Environmental and disaster biology introduction

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"The biosphere is a self-regulating entity with the capacity to keep our planet healthy by controlling the chemical and physical environment."

James Lovelock

Environment

The surroundings or conditions in which we live and operate (organic, inorganic i.e. artificial)





Environmental elements

Natural environmental elements

- Soil and geological medium (bedrock, minerals, sediments)
- Water (surface and subsurface)
- Air
- Biosphere (plants, animals, microorganisms)
- Artificial (built) environment (settlements, road networks, etc.)



A living thing is a discrete entity, or organism, that has the following characteristics:

- It can capture useful energy from its surroundings.
- It can **extract materials** from its surroundings for growth and maintenance of its structure.
- It is **responsive**—it reacts to its surroundings, using energy and material to move or grow.
- It **reproduces**—it generates other organisms like itself, preserving its own characteristics by passing them on to succeeding generations.
- It adapts and evolves—successive generations can change as the environment changes; individuals exhibit adaptations, that is, they are specialized for a particular function in a particular environment.

The hierarchy of life

- Metabolism All of the chemical reactions within an organism that sustain life.
- Organelle Subcellular structures within cells that carry out specialized functions.
- **Cell** The basic unit of life, a structure completely surrounded by a membrane, containing a nuclear region and cytoplasm; the smallest structure capable of having all of the characteristics of life.
- **Tissue** A group of similar cells having the same function in a multicellular organism.
- **Organ** A single structure of two or more tissues that performs one or more functions in an organism.
- **Organ system** A group of organs that carry out related functions.
- **Organism** An individual entity that has all of the characteristics of life described above and possessing the same hereditary information in all its cells.
- **Population** A group of individuals of the same species living in the same environment and actively interbreeding.
- **Community** A group of interacting populations occupying the same environment.
- **Ecosystem** The combination of a community and its environment.

The hierarchy of life

- **Organism** An individual entity that has all of the characteristics of life described above and possessing the same hereditary information in all its cells.
- Taxonomy of organisms:
- Biologists use the Linnaean system to classify organisms, which consists of a hierarchy of groupings and a naming convention. The lowest level of the hierarchy is the species. Similar species are grouped into a genus. The naming convention, called binomial nomenclature, assigns to each species a two-word name.
- Memory trick for the sequence from kingdom to species:
- King Phillip came over from Greece Saturday
- Kingdom Phylum Class Order Family Genus Species



- The biosphere is the 20 km thick part of the Earth's lithosphere, hydrosphere and atmosphere, where life and biological processes take place. According to the broadest geophysiological point of view, the biosphere is the global ecosystem, with all living things and all their connections, including the non-living environment. The basic feature of the biosphere is evolution, which began 3.5 billion years ago with the birth of life.
- The living material of the biosphere, the biomass, contains about 1,900 gigatonnes of carbon, which corresponds to 3.7 kg / m² of carbon on the surface of Earth (including sea and land).



This time series animation features the MODIS Terra Vegetation Index (MOD13C1) product. The MOD13C1 16-day product features data starting on February 2, 2000, through December 19, 2013, and includes 320 images.

Biosphere - Living things don't just live in the environment: To a great extent they create it.





Biogeochemical cycles

Biogeochemical cycles include a variety of metabolic pathways as well as abiotic reactions that continuously replenish the chemical ingredients of life.

Microbes are the agents of most of these reactions, often through adding electrons to and removing electrons from atoms within a sequence of redox reactions.



 Biogeochemical cycles are not maintained by any single species; rather, they represent the collective effects of diverse, yet inherently coordinated, microbial consortia as well as interactions of microbes with higher organisms.

Biogeochemical cycles

- Biogeochemical cycles are a starting point for the analysis of the effect of humans on the environment.
- Since the heat-trapping effect of atmospheric CO₂ and methane are a serious concern, it is important to understand the rate of anthropogenic (human-caused) emissions (environmental impacts) of these gases and the rates at which they are naturally removed.
 - Half of the CO₂ released by combustion of fossil fuel since the beginning of the Industrial Revolution remains in the atmosphere.
 - It is thought that the ocean has absorbed the remainder.

Environmental Impact

- Changes in the environment arising completely or partially from a natural process, phenomenon or activity of an individual or an organization, its products or its services.
- Environmental impact can be favorable or unfavorable.
- In short: any activity with an impact on the environment (or on one of the environmental elements)

Use of the environment

• An activity subject to permit for the use or exposure of the environment or an environmental element.

Use of the environment:

Environmental impact:

bring change into the environment,

the usage of environment or an element as a natural resource (e.g. ecosystem service)

release of a substance or energy into the environment

THE QUESTION IS: Where is the threshold between environmental impact and environmental contamination?

Environmental protection

- A system of activities and measures to protect, improve and prevent the deterioration of <u>natural environmental elements</u> (soil and geological medium, water, air, ecosystem, landscape) and <u>artificial environments</u> to ensure the health and survival of mankind and the ecosystem.
- The level of environmental protection is strongly connected to the stage of development (economic, technical, social, cultural, scientific, political and legal).

Environmental Protection Hierarchy (source: epa.gov)

Pollution prevention (P2) is any practice that reduces, eliminates, or prevents pollution at its source, also known as "source reduction."

- Source reduction is fundamentally different and more desirable than recycling, treatment and disposal.
- There are significant opportunities for industry to reduce or prevent pollution at the source through cost-effective changes in production, operation, and raw materials use.
- The opportunities for source reduction are often not realized because existing regulations focus upon treatment and disposal.



Environmental management

Reasonable, economical, environmentally friendly, lowwaste, pre-lanned, long-term, sustainable use of natural resources through effective conservation.

ENVIRONMENTAL SAFETY

THE CONDITION OF ENVIRONMENTAL ELEMENTS (EXOTOXICOLOGY? MONITORING?)

USAGE OF NATURAL RESOURCES

USE OF ENVIRONMENT AND RESOURCES, ENVIRONMENTAL LOAD

IPPC PERMIT

ENVIRONMENTAL REVIEW

ENVIRONMENTAL PERFORMANCE REVIEW Environmental management

Reasonable, economical, environmentally friendly, lowwaste, pre-lanned, long-term, sustainable use of natural resources through effective conservation.

ENVIRONMENTAL PROTECTION

NATURE CONSERVATION

POLLUTANTS (E.G. POP, EDC, EMP)?

MITIGATION

DAMAGE CONTROL

REMEDIATION

SEWAGE TREATMENT

WASTE MANAGEMENT

DISASTER CONTROL MANAGEMENT

PROTECTION OF CRITICAL INFRASTRUCTURES

BIOLOGICAL CONTAMINANTS

Environmental Pollution

Physical, chemical or biological <u>changes</u> in the properties of environmental elements which have an adverse effect on the health, survival or well-being of humans and/or other living organisms.

IN SHORT: SUCCEED ENVIRONMENTAL LIMIT VALUES OR THE ILLEGAL DISPOSAL OF WASTE (BELOW LIMIT: ENVIRONMENTAL LOAD) CAUTION: "DON'T BE ENCHANTED BY LIMIT VALUES" !!!







Environmental Pollutant

- Substances or mixtures of substances, their degradation products, which, if released into the environment,
- may adversely affect the condition of environmental elements or
- harmful to human health or have a negative effect on environmental use.

Primary and secondary pollutants



What is considered as environmental contaminant/pollutant (i.e. harmful substance/chemical?

Ambient air (Hungarian low):

4/2011. (I. 14.) VM decree: technological (general: 228 organic substance + 11 carcinogen and procedure-specific: 56 procedure (e.g. steel construction, fertilizer production, etc.) emission values; air load level exposure limits (20 substance with a special attention to air pollutants of major importance and carcinogen air pollutants immission values), air pollutants planning values (166 substances + sedimenting dust!), disclosure (information) and alert thresholds (sulfur dioxide, nitrogen dioxide, carbon monoxide, particulate matter PM10, ozone), critical air load levels set to protect ecosystems (sulfur dioxide, nitrogen oxides, ammonia, near-ground ozone, 9 types of aerosols including metal-containing ones)

AIR POLLUTANTS OF MAJOR IMPORTANCE 4/2011.(I.14.) VM DECREE

Sulfur dioxide, nitrogen dioxide, carbon monoxide, particulate matter PM10, lead, mercury and benzene (carcinogen air pollutant),

ozone is in a separate section

"CERTAIN CARINOGEN AIR POLLUTANTS"

Arsenic, Cadmium, Nickel, Benzo(a)pyrene, Chromium, Beryllium, 1,3-Butadiene, Dioxins and furans, Tetrachloroethylene, Trichlorethylene, Vinyl chloride,

Special item: asbestos



Surface water

 10/2010. (VIII. 18.) VM decree on surface water contamination limit values (connected to Water Framework Directive to determine the general chemical and physical properties of water affecting biological elements such as priority and other substances)

56 priority substance, 4 other harmful substance (zinc, copper, chrome, arsenic)

• 28/2004 (XII.25.) KvVM decree on emission values of water pollutants...

