption, precipitation/dissolution, complexation, redox reactions, etc. In essence, the nature of these processes determine the kinetics of natural remediation that can be parameterized by bioavailability. The next phase (B, B') involves the transport of metals to organism. The metal can be transported in soluble form and/or colloidal (i.e., particulate) form. The particulate form can play significant role in humans and animals. For example, ingestion of soil particles by children is an important exposure pathway for lead. By the same token, ingestion of soil particles by grazing livestock is an important exposure pathway for animals feeding on contaminated pastures. The next phase (C) involves passing through a biological membrane, which in many instances can serve as a bio-filter for contaminants. In plants, this is represented by the root membrane; in humans, it is represented by the GI tract. Then the last phase (D) involves circulation and assimilation in the metabolic machinery of the organisms, culminating in some form of biological response. Assay of such biological response comes in many forms, from the whole organism (e.g., growth, biomass) to the molecules (e.g., biomarkers).

Although the bioavailable approach deals with kinetics processes that are also sensitive to several major factors (e.g., pH, organic matter or DOC, redox potential, chemical speciation, time, weathering, microbial diversity, etc.), it is gaining popular support and wider acceptance because of the solid science behind it.

The emphasis in this paper is the discussion of how major abiotic factors (e.g., pH, chemical speciation, redox potential, etc) influence the various physico-biogeochemical processes, which in turn influence the extent of bioavailability.

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Sequential Extraction of Cd, Pb, and Ni in a Calcareous Soil Amended with Sewage Sludge

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INTRODUCTION

Addition of heavy metals into soil through application of sewage sludge may result in decreasing crop yield, and contamination of human and animal food chains. The bioavailability and mobility of the heavy metals depend on the forms that they exist in the soil. For example, water-soluble and exchangeable forms are readily mobile and available to plants where as adsorbed fractions are strongly fixed in the soil. In this paper data are reported on a sequential extraction of a soil that have been amended with various rates of sewage sludge since 1997.

MATERIALS AND METHODS

Soil samples were collected (0-20 cm) from plots that have been amended with sewage sludge at 0, 25, 50, and 100 t ha⁻¹ for either one, two, or three times since 1997. Nickel, cadmium, and lead were fractionated by a sequential extraction procedure that will give fractions of the heavy metals as water-soluble, exchangeable, specifically sorbed, organic, and occluded forms. Plant available heavy metals in the soil samples were also determined using 0.005 M DTPA extracting solution.

RESULTS AND DISCUSSION

The concentrations and percentages of Ni, Pb, and Cd extracted by the various extractants sequentially are shown in Table 1, 2, and 3. These results show that application of sewage sludge has resulted in pollution of the soil with Ni, Pb, and Cd, especially with 100 t ha⁻¹ sludge rates. This is based on the fact that the metal concentrations in the sludge treated plots were higher than the concentrations in the control plots. The amount of Pb and Ni extracted in water-soluble and exchangeable forms were very small. The percent of the water-soluble and exchangeable fractions combined were <3% for Pb and Ni. The levels of Cd in the water-soluble and exchangeable forms were higher and in some treatments more than 10% of the total Cd. The highest amounts of Pb and Cd were occluded mainly in carbonate and oxide phases, with a lesser concentrations in the residual and organic fractions (Tables 2 and 3), where as the highest amounts of Ni was in the residual fraction (Table 1). The levels of the metals in the organic form were positively correlated with amount of sludge added but showed a decreasing trend as the sludge application time passed. Sewage sludge application rate also significantly affected DTPA-extractable metal concentration in the soil. However, as the time of sludge application passed the plant available metal decreased approaching the levels in the control plots. The results of this study show that the accumulation and forms of Pb, Ni, and Cd in the soil are governed by content of the metals in sludge, the rate of sludge application, and the time of sludge application.

Table 1 Concentration and percentage forms of Ni assayed using the sequential extraction

Sludge (t/ha) Ni	Water-soluble		Exchangeable		Spec.sorbed-carbon		Fe-Mn oxide		Organic		Residual		DTPA-EX		Total
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
25	0.35	0.29	0.70	0.58	5.20	4.32	14.30	11.87	23.25	19.31	76.63	63.63	1.27	1.05	120.43
25+25	0.55	0.44	0.95	0.75	5.10	4.05	14.60	11.60	23.90	18.99	80.73	64.16	1.29	1.03	125.83
25+25+25	0.25	0.20	1.00	0.79	5.30	4.21	14.50	11.52	23.30	18.51	81.55	64.77	1.64	1.30	125.90
50	0.35	0.29	1.05	0.88	5.20	4.35	15.40	12.89	21.55	18.04	75.93	63.55	1.34	1.12	119.48
50+50	0.65	0.56	0.80	0.69	5.35	4.63	14.60	12.65	23.95	20.74	70.10	60.72	1.60	1.39	115.45
50+50+50	0.45	0.36	0.95	0.75	5.3	4.15	14.70	11.62	26.75	21.15	78.38	61.97	1.87	1.48	126.48
100	0.35	0.28	1.05	0.85	5.3	4.26	15	12.13	24.85	20.16	76.81	62.31	1.51	1.22	123.26
100+100	0.45	0.36	1.25	1.01	5.60	4.54	14.00	11.34	25.75	20.86	76.37	61.88	2.04	1.65	123.42
100+100+100	0.50	0.44	1.25	1.11	7.12	6.32	13.20	11.72	28.15	24.99	62.43	55.42	2.42	2.15	112.65
Control	0.39	0.33	0.45	0.38	5.09	4.29	14.29	12.03	21.98	18.50	76.60	64.47	0.98	0.82	118.81

Table 2. Concentration and percentage forms of Pb assayed using the sequential extraction

Sludge(t/ha) Pb	Water-soluble		Exchangeable		Spec.sorbed-carbon		Fe-Mn oxide		Organic		Residual		DTPA-EX.		Total
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
25	0.35	0.71	1.20	2.44	7.80	15.89	16.65	33.91	8.00	16.29	15.10	30.75	3.35	6.82	49.10
25+25	0.30	0.58	1.20	2.31	7.30	14.04	17.85	34.34	8.75	16.83	16.58	31.90	3.60	6.93	51.98
25+25+25	0.35	0.67	1.45	2.77	7.30	13.95	17.35	33.15	10.15	19.39	15.74	30.07	4.14	7.91	52.34
50	0.30	0.58	1.95	3.78	7.35	14.23	18.45	35.73	9.05	17.53	14.53	28.15	3.80	7.36	51.63
50+50	0.20	0.40	1.65	3.29	7.50	14.96	16.85	33.60	9.95	19.84	14.00	27.92	4.18	8.33	50.15
50+50+50	0.45	0.77	1.35	2.32	7.5	12.80	16.8	28.79	14.90	25.61	17.28	29.71	5.12	8.79	58.18
100	0.30	0.58	1.25	2.41	7.35	14.20	17.15	33.13	10.20	19.70	15.52	29.97	4.19	8.10	51.77
100+100	0.10	0.18	1.40	2.49	7.45	13.26	16.90	30.08	14.15	25.18	16.19	28.81	5.53	9.85	56.19
100+100+100	0.50	0.79	1.30	2.06	9.85	15.63	12.65	20.08	22.90	36.35	15.80	25.08	5.89	9.35	63.00
Control	0.40	0.82	1.34	2.75	7.94	16.28	17.13	35.12	6.98	14.32	14.98	30.71	2.76	5.66	48.78

Table 3. Concentration and percentage forms of Cd assayed using the sequential extraction

Sludge(t/ha) Cd	Water-soluble		Exchangeable		Spec.sorbed-carbon		Fe-Mn oxide		Organic		Residual		DTPA-EX.		Total
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
25	0.00	0.00	0.15	5.59	0.85	31.68	0.90	33.54	0.15	5.59	0.63	23.60	0.06	2.33	2.68
25+25	0.00	0.00	0.15	6.01	0.75	30.04	0.75	30.04	0.15	6.01	0.70	27.90	0.06	2.50	2.50
25+25+25	0.00	0.00	0.25	9.58	0.75	28.73	0.75	28.73	0.15	5.75	0.71	27.22	0.08	3.03	2.61
50	0.00	0.00	0.20	7.95	0.75	29.80	0.75	29.80	0.15	5.96	0.67	26.49	0.07	2.81	2.52
50+50	0.05	1.88	0.25	9.38	0.8	28.13	0.90	33.75	0.15	5.63	0.57	21.25	0.08	2.97	2.67
50+50+50	0.20	6.22	0.15	4.67	0.75	23.34	1.25	38.90	0.25	7.78	0.61	19.09	0.12	3.76	3.21
100	0.15	4.58	0.30	9.16	0.75	22.91	1.25	38.19	0.20	6.11	0.62	19.04	0.09	2.67	3.27
100+100	0.20	5.63	0.25	7.04	0.80	22.52	1.35	38.01	0.25	7.04	0.70	19.76	0.13	3.64	3.55
100+100+100	0.35	10.30	0.05	1.47	0.90	26.49	1.25	36.78	0.30	8.83	0.55	16.13	0.17	5.03	3.40
Control	0.20	7.64	0.13	4.77	0.60	22.92	0.85	32.47	0.15	5.73	0.69	26.47	0.04	1.46	2.62

Spatial Variability of Pb, Zn and Ni Around Two Industrial Factories Based On Wind Direction

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Introduction

Studies in recent years have shown that heavy metal pollution of soil and plants in the vicinity of ore smelters is dependent upon climate factors and distance from the smelter (1,2). Like many other geochemical parameters, trace element concentration in soils are typically regionized variables (3). A number of mathematical methods are available for analysis of this kind of data which autocorrelation, trend surface fitting and geostatistical analysis are most often used. One of the most important purposes of applying spatial analysis on environmental geochemical data is to interpolate value between sampling location based on distance to create a grid of predict estimates for mapping purpose. The objective of this study was to evaluate concentration of Pb, Ni and Zn in soils around two steel factories based on wind direction.

Material and methods

More than 208 soil (0-10cm) samples in an 300 Km² were collected around two steel factories. The samples were collected on random grid with distance ranged from 500 to 3000 m. Total concentration of Pb, Ni and Zn were determined by flame atomic absorption spectrophotometer after digested with HNO₃(4).

Result and discussion

The concentration of the heavy metals in soils around two factories are show in Tables (1, 2):

Table 1: Descriptive statistic of Pb,Zn,Ni in soils around Isfahan Steel meel factory

Element	Mean	Median	Mode	Variance	Skewness	Kurtosis	Range
Pb	50.78	47.5	40	155.07	0.831	1.17	22.5-90
Zn	57.04	55	45	273.18	0.77	0.51	27-112.5
Ni	67.15	65	62	294.50	0.324	2.53	10-122.5

Table 2: Descriptive statistic of Pb,Zn,Ni in soils around Isfahan Steel meel factory

Element	Mean	Median	Mode	Variance	Skewness	Kurtosis	Range
Pb	46.46	47	45	154.66	0.107	1.72	7.5-90
Zn	50	52	60	419	-0.2	0.02	2.5-97
Ni	71	77	85	534.79	-0.4	0.2	2.5-135

All of the metals have a normal distribution. The distribution of metals are correspond with the wind direction that show in Fig.below. The Zinc and Ni have the showed similar.

The mean concentration of all the metals are below the threshold values (5). In spite of that the total concentration of Pb, Zn and Ni were higher around the smelter factories and decreased with the distance from the factories. The results indicate that industrial activities are polluting the soil. The spatial pattern of heavy metals concentration was characterized by constructing variogram. The structure of heavy metal variograms, its parameter, kriging base maps will be presented.

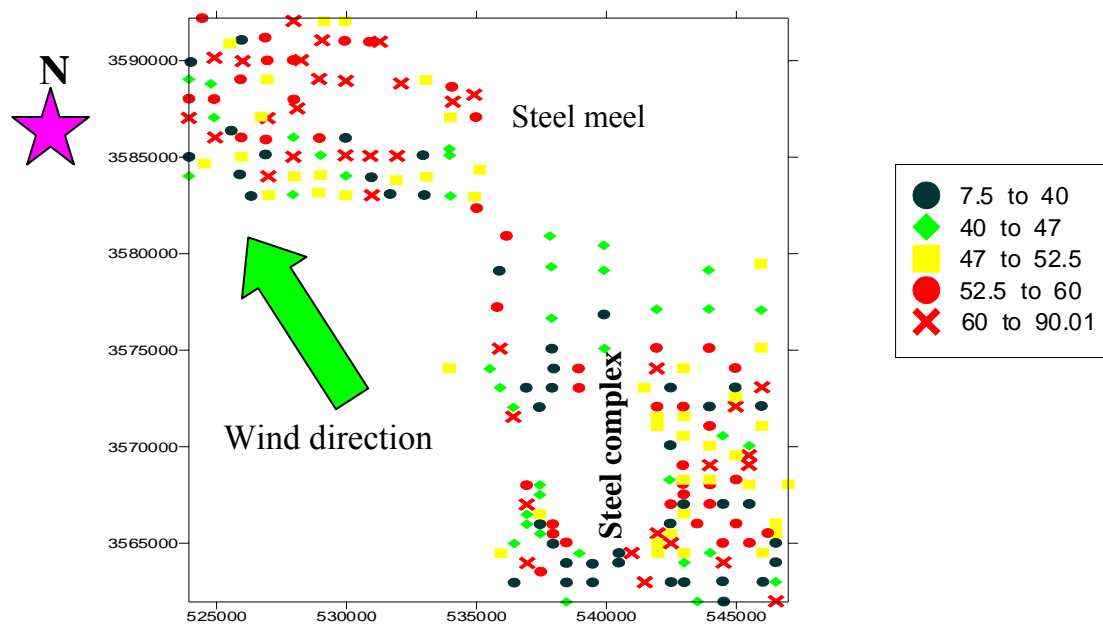


Figure 1: Symbol maps of total lead concentration around Steel Meel and Steel Complex

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**The use of biomarkers for the assessment of the bioavailability of soil pollutants –
an approach with the collembola *Folsomia candida***

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The effect of xenobiotics on soil invertebrates is mainly assessed with single species tests conducted in the laboratory. However, the evaluation of sublethal effects in soil invertebrates generally implies test designs of several weeks. Biomarkers are potential tools for more sensitive and predictive test methods characterising the risk associated with exposure to xenobiotics.

The aim of the study presented here was to investigate the effects of the pesticide dinoseb on life cycle parameters and biomarker responses of the springtail *Folsomia candida* (Collembola) in order to establish a relationship between short term (biomarkers) and long term effects (reproduction). After 6 days of exposure, the biomarker levels were increased in the exposed organisms compared to the control, indicating a stimulation. In the case of dinoseb, the selected biomarkers did not allow to detect an effect at a lower concentration than the classic endpoint reproduction, but the observed stimulation could be a pertinent early effect indicator. The advantages and limits of the use of biomarkers will be discussed.

Reductive dechlorination of alpha-, beta-, and gamma-hexachlorocyclohexane isomers by hydroxocobalamin in the presence of dithiotreitol as a reducing agent

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Introduction

Reductive dechlorination of halogenated compounds in anaerobic environments is a major mechanism for halogen removal, and results in compounds that are generally less toxic, less likely to bioaccumulate, and more susceptible to oxidative degradation. Nowadays there is great interest in the potential use of reductive dechlorination reactions mediated by abiotic reductants - such as corrins - in the treatment of waste and remediation of contaminated soils and waters. Marks et al. (1989) demonstrated gamma-HCH dechlorination by Co-containing corrinoids (cyanocobalamin, hydroxocobalamin, cobinamide dicyanide) in cell-free systems, using dithiothreitol as the external electron source. However, no such studies have been carried out to compare the reductive dechlorination of the different HCH isomers. The objective of this study was to determine and compare the kinetics of the reductive dechlorination of alpha-, beta-, and gamma-HCH mediated by hydroxocobalamin (vitamin B_{12a}) in cell-free systems, using dithiothreitol as the bulk reductive agent.

Materials and Methods

A 20-mL sample of aqueous reagent mixture, containing Tris buffer-pH 8.9 (100 mM), DTT (5 mM), and the HCH isomer under study (34.4 μ M), was placed in a 25-mL vial (preparation of the reagent mixture was carried out in a glovebox with N₂ atmosphere). A 200 μ L sample containing 73 μ M cob(III)alamin dissolved in 100 mM Tris buffer was added to the 20 mL aqueous reagent mixture and sealed before the vials were removed from the glovebox. The vials were incubated for 1 h at 37°C in the dark, without shaking. At appropriate time intervals, 25 μ L of samples from the liquid phase were taken from the reaction vials. Duplicate vials were prepared for each substrate. Identical aqueous reagent mixtures, but containing no corrinoids, were prepared and sampled at the same times as above, and used as controls. The concentrations of the HCH isomers were determined in the liquid phase after extraction of 25 μ L of sample with 5 mL of hexane. The extraction mixtures were sonicated for 20 min and then dried with anhydrous Na₂SO₄. Extracts were analyzed by gas chromatography (GC-EC). Spectrophotometric studies were also carried out, using a Jasco V-530 spectrophotometer (Jasco Corporation, Tokyo, Japan).

Results and Discussion

We used UV/Vis spectrophotometry to monitor changes in tetrapyrrole oxidation state over time, following the addition of reduced DTT. Vitamin B_{12a} [Co(III)] showed the characteristic spectrum with absorbances at 278, 356 (typically intense), and 534 nm, and that of vitamin B_{12r} [Co(II)] at 311 (typically intense), 405, and 473 nm. Complete reduction was almost immediate (100% after 2 min). Therefore, at the DTT:corrin molar

ratios used here, the reduction of vitamin B_{12a} probably did not limit the rate of dechlorination reactions of HCH isomers. Under the experimental conditions used, dehalogenation of gamma- and alpha-HCH occurred, with the gamma isomer being most degraded (92.9 and 30.8% degradation, respectively). The reaction proceeded by initial dechlorination to produce tetrachlorocyclohexene. Beta-HCH was very recalcitrant, and no degradation was observed. The results suggest that the combined use of hydroxocobalamin and DTT are potentially useful in wastewater treatment for the reductive dehalogenation of gamma- and alpha-HCH.

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Sorption of hexachlorocyclohexane isomers on soils which differ in the type of organic matter and clay fraction

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Introduction

The hexachlorocyclohexane (HCH) isomers are persistent organic pollutants (POPs) commonly present in the environment. Sorption processes in soils determine, to a great extent, the mobility and bioavailability of these contaminants. The colloidal system of the soil, specially the organic colloids, are key factors influencing the extent to which sorption processes of hydrophobic pollutants take place. The nature and amounts of soil colloids mainly depend on the type of pedogenic processes that generate them. Incipient weathering of basic and metabasic materials (e.g. amphibolites), under humid temperate conditions, gives rise to the simultaneous flocculation of organic matter and accumulation of humus through formation of Al-humus complexes. These soils often exhibit andic soil properties, and have a typically low organic acidity. In contrast, a very acidic and mobile organic matter prevail in surface horizons of forest soils developed from acid rocks, such as quartzites, on which podzolic soils are commonly formed. Moreover, the type of inorganic colloids (2:1 vs. either 1:1 phyllosilicates or metal oxyhydroxides) and their amounts (soil texture) present in soils can vary greatly. In this study, the sorption capacity of the alpha, beta, and gamma-HCH isomers on three soils which greatly differ in the characteristics of their colloidal fractions are investigated: (i) an A horizon of an Andosol (A-A), (ii) an A horizon of a Podzol (A-P), and (iii) a B horizon of a Ferralsol (B-F).

Methodology

A buffered solution 0.005 M sodium azide (added to inhibit biological activity), 0.005 M sodium tetraborate, and 0.005 M calcium chloride was prepared (Miller and Pedit, 1992). Hydrochloric acid was used to adjust the borate buffer to a pH of 8.4. The HCH isomers (alpha, beta, and gamma-HCH) were separately added into the buffer solutions using a methanol carrier solvent to reach final HCH concentrations of 5 mg L⁻¹ each. A volume of 25 mL were added to an erlenmeyer containing 2.5 g of soil. A control study was carried out in identical erlenmeyers, with no solid presents. The erlenmeyers were shaken during 19 h, and known volumes were taken out at different time intervals within this time frame. Then, HCH present in these samples was extracted with hexane. The extraction mixtures were sonicated for 20 min and then dried with anhydrous Na₂SO₄. Extracts were analyzed for HCH isomers with a gas chromatograph (model GC 8532 Mega 2 Series, FISON Instruments, Milano, Italy) with a 25 mm x 30 m column (Restek Corporation, Bellefonte, PA) and an electron capture detector (model ECD 850, Thermo Quest, Milano, Italy). Total organic C present in these soils was 91.6, 133.8, and 3.9 g kg⁻¹ for the A-A, the A-P, and the B-F soils, respectively. Clay percentages were 7.5, 10.0, and 31% for the A-A, the A-P, and the B-F soils, respectively.

Results and Discussion

The results obtained indicated that, as expected, the organic matter was the main factor governing the extent to which the sorption processes occurred. The two A horizons highly retained all three isomers (>85% of HCH added), whereas the B horizon retained up to 86% of beta-HCH, and 42 and 34% of alpha- and gamma-HCH, respectively. The influence of the type of organic matter could be inferred when the solid-water partition coefficients were normalized by the soil organic C content (K_{oc}). The K_{oc} values obtained for the alpha-, beta-, and gamma-HCH isomers retained by the A-P soil were 1068, 645, and 586, respectively, whereas for those in the A-A soil were 610, 1179, and 614, respectively (Fig. 1).

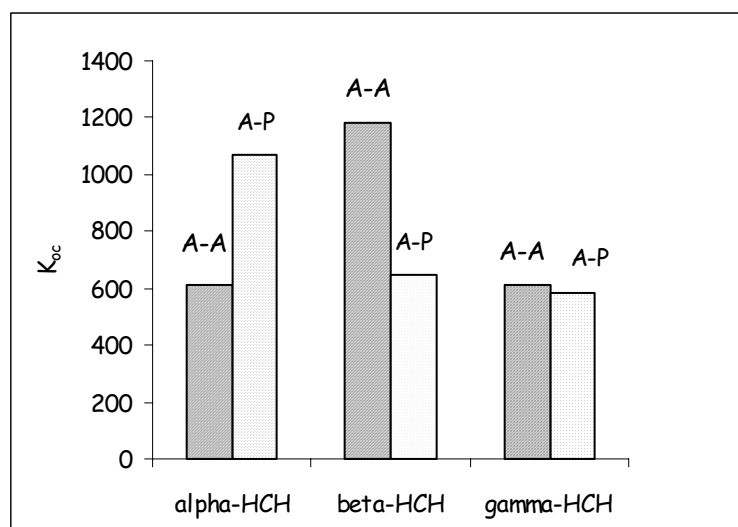


Fig. 1. Values of K_{oc} obtained for the alpha-, beta-, and gamma-HCH isomers retained by the A-A soil and by the A-P soil.

These results indicate a greater affinity of the non polar HCH isomer - beta-HCH - for organic matter of the surface horizon of the Andosol, characterized for being more recalcitrant and hydrophobic than that of the Podzol. The invers pattern was observed for the alpha-HCH. The most water soluble of the three HCH isomers studied - gamma-HCH - had similar K_{oc} values for both surface horizons. Finally, the results obtained with the subsurface horizon studied (soil B-F) (data not shown) indicated that, in spite of the smaller HCH retention capacity displayed, sorption processes of these compounds on inorganic surfaces cannot be neglected.

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Impact of vegetation on the bioavailability of HCH isomers in contaminated soils

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Hexachlorociclohexane isomers (HCHs) are hydrophobic organic compounds characterized by their toxicity and persistence. The most important anthropogenic sources of HCHs include those used for agricultural purposes and those disposed of as waste products during the synthesis of lindane (gamma-HCH). Several studies have shown that plants can enhance the biodegradation of organic pollutants in the immediate environment of the root (rhizosphere), bioavailability often being the limiting factor for biodegradation. The current study examines how plant roots and their associated rhizospheres affect HCH bioavailability in a heavily contaminated site at Porriño (Galicia, NW Spain). Two plant species growing in contaminated soil, *Cytisus striatus* and *Avena sativa*, were studied. Hexane- and water-extractable fractions of HCHs were analyzed in both the bulk and the rhizosphere soil. Chemical analysis of the bulk soil indicated the presence of four HCH isomers (alpha-, beta-, gamma-, and delta-HCH), with alpha and beta isomers being dominant. Only 1-10% of hexane-extractable beta-HCH present in the bulk soil was recovered by the water extraction, whereas for the alpha-HCH the proportion was higher (10-44%). Comparison between bulk and rhizosphere soil suggests that both species tended to reduce the total concentration of the four HCH isomers in the rhizosphere. The reduction was relatively higher for alpha-HCH than for beta-HCH, the latter becoming the dominant isomer in the rhizosphere soil (beta-HCH was generally more than 80% of the sum of the four isomers in these soils). There was an increase in the relative water-solubility of the HCH isomers present in the rhizosphere soils compared to the bulk soils (mainly for alpha-HCH); up to 70% of the total alpha-HCH in the rhizosphere soil was water-extractable. The rhizosphere effect on HCH water solubility implies an increase in the bioavailability of these compounds and, hence, a possible enhancement of their biodegradation. This could explain the lower HCH concentration in the rhizosphere compared with that in the bulk soil, although abiotic processes of dissipation, such as volatilization or leaching, may also take place. In a further study, biotic and abiotic mechanisms that may account for the decrease in HCH concentration in the rhizosphere soil will be investigated.

Evidence of Effective *in situ* Inactivation of Soil Pb Using Phosphate or Composted Biosolids in the IINERT Field Test at Joplin, Missouri.

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Summary

Reducing risks associated with Pb in soil has typically been accomplished by soil removal, covering, or dilution by mixing with uncontaminated soil. EPA's National Risk Management Research Laboratory and DuPont Co. established a collaborative effort to evaluate *in situ* remediation techniques. The In-place Inactivation and Natural Ecological Restoration Technologies (IINERT) Soil-Metals Action Team was established in November 1995 as one of several Action Teams under the US EPA Remediation Technologies Development Forum (RTDF). Its primary goal was to examine *in situ* remediation alternatives that are low cost and environmentally benign, yet provide equivalent or better protection to human health and the environment compared with conventional methods, such as excavation and capping. The IINERT Soil-Metals Action Team includes representatives from government, universities and industry who share an interest in further developing and validating *in situ* techniques as viable technologies for eliminating the hazards of metals in soils and other surficial materials. Although some sites may have such high soil Pb concentrations that removal is necessary, we anticipate that *in situ* inactivation will take care of a much larger area of intermediate contaminated soils at great public savings. A field experiment was started in the fall of 1996 at an urban Pb-smelter contaminated site in Joplin, MO, USA. Treatments were installed in March 1997 using a completely randomized; treatments were rototilled to about 12.5 cm depth, and individual plots were separated with heavy polyethylene barriers to limit soil movement during the study. The objective was to help accomplish the two-fold mission of the team: 1) develop and demonstrate *in situ* treatment technologies that reduce and eliminate the risks to human health and the environment of metals in soil and 2) achieve regulatory and public acceptance of these technologies. The data that has been developed from this cooperative research effort will be summarized and discussed. Evaluation of both the change in soil Pb geochemistry and its bioavailability [in vivo (using rats, swine, and humans) and in vitro] were measured over time. Results from this field evaluation illustrate the reduction in soil Pb, Zn and Cd availability to plants, and reduction of soil Pb bioavailability to mammals. Soil Pb was altered to pyromorphite on phosphate or compost treated plots during the 3-year test based on spectroscopic and chemical evaluation; increased adsorption also contributed to reduced Pb bioavailability of soil Pb where high Fe biosolids compost was applied. Both animal and *in vitro* chemical testing showed reduction in the bioavailability of soil Pb to mammals, with estimates of 35-40% reduction for rats and swine, and 69% for human adults. This test demonstrates that reduction in soil Pb risk can be accomplished without soil removal.

In 1994, Chaney and Ryan summarized technical evidence that soil Pb could be converted to forms with lower bioavailability. Ryan's group had developed use of phosphates to hasten formation of pyromorphite, while Chaney's group had illustrated use of high Fe biosolids compost to revegetate metal toxic soils and to reduce soil Pb bioavailability. Different compost products had different ability to reduce bioavailability of urban soil Pb to rats. And working with Freeman, Davis, Ruby et al., these principles were illustrated for Pb mine wastes. One needs to understand that this body of research was conducted to determine if Pb in all contaminated soils was 60% as bioavailable as Pb-acetate, the default assumption of the IEUBK model used by US-EPA to estimate risk from soil Pb at Superfund sites (measure total Pb and adjust to 60% based on IEUBK). Testing illustrated that soil materials varied widely in Pb bioavailability to rats. And a chemical extraction method was developed which yielded results well correlated with relative bioavailability in the animal tests. In the rat model, increasing doses of soil yielded a plateau response rather than a linear response, very different from the response to increasing Pb-acetate. These studies used highly purified casein-sucrose diets with little fiber and no phytate (these can reduce soil Pb absorption in normal diets). One suggested interpretation of the plateau response in tissue Pb with increasing dietary soil was that soil added metal adsorption capacity to the diet; greater Pb was balanced by greater Pb adsorption capacity. The implication would be that children who consumed larger amounts of soil (*pica* children) would have effectively much lower bioavailability than children who ingested small amounts of soil or dust. Superfund had assumed that children ingested 200 mg/day of soil/dust, while measurements of Pb ingestion by analysis of feces of 2-year-old children revealed that soil/dust ingestion geometric mean was 30 mg/day.

Other scientists used a different feeding protocol to feed soils to young swine (e.g., Casteel et al., 1997). They also showed that soil Pb bioavailability varied and argued that total Pb should be adjusted for relative bioavailability to estimate a site specific clean-up level. That protocol used three soil-Pb dose rates and replicate animals for each soil being tested. Blood and tissue Pb were lower for soil-Pb than found with Pb-acetate. During the IINERT study, rats were fed following a similar three dose rates protocol but the response remained a sharp plateau as dietary soil increased (Figure 1).

The chemical methods developed by Ruby et al. was modified by IINERT cooperators to a simple glycine-HCl buffer extraction at 37°C. Toxicologists argued that extractions should be conducted at pH 1.5 to evaluate the fasting condition. But stomach pH rises rapidly upon food consumption. Environmental scientists argued that extract pH should be determined based on correlation with bioavailability results. Using the IINERT soil feeding dataset, it became clear that the glycine-HCl method at pH 1.5 extracted equal amounts of Pb from untreated and treated Joplin soil; but feeding to rats, swine and humans showed a substantial reduction in Pb bioavailability from at least phosphate treatments (only phosphate treatments and control were fed to swine because of the larger cost of feeding soil to swine than rats). If the extraction was conducted at pH 2.2 or 2.5, extraction and feeding results were reasonably well correlated. Many soil treatments caused reduction in soil Pb bioavailability.

