BIOSTIMULATION AND BIOAUGMENTATION - BASIC STRATEGIES OF THE ADVANCED *in situ* BIOREMEDIATION

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Contaminated Sites Bratislava 2013

OUTLINE

- Bioremediation technologies
- Biostimulation and bioaugmentation: perspective and prospective strategies of bioremediation; how to ensure the success of such a processes
- Results on bioremediation of PCB-contaminated sediments using biostimulation and/or bioaugmentation approaches
- Conclusions

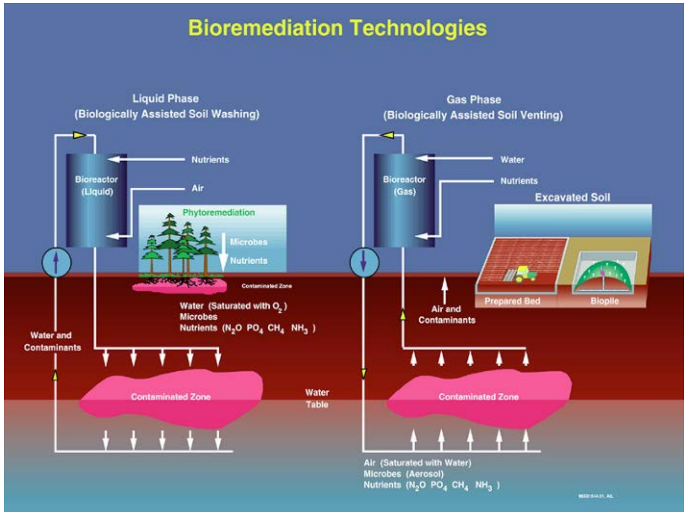
CLASSIFICATION OF REMEDIATION TECHNOLOGIES

- Combustion technology
  (at imperfect combustion toxic compounds are produced)

- Non-combustion technologies:

- 1. **Physico-chemical methods (expensive, require high energy demand and consumption of chemical reagents)**

- **2. Biological methods – BIOREMEDIATION – economical and ecological alternative of decontamination**



Why use bioremediation?

Bioremediation can be defined as any process that uses **microorganisms**, **green plants** or their **enzymes** to return the environment altered by contaminants to its original condition (transformation of harmful chemicals into the harmless ones)

- Terminal destruction - mineralization

- Detoxification
- On-site treatment
- Eco-friendly
- Cost-effective

emerged as the most advantageous clean-up technique for CS (HM+OP)

Unfortunately, being a natural process, it requires time

RISKS OF APPLICATION OF BIOREMEDIATION TECHNOLOGIES

1. **Insufficient knowledge of metabolites and byproducts of biotransformation**
2. **The possibility of appearance of pathogenic strains in the heterogennous polyculture-consortium**

↓

ecotoxicological and sanitation risks

A hierarchy of in situ treatment approaches

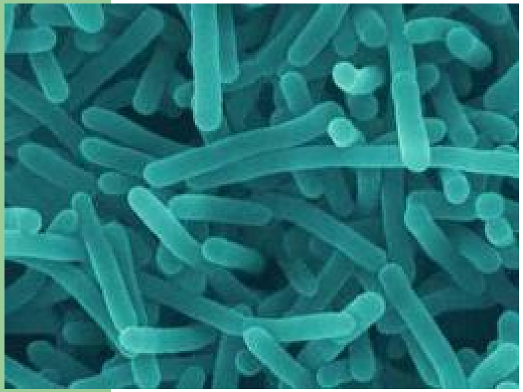
contaminant types, site-specific conditions, regulatory factors

Established practices for enhancing bioremediation:

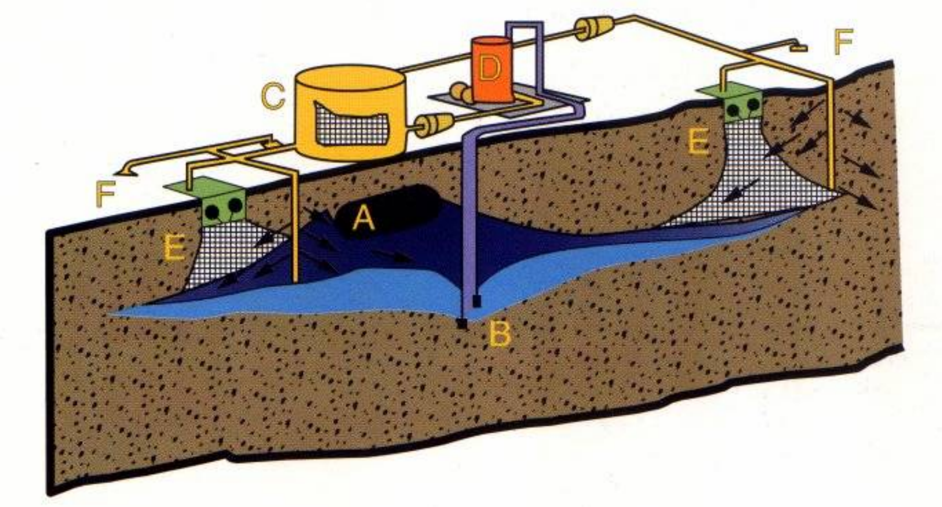
1. NATURAL ATTENUATION

2. ASSISTED BIOREMEDIATION

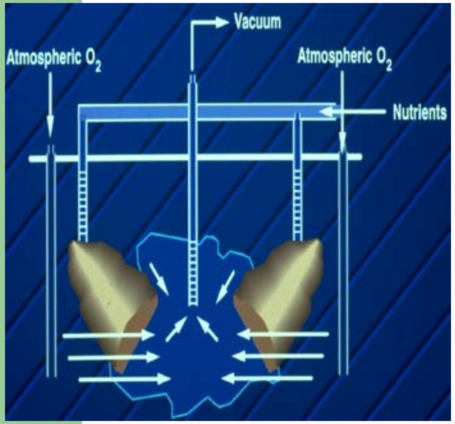
- **Biostimulation (nutrients)**
- **Bioaugmentation (inoculum of exogenous or indig. microorganisms)**



In situ Bioremediation Techniques
(involves direct treatment at the contaminated site)