(technological: 37-types of technology, areal: 35types of parameters, parameters for release to public sewer: 40 parameters, unique limits: 54 parameters)

Soil and subsurface water

219/2004. (VII. 21.) Government Regulation
About the protection of subsurface water and its implementing regulation:
6/2009. (IV. 14.) KvVM-EüM-FVM joint decree

Contamination threshold value

1. melléklet a 6/2009. (IV. 14.) KvVM-EüM-FVM egvüttes rendelethez

Anyagcsoportonként (B) szennyczettségi határértékek földtani közegre

CAS szám = Chemical Abstract Service azonosító száma

K_i = a veszélyességet jellemző besorolás, mely szerint K1 a minden esetben veszélyes anyagokat jelöli

B = (B) szennyezettségi határérték

1. Fémek ("összes" kioldható) és félfémek (mértékegység: mg/kg szárazanyag)

CAS szám		and the second	В	K.
7440-47-3	Króm összes		75	K2
	Króm VI.		1	K1
7440-48-4	Kobalt		30	K2
7440-02-0	Nikkel		40	K2
7440-50-8	Réz		75	K2
7440-66-6	Cink		200	K2
7440-38-2	Arzén		15	Kl
7782-49-2	Szelén		1	K2
7439-98-7	Molibdén		7	K2
7440-43-9	Kadmium		1	K1
7440-31-5	Ón		30	K2
7440-39-3	Bárium		250	K2
7439-97-8	Higany		0,5	K1
7439-92-1	Ólom		100	К2
7440-22-4	Ezüst	1	2	К2

2. Szervetlen vegyületek (mértékegység: mg/kg szárazanyag)

CAS szám		в	K,
	Cianid 4,5 pH	2	K1
	Cianid összes	20	K1
	Tiocianátok	1	K1

3. Alifás szénhidrogének (TPH) (mértékegység: mg/kg szárazanyag)

	в	Ki
Összes alifás szénhidrogén (TPH)	100	K1
C5C40		1 A A

4. Benzol és alkilbenzolok (BTEX) (mértékegység: mg/kg szárazanyag)

CAS szam		в	Ki
71-43-2	Benzol	0,2	K.1
108-88-3	Toluol	0,5	K1
100-41-4	Etil-benzol	.0,5	K1
1330-20-7	Xilolok	0,5	K1

Approx. 180 substance

Example for secondary contaminant in soil-groundwater system: Among chlorinated hydrocarbon substances in soil:



Which is more water soluble and more reactive with proteins and nucleic acids than the parent compound.

Carcinogen?



European Union: REACH (in force: EC 1907/2006) Annex. XVII. Carcinogen substances are listed in Appendix 1, Entry 28. (Entry 29. is listing mutagens)

Carcinogens category 1A and 1B

WHAT IS REACH? → separate slide

SAFETY SHEETS! → Hazard statements (H350 and H351)

International Agency for Research on Cancer



IARC (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER) Last update: 12 December 2019. 1017 substances

More than 40 years of data collection !!! <u>https://monographs.iarc.fr/agents-classified-by-the-iarc/</u>

EPA USA (ENVIRONMENTAL PROTECTION AGENCY)





What is REACH?

- REACH (EC 1907/2006) aims to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances.
- This is done by the four processes of REACH, namely the Registration, Evaluation, Authorization and restriction of CHemicals.
- REACH also aims to enhance innovation and competitiveness of the EU chemicals industry.
- "No data no market" philosophy: the REACH Regulation places responsibility on industry to manage the risks from chemicals and to provide safety information on the substances.
- The Regulation also calls for the progressive substitution of the most dangerous chemicals (referred to as "substances of very high concern") when suitable alternatives have been identifie



Annex XVII REACH

• The restricted substances (on their own, in a mixture or in an article) are substances for which manufacture, placing on the market or use is limited or banned in the European Union.

ANNEX XVII

RESTRICTIONS ON THE MANUFACTURE, PLACING ON THE MARKET AND USE OF CERTAIN DANGEROUS SUBSTANCES, MIXTURES AND ARTICLES

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction			
1. Polychlorinated terphenyls (PCTs)	 Shall not be placed on the market, or used: — as substances, — in mixtures, including waste oils, or in equipment, in concentrations greater than 50 mg/kg (0,005 % by weight). 			
 Chloroethene (vinyl chloride) CAS No 75-01-4 EC No 200-831-0 	Shall not be used as propellant in aerosols for any use. Aerosols dispensers containing the substance as propellant shall not be placed on the market.			

Categories	Criteria
CATEGORY 1:	Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A:	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B:	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.
	 The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: •human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or •animal experiments for which there is sufficient^a evidence to demonstrate animal carcinogenicity (presumed human carcinogen).
	In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
CATEGORY 2:	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited ^a evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

ECHA (European Chemicals Agency) table

 A table has been prepared by the European Chemicals Agency (ECHA) to facilitate the searching of restricted substances in the Annex XVII of the REACH Regulation, and the table provides additional information related to the specific restriction entry.

kibontás/összecsukás	EC No. 🗘	CAS No. 🗘	Entry no. O	Conditions
B- :triol and any of its mono-, atives	-	-	73	P
ne	-	630-20-6	36	P
ne	201-197-8	79-34-5	35	P
	201-166-9	79-00-5	34	P
	200-864-0	75-35-4	38	P
	203-400-5	106-46-7	64	P
	212-828-1	872-50-4	71	P
nol (DEGBE)	203-961-6	112-34-5	55	P
nanol (DEGME)	203-906-6	111-77-3	54	P
; salts cetate EC No.: 209-030-0 CAS	-	-	12	R

Label elements for carcinogenicity

Classification	Category 1 (Category 1A, 1B)	Category 2		
GHS Pictograms				
Signal Word	Danger	Warning		
Hazard Statement	H350: May cause cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H351: Suspected of causing cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)		
Precautionary	P201	P201		
Statement Prevention	P202	P202		
	P280	P280		
Precautionary	P308 + P313	P308 + P313		
Statement Response				
Precautionary	P405	P405		
Statement Storage				
Precautionary	P501	P501]		
Statement Disposal				

Identification criteria for persistant (P) bioaccumulative (B), toxic (T), very persistant (vP) and very toxic (vT) substances

	Persistant <mark>(P)</mark> criteria, half life (days)	Very persistant <mark>(vP)</mark> criteria, half life (days)	Bioaccumulative (B) criteria, bioconcentration factor	Very bioaccumulative (vB) criteria, bioconcentration factor		Toxicity <mark>(T)</mark> Criteria
Seawater	> 60				-	
Freshwater or estuary	> 40	> 60	Aquatic species > 2000	Aquatic species> 5000	a) b)	NOEC or EC10 is lower than 0.01 mg/l; Based on the 1272/2008/EC directive, substance is classified as a carcinogen (category I and II),
Sea sediment	>180				c)	mutagen or reprotoxic agent There are other evidences for the chronic toxicity
Freshwater or estuary sediment	> 120	> 180				e.g. The substance is classified as a Specific Target Organ Toxicant (STOT) (it produces specific target organ toxicity/systemic effects that are not specifically addressed elsewhere in the GHS). All significant health effects that can impair
SOIL	> 120	> 180				function, both reversible and irreversible, following single exposure or repeated exposure, are included.

Is this enough? What about production and sale?

- The annual global production of chemicals has risen from 1 million tonnes (1930) to over 400 million tonnes.
- On the community market, slightly more than 100 000 different substances are traded of which 30 000 succeed the annual volume of 1 tonne. Internationally, approx. 200-1000 new substances are introduced in a year.
- According to World Wildlife Found (WWF) 80% of chamicals used in bulk have never been tested for human health (...what about ecological effects???)


