European Journal of Science and Theology, *August 2013, Vol.9, No.4, 211-224*

SYMBIOSIS IN THE ENVIRONMENT BIOMANAGEMENT OF SOILS CONTAMINATED WITH HEAVY METALS

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(Received 24 May 2012, revised 31 May 2013)

Abstract

Anthropogenic activities have considerably altered the composition and functions of the soil. Remediation of contaminated soils using plants, microbes and other biological systems to degrade/convert environmental pollutants under controlled conditions to a level up to which they are harmless and their concentrations get below the concentration limit set by regulatory authorities is an ongoing challenge for researchers and authorities regulators in domain. This paper analyses bioremediation techniques and their biomanagement for the rehabilitation of soils contaminated with heavy metals, which could offer sustainable alternatives for ecological reconstruction of contaminated soils, feasible and economically sustainable. In this context it was shown that a number of biological agents that include both microbial communities and plants with different origins can be applied. Both biological systems can play a significant role in the management of soils polluted with metals.

Keywords: bioavailability, bioremediation, decontamination, phytoremediation, toxicity

1. Introduction

Humanity depends in an incommensurable degree on soil. Soil is the support of life since it acts likewise a home to a numerous community of microscopic and macroscopic plants and animals. As a composite living entity, soil contains a large diversity of microorganisms, which play a vital role in soil properties and transformations, due to the interactive biochemical functions of microbes [1-4]. On the other hand, soil is characterized by a high porosity, which confers it a huge surface area, so that it can retain various compounds. Ancient peoples, who lived in a powerful relationship with nature, used to

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venerate the soil, since it was not only their resource of living, but also the material for their homes and vessels, as well as for communication (on writing tablets).

The *Encyclopaedia of Soils in the Environment* mentions that „*In the Bible, the name assigned to the first human was Adam, derived from 'adama,' meaning soil. The name given to that first earthling's mate was Hava (Eve, in transliteration), meaning 'living' or 'life-giving.' Together, therefore, Adam and Eve signified quite literally 'Soil and Life'*". The same Encyclopaedia enlightens that "*the Latin name for the human species – Homo, derived from humus, the material of the soil. Hence, the adjective 'human' also implies 'of the soil'*". [http://www.sciencedirect.com/science/referenceworks/9780123485304]

Soil logically behaves as a living filter, since pathogens and toxins that could otherwise collect leading to polluted terrestrial environment are turned into harmless. Unfortunately, during time, the humanity development has led to an altered and frequently amplified environmental impact. The challenge we are facing today is to ensure sustainability, by combining economic development with a reducing in environmental pressure. Environmental problems are one of the most critical concerns in the 21th century, considering the pressure that humanity continues to pose on the planet resources and its capacity to assimilate wastes released in the environment. The understanding of the mechanism of ecological systems in relation with human activities is a prerequisite in the development of theoretical and practical approaches on the symbiosis between anthropogenic and natural environments. Both site-specific and organism–environment exchanges influence the character of symbioses.

Any resource that is taken out and exploited in economy and society induces potential environmental impacts. This is true in particular for heavy metals, whose environmental footprints are expected to develop a more evident impact in the future owing to increasingly requirements for metals. Balancing metals use and life-cycles from environmental impacts point of views is consequently a central element of a directly desirable symbiosis with the ecological systems as well as for sustainable environmental management.

This paper gives the basic elements for an extensive reporting of the natural and sustainable alternatives in the removal/detoxification of soil polluted with heavy metals, using a large variety of biological materials therefore going ahead towards the improvement soils quality in the view of effective crops.

2. Bioavailability of heavy metals in soil

Plants possess the ability to extract, immobilize and/or detoxify heavy metals from polluted soils. Bioaccumulation is one of the most common ways in which living organisms retain heavy metal ions [1-5]. A number of plants possess the ability to grow on metalliferous soils and to accumulate extraordinarily high amounts of heavy metals in the aerial organs, at higher levels than those found in the majority of species, without suffering phytotoxic effects [6-8].

Plants that grow in soils containing metals can be grouped into three categories [9-11]:

- **excluders**, where metal concentrations are maintained until a critical value at a low level through shoots;
- **accumulators**: that concentrates metals in the aerial part of the plant and can be applied to a wide range of concentrations of metals in the soil;
- **indicators** where the internal concentration of the metal is a reflection of the external concentration.