Bioventing

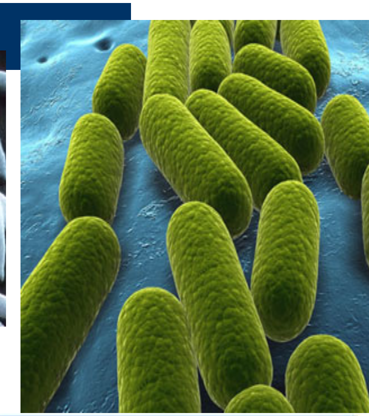


Biosparging

Permeable Reactive Barriers

Organisms used for biotreatment

bacteria BIO-REMEDIATION

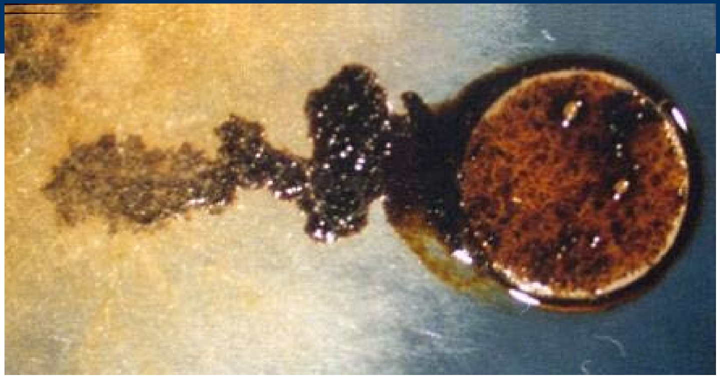


plants PHYTO-REMEDIATION

fungi MYCO-REMEDIATION

Application of mycoremediation at oil spills and accidents

- **Mexican Bay**
- **Amazon – Equador – decontamination of oil lagoons**



degradation and sorption ability of fungi

a) BIOSTIMULATION

injection of **nutrients** (and other **supplementary components**) - to increase populations of indigenous, naturally occuring microorganisms, to encourage their growth and to maximize degradation rate of target pollutant

Applied when degradative population exists but concentration of nutrients (or other conditions) are insufficient for microbial activity

b) BIOAUGMENTATION

Application of indigenous or exogenous microorganisms to polluted sites in order to improve degradation ability and/or to accelerate the removal of undesired compounds.

Applied:

1. Competent degradative organisms are not present among the indigenous population, or **their amount is insufficient**
2. Competent degradative organisms are present - **to increase the rate of degradation** and thereby **shorten the time frame** for full scale remediation

SUCCESSFUL BIOAUGMENTATION DEPENDS ON THE CRITERIA

1. Colonization ability – exogenous MO must be able to survive and maintain population, compete with indigenous MO, adapt to the environment, resist toxins
2. Degradation ability – to preserve degradative ability, to express appropriate enzymes capable of degrading target compounds
3. Bioavailability of contaminants – MO must have contact with the contaminant for degradation reaction to take place

Limitation:

microorganisms introduced into a foreign environment may have sometimes a hard time surviving

Positive aspects:

it probably supplies the plasmids containing the relevant genes, which can be spread through the soil population even if the inoculum itself does not survive! (HTG)

The enhancement of bioaugmentation

in soil, sediment, and groundwater can be performed by delivering:

- individual strains (adapted allochthonous and autochthonous MO)
- microbial consortium
- immobilized cells (encapsulated into PVA gel or immobilized by adsorption on various carriers)
- activated soil (soil exposed previously by contaminants)
- genetically modified organisms (GMO)
- genetically mobile elements (GME - plasmids) (HTG)

Technologies for delivering microorganisms to polluted areas

Bacteria introduced in liquid culture stage – does not guarantee proper distribution, their shelf life, and activity

Carriers	Microorganisms	Pollutants degraded
κ-Carrageenan	Pseudomonas sp. UG30	Pentachlorophenol
Polyvinylalcohol	hydrocarbon-deg. bacteria	Diesel oil
Polyurethane foam	Rhodococcus sp. F92	Petroleum products
Chitin, Chitosan	hydrocarbon-deg. bacteria	Crude oil
Alginate, Agar,	Pseudomonas fluorescens	Ethylbenzene
Zeolite, Activ. carbon	hydrocarbon-deg. consortium	Crude oil

CARRIER MATERIALS

- **maintain sufficient activity of inoculants** over a prolonged period

- **provide protective niche and temporary nutrition** for introduced microorganisms being non-toxic to inoculants and soil organisms

Biocarriers:

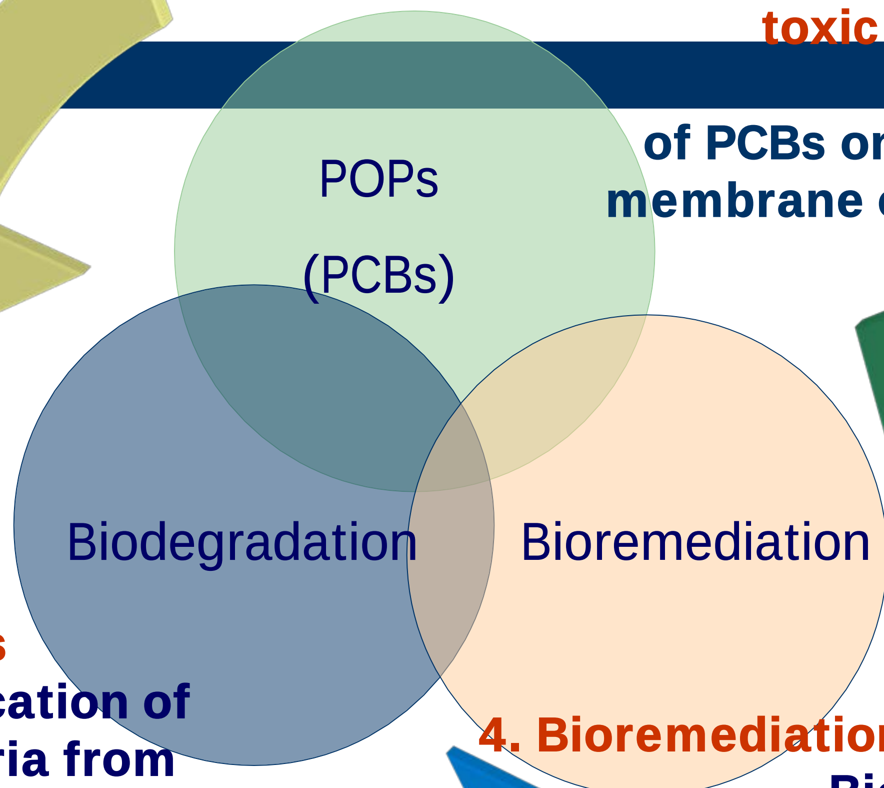
- stimulate - microbial colonization, enhanced degradation
- improve - diffusion of oxygen, nutrient mass transfer, and water holding capacity – limiting factors of pollutant biodegradation