~ 22 600 chemicals with a use over 1 tonne per year

~ 4 700 chemicals with a use over 100 tonnes per year prioritised in hazard characterisation and evaluation

~ 70 000 chemicals with poor characterisation of their hazards and exposures

~ 500 chemicals



• Short answer: No, it is not enough

New challenges

- Climate change
- Plastics and microplastics
- Emerging micro-pollutants (pesticides, pharmaceuticals)









Health Risks Associated with Synthetic Pesticides

Because pesticides end up virtually everywhere instead of remaining on crops, their existence in our environment has been linked to the following health problems:



Skin, Eye, and Lung Irritation

Nerve Disorders



Hormone Disruption



Birth Defects / Toxicity to a Fetus



Brain and Nervous System Toxicity



Reproduction Effects



Cancer



Blood Disorders

Children living in areas with heavy pesticide use had strikingly impaired hand-eye coordination, decreased physical stamina, short-term memory impairment, and trouble drawing

The Natural Resource Defense Council (2014)

What can we do? - ENVIRONMENTAL PROTECTION

- THE PROTECTION OF NOT ONLY THE NATURAL ENVIRONMENT BUT BUILT (I.E. ARTIFICIAL) ENVIRONMENT AS WELL.
- AIM: TO ORGANIZE HUMAN ACTIVITIES (INDUSTRIAL, AGRICULTURAL, TRANSPORTATION, ETC.) IN A WAY TO PRESERVE THE NATURAL AND ARTIFICIAL ENVIRONMENT OF HUMANITY.
- BALANCING COMPROMISE SEARCH, BUT CONSERVATIVE ESTIMATION AND APPROACH! (in environmental protection, a conservative risk/management approach is when the worst-case scenario is taken into account during planning or permitting environmental use)
- In the long run, nature conservation areas can be only maintained with the elimination or reduction of harmful effects!

The basics and the role of environmental biology in environmental protection

• The concept map on the role of biology in Engineering and Science



ENVIRONMENTAL

BIOLOGY FOR ENGINEERS AND SCIENTISTS

Biological applications for environmental control

- Applied biology plays a vital role for all humans, in terms of sustaining both human life and environment quality.
- **Production**: high level of dependence (cultivated crops, managed livestock, managed forests, biochemical products e.g. foods and beverages, bulk chemicals, e.g. acetic acid, ethanol, advanced pharmaceuticals).
- Biological systems inherently qualify as **environmentally friendly**, and as a naturally renewable resource they tend to offer **significant economic advantages**.
- Most of these systems can even self-adjust (i.e., acclimate) to their circumstances (environmental conditions, competitive pressures, etc.), in an attempt to maintain optimal performance while handling metabolically an increasingly complex array of industrial chemicals, which may be anthropogenic (human-made) or xenobiotic (foreign to living things).

Biological applications for environmental control



• Some environmental pollutants occurs naturally and they are known for living organisms (e.g. hydrocarbons).

The possible reactions of living organisms to environmental pollutants



Biological applications for environmental control

Applied Realm of Environmental Management	Specific Process Application	Biological Level	
		Micro-scale ^a	Macro-scale ^b
Wastewater	Attached growth	×	
	Suspended growth	×	
	Stabilization lagoons	×	
	Constructed wetlands	×	×
Sludge	Anaerobic digestion	×	
	Aerobic digestion	×	
	Composting	×	
Water	Potable water	×	
Disinfection	Water and wastewater	×	
Solid waste	Landfill degradation	×	
Air	Biofiltration	×	
Soil and groundwater	Phytoremediation	×	×
	Bioremediation	×	×

^aMicroscale applications involve various combinations of bacteria, protozoms, rotifers, fungi, algae, etc. ^bMacroscale applications involve higher plants.

Vaccari et al., 2006

Why the microorganisms?

- They are detectable in all environmental elements all around the world
- They can adapt to extreme environmental conditions
- The final products of the degradation of organic environmental pollutants are ideally non-hazardous metabolites (e.g. CO₂, H₂O)
- → Bio-conversation, biotransformation



https://www.nsf.gov/news/special_reports/sfs/





INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Oxidoreductases
Transferases
Hydrolases
Lyases
Isomerases
Ligases
Translocases

https://www.enzyme-database.org/class.php

Enzymes with relevance in environmental biotechnology

- Oxigenases e.g. the degradation of aromatic hydrocarbons
- Proteases keratin degradation e.g. feather, fur
- Amylases starch degradation and transformation
- Cellulases cellulose and lignocellulose degradation
- Esterases
- Lipases pl. biodiesel production



Demonstration of enzyme reactions through the example of pesticide degradation

- **Hydroxylation**: introduction of OH group (increased biological activity, more polar, more water soluble (catalyzed by oxygenase enzyme)
- **Desalkylation**: many pesticides contain alkyl groups responsible for toxic effects (detoxification! Mixed-function dehydrogenases)
- Cleavage of ether bond: easier metabolism (mixed oxygenates)
- Oxidation of aromatic ring: dioxygenases
- Beta-oxidation: aliphatic side chains → fatty acid, extraction of 2 C atomic fragments

Demonstration of enzyme reactions through the example of pesticide degradation

- Epoxidation: double bond O atom → toxic intermediates! (e.g. aromatic CH oxides → mercapturic acids
- **Sulphoxidation**: 2-valency sulfur \rightarrow sulfoxide, sulfone
- Reduction reactions: rare. Reductive dehalogenation is important for chlorinated CH
- Hydrolysis: lipophilic → OH group. incorporation, hydrophilic (amidase, esterase, nitrilase, phosphatase) → acid, alcohol, or amine
- **Dehalogenation**: C-halogen bond stable!
- Hydrolytic dehalogenation (OH group instead of halogens)
- **Reductive dehlogenation** (H instead of halogens) e.g. DDT \rightarrow DDD
- Dehydrohalogenation (Instead of H and halogen = bond) DDT → DDE
- Synthetic reactions: conjugate, condensate formation

Bio-deterioration relates to the biofilm growing on the surface and inside the plastic, which increases the pore size and provokes cracks that weaken the physical properties of the plastic (physical deterioration) or releases acid compounds that modify the pH inside the pores and results in changes in the microstructure of the plastic matrix (chemical deterioration).

Mineralization is the ultimate step in the biodegradation of a plastic polymer and results in the excretion of completely oxidized metabolites (CO_2 , N_2 , CH_4 , and H_2O).



Bio-fragmentation corresponds to the action of extracellular enzymes (oxygenases, lipases, esterases, depolymerases and other enzymes that may be as diverse as the large spectrum of polymer types) released by bacteria colonizing the polymer surface. These enzymes will reduce the molecular weight of polymers and release oligomers and then monomers that can be assimilated by cells.

Assimilation allows oligomers of less than 600 Daltons to be integrated inside the cells to be used as a carbon source, thus increasing the microbial biomass.

Schematic form of biodegradation and phytoremediation

- It is not like this ⊗
- → we will discuss in details the challenges of biological processes during environmental interventions in another time ⁽¹⁾



PHYTOREMEDIATION RHIZOREMEDIATION

Edit Kaszab

Phytoremediation

- Remediation methods have several limitations and, sometimes, they are disruptive to the environment. Look for environmentally friendly treatment technologies to complement or substitute the conventional ones.
- Phytoremediation is a broad term that has been in use since the early 1990s
- Phytoremediation is the use of green plants and their associated microbiota, enzymes, water consumption, soil amendments, and agronomic techniques to remove, contain/retain, immobilize, transform/degrade environmental contaminants from soil, sludges sediments, water wastewater and even atmosphere.
- It is an emerging technology which offers a potentially cost-effective and environmentally sound (ecofriendly) alternative to the environmentally destructive physical methods.
 - independent of an external energy supply,
 - have more public acceptance
 - non-invansive technology
 - plants increase the amount of organic carbon in the soil, which can stimulate microbial activity and augment the rhizospheric degradation of the pollutants

- Plants ideal for phytoremediation should fulfil four main requirements (Schnoor, 1997):
- 1. they must be fast growing and have high biomass,
- 2. have deep roots,
- 3. have easily harvestable aboveground portion,
- 4. accumulate large amounts of metals (~ 1000 mg/kg) in aboveground biomass.
- Plants can be used to treat most classes of contaminants—
 - toxic metals,
 - radionuclides
 - recalcitrant organic pollutants,
 - chlorinated pesticides,
 - organophosphate insecticides,
 - petroleum hydrocarbons (BTEX),
 - polynuclear aromatic hydrocarbons (PAHs),
 - sulfonated aromatics,
 - phenolics,
 - nitroaromatics and explosives,
 - polychlorinated biphenyls (PCBs),
 - chlorinated solvents (TCE, PCE).

Phytoremediation

Phytoremediation can be performed both in situ and ex situ. Different strategies:

- phytoextraction,
- phytodegradation,
- Phytovolatilization
- $\circ~$ Rhizodegradation
- Selection, traditional breeding and genetic engineering focus on the optimization of pollutant tolerance, root and shoot biomass, root architecture and morphology, pollutant uptake properties, degradation capabilities for organic pollutants etc.