Studies on hyperaccumulation of metals by plants are preponderantly developed after 1990, where hyperaccumulators proved to be small plants such as: *Alyssum murale*, which can grow on metamorphic rocks, *Brassica juncea* (Indian mustard), that extract lead, or *Thlaspi*, which extract and accumulate zinc and nickel [1, 8, 12-15]. Table 1 presents a series of plants and species of hyperaccumulators and metal species that can accumulate [6, 11, 14, 16, 17].

Table 1. Hyperaccumulators plants for metal species [6, 14, 16].

Metal	Species
Zinc	*Typha caerulescens*
Cadmium	*T. caerulescens*
Nickel	*Berkhya coddii*
Selenium	*Astragalus racemosa*
Copper	*Ipomoea alpina*
Cobalt	*Haumaniastrum*
Arsenic	*P. vittata*
Zinc, Nickel, Cadmium	*Thlaspi caerulescens*
Zinc/Cadmium	*T. caerulescens, Arabidopsis halleri*
Nickel	*Hybanthus floribundus* subsp. *Adpressus, H. Floribundus* subsp. *Floribundus, Pimelea leptospermoides*

In general, accumulation efficiency is not always very high, since there are not yet known plants to bioaccumulate some metals such as silver, mercury and arsenic. However, it has been demonstrated that some plants can tolerate and accumulate arsenic, while having the advantage that it has high growth rate and biomass abundance [18-20]; others can accumulate silver [21].

Bioavailability of metals in the plants refers to the amount of metal available for plant uptake from the environment [22-24]. Bioavailable fraction of metal refers to the proportion of metal usually available in free ionic form [25]. This is a function of the total concentration of the metal and the prevalence of physical, biological and chemical factors (pH, redox potential, the proportion of clay mineral components - carbonates and oxides - soil organic matter) [26]. Bioavailability is related to biological processes such as biosorption, bioaccumulation, solubilization [27]. Plants are exposed to metals mainly through soil aqueous phase [16]. Metal availability to plants is generally higher in low soil pH, but depends on speciation of metals according to the pH and redox potential. For example, the absorption of Cadmium in soil decreases with increasing pH values, from acidic to neutral values [5, 28-30], but for some metal species adsorption of metals increases in alkaline medium [31-34].

Bioremediation by plant-metal interactions can occur through the following mechanisms [2, 20, 35-38]:

1) *phytoextraction*, which refers to the use of plants that can accumulate pollutants (metals and organic compounds) by concentrating them in parts to be harvested;
2) *rhizofiltration*, which is use of plant roots to absorb pollutants (particularly metals);
3) *phytostabilization*, which involves the use of plants to reduce bioavailability of pollutants in the environment;
4) *phytovolatilization*, which means that plants are used to convert pollutants through volatilization.

To overcome the stress induced by the presence of metals, plants develop mechanisms to allow tolerance and detoxification of high levels of metal concentrations [39-41]. For example, metals can be linked by organic ligands in the extracellular cell wall, or can be detoxified within the cell [2, 33, 42, 43]. The enzymes are producing antioxidants that may reduce the effects of oxygen-reactive species (ORS).

3. Phytotoxicity of heavy metals in soil

Metals affect plant growth by three mechanisms [2, 16, 33]:

- **generation of oxygen reactive species** (as a result of redox active transition metals);
- **binding to functional groups in biomolecules** (metal redox-inactive);
- **displacement of metals in biomolecules**.

These mechanisms may act independently or simultaneously generating a specific area of toxic effects. Some of these are manifested as visible symptoms; others cause changes in cell structure in plants and interfere with normal physiological processes. Roots are the primary organs of plants which are in direct contact with contaminated soil and are sensitive to the toxic effects of heavy metals in the soil (Figure 1) [41, 44]. For this reason, the length of root is often used as an indicator of plant sensitivity to the toxic effects of metals [4, 16, 45].

A subject of particular relevance to heavy metal toxicity is the effect on seed germination processes in soil [29, 32, 46, 47].

In this context, phytotoxicity is seen as a delay in seed germination process, inhibition of plant growth together with any other adverse effect on plants caused by specific substances (phytotoxines) or growth conditions [42, 48, 49].