Scheckel and Ryan evaluated the chemical forms of Pb in untreated and treated soils as part of their research on whether phosphate or other treatments can cause a persistent reduction in soil Pb bioavailability. Study of the IINERT samples offered a test of samples with measured bioavailability. Using EXAFS and other spectroscopic methods, they found that high phosphate caused transformation of soil Pb to chloropyromorphite. In the field. Transformation increased somewhat over time. The high Fe of the

composted biosolids reduced transformation of soil Pb to pyromorphite, but simultaneously reduced soil Pb bioavailability nearly as well as phosphate and at lower phosphate additions. Their work also has illustrated that transformation of Pb in soil samples can occur during extraction. When acidic soil extracts are used, both Pb and phosphate are converted from precipitated or adsorbed forms to soluble forms and can rapidly react. So the fraction of transformation measured by sequential extractions may not represent environmental soil. The spectroscopic examination of field soil samples does not suffer that artifact, so it is clear that when high phosphate is applied, soil Pb is increasingly transformed to chloropyromorphite and the treated soils have lower relative Pb bioavailability than untreated soils.

An opportunity occurred to feed the same untreated and phosphate-treated Joplin soils to human volunteers. Maddaloni et al. (1998) have reported feeding high Pb soil to both fasting and non-fasting humans. For the fasting individuals, the absolute Pb bioavailability (based on measurement of Pb stable isotopes in soil and blood) was about 26.2%, while for the fed individuals, absorption averaged 2.5% [this agrees well with the effect of meals on absorption of soluble Pb isotopes by James et al. (1985)]. The same team fed the Joplin soil during fasting and found 42.2% bioavailable for the untreated soil and only 13.2% for the soil treated with 1% P using H_3PO_4 . This last was 69% reduction in bioavailability.

Although data are not included in this Abstract, we measured metal uptake by the lawn grasses established at the site. After incorporation of the several treatments [untreated; 1% P as H_3PO_4 ; 1 and 3.2% P as triple superphosphate (TSP); 1% P as rock phosphate; 1% Fe plus 1% P as TSP; 2.5% Fe plus 1.0 or 3.2% Fe as a precipitated Fe byproduct; 10% composted biosolids rich in limestone equivalent with 0, 0.32, or 1.0% additional P as TSP], and incubation for several months, hydrated lime was applied to return all plots to above pH 7, fertilizer needed to establish a new lawn was applied, and tall fescue was seeded. Fescue shoots were harvested annually. The soil contained on average about 2200 mg Pb, 4300 mg Zn and 17 mg Cd/kg dry weight in the fraction sieved < 250 μm . Fescue shoots harvested in fall 1997 contained over 250 mg Zn/kg, 4.8 mg Cd/kg and 13.4 mg Pb/kg, but this level declined over time after treatment incorporation, reaching about 105 mg Zn, 1.0 mg Cd and 6 mg Pb/kg dry fescue. Because most Pb contaminated sites, both mining and smelting sites and urban soils are co-contaminated with Pb, Zn and Cd, treatments to inactivate soil Pb should also deal with high soil Zn and Cd. Making soils calcareous along with added compost or phosphate clearly remediated any Zn phytotoxicity or food chain Zn or Cd risks at the site. This low Cd:Zn ratio (0.004) would limit food-chain transfer of Cd by reducing crop Cd bioavailability as well. Brown et al. (2003) have previously discussed the need to make Zn-rich contaminated soils calcareous to prevent future Zn phytotoxicity if soil pH were allowed to decline substantially. Keeping the site calcareous can persistently limit Zn and Cd uptake by crops and prevent Zn phytotoxicity. Barren Zn-Pb contaminated industrial sites may also be severely infertile and P deficient, so amendment combinations such as the composted limed biosolids or other byproduct mixtures can provide all the treatment and fertilizers required to persistently remediate long barren sites.

Although scientists may want to see more field tests of *in situ* Pb remediation, and more tests of factors which affect soil Pb bioavailability, these results strongly support use of these simple and inexpensive treatments with phosphates, or with composts rich in P and Fe and/or Mn, to provide public savings while achieving the level of reduction of lead risk required to protect children and wildlife. Revegetation of such sites allows

biomass to be produced to support new ecosystems which show diversity of plants and animals, while animals have been found to be protected from adverse effects of soil Pb, Zn and Cd. Such remediated sites should not be playgrounds, but the treatments appear to provide the safety required for ecosystems at great public savings. The treatments are so simple that they can be applied by homeowners tired of waiting for government to decide what to do when we all know that urban soils comprise most of the Pb contaminated soils to which children are exposed yet only industrial sites can become Superfund sites in most countries.

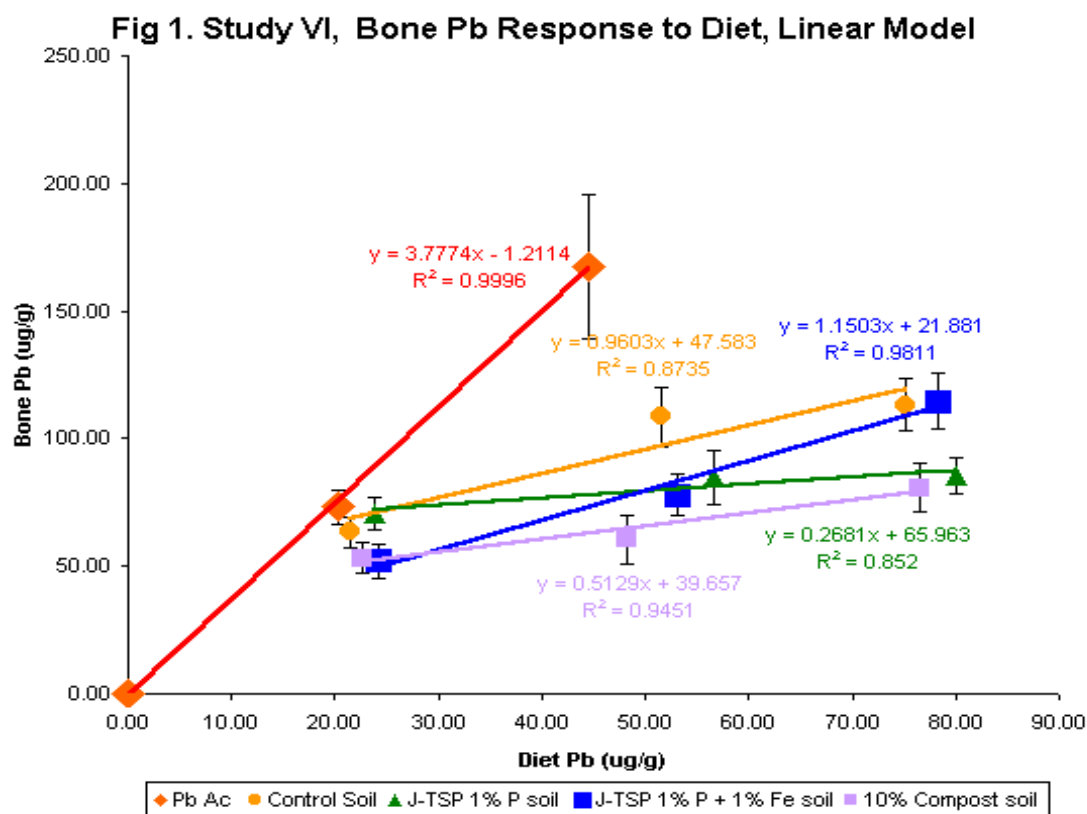


Figure 1. Bone Pb concentration for rats fed Pb acetate, untreated Joplin soil, and 3 treated soils (1% P as TSP; 1% P + 1% Fe; or 10% composted biosolids; soil samples collected after three years incubation in the field, sieved < 250 μ m, and mixed with AIN purified diets for feeding to rats.

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Changes on lead bioavailability after induced phytoextraction

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Induced phytoextraction is a cleanup technology, especially useful for lead polluted soils, based on the use of non-accumulator plants with high biomass production and fast growth, were synthetic chelating agents, such as ethylenediaminetetraacetic acid (EDTA), currently used in agriculture, are added to the soil to increase metal mobility and thus plant uptake of heavy metals from the contaminated soil (Salt et al., 1998).

Lead bioavailability, especially in aged contaminated soils, is limited due to stable complexation with organic matter, sorption on clay minerals and iron and manganese oxides, and precipitation as carbonates, hydroxides and phosphates.

Lead bioavailability is known to be dependent on lead speciation/fractionation and on site-specific soil chemistry, so it is necessary to adapt any study to specific circumstances.

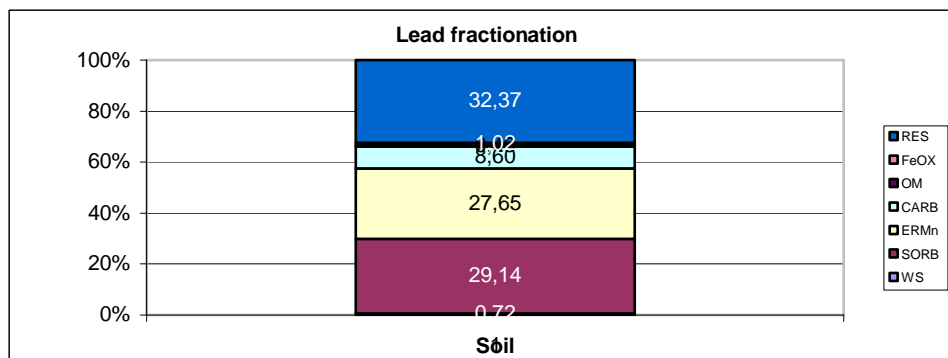
A calcareous area contaminated by long-term lead deposition processes, at the vicinity of a highway, near Barcelona, was sampled that contain 390 ppm of aqua regia extractable lead. Surface samples were collected (0-5 cm depth), air dried and passed through a 2 mm Ø sieve to be characterised and used in batch experiments.

Soil characterisation was done by soil standard methods. Aqua regia extractable lead was determined by ISO Norm 11466. Sequential extraction of lead was achieved in 7 steps (Ma et Uren, 1995): 1) water soluble, 2) sorbed, 3) easily reducible Mn bound, 4) carbonate bound, 5) organic matter bound, 6) iron and aluminium oxides bound, and 7) residual fraction were analysed for lead.

Table 1. Soil characterization and lead fractionation.

total Pb, mg kg ⁻¹	390
pH	7.60
conductivity, dS/m	0.27
texture ISSS <2-mm fraction	loamy sand
organic carbon, %	7.87
carbonates, %	10.3
P ₂ O ₅ bioavailable, mg kg ⁻¹	87
CEC, cmol/kg dry soil	75
CaO bioavailable, mg kg ⁻¹	10102
MgO bioavailable, mg kg ⁻¹	330
Na ₂ O bioavailable, mg kg ⁻¹	29

Table 1. Soil characterization and lead fractionation. (continuing)



Lead water soluble and sorbed fraction are bioavailable forms, whereas easily reducible Mn bound, carbonate bound, organic matter bound, and iron and aluminium oxides bound fractions are supposedly bioavailable, depending on media conditions and chelant capacity.

Substantial removal of Pb requires a chelant concentration at least equimolar with the total lead that can potentially be solubilized. Experiments were done with different concentrations of K_2H_2EDTA : 1, 5, 10, 25, 50, and 100 mmol L^{-1} that assured a good ratio K_2H_2EDTA/Pb .

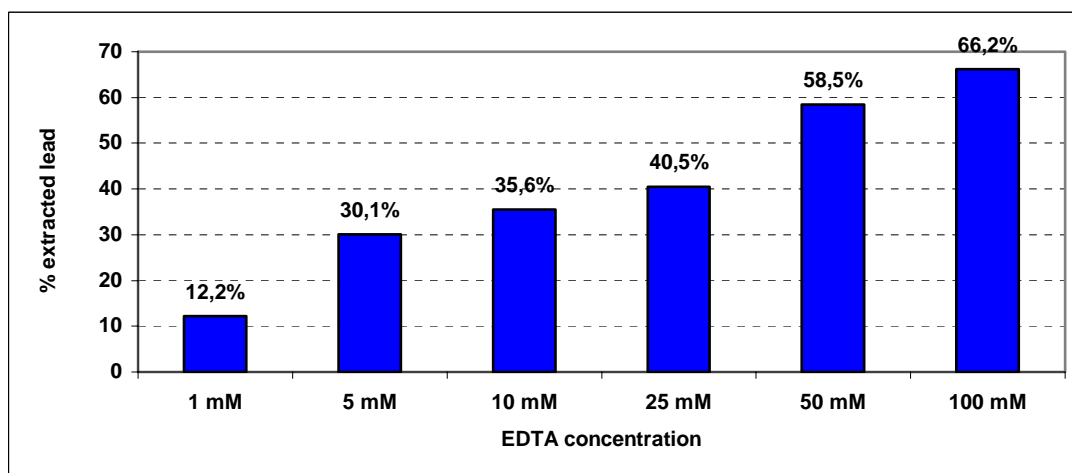
For this calcareous soil, an acidic form of chelant, such as K_2H_2EDTA , is expected to increase the amount of available lead. Since EDTA solutions have low pH (Table 2), and lead removal is very sensitive to pH changes, extracted Pb levels are high (Figure 1).

Table 2. EDTA solution's pH.

K_2H_2EDTA	pH	K_2H_2EDTA	pH
1 mmol	5.18	25 mmol	4.52
5 mmol	4.72	50 mmol	4.34
10 mmol	4.72	100 mmol	4.62

Lead desorption from contaminated samples was determined through batch extractions of the fine fraction ($< 2mm \varnothing$). Samples were allowed to equilibrate with chelant solutions for 2 hours and extraction solutions filtered (Whatman n° 42). Experiments were done at saturation moisture content, at a ratio of 1:10 (w/v) (soil : K_2H_2EDTA solution). All the extractions were performed in triplicate. Lead desorption levels are shown in Figure 1.

Figure 1. K_2H_2EDTA lead extraction.



The buffering capacity of soil is exceeded with K_2H_2EDTA concentrations above 10 mmol as can be seen in Table 3.

Table 3. Comparison of soil pH before and after treatment.

* soil pH	1 mM	5 mM	10 mM	25 mM	50 mM	100 mM
7.6	6.8	6.9	6.8	4.9	6.7	4.9

Drawbacks associated with chelating application include changes in soil properties that must be forewarned adjusting chelant concentration.

A second approach is necessary to measure lead bioavailability from a physiological point of view. Gastric pH in children is quite variable among individuals, and depends strongly on nutritional status. Measurements of gastric pH resulted in mean values of 1.75 that rise to more than 4 following ingestion of food. The return to basal values is achieved after two hours (Ruby, 1996).

A physiologically based extraction test was performed that reproduce availability of Pb in the acidic stomach conditions in children (pH 1-4). Soil samples were allowed to equilibrate with the corresponding acidic solution (prepared adjusting pH with HCl 42.6 %) for 1 hour and filtered (Whatman n° 42). Extraction was done at a ratio of 1:100 (w/v) (soil : HCl solution). All the extractions were performed in triplicate. Lead available levels are shown in Table 4.

Table 4. Lead extraction in very acidic conditions.

pH	ppm Pb	% total Pb
1	216 ± 29	55
4	11.93 ± 2.37	3

Standard procedures of assessing risk associated with lead contaminated soils recommended by USEPA (United States Environmental Protection Agency) assume that 30% of ingested soil Pb is sorbed in children stomach. However this general assumption may not be appropriate for all circumstances.

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Effect of different N fertilizers on heavy metals bioavailability in soil

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Soil pH is a master variable in determining heavy metals solubility; for most trace elements, such as Zn, Cu, Pb and Cd, acidification of soil solution leads an increase in their amounts as free ions and a consequent increase in their bioavailability.

In this work was studied the possibility of improving phytoextraction of heavy metals in con-taminated soils through a lowering in soil solution pH due to application of different N fertilizers evaluating the effects of acidification of the whole (bulk) soil and of the root zone soil only.

In particular, the degree of bulk soil acidification due to ammonium based fertilizers (consequently to the dissolution of ammonium sulphate, biological oxidation of thiosulphate and nitrification of ammonium) was investigated by addition of ammonium sulphate, ammonium sulphate plus the nitrification inhibitor DMPP, ammonium thiosulphate and urea to a neutral, poor in organic matter and unpolluted soil. Soil acidification effectiveness of N-fertilizers was evaluated and compared with pH changes in unfertilised and $\text{Ca}(\text{NO}_3)_2$ treated soil.

The different behaviour of N-fertilizers was studied by checking pH and mineral N-forms ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ KCl sol.) in a 30 days incubation trial. Treatments with urea and ammonium sulphate showed the most relevant lowering of pH.

In the meantime a trial with sunflower grown in small pots (300 cm^3) was conducted with the same experimental design of the incubation one, in order to observe the influence of root activity on soil pH variation in rhizosphere. After 14 days growth, the effect on rhizosoil pH due to plant uptake of different N forms is clearly evidenced. In comparison with the values observed in the incubation experiment alkalization of rhizosoil (0.4-0.5 pH units) were obtained in all the treatments except in the $(\text{NH}_4)_2\text{SO}_4$ plus DMPP treatment, that showed a decrease in soil pH (0.3 pH units). As pointed out by the results of the incubation experiment nitrate is the main inorganic N form in $\text{Ca}(\text{NO}_3)_2$, ammonium sulphate, ammonium thiosulphate and urea treatments while ammonium is lasting in soil by DMPP in ammonium sulphate plus the nitrification inhibitor treatment. Therefore the results of soil pH may be explained by plant uptake.

This physiological mechanism may be exploited in polluted soils to increase the solubility of heavy metals in soil sites directly involved in metal uptake by roots, so preventing leaching losses of heavy metals due to their mobilization in the whole soil.

In this aim a preliminary study was carried out in order to examine the effectiveness of the two acidification strategies – localized and diffused – in heavy metal phytoextraction on a high polluted soil.

Integrating Bioavailability of Metals into Life Cycle Impact Assessment of Terrestrial Ecotoxicity

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Assessment of terrestrial ecotoxicity in Life Cycle Impact Assessment (LCIA) uses multi-media fate and exposure models to evaluate which part of an emission would result in an intake of contaminant by living organisms. Most currently used multi-media models are Caltox, EUSES, USES-LCA and recently, IMPACT 2002. These models are divided into three modules: the fate, which is the distribution of an emission through different media; the exposure, characterizing the fraction taken up by organisms; and the effect, which is the quantified toxicological response to this intake of toxic based on NOEC or EC₅₀ laboratory data.

In a general way, the ecotoxic impact (I) per unit emission (\mathcal{M}) is expressed by the following equation:

$$(1) \quad \frac{I}{\mathcal{M}} = \frac{C_{\text{soil}} * V_{\text{soil}}}{\mathcal{M}} * EF$$

where C_{soil} : total metal concentration in soil
 V_{soil} : volume of soil compartment
 EF : effect factor based on NOEC or EC₅₀

In the context of Life Cycle Assessment (LCA), the time-integrated concentration in soil must be considered in the potential impact calculation, so that equation (1) becomes:

$$(2) \quad \frac{I}{M_0} = \frac{\int C_{\text{soil}} * dt * V_{\text{soil}}}{M_0} * EF$$

$$(3) \quad = \frac{f_{\text{m} \rightarrow \text{soil}}}{k} * \frac{1}{k} * EF$$

where M_0 : total mass released in the environment (kg)
 $f_{\text{m} \rightarrow \text{soil}}$: mass fraction transferred from the initial media to soil
 k : rate constant (yr⁻¹)

Up until now, multi-media models have not included the concept of metal bioavailability through the soil media to the terrestrial ecosystem. In fact, site-specific variations of metal behavior have not yet been expressed in LCIA.

In this context, an approach to integrate the bioavailable fraction of metals in the currently under-development IMPACT 2002 multi-media model is proposed here. The general expression of this new approach including the AMI method for the effect factor (EF) is :

$$(4) \quad \frac{I}{M_o} = f_{m \rightarrow \text{soil}} * \frac{1}{k} * F_{\text{bio}} * \frac{0.5}{\text{HC}_{50 \text{ bio}}}$$

where $(f_{m \rightarrow \text{soil}} * 1/k)$: fate factor

F_{bio} : ratio of bioavailable concentration on total soil concentration

$\text{HC}_{50 \text{ bio}}$: median of EC_{50} for terrestrial organisms expressed in bioavailable concentration

Considering the lack of general correlation suitable for LCIA, between metal bioavailable fractions, soil characteristics and ecotoxicological data from literature, the exposure model proposed in equation (4) for metals is to be validated through a set of experiments with copper. The identification of the fraction of metal inducing an effect on terrestrial organisms (F_{bio}) and its correlation with generally well-known soil characteristics are the main objectives of the on-going experiments. Moreover, as opposed to the aquatic ecosystem, few ecotoxicological databases are available for terrestrial organisms. In this perspective, proposed experiments contribute to the improvement of available and complete ecotoxicological data.

On this basis, ten different soils spiked in laboratory were generated to cover most of the variability of North American and European soils for three main soil characteristics: organic matter soil content, clay soil content and pH. The range of pH varies from 5 to 8, clay content from 5 to 35% while organic matter content ranges from 1 to 30%. Experiments are being conducted in order to find a correlation between terrestrial ecotoxicity of copper and soil characteristics via the measurement of several soil metal pools and toxic response of organisms which are directly in contact with the soil matrix: *eisenia fetida* (compost worm) and *hordeum vulgare* (barley). Germination and growth tests are being assessed for barley, while mortality and avoidance are tested on compost worm. For all the experiments, chemical extractions are performed in order to characterize the soluble fraction and the readily exchangeable fraction (1M MgCl_2). The free ions and other soluble complexes are also analyzed.

Furthermore, a set of wetting and drying cycles are performed to evaluate the way bioavailable fractions behave with time in a realistic aerobic-anaerobic cycle scheme. Ultimately, these kinetics measurement could be used to validate the rate constant (k) used in equation (4).

Finally, including regional soil characteristics in LCA studies would give more information of what is really taken up by the terrestrial regional ecosystem (bioavailability). Consequently, a proposal for including site-specific soil data offers promising perspectives for the improvement of exposure models to be used in the assessment of toxicological impacts of metals on terrestrial ecosystem.

Distribution and plant availability of selenium fractions in seleniferous soils of northwest India

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Introduction

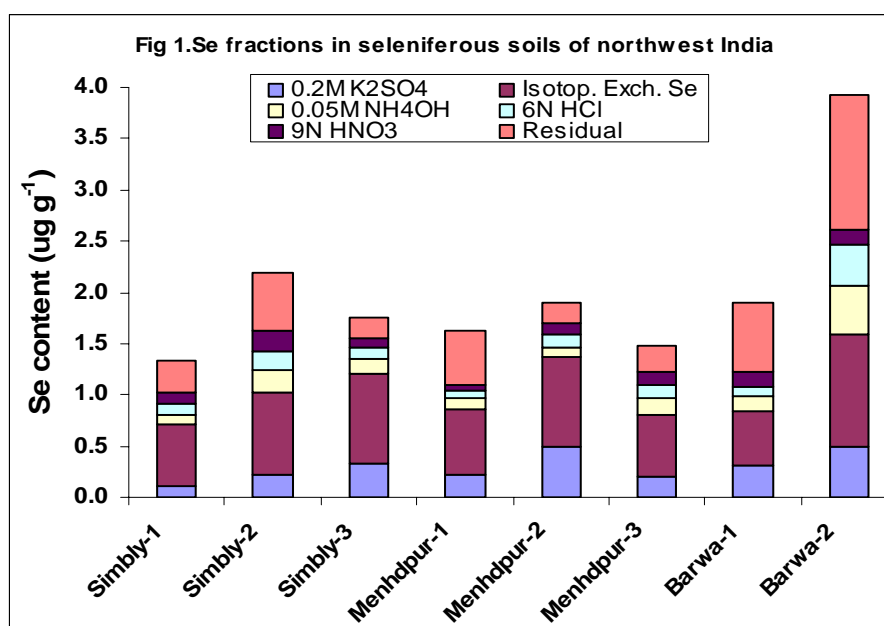
The soils containing as low as $0.5 \text{ mg Se kg}^{-1}$ are considered as seleniferous because the forages grown on such soils contain $> 4 \text{ mg Se kg}^{-1}$ – the maximum permissible level for animal consumption (Dhillon et al., 1992). The management of seleniferous agricultural land requires a good understanding of the nature and behaviour of Se in affected soils. Selenium exists in soils in several chemical forms that differ widely in their solubility and availability to plants. The present investigation was undertaken to understand the soil-phase association of Se in seleniferous soils using sequential extraction procedure proposed by Cary et al. (1967). Availability of different soil Se fractions to corn was also evaluated.

Methods and Materials

Eight surface soil samples were collected from the seleniferous region and equilibrated with carrier free ^{75}Se @ $0.25 \mu\text{Ci g}^{-1}$ soil by subjecting to alternate wetting and drying cycles. Separation of soil Se into different fractions was achieved by sequentially extracting the samples with $0.2\text{M K}_2\text{SO}_4$ followed by $0.1\text{M Na}_2\text{SeO}_3$, $0.05\text{M NH}_4\text{OH}$, 6M HCl and 9M HNO_3 and measuring the radioactivity in each extract. In the greenhouse, corn (*Zea mays* L.) was grown for 50 days in these soils and uptake of Se was worked out. Correlation coefficients between different Se fractions and total Se uptake by corn were determined. In another greenhouse experiment, the critical levels of Se in the soil-plant system were determined. Corn was grown in the soil supplied with different levels of Se ranging from 0 to 25 mg kg^{-1} as sodium selenite. Plants were harvested 50 days after sowing and analyzed for Se.

Results and Discussion

Large variations were observed in the distribution of Se in different fractions in the seleniferous soils (Fig 1). From 8.8 to 26.1% of total Se was present in readily available form ($0.2\text{M K}_2\text{SO}_4$ extractable). The bulk of the total Se was found to be in the isotopically exchangeable form (extractable with 0.1M NaHSeO_3) and ranged from 27.6 to 49.0%. Organic Se extracted by ammonium hydroxide constituted 5.3 to 12.0% of total Se. The amount of Se extracted by HCl (4.8 to 10.4%), HNO_3 (3.7 to 8.8%) and residual (10.9 to 33.2%) were considered to be difficultly available forms of Se. Significantly positive relationship between isotopically exchangeable and K_2SO_4 extractable Se ($r=0.741^{**}$) suggests that soil solution Se concentration is governed by exchangeable Se content in the soil. Significantly positive correlation coefficients have been observed between total Se content of seleniferous soils and amount of Se extracted through $0.2\text{M K}_2\text{SO}_4$ ($r = 0.660^*$), isotopically exchangeable Se ($r=0.813^{**}$) and organic form of Se ($r=0.954^{***}$).



Dry matter yield and total uptake of Se by corn plants grown in seleniferous soils in the greenhouse varied between 10.9 to 15.0 g / pot and 63.9 and 103.5 µg / pot, respectively. Highly significant positive correlation coefficient ($r=0.800^{**}$) between exchangeable Se and Se uptake indicates that the exchangeable form of Se in seleniferous soils determines Se uptake by corn plants.

In an artificially selenized soil, significant decrease in dry matter yield of corn was observed only beyond a level of 5 mg SeO₃²⁻-Se kg⁻¹ soil. Using statistical procedure given by Cates and Nelson, critical level of Se in corn plants (dry weight basis) was found to be 39.2 mg kg⁻¹ and beyond this level significant decrease in dry matter accumulation was noticed.

Conclusions

- Isotopically exchangeable Se was the dominant form of Se in seleniferous soils. It ranged from 27.6 to 49.0% of total Se and was positively correlated with K₂SO₄ extractable Se
- Isotopically exchangeable fraction of Se should prove as better index of bioavailable Se in seleniferous soils of northwest India
- Critical level of Se beyond which significant reduction in dry matter yield of corn takes place was 39.2 mg kg⁻¹ in plants and 5 mg Se kg⁻¹ in soil

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Bioavailability of heavy metals to plants: Restricted options for generalization

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Metal availability to plants is a process strongly controlled by plant characters, and not per se by soil chemistry. Plant species control their rhizosphere by exudation of protons and organic acids via their roots, and during a vegetation cycle by modifying metal complexation via litter decomposition. The root activities may enhance or diminish metal availability in a species-and/or ecotype-specific manner. The degree of adaptation of plants to a surplus of metals is often expressed by down- and/or upregulation of the activity of metal transporters which determine metal access to the plant. Cellular compartmentation and metal transfer from root to shoot are the next steps which are responsible for the degree of toxicity of a surplus of a metal and thus for the impact of metal availability on plant survival.

These general patterns are further complicated by the symbiosis of higher plants with various types of mycorrhizal fungi. These fungi are able to modify metal availability to plant roots and may strongly modify metal toxicity.

The consequences of these species- and ecotype-specific reaction and association patterns will be highlighted. It will be concluded that chemical extractants can not simulate bioavailability of heavy metals to plants.

Forecast methods to assess the contamination of edible vegetables growing on soils polluted by organic chemicals.

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Key-words : bioavailability, transfer, organic pollutants, edible vegetables

Introduction

Human exposure to organic pollutants can occur through different environmental pathways, including internal absorption through food and water consumption. Former industrial sites may contain variable concentrations of chemicals, more than 70% of them concerning polycyclic aromatic hydrocarbons and/or chlorinated solvents, since these organic products are largely used as combustible, fuel or solvents. These sites represent a potential source for food chain contamination if they are converted to residential zones with gardening practices [1], or when they can contaminate water used for crop irrigation. This work was undertaken to test four forecast methods evaluating the bioavailability of PAHs (Polycyclic Aromatic Hydrocarbons) and OHVs (chlorinated solvents) and their potential transfer to edible parts of different vegetables cultivated on polluted soils. The main objectives were to detect edible vegetables which represent a risk for human health in scenarios of industrial site rehabilitation, and to elaborate an advice guide in order to help responsible of these polluted industrial sites to evaluate easily the risk.

Materials and methods

Four forecast methods were investigated to evaluate the contamination of four vegetables (lettuce, carrot, bean, tomato) and an aromatic plant (sorrel) cultivated on soils polluted by two organic pollutants, PAHs and OHVs. The first method consisted to identify, sample and analyse wild plant species analogous to cultivated vegetables and naturally present on industrial polluted soils, in order to compare their chemical bioaccumulation factors with edible vegetable bioaccumulation factors. The second method consisted to cultivate vegetables on polluted soils taken up from previous sites; chemical bioaccumulation factors were calculated and the effect of endomycorrhizal fungus on chemical transfer in plants was studied. The third method consisted in the validation of different models of soil-to-plant transfer of pollutants by comparison between values predicted by models and experimental results. The fourth predictive method aimed to develop protocols of selective extraction of pollutants from soil, and to determine if there was a relation between these values and the plant uptake.

