Chapter 2
Biogeosciences in Heavy Metal-Contaminated Soils

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2.1 Introduction

The development of the strongly interdisciplinary and highly applied research area of biogeosciences has been carried on to many fields, specifically those related to climate change or anthropogenic pollutions, where only the understanding of the interrelationships between both compartments allows prediction of changes introduced by mankind. This comparably young field of research integrates many different disciplines including hydrochemistry, plant physiology or microbiology and bacterial genetics, generally aiming at integration of effects of life ($\beta\acute{\iota}o\varsigma$) on Earth ($\gamma\varepsilon o\varsigma$). Facing global change phenomena, we can make the general statement that the interference of humans with biogeochemical cycles leads to a dangerous imbalance in the overall mass balance of nature. The outcome of this disordered matter cycling for agricultural use of soils in Europe and subsequently for food security has recently been outlined (Miraglia et al. 2009). Biogeoscience arose as an answer to the alteration of our environment and is thought to deliver the scientific approach for measures to be taken to alleviate these deleterious effects of disequilibrium.

Aside from atmospheric changes and global climate change, the effects of pollution and their remediation are a key subject within biogeosciences. While organic pollution, including for example oil spills, may be accessible to microbial degradation as a remediation measure, the pollution, especially of soil, with (heavy) metals is not easily remediated since the pollutant cannot be degraded. Thus, one aim of biogeochemistry as a center piece within the biogeosciences is the understanding of the processes by which microbes influence pedogenesis and movement of metals in soil and water (Borch et al. 2010). This is important in any mining operation, where alteration and dissolution of bedrock and low-grade ore material

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are seen. The mechanisms by which bacteria and fungi impact the fate and transport of contaminants are yet to be completely understood. This is necessary to allow for modeling approaches of contaminant fate and distribution (Wiatrowski and Barkay 2005). Only with a sound scientific basis of contaminant transport and metal introduction into food chains, remediation strategies can be developed. Therefore biogeochemistry could also be seen as conception of an open-ended system: the metabolic diversity of (micro)organisms is extended by the network of material transport trails and especially metal trafficking and distribution pathways in any kind of habitat. A successful application of knowledge gained in biogeochemistry has been realized in biorecovery (also known as bioleaching or biomining), the exploitation of microbial metabolism in order to mobilize and enrich metals from low-grade ores and sewage sludge (Pathak et al. 2009). For example, approximately 25% of copper originates from bioleaching processes (Stabnikova et al. 2010). This may demonstrate the impact microbial metabolism can have on metal mobility.

Bioremediation is at least of the same importance as biorecovery in applied biogeochemistry. In bioremediation, single organisms – mainly plants, bacteria and fungi – or organisms in their interaction are adopted to convert contaminated soil and water to a condition which is not deleterious to plants, animals or humans. The synergistic effects of interacting organisms may involve, for example, iron oxidizing microorganisms in the rhizosphere of wetland plants to precipitate the excess iron (Laanbroek 2010). Essentially, every type and method of bioremediation benefit from the huge metabolic diversity of organisms. Making use of the potential of very specific metabolic adaptations of adequately niched organisms, the main advantages of bioremediation lies in the comparatively moderate investment necessary, low energy demand, inherent safety of biological processes to the environment, low waste production and, in optimal cases, self-sustainability (Haferburg and Kothe 2010). However, the process needs time, and thus a substantial amount of monitoring is required to allow for optimized bioremediation. Instruments for field applicable biological and chemical monitoring such as microarray analysis systems or reporter gene assays become more and more common (Chandler et al. 2010; Alkorta et al. 2006). In order to decide on the optimal strategy and the best possible monitoring of success, a large set of data from both biological and (hydro) geochemical/physico-chemical parameters is necessary. This initially high investment of site-specific research is then balanced in bioremediation by the long-term low-input strategy which often can be achieved in enhanced natural attenuation,[1] if an optimized strategy is developed. Such a strategy needs to be site specific since climatic condition, hydrogeochemical

[1] The term "natural attenuation" often causes misunderstanding. Here it reflects on the whole of all physical, chemical and biological processes which are active in a particular postindustrial area without any anthropogenic intrusion. Under particular conditions, the interplay of all these processes results in a decrease of mass, toxicity, mobility, volume or concentration of pollutants in soil and groundwater. "Enhanced natural attenuation" also comprises approaches of biostimulation and bioaugmentation.

settings and metal contaminations are different for every site necessitating different (micro)biological amendments for bioremediation. In contrast to conventional soil remediation standards (so-called Dutch standards) there are no harmonized standards in Europe for the potential application of bioremediation strategies yet. It will be both a challenge and a virtue to work on standardization within and for the European Union.