RESEARCH TOPICS

1. Characterization of PCB-contaminated sediments

Bioavailablity of PCBs
Ecotoxicity and genotoxicity of sediments

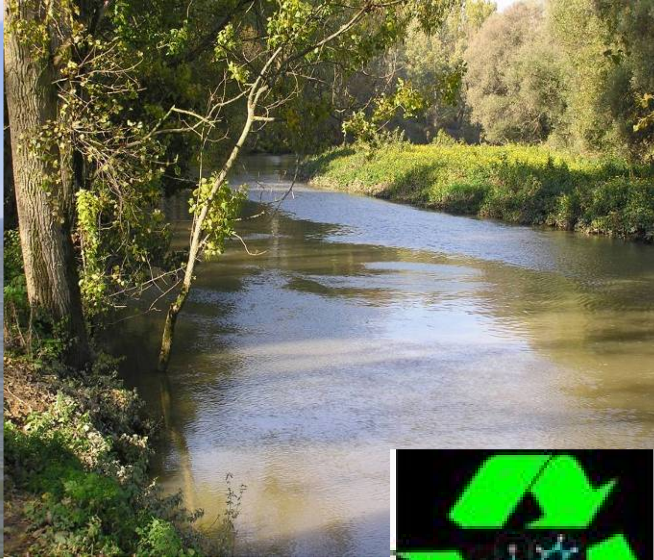
3. Adaptation responses of bacterial cell membrane on the toxic environment –
the effects of PCBs on fluidity of cell membrane of the bacterial degraders



2. Bacterial degraders
Isolation and identification of PCB-degrading bacteria from contaminated sediments;
Detection of *bphA* gene

4. Bioremediation of sediments
Bioaugmentation
Biostimulation
approaches






PCB-CONTAMINATED SEDIMENTS

- Sediments - essential, integral, and dynamic part of the hydrological system
- Ultimate reservoir for the chemical (and biological) contaminants posing ecological and human health risks
- Several hundred tons of PCBs have been released into the environment in past due to hydrophobic properties - PCBs tend to be adsorbed by the sediments – they represent an abundant source causing contamination of the waters of Eastern Slovakia

Polychlorinated biphenyls (PCBs)

low water solubility, high hydrophobicity, low degradability, bioaccumulation, high toxicity, semi-volatility, persistence in the environment

PCBs are considered **„endocrine disruptors“**

3,500 tons of PCBs may be found at the territory of SR

Production:

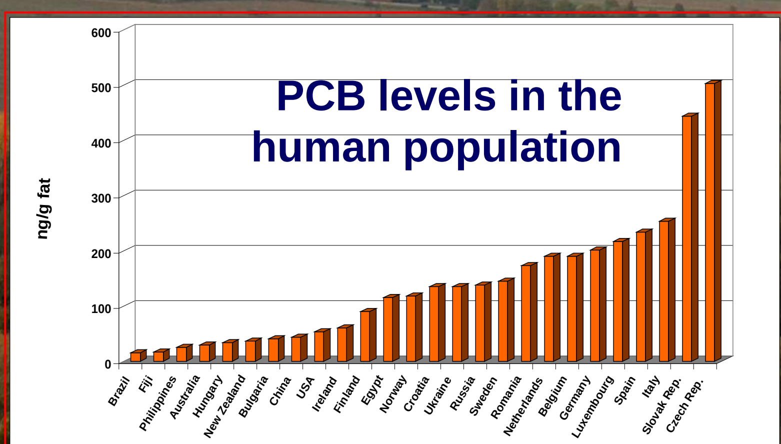
21,500 tons

1959-1984

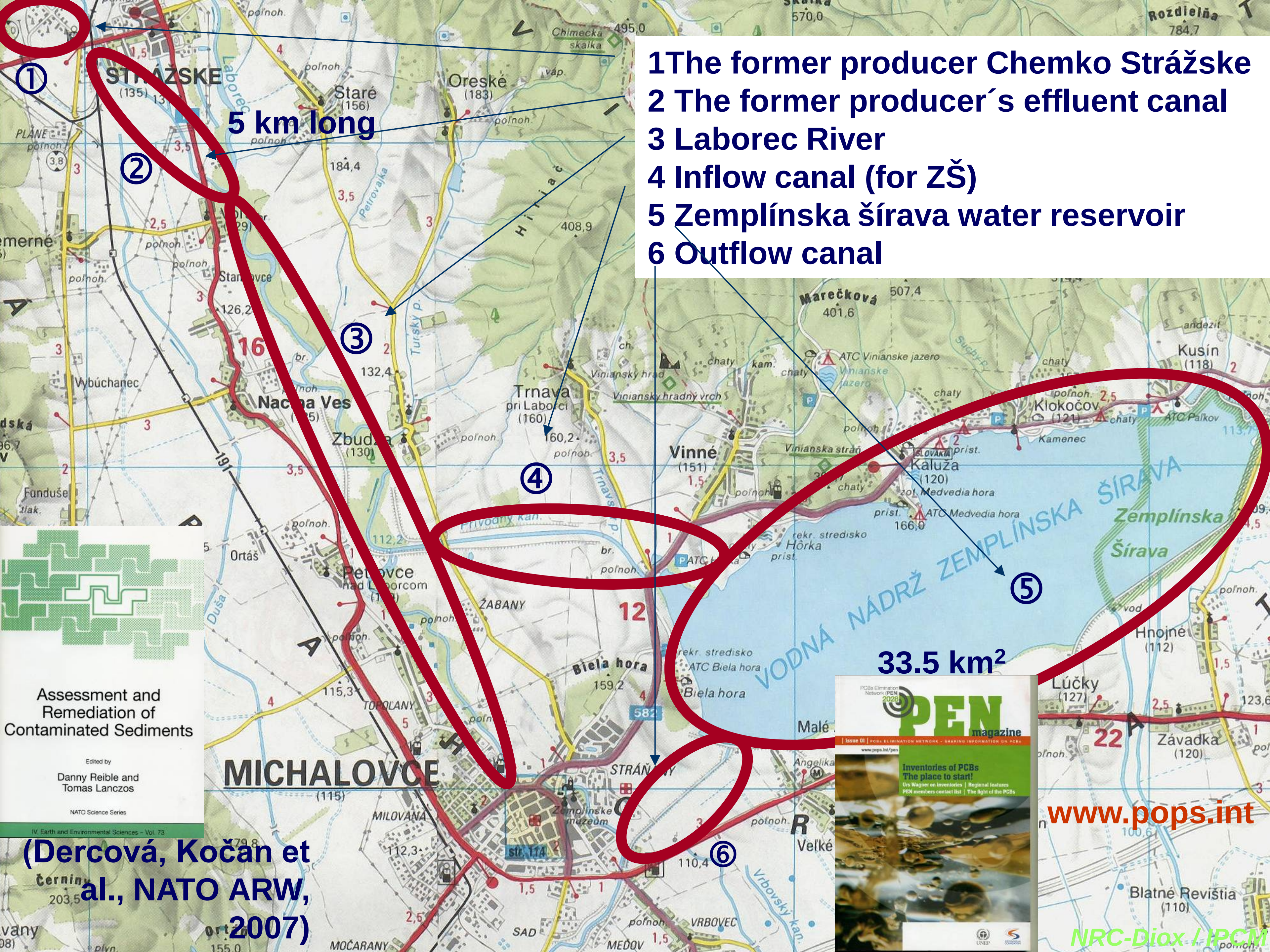
„Old Environmental Burdens“
Hot spot and urgent problem in Slovakia

Chemko Strážske – former PCB producer

Strazske Town



(Holoubek, 2006)


1The former producer Chemko Strážske
2 The former producer´s effluent canal
3 Laborec River
4 Inflow canal (for ZŠ)
5 Zemplínska šírava water reservoir
6 Outflow canal
5 km long
33.5 km2
①
②
③
④
⑤
⑥
Assessment and Remediation of Contaminated Sediments
Edited by
Danny Reible and Tomas Lanczos
NATO Science Series
PEN magazine
Inventories of PCBs The place to start!
(Dercová, Kočan et al., NATO ARW, 2007)
www.pops.int
NRC-Diox / IPCM

Sampling sites:

Strážsky canal: 1 – 10 g/kg of sed.d.w.