The land approach was conducted on four industrial brownfields, two of them presenting a pollution by hydrocarbons, the two other sites showing a contamination by chlorinated solvents. On each site, a cartography of the vegetation was realised. Plants and rhizospheric soil were then collected. Shoots were separated to roots, and all plant parts were washed to remote adherent soil particles. The various plant parts were dried at 35°C to avoid chlorinated solvent volatilisation, weight, and mixed prior to organic pollutant analysis. After extraction, PAHs were detected by HPLC (High Performance Liquid Chromatography), and OHVs by atomic emission. For the second method, plants were cultivated in pots, under controlled conditions, and in bigger containers, under natural conditions. Soils were sampled in the upper horizon (0-20 cm) of two of the previous industrial brownfields, one presenting a pollution of 207 mg PAHs kg⁻¹ dry soil, and the other showing a contamination of 50 µg OHVs kg⁻¹ dry soil. Soils were sieved to 5 mm for pot experiment, and to 5 cm for container experiment. An amount of 5 kg dry soil was introduced in 10-L plastic pots and of 280 kg in containers, under a 3-cm gravel layer. Plant seeds were sown directly in pots and containers. Pot experiment was conducted in a greenhouse (12-16 hours of photoperiod ; 20-22°C of day temperature and 15-16°C of night temperature), and container experiment in natural conditions, with four replicates for each treatment. In the pot experiment, soils were irrigated daily to maintain the moisture content at 80% of the field moisture capacity. At maturity, plants were harvested and prepared to analysis following the same protocol than wild plants. For the third method, 5 models was tested : HESP, PlantX, CEMOS-PLANT, Mackay 1994 and Mackay 1997. Selective extractions of pollutants were conducted with different liquid/solid fractions and different solvents (water, methanol, acetonitrile) at different gradients.

Results and discussion

Various wild plant species colonised naturally the contaminated soils of former industrial sites, and five wild species analogous to edible vegetables were identified. PAHs were detected in all plant parts, at a concentration varying from 0.1 to 0.5 mg kg⁻¹ DM in aerial parts, and from 1.5 to 5.0 mg kg⁻¹ DM in roots, probably due to an high adsorption of PAHs on the root epidermis (*figure 1*). OHVs were present only in traces.

Edible vegetables grew on these polluted soils without symptoms of phytotoxicity. However, at harvest, biomass of plants cultivated on OHVs polluted soils was significantly lower than biomass of control plants. Inversely, the foliar and the fruit biomass of plants cultivated on PAHs polluted soils tended to be higher than biomass of control plants (*figure 2*).

Figure 1.

PAH content of above- and belowground parts of wild analogous plants growing on a PAHs polluted soil (207 mg PAHs kg⁻¹ dry soil)

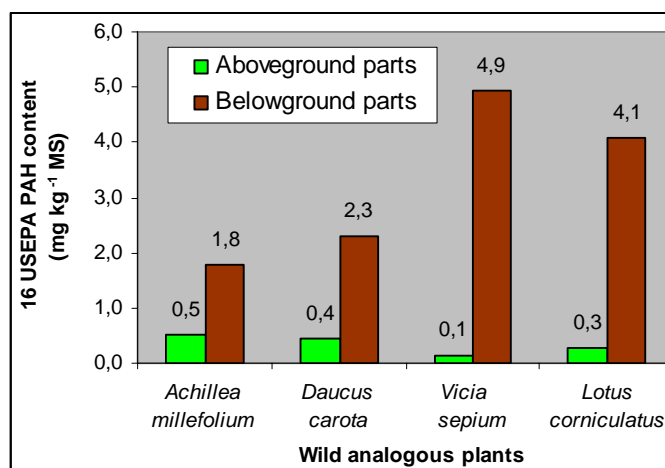
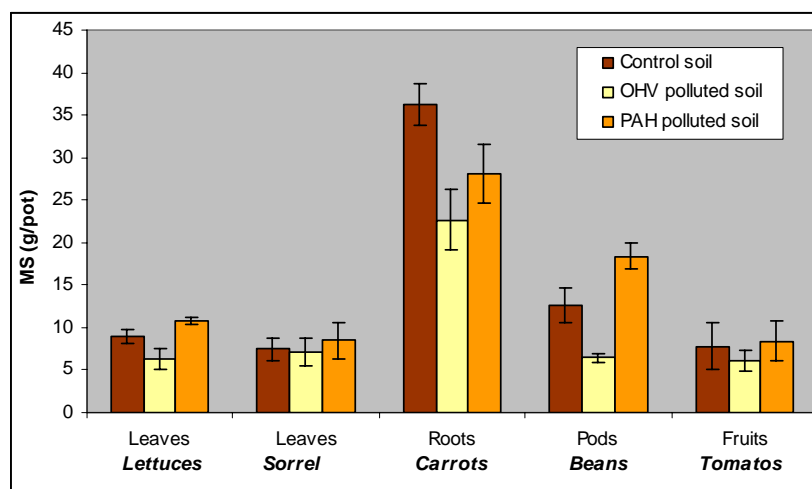


Figure 2.

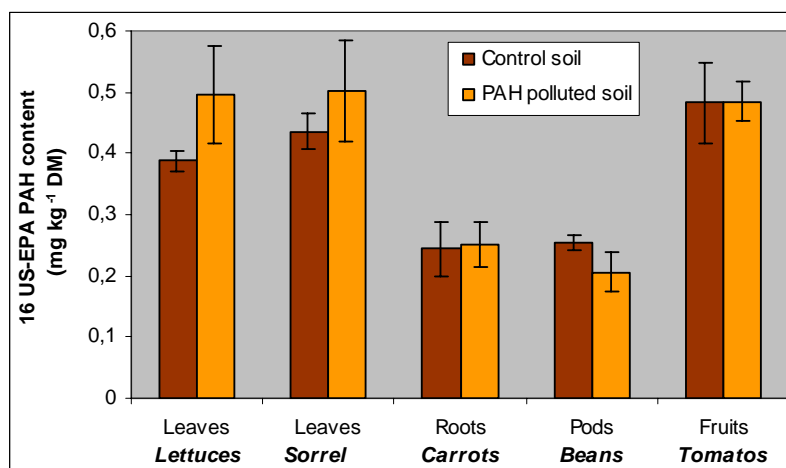
Biomass of edible parts of vegetables cultivated on a control soil and on two polluted soils.



When soils presented concentrations of pollutants compatible with a residential land-use (about 200 mg PAHs kg⁻¹ of dry soil and 50 µg chlorinated solvents kg⁻¹ of dry soil in our study), no significant concentrations of organic pollutants were measured in the different edible tissues of the plants as compared to concentrations in plants grown on uncontaminated soils, though content of PAHs in leaves of vegetables grown on polluted soils tended to increase (figure 3) [2]. Except in tuberised roots of carrots, root PAH content of vegetables cultivated on PAH polluted soils was about ten times higher than root PAH concentration of plants grown on control soil, as observed with wild analogous plants. Since variability of these results were very high, the most probable explanation is that particles of polluted soil were strongly adherent to root epidermis. No significant PAH content was observed in tuberised roots of carrots, probably due to the very low transfer of high molecular weight chemicals from leaves to belowground storage organs (figure 3) [2].

Figure 3.

PAH content of edible parts of vegetables cultivated on a control soil and on a PAH polluted soil (207 mg PAHs kg⁻¹ dry soil)



Moreover, we noted a similar behaviour of cultivated vegetables and of their wild analogous plants facing organic pollutants [3]. This would allow the use of wild plants naturally present on polluted soils to assess the risk of contamination of the food chain, complementary to extraction methods.

Results of modeling and analytic approach are not yet available.

Conclusions

This work shows that no risk for human health through vegetable consumption was evident in scenario of industrial site reconversion when soils presented concentrations of pollutants compatible with a residential land-use (about 200 mg PAHs kg⁻¹ of dry soil and 50 µg chlorinated solvents kg⁻¹ of dry soil in our study). However, in case of significant transfer of PAHs in plants, this study underlines the interest of wild analogous plants spontaneously present on polluted soils to forecast the transfer of organic pollutants in edible vegetables.

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The use of microbial bioassays and activity measurements for the evaluation of metal-contaminated soil quality

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The objective of this research was (i) to estimate metal bioavailability in a metal contaminated soil using microbial biosensors and (ii) to assess adverse effects on the soil microorganisms in relation to metal bioavailability.

Heavy metals were applied in model forest ecosystems as filter dust from a secondary metal smelter at the beginning of the experiment and mixed with the 15 cm upper soil layer (pH 6.4). The added concentration was 2700 mg / kg Zn, 385 mg / kg Cu and 63 mg / kg Pb. Cadmium was added additionally as CdO at a concentration of 10 mg / kg. The treatments in 4 chambers each are control, acidic irrigation, heavy metals in the upper soil and the combination of acidic irrigation and heavy metals.

Soil toxicity was assessed using the MetPlate bioassay (Bitton et al. 1994) and metal-specific recombinant bacterial sensors BIOMET (Tibazarwa et al. 2001) were used to detect bioavailable heavy metals in the soil. The level of soil microbial activity was measured by heterotrophic respiration and dehydrogenase activity. The structure and function of microbial populations was assessed by the carbon substrate utilization pattern (Biolog) and by terminal restriction fragment length polymorphism (T-RFLP) of DNA directly extracted from soil.

Metal contaminated soils had significantly reduced the respiration rate and the dehydrogenase activity. Similarly, total DNA content (as a measurement for the microbial biomass) was significantly reduced by the metal treatment. In addition, heavy metals affected the structure of the microbial population in the soil shown by the differences in the carbon source utilization pattern and RFLP fingerprints. The numbers of carbon sources oxidized, the rate of color development and the metabolic profiles were changed by the metals in the soil (reduced inoculum size, viable cells or change in the bacterial community). The strongest effect on microbial indicators was shown in the combined treatment (metal and acid irrigation). The bioavailable metal concentrations measured by the bacterial sensors were in good accordance with the concentrations in the exchangeable metal fraction (1 M NH₄NO₃ extraction). This approach appeared to be very useful to quickly quantify the concentration of bioavailable heavy metal or to evaluate the efficacy of soil remediation techniques.

The toxic effects of arsenic on growth of sunflower

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1 Introduction

Arsenic, chemically very similar to phosphate, is not essential for plant growth. Plant responses to both, arsenate and phosphate depend on growth conditions. Hurd-Karrer (1936) found that in nutrient solution a P:As ratio [mg kg^{-1}] of at least 4:1 was needed to protect wheat roots against As toxicity. Rumberg et al. (1960) reported that phosphate addition improved plant growth in nutrient solutions containing sufficient arsenate to be toxic at low P concentrations. In soil systems, results were ambiguous; deleterious, negligible or even beneficial effects of P addition on As uptake and plant growth have been reported (Hurd-Karrer 1936; Jacobs and Keeney 1970; Woolson et al. 1973). One reason for this diversity may lie in the way how the experiments were designed. In most studies investigating the effect of P on As toxicity and plant uptake in soils, P and As were added simultaneously to the soil and effects are related to the rates and ratios of P and As added. As has been demonstrated already by Woolson et al. (1971), however, As and P uptake by plants is determined by their concentrations in the soil solution rather than by the total amount added. For several reasons concentrations in solution do not simply depend on the latter. Due to the chemical similarity, sorption of phosphate and arsenate occurs by the same mechanisms, but arsenate is generally sorbed less strongly than phosphate. In addition, P forms organic complexes with organic soil matter, while As does not form organic complexes, with the result of higher soluble As concentrations in soils with a high organic matter content. Also kinetic effects may influence As and P in the soil, due to different time necessary for equilibration. Additionally, the amount of As and P already present in the soil and sorbed on soil particles may influence the competition of added As and P.

The objective of this study was to investigate the effects of phosphate addition on As availability in two different As contaminated soils as well as on the As accumulation and growth of sunflower (*Helianthus annuus*, L.).

2 Materials and Methods

The two soils used in this study were the plough layer of an arable field of the surrounding from a brass smelter (silty loam) and glass manufacture (sandy loam). Aliquots of both soils were have been spiked one year before with various amounts of As (Na_2HAsO_4) so that four different As treatments (As_0.7, As_1.3, As_3.4, As_6.8) in the silty loam and two in the sandy loam (As_3.1, As_6.9) were designed. The number in the designation of a treatment refers to the soluble As concentration [mg kg^{-1}] of the soil at the beginning of the greenhouse experiment. Aliquots of 7 kg of sieved and homogenized soil were filled into plastic pots. Each As treatment level was carried out in triplicates. Sunflower (*Helianthus annuus* L., San Luca) was seeded at a density of 5 grains per pot and reduced to two plants ten days after germination. Phosphorus was applied in form of $(\text{NH}_4)_2\text{HPO}_4$. Four different P treatments (28 mg P kg^{-1} soil (P_28), 56 mg P kg^{-1} (P_56), 84 mg P kg^{-1} (P_84) and 140 mg P kg^{-1} (P_140),

respectively) were applied to each As pre-treatment. A control treatment without any P addition (P_0) was also included. The first P application started 30 days after seeding and was then repeated two times at intervals of 21 days. After four months sunflower was harvested, separated into roots and shoots and extracted by 5 ml HNO₃ and 3 ml H₂O₂. Soil samples were analyzed for pH, soluble (0.1 M NaNO₃) and total (2 M HNO₃) metal and As concentrations. Plant and soil extracts were analyzed for As content by AAS–GF–HG and for Cu, P and Zn content by ICP–AES.

3 Results

3.1 Effect of P addition on soluble P and As concentrations

Increasing P addition resulted in increasing soluble P concentrations in both soils (Fig. 1). The increases were, however, much less than proportional to the amounts of added P. They were larger at higher than at lower As pre-treatment levels and also much larger in the sandy loam than in the silty loam. This pattern of differences was already present in the background P concentrations without P addition (P_0). It can be explained by competition between arsenate and phosphate for binding sites.

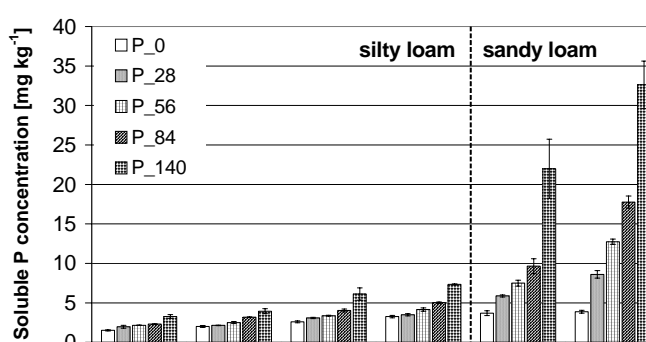


Fig. 1 Soluble P concentrations [mg kg⁻¹] after P treatment

Phosphate addition did have the same effects on As in the two soils (Figure 2). In the sandy loam, all P treatments increased soluble As concentrations. The mobilization effect increased with the rate of P addition. In As_3.1 As concentrations increased from 3.1 to 4.7, 5.5, 6.1 and 9.7 mg As kg⁻¹ in the sequence of P treatments from P_0 to P_140. This corresponds to an increase of 50, 75, 100 and 200 % with respect to P_0. In proportion, the mobilizing effect decreased with increasing level of As pre-treatment. In As_6.9 As concentrations were only doubled in P_140. In the silty loam, on the contrary, low addition of P led to a slight decrease in soluble As. This 'immobilization' effect decreased with increasing rate of P addition and at the highest levels of P applications, no significant difference between soluble P concentrations in P_0 and P_140 were observed. At higher rates of P addition, a mobilizing effect would have occurred also in this soil, if this trend continued.

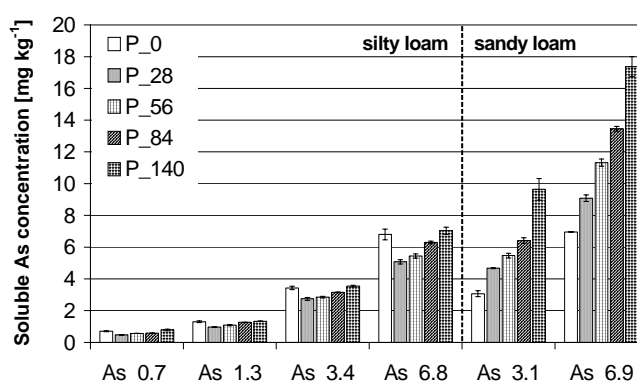


Fig. 2 Soluble As concentrations [mg kg⁻¹] after P treatment

3.2 Arsenic and phosphate accumulation by sunflower

Roots accumulated the highest As concentrations in the sunflower plants. Without P addition, accumulation increased with increasing As pre-treatment level. In the silty loam, roots contained 100 mg As kg⁻¹ in As_0.7 and 340 mg As kg⁻¹ in As_6.8 (Fig. 3). In the

sandy loam, As accumulation by roots was higher. Root As concentrations were 410 mg As kg⁻¹ in As_3.1 and 600 mg As kg⁻¹ in As_6.9.

Phosphate addition increased As accumulation by roots in all As pre-treatment levels and both soils. On the silty loam, P addition resulted in increased As accumulation, although soluble As in the soil was decreased compared to controls at low P treatment levels and were not different from controls at the highest P treatment level. Increases of root As concentrations due to different P treatment levels within the same As pre-treatment were only significant in As_0.7 and As_1.3. In As_3.4 and As_6.8 a doubling of As uptake in roots was found at the lowest rate of P addition, whereas further increases of P had negligible effects relative to the lowest P level. The same pattern of P effects on As accumulation by roots was observed on the silty sand.

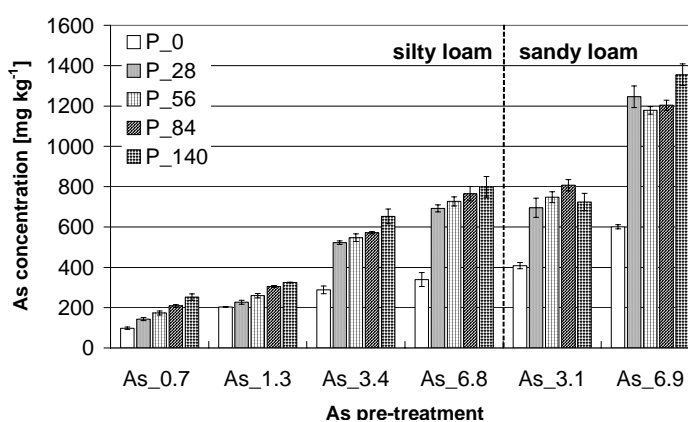


Fig. 3 As accumulation [mg kg⁻¹] in sunflower roots depending on soil and P treatments. Error bars denote the standard error, n=3.

In As_3.1 treatment of the sandy loam As concentrations in the roots were 80 % higher with than without P addition, independently of the applied rate. In As_6.9 As uptake by roots was about 1200 mg As kg⁻¹ for all P rates, which was twice the amount taken up in P_0. Comparing the arsenic uptake by roots on the sandy loam with that at the corresponding As pre-treatment in the silty loam, significantly higher As accumulation was found on the sandy loam. Arsenic accumulation by shoots increased without P addition (P_0) with increasing As pre-treatment level (Fig. 4). In the silty loam, shoots contained 21 mg As kg⁻¹ in As_0.7 and increased to 69 mg As kg⁻¹ in As_6.8. In the sandy loam As accumulation by shoots was higher and As uptake was 87 mg As kg⁻¹, independently on the As pre-treatment level. Arsenic concentrations in the shoots were positively affected by P addition in all As pre-treatment levels, except for As_6.9 (Fig. 4). Phosphate induced increase of As accumulation in the shoots was lower in the silty loam than in the sandy loam. While all P treatments increased As in the shoots on the silty loam, significance was only reached for P_84 in As_1.3 and As_3.4 and for P_140 in As_0.7, As_1.3 and As_3.4. The highest increase of As accumulation by shoots in that soil was observed in As_3.4. Here, As concentrations increased from 54 mg kg⁻¹ in P_0 to 91 and 96 mg As kg⁻¹ in P_84 and P_140, respectively. This corresponds to an increase of 75 %, compared to the controls. In the sandy loam, all rates of P addition led to a significant increase in shoot As at both As pre-treatment levels. Arsenic shoot concentrations increased in As_3.1

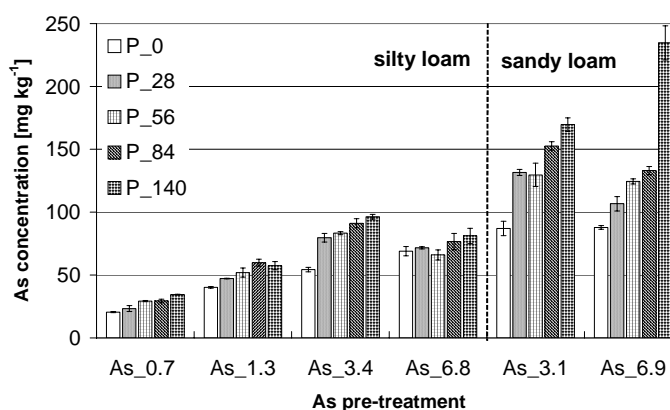


Fig 4. As accumulation [mg kg⁻¹] in sunflower shoots depending on soil and P treatments. Error bars denote the standard error, n=3.

from 94 mg kg⁻¹ (P_0) to 131, 140, 152 and 170 mg As kg⁻¹ in P_28 to P_140 and in As_6.9 from 88 mg kg⁻¹ (P_0) to 235 mg As kg⁻¹ in P_140. Comparing similar As pre-treatment levels, the same accumulation pattern was observed as for the roots; in the sandy loam arsenic accumulation by shoots was twice the amount of As accumulation in the silty loam.

Phosphate uptake by roots was not affected by the As pre-treatment level and not significantly different in the two soils. On the silty loam, a tendency of increased P uptake with increasing P treatment level was observed, but this increase was only significant for P_56, P_84 and P_140 in As_0.7 and As_1.3 and for P_140 in As_3.4 and As_6.8. On the sandy loam, however each P treatment level improved P uptake by roots.

P concentrations of the shoots were significantly higher than corresponding P concentrations of the roots. Increasing As pre-treatment levels decreased P uptake only in As_6.8 and As_6.9, respectively. P addition increased P concentrations of the shoots, but the uptake pattern was different on the two soils. On the silty loam, P addition increased shoot P concentrations but this effect decreased with increasing As pre-treatment level. In the As_0.7, As_1.3 and As_3.4 pots P_140 enhanced P uptake by a factor of 2.5, while the other P treatments doubled P uptake, independently of the P amount added to the pots. In As_6.8 P addition increased P uptake only by 50 %. On the sandy loam, P addition increased P uptake by shoots significantly and already P_56 led to higher P concentrations in the shoots than P_84 and P_140 on the silty loam. As on the silty loam, P uptake increased with increasing P addition and the P uptake patterns in As_3.1 and As_6.9 were very similar.

4 Discussion

Phosphate, a chemical analogue of arsenate, is able to displace sorbed arsenate and thus increase bioavailable (soluble) As in the soil. Our results show, that phosphate addition to As contaminated soil in fact significantly enhanced As accumulation in roots and shoots of sunflower. On the calcareous silty loam this effect occurred although soluble As concentrations were not increased as on the sandy loam, but even decreased at low rates of P addition. This again showed that the soluble concentration of As alone is insufficient to characterize its phytoavailability in the soil. Addition of P did not only increase the accumulation of As in the plants, but also reduced its toxicity. At low As concentrations, i.e. soluble soil concentrations up to 3.4 mg As kg⁻¹ soil, growth was significantly increased. Higher soluble As concentrations led to heavy root damages, significantly decreasing root and shoot growth.

Acknowledgements

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Model forest ecosystems to test heavy metal effects in an interdisciplinary approach "From Cell to Tree"

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Scope

Areas moderately contaminated by heavy metals may be reafforested for landscape improvement and for an environmentally sound and cost effective soil reclamation using indigenous tree species. Before considering genetically modified organisms, more should be known about the potential of our native plants. The interdisciplinary framework 'From Cell to Tree' was designed to study heavy metal availability, uptake and allocation relative to cellular defence reactions and plant growth in near-natural model ecosystems involving interorganismic interactions with different combinations of soil and rain acidity. Preliminary results from the first two and a half years are presented here.

Experimental layout

Thirty two model ecosystems were established in April 2000 with trees (*Picea abies* (L.) Karst., *Populus tremula* L., *Salix viminalis* L.) and understory plants (particularly *Tanacetum vulgare* L., and *Carex sylvatica* Hudson) in 16 large (6 m²) open-top chambers (OTCs). Plants grow under competition on slightly acidic (pH in 0.01M CaCl₂ 6.4) topsoil (0-15cm) and natural forest subsoil (15 - 95cm). Below is a 50cm drainage layer of quartz sand. Each chamber is split in two compartments (two model ecosystems), one with an acidic loamy sand pH 4.2, the other with a calcareous sandy loam pH 7.4 subsoil. The OTCs have automatically closing roofs to exclude natural precipitation. Four treatments are applied: control with rain-like buffered solution pH 5.5 (CO) applied by means of sprinklers, heavy metals (HM), acid rain pH 3.5 (AR) established with HCl, and their combination (HMAR), with four replicates each. The treatment with HM (filter dust from a heavy metal smelter) was applied manually in the topsoil resulting in Zn 2700mg/kg, Cu 385mg/kg, Pb 63mg/kg and Cd 10mg/kg, to represent a rough average of a moderately contaminated soil. The experiment is repeated in the field (field plots = FPs) with acidic subsoil and irrigation only during dry periods. A pathogen-saprophyte-system (*Armillaria ostoyae* und *A. cepistipes*) was inoculated between the spruce trees, each in one separate quarter per soil compartment.

Above ground biomass is determined yearly at the end of the vegetation period by coppicing the deciduous trees and estimating the tree biomass for spruce by harvested branches. Below ground and above ground samples are analysed in 23 projects. Results here refer to HM content (ICP-AES, aliquots out of the mixed whole plant foliage and wood mass), pH (0.01M CaCl₂), HM speciation in the topsoil (sequential extraction according to Zeien 1995), water household (8 tensiometers per OTC), cellular reactions (Günthardt-Goerg & Vollenweider 2003), visible leaf symptoms and interorganismic

influences (bacterial community analysed by denaturing gradient gel electrophoresis of the 16s rDNA with eubacterial primers, insects and fungi).

Results

At the beginning of the experiment, the topsoil acidity was raised by 0.7 pH-units with the addition of the alkaline heavy metal dust. Topsoil pH decreased with time, AR and HM (pH HMAR > HM > AR > CO), resulting in small differences by autumn 2001.

The total contents of the heavy metals in October 2001 were 2753 ± 679 mg/kg Zn, 570 ± 114 mg/kg Cu, 110 ± 20 mg/kg Pb, 7 ± 2 mg/kg Cd. The respective values for the uncontaminated soil were 97 ± 1 mg/kg Zn, 28 ± 4 mg/kg Cu, 37 ± 3 mg/kg Pb and 0.1 mg/kg Cd. Initially most of the Zn (83 %) was in easily available fractions. These fractions decreased to 63% after 18 months. The strongly bound fractions increased but never reached their level in the uncontaminated soil. The Zn-oxide from the filter dust was rapidly dissolved but the Zn remained relatively weakly bound in the soil. Cu shows a similar behaviour. However, an increase of the organic fraction was observed with time.

At the end of the first vegetation period (autumn 2000) the model ecosystem biomass was dominated by the understory plants particularly in the FPs. In the OTCs the trees profited from better conditions due to the partly protected situation and confined therefore the understory plant growth. In the OTCs the HM treatment led to a small but significant decrease of understory plants and spruce, but an increase in deciduous tree biomass production, particularly on acidic subsoil. In the FPs plant competition was different. Here the understory plant and deciduous tree biomass showed a small but significant increase, whereas that of spruce decreased in the HM treated model ecosystems (Fig.1).

Among the deciduous trees, the HM treatment induced visible leaf symptoms in poplar, namely light-green stipples on the adaxial leaf side, which during the vegetation season developed into leaf edge necroses. The slower growing young birch and sycamore trees only developed clear visible leaf symptoms in the third year (2002). Most consistent and specific microscopical symptom of HM stress in symptomatic leaves was the extended necrosis of lower epidermis, which could be the determining factor for the observed leaf edge necroses. Cell wall reactions were observed along the pathway taken by the HM from the veins through the leaf blade. New methods, developed for visualisation of Zn in leaf tissues by light microscopy, may confirm that the cell injury corresponds to Zn allocation.

In all species HM concentrations were significantly increased for HM treatment vs. CO, in the tree leaves/needles as well as in the wood (on average a third of the leaf conc.) with large interspecific differences. On a model ecosystem level (sum of all plants per 3m² compartment), Zn extraction in the first year (2000), in above ground plant parts (as a product of Zn concentration and biomass production) reached a maximum of 1g in the FPs HM treatment (Fig. 2). As expected in the first year of a reafforested system, the light demanding understory plants were dominating the extraction process (Fig.2). In the second year (2001) the trees have taken root, which disfavours the growth of the understory plants. Compared to Zn, Cu was less mobile in the topsoil and less extracted by the plants in the first year (2000). Given a roughly initial Zn:Cu content of 7:1 in the HM treatment, the resulting uptake of the model ecosystems was about 20:1 respectively. In contrast to the Cd uptake into deciduous tree foliage up to a concentration of 10 mg/kg dry mass, the Cd uptake in spruce and understory plants was often below the detection limit of 0.6 mg/kg. Work has begun on analysing the HM content of plants harvested in autumn 2001.

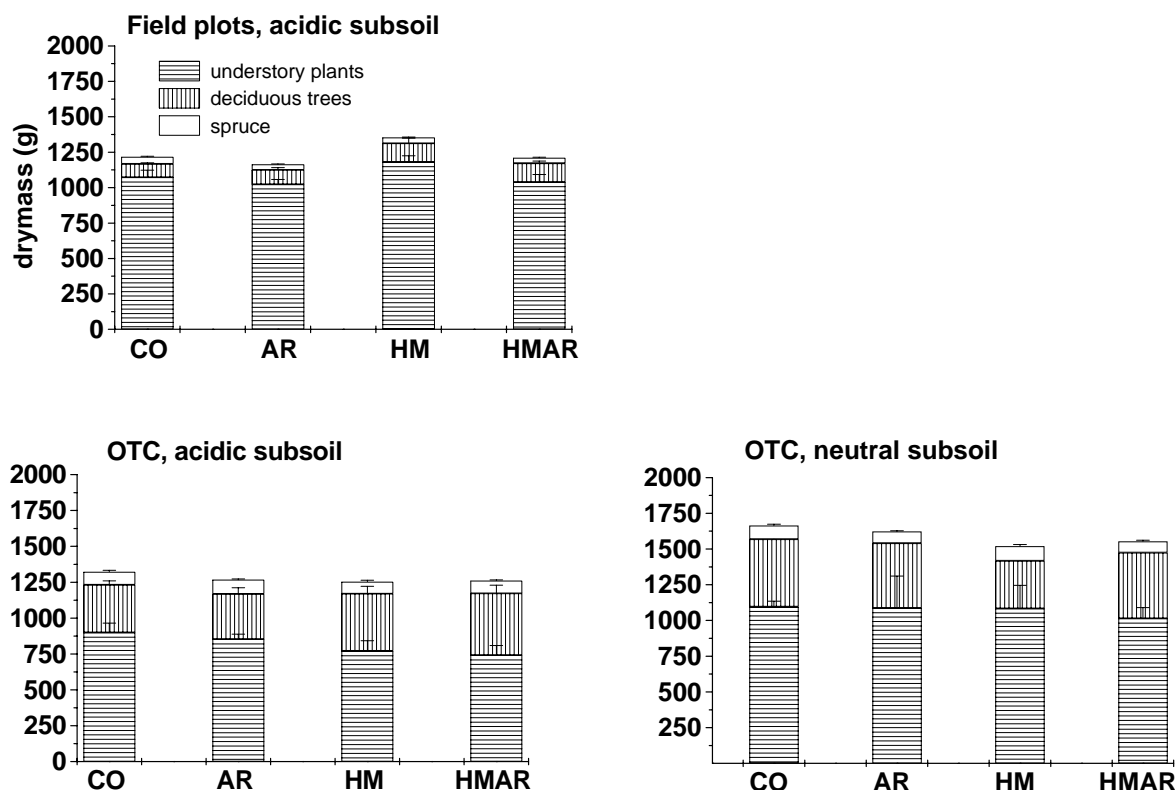


Fig. 1: Above ground biomass in model ecosystems (first year, means + SE). ANOVA $P > F$ 0.0001 for HM in FPs and OTCs both soil compartments, each for understory plants, deciduous trees and spruce. Understory plants in OTCs < FPs, trees in OTCs > FPs.