2.2 Metal Contaminated Soil

After estimation from 1995, a total amount of over 700 million kg of metals is being dumped in mine tailings worldwide annually (Warhurst 2002). Depending on the metal (As, Cd, Cu, Ni, Pb and Zn), the volume of tailing material ranges from 10,000 to 600,000 metric tons (ib.), illustrating the negative consequences of ore processing. When large volumes of geogenic substrate are excavated, waste rock material is often still rich in metals after the extraction process. The reallocated geogenic material is prone to weathering and source of continuous metal release. Usually, the leached residues are dumped onto waste piles. Under irrigated and aerobic conditions, acid mine drainage ensues, often seen as seepage effluent with high-metal load and low pH. This contamination of the water path (often running through arable land) leads to soils with an increasing amount of metal and, subsequently, to a slow and continuous toxification of plants and animals, thus allowing for introduction in food chains and intoxication of humans through food or drinking water. In addition, the dilution leads to three-dimensional expansion of contamination which makes re-concentration and removal of metals impossible, resulting in both losses of metals and arable land.

In 2008, 1.4 billion tons of metals was produced globally which is a production rate sevenfold higher than in 1950. In 1950, metal consumption was 77 kg per person and year, which increased to 213 kg in 2008, varying tremendously among countries. While the benefits of metal production are easy to recognize, the negative impact is less obvious. Global mining occupies a territory of approximately 37,000 km^2 which equals approximately the area of Belgium or 0.2% of the world's land surface (Dudka and Adriano 1997). In addition, approximately 240,000 km^2 (approximately the size of the UK) is influenced by metals released from waste dumps and open mines (Furrer et al. 2002). Estimates of the European Environment Agency listed 1.4 million contaminated sites (Prasad et al. 2010). Since metal contamination cannot be detoxified by degradation, metal contaminated soils have to be either remediated by removal of the metals from the arable land with subsequent safe deposition, or by changing land use after metals have been immobilized on the spot.

An issue closely linked to the health hazards of metal contaminated land is soil erosion and land degradation. Estimations of the annual loss of farming land predominantly by industrialization, contamination, urbanization and desertification range between 70 and 140,000 km^2. 4.3 million km^2 of arable land became abandoned during the last 40 years. Globally, 100 billion tons of topsoil is lost every year (Döös 2002). Natural pedogenesis proceeds five times slower than devastation of soil. Especially, scarcely vegetated, metalliferrous soils are prone to whatsoever mechanism of erosion. With the given numbers, it seems evident that soil protection, soil remediation and soil recovery are of ultimate importance, especially when relating this to the growing world's population. Biogeosciences is meant to deliver a scientific understanding of the environmental changes and elaborate proposals how to counteract.

2.3 Soil Analyses

All bioremediation strategies depend on the geological and hydrogeochemical description of the site to be remediated. Both the contaminated soil as well as the bedrock need to be analyzed in terms of occurrence and distribution of mineral phases (petrology and lithology), concentration and bioavailability (using sequential extraction to determine the mobile and easily mobilized fractions of metals), adsorptive capacity of the soil (e.g., for clay minerals and humic acids), metal complexion and oxidation state (to determine factors for reactive transport) and mobility (in ground- and surface waters including hydrogeology and capillary flow). For reactive transport in acid mine drainage impacted landscapes, the use of rare earth elements has proven helpful to determine source and sink relationships (Haferburg et al. 2007). Stable isotopes have been used for some time now to follow distribution paths of elements (Miljević and Golobocanin 2007). Since biological system shows a strong selection for lighter isotopes in some cases, depending on the transport proteins and enzymes involved in uptake and metabolism, this methods seems specifically well suited to show the role of (micro)biology in changes of soil types and metal transport over time.