- phytoextraction/phytoaccumulation this process involves the removal of pollutants by the roots of plants and subsequent transport to aerial plant parts; pollutants accumulated in stems and leaves are harvested with accumulating plants and removed from the site;
- phytodegradation this consists on the conversion of organic pollutants into compounds with reduced toxicity through the action of internal or secreted enzymes;
- phytovolatilization through this process soluble pollutants are taken up with water by the roots, transported to the leaves and volatilized into the atmosphere through the stomata; amounts of pollutants transpired are proportional to the water flow and usually relatively low;
- rhizodegradation breakdown of organic pollutants through microbial enzymatic activity is called rhizodegradation; the types of plants growing in the contaminated area influence the amount, diversity and activity of microbial populations;
- rhizofiltration this mechanism of pollutants retention involves either adsorption or absorption by plants roots; consequently, large root surface areas are usually required for these processes;
- phytostabilization through this process, accumulation by plant roots or precipitation in the soil by root exudates immobilizes and reduces the availability of soil pollutants; plants growing on polluted sites also stabilize the soil and can serve as a groundcover thereby reducing wind and water erosion and direct contact of the pollutants with animals;
- hydraulic control containment of pollutants within a site can also be achieved by limiting the spread of a contaminant plume through plant evapotranspiration.



In the soil/water-plant-atmosphere continuum, a specific contaminant can be remediated at specific points along this continuum by different phytoremediation mechanisms



2025.02.19.

Advantages of phytoremediation

- Phytoremediation installations provide improved aesthetics and receive a better acceptance from the public as they are less invasive and destructive than other technologies. They additionally may provide habitat to animals and promote biodiversity.
- Phytoremediation is a low-cost technology compared to other treatment methods. Studies have indicated that implementing phytoremediation may result in cost savings of 50 to 80 % over traditional technologies.
- It is easy to implement and maintain, and it is effective for a variety of organic and inorganic compounds.
- It reduces the amount of dust emission and may promote better air quality in the vicinity of the site.
 Vegetation also helps reduce erosion by wind or water.

Disdvantages of phytoremediation

- Extremely high concentrations of pollutants may not allow plants to grow or them to die. So, phytoremediation is more effective or limited to lower concentrations of contaminants.
- For phytoremediation to be successful, contaminants must be within reach of plants roots, therefore it is restricted to sites which shallow contamination, with effective depth limited by the size of the rooting zone.
- Phytoextraction can cause contaminants to accumulate in plant tissues. The potential for ecological exposure through consumption of contaminated plants by animals and possible effect on the food chain is an environmental concern. Harvesting of contaminated biomass may be required.
- Phytovolatilization may remove contaminants from the subsurface but might then cause increased airborne exposure. In general, the fate of contaminants is often unclear, which may raise important issues with the potential for the dissemination of some pollutants in the environment.
- If non-native species are selected for phytoremediation, the consequences of introducing them in the ecosystem may be unknown or unexpected.
- Phytoremediation may take a longer time to achieve remediation goals (sometimes several year) than is required by other treatment technologies. For instance, a tree stand may take several growing seasons to be established and for contaminant concentrations to be reduced.
- The success of phytoremediation may be seasonal, depending on location. Its effectiveness may be variable, affected by climate conditions.

Application of phytoremediation methods

- Shallow contamination of organic, nutrient or metal pollutants
- Large scale pollution, where other environmental implementations would by very expensive
- Areas with a low level of pollution
- Simultaneous application with other remediation methods
- Plant species:
 - Trees e.g. poplar
 - Grasses e.g. Festuca spp.
 - Nitrogen fixing e.g. Trifolium spp., Medicago sativa
 - Aquatic plants e.g. Myriophyllum spicatum, Saggitaria latifolia





Scale of phytoremediation



Metabolism of organic xenobiotics in plants

- Several enzyme systems, not necessarily physiologically connected, form a metabolic cascade for the detoxification, breakdown and final storage of organic xenobiotics. In plants, detoxification resemble the reactions in the animal liver than the bacterial mechanism (called "green liver metabolism) (Sandermann, 1994).
- This network of reactions can be subdivided into three distinct phases:
- tranformation (phase I),
- conjugation (phase II)
- compartmentation (phase III) transport and store in the vacuole and the final step is cell wall binding or excretion



Phytovolitilization Translocation Phytodegradation Insoluble conjugates of contaminant in CELL WALL Uptake Deep Soluble conjugates of contaminant in Rhizodegradation oxidation VACUOLE Rhizostabilization Organic Phase I Contaminant with Phase III functional group contaminant Phase II Conjugate of contaminant PLANT with cell compounds CELL

Major removal processes and transformation pathways of organic xenobiotics in plants (adapted from Kvesitadze et al. (2006) and Abhilash et al. (2009)

Phase I

- Functionalization: involves a conversion/activation (oxidation, reduction or hydrolysis) of lipophilic xenobiotic compounds
- In this phase the molecules of the hydrophobic compound acquire a hydrophilic functional group (e.g. hydroxyl, amine, carboxyl, sulphydryl) through enzymatic transformations.
- The polarity and water solubility of the compound increase
- Increased affinity to enzymes catalyzing further transformation (conjugation or deep oxidation by the addition or exposure of the appropriate functional groups.
- Plant cell not only detoxifies the compound but also assimilates the resulting carbon atoms for cell needs
- In plants, oxidative metabolism of the xenobiotics is mediated mainly by cytochrome P450 monooxygenase which is of crucial importance in the oxidative processes to bioactivate the xenobiotics into chemically reactive electrophilic compounds which subsequently form conjugates during Phase II

Cytochrome P450 enzyme system

- Cytochrome P450s (CYPs) are heme-thiolate proteins that plays a key role in the metabolism of drugs and other xenobiotics (Estabrook, 2003).
- Cytochrome P450 proteins, named for the absorption band at 450 nm of their carbonmonoxide-bound form, are one of the largest superfamilies of enzyme proteins. The P450 genes (also called *CYP*) are found in the genomes of virtually all organisms, but their number has exploded in plants.



Their most conserved structural features are related to heme binding and common catalytic properties, the major feature being a completely conserved cysteine serving as fifth (axial) ligand to the heme iron.

They contribute to vital processes such as carbon source assimilation, biosynthesis of hormones and of structural components of living organisms, and also carcinogenesis and degradation of xenobiotics.

CYP P450 gene family

Until 22.08.2012: 18687 protein-encoding P450 gene:

- In humans, 57 and 58 pseudogeneic

Smaller families and subfamilies are subdivided based on DNA sequence homology.

Their amino-acid sequences are extremely diverse, with levels of identity as low as 16% in some cases, but their

structural fold has remained the same throughout evolution.

Based on protein similarity:

40% similarity - one family (denoted by a number, CYP1)

55% similarity - one subfamily (denoted by a letter, CYP1A)

marking of a given gene (number, CYP 1A1)

Gene family	Number of genes	Main function
CYP1	3	Biotransformation
CYP2	16	Biotransformation
CYP3	4	Biotransformation
CYP4	12	Fatty acids metabolism
CYP5	1	Thromboxane A2 synthesis
CYP7	2	Bile acid biosynthesis
CYP8	2	Bile acid and prostacyclin biosynthesis
CYP11	3	Steroid biosynthesis
CYP17	1	Steroid biosynthesis
CYP19	1	Steroid biosynthesis
CYP20	1	Unknown
CYP21	1	Steroid biosynthesis
CYP24	1	Vitamin D degradation
CYP26	3	Retinoic acid hydroxylation
CYP27	3	Vitamin D3 and bile acid synthesis
CYP39	1	Cholesterol synthesis
CYP46	1	Cholesterol synthesis
CYP51	1	Cholesterol synthesis

• P450 enzymes catalyze many reactions that are important in drug metabolism or that have practical applications in industry; their economic impact is therefore considerable.

In prokaryotes:

 P450s in prokaryotes are soluble proteins. Class I P450s require both an FAD-containing NAD(P)Hreductase and an iron-sulfur redoxin as electron donors. Prokaryotic class II require only an FAD/FMNcontaining NADPH-P450 reductase, which is fused to the P450 protein. P450s often confer on prokaryotes the ability to catabolize compounds used as carbon source or to detoxify xenobiotics. Other functions described for prokaryotic P450s include fatty acid metabolism and biosynthesis of antibiotics.

In eucaryotes:

- Eukaryotic class I enzymes are found associated with the inner membrane of mitochondria and catalyze several steps in the biosynthesis of steroid hormones and vitamin D3 in mammals.
- Class II enzymes are the most common in eukaryotes. Class II P450s from plants are involved in biosynthesis or catabolism of all types of hormones, in the oxygenation of fatty acids for the synthesis of cutins, and in all of the pathways of secondary metabolism.

Class I and class II P450s from all organisms participate in the detoxification or sometimes the activation of xenobiotics.

Phase II

- Conjugation: involves conjugation of xenobiotic metabolites of Phase I (or the xenobiotics themselves when they already contain appropriate functional groups) to endogenous molecules (proteins, peptides, amino acids, organic acids, mono-, oligo- and polysaccharides, pectins, lignin, etc.);
- As result of conjugation, compounds of higher molecular weight are formed with greatly reduced biological activity and usually reduced mobility.
- The end products of Phase II are usually less toxic than the original molecules or Phase I derivatives exceptions!
- Conjugation is catalyzed by transferases. Enzymes such as **glutathione-S-transferases**, glucosyl transferase and N-malonyl transferases are associated with Phase II (Eapen et al., 2007).
- Conjugation of Phase I metabolites takes place in the cytosol, but it is harmful to accumulate these compounds in cytosol (Eapen et al., 2007).