The effect of the AR treatment on HM uptake and biomass was negligible (although sometimes significant on a single species level). In contrast, the subsoil acidity modified HM uptake and growth parameters. Although in 2000 the aboveground biomass was reduced in the acidic subsoil type (Fig. 1), more Zn was taken up particularly in the FPs (by increased Zn concentration) compared to the calcareous subsoil type (Fig.2). The nutrient rich calcareous subsoil type may have favoured the trees root growth in deeper soil layers. The latter supposition is supported by the measurements during 2001 indicating significantly lower water potentials in the calcareous than in the acidic subsoil.

Aside from plant competition, the following interorganismic effects may become more important towards the end of the experiment. The bacterial community showed clearly different microbial patterns between the CO and the HM contaminated topsoil. The selected pathogen-saprophyte-system was supposed to infect the spruce roots and kill the trees after a four years period. In the third year of the experiment from 96 spruce trees in OTC five trees died from pathogen infections. The pathogen was therefore shown to be active. However with only a few dead trees (2 in AR, 2 CO, 1 HMAR) we cannot yet substantiate, if the HM was more injurious to the pathogen than to the trees, or if the AR was increasing infection. HM indirectly influenced spontaneous insect infection (more aphids were counted on willow trees in the HM treatments). The proposed exposure of insects on the plants will show if subjecting the plants to the HM treatment favours insect development.

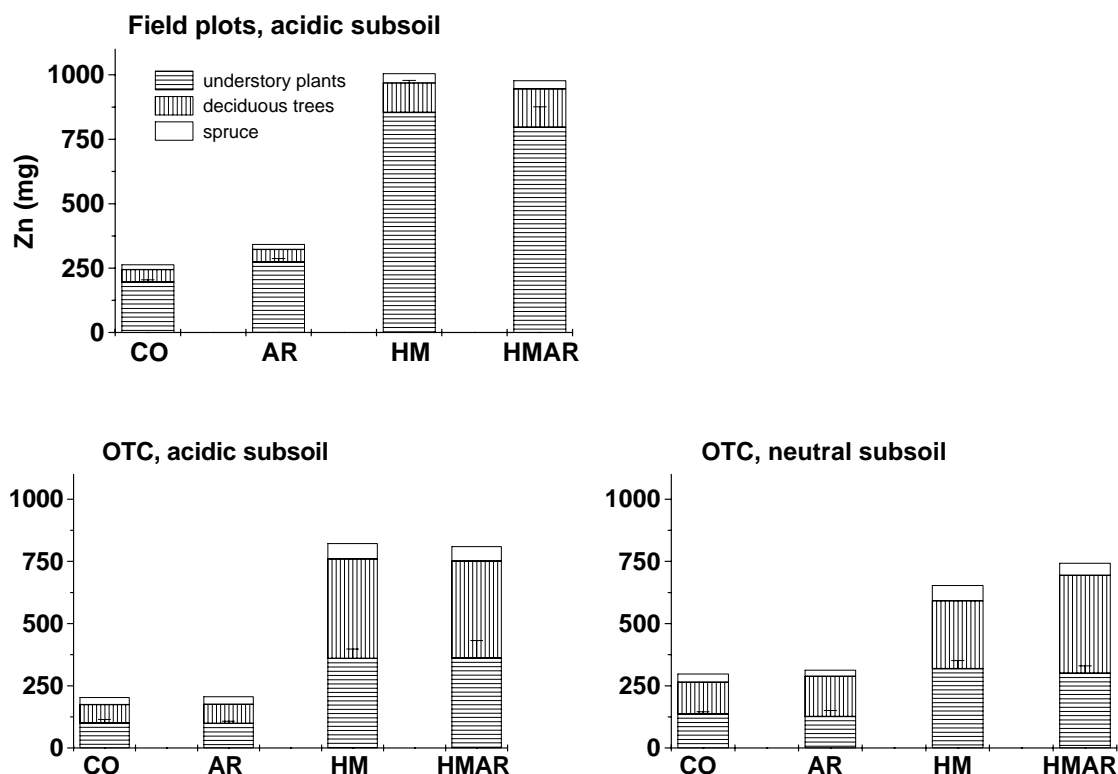


Fig. 2: Zn in aboveground biomass in model ecosystems (first year, means). ANOVA $Pr > F$ 0.0001 for HM in FPs and OTCs, both soil compartments, each for understory plants, deciduous trees and spruce. Understory plants in OTCs < FPs, trees in OTCs > FPs. Deciduous trees: for subsoil acidity in OTCs $Pr > F$ 0.05.

Conclusion

The results show, that communities of trees, understory plants and associated organisms living together in different edaphic conditions greatly modify HM availability, uptake and effects on the vegetation. In contrast to observations in nature (without control treatment) and experiments with potted plants (without competition and interorganismic influences) a range of different contributing factors will be detected in this interdisciplinary approach using near-natural model ecosystems with an experimental layout allowing a scientifically sound interpretation.

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“Role of Bio-Available Metals in Contaminated Soils to Formulate Ordinance on Risk Assessment and to Develop Risk Management Techniques

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In the past, mining manufacturing, urban and agricultural activities have all contributed to extensive soil contamination. A particular problem is the widespread pollution of agricultural and other cultivated land by contaminants e.g. Heavy metals. Elevated heavy metal concentration in soil may have toxic effects on soil organisms, impair plant growth and by entering the food chain, threaten human and animal health. Depending on soil properties heavy metals may also be leached into ground waters.

The soil system is mainly composed of three phases Gas, Solid and Liquid and these three phases constitute around, 25%, 50% and 25% of the total soil volume, respectively.

The metal concentration in gaseous phase of soil is insignificant except a few metals such as Hg. Total metal content in solid phase expresses the metal capacity factor of a given soil.

On the contrary, metal concentration in Soil Solution (simulated with neutral salt extracting solutions such as NaNO₃ or just water) expresses the metal intensity factor, which is independent of soils and soil properties.

With the help of linear equation, it is shown that in different soils Metal Transfer factor (TF) are not constant. The TF is calculated by taking the ratio between the Total Metal Concentration in Plants and the Total Metal Concentration in Soil. The total metal content is an excellent criterion to define the extent of metal build up or contamination (degree of contamination) in soil. It provides an estimate of the extent or degree of saturation of total cation exchange capacity (CEC) of soil colloids through given metal or metals. Further, it is very difficult if not impossible to clarify the usually observed differences in the metal concentration in plants grown in different soils alone with the help of total metal content in soil. The total metal content does not express in any way the changes either in soil pH or in binding capacity of soil that could occur due acidification or addition of binding materials.

On the contrary, there are theoretical and experimental evidences that the **Metal Concentration in Soils Solution** for some metals and metalloids can give approximately constant Transfer Factor (TF) for different soils. There are theoretical reasons for experimental finding. Firstly metal concentration in soil solution is a mirror picture of soil properties. Secondly, the metal intensity quantity calculated from the soil solution concentration ranges usually between 2-3 % of the total metal concentration in soil, which corroborates with the general plant uptake data ranging between 2-5%. Thirdly, it has been established that soil solution concentration in soil predicts the effect of metal on growth and activities of micro-organisms significantly better than the total metal concentration. Thus, the metal concentration in Soil Solution is only an analytical tool for prediction of ecological and eco-toxicological problems in relation to soil metal concentration. (Fig 1)

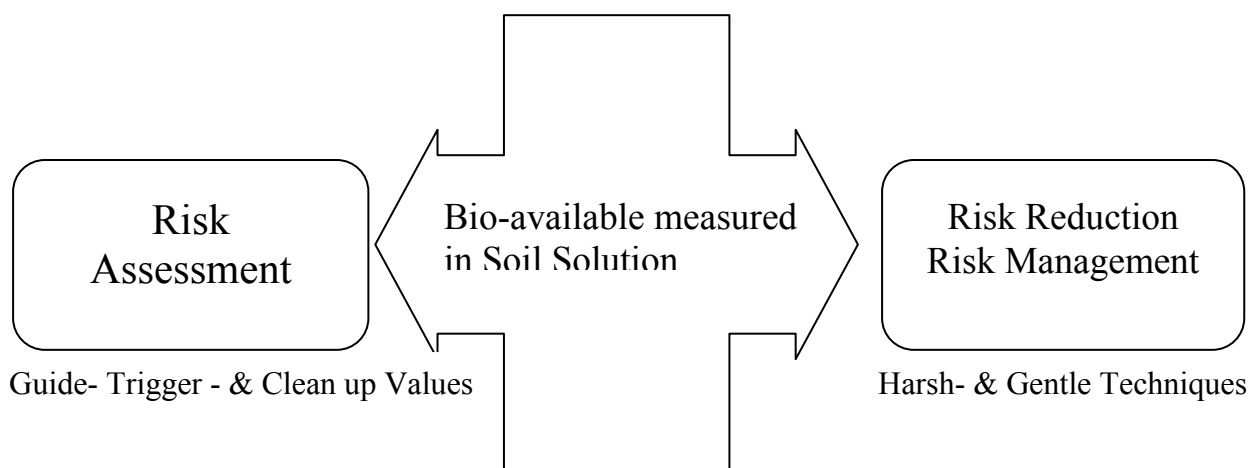


Fig. 1 Relationship among Bio-Available Metal concentration in Soil and Risk Assessment and Risk Reduction

1 Role of Soluble Metal Concentration in Soil for Risk Assessment

The decision to remediate a site or not is made on the basis of the degree of the risk posed by a further spread of heavy metals. In Swiss Ordinance on Soil Impacts (VBBo, 1998) a scheme has been accepted. This schema's assess the ecological hazard with three metal concentrations in soils: 1. Tolerable levels or "Guide values" and 2. Trigger value 3. Harmful level or «Clean up values».

In soils exceeding "Guide values", the long-term multifunctionality of the soil (e.g. soil quality and soil fertility) is no longer assured. In this case, the source of heavy metal must be identified and later on appropriate measures to reduce the emissions of metals at source must be initiated.

Soils with heavy metal concentrations above the clean up values are a hazard and such situation must be tackled with quick, efficient and if needed stringent risk reduction measures need to be initiated.

2 Role of Soluble Metal Concentration in soil for Risk Reduction

In order to restore contaminated soil as a medium suited for plant production, the following two categories of Risk Reduction techniques are used

2. 1 Harsh Risk-Reduction techniques

Harsh Risk reduction Techniques, which are generally *ex-situ* soil washing incineration, thermal treatment, electro-migration, vetrification, disposal of contaminated layers destroy the ecological soil quality and are also in general too expensive but for agricultural land and, thus could be applied on agricultural land in cases of extreme pollution.

2. 2 Gentle Risk Reduction Techniques.

The "Gentle Remediation techniques (*in-site*) remediation of heavy metal polluted soils by a combination of bio-chemical treatments. These techniques are named as. For large areas of contaminated land through diffuse sources, these techniques are economically and ecologically reasonable alternatives. The gentle remediation strategies will be

specific for each site. The choice of the principal category is mainly made on different site factors such pH, soil types, nature and type of pollutants, and clean up goals.

There are principally two groups of gentle Risk reduction techniques for contaminated soils: Usually, only the mobile fractions of heavy metals in soil can be directly influenced by any of the categories of gentle remediation techniques. The equilibrium between soluble and insoluble fractions may either be shifted toward more insoluble or toward more soluble heavy metals for easy and quick removal through plants.

2.2.1 Stabilisation (immobilisation)

The strategy aims to reduce the immediate risk of uncontrolled heavy metal transfer to the to the biosphere or to ground water. To attains this aim, the heavy metal fraction available to plants in the soil has to be reduced, which means that heavy metals are immobilised and the equilibrium between soluble and insoluble fraction is intentionally shifted toward more insoluble forms either by increasing soil pH or by increasing the binding capacity of the soil. The plants mediate a special form of stabilization. This requires heavy metal-tolerant living plants that reduce the mobility of heavy metals in soil by uptake and storage in the roots. Nevertheless, the heavy metals remain in the soil.

2.2.2 Decontamination by phytoextraction

Phyto-extraction, as proposed by a number of authors, is a “gentle” in situ decontamination technique making use of the natural ability to extract heavy metals from soils. To remove sufficient amounts of heavy metals by this technique, plants have to be highly efficient in metal uptake and translocation into their aboveground parts. Plants, known as hyper-accumulators, take up extraordinary quantities of metals. The fact is that the dry matter yield per hectare of these plants is usually low limiting the total amount of heavy metals that can be extracted within an economically affordable time period. On the other hand, fast growing crop plants such as tobacco, maize having high biomass but have low metal uptake.

To overcome the limitations of Phyto-extraction several approaches have been undertaken such as development of crossbreed of hyper accumulator that produce high biomass or new transgenic-plants or improving metal uptake capabilities of high biomass plants by somaclonal variation and selection techniques.

A different approach is to increase the metal concentration in soil solution to increase the metal phytoavailability. In order to achieve successful and time bound phyto-extraction, two principle ways have been proposed and investigated: artificial soil acidification and by solubilization through ligands addition in particular chelators. The results of several authors substantiate the hypothesis that increasing metal solubility by applying chelates to the soil can enhance metal removal from soil by common plants.

The possibility of a dual use of remediation plants - economically as crops and to decontaminate the soil – might make longer time periods for decontamination more acceptable. The investigators have found that the edible and economical part of crop plants does not accumulate severe amounts of heavy metals. On the contrary, the metal concentration in vegetative parts is recorded below the critical concentration set for any compartment of food chain. It can also be suggested that even two biomass rich crops can be tried in an agricultural year. This will again reduce to half the expected time. This information is very important and useful in risk reduction of contaminated soil and sites.

The recent studies in lab and in field suggest that ligand-assisted extraction-extraction for few target metals are promising. It suggests the use of NTA. There are still chances of finding more

efficient and biodegradable ligands so as to achieve the goal of decontamination of contaminated soils in an economically viable time period.

It must be mentioned that with phytoremediation there are still problems and bottlenecks. It is difficult to suggest on one hand a field applicable technique and on the other hand an efficient and economically viable technique. However, the phytoremediation is not utopia but with constant sincere efforts it can be an economical viable alternative to other harsh in-situ or ex- situ techniques.

Which influence have heavy metal pollution, soil type and acidic irrigation on physiological activities and biomass production on a young forest ecosystem?

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Abstract

During the last 60 years, an increasing area has been polluted with heavy metals through agricultural and industrial activities. More knowledge on heavy metal uptake and physiological influence on the vegetation under near-natural conditions is needed to develop solutions for stabilisation, decontamination and the re-use of these sites.

The framework "From cell to tree" is designed as an interdisciplinary experiment using near-natural model ecosystems to study the influence of heavy metal polluted topsoil on plants under competition with the modifying influences of subsoil and precipitation acidity. In 16 large (6 m²) open-top chambers (OTCs) model ecosystems were established in 2000 with different tree species (*Salix viminalis*, *Populus tremula*, *Betula pendula*, *Picea abies*). Understory was formed by *Tanacetum vulgare*, *Carex sylvatica*, *Fagus sylvatica* & *Picea abies* from seeds, and additionally in 2001 *Quercus pubescens* from seeds & *Allium ursinum*). The OTCs (with roofs which close automatically to excluding rain), are arranged in a Latin square and receive four treatments (control with rain-like buffered irrigation pH 5.5, heavy metals, acidic irrigation pH 3.5 established with HCl, and their combination) with four replicates each. Each chamber is split in an acidic pH 4.2 and a neutral pH 7.4 (15cm-150cm) natural subsoil compartment. The treatment with heavy metal dust was applied manually in the topsoil layer (15 cm, pH 6.4) resulting in Zn 2700mg/kg, Cu 385mg/kg, Pb 63mg/kg and Cd 10mg/kg which represent a moderately contaminated soil. Transpiration and stomatal conductance of *P. tremula* and *S. viminalis* were measured on four leaves per plant with a LiCor-6400 infrared gas analyser (open system) under ambient light conditions during June 2001 between 9am and 4pm with a given CO₂ concentration of 400µmol/mol and a flow rate through the cuvette between 350µmol/s and 500µmol/s. Annual (2000 and 2001) herb and tree foliage and wood mass was determined (estimated for *P. abies*) by coppicing the deciduous trees and harvesting branches from the coniferous species. Heavy metal (Cd, Cu, Zn, Pb) concentrations in leaves/twigs, wood and flowers (only *T. vulgare*) were analysed in aliquots taken from the total leaf and wood mass per tree or branch (analyses from 2001 are in process).

Biomass produced by deciduous trees and understory plants in 2001 was increased on calcareous vs. acidic subsoil type, in contrast to *P. abies* (no effect). On the calcareous subsoil type, the deciduous trees showed a significant biomass reduction caused by the heavy metal treatment. The transpiration of the trees, estimated on a surface base (soil compartment = 3 m²), was lower on the acidic subsoil compartment due to smaller leaves and a reduced foliage mass vs. the calcareous soil compartment. On a single tree and species base *S. viminalis* had the highest transpiration rate, whereas *P. abies* showed a significant decline under heavy metal stress. Stomatal conductance of *S. viminalis* was increased on the acidic subsoil compartment and by the heavy metal treatment.

Further analysis of the harvest data 2002 will show how the model ecosystems develop under heavy metal stress over time.

Phytoremediation: using Bioavailability to improve estimates of uptake rates by crops

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Uptake rates of metals by crops are often crucial (next to biomass production rates) to obtain realistic estimates of duration times of phytoremediation projects. Currently uptake rates are often estimated from laboratory experiments or taken from literature. In both cases, erroneous estimates can be obtained which hampers the applicability of the technology as such.

In recent years progress has been made in our understanding of the processes that are in control of the uptake of metals by crops. Although the ultimate uptake rate by crops is highly plant specific and controlled by plant physiology, several observations have been made that allow us to link aspects of soil chemistry to uptake rates by crops:

1. the plant metal content at harvest of metals like Cd, Zn, Ni and to a lesser extend As, Pb and Cu by various crops is related to the dissolved metal concentration (for Zn, Cd and Ni) or the free metal activity in the soil solution for metals like Cu and Pb;
2. the concentration and or free metal activity, also often referred to as the *actual* bioavailable fraction (in contrast to the potential available fraction, can be assessed by either an extraction with a specific dilute salt solution (e.g. CaCl₂) or calculated by models (either soil solution speciation models or solid phase adsorption models). For metals like Cd, Cu, Pb and Zn, reasonable to good models are available to calculate either the concentration or the free metal ion activity;
3. the combination of 1 and 2 can be used to derive practical soil to plant uptake models. Although these models *do not* describe what actually happens at the root surface and in the interior of the plant (since these are not plant physiological models) they enable the user to obtain rather accurate estimate of uptake rates of metals from soils.

During phytoremediation, however, the soil metal content will decrease due to removal of plant biomass, and the observed relation between the soil and the metal content in the crop will change as well. Since actual measurements of changes in the soil metal content due to crop uptake are lacking (this would require a rather long-lasting experiment.....), an alternative method was tested aimed at the derivation of a model that links uptake rates by selected crop species to a range in levels of the actual (bio)availability (here solubility) in soils.

Description of experimental approach

The aim was to study whether uptake rates by crops can be described by a single model concept within the range of soil conditions that occur during the removal of metals from the soil. To determine the relationship between the actual bioavailable fraction (dissolved concentration or activity) and uptake by crops, a pot experiment was conducted. To obtain the desired range in soil heavy metal levels - comparable to those at various stages of phytoremediation- two soils (heavily polluted soil from Katowice, Poland, and Milano, Italy) were “diluted” with clean soil (with approx. the same pH,

organic matter content and clay content) in different mixing ratios and pre-equilibrated for six months. Plants were grown on the soil mixtures under greenhouse conditions heavy metal concentrations were determined in the soil, the soil solution and in the harvested crops.

Results

In figure 1, the observed relation between the dissolved metal concentration and the heavy metal content in the crop is shown. In total crops were harvested three times (together with analysis of the corresponding soil solution obtained by Rhizon Soil Solution Samplers). The data are average values of the three rounds (levels of the standard error of the triplicates are also shown), no significant differences in soil solution concentration and plant metal content were observed between sampling events. The solid lines indicate predicted plant metal levels based on the dissolved concentration (obtained by regression)

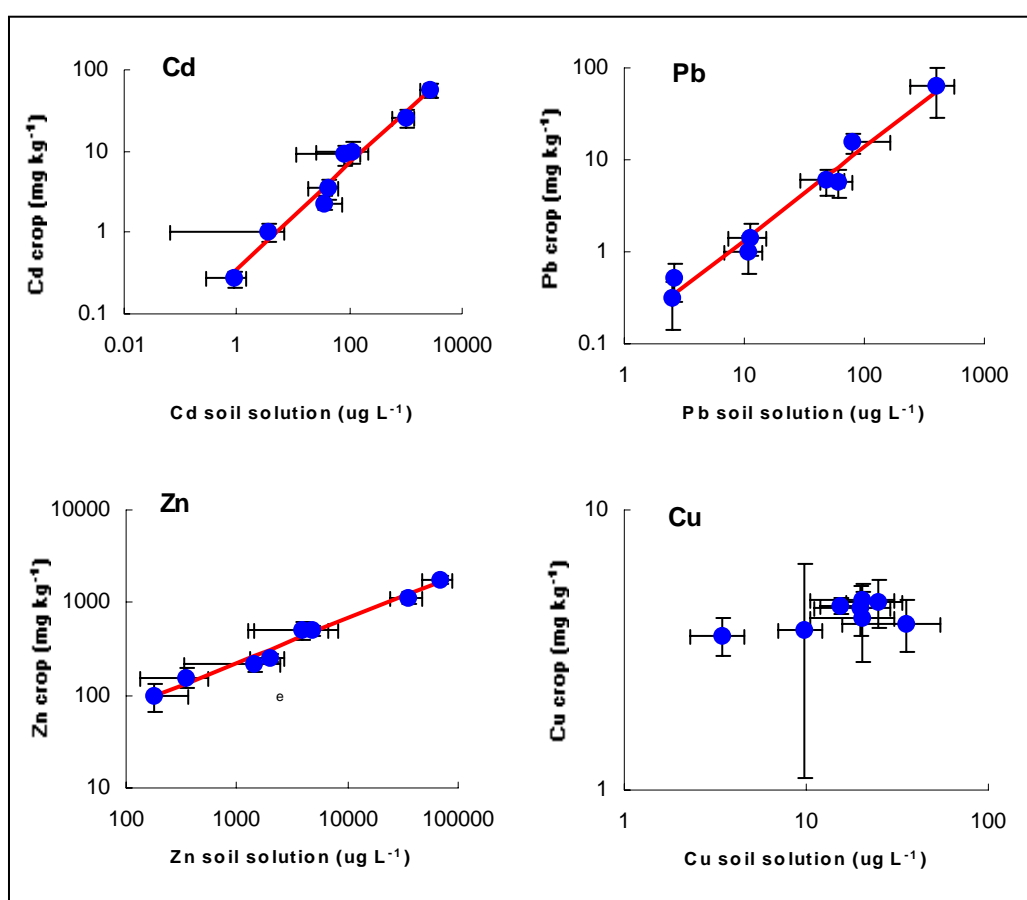


Figure 1. Relation between measured concentration in the soil solution of Cd, Cu, Pb and Zn and the plant metal content in *Lupinus Alba*. Error bars indicate standard errors of the measured concentrations and plant metal content.

For Cd, Pb and Zn log - log relationships between the measured solution concentration and the plant metal content were observed. For Cu and As (data not shown) this appeared not to be the case. Whether this was due to the limited range in the measured plant metal content (for Cu) or highly variable dissolved metal concentrations (for As) remains yet unclear. The result for Pb was somewhat surprising since it can be assumed

that a large part of the dissolved Pb in solution is bound to dissolved organic carbon (DOC). Levels of DOC ranged from 9 to 106 (median value 58) mg C L⁻¹ at a pH level of 5.7 ± 0.4. This results suggests that either organically bound DOC is plant available (for *Lupinus Alba* that is) or that the free metal ion activity varies accordingly as well. The relation between the dissolved concentration and the plant metal content can be used to obtain more accurate estimates of the duration of phytoextraction times. In figure 2 the schematic calculation of the changes in the soil metal content are shown.

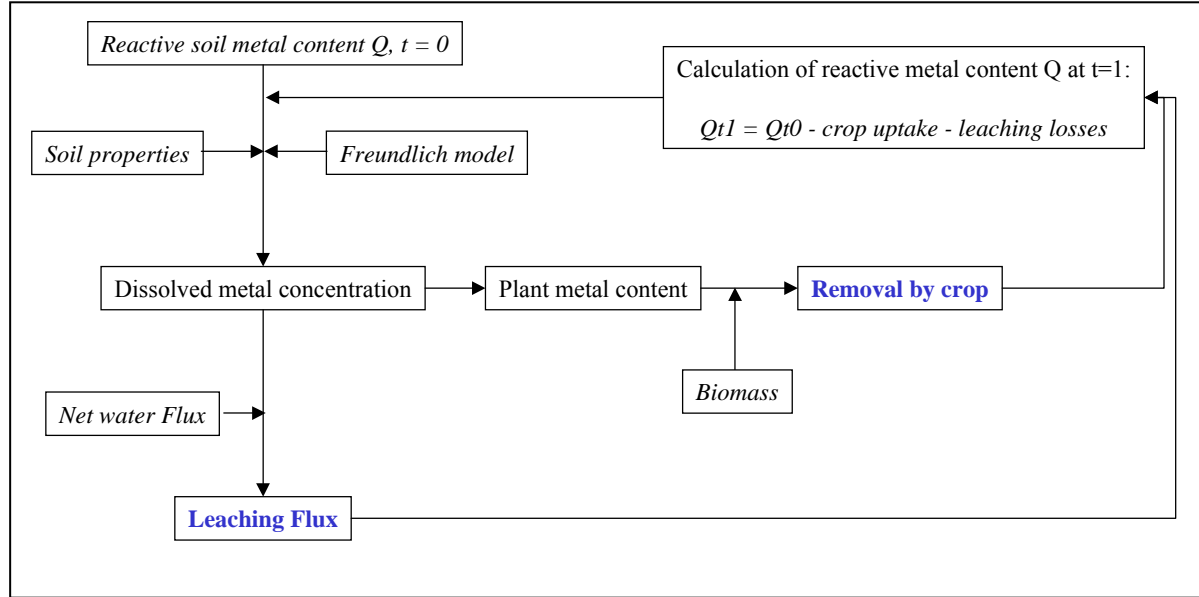


Figure 2. Schematic representation of the calculation of changes in the soil metal content resulting from plant uptake and leaching. Additional sources of metals (atmospheric deposition, manure, fertiliser can be included if relevant. Input data are printed italic, fluxes used to calculate the change in the reactive metal content are marked blue.

However, to calculate uptake rates in a certain soil, an estimate of the dissolved metal concentration is needed from which the uptake can be calculated. Here, an extended Freundlich model is used that enables the user to calculate the dissolved concentration from soil pH, organic matter content, clay content and the reactive metal content according to:

$$\log[Me_{\text{solution}}] = \alpha + \beta \cdot pH + \gamma \cdot \log[OM] + \delta \cdot \log[\text{clay}] + \varepsilon \cdot \log[Me_{\text{reactive soil}}] \quad [1]$$

From this, the plant metal concentration can be estimated (using data shown in figure 1) from any given starting point (ie metal levels in soil):

$$\log[Me_{\text{plant}}] = \omega + \xi \cdot \log[Me_{\text{solution}}] \quad [2]$$

It should be noted that the values for ω and ξ are highly plant specific and can vary considerably. However, with the approach as described here, the parameters can be obtained for a range of crops in a relatively short period.

The duration of the phytoextraction period now can be calculated using the following target parameters: initial metal content, target metal content, annual biomass production, and depth of layer to be remediated. Of course eq. 2 can only be applied for soil - crop combinations for which the parameters have been established. In figure 3, a model calculation of Zn extraction from soil is shown using the concept from figure 2 and data from the pot experiment together with the Freundlich isotherm for Zinc. In table 1 the effect of soil pH on the net leaching loss, crop uptake and final Zn content of the soil after 50 years ($Q_{t0} = 2000 \text{ mg kg}^{-1}$)

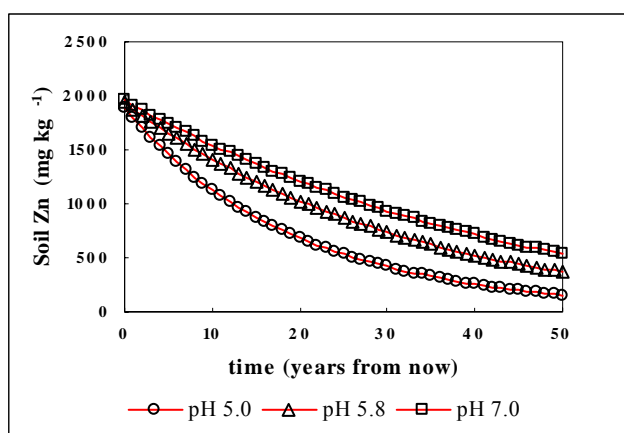


Figure 3. Changes in the soil Zn content during 50 years of phytoextraction (leaching included)

Table 1. Cumulative plant uptake, leaching losses and heavy metal content after 50 yrs (data from curves shown in figure 3)

	<i>pH 0 - 60 cm</i>		
	5.0	5.8	7.0
Plant uptake (kg ha^{-1})	4486	5858	6615
Leaching loss 0-40 cm (kg ha^{-1})	4380	1991	421
Leaching loss bottom of profile (kg ha^{-1})	1257	659	428
Reactive metal content after 50 yrs in the 0 - 40 cm layer (mg kg^{-1})	154	366	536

Conclusions

Although phytoremediation needs further study in order to fine-tune all fluxes and parameters to obtain reliable estimates of the duration under field conditions, the link between soil chemistry and plant uptake as illustrated in this paper will increase our ability to judge whether or not this technology can serve as an appropriate alternative to more conventional technique.