Laborec River: 0.1 - 1 g/kg

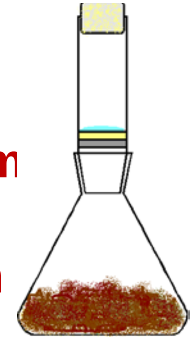
Zemplínska Šírava Lake:0.001 - 0.1 g/kg

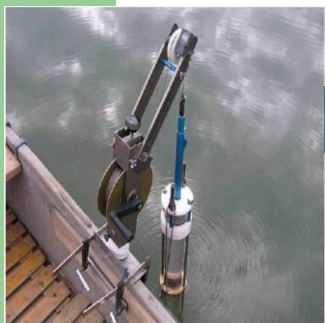
The sediment corer sampler (UWITEC Corp. Austria), STN

Initial approximation assumes: 40,000 tons of PCB-contaminated sediments

Experimental design

Sediment
Mineral medium
Inducers
Microorganism



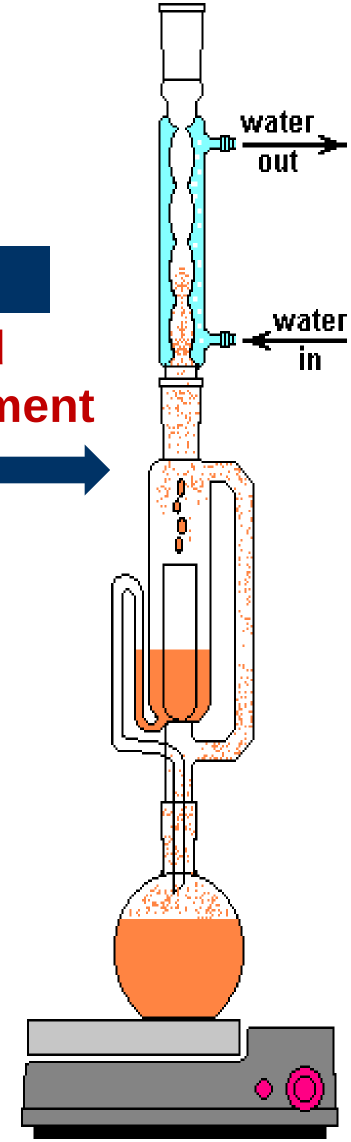


Sampling of sediments

Biodegradation
7,14, and 21 days

Centrifugation

Dried
sediment



Soxhlet apparatus - extraction

GC-ECD analyses

Evaluation of
degradation (%)

Isolation of DNA and identification of *bphA1* gene determined in the genome of selected strains

980 bp

Gel electrophoretic proof – visualization of bands of ***bphA1*** gene encoding BPDO – biphenyldioxygenase *

15 new strains were isolated and 11 from them were identified by the 16S rRNA gene sequence phylogenetic analysis as follows:

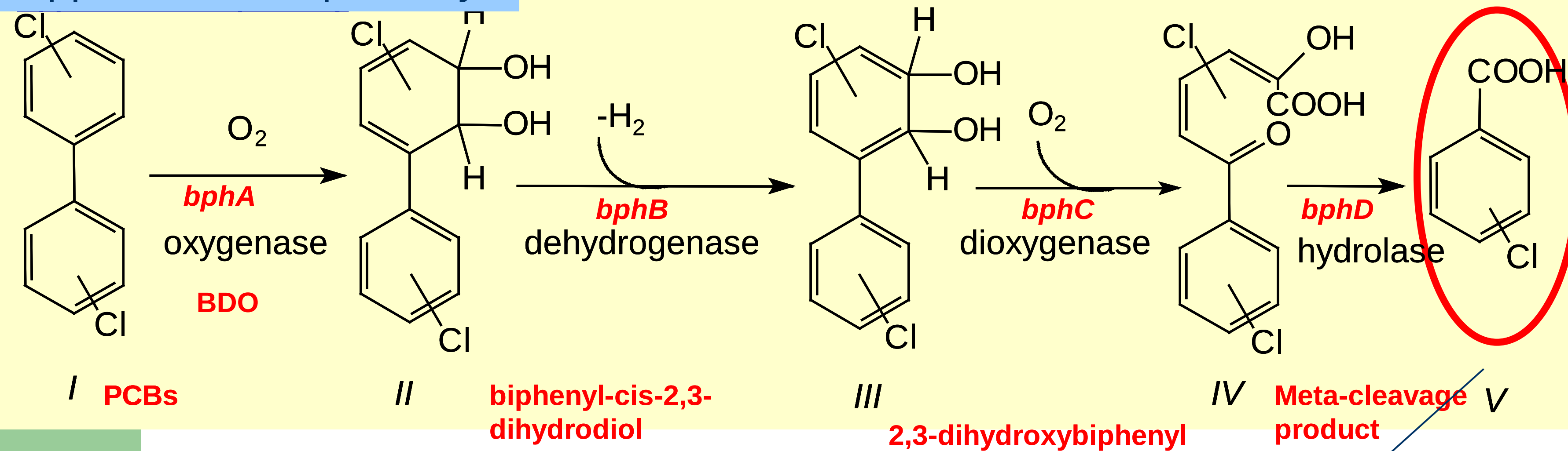
Microbacterium oleivorans
Brevibacterium sp.
Pseudomonas mandelii
Stenotrophomonas sp.
Pseudomonas aeruginosa

Stenotrophomonas maltophilia
Ochrobactrum anthropi
Rhodococcus sp.
Ochrobactrum sp.
Starkeya novella
Achromobacter xylosoxidans *