- Of the different Phase II reactions that are most commonly involved in pollutant metabolism in trees, conjugation with GSH is one of the most important reactions and often the rate limiting step in the detoxification of an organic compound
- GSH transferases mediate the GSH-conjugation of chloroacetanilide herbicides in poplar trees according to the reaction:

GSH + X-R
$$\xrightarrow{GST}$$
 GS-R + XH
X-R = pollutant
GS-R = glutathione conjugate of the pollutant

Glutathione-conjugation reaction of a pollutant containing an X- leaving group

 Although the Phase II conjugation system is regarded as a detoxification process of xenobiotics, GSH conjugates are not devoid of biological activity. → reducing their concentration is important in detoxification

2025.02.19.

Phase III

- Compartmentalization: involves modified xenobiotics getting compartmentalized in vacuoles or getting bound to cell wall components such as lignin or hemicellulose (Coleman et al., 1997; Dietz and Schnoor, 2001; Eapen et al., 2007).
- In this phase (a potential final step in the non-oxidative utilization of xenobiotics) the conjugates are removed from vulnerable sites in cytosol and transported to sites where they may not interfere with cellular metabolism: soluble conjugates (with peptides, sugars, amino acids, etc.) are accumulated in vacuoles, whereas insoluble conjugates (coupled with pectin, lignin, xylan and other polysaccharide) are taken out of the cell and accumulated in plant cell walls (Sandermann, 1992; Sandermann, 1994; Eapen et al., 2007).
- Phase III reactions are unique to plants because they do not excrete xenobiotics as animals do. Plants therefore, need to somehow remove the xenobiotic within their own system. ATP driven vacuolar transporters are the main enzymes involved in this phase and further processing of conjugates may take place in the vacuolar matrix (Eapen et al., 2007).

Metabolization = \neq mineralization

Phytotoxicity

- Plant response for the foreign compounds (plant's physiological, biochemical and molecular responses)
 - Growth reduction
 - Chlorosis
 - Necrosis of tissues
 - Xenobiotic stress quantitative parameters
 - Relative growth rate (RGR)
 - Photosynthetitc pigments concentration in plant tissues
 - \circ Chlorophylls (total, a and b), carotenoids \uparrow
 - Biochemical alterations production of reactive oxygen species (ROS) (reduced forms of atmospheric oxigen) by products of various metabolic pathways. The rapid and transient production of high quantities of ROS consequently results in what is called —oxidative burst.


Effect of various *Pseudomonas* strains on sorghum development in a soil containing naphthalene (1 g/kg), phenanthrene (0.2 g/kg) and sodium arsenite (50 mg/kg): (1) bacteria-free plants in clean soil; (2) bacteria-free plants in contaminated soil. Plants inoculated with (3) *P. chlororaphis* PCL1391(pBS216,pKS1); (4) *P. chlororaphis* PCL1391(pBS216); (5) *P. aureofaciens* BS1393(pBS216,pKS1); (6) *P. aureofaciens* BS1393(pBS216) in contaminated soil.

Phytoremediation of Heavy Metal Contaminated Soils

- Remediation of inorganic contaminants differs from the case with organic compounds (organic compounds can be mineralized)
- Inorganic contamination must either physically remove the contaminant from the system or convert it into a biologically inert form.
- Phytostabilization, phytoextraction, rhizofiltration and phytovolatilization -Selection of plants for phytoremediation of metals depends on the particular application
- Besides plants rhizospheric microorganisms play an important role in metal removal from soil.
- Plants that accumulate toxic metals can be grown and harvested economically, leaving the soil or water with a greatly reduced level of toxic metal contamination.
- Dried, ashed or composted plant residues, highly enriched in heavy metals may be isolated as hazardous waste or recycled as bio-metal ore

Plant Adaptation to Metal Stress

- Two responses can be distinguished in the presence of metals metal sensitivity and metal resistance
- Resistance means that in spite of metal toxicity plant reacts in a way that allows it to survive high concentration of metals, and to produce the next generation of plants
- Resistance:
 - Avoidance (mechanisms for external protection of the plant from metal stress)
 - Tolerance (the plant is able to survive internal stress imposed by high metal concentrations)
- Plants that are tolerant to metalliferous soils can be divided into three groups according to the metal concentration found in their tissues (Baker, Brooks, 1989):
- 1. accumulator (uptake of very high levels of ions to the extent of exceeding the levels in the soil)
- 2. indicator (take up metals at a linear rate relative to the concentration of metal in the soil)
- 3. excluder (takes up metals but restricts increased concentrations in the shoots until a critical level is reached, above which metal concentrations start to increase)



Hyperaccumulators

- Hyperaccumulation: Content of specific metals in plant tissues exceeds levels that are actually required for normal growth and development.
- Hyperaccumulators can concentrate metals in their aboveground tissues to levels far exceeding those present in the soil or in the non-accumulating species growing nearby.
- A plant containing more than 0.1% of Ni, Co, Cu, Cr and Pb or 1% of Zn in its leaves and stems on the dry weight basis, can be considered a hyperaccumulator, if the accumulated amount exceeding the metal concentration in the soil
- Basic characteristics:
 - plant must be able to tolerate high levels of the element in root and shoot cells: hypertolerance is the first property that makes hyperaccumulation possible,
 - plant must have ability to translocated an element from the roots to the shoots at high rates,
 - there must be a rapid uptake rate for the element at level that occur in soil solution.

Agrostis stolonifera accumulates 300-times more of arsenic from soil than other plants growing in the same area





Sedum alfredii was found to be a hyperaccumulator of Cd

Alyssum and Thlaspi are very good nickel hyperaccumulators







Minuartia verna contained 1000-times more of cadmium than was in soil



Sonchus asper has hyperaccumulation capacity to Pb and Zn

Steps of metal accumulation

- Mobilization, root uptake and sequestration
 - Cation exchange by roots
 - Transport inside cells by chelating agents or other carriers
 - Rhizosphere effects
- Translocation: Metal absorbed by roots are translocated to different plant organs by the same
- physiological processes as are used to transport nutrients
- Tissue distribution and storage: The distribution pattern varies with plant species and element.



Schematic diagram shows the uptake, translocation, and sequestration of heavy metals in plants.



The organic compounds involved in heavy metal ion chelation include organic acids, amino acids, phytochelatins (PCs), metallothioneins (MTs), and cell wall proteins/pectins/polyphenols

Increasing Bioavailability of Heavy Metals

 The strategies used to improve heavy metal phytoremediation, including genetic engineering, microbe-assisted, and chelateassisted phytoremediation



Metal hyperaccumulation

- Hyperaccumulation provides a defense against herbivores and pathogens, an idea termed the 'elemental defense' hypothesis
- The molecular mechanisms of metal hypertolerance and hyperaccumulation are likely derived from alterations in the basic mechanisms involved in general metal homeostasis.
- Genes involved in metal transport, synthesis of metal chelators and oxidative stress responses are constitutively and highly expressed in metal hypertolerant and hyperaccumulator species.