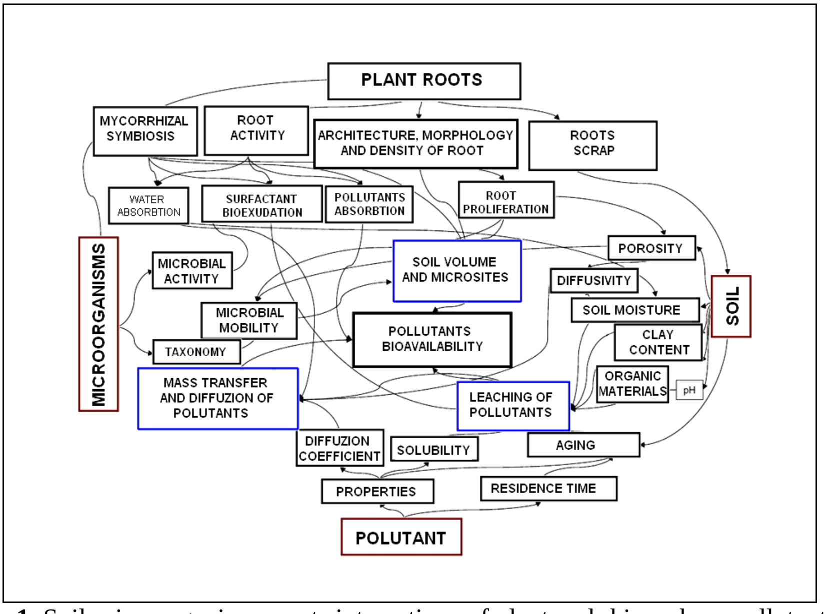

Figure 1. Soil-microorganisms-roots interactions of plant and rhizosphere pollutants responsible for the monitoring of pollutant bioavailability (adapted upon [49]).

3.1. Inhibition of germination

Some studies revealed the inhibitory effects of heavy metals during the germination of seed belonging to some species [32, 46, 50]. Atici et al. [50] studied the germination of the species *Cicer arietinum* (chickpeas) and found that it is significantly reduced by 54% in the presence of Zn (10mM) and by 73% in the presence of Pb (5mM). Germination of species *Oryza sativa* (rice) was 75% affected when Cu (1.5 mM) was presented in the soil, or completely inhibited at a concentration of 2 mM Cu [46]. In another study of germination, metals phytotoxicity has been ranked as follows: Cd> Cu> Ni> Pb for *Lactuca sativa* (lettuce), *Brassica oleracea* (broccoli), *Lycopersicon esculentum* (tomato), while for *Raphamus sativus* (radish), the order was: Cd = Ni > Pb > Cu [45].

3.2. Disturbance of plant growth

High levels of heavy metals concentration in the soil changes the usual way of growth and development of plants directly or indirectly. For example, Murch et al. [51] found experimentally that the nickel present in the soil can cause a reduction in growth rate of the plant *Hypericum perforatum* with 30-80% if the concentration of 25 mM, 50 mM respectively. Cell division and growth in length of roots are inhibited by excessive levels of Cu and Zn [16]. Heavy metals can influence plant development stages. High levels of concentration of Cu, Cr, Pb, Zn in the soil can cause side effects to large plants, including trees [52]. Metals can interfere with the use of energy in plants by altering enzyme activity, respectively, and ATP levels [53].

Therefore, the major components of plant cells can be affected by the presence of metals in the soil, observing a number of physiological effects.

3.3. Disturbance of cell membrane functions

Cell membranes are the first elements of plants affected by the toxicity of heavy metal ions, both structurally and that of system functions. Biological membranes consist of a double layer represented by lipidic components where proteins with different functions are included. Peroxidation of lipids induced by the presence of metals affects membrane structure and therefore, their capability to ensure the homeostasis of ions in the cytoplasm [54, 55]. Metals binding to sulfhydryl groups and active groups of proteins and enzymes induce deactivation of membrane associated proteins. Luna et al. [56] found that a high concentration of Cu intensifies reactions of protein peroxidation and membrane permeability in leaves of species *Avena sativa* (oats). In this context it was found that in the leaves of the *Triticum astivum* (wheat) lipid peroxidation, indicators of high levels of malonic dialdehyde concentration, is enhanced by exposure to Cr and Zn [57].