Dudášová et al., JBM 2013

BIODEGRADATION OF PCBs

Upper catabolic pathway



+2-hydroxy-penta-2,4-dienic acid

Chlorobenzoic acid

Lower catabolic pathway

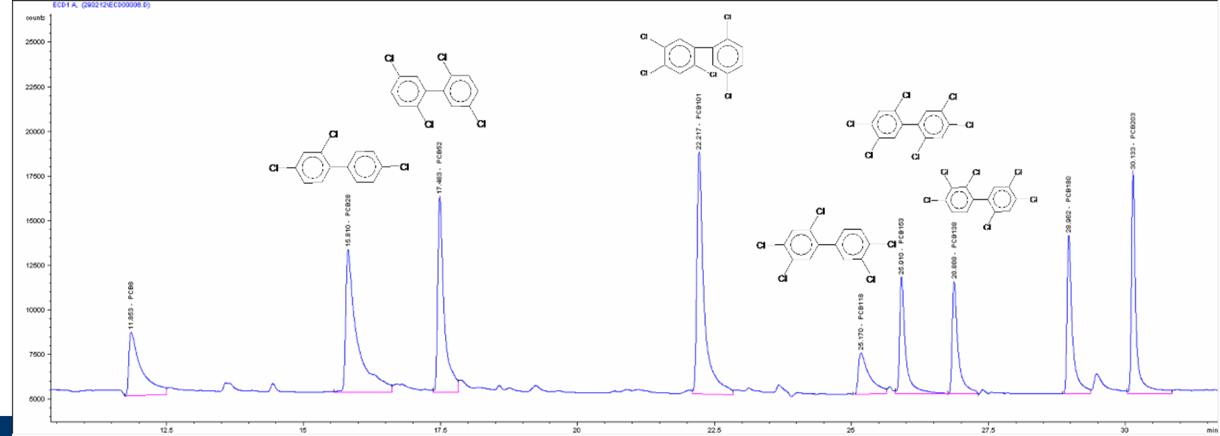
Meta-cleavage pathway (end-product protocatechuate)

Oxo-adipate pathway (toxic end-product protoanemonin)

Chloro-catechol pathway (end-product catechol)

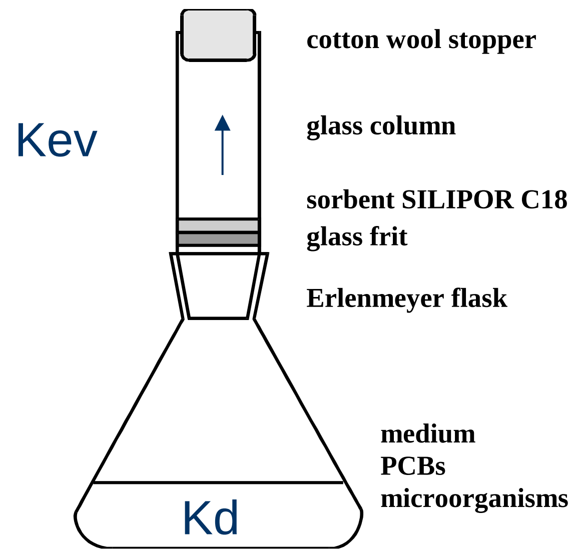
Bioacumulative product

GC ECD Analysis of PCBs



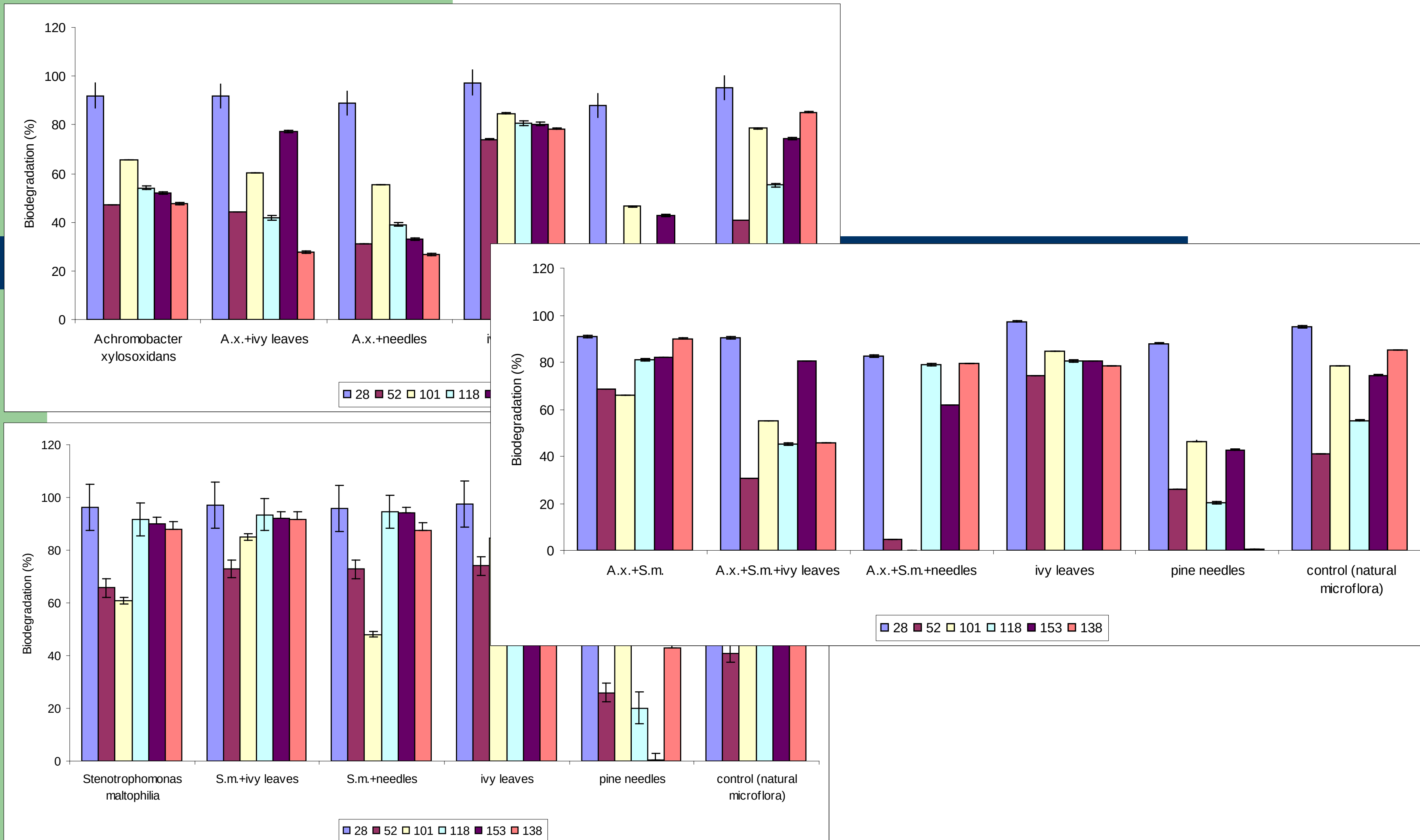
IUPAC	Chlorine position
PCB 8	2, 3, 6; 2, 3´, 6
PCB 28	2, 3´, 4, 5
PCB 52	2, 2´, 3, 4´, 5, 5´
PCB 101	2, 2´, 3, 4, 5, 5´
PCB 118	2, 3´, 4, 4´, 5
PCB 153	2, 2´, 4, 4´, 5, 5´
PCB 138	2, 2´, 3, 4, 4´, 5
PCB 180	2, 2´, 3, 4, 4´, 5, 5´
PCB 203	2,2´,3,4,4´, 5,5´, 6