J. Environ. Sci. Technol., 4 (2): 118-138, 2011

Plants	Metal	Bioaccumulation	References	
A. racemosus	\mathbf{Se}	$14,900 \ { m mg \ kg^{-1}}$	Beath <i>et al.</i> (1937)	
Sebertia acuminate	Ni	25% by wt. dried sap	Jaffre <i>et al</i> . (1976)	
Ipomea alpine	Cu	$12,300 \text{ mg kg}^{-1}$	Baker and Walker (1989)	
Berkheya coddii	Ni	$5,500 \ { m mg \ kg^{-1}}$	Robinson <i>et al.</i> (1997)	
Eichornia crassipes	Cr (Vi)	$6000~\mathrm{mg~kg^{-1}}$	Lytle <i>et al.</i> (1998)	
Iberis intermedia	Ti	$3,070 \ { m mg \ kg^{-1}}$	Leblanc <i>et al.</i> (1999)	
Alternanthera sessilis	Cv	$1017 { m ~mg~kg^{-1}}$	Sinha et al. (2002)	
Zea mays L. Cv Ganga 5	\mathbf{Cr}	$2538~{ m mg~kg^{-1}}$	Sharma et al. (2003)	
Pteris vittata	As	$23,000 \ \mu g \ g^{-1}$	Dong (2005)	
Sesbania drummondi	$\mathbf{C}\mathbf{d}$	$1687~{ m mg~kg^{-1}}$	Israr <i>et al</i> . (2006)	
Thlaspi caerulescens	Zn	$19410~{ m mg~kg^{-1}}$	Banasova and Horak (2008)	
Thlaspi caerulescens	$\mathbf{C}\mathbf{d}$	80 mg kg^{-1}	Banasova and Horak (2008)	
Myriophyllum heterophyllum	Cd	$21.46~\mu g~g^{-1}$	Sivaci <i>et al.</i> (2008)	
Potamogeton crispus	$\mathbf{C}\mathbf{d}$	$49.09 \ \mu g \ g^{-1}$	Sivaci <i>et al.</i> (2008)	
Sorghum sudanense	Cu	$5330~\mathrm{mg~kg^{-1}}$	Wei et al. (2008)	
Phragmites australis	\mathbf{Cr}	$4825~\mathrm{mg~kg^{-1}}$	Calheiros et al. (2008)	
Arabis paniculata	Cd	$1127~{ m mg~kg^{-1}}$ in the shoots	Zeng et al. (2009)	
Atriplex halimus subsp. schweinfurthii	$\mathbf{C}\mathbf{d}$	$606.51~\mu g~g^{-1}~DW$	Nedjimi and Daoud (2009).	
Sedum alfredii	$\mathbf{C}\mathbf{d}$	2,183	Jin and Liu (2009)	
Sedum alfredii	Zn	$13,799 \ { m mg \ kg^{-1} \ DW}$	Jin and Liu (2009)	
Phytolacca americana	Mn	$32,000 \ \mu g \ g^{-1}$	Pollard <i>et al.</i> (2009).	
Brassica juncea	Ni	$3916 \text{ mg kg}^{-1} \text{ dry wt.}$	Saraswat and Rai (2009)	
Potentilla griffithii	Zn	leaves (19,600) mg kg ⁻¹ dry weight	Hu et al. (2009)	
Rorippa globosa (Turcz.)	$\mathbf{C}\mathbf{d}$	218.9 $\mu g~g^{-1}~dry~weight~(DW)$	Sun et al. (2010)	
Thlaspi praecox Wulfen	Cd	> 1,000 µg g ⁻¹ dry weight in seeds	Vogel-Mikus et al. (2010)	

Table 3: Examples of some metal hyperaccumulator and their bioaccumulation potential



frontiers in Environmental Science

published: 13 November 2013 doi: 10.3389/fenvs.2018.0012



Ecological Potential of Plants for Phytoremediation and Ecorestoration of Fly Ash Deposits and Mine Wastes

Gordana Gajić*, Lola Djurdjević, Olga Kostić, SneŽana Jarić, Miroslava Mitrović and Pavle Pavlović

- Strategies for As, Cu, Zn, Mn, Fe, Cd, Ni and Pb uptake by plant roots from soil through different active transporters (ATs);
- ZIP/IRT, zinc, iron regulated protein; COPT, copper putative transporter; NRAMP, natural resistance – associated macrophage protein; HMA-heavy metal P-type ATPase; H⁺ATP, HA-proton efflux transporter; FRO-ferric reductase/oxidase; PEZ1, phenolics efflux zero1; TOM1, efflux transporter; YS1, yellow stripe-like1; NA, nicotinamine; MA, family of mugineic acids; LCT1, lowaffinity cation transporter; GNGC channels, putative transition metal transporter
- (B,C); Tolerance mechanisms for metal(loid)s in plants:
 (1) binding of metal(loid)s to the cell wall, (2) chelate of metal-organic acid on the outside of the cell membrane,
 (3) active metal efflux from cells, (4) metal-chelator complexes (5) compartmentalization or sequestration in a vacuole;
- Ats, active transporters; MTs, metal(loids); OA, organic acid; NA, nicotinamine; AA, amino acids; GSH, glutathione; PC, phytochelatines **(D)**.

Heavy metal	Soil (mg kg^{-1})	Shoot (mg kg^{-1})	Root (mg kg^{-1})	Shoot/root (%)	Shoot/total (%)
As	620.00	11.20	268.0	4.2	4.0
Cd	1.66	0.31	14.2	2.2	2.1
Cu	50.00	13.00	68.0	19.0	16.0
Cr	600.00	18.00	1750.0	1.0	1.0
Pb	730.00	78.20	87.8	87.0	47.0
Hg	6.17	0.12	10.8	11.0	6.0
Ni	300.00	448.00	1040.0	43.0	30.0
Se	74.30	11.30	24.8	46.0	44.0



Vetivera zizanioides: has an ability to accumulate multiple heavy metals in roots and shoots – good candidate for a wide range of phytoremediation purposes.



The root system of Vetiver grass in a polluted wetland under natural conditions (Darajeh et al., 2019)



50 cm in diamet

GRASS

CONVERTED INTO BRIQUETTES FOR COOKING

USED AS THATCH FOR ROOFING

ESSENTIAL OIL AND CRAFT PRODUCTION FOR MARKET

LIVESTOCK FEED, GROUND MULCH, AND SOIL RECONDITIONING

ROOTS

SOIL STABILIZATION, EROSION CONTROL, AND GROUNDWATER RETENTION

REMOVE NITRATES, PHOSPHATES AND HEAVY METALS CONTAMINANTS

TOLERANT TO SOILS WITH HIGH AND LOW PH, SALINITY, AND HEAVY METALS

DROUGHT AND FIRE RESISTANT

CARBON SEQUESTERING

(a)



Thlaspi caerulescens the only perennial weed that can survive in zinc and nickel contaminated areas, adsorbing these metals by phytoextraction

OPEN OACCESS Freely available online

PLOS pathogens

Metal Hyperaccumulation Armors Plants against Disease

Helen Fones, Calum A. R. Davis, Arantza Rico, Fang Fang[¤], J. Andrew C. Smith*, Gail M. Preston*

- *Thlaspi caerulescens*, a hyperaccumulator of zinc, nickel, and cadmium, vs. the bacterial pathogen *Pseudomonas syringae*
- *pv. maculicola* (Psm) → hyperaccumulation of any of the three metals inhibits growth of Psm in planta.
- Metal affects the pathogen directly.



REVIEV published: 13 November 201 loi: 10.3389/ferrys.2018.0012



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Gordana Gajić*, Lola Djurdjević, Olga Kostić, SneŽana Jarić, Miroslava Mitrović and Pavle Pavlović

Mine waste in Serbia

- Plant growth promoting bacteria (PGPB) present a consortium of bacteria that colonize different niches of plant roots and they can degrade pollutants more efficiently than a single species/strain.
- Rhizobacteria, such as Achromobacter, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Enterobacter, Pseudomonas, Serratia, and Streptomyces have been found to have beneficial effects on plant species in metal-contaminated environments because they can reduce the metal toxicity by biosorption and bioaccumulation.



TABLE 2 | Plant species grown on fly ash deposits and mine waste worldwide.

Plant species

FLY ASH DEPOSITS

Europe

Achillea millefolium, Agrostis capillaries, Calamagrostis epigejos, Chenopodium rubrum, Cirsium arvense, Crepis setosa, Cynodon dactylon, Dactylis glomerata, Daucus carota, Echium vulgare, Erigeron canadensis, Equisetum sp., Epilobium hirsutum, Festuca rubra, Hypericum perforatum, Linaria vulgaris, Lolium perenne, Lotus corniculatus, Medicago sativa, Melilotus officinalis, Oenothera biennis, Phragmites communis, Plantago lanceolata, Reseda lutea, Rumex acetosella, Polygonum lapathiholium, Pycreus glomeratus, Sonchus arvensis, Sysimbrium orientale Rumex obtusifolia, Salsola kali, Silene vulgaris, Sinapis arvensis, Solidago serotina, Senecio vulgaris, Sonchus oleraceus, Sorghum halepense, Trifolium repens, Tussilgo farfara, Vicia villosa, Verbascum phlomoides, Xantium strumarium, Acer pseudoplatanus, Amorpha fruticosa, Gleditschia triacanthos, Eleagnus angustifolia, Morus alba, Morus nigra, Robinia pseudoaccacia, Rosa canina, Rubus caesisus, Populus alba, Salix alba, Tamarix tetandra Hodgson and Townsend, 1973; Mulhern et al., 1989; Shaw, 1996; Djordjević-Miloradović, 1998; Pavlović et al., 2004; Djurdjević et al., 2006; Mitrović et al., 2008; Gajić and Pavlović, 2018; Gajić et al., in press

 Microorganisms in the rhizosphere significantly increase heavy metal availability and uptake by plants (Vamerali et al., 2010; Sheoran et al., 2011). These microorganisms can secrete enzymes and chelate into the rhizosphere, which lead to the formation of heavy metal-chelate complexes, thus improving heavy metal uptake and translocation (Clemens et al., 2002).

2025.02.19.