Membrane associated enzyme activity can be strongly disturbed by high concentrations of heavy metals. H^+-ATP enzyme activity in roots of *Cucumis sativus* decreased after exposure to 10 or 100 μM Cd, Cu or Ni [55]. Cu and Cd exposure caused a reduction in the amount of H^+-ATP in *Helianthus annuus* (sunflower) and wheat [54]. Also metal contamination can alter the biosynthesis of secondary metabolites in plants. In herbal medicine, Murch et al. [51] found a reduction of about 15 times the production of components with therapeutic role (hypericin) in the presence of 25 mM Ni.

3.4. Plants and limit concentrations of toxic metals

Heavy metals play a vital role in the metabolic processes of living organisms. Some heavy metals are essential for organisms with role of micronutrients (cobalt, copper, chromium, nickel, iron, manganese, zinc, etc.) and are known as trace elements. They are involved in redox processes, as catalysts in enzymatic reactions as well as osmotic balancing. On the other hand, some metals have no biological role and are toxic to living organisms even at very low concentrations (cadmium, mercury, lead). However at high concentrations the essential metals are toxic to living organisms [45, 58].

Metal toxicity to plants varies depending on the species of which they are part. Also, other factors that contribute to achieving the relations are: plant age, developmental stage, the amount of nutrients, stress levels [13, 16, 59]. In this complex context is difficult to determine the relative toxicity of different metals for any given plant.

4. The mechanism of metal tolerance

There are two strategies developed by plants to avoid metal toxicity [4, 10, 16, 60]:

- *exclusion*, when plants limits movement and penetration of metals into cells;
- *accumulation*, when permeated metals in plants are subjected to detoxification mechanisms such as chelating, active efflux, sequestration in vacuoles, cell wall binding matrix.

Other mechanisms refer to: increased production of antioxidants, reducing metals bioavailability by modifying the soil pH, cell wall binding metals, precipitation [16, 43]. Plants able to accumulate large quantities of metals - hyperaccumulators - are characterized by thresholds of hyperaccumulation that vary by species of plants and metals involved (Figure 2) [8, 61]. Johnson et al. [16] provided an analysis of these correlations (Table 2).

Some metals are able to accumulate in different plant species due to some mobile species in soil [47, 62]: for example there are several hundred hyperaccumulators of Ni, but less for Pb Cr, because these metals are mainly in insoluble forms in soil solution. Hyperaccumulators may play a role in phytoremediation of metals from soil by phytoextraction plan [63, 64]. Hyperacumulator plant species are often able to survive in soils where other crops can be affected. However, hyperaccumulator plants have low biomass, slow-growing, with a long period of germination [17, 65].

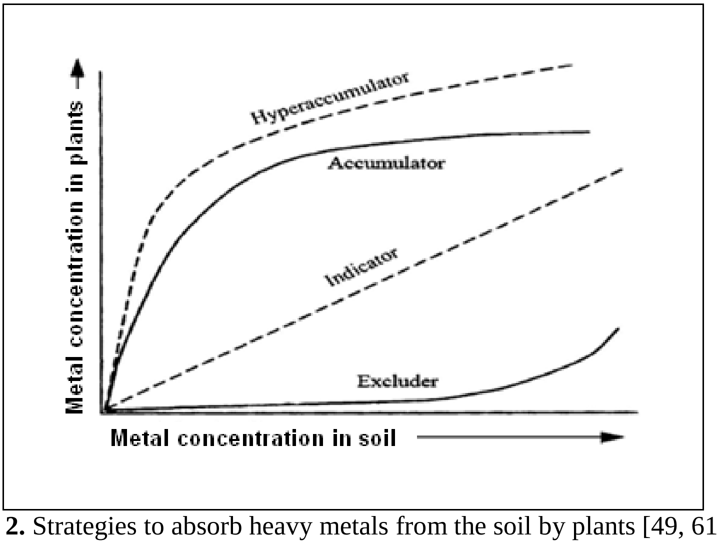

Figure 2. Strategies to absorb heavy metals from the soil by plants [49, 61].