6 indicator PCB congeners (+3)



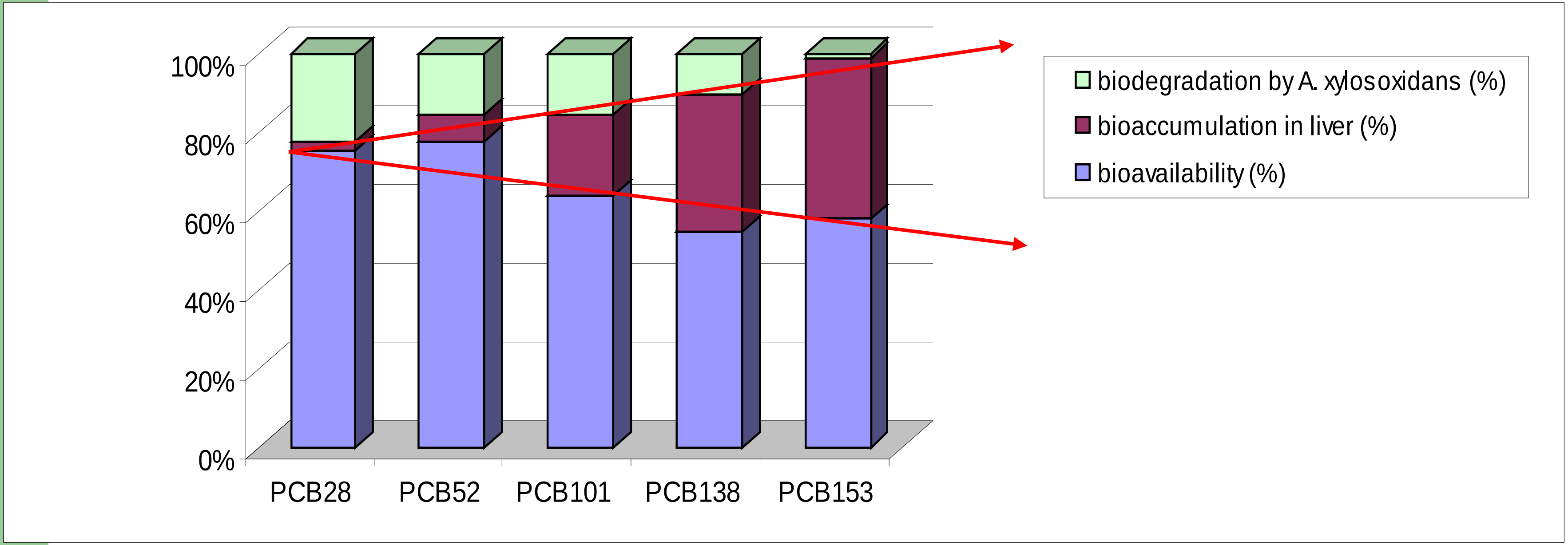
Apparatus for simultanneous monitoring of evaporation and biodegradation of PCBs (Dercová et al. 1996).

Bioaug. Bioaug.+Biostimul. Biostimul. IM



Conditions: 28 °C, in the dark, occasionally aerated by shaking at 180 rpm.
100 ml of mineral medium, 20 g of dry contaminated sediments (from Strážsky canal), biomass concentration of individual bacterial strain 1 g.l-1/ biomass concentration of individual bacterial strain for combination of two strains 0,5 $g.l^{-1}$, ivy leaves/pine needles 0,75 g.

Bio-availability
Bio-accumulation
Bio-degradation



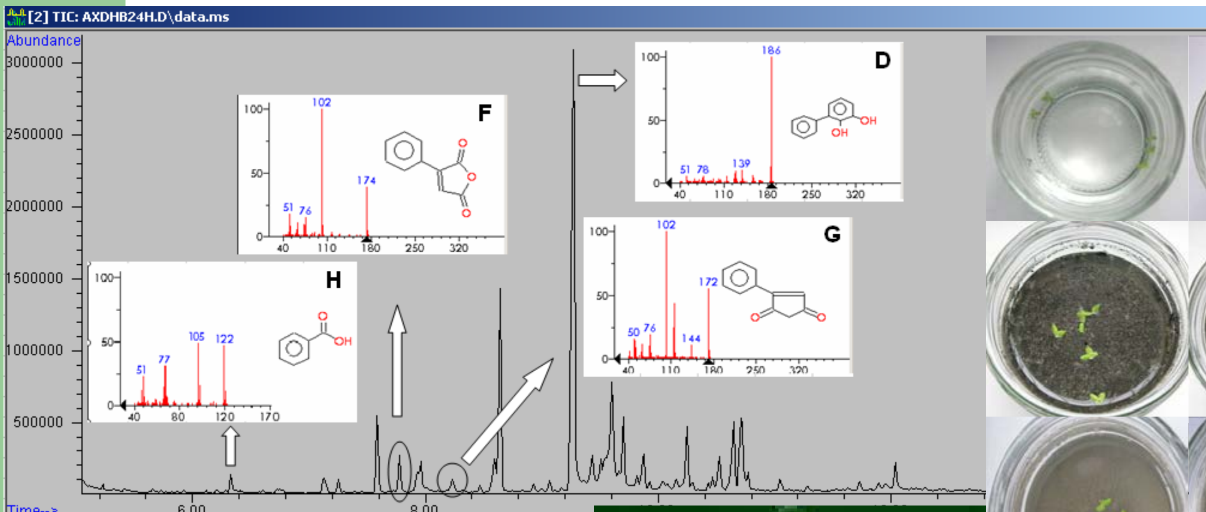
Source of bioaccumulation data:

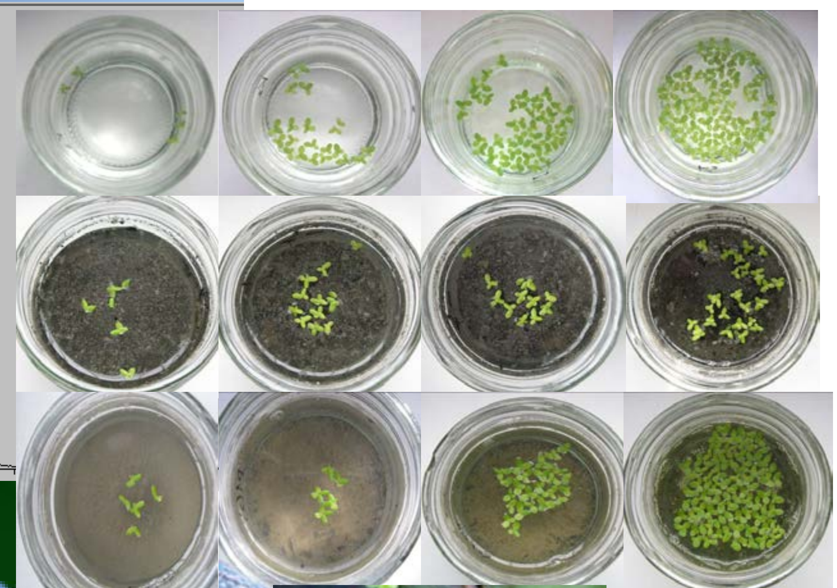
Bioavailability: measured in cooperation of MU Brno (2012)