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Modelling carbon turnover and cadmium bioavailability and leaching in sludge-amended soil

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Data obtained from a long-term field trial (41 years) on the effects of sewage sludge amendments on soil physical and chemical properties and cadmium distribution in the profile, and bioavailability (Bergkvist et al., these proceedings) has been used to calibrate and validate a simple deterministic model of organic carbon turnover and cadmium fate and mobility. The conceptual model (called SLAM) includes four carbon pools (humified and fresh organic material derived from crop residues and sludge respectively) and four cadmium pools in soil: the free ion in solution, DOC-adsorbed Cd in solution, a matrix-adsorbed but bioavailable pool, and an adsorbed ‘occluded’ pool in the matrix that is not available for plant uptake. Cd transport is simulated with the convection-dispersion equation, assuming steady water flow, accounting for DOC-facilitated transport. The DOC concentration is estimated as a function of the mass fractions of fresh and humified organic material. Adsorption to the bioavailable matrix pool is calculated using an extended Freundlich isotherm, accounting for pH, and the differing adsorption affinities of six components: mineral matter derived from sludge, fresh and humified organic matter derived from crop residues and sludge respectively, and an indigenous soil mineral component. First-order exchange between the two matrix-adsorbed pools is assumed. Crop uptake is calculated as a first-order function of the bioavailable pool concentration and the crop biomass production. Carbon turnover is modelled using an extended version of the ICBM model (Andrén & Kätterer, 1997), which assumes first-order kinetics for decomposition of fresh and humified material, with a humification constant describing the efficiency of microbial respiration, and a lumped ‘climate factor’ controlling C flows.

The model was parameterised for the long-term field experiment, using a combination of direct measurements, calibration, and literature estimates where data was missing. The model adequately represented the depth distributions of organic carbon, and HNO_3 and EDTA-extractable Cd contents after 41 years of sewage sludge application to the soil. Leaching losses were negligible in both measurements and modelling, despite the inclusion of DOC-facilitated transport in the simulations. A sensitivity analysis is performed to identify critical parameters for predictions of bioavailability, while scenario simulations predict trends in Cd availability following cessation of sludge applications. Long-term effects of sludge applications (100+ years) are investigated in Monte Carlo simulations, utilizing information contained in two national databases to parameterize the model, i.) a soil quality monitoring database containing (besides other data) information for topsoil (n=3146) and subsoil (n=1776) on soil pH, organic carbon and Cd contents, and ii.) a soil physical database (n=168) containing information on clay content, bulk density, field capacity water content, and organic matter content.

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Long-term effects of sewage sludge applications on cadmium bioavailability, distribution and leaching in an arable field soil.

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Sewage sludge represents a potentially valuable source of nutrients and organic matter to arable land, but its use may be limited due to the presence of toxic heavy metals, especially the more mobile elements such as cadmium. Studies have shown that sludge application to arable soils results in an increased bioavailability and crop uptake of cadmium (McGrath et al. 2000). However, there is controversy over the likely long-term effects of sludge-applications on metal uptake, especially with regard to the role of organic matter decomposition following cessation of applications (Chang et al. 1997). With respect to leaching risks, most field studies have shown only a limited mobility of the added cadmium below the plough depth (McGrath, 1985), although some shorter-term studies have indicated a potential for preferential and accelerated migration of DOC-complexed cadmium in soil macropores (Richards et al., 1998). Questions concerning the long-term effects (>40 years) of sludge application on cadmium mobility and availability remain unresolved largely because experimental information is still lacking. The objective of this study was to investigate the long-term effects of sludge applications on cadmium bioavailability and mobility in soil. A field trial, ongoing since 1956, on the effects of organic and inorganic fertilizers on soil chemistry and fertility, was utilized for this purpose. Measurements were made in 1997 in plots from a control treatment (calcium nitrate fertilizer) and a treatment that has received sewage sludge biennially for 41 years. The data includes distributions of cadmium (HNO_3 and EDTA-extractable), soil organic carbon and DOC concentrations, crop Cd concentrations, and results of batch adsorption experiments to investigate the influence on sludge on Cd solubility.

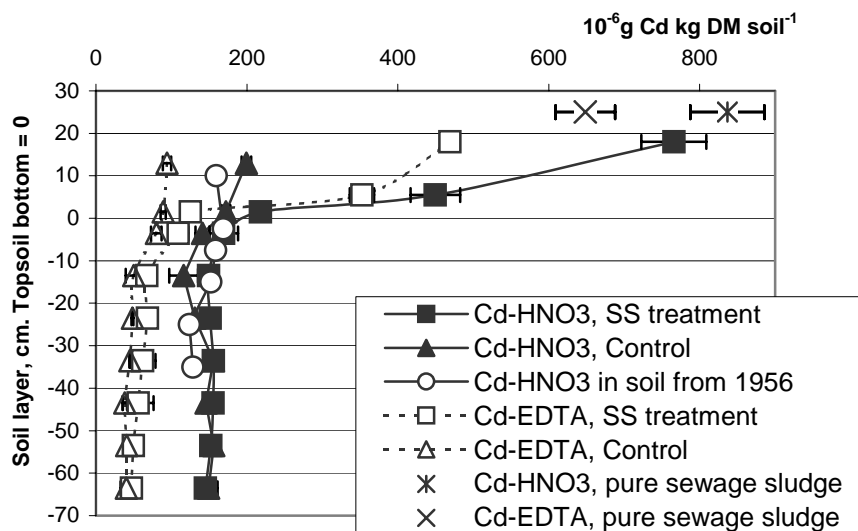


Fig.1 Distributions of HNO_3 and EDTA extractable Cd in control and sewage sludge treatments

Calculated mass balances showed that 91% of the accumulated Cd loading was retained in the topsoil after 41 years, while no influence of the Cd loadings was detected deeper than 17 cm below the base of the topsoil (see Fig.1), even though enhanced DOC concentrations were found throughout the profile of sewage sludge treated plots. Crop Cd

concentrations were highly variable and no significant trends with time could be discerned. However, compared to the control, the Cd solubility measured in batch experiments was 20 times larger in the sludge-amended topsoil, due to lower pH, and the straw Cd concentration was also c. 2 times larger on average. Batch experiments confirmed that soil pH was the dominant factor controlling Cd adsorption at this site. Effective K_d values for cadmium in the sludge treatment were smaller at any given pH, despite much larger organic carbon contents compared to the control (Fig. 2), due to the presence of DOC.

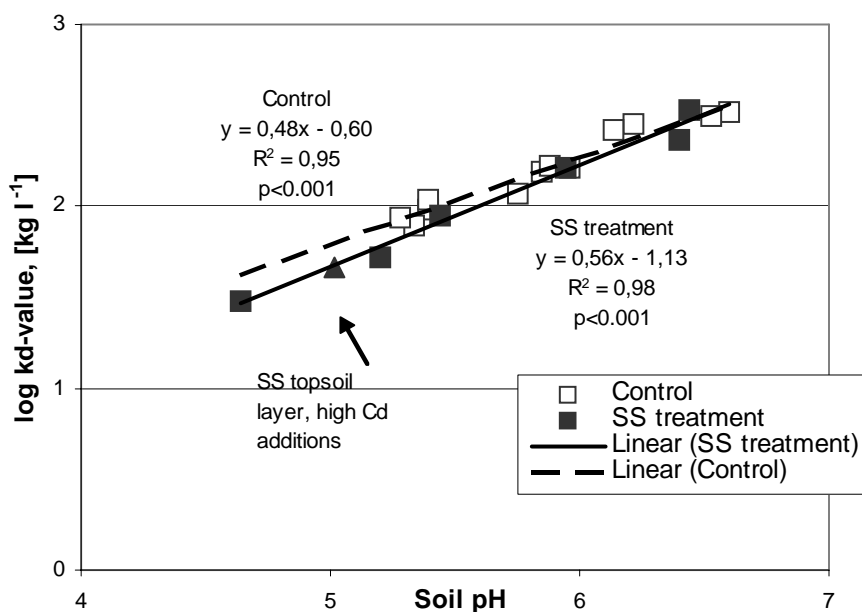


Fig. 2. Adsorption k_d values as a function of soil pH

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**FORMATION AND STABILITY OF RESIDUES DERIVED FROM
MICROBIAL DEGRADATION OF ORGANIC POLLUTANTS: - bound residues
versus refractory soil organic matter**

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Microbial degradation of organic compounds in soil is generally accompanied by the distribution of carbon to mineralisation products (CO₂ and H₂O), metabolites and biomass. Possible modes of residue formation from radiolabelled organic compounds in soils and sediments thus depend on the type of bound components (parent molecule, metabolites, carbon assimilated into microbial biomass or even fixed CO₂) and on the binding matrix (organic, inorganic soil matrix or xenobiotic matrix). Therefore, processes of residue formation of organic pollutants have to be differentiated carefully into bound residue formation in terms of the IUPAC definition for pesticides (parent xenobiotic compounds or toxicologically relevant metabolites) and in terms of biogenic residue formation (Kästner, 2000).

Carbon partitioning and residue formation during microbial degradation of polycyclic aromatic hydrocarbons (PAH, 100 mg/kg) in soil and soil-compost-mixtures were examined applying ¹⁴C-anthracenes labelled at different positions. In native soil 43.8% of [9-¹⁴C] anthracene were mineralized by the autochthonous microflora and 45.4% were transformed into bound residues within 176 days. Compost addition increased the metabolism (67.2% mineralisation) and lowered the residue formation (20.7%). Thus, the higher content of organic carbon after compost addition did not enhance the residue formation. ¹⁴20C-anthracene labeled at position [1,2,3,4,4a,5a] was metabolised more rapidly but revealed higher amounts of residues (28.5%) by the soil-compost-mixture than the radiolabel at C-9 (20.7%; Kästner et al., 1999). Pure cultures with the anthracene degrading *Sphingomonas* sp. BA2 showed that the C-9 carbon of anthracene was not incorporated into the biomass. Thus, the position of the ¹⁴C-label is an important factor which controls the mineralization and the residue formation from metabolised labelled compounds (Kästner et al., 1999).

Two phases of residue formation were observed in the experiments: in the first phase the original compound was sequestered in the soil indicated by limited extractability. In the second phase metabolites were incorporated into humic substances after microbial degradation of the PAH (biogenic residue formation). PAH metabolites undergo oxidative coupling reactions to phenolic compounds to form non-hydrolysable humic like macromolecules. However, hydrolysable ester bonds or sorption of the parent compounds play a minor role within the residue formation (Richnow et al., 1999). In addition, experiments with ¹⁴CO₂ revealed that residues may arise from CO₂ within the soil in amounts typical for anthracene biodegradation. The label of the CO₂ was found to be incorporated into biomolecules such as lipids, amino acids but not into amino sugars (Miltner et al., 2003).

Many fungal species of all taxonomic groups are able to reduce TNT to the corresponding amino dinitrotoluenes. However, the ability to mineralize the explosive

is limited to wood and litter decaying basidiomycetes. The litter decaying fungus *Stropharia rugoso-annulata* was found to be a highly active strain and a static labor-pile reactor contaminated with ^{14}C -labeled TNT was inoculated with the fungus pregrown on wheat straw. Within one week the concentration of TNT decreased from about 200 mg/kg soil dry weight down to < 0.1 mg/kg soil dry weight after 84 days. Accumulation of known metabolites of TNT was observed only during first 28 d of the remediation process. The parent TNT could not be detected even after three weeks of operation. 86 % of the applied radioactivity was fixed as nonextractable residue within the soil material. Only 1 % of the radioactivity was mobilized by water extraction whereas about 10 % were found to be extractable by organic solvents. 3 % of the applied radioactivity was mineralised to $^{14}\text{CO}_2$. However, only 50 % of the applied TNT were metabolized and fixed by the indigenous soil microflora in the control culture without fungal inoculation on wheat straw. The addition of the fungi *Stropharia rugoso-annulata* to the soil leads to the aerobic fixation of the TNT metabolites within the organic matrix and cause significant detoxification and decrease in bioavailability. In soil systems the TNT metabolites, generated by radical processes of ligninolytic enzymes, may be coupled to soil components much faster than further mineralisation steps will occur (Dawel et al., 1997).

The stability of the residues derived from PAH and TNT was examined to assess the remobilization potential of the bound transformation products in soil organic matter (Kästner and Richnow, 2001). The experiments were conducted under environmental "worst case" conditions simulating natural stress factors such as (I) mechanical disruption of the soil texture, (II) withdrawal of multivalent complex forming metal cations, and (III) exposition of the soil to plant seeds, humic acid degrading fungi and enzymes. The distribution of ^{14}C -activity in the contaminated soils was similar before and after disruption of soil the texture. The amount of extractable non-identified ^{14}C -labelled compounds from all soils increased generally in the same range with increasing concentrations of EDTA. No release of sequestered PAH and TNT or remobilization of bound residues occurred. The application of radical generating enzymes showed only a slight remobilization for the soils from the aerobic/anaerobic processes but not for the soils with aerobically generated residues from the fungal process. Significant incorporation or mobilization of the residues by plants were only observed after incomplete elimination of the parent compounds. However, none of these treatments caused a significant remobilisation of the residual radioactivity from the soils. This clearly demonstrates the increased stability of the bound residues derived from microbial degradation of the pollutants in soil (Weiss et al., 2003).

Biogenic residues have to be assessed as result of humification processes because this type of residue is derived from microbial products of metabolism (non-toxic, central metabolites of *microbial* metabolism) or from carbon which has been incorporated into the biomass by assimilation processes. Therefore, the term "bound carbon" instead of bound residues should be used *for* this type of contribution to the soil organic matter. Once incorporated into the organic soil matrix, the bioavailability of the residue components is decreased by orders of magnitude and only minimal transfer into plants was observed. Compounds which are incorporated by covalent bonds can no longer be identified and have lost their chemical identity. Thus, it can be concluded that the general microbial turnover of humic matter is the essential factor controlling the further fate of the carbon from the residues (Kästner and Hofrichter, 2001). Biphasic degradation kinetics were observed during microbial metabolism of organic compounds in soil which is first dominated by degradation of the parent compound. In the second

phase the fixation products and residues within the organic matrix are metabolised with 1/10 of the turnover rates of initial degradation. With increasing age of the residues and especially the biogenic residues, the molecules become more and more inert, leading to soil organic matter with high long-term stability (Kästner et al., 2000).

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Bioavailability of contaminants- relation to risk and bioremediation potential

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The bioavailability of contaminants in soils not only determines the risk to receptors, including environmental organisms and humans, but is also a critical determinant of the remediation potential of many soils polluted by urban and industrial activities. Because of this pivotal role of contaminant bioavailability, environmental legislation (e.g. in the U.K.) is increasingly focused to regulate whether soil contaminants are causing harm rather than continuing to only consider absolute pollutant concentrations. Furthermore, the importance of relating the structure of a contaminant to both potential effects on receptors (QSARs) and to effects on degradation of the compound (QBARs) is now being recognised (Bundy et al., 2000 & 2001). However, conventional chemical techniques cannot fully address the complexity and dynamics of contaminant bioavailability in soils and new technologies are now being used to complement the analytical approach to provide practical support for both site assessment and remediation.

Biosensors are biological materials that give diagnostic signals of analyte quality, via a transducer. Microbial biosensors, based on luminescence and fluorescence reporter genes introduced into environmental bacteria, appeared as a research tool some ten years ago (Killham, 1992). The biosensors developed are of two main types. Contaminant specific biosensors are constructed by placing the reporter genes under the control of either catabolic (in the case of organic contaminants, where catabolic induction is then linked to reporter gene expression) or resistance (in the case of metals where resistance induction is linked to expression) promoters. Non specific toxicity biosensors are constructed by placing the reporter genes under the control of general metabolic promoters and so reporter gene expression (light output) reports on metabolic activity of the bacteria and light reduction to contaminant exposure is a measure of toxicity. These two types of biosensor have generated a raft of technology that is now offering a powerful way forward to not only assess the bioavailability of contaminants, but to also investigate the factors (soil and contaminant properties) controlling in situ bioavailability and to design/monitor strategies for the bioremediation of soils (Ref; Sousa et al., 1998a; Killham et al., 1999). Furthermore, the biosensors can be coupled to specialised extractants to further examine bioavailability and toxicity issues (Tiensing et al., 2001). Data are presented, for example, for biosensor based screening of cyclodextrin extracts of PAH contaminated soils.

Reliable monitoring of contaminant bioavailability, both as a hazard assessment tool and as a predictive measure of bioremediation potential, requires validation. The best approach to do this is through the use of ¹⁴C labelled compounds, so that their mineralisation can be compared to biosensor response to appropriately contaminated samples. Such data are presented for a range of contaminants in different soils and sediments and where the contaminants of interest are non chlorinated and chlorinated hydrocarbons. Having calibrated the biosensors in this way, investigating their response to sample manipulations can be used to optimise the activity of bioremediating microbes. Data are also presented, therefore, where biosensor screening of contaminated

samples is linked to both assessment of the microbial community structure (using physiological profiling and molecular based techniques) of the samples and to their mineralisation/remediation.

Bioremediation is frequently hampered by the degree of bioavailability of the target contaminant and indeed, the stable, negligible bioavailability of a contaminant is increasingly becoming an argument

for non-intervention based on negligible risk. Contaminant specific and toxicity based biosensors are ideal tools to assess bioavailability issues such as these. The biosensors can also identify other constraints (e.g. the presence of contaminant toxicities, nutrient limitations, adverse redox/pH

conditions etc) to the activity of bioremediating microbial populations and remediation strategies can therefore include operations aimed at alleviating these constraints (Sousa et al., 1998b; Ferguson et al., 1998). This has now been achieved successfully at a wide range of industrial sites and the sites successfully remediated as a consequence.

Solid phase biosensor-based assays of contaminant bioavailability in soil involve bringing the biosensor into molecular contact with the solid phase, either by centrifugal or suctional force. These assays are therefore able to test established views regarding the bioavailability of contaminants associated with the solid phase with varying degrees of intimacy. Solution phase assays are able to reveal bioavailability of contaminants in interstitial pore water and have already shown, for example, that chlorinated phenolics often have reduced bioavailability due to complexation by labile humics (Shaw et al., 2000). Solid and solution phase biosensor assays are therefore increasingly being applied to further understand the nature of contaminant bioavailability in soils. Data from these biosensor assays are presented for a range of contaminants to demonstrate some spatial aspects of contaminant toxicity to microbes in soils where contaminant toxicity is a potential constraint to bioremediation and where contaminant availability on the solid phase is the rate limiting step to bioremediation. Radiolabelling experiments are presented to assess the degree to which heavy metal availability/toxicity is a constraint to microbial degradation of organic pollutants and to assess the correlation between biosensor prediction and actual mineralisation of ^{14}C organopollutants by the indigenous soil microbial population.

Bioremediation of contaminated soils is increasingly being recognised as a sustainable alternative technology, where appropriate, to dig and dump. The success of this technology will to a large extent depend upon the reliable assessment of the relationships between contaminant bioavailability and both risk and bioremediation potential. This paper has highlighted some ways in which those relationships can be quantified.

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THE ROLE OF BLACK CARBON IN BIOAVAILABILITY OF ORGANIC CONTAMINANTS IN SOILS

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Sorption largely governs the mobility and bioavailability of non-ionic organic contaminants in soils. On the basis of partitioning theory, sorption of organic compounds is expressed per unit mass of organic carbon (K_{oc}) with little consideration of the nature of soil organic matter (SOM). SOM is highly heterogeneous and its chemistry varies with soil type as a function of vegetation, hydrology, geomorphology and land use. Many soils contain black carbon, especially in regions where fires (forest, grass) are a common occurrence. Black carbon includes partially charred material, char and recondensed soot particles. In order to understand the sorption characteristics of black carbon, we studied the sorption behaviour of two organic compounds (carbaryl and ethion pesticides) on a range of charred materials. Charred materials were collected from the field after fires as well as prepared under laboratory conditions at different temperatures. The chemistry of carbon was characterised by solid state C¹³ NMR. The sorption affinity varied markedly among different types of charred materials collected from the field. While some materials had relatively low affinity to the two organic compounds, the others sorbed 100% of the applied amount. Studies on char that was synthesized under laboratory conditions showed a positive correlation between sorption and the temperature at which char was formed. It was concluded from the study that the heterogeneity in sorption affinity of natural charred material is related to the temperature and the parent material (grass, wood) from which it is formed. The high sorption affinity of black carbon can strongly influence the bioavailability for organic compounds in soils and sediments.

ARBUSCULAR MYCORRHIZAL FUNGI CAN AFFECT RADIONUCLIDES TRANSPORT INTO HERBACEOUS PLANTS

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Among the rhizospheric organisms involved in plant interactions with the soil milieu, the arbuscular mycorrhizal (AM) fungi (*Glomales. Zygomycetes*) deserve special attention because 95% of the world's plant species belong to characteristically mycorrhizal families and potentially benefit from AM fungus-mediated mineral nutrition. Early phytoextraction studies have focused on metallophytes from predominantly nonmycorrhizal plant families, e.g., *Brassicaceae* or *Caryophyllaceae*, so AM have not been considered an important component of phytoremediation practices. Recently, plants capable of forming an association with AM fungi have been shown to accumulate considerable amounts of metals and radionuclides. We have examined at pots experiments the ability of two AM isolates (*Glomus sp.*) to modify the transport of radiocesium and radiostrontium in maize (*Zea mays*), rye grass (*Lolium italicum*) and sorgo (*Sorgum vulgare*) plants. The obtained results have shown that GV isolate had some potential to be used with *Z.mays* plants for 90-Sr and 137-Cs phytoextraction. Further progress with AM use for radionuclides phytoremediation is achieved with inoculum produced with endogenous fungi samples collected in the Chernobyl zone. Different plant samples (41 in total) were analysed both for AM colonisation level and radionuclides content. Those plants, which had extremely high accumulation abilities and colonisation level as well, were selected for the forthcoming investigations. These are the following plant species: *Plantago major*, *Plantago lanceolata*, *Trifolium repens*, *Viola tricolor*, *Hypericum perforatum* and *Crepis tectorum*. The highest value for 90-Sr plant/soil ratio was as 93,8 in case of *Crepis tectorum* plant and the maximal level of plant/soil ratio in case of 137-Cs was about 5,2 as it was calculated for the *Hypericum perforatum* plants. It was shown for the wild plants also that AM colonisation level and radionuclides concentration in plant tissues depend on the plant phenophase and time when samples have been collected and analysed.

**Approaches to study effects of heavy metals on soil microbial communities:
a conceptual work**

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In order to make adequate environmental policies concerning the reduction of the load of heavy metals to the environment, effort for an assessment of the effects of heavy metals and the identification of those loads of heavy metals which harmful effects no longer occur, the so-called 'critical loads', is needed. In this risk approach a critical limit of a receptor of concern is essential for the critical load calculations. Soil microorganisms are considered as the most important receptors for metal effects in non-agricultural soils. The proposed project is based on effects of heavy metals on soil organisms in forest soils. Most soil microbial toxicity tests have been related to total contents, however, a major route of exposure for the soil ecosystem is the dissolved metal concentration. Therefore, information on the bioavailability of the test metal, which is influenced by soil properties, is needed. In this conceptual work we present an overview of ecotoxicological assessments to estimate critical limits of heavy metals in forest soils.

EFFECTS OF ORGANIC AND INORGANIC AMENDMENTS ON CADMIUM, ZINC AND LEAD FRACTIONATION IN CONTAMINATED SOILS.

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Plant-based phytostabilization reduces the risk presented by un- or sparsely-vegetated contaminated soil by the use of a combination of plants and soil amendments to establish a stable vegetation cover which may progressively reduce the soil labile metal pool. This technology does not achieve a clean up of the soil, but changes the mobility of potentially toxic elements by either reducing concentrations in the soil water and other freely exchangeable sites within the soil matrix or by reducing re-entrainment of toxic particulates following the development of a stable and permanent vegetation cover. Both processes alter the speciation of soil metals, reducing potential environmental impact. Sorption, ion exchange and precipitation can be responsible for the conversion of soluble and pre-existing potentially soluble solid phase forms to more geochemically stable solid phases, reducing the metal pool for root uptake.

The aim of this study was to evaluate if the amendments used in a phytostabilization experiment changed the association of the metals in a contaminated soil to the less mobile phases. For that purpose, a fractionation scheme according to Tessier et al (1979) was applied to soil samples from a long-term experiment. Five treatments consisted with: (1) *untreated*; (2) *soil + compost (5%)*; (3) *soil +compost (5%) + beringite (5%)*, (4) *soil+compost (5%)+beringite (5%)+steel shots (1%)* (5) *soil+beringite (5%)+steel shots (1%)*.

Results show significant differences between treatments and fractions. In general, Cd, Zn and Pb were associated to the carbonate and oxide fractions in untreated soil. Different trends were found in relation to the exchangeable fractions where no Pb was found, but Zn and Cd show high values.

On the other hand, the most effective treatment to immobilize Cd was soil amended with compost, beringite and steel shoots. Similar results were found for Pb. With regards to Zn, the presence of organic amendments increased the value of this metal on the interchangeable fraction. Thus, the best treatment to immobilize Zn was the mixture of Beringite and Steel Shoots. In this case, the proportion of Zn in the interchangeable fraction was redistributed to the reducible one.

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Plant water uptake in heavy metal contaminated soil

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Abstract

We are investigating the impact of heavy metal stress on the water regime of young forest ecosystems grown in 32 open top lysimeters (3 m² in diameter by 1 m depth). The factorial treatments of the lysimeters include variations of rainwater acidity (acidic, ambient rain), subsoil type (acidic, calcareous), and soil contamination (with and without copper, zinc and cadmium in the top 20 cm). Each lysimeter was planted in spring of 2000 with the same selection of trees and herbaceous plants. All lysimeters are equipped with tensiometers for monitoring of pressure head and time domain reflectometry for measuring of water content. Irrigation is applied equally to all lysimeters through sprinkler devices. Drainage water is collected by means of canisters installed at the bottom of the lysimeters. Evapotranspiration is calculated through water balancing. We monitored the water regime for two years including an imposed drought period. Significantly more water was extracted from the calcareous than the acidic subsoil. The water potential measurements show that also the heavy metal polluted topsoil had a significant influence on the water regime. Metal stress was particularly evident under reduced irrigation. We suspect that the roots were damaged in the contaminated topsoil. In contrast to the subsoil type, heavy metal pollution did not produce a significant effect on evapotranspiration (ET) though. Neither did and acidic rain. Pot experiments confirmed that in presence of clean subsoil plants compensated for metal stress in contaminated topsoil by shifting their root activity from contaminated to uncontaminated zones.

Bioavailability of soil pollutants and risk assessment: Abiotic factors

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Abstract: The most exciting technical area in the risk assessment and remediation field today is contaminant bioavailability- its ex-situ and in-situ assessment and its implication to risk based land management. Contaminant bioavailability is increasingly being used as the key indicator of potential risk that contaminants pose to both environmental and human health. However, bioavailability is often assumed to be static in nature where most decisions on risk and remediation are based on laboratory estimations of the bioavailable fraction, which may vary with time, nature of species as well as with temporal variations in environmental factors. Such determinations are even more complicated for organic contaminants where most estimates are based on total organic content rather than the bioavailable fraction. Consequently, the risk such organic contaminants pose to end users may be overestimated. Given that ‘Risk Based Land Management’ is increasingly being adopted as a cost-effective management strategy for contaminated sites, our research team has focussed on ‘micro-scale processes controlling chemical bioavailability in the terrestrial ecosystem’ in order to assist our regulatory bodies better define risk posed by contaminated sites. In this paper, we define bioavailability as the fraction of the total contaminant in the interstitial water that is available to receptor organisms (microorganisms, plants, earthworms, pigs etc). The bioavailable fraction may vary with time, soil type, capacity of the soil to replenish the bioavailable pool in the interstitial water, chemical speciation, organisms present and other environmental factors (pH, Eh etc).

** Effect of time:* Long-term incubations of contaminant spiked soils, simulating field conditions in our laboratory, showed an exponential decline in contaminant bioavailability with aging. The partition coefficient of contaminants increased with aging and this seemed to have a direct impact on chemical toxicity to microorganisms and earthworms. The reduced toxicity of contaminants (As, Cr) to earthworms was attributed to increased binding of chemicals to soil colloids and consequent reduction in the bioavailable fraction in soil interstitial water. Detailed investigation of the aged soils in relation to contaminant desorption revealed a significant relationship between soil sorption capacity and the density of micro-pores.

Effect of soil type: Bioavailability is directly related to soil type through its capacity to bind chemicals. The binding capacity of soils is often defined by the partition coefficient. Detailed studies in our laboratory using Australian soils spiked with Cd and Pb and in Poland using long-term Cd-contaminated soils revealed a close relationship between soil partition coefficient and contaminant bioavailability as reflected by both toxicity to microorganisms and plant uptake of contaminants

Replenishment capacity of soil interstitial water: Chemicals present in the interstitial water are defined as the most readily available fraction in the terrestrial ecosystem. As chemicals

from this pool are utilised by living organisms or leached by physical gravitational forces, the capacity of soils to replenish this pool dictates chemical bioavailability. Our study showed that the replenishment factor is controlled by mineral and organic matter composition, partition coefficient and the chemical saturation index. In soils with high reaction capacity but low saturation index, the bioavailable fraction is low. The replenishment factor can be manipulated by changing the chemistry of the soil; data on the kinetics of metal release from different mineral pools in relation to metal bioavailability will be discussed.

Chemical speciation: From a purely chemical perspective, chemical reactions are controlled by speciation. We show evidence that organically complexed metal is bioavailable, contrary to the general notion that free-metal ion is the only bioavailable form. We present results demonstrating the role of speciation on contaminant uptake by microorganisms and implications to soil health. The replenishment capacity of free ion in interstitial water and its implications to contaminant bioavailability are also be discussed.

Sodium enhances the bioavailability of contaminant in soil: Studies in our laboratory show for the first time, that the addition of sodium to a long-term organically contaminated soil could enhance the transformation of a contaminant by increasing its bioavailability through dispersion of the clay followed by an increase in dissolved organic matter content. This finding has greater implications for the remediation of long-term contaminated soils and the results will be presented at the workshop. Based on the above, the implications to monitored natural attenuation of contaminants in terrestrial ecosystems in relation to risk assessment are discussed.

Metal-ligand complexes: a coin with two sides

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The chemistry of metals in the environment is strongly influenced by the presence of ligands. Metal bioavailability, uptake, toxicity, transport, adsorption, distribution and fate are all significantly altered by metals. And the ligand? A metal-ligand complex can be depicted as a coin that has two sides: the metal and the ligand side (Figure 1). Most studies have been centered on the metal and therefore the view of the coin was from the *metal* side. However, we can also look at the coin from the *ligand* side and we find that ligands are also influenced by the presence of metals. The formation of complexes with different metals strongly affects the chemical behavior of the ligand. We have to look at the metal-ligand coin also from the ligand side and also set the focus on the influence of metals on ligand behavior. The picture of the metal-ligand system is complete when we acknowledge, that the coin cannot be separated into the two sides and that we have to look at metal and ligand simultaneously.