Plants sensitive to metal toxicity can be appreciated by endogenous synthesis of metal-binding proteins, particularly metalotioenine (MTs) and fitochelatine (PCs) [16], in response to high levels of metals concentrations. Plants can also change the oxidation state of redox-active metals, thereby reducing toxicity. For example, *Larrea tridentate* species can reduce Cu^{2+} to Cu^{+} as a result of translocation from roots to leaves, using PC [66]. To reduce a disruptive high concentration of heavy metals, plants can produce antioxidants, which represent an indicator of metals toxicity [12].

Table 2. Threshold levels of some metals in some plant species hyperaccumulators [16].

Metal	Threshold concentration in leaves (mg/g dry weight)	Hyperaccumulator plants
Cd	0.1	*Thlaspi caerulescens* *Sedum alfredii*
Co	1	*Aeolanthus biformifolius* *Berkheya coddii*
Cr	1	*Sutera fodina* *Leptospermum scoparium*
Cu	1	*Aeolanthus biformifolius* *Commelina communis* *Crassula helmsii*
Mn	10	*Macadamia neurophylla*
Ni	1	*Alyssum bertolonii* *Alyssum murale* *Sebertia acuminata* *Berkheya coddii*
Pb	1	*Hemidesmus indicus*
Zn	10	*Thlaspi caerulescens* *Sedum alfredii*

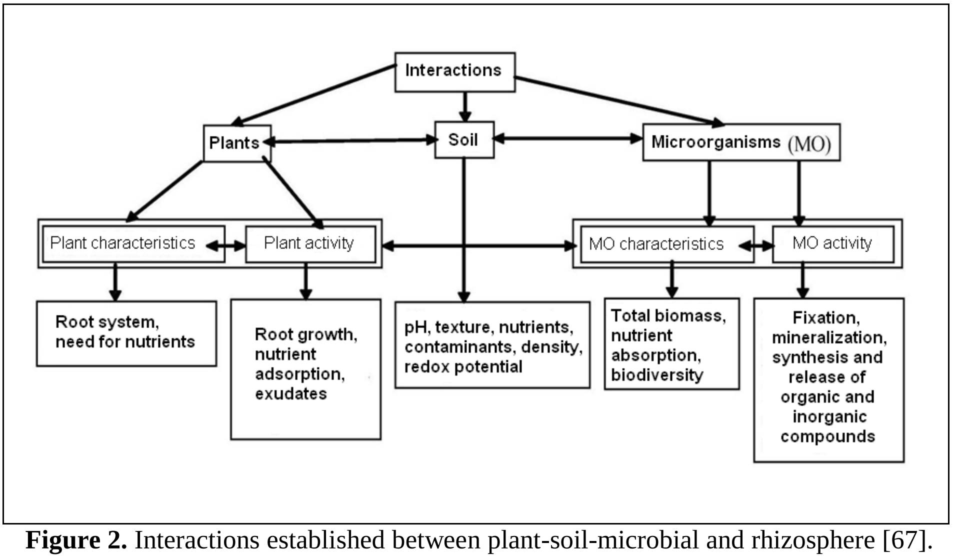

Figure 2. Interactions established between plant-soil-microbial and rhizosphere [67].

5. Metal - microorganism interaction: the role of microorganisms in the behaviour of heavy metals in the environment

Microbes have a variety of properties that can induce changes in metal speciation, their toxicity and mobility (Figure 3).