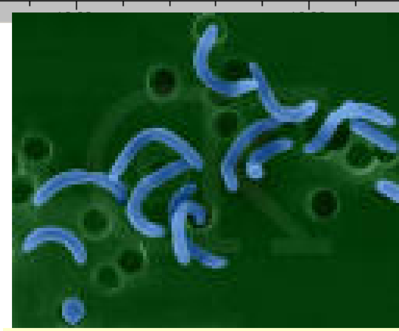
Brázová et al.: Tissue-specific distribution in fish ((2012)

Murínová et al., Biodegradation, 2013

DEGRADATION PRODUCTS







Unsufficient knowledge of metabolites of biotransformation may cause ecotoxicological and health risks

ECOTOXICITY OF SEDIMENTS

Dercová et al., Int. Biodeter. Biodegr. 2008 and 2009

CONCLUSIONS

1. In long term contaminated sediments sampled from Strážsky canal **PCB-degradative bacteria** were isolated and identified
2. **Biostimulation** using plant terpenes seems to be effective aproach of bioremediation.
3. The obtained results indicated beneficial effect of both **biostimulation** and **bioaugmentation** approaches on biodegradation of PCBs. Data obtained in sediments might be useful in the preliminary design of a site-specific bioremediation approach.
4. Efficiency of biodegradation is **conditioned** by **selection** of appropriate **bacterial strain** (or consortium) and **selection of technology approaches** (and **system**, where the experiment is performed).
5. More field experience with biotreatment will help to better define their limitations as well as to broaden their range of applications.

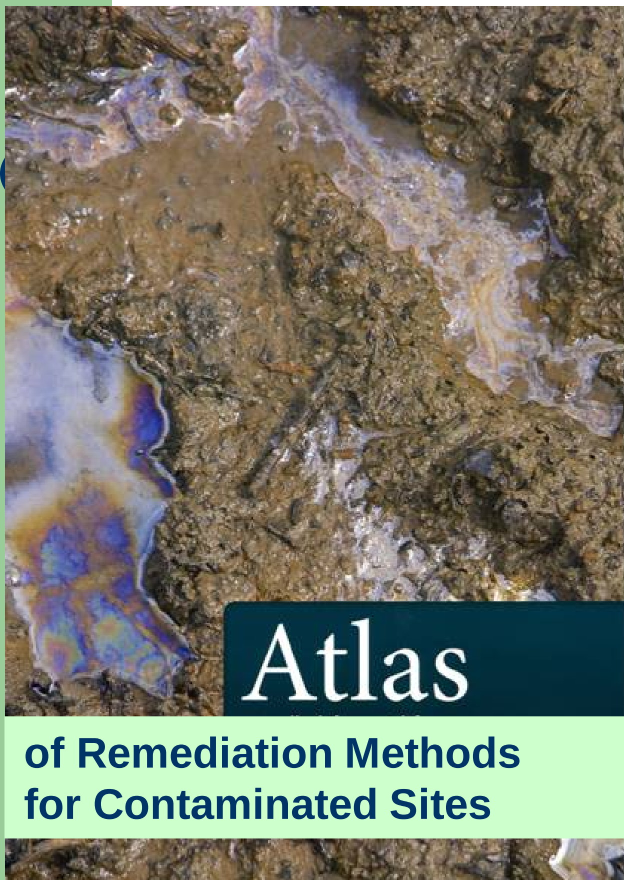
Comments

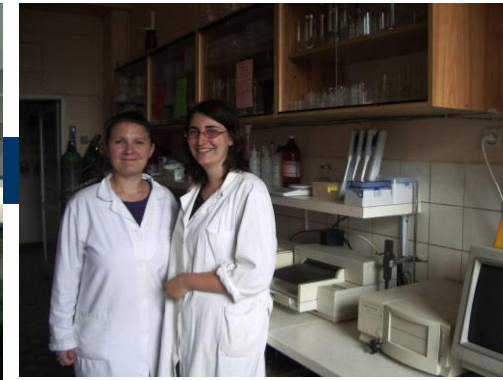
- **Bioaugmentation** – **the less easy to control**, but at the same time the one exhibiting the **great potential for the soil cleaning-up.**

- **Bioaugmentation** - remains **debatable** as a scientific and technological endeavour. Despite many reports that demonstrate the **usefulness** of bioaugmentation **in the field application**, there have been cases in which it **failed.**

- **Biostimulation -** using **addition of nutrients** seems to be **effective approach** eliminating problem with survival of inoculum and competition with indigenous microflora.

Thank you for your attention!





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Published results – about 60 (CC) papers

International Biodeterioration & Biodegradation 65 (2011) 1019–1023

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

The effect of polychlorinated biphenyls (PCBs) on the membrane lipids of *Pseudomonas stutzeri*

Slavomíra Zorádová*, Hana Dudášová, Lucia Lukáčová, Katarína Dercová, Milan Čertík

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ABSTRACT

In this study we examined the effect of polychlorinated biphenyls (PCBs) on biomass production of
lipids. Growth
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us addition of
of biphenyl or
'CBs alone the
tutzeri in total
ne (carvone at
tty acids was
reased. When
-vaccenic, and
oic acid led to
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Effects of plant terpenes on biodegradation of polychlorinated biphenyls (PCBs)

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Terpenes

ABSTRACT

Effects of four plant materials conta
leaves, and two synthetic terpene
a commercial mixture of polychlor
from long-term PCB-contaminated
minimal mineral medium with the
of ivy leaves (52.8%) and pine needl
cases even without the increase c
carvone, resulted in an effect simil
dation. It is concluded that the role
evaluate exactly if terpenes serve
PCB-biodegradation pathway or as
carbon source.