Figure 1. Representation of a metal-ligand complex as a coin with two sides.

Many chelating agents are under scrutiny due to their influence on metal availability and mobility and in particular due to their persistence in the environment. Chelate adsorption, metal-mobilization, metal-exchange, mineral dissolution, reactive transport, photodegradation, chemical degradation, toxicity and bioavailability are all shown to be substantially affected by the chelated metal ion. Knowing the speciation of chelating agents in natural waters is therefore crucial for predicting their environmental fate. Despite this importance, only a few speciation measurements have been reported for natural waters and model calculations have been frequently used instead. These calculations are however, complicated by slow metal-exchange reactions that result in a non-equilibrium speciation and by the presence of naturally occurring ligands that compete with the chelating agents for available metals.

Chelating agents not only enter the environment through wastewater treatment plants or are released from waste sites, they are also avertedly added to natural systems. Many fertilizers for example contain Fe(III)-complexes of strong chelating agents to enhance iron availability to plants. These complexes are then distributed over the fields and enter the environment through the soil. Chelate-assisted remediation of metal polluted soils is another possible way how chelating agents can enter the soil.

The influence of speciation on the behavior of the metal and the ligand is not only important for anthropogenic ligands but also for natural ligands. An example is shown in figure 2 for the microbial siderophore desferrioxamine B (DFOB). It shows both the influence of the ligand on metal behavior as the influence of the metal on ligand behavior. DFOB enhances metal adsorption at low pH compared to the ligand-free system. Figure 2a shows this for Zn as an example. At higher pH, DFOB diminishes metal adsorption by formation of soluble ZnDFOB complexes. But also the metal influences DFOB adsorption. Zn has only a minor influence on DFOB adsorption, Cu, however, increases DFOB adsorption from pH 4 to 9 considerably (Figure 2b). Although the mechanism that is responsible for adsorption is different (ion exchange for DFOB and surface complexation for polycarboxylate chelating agents), the effect of the metal on ligand behavior is significant in both cases.

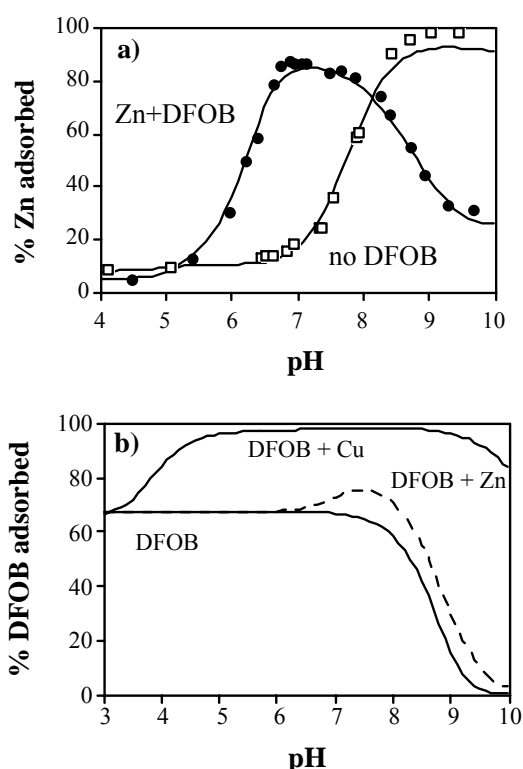


Figure 2 Influence of desferrioxamine B (DFOB) on Zn adsorption onto the clay mineral montmorillonite (a) and influence of metals on DFOB adsorption montmorillonite (b).

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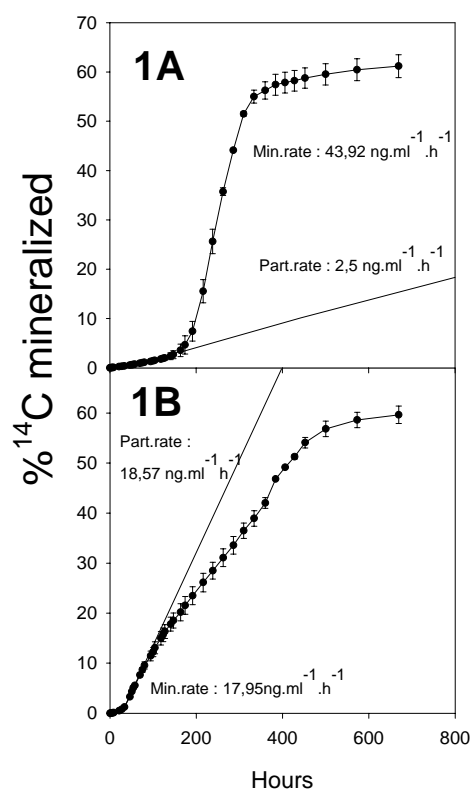
Physiological promotion by bacteria of bioavailability of polycyclic aromatic hydrocarbons

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Bioavailability is one of the bottle-necks in bioremediation of soils and sediments polluted with polycyclic aromatic hydrocarbons (PAHs), and it is also a relevant parameter in evaluating environmental quality. However, the physico-chemical and biological basis for the bioavailability to microorganisms of these and other hydrophobic compounds still remain to be completely understood. The present talk will summarize our recent research efforts in this issue. The accessibility of a compound for its metabolism may be limited due to its association to clay and organic matter fractions or to non-aqueous phase liquids (NAPLs), but degrading microorganisms may physiologically promote bioavailability. The mechanisms involved include adhesion to the pollutant interface, utilization of multiple substrates, production of biosurfactants, and an increased rate of transport by water flow or through chemotaxis.

Nonaqueous-phase liquids (NAPLs), such as creosote or coal tar, often remain as a free phase in the soil or sediment structure after massive contamination, and also influence the bioavailability of PAHs. The biodegradation of PAH present in NAPLs has been found to be severely limited by the slow kinetics of abiotic mass transfer or partitioning of these hydrophobic compounds into the aqueous phase. However, adhesion to the NAPL-water interface seems a relevant mechanism by which degrading bacteria can accelerate the already slow release of these hydrophobic pollutants. An example of the role of microbial adhesion to the NAPL-water interface is given in Figure 1. The results showed that strain *Sphingomonas* sp. LB128 is capable of mineralizing phenanthrene initially present in a NAPL (heptamethylnonane) at a higher rate than the predicted by abiotic partitioning (Fig. 4A, solid line). The addition of the adhesion-inhibiting agent Triton X-100 suppressed this phase of maximum mineralization rate, and induced maximum mineralization rates that were not statistically different to partitioning rates (Fig. 1B).

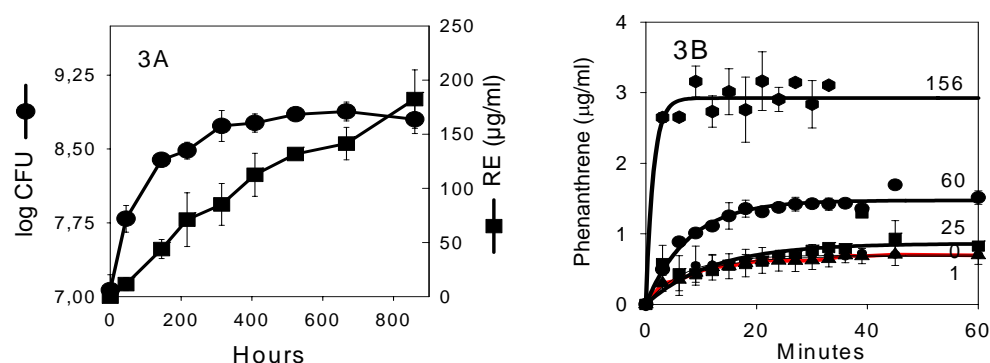


There are other situations in which bacteria can also enhance bioavailability. For example, the multiple-substrate physiology of PAH biodegradation by *Mycobacterium* sp. VM552 was studied in a series of ^{14}C -pyrene mineralization experiments, performed

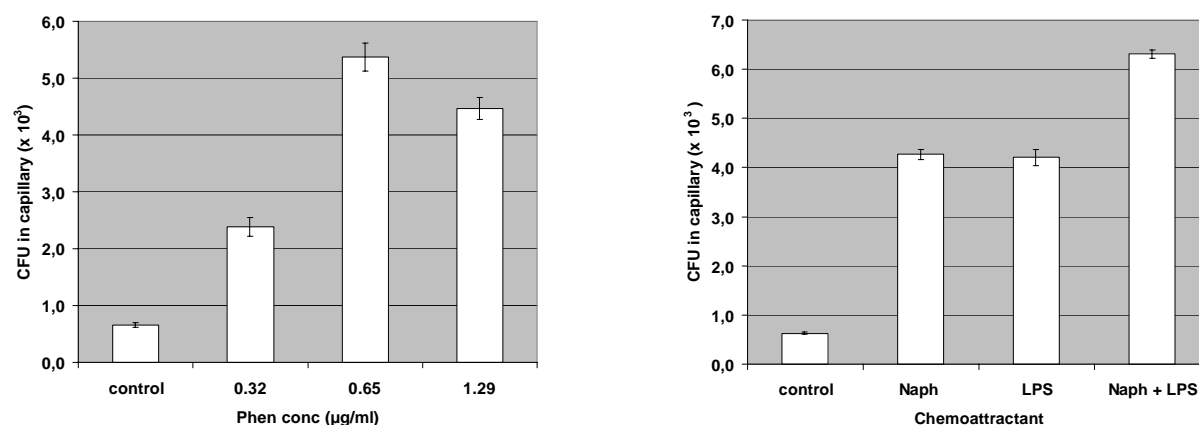
in model systems that restricted bioavailability. Biodegradation of small quantities (ng/ml) of the test compound was measured in the presence and absence of alternative carbon and energy sources for this organism (phenanthrene, acetate and glucose, all supplied at 0.2 %). The production of $^{14}\text{CO}_2$ from 453 ng of ^{14}C -pyrene completely dissolved in the water phase (87 ng/ml) was not significantly affected by the presence of the alternative carbon sources tested, glucose and acetate (Fig. 2A). However, acetate clearly stimulated the transformation when the compound was initially sorbed to 10 mg of solid humic acid (fig. 2B). In the absence of acetate, only 4 % of the sorbed compound was transformed to CO_2 after 120 h. The transformation was again stimulated, in comparison to the control without an alternative carbon source, with most of the compound partitioned in a NAPL, dibutyl phthalate (fig. 2C). Acetate did not stimulate the mineralization of 5 $\mu\text{g/ml}$ solid pyrene (fig. 2D). The mechanism responsible for the observed effect of acetate on pyrene mineralization may be related to the existence of a threshold concentration in the water phase for pyrene mineralization. The biodegradation of phenanthrene by the biosurfactant-producing strain *Pseudomonas*

aeruginosa 19SJ, isolated from a PAH-contaminated soil, was investigated in experiments with the compound present as crystals. Growth on solid phenanthrene (estimated by colony forming units, CFU) exhibited an initial phase not limited by dissolution rate, and a subsequent, carbon-limited phase caused by exhaustion of the carbon source (Fig. 3A). Rhamnolipid biosurfactants (measured as rhamnose equivalents) were produced from solid phenanthrene and appeared in solution and particulate material (cells and phenanthrene crystals). During the carbon-limited phase, the concentration of rhamnolipids detected in culture exceeded the critical micelle concentration (CMC) determined with purified rhamnolipids. The biosurfactants caused

a significant increase in dissolution rate and pseudosolubility of phenanthrene (Fig. 3B – numbers denote biosurfactant concentrations). Externally added rhamnolipids at a concentration higher than the CMC increased the biodegradation rate of solid phenanthrene. Our results suggest that the biodegradation of solid phenanthrene by *P. aeruginosa* 19SJ is not exclusively governed by abiotic mass transfer of the compound into the water phase, but changes in this mass transfer, derived from the physiology of the organism, lead to an increased bioavailability.



Microorganisms can also mobilize through the porous matrix of soils and sediments to degrade pollutants localized at distant places, increasing therefore bioavailability. This enhanced mobilization can be caused by water flow (Lahlou et al., 2000) or, as revealed recently, by chemotaxis (Ortega-Calvo et al., 2002). In that study, strains capable to degrade different PAH (naphthalene, phenanthrene, anthracene, pyrene and fluoranthene) were isolated from rhizosphere soils contaminated with coal tar and oil. The chemotactic attraction towards different seed extracts of gramineae (wheat, oat, and meadow fescue) and legumes (alfalfa, pea, white clover and red clover) was quantified by capillary and densitometric assays. A total of 20 chemotactic, PAH-degrading strains were isolated. From those, a significant fraction showed a positive response to the plant extracts tested. The indication that many PAH-degrading rhizosphere bacteria respond chemotactically towards plant exudates, suggests a possible way of optimisation of PAH phytoremediation, by the use of selected plants and microbial inoculants.



Rhizosphere bacteria were also capable of responding chemotactically to PAH. In figure 4A is shown the attraction of *Pseudomonas* sp. 10D to phenanthrene, as revealed by capillary assays. The strain was also attracted towards its own LPS, a component of the bacterial cell wall (Fig. 4B). This bacterial component still promoted chemotaxis in the presence of 30 µg/mL naphthalene, possibly due to an increased bioavailability of naphthalene in the presence of these microbial lipids

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Modelling kinetic phenol biodegradation processes and the associated hydrogen and acetate concentrations in a batch microcosm

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Organic contaminants like phenol may be degraded by microbes in the sub-surface at oxygen deficient conditions via a range of other terminal electron accepting processes (TEAPs), for example iron and manganese reduction. Fermentation can be the first step of the degradation process producing intermediate species, which are subsequently consumed by TEAPs. A numerical model of an aquifer-derived laboratory microcosm is developed to simulate the dynamic behaviour of fermentation and respiration in an aqueous phase in contact with mineral and anaerobic gas phases. Microbial growth and substrate utilisation kinetics are coupled via a formulation that also includes aqueous speciation and other geochemical reactions including surface complexation, mineral dissolution and precipitation. Competitive exclusion between TEAPs is integral to the conceptual model of the simulation, and the results indicate that exclusion is not complete, but some overlap is found between TEAPs. The model was used to test approaches like the partial equilibrium approach that currently make use of hydrogen levels to diagnose prevalent TEAPs in groundwater. The observed pattern of hydrogen and acetate concentrations were reproduced well by the simulations and showed for example that steady state hydrogen concentrations during different TEAPs do not differ significantly.

Residues of atrazine in rhizosphere and non-rhizosphere soils

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It is known that significant proportion of pesticides or their degradation products remain in soil as bound (non-extractable) residues. Radiotracer techniques offer quick and efficient way of determining bound residues.

A soil biometer flask was used to screen rhizosphere soils from plants for their ability to degrade ^{14}C atrazine. Ten grams of soil was treated with ^{14}C atrazine. The mineralization of ^{14}C was monitored by trapping $^{14}\text{CO}_2$ in NaOH. At the end of the experiment, the soils were extracted with solvents. The unextracted soils were combusted in biological material oxidizer in order to estimate ^{14}C bound residues. All the counts were made using liquid scintillation counter and appropriate quench corrections were made.

In one set of experiments, when sugarcane rhizosphere soils were used, considerable amount of bound residues (40.5%) were formed as compared to non-rhizosphere soils (23.4%) with negligible mineralization. In subsequent experiments, rhizosphere soils from five non-agricultural plants growing in JRF farm, Vapi, were used. Considerable mineralization occurred ranging from 29.3% to 35.9%. Interestingly, bound residues were anywhere between 30.7% to 78%.

Ecotoxicological risk analysis of soil polluted from pyrotechnical activities

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An industrial area close to Göteborg, Sweden, was investigated with regard to toxic effects resulting from the handling of pyrotechnical products. Potential pollutants, resulting from the industrial processing, besides acidifying components, were metals (strontium, barium, lead, cadmium) and some organic compounds (phenols).

The area under study measured approximately 250 x 600 m and was spot-sampled using a 30x30m grid. Surface soil collected from totally 46 spots, were extracted using pressurized liquid extraction (Dionex ASE 200). Extract toxicity was assessed using three biological models: Bioluminescence assay in *Photobacterium fischerii*; Neutral Red Incorporation assay using a mouse cell line L929; Water flea acute toxicity test on *Ceriodaphnia dubia*.

Toxicity levels at 50% effect were calculated for each model. Results showed good correlation between the models, all indicating the most critical spots within the sampling area. Linear correlation coefficient ($r^2 = 0.88$) between *Photobacterium fischerii* and L929 was based on 19 spots showing more than 50% effect (see Figure A). The spots showing highest toxicity in initial tests were confirmed by validation tests on *Ceriodaphnia dubia*.

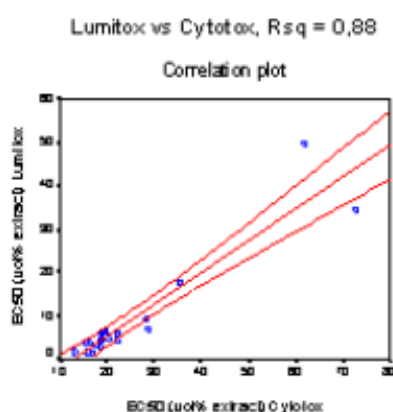


Figure A

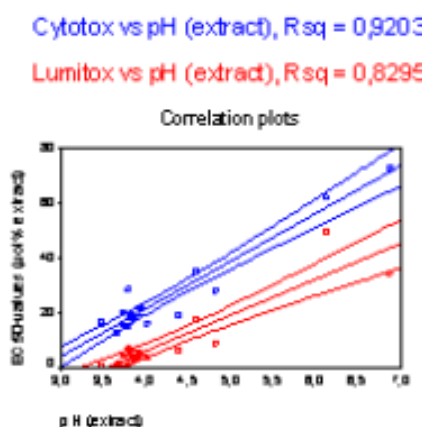


Figure B

Measured toxicity revealed differences in test model sensitivity, although in each instance the same spots were indicated by *Photobacterium fischerii* and L929. The influence of pH on toxicity of the extracts was obvious, as shown in Figure B. However, pH itself could not have contributed to toxicity in the L929 assay where extracts were

buffered by the cell cultivation medium. Thus it was suggested that toxicity emerged from other extract components than pH. Furthermore, pH may still have a major influence on the mobility of certain pollutants in soil, especially metals, as well as on the extractability of soil components, both factors making pollutants available for uptake in the test models. Other explanatory factors on the bioavailability of pollutants will be discussed.

Risk assessment of heavy metal leaching by soil solution monitoring : Chemical behaviour of different soil water samplers

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Introduction

Monitoring the chemical composition of the soil solution at different depths is a direct way to assess the risk of heavy metal (HM) leaching to the groundwater from contaminated soils. In particular, temporal variations in metal concentrations caused by the seasonally varying plant activity can be detected. Such information is of utmost importance to evaluate soil remediation strategies like the phytostabilization with trees or other plants.

Soil solution monitoring requires the use of methods to extract soil solution. The method that is chosen and the probes that are used can have a great effect on the measured concentration of major and trace constituents in the soil solution. Results found in the literature are sometimes contradicting on the suitability of different materials used to build tension soil water samplers for heavy metal monitoring.

Laboratory tests were conducted to assess and compare different pre-selected commercially available soil water samplers to test their influence on the chemical composition of soil solution. The different types investigated were two plastic soil water samplers (nylon and teflon), one made of borosilicate glass, and one made of ceramic.

Material and methods

Nylon cups used are manufactured by ecoTech GmbH (Bonn, Germany). The nylon membrane is supported by a polyethylene substructure and cover by a polyethylene shield. Teflon cups (Prenart ApS, Copenhagen, Denmark) are manufactured by mixing PTFE and quartz flour. The borosilicate glass cups come from ecoTech GmbH and the ceramic ones from Soilmoisture Corp. (Santa Barbara, U.S.A.).

The chemical tests conducted on the above mentioned soil water samplers were preceded by washing firstly the samplers with HCl to analyse the contaminants released and secondly with NaOH to investigate the loss of DOC especially from the plastic cups (Grossmann et al., 1990). Samples were taken at intervals during the rinsing procedure with deionised water to control the effectiveness of the procedure.

Then, each type was tested by percolating acidic (pH 4.5) and alkaline (pH 7.5) synthetic soil solutions through the sampler by means of a peristaltic pump at a low flow rate (comparable as to field conditions) of 20 ml/h. After an initial pre-conditioning step (1) (figure 1) different solutions with different metal concentrations, with and without dissolved organic matter were used in a stepwise protocol (figure 1) adapted from Grossmann et al. (1990).

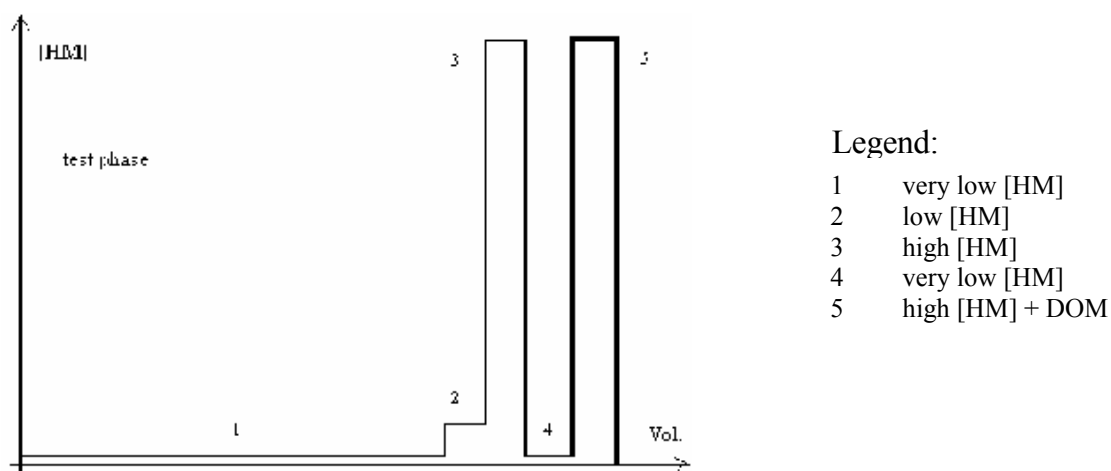


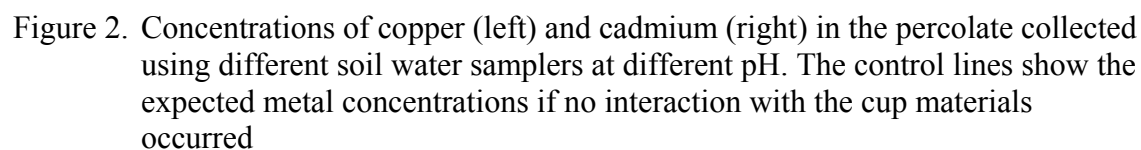
Figure 1. Schematic overview of HM concentrations in the different steps of the percolation test

The composition of the test solutions were chosen to be representative of those typically found in soil solutions of a model forest ecosystem with artificially contaminated topsoil (20cm), uncontaminated subsoil (acidic and calcareous) and neutral or acidic rain.

At different time steps (high sampling rate), the concentrations in the percolate were compared with the initial concentrations. The measured parameters included heavy metals (Zn, Cd, Cu, Pb), other cations (Al, Ca, K, Mg, Mn, Na, Fe), inorganic anions (Cl, NO₃, PO₄, SO₄), dissolved organic carbon (DOC), pH and conductivity.

Results and discussion

For nylon and ceramic cups, the results obtained by Grossmann et al. (1990) and Wenzel et al. (1993, 1997) without DOC are generally confirmed. The nylon cups reflected quite accurately the effective heavy metal concentrations in the acidic solutions (see Cd and Cu results in figure 2) In the alkaline solutions, some adsorption of Cu and strong adsorption of Pb with a memory effect were noted. The ceramic cups were found to be suitable at pH 4.5 for Zn and Cd, but partly adsorption of Cu and complete adsorption of Pb were observed. At pH 7.5, the adsorption of all heavy metals onto the ceramic material was complete. The teflon cups exhibited no adsorption of Zn and Cd at pH 4.5, but showed strong Cu adsorption with a memory effect and complete Pb adsorption. At pH 7.5, the adsorption and memory effects were significant and increased in the sequence Cd, Zn, Cu, Pb. These results for teflon, incomplete and scarce in the literature, are in contradiction with Morrison (1982) but in some accordance with Andersen et al. (2002). The glass cup expected to give good results in presence of DOM revealed a complete HM adsorption at pH 7.5. For Cu, somewhat less adsorption to nylon and teflon cups was found in the presence of dissolved organic matter.



These results show that nylon cups are the most suitable samplers for use in the monitoring of soil solution with respect to heavy metals. At acidic pH, no adsorption of the studied metals was observed. However, the performance at higher pH is different for different metals and depends on the concentration of dissolved organic matter. In particular it could be shown that in the presence of DOM the adsorption behaviour at $\text{pH} > 7$ with respect to Cu is reduced.

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Bioavailability in soil: The role of invertebrate behavior

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Bioavailability is usually defined as a concept that describes the interaction between a chemical and a biological entity. Individual steps of this interaction (e.g. the route of entry, the time of exposure, the type and concentration of the chemical as well as the influence of the properties of the surrounding soil) are well investigated or are at least identified as being important. The biological receptor with which a specific chemical is interacting has also been covered in recent research projects. However, from a biological point of view it seems that the behavior of individual organisms has been rarely taken into consideration in this interactive process. In fact there are various ways in which an organism can actively (directly by its own behavior or indirectly by changing its environment) influence the process of coming into contact with a chemical or taking up a chemical.

First indications how organisms can change their environment in a way that the bioavailability of chemicals is affected came from marine studies with polychaete worms living in self-created tubes. Later the same problem was studied in soils, too, using oligochaetes as examples. By building mucus-lined permanent burrows the nightcrawler *Lumbricus terrestris* is creating areas in which many chemicals are enriched accumulated in comparison to the surrounding soil. On the other hand, the same species is able to distinguish between “clean” and chemical-contaminated litter (depending on the type of chemical), thus decreasing actively the chance of taking up chemicals. The latter behavior is currently used for the development of a new type of laboratory test, using the “avoidance behavior” as a sensitive endpoint when assessing the bioavailability of chemicals. However, the advantages and disadvantages of such tests and their role within accepted environmental risk assessment procedures are not clear yet.

Bioavailability: from Scientific Concept to Applicable Approach: Development of Soil-specific Protection Levels

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Introduction: need for improved soil quality standards

To evaluate soil quality, standards have been imposed. As of now these are mostly based on the total contaminant level in soil. Research from the last two decades (and before that) clearly has shown that risks associated with contaminants in soil do not depend (or only in a few cases) on the total level in soil, but merely on a fraction of the total level. The question that arises is how to quantify this fraction so that new soil quality standards are linked to actual and potential risk levels.

However, risks of contaminants in soil can vary according to the compartment (including groundwater, soil ecosystem or arable crops) one is dealing with. For each of these compartments the link between what is in the soil and effects that can occur may be different.

However, in order to convince policy-makers that a shift from total levels (for quality standards) to what is commonly referred to as 'bio-available' levels is needed, a comprehensible approach is needed that is able to grasp some of the variability not accounted for by the total level. The question then is if we are able to define 'bio-availability' in such terms (*and measurable quantities!*) that indeed a consistent set of soil quality standards can be derived from that.

Here we will present the general idea, which is currently being applied to derive new soil quality standards to protect groundwater quality, soil ecosystems and arable crops.

General Concept behind the incorporation of the Bioavailability concept into legislation

The general idea behind the concept is that in soils the link between the total level present in the soil and the ultimate effect can be described as is shown in figure 1. Here the concept is schematically presented for the derivation of soil protection standards for a single organism or crop. For ecosystems, the concept is more complex since food-web interactions have to be considered as well.

Underlying assumptions to this concept include:

- The different pools can be quantified chemically;
- There is measurable link between a measurable exposure and effect in the target organism;
- The different pools can be related to each other, e.g. a conversion of the actual available pool to the potentially available pool can be described using a model;
- The relation between exposure and effect can be quantified based on a limited number of parameters, which are more or less the same for a large group of organisms.

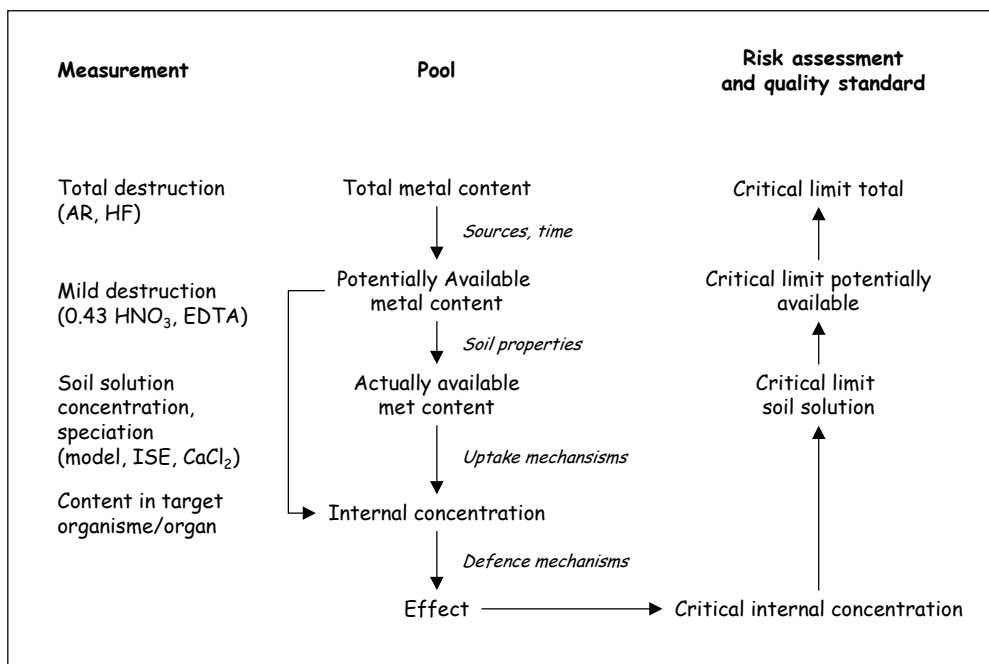


Figure 1. Assumptions underlying the concept of the derivation of soil quality standards based on bioavailability

Currently the approach as described in figure 1 is being used to derive so-called Soil Use Values (In Dutch: Bodem Gebruiks Waarden, de Vries et al., 2002). Based on the protection of soil organisms, arable crops and groundwater, the following steps are being made:

1. For each category, specific *critical limits* are derived. These include for example maximum permissible levels in groundwater, maximum permitted levels in arable crops (food quality criteria) or No Observed Effect Levels (NOEC) or LC_x values for specific organisms.
2. Based on the composition of the soil (pH organic matter content, clay content), this critical limit is converted to a critical metal content in the soil at which the critical limit in the target organism or groundwater is exceeded. To do so, model concepts are use to achieve this conversion.