References

Plant species	Bacterial strain	References	Plant species	Bacterial strain/Fungi	References		
PLANT PROMOTING	PLANT PROMOTING BACTERIA (PGPB)/MYCORRHIZAL FUNGI						
Lolium perenne Trifolium repens	Rhizobium leguminosarum	Johnson et al., 2005	Lotus edulis Lotus ornithopodioides	Variovorax paradoxus5C-2 + Mesorhizobium loti Le2 V. paradoxus 5C-2 + Sinorhizobium sp. Mc1 Bacillus megaterium (633) + Azozobacter chroococum	Safranova et al., 2012		
			Elymus repens Bromus tectorum Cardaria draba		Petrisor et al., 2004		
Sorghum bicolor	Pseudomonas montellili Bacillus subtilis Bacillus pumilis Pseudomonas pseudoalcaligenes Brevibacterium halotolerans	Duponnois et al., 2006; Abou-Shanab et al., 2008	Sedum alfredii + Medicago sativa	Phyllobacterium myrsinacearum RC6b	Liu et al., 2015		
Salix sp.	Pseudomonas sp. Janthinobacterium lividum	Kuffner et al., 2008	Veronica rechingeri	Glomus sp.	Zarei et al., 2008		
	Serratia marcescens Flavobacterium sp. Streptomyces sp.		Zea mays Plantago lanceolata	Glomus intraradices	Turnau et al., 2012		
	Agromyces sp		Salsola kali Panicum virgatum	Glomus etunicatum	Johnson, 1998		

What can we do with biomass?



Helena I Gome

- One of the main concerns regarding phytoremediation is the crop disposal after phytoextraction processes.
- Sas-Nowosielska *et al.* examined various strategies:
 - composting, compaction, incineration, ashing, pyrolysis, direct disposal and liquid extraction
 - Incineration (smelting) the most feasible, economically acceptable and environmentally sound.
 - Other authors have defended the pyrolysis of hyperaccumulator biomass, since char can be considered a rich 'ore' or metal concentrate, which can be processed for possible separation of the metal in a conventional ore-processing unit
 - The addition of biochar (the product of pyrolysis) to soil has been also suggested as a means to sequester carbon, thereby reducing the effects of human-induced climate change caused by CO₂ emissions



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Coupling phytoremediation of cadmium-contaminated soil with safe crop production based on a sorghum farming system

Zhi-Quan Liu ^{a, b, 1}, Hong-Li Li ^{a, c, 1}, Xian-Jie Zeng ^d, Cheng Lu ^{a, c}, Jing-Ying Fu ^{c, e}, Li-Jun Guo ^d, Wilson Mwangi Kimani ^{a, c}, Hui-Li Yan ^a, Zhen-Yan He ^a, Huai-Qing Hao ^{a, b, *}, Hai-Chun Jing ^{a, b, c}

Case study

- Approximately 5.6-38 103 tons of cadmium (Cd) are discharged annually.
- Easy transfer through the food chain and a long-lasting biological halflife → terrestrial-borne Cd has
 potentially become one of the most hazardous metallic pollutants to human beings and the
 environments
- Globally, nearly 12 million hectares of croplands are contaminated by cadmium (Cd)
- $^{\circ}\,$ Generally, Cd is absorbed by the roots of plants and then transported to the above ground parts
 - Root-to-shoot translocation via the xylem and xylem to phloem at nodes, then eventually into the grain.
- $^{\circ}\,$ Availability of Cd in the soil & genetic background of the plant



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Soil phytoremediation and biofuel industry



Type II Sorghum in Cd-polluted soil

Cd-contamination free grains



Safe use for food



Type III Sorghum in Cd-polluted soil

Cd-contamination free leaves and stems



Safe use for forage 2025.02.19.



Novel methods for microbial source tracking, pathogen flow modelling and quantitative microbial risk assessment

Edit Kaszab, PhD Department of Environmental Protection & Safety

Waterborne diseases

842,000 diarrhoeal deaths in low and middle-income countries are caused by inadequate drinking-water, sanitation and handwashing practices (WHO, 2014)

USA:

- 7.2 million cases/year
- 120,000 hospitalizations
- 7000 deaths
- \$3.2 billion healthcare costs



• Over 80% of our wastewater flows back to nature untreated.





Reactive vs. Proactive risk assessment

- For water quality, traditional coliform/*E. coli* treatment verification
 - For last 100 years based on controlling bacterial diseases (cholera /typhoid) from raw sewage contamination of waters
 - Problems include:
 - Pathogens are acute hazards, outbreaks often from short-duration events
 - Events can be missed with weekly or even daily sampling
 - Enteric bacteria easiest to remove/kill, enteric viruses and protozoa largely the issue today
- Solution, Proactive Management: **Health Target** of 'tolerable' risk to estimate reduction in enteric viruses, bacteria and parasitic protozoa



- <u>www.waterpathogens.org</u>
- 254 contributors from 49 countries
- Focus on pathogens and pathogen risks from excreta.
- Provide an updated review of the efficacy of sanitation technologies and serve as a compendium of waterborne pathogen information and quantitative data to support risk assessment to protect water safety.
- The project contribute to the implementation of SDG 6 on water and sanitation by:
 - Providing maps showing pathogen emissions to surface water from untreated and treated wastewater to support decision making (<u>target 6.1</u>);
 - Providing information on small and large systems for excreta and wastewater treatment (<u>target 6.2</u>);
 - Evaluating available wastewater treatment and sanitation technologies to achieve the needed removal of pathogens to protect other water resources and public health (target 6.3);
 - Providing information on water quality diagnostics (source tracking markers), which can be used to formulate Water Quality Agreements (<u>target 6.5</u>);





The Water Pathogen Knowledge to Practice (Water-K2P) project

 Support water and sanitation safety planners in using an evidence-based approach for managing health outcomes by improving access to scientific data on the efficacy of sanitation technologies and the occurrence and persistence of pathogens in human excreta and sewage.



Quantification of Microbial Source Tracking and Pathogenic Bacterial Markers in Water and Sediments of Tiaoxi River (Taihu Watershed)



Microbial source tracking (MST)

- Microbial source tracking (MST) is an emerging field that seeks to predict the source of microbial contamination in the environment. The field has been developing rapidly from a growing need to determine the source(s) of faecal contamination in aquatic environments.
- It is based on the assumption that, given the appropriate method and faecal source identifier, the source of faecal pollution (such as human, cattle, or bird) can be detected (US EPA 2005).
- Source identifiers are the genotypic or phenotypic traits used as targets to detect host specific microbial populations in environmental waters

Concept of Microbial Source Tracking (Image Courtesy – United States Environmental Protection Agency Great Lakes National Program Office and Rachael McNinch)





Steps involved in some of the different types of MST methods. Source: adapted from USEPA 2005. <u>https://www.who.int/water_sanitation_health/emerging/identification.pdf?ua=1</u>

NTERNATIONAL	JOURNAL	OF	ENVIRONMENTAL	HEALTH	RESEARCH
ttps://doi.org/1	0.1080/09	603	123.2019.1691719		

ARTICLE

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Taylor & Francis

Groundwater, soil and compost, as possible sources of virulent and antibiotic-resistant Pseudomonas aeruginosa

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- PFGE (Pulsed-field gel electrophoresis (Bannerman et al. 1995; Deplano et al. 2005)
- spel rare cutting enzyme for digestion
- CHEF DRII System (Bio-Rad) for the separation of fragments in 1% agarose gel.
- Electrophoresis for 25 h at 5.8 V, 14°C with the initial switch time of 10 sec and the final switch time of 45 sec.
- quantitative differences among the banding patterns were defined by the Dice coefficient.
- Unweighted pair group method with arithmetic mean (UPGMA) was used for cluster analysis of the PFGE patterns.
- Interpretation of chromosomal DNA restriction patterns based on the criteria of Tenover et al. (1995) using the following categories:
 - indistinguishable (homology higher than 95%),
 - closely related (homology between 85-95%),
 - possibly related (homology higher than 85%, but the criteria of Tenover were not fulfilled) and
 - different (homology lower than 85%).
 - PFGE results of environmental isolates were compared to the surveillance database of the National Center for Epidemiology, Hungary on hospital outbreaks.

An example



• Source of flowchart: Radó Júlia

https://www.youtube.com/watch?v=k_QAXLGuQ5w

9812-13 9812-13

85-95% homology in 5 cases with environmental-hospital strains - common origin?

95% homology (same PFGE type) in 7 cases between environmental strains



Promising barriers to environmental dissemination of antimicrobial resistance along the water and sanitation continuum and ultimately to human exposure



FEMS Microbiology Ecology, 2018, Vol. 94, No. 9

Quantitative Microbial Risk Assessment (QMRA)

What is risk and risk assessment?