Appl Microbiol Biotechnol (2012) 94:1375–1385
DOI 10.1007/s00253-011-3763-8

ENVIRONMENTAL BIOTECHNOLOGY

Adaptation mechanisms of bacteria during the degradation of polychlorinated biphenyls in the presence of natural and synthetic terpenes as potential degradation inducers

Slavomíra Zorádová-Murínová · Hana Dudášová · Lucia Lukáčová · Milan Čertík · Katarína Šilharová · Branislav Vrana · Katarína Dercová

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Abstract In this study, we examined the effect of polychlorinated biphenyls (PCBs) in the presence of natural and synthetic terpenes and biphenyl on biomass production, lipid accumulation, and membrane adaptation mechanisms of two PCB-degrading bacterial strains *Pseudomonas stutzeri* and *Burkholderia xenovorans* LB400. According to the results obtained, it could be concluded that natural terpenes, mainly those contained in ivy leaves and pine needles, decreased adaptation responses induced by PCBs in these strains. The adaptation processes under investigation included growth inhibition, lipid accumulation, composition of fatty acids, *cis/trans* isomerization, and membrane saturation. Growth inhibition effect decreased upon addition of these natural compounds to the medium. The amount of unsaturated fatty acids that can lead to elevated membrane fluidity increased in both strains after the addition of the two natural terpene sources. The cells adaptation changes were more prominent in the presence of carvone, limonene, and biphenyl than in the presence of natural terpenes, as indicated by growth inhibition, lipid accumulation, and *cis/trans* isomerization. Addition of biphenyl and carvone simultaneously with PCBs increased the *trans/cis* ratio of fatty acids in membrane fractions probably as a result of fluidizing effects of PCBs. This stimulation is more pronounced in the presence of PCBs as a sole carbon source. This suggests that PCBs alone have a stronger effect on bacterial membrane adaptation mechanisms than when added together with biphenyl or natural or synthetic terpenes.

Keywords Membrane lipids · Polychlorinated biphenyls · Adaptation · Bacteria · Environmental stress · Terpenes · Stimulators

Introduction

PCBs constitute a class of 209 congeners that are structurally related. They comprise a biphenyl core with varying number of chlorine atoms and are produced by catalytic chlorination of biphenyl to various degrees (Furukawa et al. 1978; Arensdorf and Focht 1994; Tříska et al. 2004). PCBs have had a wide range of industrial applications, particularly as lubricants, cooling agents, and electric insulants. Between 1930 and 1979, over 600 million kg of PCBs were used in North America alone (Sylvestre 1995). Although the manufacture of PCBs has been forbidden (in USA in 1970; in Slovak Republic in 1984) they still pose an

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Isolation and identification of PCB-degrading microorganisms from contaminated sediments

Katarína Dercová[a,*], Jana Čičmanová[a], Petra Lovecká[b], Kateřina Demnerová[b], Martina Macková[b], Pavel Hucko[c], Patrik Kušnír[c]

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ABSTRACT

PCBs represent a serious ecological problem due to their low degradability, high toxicity, and strong bioaccumulation. The goal of this study was to analyze the PCB-contaminated sediments from Strážsky canal and Zemplínska šírava water reservoir from several points of view. The study of ecotoxicity confirmed that both sediments were toxic for various tested organisms. The genotoxicity test has not proved the mutagenic effect. The subsequent step included microbiological analysis of the contaminated sediments and isolation of pure bacterial cultures capable of degrading PCBs. In order to determine the genetic potential for their biodegradability, the gene *bphA1* encoding the enzyme biphenyldioxygenase, responsible for the first step of PCB aerobic degradation, was identified using a PCR technique. The ultimate goal of the work was to perform aerobic biodegradation of PCBs in the sediments. The bacteria present in both sediments are able to degrade certain low chlorinated congeners. The issue of biodiversity is still open and has to be studied in more detail to reveal the factual interactions between bacteria.

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Characterization of the bottom sediments contaminated with polychlorinated biphenyls: Evaluation of ecotoxicity and biodegradability

Katarína Dercová[a,*], Jana Šeligová[a], Hana Dudášová[a], Mária Mikulášová[b], Katarína Šilhárová[c], Lívia Tóthová[c], Pavel Hucko[c]

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Sediments

ABSTRACT

At the locality of the former producer of PCBs Chemko Strážske in East Slovakia, a large amount of PCBs (the commercial mixture DELOR 103, an equivalent of AROCLOR 1242) is still persisting in sediments and negatively influences health of the population. The objective of this work was to provide a study of ecotoxicity and genotoxicity of PCBs in contaminated sediments. Toxicity of the PCB-contaminated sediments sampled from Zemplínska šírava and Strážsky canal (surroundings of the former producer of PCBs) was determined applying a standard aquatic plant toxicity test using *Lemna minor*. The endpoints for the test were frond numbers and frond areas. The sediment sampled from Zemplínska šírava was more toxic to *L. minor* than the one sampled from Strážsky canal. The results on genotoxicity showed that both sediments were not mutagenic toward the standard strains of the Ames test, *Salmonella typhimurium* TA98 and TA100. This work deals also with biodegradation of PCBs in two samples of the above mentioned contaminated sediments: a) in the natural sediments by autochthonous microbial consortium and b) in the bioaugmented sediments inoculated by allochthonous bacterial strains, two bacterial isolates from long-term PCB-contaminated soil *Pseudomonas stutzeri* and *Alcaligenes xylosoxidans*. Both approaches were applied under the biostimulation conditions, with addition of glucose or biphenyl as co-substrates, as well. The highest PCB degradation was observed in the bioaugmented sediment inoculated with bacterial strain *P. stutzeri*. Addition of biphenyl, as the co-substrate and the inducer, positively affected degradation of PCBs. The *bphA1* gene, encoding enzyme biphenyldioxygenase, responsible for the start of PCB degradation, was identified in genome of *P. stutzeri*, a potential PCB-degrader isolated from long-term PCB-contaminated soil, but not in genome of *A. xylosoxidans*.

Current results - Publications 2013

1. Tandlich R., Martišková M., Dercová K., Baláž Š.: Characterization of the chlorobenzoate hydrophobicity using the 1-octanol/water partition system. *Fres. Environ. Bull.* (2013)
2. Dudášová H., Lukáčová L., Murínová S., Puškárová A.: Pangallo D., Dercová K.: Biodegradation ability of bacterial strains isolated from long-term PCB-contaminated sediment. *J. Basic Microbiol.* (2013)
3. Murínová S., Dudášová H., Lukáčová L., Lászlová k., Dercová K.: Adaptation responses of bacterial strains on environmental stress caused by the presence of toxic compounds. *Chem. Listy* (2013)
4. Lukáčová l., Dudášová H., Murínová S., Tóthová L., Dercová K.: Ecotoxicity and genotoxicity of PCB and PCB contaminated sediments. Int. *J. Soil Sediment* (2013)
5. Murínová S., Dudášová H., Lukáčová L., Dercová K.: The adaptation responses of bacterial degraders on the environmental stress mediated with polychlorinated biphenyls (PCBs) and 3-chlorobenzoic acid (3-CBA). *Mol. Cel. Biochem*. (2013)
6. Murínová S., Dercová K.: Bacterial cell membrane adaptation responses on stress caused with the environmental pollutant. *Acta Chimica Slovaca* (2013)
7. Murínová S., Dercová K., Tolgyessy P., Tarábek J., Dudášová H.: Metabolic pathway and degradation ability of four bacterial strains toward polychlorinated biphenyls (PCBs) in the presence of various co-metabolites. *Biodegradation* (2013)