Pedogenetic parameters such as soil type, soil texture and particle size, organic matter content, humus layer, soil density and soil morphology thus need to be determined to provide input parameters for prognoses of development of a site. Pedogenesis connects the geological site description with the inventory of (micro) biological data. In order to evaluate the fertility of the soil substrate present, it is inevitable to analyze the microbial colonization, composition of microflora, soil respiration and microbial activity with respect to metal solubilization. For example, organic carbon content strongly influences the mobility of metals. The analysis of the organic carbon content thus helps to decide on the use of organic amendments to stabilize metals in the soil and to decrease metal mobility.

Finally, evaluation of ecotoxicological hazards can follow from an integration of all site-specific compiled data. An important step forward is provided by modeling approaches which address the expansion of contaminants at landscape level and provide a prognosis for the success of specific remediation measure. Since modeling can be used to determine the amount of monitoring necessary, the potential of bioremediation depends not only on the geological and pedological character of the site but also on data processing.

2.4 Microbial Communities

For the use of microbes in bioremediation actions, it is mandatory to isolate, cultivate and select strains. Soil bacteria in general can be described as metabolically very heterogenous. At least 150 diverse metabolic pathways and 900 different reactions of bacteria have been revealed (Scheffer and Schachtschabel 2010). Hence, it seems rather a problem of the appropriate screening assay to derive isolates for the desired bioremediation application than to find the corresponding biochemical feature. Bioaugmentation is the insertion of living microbial biomass into soil in order to make use of the strain(s) properties in a clean-up attempt. As a prerequisite for a bioaugmentation application, these bacterial strains need to be resistant against the metals in bioavailable concentrations present at the site of interest. The combination of soluble and easily remobilizable metals varies at each site which may indicate that the isolation of strains from the site investigated is the optimal strategy. However, strains resistant to metals may also be derived from nonpolluted environment, albeit with lower efficiency. Nevertheless, strains originating from other sites may be used in bioaugmentation if they are capable of withstanding the multimetal stress prevalent at the current site. There is a long-lasting discussion on advantages and disadvantages of biostimulation versus bioaugmentation. In biostimulation the remediation progress is triggered by external supply of nutrients whereas in bioaugmentation microbial biomass is introduced into the site to remediate. Biostimulation is based on the assumption that the remediation effect can be performed with the well-adapted microorganisms already present in the ground but they depend in their metabolic activity on an external supply of nutrients. Bioaugmentation in contrast seems indicated if the autochthonous microflora cannot exert the necessary remediation function alone. In general, this discussion shows how much more research is needed on the theory of “everything is everywhere, but, the environment selects” (Becking 1934; de Wit and Bouvier 2006).

Until now bioaugmentation plays a far greater role in bioremediation of soils contaminated with aliphatic, aromatic and halogenated hydrocarbons. Introduction of microorganisms in organically polluted soils results ideally in the overall metabolisation of the pollutant by the microbial cell. The microbially driven processes of soil decontamination from hydrocarbons are entirely different from those essential in bioremediation of metal contaminated soils. In general, two types of organisms are applied to treat soils with a metal burden: plant growth promoting (rhizo) bacteria (PGPR) and metal (im) mobilizing bacteria (MB). The combination of both groups seems to be the key for phytoextraction or phytostabilization.

Meanwhile production and trade of PGPR became an important market. Plenty of small biofertilizer enterprises were founded especially in India and China long before the application of PGPR (often combined with mycorrhiza inocula) reached the market for agriculture and gardening elsewhere in the rest of the world. Interestingly, the first biofertilizers (also named bioinoculants) were already applied in the nineteenth century, most notably with nitrogen-fixing *Rhizobium* species. Owing to the continuous research and development on plant growth promoting properties of microorganisms the biofertilizer market has amazingly grown and was estimated with a volume of $690 million for the USA alone in 2001(de Freitas 2002).

Primarily PGPR were applied to strengthen plant health and to increase crop yield. Subsequently strategies on PGPR supported phytoremediation of organically or inorganically polluted soils were developed from the knowledge gained in sustainable agriculture. Originally the group of PGPR has been described for *Pseudomonas* strains active as biocontrol organisms (Kloepper and Schroth 1978). Biocontrol has huge power to contain plant pathogens that threaten crop yield in disturbed but agriculturally used land. Especially in environments that provoke stress in plants, the use of biocontrol microorganisms seems to be an advantageous alternative to the application of pesticides. Intensified pest infestation on stressed plants and subsided symptom development due to PGPR activity are well investigated (Han et al. 2005; Babalola 2010). Nevertheless the term PGPR, coined by Kloepper and Schroth, was later extended to be applied for any bacterial isolate actively or passively promoting plant growth. Table 2.1 shows examples of PGPR.