• Risk = probability (likelyhood) x consequence



- Risk assessment (RA) is used to quantify potential health consequence(s) from pathogen(s)
 - Haas (1983) first adapted single-hit dose-response models for waterborne pathogens, which
 - Led to U.S. EPA being the first to use QMRA to consider pathogens for surface drinking water treatment rules (1989, 1991, 1998, 2006)
 - USDA formalized QMRA in 1995

Nicholas J. ASHBOLT: Introduction to QMRA and relevance to circumpolar household water uses. Alberta Innovates Translational Research Chair in Water
QMRA – Quatitative Microbial Risk Assessment





Table 2.4 Summary of the four-step framework for water-related QMRA

Step	Description
Problem formulation	The overall context (reference pathogens, exposure pathways, hazardous events and health outcomes of interest) of the risk assessment is defined and constrained in order to successfully target the specific risk management question that must be addressed.
Exposure assessment	The magnitude and frequency of exposure to each reference pathogen via the identified exposure pathway(s) and hazardous events are quantified.
Health effects assessment	Dose-response relationships (linking exposure dose to probability of infection or illness) and probability of morbidity and mortality (depending on the health end-point of the assessment) are identified for each reference pathogen.
Risk characterization	The information on exposure and the health effects assessment are combined to generate a quantitative measure of risk.

Quantitative microbial risk assessment (QMRA)



An example for QMRA – AquaNES webtool





Select the water source used in the system for which you want to assess the risk. The range of expected concentrations of pathogenic micro-organisms is then displayed under Pathogens. This range will be used in the risk assessment. The ranges are based on guidelines and references, which will be displayed on the Summary page. The 'i' button displays an introduction for each pathogen group and the index pathogen for which the risk is calculated.

Change values



Bacteria Min Value: 0.00e+00, Max Value: 1.10e+03 (CFU/liter) Viruses Min Value: 0.00e+00, Max Value: 3.00e+00 (PFU/liter) Protozoa Min Value: 2.00e+00, Max Value: 2.40e+02 ((oo)cyst/liter) Image: Comparison of the state of th	Pathogens 🙃				
	Bacteria 🔶	Min Value: 0.00e+00, Max Value: 1.10e+03	(CFU/liter)		
Protozoa Min Value: 2.00e+00, Max Value: 2.40e+02 ((oo)cyst/liter)	Viruses 🔶	Min Value: 0.00e+00, Max Value: 3.00e+00	(PFU/liter)		
	Protozoa 🔸	Min Value: 2.00e+00, Max Value: 2.40e+02	((oo)cyst/liter)		



Drag the treatment processes that are used into an empty box in the process scheme field. To delete a box click 'x' to add a box click '+' or drag and drop boxes to change the order. Point at the selected process to display a brief introduction. The range of log removal of each pathogen group by this process is shown. These log removals are used in the risk calculation. The references for the log removal values are provided on the summary page. If you have monitoring data for your processes, add the process here and then adapt the log removal values on the Summary page.



Date Created : 2020-04-17 Date Modified : 2020-04-17 Description: Irr. water, protected User: Takács Rita « Back Next »

Select the intended water use. If multiple uses are considered, select the one with the highest exposure. The exposure of the chosen water use is shown as the number of times the water is used this way and the amount of water that is ingested each time. Literature reference for the selected data is shown on the summary page.

Water Use

Irrigation, Unrestricted

Irrigation, Unrestricted Irrigation, Unrestricted Domestic Use, Car Washing Irrigation, Restricted Domestic Use, Toilet Flushing Drinking Water Irrigation, Public Irrigation, Garden Domestic Use, Washing Machine

Selected Water Use Information

Description:

100 g of lettuce leaves hold 10.8 mL water and cucumbers 0.4 mL at worst case (immediately post watering). A serve of lettuce (40 g) might hold 5 mL of recycled water and other produce might hold up to 1 mL per serve. Calculated frequencies are based on Autralian Bureau of Statistics (ABS) data

Water Use events per Year equal to 70 (events) Water Use per Event equal to 0.005 (liter)

Change values

Summary of Data

User Treatment Data Export PDF

Number of Monte Carlo Simulation: 1000

Click on a heading below to display a full overview of the default data that is used for the risk calculation based on your selections. The user can adapt these data based on their own microbial monitoring of treatment performance. Click on 'User Treatment Data' to open up a spreadsheet where you can calculate these values for each treatment process based on your data. Click on 'Enter Treatment Data' and adapt the numbers and distribution in the treatment table accordingly (set the distribution from 'uniform' to 'norm'). Replace the Reference by 'User data'. Click 'Export PDF' to save a copy of the data in a PDF file. Click 'Calculate' to perform calculation

Water Source: Danube							
Treatment Process							
Treatment Process	Order	Data	Bacteria	Viruses	Protozoa		
Bank filtration	1	Minimum Log Removal	2.0	2.1	1.0		
		Maximum Log Removal	6.0	8.3	2.0		
		Distribution	uniform	uniform	uniform		
		Reference	<u>WHO (2011): Drinking water guideline,</u> <u>Table 7.7</u>	<u>WHO (2011): Drinking water guideline,</u> <u>Table 7.7</u>	<u>WHO (2011): Drinking water guideline,</u> <u>Table 7.7</u>		

Results after Monte-Carlo-simulation



Infection risk per person per year





QMRA webtool

http://5.153.252.94:8080/QMRA/login.do



Pathogen flow model concepts



disability-adjusted life years (DALYs) Quantifying the Burden of Disease from mortality and morbidity Definition

One DALY can be thought of as one lost year of "healthy" life.

Pathogen Flow & Mapping Tool

- Evaluate areas with high emissions of pathogens to surface waters and evaluate the potential impact of changes in population growth, access to improved sanitation facilities, and increased conveyance and treatment of wastewater and fecal sludge.
- <u>https://tools.waterpathogens.org/maps/</u>
- <u>https://www.waterpathogens.org/k2p-demo/index.html</u>





- The frequency and intensity of floods are increasing
- Floods mean a serious public health risk due to the spread of pathogen microorganisms (E. coli and faecal coliforms, Salmonella, Hepatitis A, calicivirus...
- Dead animals, sewage (manholes, canals, court toilets)
- Lack of disinfection, consumption of contaminated water, food (fruit, vegetables)
- Infected mosquitoes the Korean mosquito (Aedes koreicus), the Asian bush mosquito (Aedes japonicus) and the Asian tiger mosquito (Aedes albopictus) have appeared in Hungary in recent years
- West Nylus fever, Chikungunya fever, nematodes (Difilaria, Brugia)
- A phenomenon directly affecting the quality of surface waters

Climate sensitive agents of water related illness (globalchange.gov)

Pathogen or Toxin Producer	Exposure Pathway	Selected Health Outcomes & Symptoms	Major Climate Correlation or Driver (strongest drivers listed first)
Algae: Toxigenic marine species of Alexandrium, Pseudo-nitzschia, Dinophysis, Gambierdiscus; Karenia brevis	Shellfish Fish Recreational waters (aerosolized toxins)	Gastrointestinal and neurologic illness caused by shellfish poisoning (paralytic, amnesic, diarrhetic, neurotoxic) or fish poisoning (ciguatera). Asthma exacerbations, eye irritations caused by contact with aerosolized toxins (K. brevis).	Temperature (increased water temperature), ocean surface currents, ocean acidification, hurricanes (Gambierdiscus spp. and K. brevis)
Cyanobacteria (multiple freshwater species producing toxins including microcystin)	Drinking water Recreational waters	Liver and kidney damage, gastroenteritis (diarrhea and vomiting), neurological disorders, and respiratory arrest.	Temperature, precipitation patterns
Enteric bacteria & protozoan parasites: Salmonella enterica; Campylobacter species; Toxigenic Escherichia coli; Cryptosporidium; Giardia	Drinking water Recreational waters Shellfish	Enteric pathogens generally cause gastroenteritis. Some cases may be severe and may be associated with long-term and recurring effects.	Temperature (air and water; both increase and decrease), heavy precipitation, and flooding
Enteric viruses: enteroviruses; rotaviruses; noroviruses; hepatitis A and E	Drinking water Recreational waters Shellfish	Most cases result in gastrointestinal illness. Severe outcomes may include paralysis and infection of the heart or other organs.	Heavy precipitation, flooding, and temperature (air and water; both increase and decrease)
Leptospira and Leptonema bacteria	Recreational waters	Mild to severe flu-like illness (with or without fever) to severe cases of meningitis, kidney, and liver failure.	Flooding, temperature (increased water temperature), heavy precipitation
Vibrio bacteria species	Recreational waters Shellfish	Varies by species but include gastroenteritis (V. parahaemolyticus, V. cholerae), septicemia (bloodstream infection) through ingestion or wounds (V. vulnificus), skin, eye, and ear infections (V. alginolyticus).	Temperature (increased water temperature), sea level rise, precipitation patterns (as it affects coastal salinity)