The models used here should 'obey' the afore-mentioned 4 criteria in order to be applicable on a national scale. Here we will present two examples that illustrate how the new soil quality standards (abbreviated: BGW) are derived from available data and model concepts that take into account bioavailability as the central issue. This concept has been developed in co-operation with RIVM (National Institute for Public Health and the Environment) and RIZA (Dutch Institute for Inland Water Management) under supervision of the ministries of the Environment, Spatial Planning and Housing (VROM) and Agriculture, Fisheries and Nature Conservation (LNV).

Example 1. Derivation of soil quality standards for crop protection: Cd in wheat

To protect human health food quality criteria for Cd are imposed by the national government and by the EU. The proposed maximum Cd content in wheat is 0.1 mg kg⁻¹ (fresh weight). To describe the transfer of Cd from soil to crop, a Freundlich type model has been fitted to the data which takes into account the effect of acidity and the organic matter content together with the potentially available metal content in the soil:

$$[\text{Me}]_p = K_{sp} \cdot [\text{Me}]_s^n \quad (\text{i})$$

with $[\text{Me}]_p$ = plant metal content (in mg kg⁻¹ dm); K_{sp} soil specific uptake coefficient; $[\text{Me}]_s$ = potentially available metal content in the soil (in mg kg⁻¹ dm). K_{sp} can be expressed as a function of soil properties (pH, clay content and organic matter (OM) according to:

$$\text{Log } K_{sp} = a + b \cdot \text{pH} + c \cdot \log(\text{clay}) + d \cdot \log(\text{OM}) \quad (\text{ii})$$

In fact this type of model is used by several authors also to predict the dissolved metal concentration in the soil, and based on the assumption that uptake is related to the dissolved metal concentration or free metal activity, the direct soil to plant model should be able to explain part of the measured variability in the crop Cd content as well. Once the model parameters are known, the critical soil concentration at which the crop quality standard will be exceeded can be calculated. This was done recently using soil maps on a national scale (Brus et al., 2002). Figure 2 presents some of the results based on eq. 1 and 2. Figure 2a contains the actual Cd content in the soil whereas figure 2b presents the critical Cd content at which the 0.1 food quality criterion will be exceeded.

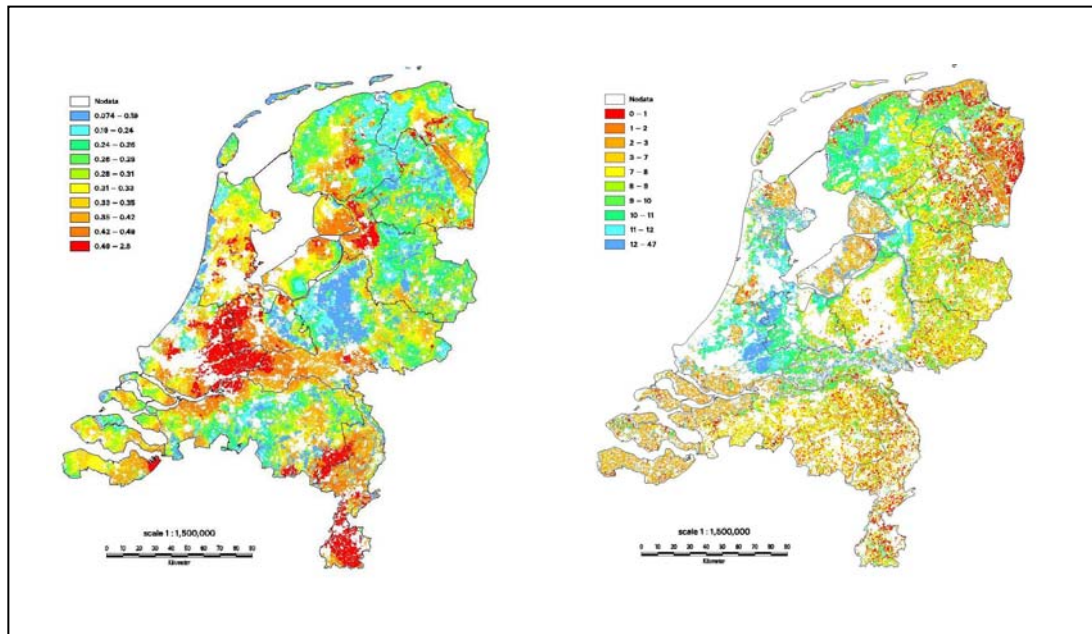


Figure 2. Cd content in Dutch soils (left) and calculated critical Cd content at which the 0.1 mg kg Cd food safety limit will be exceeded (right)

Example 2. Derivation of soil critical levels for soil dwelling organisms

To derive critical metal levels in soils for soil dwelling organisms an additional factor has to be included: food-web interactions. An example for various organisms is shown in figure 3.

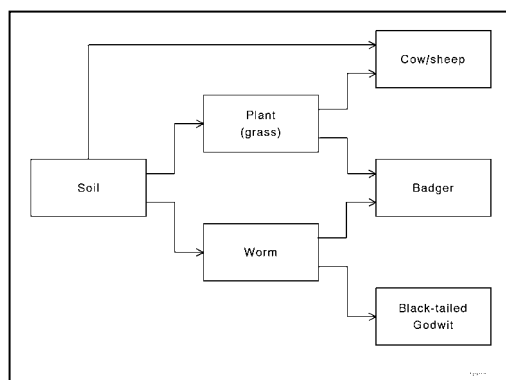


Figure. 3. Simplified food-web structure for soil dwelling organism

In this case not only the uptake from soil to crop has to be included but also the intake of both soil and crops by various organisms has to be considered based on the feeding habits of the organism. For example, cows eat both grass and soil whereas an animal like the badger eats both worms, herbs and grass but little soil. The criterion used here as a starting point is the internal critical level in target

organisms like kidney and liver, which accumulate metals.

To predict the critical soil metal content data on both the amount of food consumed and the metal content in the food itself have to be known or predicted using model concepts as presented in example 1. In table 1 the critical Cd content for the organisms mentioned here are calculated for three soil types.

Table 1. Overview of critical Cd and Pb contents in the soil based acceptable daily intakes of those metals by the godwit and badger.

Soil use	Soil type	Cd critical - soil (mg.kg ⁻¹)	
		Black-tailed godwit	Badger
Agriculture	Sand	0.14	0.28
Agriculture	Clay	0.66	1.3
Agriculture	Peat	1.0	2.0
Nature	Sand	0.067	0.13
Nature	Clay	0.47	0.92
Nature	Peat	0.33	0.65

The examples presented here show that improvement of soil quality standards seems possible taking into account aspects of bioavailability. However, it should be stressed that the approach used here sometimes fails to explain the measured variability also. For example uptake of Pb by arable crops is controlled largely by internal plant storage and defence mechanisms. For some elements no 'functional' relationship between soil and crop can be derived from available data which limits the application. Better insight in the processes controlling the actual relationship between exposure and effect is needed in those cases.

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Phytoextraction capacity of trees growing on a metal contaminated soil

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Phytoremediation is an innovative biological technique to reclaim land contaminated by heavy metals or organic pollutants. It aims to select the appropriate plant species to decontaminate polluted areas by contaminant extraction.

A vegetative cover on a heavy metal polluted soil may help to avoid lateral dispersion of the contaminants by slowing the wind erosion and reducing the volume of water percolating through the soil. This may keep the contaminants away from the underlying ground water, stabilising them in the soil profile. Some plants can further phytostabilise heavy metals in the rhizosphere through root exudation. Other species are known to phytoextract metals from the soil by incorporating them into root tissues. From the roots, some plant species are able to transfer metals to their superior tissues, potentially allowing the soil to be decontaminated by harvesting the aerial parts of the plants. In extreme cases, such as mine spoils, revegetation of contaminated soils may raise problems, due to the phytotoxicity of heavy metals, requiring the use of metal-tolerant plants.

A vegetative cover on a heavy metal polluted soil may help to avoid lateral dispersion of the contaminants by slowing the wind erosion and reducing the volume of water percolating through the soil. This may keep the contaminants away from the underlying ground water, stabilising them in the soil profile. Some plants can further phytostabilise heavy metals in the rhizosphere through root exudation. Other species are known to phytoextract metals from the soil by incorporating them into root tissues. From the roots, some plant species are able to transfer metals to their superior tissues, potentially allowing the soil to be decontaminated by harvesting the aerial parts of the plants. In extreme cases, such as mine spoils, revegetation of contaminated soils may raise problems, due to the phytotoxicity of heavy metals, requiring the use of metal-tolerant plants.

In the present work, we studied the ability of five woody tree and shrub species to extract heavy metals from a polluted soil to their superior parts. The metal content in leaves and twigs was determined. *Salix* and *Betula* extracted heavy metals from the contaminated soil and transferred them to their superior tissues. On the other hand, *Alnus*, *Fraxinus* and *Sorbus* excluded them from their superior tissues.

Concentrations of copper and zinc in leaves and twigs (in mg kg⁻¹ of dry matter) of 5 species planted on the sewage sludge contaminated compost area and non-contaminated arable soil area (control area). Uptake and transfer to the aboveground tissues succeeded in *Betula* and *Salix*.

Plant species	Tissue	Cu [mg kg ⁻¹]		Zn [mg kg ⁻¹]	
		control area	contaminated area	control area	contaminated area
<i>Alnus incana</i>	Leaves	13	29	47	42
	Twigs	11	14	41	38
<i>Betula pendula</i>	Leaves	15	10	170	231
	Twigs	5	14	124	196
<i>Fraxinus excelsior</i>	Leaves	8	8	11	14
	Twigs	29	21	44	32
<i>Salix viminalis</i>	Leaves	9	8	129	228
	Twigs	8	15	77	174
<i>Sorbus mougeotii</i>	Leaves	14	8	24	16
	Twigs	18	9	35	31
Average in soil		21	557	65	620

All species analysed were able to grow on a heavy metal-polluted soil, whether or not the pollutant was taken up into their superior tissues. The concentration of copper that was found in the aboveground plant tissues was similar in the samples collected from the contaminated or non-contaminated soil.

The zinc concentrations for *Betula* and *Salix* growing in non-polluted conditions were higher in leaves than in twigs and the highest Zn-transfer factor to the superior parts among the species analyzed. This may be a mechanism to eliminate the metal from the organism, via autumnal leaf fall.

Bioconcentration factors to the aboveground plant tissues for copper and zinc for the trees growing on the contaminated area.

Plant species	Bioconcentration factor for Cu		Bioconcentration factor for Zn	
	Leaves	Twigs	Leaves	Twigs
<i>Alnus incana</i>	0.05	0.03	0.07	0.06
<i>Betula pendula</i>	0.02	0.03	0.37	0.32
<i>Fraxinus excelsior</i>	0.01	0.04	0.02	0.05
<i>Salix viminalis</i>	0.01	0.03	0.37	0.28
<i>Sorbus mougeotii</i>	0.01	0.02	0.03	0.05

In a phytoremediation approach of soil contaminated by heavy metals, the plants must be chosen among the species that are able to grow on contaminated soils. This is true for phytoextraction and phytostabilisation. When they can also take up large quantities of heavy metals through high concentrations in their shoots and/or a high biomass production, then phytoextraction can be considered. Ultimately, the option to be taken will depend on the physico-chemical characteristics of the soil to be decontaminated as well as on local laws and further use of the soil.

New methods to measure and estimate the bio-availability of ^{14}C -organic chemicals in soils

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New analytical methods as well as a new (simple) mathematical model were used to get direct and indirect information about the bio-availability and the formation of non-extractable residues ("bound residues") of organic chemicals in different soils. Experiments were done with three different organic ^{14}C -labeled compounds representing different classes of chemicals (isoproturon and benazolin-ethyl as herbicides; benzo-a-pyren as a member of the PAH-chemical group) and with 8 different types of soil. The degradation/mineralization and the volatilisation of the chemicals as well as the formation of metabolites and of non-extractable ^{14}C -chemicals-residues were measured in native and sterilized soils under controlled laboratory conditions and in native soils under outdoor conditions in lysimeters (depth 2 m, 1m² soil surface). Volatilisation and , mineralization were measured "on-line" during the test periods; formation of chemical metabolites and the formation of non-extractable residues were determined shortly after the application of the ^{14}C -chemicals followed by further determinations up to the end of the experiments.

In a first step the bio-availability of the ^{14}C -labeled chemicals in the native soils was estimated from these data. These results were compared with a simple model for bio-availability and degradation of organic xenobiotics in soils. Depending on physico-chemical parameters of the soils time courses of mineralization were simulated. First results of this study will be shown.

Modelling leaching and root uptake of heavy metals in the presence of organic ligands

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In response to various environmental stimuli, plant roots produce organic acids which may change the (bio)availability of metals. For remediation purposes, synthetic chelating agents such as NTA and EDTA are added to enhance the uptake of heavy metals by plant roots. Models that integrate rhizosphere processes of organic acid exudation and degradation, metal uptake, and transport of metals and ligands to soil layers below the root zone, are lacking. The hydrogeochemical model PHREEQC was used to calculate Cu transport and root uptake in a goethite-coated sand in the presence of organic ligands. Processes included were solution- and surface-complexation, (kinetic) goethite dissolution, metal diffusion towards root, root uptake, root exudation, ligand degradation and convective-dispersive transport of the soluble species. A first example calculation showed the ability of EDTA to mobilize Cu. EDTA formed complexes with Cu present in the contaminated root zone. At a slightly acidic pH (pH 6), CuEDTA was strongly adsorbed to the goethite surface as a ternary surface complex. The addition of EDTA lowered the dissolved Cu concentration in the root zone and slightly retarded the movement of the Cu-front. This means that under acidic conditions –and for the defined soil system– EDTA was *stabilizing* Cu in the contaminated root zone. On the other hand, at a slightly alkaline pH (pH 7.5), EDTA was *mobilizing* Cu. It was further shown that FeEDTA, resulting from goethite dissolution, moved faster than CuEDTA due to weaker sorption of FeEDTA. Goethite dissolution kinetics also affected the position of the moving EDTA front. In a second numerical experiment, the effect of oxalate exudation was modeled in the presence and absence of organic matter, represented by chelidamic acid. While oxalic acid is exuded, Cu is strongly sorbed to the goethite surface as a ternary Cu-oxalate surface complex at pH 5, disabling the migration and uptake of Cu. In the presence of chelidamic acid and at pH 7.5, the stabilizing effect of oxalate is counteracted by an enhanced Cu mobilization by dissolved chelidamic acid. The model can be extended to other sorbents, heavy metals and ligands for which consistent thermodynamic data are available. The model will be used for the design of future studies dealing with ligand-assisted metal uptake by plant roots.

Assessment of exchangeable cadmium in soils using Isotope Exchange Kinetics

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Introduction

Cadmium is a biotoxic heavy metal regarded as an important environmental pollutant in agricultural soils because of the potential adverse effects it may pose to food quality, soil health and the environment. It is generally recognised that it is the labile fraction rather than the total soil Cd content which is critical when assessing Cd bioavailability in soils. In response to this concept, there has been a great deal of research conducted to find suitable methods for the determination of bioavailable Cd in contaminated soils. Some of these methods include chemical extractants such as dilute salts, complexing agents and mild acids, ion-exchange resins, sequential extraction procedures and isotope dilution techniques.

Cadmium bioavailability to plants and soil micro-organisms like other ions is a function of the availability of the dissolved metal species in the soil solution and the ability of the soil solid phase to replenish the soil solution. These two components are described as the Intensity factor (I) and Quantity factor (Q) and the relationship between the two as the buffering Capacity (C) of the soil. While many of the techniques proposed above provide information on either one or two of the factors, often estimation of the all 3 factors is not achieved by these techniques.

In recent years, an isotope exchange kinetic (IEK) technique has been developed and tested to describe the kinetic transfer of $^{32}\text{PO}_4$ ions from the soil solution to the soil solid phase (Fardeau 1985; Frossard and Sinaj 1997). It has been shown that the IEK technique was very useful in describing P availability in terms of the concepts of intensity, quantity and capacity factors. More recently, the IEK technique has been applied successfully to study soil Zn exchangeability in polluted and non-polluted soils (Sinaj et al. 1999) and Echevarria et al. (1998) used IEK to assess Ni phytoavailability in 2 Ni polluted soils.

The objectives of this study were to estimate cadmium (Cd) exchangeability in a range of contaminated soils using the IEK technique.

Materials and Methods

Topsoils were sampled from a range of sites which have had different histories of Cd contamination, which included long-term land application of biosolids and from P-fertiliser application.

An IEK batch technique was used to determine Cd exchange kinetic parameters and exchangeable Cd pools (E-values) in the different soils. It has previously been shown that when carrier free ions such as ^{32}P or ^{65}Zn are added to a soil solution system at

steady state, the radioactivity in solution decreases with time according to Equation (1) (Fardeau *et al.* 1985):

$$r_t/R = (r_1/R) \times [t + (r_1/R)^{1/n}]^n + r_\infty/R \quad (1)$$

Where R is the total introduced radioactivity, r_1 and r_∞ are the radioactivity remaining in the solution between 1 minute and infinity and n is a parameter describing the rate of disappearance of the radioactive tracer from the solution after 1 minute of exchange.

Short term kinetic parameters were obtained by plotting the slope of the linear regression between $\log [r_t/R]$ and $\log (t)$ for an exchange time of up to 60 minutes. The parameter n is calculated from the slope of the plot while r_1/R is the inverse log of the intercept along the y-axis. The ratio r_∞/R , is the maximum possible dilution of the isotope and is approximated by the ratio of water soluble Cd to the total soil Cd concentration Equation (2).

$$r_\infty/R = 10 \times C_{Cd} / Cd_{Total} \quad (2)$$

Where C_{Cd} is the water soluble Cd ($mg\ Cd\ L^{-1}$) and Cd_{Total} is total Cd expressed in $mg\ kg^{-1}$.

By analogy to IEK studies of P and Zn (Frossard and Sinaj, 1997; Sinaj *et al.*, 1999) based on the results obtained by IEK for Cd, we propose a three compartmental model to describe soil exchangeable Cd; E_{1min} , which is Cd ions exchangeable within 1 min; $E_{1min-24h}$, Cd ions exchangeable between 1 min and 24 h; and $E_{>24h}$, Cd ions not exchangeable within 24 h.

Results and Discussion

Prediction of exchangeable Cd pools (E-values) for the various soils were made using equations (1). The compartment analysis conducted revealed that there were clear differences in the distribution of Cd in exchange pools between those soils where Cd was derived from phosphate fertiliser or from biosolids (Table 1).

Table 1. Isotopically exchangeable kinetic parameters and isotopically exchangeable Cd [$E(t)_{pred}$] for P-fertiliser and biosolids amended soils

Treatments	P-fertiliser				Biosolids				
Mean total Cd (mg kg ⁻¹)	0.44				1.77				
C _{Cd} x 10 (mg kg ⁻¹)	0.0012				0.0088				
<i>r</i> 1/ <i>R</i>	0.022				0.045				
<i>n</i>	0.223				0.325				
	Cd pools (% total Cd)								
	range			mean		range			mean
<i>E</i> _(1 min) pred	6	–	51	21	<i>E</i> _(1 min) pred	7	–	35	13
<i>E</i> _(1 min – 24h) pred	16	–	38	25	<i>E</i> _(1 min – 24h) pred	32	–	49	42
<i>E</i> _(^{>} 24h) pred	33	–	70	57	<i>E</i> _(^{>} 24h) pred	33	–	55	46

For example, in the P-fertiliser soils, the percentage of Cd located in the $E_{(1\ min)}$ pool, which estimates soil solution and instantaneously exchangeable Cd, was on average 21%, compared to 13% for the same pool for the biosolids-amended soils. This may in

part be a result of Cd being supplied in relatively more soluble forms via superphosphate fertiliser compared to the biosolids-amended soils which likely had part of the Cd added in recalcitrant forms as a consequence of the processing of biosolids prior to land application.

By contrast, the biosolids-amended soils had on average 42% of the total Cd located in the $E_{(1\text{min} - 24\text{h})}$ pool compared to 25% for the P-fertiliser soil. Cadmium in this pool is considered essentially labile, and has the potential to replenish soil solution Cd when it is removed by processes such as root uptake or leaching. The kinetic parameters r_1/R and n that give information on the soil Cd fixing capacity were higher for the biosolids-amended compared to the P-fertiliser soils, indicating a higher buffering capacity. This may be in part related to the significantly higher total carbon values found in the biosolids-amended soils which have been shown in previous studies to be very important with regards Cd sorption and desorption, especially in soils with large amounts of total carbon.

The $E_{(>24\text{h})}$ pool contains Cd not immediately isotopically exchangeable and averaged 57% for the P-fertiliser soil compared to 46% for the biosolids-amended soil. These values for slowly or non-labile Cd are similar to results found in other investigations of Cd availability using isotope dilution techniques. For example, Stanhope *et al.* (2000) found that 52% of the Cd was in non-labile forms in a sludge amended soil in their investigation of Cd mobilisation. While Nakhone and Young (1993) measured Cd availability for 33 soils that had been contaminated with either mine spoil or sewage sludge and found for the sludge amended soils non-labile Cd ranged between 11 – 71% with an average of 52%.

Clearly, the proportion of non-labile Cd in soils varies depending upon the source of the Cd, the soils physical and chemical characteristics and the length of time the Cd has been in contact with the soil.

Conclusions

The IEK technique simultaneously provided information on the intensity, quantity and buffering capacity of Cd in soils. It also indicated that there were differences in Cd availability between soils contaminated by P-fertiliser and biosolids. It appears that the IEK technique has potential as a method of estimating Cd availability in different soils.

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Assessment of Phytoavailability of Soil Zinc

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Zinc is an essential element for plants, animals as well as human. However, at high concentrations Zn can cause toxicity to plants, soil microorganisms and fauna. Prediction of phytotoxicity or ecotoxicity of soil Zn and phytoremediation (revegetation or phytoextraction) of Zn polluted soils need to assess bioavailability of soil Zn to plants.

Due to complex plant root-microorganism interactions in the rhizosphere, phytoavailability of soil metals is believed to be plant-specific. Therefore, plant test is the most direct method for assessment of phytoavailability of soil metals. However, plant test is generally time-consuming and thus expensive. Over the past 20 or more years, scientists have been trying to identify a substitute measurement suitable for routine assessment of phytoavailability of soil metals that correlates with plant uptake. Methods that have been tested include single or sequential chemical extractions, concentration of soluble or free metal ions in soil solution, and isotopically exchangeable pool. More recently, effective concentration C_E value determined by the DGT technique was introduced which considered both the soil solution concentration and metal re-supply from the solid phase.

To identify a soil test with a general applicability, an appropriate soil set comprising of different soil types, physiochemical properties, degree of contamination and contamination history should be used in such studies.

In the present study, 33 soils from diverse sources in the UK, France, Germany, Belgium and China varying widely in soil pH (4.3-7.7), total carbon (0.34%-10.02%) and total Zn (59-27413 mg kg⁻¹) were used in pot trials. *Lepidium heterophyllum* Benth was grown under controlled conditions for 40 days. Six soil tests including soil total Zn, soil solution Zn, the mean interfacial Zn concentration determined by DGT (Diffusive Gradient in Thin Film) device (C_{DGT-Zn}), 1M NH₄NO₃ extractable Zn and 0.05M EDTA extractable Zn and radio-labile Zn (E value) were evaluated in terms of their correlation to plant uptake.

The results showed that correlation was not significant between Zn concentration in *L. heterophyllum* shoots and soil total Zn, Zn E value or 0.05M EDTA extractable Zn. In contrast, 1M NH₄NO₃ extractable Zn, total Zn concentration in soil solution and C_{DGT-Zn} gave good estimation of Zn concentration in the shoots of *L. heterophyllum*. Among them Zn concentration in soil solution and 1M NH₄NO₃ extractable Zn were also significantly correlated with the dry weight of *L. heterophyllum* shoots.

Considering the simplicity and low cost of 1M NH₄NO₃ extraction it can be recommended as a routine method for assessment of phytoavailability of soil Zn to plants.

THE USE OF PLANT TECHNOLOGIES FOR THE CLEAN-UP OF SOILS AND AQUATIC SYSTEMS CONTAMINATED WITH RADIONUCLIDES IS LIMITED WITH POLLUTANTS' BIOAVAILABILITY

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The phenomenon of radionuclides hyperaccumulation by plants, which has been of considerable interest academically may be also used economically in the clean-up of contaminated soils and water. The use of plants for environmental clean-up is an emerging technology, which is called Phytoremediation. The need for radionuclides excluding crop plants is particularly actual in the large areas contaminated after the Chernobyl accident. For example following the close of the Chernobyl Nuclear Power Plant (ChNPP), the cooling pond (22 km²; depth between 1,5 and 15 m) which contain about 111 TBq ¹³⁷Cs and 37 TBq ⁹⁰Sr will have to be dried out and the contaminated sediments should be stabilized. Natural willow vegetation systems and artificial planting of fast-growth willow clones seems to be the most acceptable approach to dry the pond and to stabilize it. We have examined during the few last years the efficiency of that plant technologies for the soils' and water clean-up at the different part of ChNPP exclusion zone. A number of trials were set-up to test the survival of newly planted agricultural herbaceous plants and short-rotating coppice (*Salix sp.*) clones. These are the lessons of our study: *i.* Phytostabilization may effectively prevent the vertical and horizontal migration of radionuclides in some critical cenoses. *ii.* Plant technologies (Rhizofiltration) are very effective for the decontamination of aquatic systems and bottom sediments polluted with different radionuclides. *iii.* The Phytoextraction may be effectively used for the removal of Sr-90 from the contaminated soils because on high availability of this radionuclides. *iv.* Cs-137 pollutions are not appropriate for the phytoextraction practice because on low bioavailability of radiocesium in soil complex. *v.* Additional biotechnological treatments especially with selected soils microorganisms may increase significantly the Phytorextraction efficacy of ¹³⁷-Cs from soil complexes.

The role of NTA in Copper and Zinc Uptake by Sunflowers

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Chelate assisted phytoextraction is seen as a way to improve the efficiency of conventional phytoextraction of metal polluted soils. EDTA has been the most commonly used chelating agent for this purpose but due to its persistence in the environment it is now considered not suitable for field use in many countries including Switzerland. In this study NTA was investigated to see if it could enhance the uptake of Cu and Zn by sunflowers in a hydroponic environment.

Helianthus annuus cv Iregi were grown in a modified 0.1% Hoaglands nutrient solution prior to starting the experiment. Treatments included Cu and Zn as single elements with and without NTA and combinations of both Cu and Zn with and without NTA, along with the control treatments.

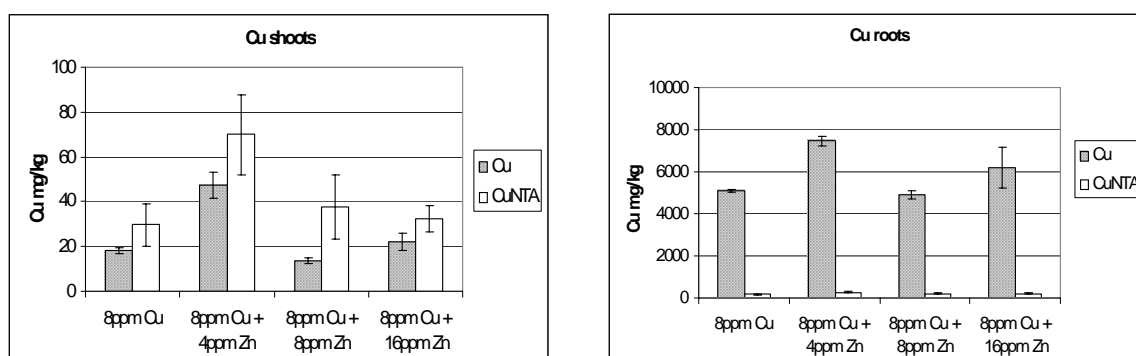


Fig 1. Copper accumulation by shoots and roots of sunflowers with and without NTA in hydroponic cultures

From figure 1 it can be seen that in general, more Cu was taken up by sunflower shoots when accompanied by NTA than without. Speciation of the hydroponic solution however found that when NTA was not present up to 70% of the Cu was precipitated as Copper Phosphate so reducing the copper available in solution considerably. The majority of Cu left in solution was mainly in the free form however which was probably bound in the cell wall of roots due to the positive charge. The addition of NTA also reduced the accumulation of Cu in the roots. In treatments with and without NTA it appears that combining Cu with Zn seems to increase the uptake of Cu into the shoots. The picture for Zn differs quite considerably from that of Cu (Fig 2). In treatments without Cu, Zn is taken up into the shoots in much greater concentrations when not associated with NTA. Speciation of the hydroponic solutions showed that in treatments of solely Zn, the main species are free Zn^{2+} and $Zn_3(PO_4)_2$ which unlike Cu is not precipitated but remains in solution. It appears that the sunflowers have a preference for uptake of free Zn^{2+} and $Zn_3(PO_4)_2$ over ZnNTA.

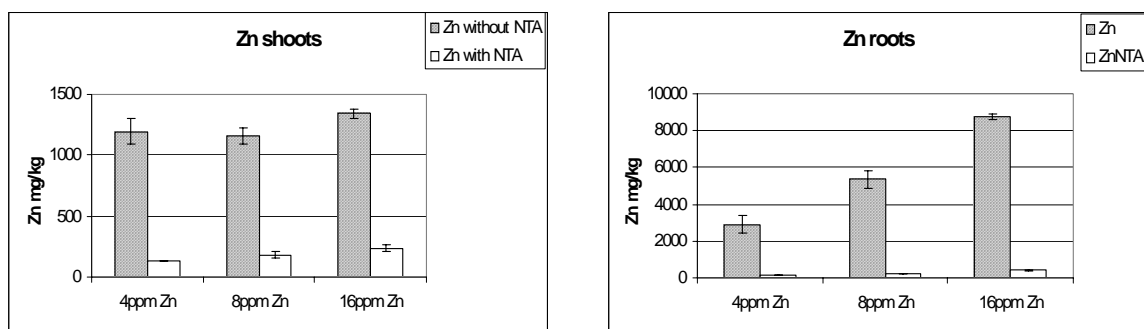


Fig 2. Zn uptake by sunflowers with and without NTA in hydroponic cultures.

In treatments that also contain Cu (Fig 3.) this situation proves more complex, the addition of Cu to the Zn treatments reduces the uptake of Zn to the shoots considerably. This may be due to competition between the two elements for uptake by the roots and transfer to the shoots. The root accumulation of Zn (figure 3) in treatments without NTA also appears to be reduced by the presence of Cu. This shows that it is possible that less Zn in total is taken up by the roots so less can be translocated to the shoots.