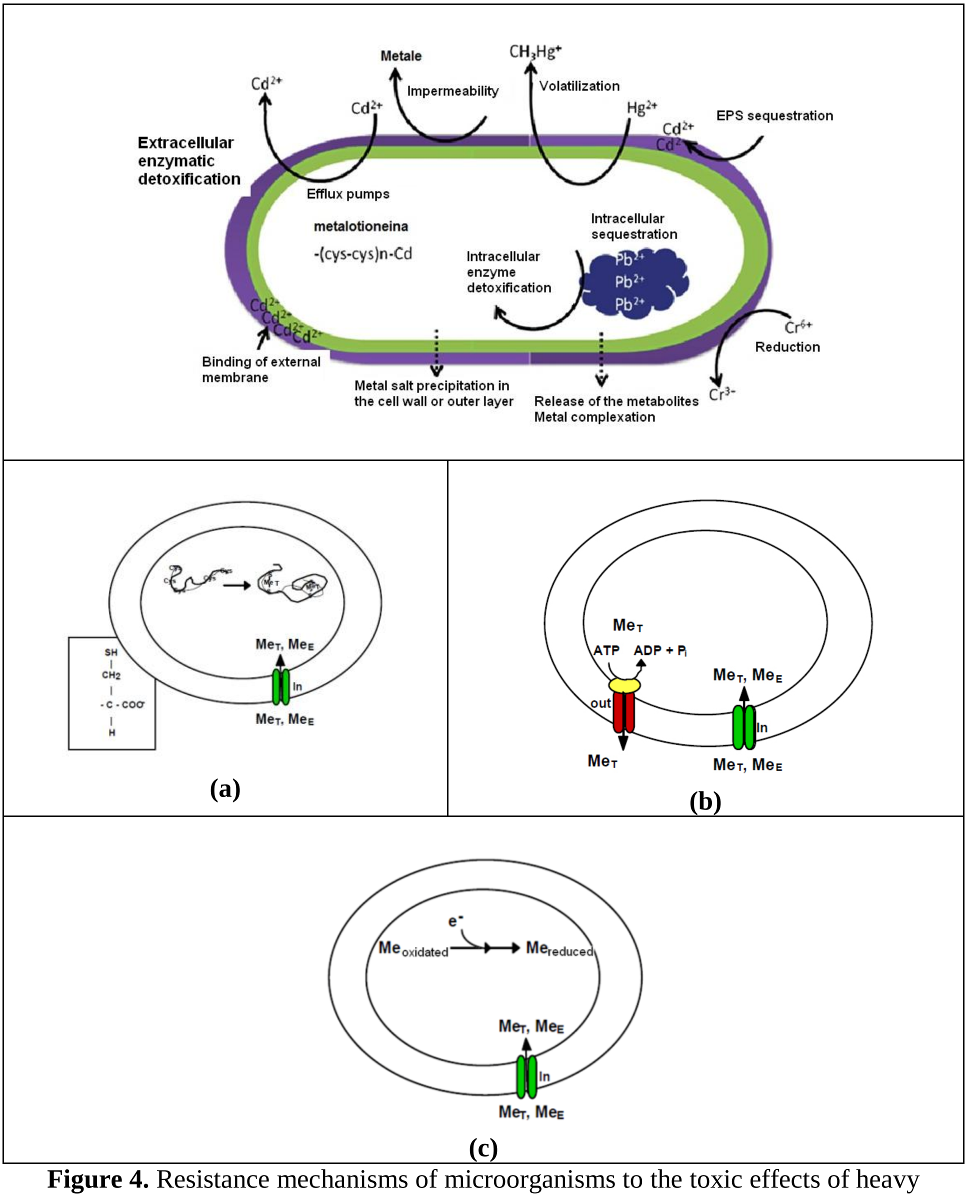

Figure 4. Resistance mechanisms of microorganisms to the toxic effects of heavy metals: (a) intra and extracellular sequestration, (b) active efflux pumps, (c) enzymatic reduction (adapted from [20]).

They are associated with biogeochemical cycle of metals and associated elements and their action is manifested through mobilization and immobilization of metals and radionuclides, depending on the mechanisms involved and the microclimate where the microorganisms are located [2, 19, 67-70]. Many microorganisms can adsorb, absorb and concentrate heavy metals, acquiring resistance to their toxic effects, thus having the potential to eliminate them from contaminated sites [15, 71-73].

Although microorganisms have a specific system of metal absorption, inside the cells can be transported considerable amounts of essential metals through a nonspecific mechanism. This 'open door' is one of the reasons why microorganisms are required to develop mechanisms that determine resistance to the toxic effects of heavy metals [20, 74]. Because metal ions cannot be degraded as well as toxic organic compounds, there are a number of possible mechanisms developed as a resistance systems for metal toxicity [20] (Figure 4):

- *exclusion through permeable barriers;*
- *intra-and extracellular sequestration (Figure 4a);*
- *active efflux pumps (Figure 4b) ;*
- *reduction of the enzyme (Figure 4c);*
- *reduction of cellular sensitivity.*