Table 2.1 Characteristics and examples of plant growth promoting bacteria applied in bioremediation strategies

Characteristics of PGPR	Bacteria	Plant partner	References
Nitrogen fixation			
Freely associated bacteria	*Azotobacter chroococcum*	*Brassica juncea*	Wu et al. (2006)
Symbiotic bacteria	*Sinorhizobium meliloti*	*Medicago truncatula*	Bianco and Defez (2009)
Phosphate mobilization			
Inorganic P source	*Pseudomonas aeruginosa*	*Vigna mungo*	Ganesan (2008)
Organic P source	*Bacillus amyloliquefaciens*	*Zea mays*	Idriss et al. (2002)
Siderophore release			
Hydroxamates	*Streptomyces acidiscabies*	*Cicer arietinum*	Dimkpa et al. (2008)
Phenol catecholates	*Rhizobium* sp.	*Sesbania procumbens*	Sridevi et al. (2008)
Carboxylates	*Pseudomonas fluorescens*	*Arachis hypogaea*	Dey et al. (2004)
Salicylic acid	*Arthrobacter oxidans*	*Pinus* sp.	Barriuso et al. (2008)
Auxin production			
Indole acetic acid	*Enterobacter chloacae*	*Oryza sativa*	Mehnaz et al. (2001)
Cytokinin	*Pseudomonas fluorescens*	*Glycine max*	de Salamone et al. (2005)
Gibberellin	*Bacillus pumilus*	*Alnus glutinosa*	Gutierrez-Mañero et al. (2001)
Influence on metal toxicity			
Increased Ni accumulation	*Bacillus subtilis*	*Brassica juncea*	Zaidi et al. (2006)
Increased Cd accumulation	*Xanthomonas* sp.	*Brassica napus*	Sheng and Xia (2006)
Reduction of Cr(VI) to Cr(III)	*Ochrobactrum intermedium*	*Helianthus annuus*	Faisal and Hasnain (2005)

From an ecological perspective, in order to reach a stable colonization of plant roots it seems more reasonable to work with consortia instead of single strain inocula. However, it remains difficult and challenging to monitor the metabolic activity of the different inoculated strains in situ. Fluorescence in situ hybridization (FISH) is a method becoming more and more popular in soil microbiology. It combines microscopy of habitat samples as, e.g., root hairs and soil particles with the specific detection of particular microbial populations. The detection of the cells of interest in a cell mixture is based on fluorescence signals of labeled oligonucleotides priming only sequence-specific targets within the DNA (Dubey et al. 2006). This method was, for instance, successfully applied to discover the physiologically active bacteria in heavy metal contaminated sites (Margesin et al. 2011). Application of the BIOLOG identification system is an indirect method to profile changes in carbon metabolism of a microbial community during a remediation operation (Miller and Rhoden 1991; Alisi et al. 2009). This type of profiling metabolic activity of rhizo-bacterial communities could help to estimate the success and stability of root colonization but it hardly answers questions on competition among single strains or establishment/survival of particular strains. Nevertheless indirect methods of pheno- and genotyping of inoculated consortia are favorable over direct plating methods due to the loss of cells in any (re-)isolation campaign, first described as great plate count anomaly (Staley and Konopka 1985).

The isolation and screening procedure for PGPR follows the classical scheme (for an extended survey, see Steele and Stowers 1991). The screening for the most common PGBR comprises assays on phosphate solubilization, nitrogen fixation, siderophore release and phytohormone production. More specific tests are performed on the reduction of metal toxicity for the plant in combination with a higher metal accumulation in the above ground plant tissue. Usually PGPR are isolated from the rhizosphere or even more specifically from the rhizoplane. Bacteria of the rhizosphere seem to be highly adapted to the conditions of that particular microhabitat, especially as a result of the continuous flux of nutrients from the root, as, e.g., sugars, amino acids, lipids, phenolics and even vitamins or enzymes. It has been hypothesized that the strongly regulated secretory system of plants supports, restricts or terminates bacterial colonization in the rhizosphere (Bednarek et al. 2010). Nevertheless, plant growth promoting bacteria can also easily be retrieved from bulk soil in many cases.