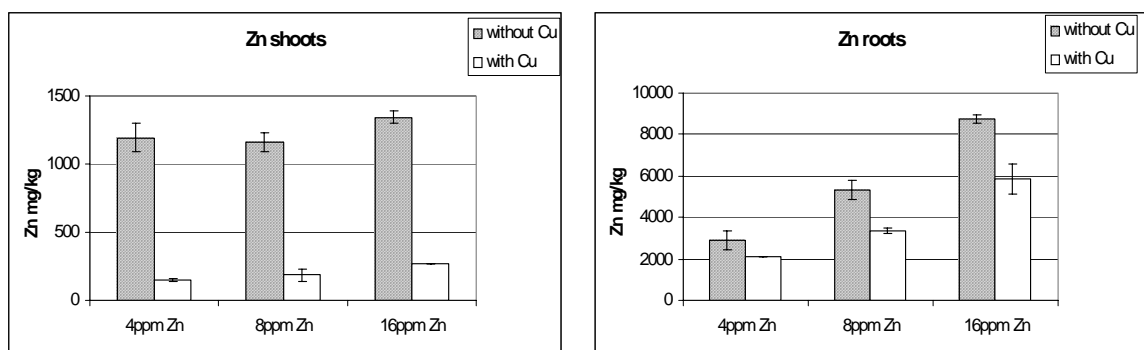


Fig 3. Zn accumulation by sunflowers with and without Cu in hydroponic cultures.

From figure 4 it can be seen that both in the presence of Cu and without it, the uptake of Zn into sunflower shoots shows no relationship with free Zn. The same can be said for the relationship between $\text{Zn}_3(\text{PO}_4)_2$ and Zn shoot uptake. However there is a relationship between both these species and root accumulation of Zn. This therefore shows that another factor comes into play when translocation Zn from the roots to the shoots.

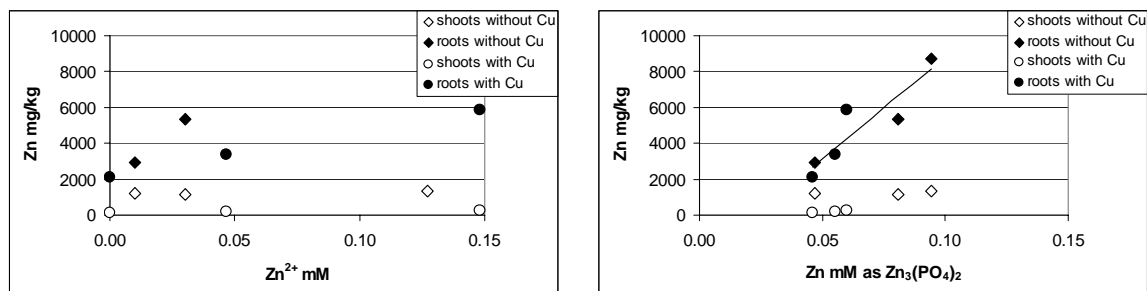


Fig 4. Accumulation of Zn by sunflowers in relation to Zn^{2+} and $Zn_3(PO_4)_2$ in solution.

From this data it is also not possible to say which Zn species is taken up into the shoots although it is obvious from figure 2 that one or both of these species is preferred over ZnNTA. Further work will look at a phosphate free system to try to establish what role $Zn_3(PO_4)_2$ plays in shoot uptake.

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Contamination, bioavailability, and risk assessment

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Risk assessment is a complex issue, because it implies that for comparison risks are classified on a common scale, despite that the adverse effects may differ widely in type, importance, and probability. In view of the latter, the concept of risk is a concept of uncertainty, which helps neither the assessment nor the practical consequences we connect to the assessment. In this presentation, the complexities of risk assessment are discussed without intention to be complete. Different types of risks are mentioned, using different classification schemes, to illustrate that a common scale is not simple. Emphasis is given also to uncertainty, which may be due to a large number of factors. One important factor is incomplete science (but we should recognise that uncertainty and lack of robustness are different concepts), and another one is related with the scale problem.

Risk assessment is sometimes conducted in full agreement with the reductionist science tradition, but of course it should not end there: the synthesis after the analysis of various simple elements is an integral part, and equally important, and cause for hilarious practical results.

The above statements are discussed in the presentation by means of examples. For instance, the value of techniques such as geostatistics in relation with bioavailability and risk is considered, because it combines several of the above complications. Also the concept of bioavailability warrants attention, because it has been interpreted in so many different ways, that have profound implications for the outcome of risk assessment. Both examples have heterogeneity as an example, and though a complicating factor, heterogeneity keeps us alive. It is logical to give it due attention in the presentation. Besides drawing attention to shortcomings, gaps in knowledge, etcetera, suggestions for improvement of risk assessment (how to do it, what is needed,...) are provided also inasfar as natural sciences aspects are concerned.

Having done that, it should be lunchtime.

Speciation dynamics and bioavailability of metals in aqueous systems

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In principle, the bioavailability of metals in complex media is not only involved with equilibrium speciation but also with kinetics of the complex formation/dissociation reactions. Simple equilibrium schemes like the free ion activity model (FIAM) and the biotic ligand model (BLM) only apply if mass transfer towards the biosurface is not flux-limiting and depletion of the medium is negligible. For systems with labile metal complexes this would require that the magnitude of the unsupported flux of free metal ions towards the biosurface is well above that of the maximum uptake flux. More generally speaking one can say that the selective consumption of one particular metal species in an interfacial process calls for careful analysis of the dynamics of the various complexation equilibria and their coupling with mass transport from the bulk medium.

A useful conception for the ability of complexes to maintain equilibrium with the free metal ion is their so-called lability. On 3D level, lability is straightforwardly derived from the pertaining volume reaction rate constants and the relevant time scale. On the level of an interfacial flux of the free metal species, lability also depends on the magnitude of the dissociation-limited flux as compared with the mass transport-limited flux. As a consequence of this, different types of non-equilibrium techniques for speciation analysis usually have different lability windows, i.e. they may provide different measures of the fraction of labile complexes in the sample.

If the formation of a complex species in an aqueous system follows the Eigen mechanism, the rate constant is determined by the rate of dehydration of the inner coordination sphere of the “free” metal ion. For a given metal the complex formation rate constant may thus be estimated on the basis of the tabulated dehydration rate constant. Then for complexes with known stability, the dissociation rate constant and the lability can be readily computed. Lability changes drastically with time and with changing spatial scales. E.g. for microorganisms the lability and bioavailability of complex species are greatly reduced compared to macroscopic biosurfaces. Such aspects, together with hydrodynamic conditions, are of primary importance in the translation of analytical speciation data into predictions of bioavailabilities of complexes towards biota.

Indicators of bioavailability: uptake fluxes or residues?

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It is often argued that the concentration of a pollutant inside an organism is the best indicator of its bioavailability. Extensive monitoring programmes have been developed based on this idea and the toxicokinetic framework for this model is well developed. According to the model, environmental risk of a pollution can be expressed as the ratio between the actual residue and the critical body burden, which is derived from toxicity experiments. Obviously, if a pollutant is detected inside an organism, it must be bioavailable, however, the residue inside an organism is not always the best predictor of its toxicological effect. In this paper I review examples from animal ecotoxicology (studies of terrestrial and aquatic invertebrates) in which toxicity of metals can be better explained from the external than from the internal concentration. The reason is that it is not the residue itself but the uptake flux which determines toxicity. The major part of the internal concentration is in a stored form which is not physiologically active. For these cases, ecological risk is related to the difference between the actual uptake flux and the maximum flux which can be handled by the physiology of the organism. Based on this view, bioavailability should be framed in terms of rates (fluxes) rather than in terms of concentrations. Unfortunately, there does not seem to be an easy way out for risk assessment.

Measures to control and reduce pollutant bioavailability and risk management

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‘Bioavailability’ can be defined as that fraction of the total contaminant content that can interact with a biological target. The bioavailability of a metal in the soil depends on the physical and chemical characteristics of the matrix, the species of the metal in the soil, the biotic components in the soil and the biological organism itself.

In many countries, remediation goals and guidelines still are based on the total metal analysis of the soil. This old paradigm is changing in favor of a more realistic approach which differentiates the ‘bioavailability’ of heavy metal contaminants. To a large degree, public and regulatory acceptance of these new remediation strategies depends on the ability to recognize and quantify this physical, chemical, and biological difference. Such a change in remediation goals and guidelines strongly encourages the development of remediation techniques which remove or convert the bioavailable fraction in the soil to non-available forms.

In order for a technique to be widely adopted at field sites, it must have a strong theoretical background, be demonstrated in the lab, and be successfully translated into a field scale program, and gain regulatory acceptance. For the most part, the technologies that will be discussed in this presentation have accomplished the first two objectives, however field scale validation has been relatively elusive. One of the reasons for the paucity of field scale tests has been uncertain regulatory acceptance of bioavailability arguments. Regulatory acceptance is coupled to the still evolving scientific consensus over how bioavailability should best be measured, and which organisms do we specifically seek to model and protect. These points also will be covered in other papers of this meeting.

A remediation technique that is low cost, environmentally sound, and at the same time sufficiently protective for human health and the environment, would be a valuable alternative to current remediation methods. In this paper an overview will be given of efforts in exploiting biological and chemical processes to reduce the inherent risk associated with metal contaminated soils. Examples of this type of remediation include ‘phytostabilization’ and ‘immobilisation’ (also termed ‘in-place inactivation’ or ‘agronomic stabilization’) and ‘phytoextraction’. All of these different terms refer to the use of soil amendments and plants to extract or alter the chemical and physical form of the heavy metal contaminants in the environment thereby decreasing the chemical and biological ability to cause harm. These technologies draw upon fundamental soil and plant science and well-established agricultural practices.

The fundamental premise of metal immobilisation and phytostabilisation techniques is to reduce the relative bioavailability of a metal in the soil by the addition of soil amendments and by the establishment of biological communities which further inactivates the metals in soil.

Many natural or synthetic materials have been screened in batch test programmes for their ability to decrease trace element mobility and phytoavailability: e.g. lime, phosphates and apatites, iron and manganese materials, aluminosilicates (zeolites, beringite, clays). The effectiveness of these ameliorants has been assessed in several different ways including chemical methods (e.g., selective or sequential chemical extractions, adsorption-desorption isotherms) and biological (e.g., plant growth and dry-matter yield, plant metabolism, ecotoxicological assays and microbial populations).

Phytoextraction removes metal contaminants from the soil through planting and harvesting plants that accumulate metals. This technique exploits plants' unique ability to take up, translocate, and accumulate metal ions from the soil. Initially, researchers thought to use pre-existing plants which accumulate large quantities of metal called hyperaccumulators. Due to their generally small biomass, slow growth rates, and unknown agronomic potentials, the initial phytoextraction field trials have concentrated on other plants, however the concept remains the same. Harvested plants may be composted, landfilled, incinerated, or extracted to recover economically important metals. This technique is often attractive because of its potential low cost and low site input and the eventual production of products that can be economically valorized (f.i. for fiber, oil and/or energy production). Phytoextraction is still developing, and has not yet resulted in the closure of any site, however multiple field demonstrations are ongoing. In many cases, plant availability of metals was reported to be a rate limiting factor. Therefore methods to increase this plant-availability are investigated: use of organic chelates (organic acids, EDTA, DTPA, HEDTA, ...) and manipulation of the rhizosphere.

Phytoextraction may not be a practical remediation technique in all sites. Even in the best case, this technique appears to remove only limited amounts of metal per kg of soil and is limited to the rooting zone. Therefore it may require several centuries to decontaminate contaminated areas. In some cases, careful site management may be necessary to prevent more soluble chelated metals from leaching and off-site migration.

Bioavailability of atrazine and metabolites to maize plants

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Atrazine is a selective and systemic herbicide used in agriculture since 1959 in maize, sorghum, sugar cane, coffee cultures and forestry. About 100'000 tons per year were used in the 1980's. Due to resistance problems and high persistence in environment, atrazine was banned in a few European countries and in others its use has been limited, but residues are still causing serious problems for surface soil and groundwater quality. Some metabolites of atrazine, namely deethylatrazine (DEA) and deisopropylatrazine (DIA), can be accumulated in soils and are still showing herbicide activities (Loiseau, 2001).

In a joint project (Indo Swiss Collaboration in Biotechnology) possible remediation techniques are being investigated to remediate soils contaminated with residues of atrazine by means of plants, which metabolize the pesticides, stimulate their microbial degradation or inactivate them in the rhizosphere. The concept of using plants in association with microorganisms to remediate soils contaminated with organic pollutants is based on observations that microbial degradation of organic chemicals is accelerated in vegetated soils compared to surrounding non-vegetated bulk soils. To further enhance decontamination, high biomass crop plants and non-agricultural plants are screened for their potential to extract and/or transform the targeted pesticides in hydroponic systems and pot experiments.

In this study we investigated the ability of maize plants either to extract atrazine or desethyl-atrazine (DEA) from nutrient solution or to stimulate the degradation of the two compounds in the solution. Maize seedlings were exposed for 15 days to either 2 ppm atrazine or 2 ppm DEA. A modified Hoagland solution was used, which was produced from pure salts and NANOpure water with pH adjustment to 6.

Preliminary results showed that maize was resistant to 2 ppm atrazine, even with a maximum bioavailability create by the model plant system with hydroponic solutions. Furthermore, atrazine decreased in the nutrient solution to 20% of the initially added concentration during the experimental time of 15 days. At the same time a development of the metabolites DEA and hydroxyatrazine (HO-Atz) was observed (Figure 1). In the DEA experiment (Figure 2), however, 80% of DEA was removed after 15 days, while no metabolites could be observed in the solution.

Because we did not work under totally sterile condition, the development of DEA in the atrazine experiment might be explained by microbial activity. While the concentration of DEA remained about the same for the first 10 days of the experiment it considerably decreased between 10 and 15 days of experimental time. A decrease of DEA was also observed in the DEA experiment without development of further metabolites in the solution. The results indicate that DEA might be taken up by plants.

HO-Atz concentrations increased between 5 and 10 days of experimental time and remained then constant until the end of the experiment. One possible hypothesis

explaining the increase of HO-Atz is that maize might have exuded a substance causing the hydroxylation of atrazine.

It has been shown that maize exudes a cyclic hydroxamic acid (Petho 1992), which was shown to be able to transform atrazine to HO-Atz (Raveton et al. 1997).

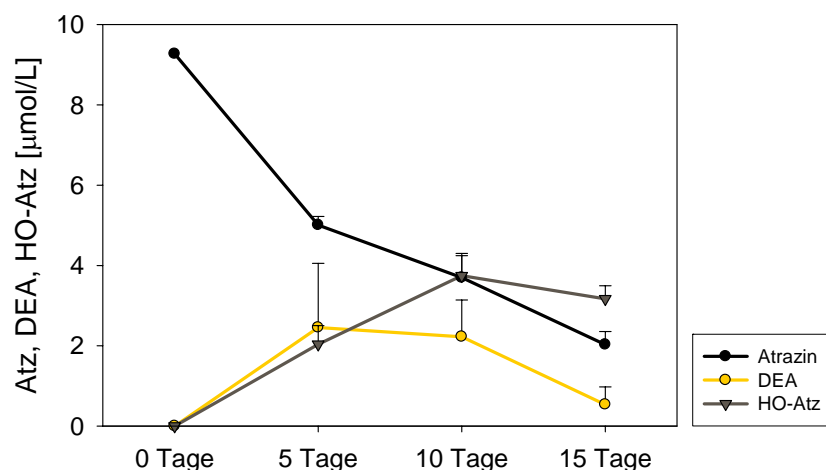


Figure 1: Effect of maize plants on atrazine removal and formation of metabolites in nutrient solution spiked with 2 ppm atrazine

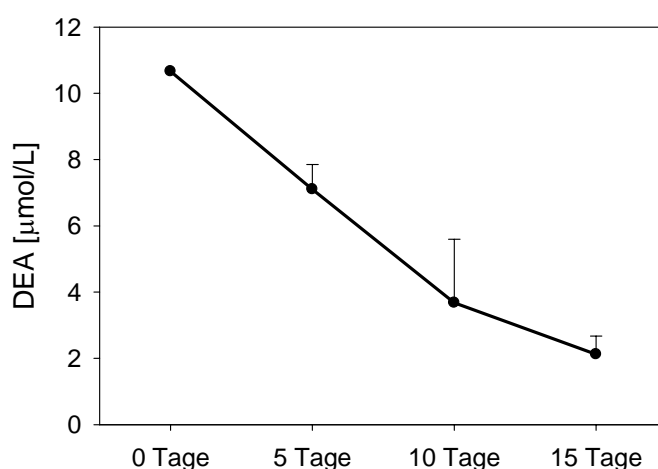


Figure 2: Effect of maize plants on DEA removal from spiked nutrient solution spiked with 2 ppm DEA

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Biotic Factors Controlling Pollutant Bioavailability: Rhizosphere Processes

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Based on a review of concepts of bioavailability we propose a comprehensive definition, including aspects related to metal / metalloid uptake and toxicity. Bioavailability is understood as a potential rather than the realization thereof by the plant. Major controls of bioavailability in the soil include soil physical (transport via mass flow and diffusion), chemical (concentration in solid phase, soil buffer power) and biological (turnover by microorganism) features. The combination of these factors is constituting the generic bioavailability of a metal / metalloid in a given soil. At the root-soil interface and in the adjacent rhizosphere soil, this generic bioavailability is typically altered as a consequence of root and microbial activities. Actual plant uptake and toxicity will further depend on plant-internal factors such as metal / metalloid demand and tolerance. Feed-back loops between the plant physiological status and the bioavailability in the rhizosphere are thought to dynamically modify the effect (uptake or toxicity) on the plant.

Modifications of pollutant bioavailability in the rhizosphere can be studied using field, pot and rhizobox techniques. We have studied the root-induced changes of pollutant concentrations in soil solution and the labile pool (1 M NH_4NO_3 extractable) in the rhizosphere of metal / metalloid hyperaccumulator species, including *Pteris vittata* (As), *Thlaspi goesingense* and *T. caerulescens* (Cd, Zn, Pb, Ni) and various willow and poplar species (Cd, Zn, Pb).

In agreement with literature data, we found little evidence for pH-induced changes in metal solubility and lability in rhizosphere soil. The role of organic chelates exuded by hyperaccumulator roots is less clear, even though most studies do not support a significant contribution to the hyperaccumulation phenomenon. However, in contrast to some hydroponic studies, some of our field and rhizobox experiments give evidence for enhanced DOC and metal solubility in hyperaccumulator rhizospheres.

Numerous observations solicit that the labile, potentially bioavailable metal pool (1 M NH_4NO_3) and the kinetics of replenishment (measured using DGTs) are typically decreased within few weeks of excessive exploitation by hyperaccumulator species, whereas the metal concentration in soil solution is often maintained or even enhanced. Despite the lack of direct evidence for chelate exudation and formation of metal-organic complexes in the rhizosphere, it is obvious that some mechanisms of replenishment from fixed metal pools must exist. This is discussed using experimental results and modeling.

Finally, the dependence of bioavailability of metals on soil-plant-microbe interactions is emphasized and the significance for natural attenuation and phytoremediation is discussed.

Bacteria as Bioavailability Enhancing Agents

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Although limited bioavailability appears to be primarily a physical, i.e. mass transfer-controlled process, different recent observations indicate that organism-specific bioavailability-enhancing strategies may exist and that generalizations about the bioavailability of sorbed, solid or dissolved substrates are inappropriate. Several reports, using polycyclic aromatic hydrocarbons (PAH), indicate that sorption-limited bioavailability plays an important role in the selection of PAH-degrading bacteria and that different PAH-degrading bacteria inhabiting the same soil may be adapted to different degrees of PAH-bioavailability. In these studies, different bacterial strains or microbial assemblages were selected depending on how the compound was provided to the bacteria (Tang et al., 1998; Bastiaens et al., 2000; Friedrich et al., 2000). This indicates that the physiology of a bacterium or its particular life style might play an important role in its adaptation to degrade HOC by providing a strategy for enhancing the compound's bioavailability.

Bioavailability for degradation of hydrophobic organic pollutants is an interactive process that is determined by the rate of physical mass transfer to microbial cells relative to their intrinsic catabolic activity (Bosma et al., 1997). Bioavailability can be conveniently described by the bioavailability number, B_n , (Koch, 1990), which is defined as the capacity of an organism's or a population's environment to provide a chemical, divided by the capacity of the organism or population to transform that chemical. This definition points at the importance of continuous substrate supply for consumptive processes and distinguishes bioavailability for degradation from bioavailability for basically 'non-consumptive' processes such as poisoning or inhibition. At high mass transfer rates, the overall degradation rate is controlled by the metabolic activity of the bacteria, i.e. the specific activity of the cells and the population density ($B_n > 1$). Conversely, when the transport of the substrate decreases or the bacterial population grows, mass transfer may become the factor that controls the degradation ($B_n < 1$). As bacteria appear to degrade chemicals only when they are dissolved in water, mass transfer to the cells in the aqueous phase is best described by Fick's first law of diffusion (eq. 1), whereas bacterial transformation can be described by the whole-cell Michaelis-Menten kinetics. From Fick's law it becomes clear that the substrate solubility is one of the keys to understand bioavailability as it limits the potential size of the diffusion gradient and concomitant diffusive mass transfer to the cell.

$$Q/t = D \cdot A \cdot (C_0 - C_x) / x \quad (1)$$

In equation 1 Q is the quantity of substrate (mol) diffusing through area A (m^2) per unit of time t (s). $(C_0 - C_x) / x$ is the concentration gradient where C_0 is the concentration at the source ($mol\ m^{-3}$), C_x is the concentration at the bacterial surface ($mol\ m^{-3}$) and x is the diffusion path length (m) i.e. the distance between source and sink. D is the diffusion coefficient ($m^2\ s^{-1}$) expressing the resistance to diffusion. In presence of bacteria, steady state is to be expected when solid or non aqueous phase (NAPL)-bound PAHs provide a

source and bacteria act as a sink, i.e., when the bacterial consumption of PAH drives the dissolution of PAH from the source.

Experimental evidence and theoretical considerations show that specific HOC-degrading bacteria may adapt to low substrate regimes by the utilization of bioavailability enhancing strategies, in order to create steep concentration gradients and concomitant fast diffusive substrate transfer. Bacteria may increase the substrate bioavailability (i) by reducing the mean distances between pollutants and bacteria, e.g. by adhesion to sorbents (shortening of the diffusion path length (x)), (ii) by causing active transfer of pollutant into the aqueous phase by production of biosurfactants and bioemulsifiers (optimization of the diffusion coefficient (D) and the area (A)), and (iii) by using uptake systems with high specific affinity, i.e. exhibiting efficient substrate degradation at low aqueous concentrations (reduction of the concentration at the sink (C_x)).

By using the poorly water-soluble solid polycyclic aromatic hydrocarbon anthracene as sole carbon source, we examined the possible role of bioavailability-promoting strategies in anthracene degradation by *Mycobacterium* sp. LB501T in batch cultures (Wick et al., 2002b). This strain had been isolated from PAH contaminated soil using a new Teflon membrane-based extraction method that appeared to select strongly hydrophobic bacteria (Bastiaens et al. 2000). *Mycobacterium* sp. LB501T exhibited a high specific affinity ($a^{\circ}_A = 32500 \text{ L g}_{\text{protein}}^{-1} \text{ h}^{-1}$) for anthracene. a°_A is defined by the slope of the first-order part of the whole-cell Michaelis-Menten uptake-rate versus concentration plot (Button, 1985). A high specific affinity allows to reduce the substrate concentration at the bacterial surface in relation to the bulk aqueous substrate concentration and thus leads to steep concentration gradients and fast diffusive substrate transfer. The observed value is up to 1000 times enhanced as compared to other PAH-degraders and indicates that *Mycobacterium* sp. LB501T is well-adapted to the naturally occurring low range of aqueous anthracene concentrations ($C_w^{\text{sat}} = 3.47 \times 10^{-7} \text{ mol L}^{-1}$). Furthermore *Mycobacterium* sp. LB501T exhibited low requirements for cell maintenance. A low maintenance rate characterizes the ability of a microorganism to survive and to grow on a very low amount of substrate consumed per unit time. Although low ‘maintenance coefficients’ do not directly affect the bioavailability, i.e. increase mass transfer to and into the cell, respectively, they may form a specific ecological advantage of a pollutant degrading community to build up an active biomass (Pirt, 1965). The less substrate an organism needs to survive, the better it is equipped to survive periods of famine and the better it is also capable of building up active biomass under a low-substrate regime, which may respond quickly to newly available pollutants and thus enhance the bioremediation efficiency.

When solid anthracene served as sole carbon and energy source, *Mycobacterium* sp. LB501T grew as a confluent biofilm on the anthracene crystals. Based on Fick’s law, a small distance between a biofilm and a solid substrate favours the diffusive mass transfer and, consequently, the bioavailability of anthracene, by giving rises to steep aqueous concentration gradients. Moreover, biofilm cells appeared to consume most of the dissolving solid anthracene as was seen from very low dissolved anthracene concentrations in the bulk medium. Cells at the crystal surfaces “etched” craters in the crystals due to consumption-driven PAH-dissolution on a micro-scale. Biofilm formation by strain LB501T seems to be a well-regulated process as no biofilm was formed on anthracene in the presence of alternative, soluble carbon sources or when high amounts of solid anthracene, leading to high substrate fluxes, were supplied.

No production of biosurfactants was observed. However, altered cell wall compositions (mycolic acids, phospho- and glycolipid fatty acids (PLFA/GLFA)) and physical

surface properties of the bacteria and no biofilm formation on anthracene was detected when better available substrates such as glucose were present: Anthracene-grown bacteria were significantly more hydrophobic and more negatively charged than glucose-grown cells. In batch and column adhesion experiments, anthracene-grown cells adhered 1.5 - 8-fold better to hydrophobic Teflon and up to 70-fold better to anthracene surfaces than glucose-grown cells. It was further found that *Mycobacterium* strain LB501T exhibited specific biochemical modifications of the cell envelope in response to solid anthracene, such as changes in its mycolic acid profile and a shift to more hydrophobic mycolic acids, respectively (Wick et al., 2002a). This finding is of interest as mycolic acids are believed to stimulate attachment to hydrophobic surfaces and hence increase the access to hydrophobic substrates (Bendinger et al., 1993). Studies on membrane-bound PLFA- and GLFA of *Mycobacterium* sp. LB501T further indicate that *Mycobacterium* sp. LB501T is specifically adapted to the growth on this poorly water soluble substrate. Despite of the low aqueous solubility of anthracene no distinct signs of stress, such as increased fractions of cyclopropyl or saturated fatty acids, of anthracene-grown cells as compared to glucose-grown cells were detected. Seen the observation that mycobacteria belong to one of the few taxonomic groups responsible for PAH degradation in soils, this finding thus is not far-fetched. In conclusion, our results indicate that attachment and biofilm formation may be a specific response of *Mycobacterium* strain LB501T to optimise substrate bioavailability.

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Influence of root exudates on transport of heavy metals in soils: assessment by combining unsaturated column experiments with spatially resolved soil solution monitoring

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Soils in general have a large storage capacity for heavy metals, but due to the sensitivity to many chemical, physical and biological factors, the leaching of heavy metals from the polluted soils is a serious problem. The effects of heavy metals on plants have been studied extensively. Especially the uptake of heavy metals by plants has received enormous attentions because of the importance for food production and human and animal health. Nevertheless, the influence of plants on heavy metals has received much less attention. Only few studies have examined the effect of plant on heavy metals transport. Both increased and decreased leaching of metals compared to plant-free systems is found. Low-molecular-weight organic acids (such as citrate or oxalate) which are exudated from plant roots into the rhizosphere may play an important roll in metal mobilization and transport in soils.

The aim of this study is to investigate the mobilization and transport of heavy metals through a soil profile affected by the plant exudates. This will provide us the useful information to evaluate the potential long-term risks on leaching and mobility of heavy metals in soils.

In unsaturated column experiments, leaching and transport of heavy metals (Zinc and Copper) were studied in the presence of *Lupinus albus* growing in polluted soil (Figure 1). A nylon mesh fixed in the column separates the unpolluted soil from the polluted soil and prevents the roots to grow into the unpolluted soil. Thus, the mobilization of metals can be observed in the upper part, and the transport and biodegradation of ligands and the immobilization of metals in the lower. To get the soil solutions, a set of suction cups are installed along the column.

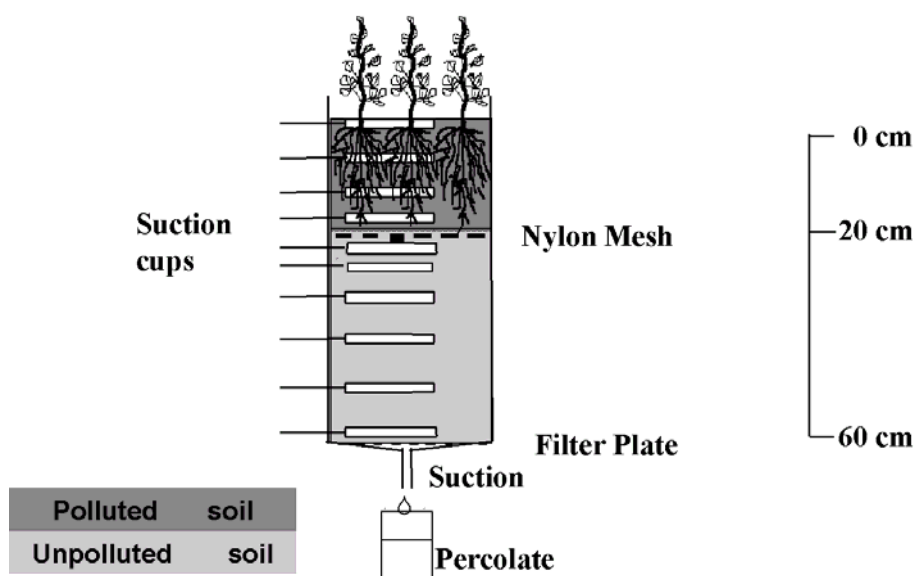


Figure 1. The set-up of the column experiment.

The soil solutions from the suction cups are taken in bi-weekly intervals. The concentrations of labile metals, total dissolved metals and low-molecular-weight organic acid are measured by differential pulse anodic stripping voltammetry, atomic adsorption spectrometry, and ion chromatography respectively.

The experimental set-up will allow us to study both mobilization and transport of heavy metals in soils under the influence of plant exudates.

Vulnerability of a calcareous soil to further Cd-input compared to its calculated critical load for Cd

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Calculating critical loads for cadmium in terrestrial ecosystems (according to DeVries et al., 2002) results in very low values in soils developed on limestone and very high values in soils developed on gneiss (Rihm and Thoeni, 2002). This suggests a high vulnerability of calcareous soils to further input of Cd.

We tested the hypothesis of a high vulnerability of these calcareous soils in a lab-experiment with a forest soil from Breitenbach (Swiss Jura). This soil has a calculated critical load which is $< 3 \text{ g Cd ha}^{-1} \text{ y}^{-1}$ and falls within the category of soils with the highest vulnerability in Switzerland (Rihm and Thoeni, 2002). Undisturbed soil cores from the top soil were percolated with solutions of increasing Cd-concentrations until a quasi-equilibrium was established. Various chemical properties were measured in the percolating solution: pH-value, electrical conductivity, content of organic C, total concentration of Cd, and concentration of the labile Cd-species. The effect of the various percolating solutions on the growth of a fresh water microalgae (*Scenedesmus vacuolatus*) was tested according to the method developed by Knauer et al. (1997).

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