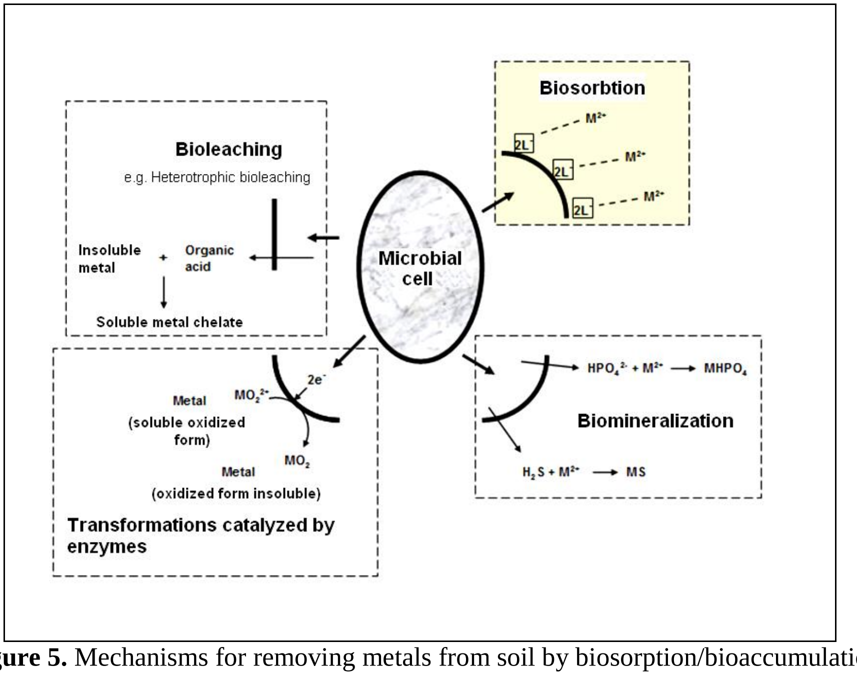

Figure 5. Mechanisms for removing metals from soil by biosorption/bioaccumulation [2, 75].

Microorganisms can exist and operate in environments contaminated with heavy metals through the development of one or more of these mechanisms. Mechanism of resistance against heavy metals depends on the genetic basis and thus varies with the species of microorganisms and heavy metal in question. Under certain conditions, even if the microbes are able to perform transformations of metals in redox reactions, precipitation, complexation, ion exchange, high concentrations can have harmful effects on soil microbial activity [75].

Numerous laboratory and field studies have demonstrated adverse effects of metals on soil ecosystems, such as reduction of microbial biomass [76, 77], decreased breathing capacity of the soil [62, 78].

Mechanisms of interaction between metal ions and microorganisms are now elucidated to a large extent. Phenomena such as biosorption (sorption of metal ions by biomass), bioprecipitation (metal ions precipitate as insoluble species induced by the biomass metabolism), biotransformation (biological oxidation-reduction biologically assisted or alkylation of metal ions by biomass) were evidenced in numerous studies and they are concisely illustrated in Figure 5 [2, 74, 75, 79, 80]. In the same context various models of these large-scale phenomena in the natural medium were developed and applied for the design of biological reactors to remove metals from soil [14, 73, 81, 82].

6. Conclusions

All processes heavy metals could suffer in the environment are important in controlling metal bioavailability in soils, sediments and water. The presence of metals in soils at toxic levels can draw a wide range of noticeable physiological symptoms in plants, which are able to respond to toxicity by either producing metal-binding compounds, sequestering metals into specific tissues, or by means of antioxidant systems.

Understanding the role of microorganisms can contribute to the development of feasible and effective processes for detoxification of contaminated sites. Therefore, the exploration and use of the natural mechanisms to detoxify and metal accumulation is the scientific basis for the application of bioremediation strategies by phytoremediation using hyperaccumulator plants, or biosorption and bioaccumulation using microorganisms on soils polluted with heavy metals. The screening and characterization of metal resistant microorganisms and plants are determinant in design and development of sustainable and feasible bioremediation processes.

The main strategies of pollution control are to reduce the bioavailability, mobility, and toxicity of metals, since it is known that excessive exposure to these heavy metals can cause toxic effects for microbes (bacteria, fungi) and plants.

Acknowledgement

Part of this paper has been elaborated with the support of a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0559, Contract 265/2011.

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