It is impossible to completely describe the entire microflora of any soil in terms of biodiversity, quantity, ecological functions and microbe–soil, microbe– microbe, microbe–plant interrelationships. Nevertheless, the typical composition of the microbial community is used to derive conclusions on soil fertility and plant health. Without its microbial content, soil would be the simple anchoring matrix for plants. Even the nutrient supply grinds to a halt if replenishment of nutrients by microbial activity would not continue. Biological parameters seem to be the more reliable indicators to follow the progress of soil treatment (Yakovchenko et al. 1996). There are at least two reasons why evaluation of the microflora is the pivot of soil status monitoring: (1) Microbial communities respond fast to environmental changes and possess a high potential for adaptation. (2) Microbial communities act as bottleneck of metals on the their way from the soil matrix into the plant biomass. Thereby the microbial cell ties the abiotic soil sphere onto higher organisms (Hargreaves et al. 2003).

Both, autochthonous microorganisms of the remediation site as well as allochthonous strains that originate from nonmetal contaminated habitats can be adopted for a remediation treatment. It was found that several isolates of actinobacteria originating from undisturbed nonmetalliferrous soils can exhibit the same remarkable resistance patterns towards a range of heavy metals such as strains derived from mining areas (Haferburg et al. 2009). The more interesting question is if isolates that follow r-selection or K-selection are more likely to support the remediation progress. r-strategists (in which "r" stands for growth rate) or synonymously zymogenous bacteria exhibit a high growth rate and occur rather in unstable environments disturbed only for a short period of time. (Micro) organisms of the r-strategy are characterized by occupying a small ecological niche due to a highly specialized metabolism. They are less well adapted to cope successfully with a broad range of widely varying soil factors such as, e.g., carbon content, nitrogen source, pH or temperature. Zymogenous bacteria have been reported to be very efficient in bioremediation of land contaminated with hydrocarbons (Beškoski et al. 2011). Autochthonous microorganisms, in contrast, are thought to be the better candidate for application in a remediation strategy to immobilize or extract metals from soil. The most important criterion for their selection seems adaptability to the occurring soil conditions. Metal resistance and adaptation to plant promoting life in the rhizosphere are essential for phytoremediation. For the characterization of well-adapted organisms the term K-selection has been coined.[2] In terms of nutrition, they are considered as oligotrophic with a broad range of accepted carbon and energy sources. It has been found that populations of K-strategists are more likely to be stable in metal contaminated soils (Kozdrój 1995). The widespread confusion in the pairs of concepts "autochthonous/zymogenous", "r- and K-selection" and "oligotrophic/copiotrophic" is nicely dissolved by Langer et al. (2004).

Turning away from ecologically important concepts in a bioremediation approach one further application-related aspect of biogeoscience should be addressed. Biogeoscience can also be seen as the science of the soil/cell interphase. The investigation of microbe–mineral interaction gives an insight into metal (im) mobilization in rhizosphere habitats and oxic/anoxic interphases of soils. Elements such as As, Cr, Mn, Se and U can be used by anaerobic organisms as terminal electron acceptor (Borch et al. 2010). Some of these elements are toxic to the majority of soil microorganisms and plants. The toxicity always depends on mobility and availability of the metal. As, for example, in the case of the soluble and highly poisonous U^{6+} some microorganisms can use it as electron acceptor. The reduced form (U^{4+}) is insoluble, precipitates and is barely bioavailable. Those

[2] K stands for the carrying capacity in the Verhulst equation to calculate population dynamics.

microorganisms using U to gain energy, for example, members of the genera *Desulfosporosinus* and *Clostridium* should possess resistance mechanisms to avoid intoxication. In general, U precipitation due to microbial energy metabolism leads to a shift of the U-rich liquid phase to the solid (Wall and Krumholz 2006). The decrease of metal-rich ground waters is related to an increase of Uranium in the sediment.

Besides oxidation/reduction reactions there are further routes for the immobilization of metal contaminants using physiology and biochemistry of soil microorganisms. Sorption benefits from the metal binding capacity of biomass. The cell envelope of the microbial cell is commonly negatively charged, also depending on the pH, and delivers binding properties to metal cations. In a so-called biocurtain, growing microbial biomass is applied as a filter in the migration way of the contaminant. This filter supports mitigation of the metal expansion. The metal load in the water path could be both concentrated and located for a subsequent removal. Microbial geotechnology (also called biogeotechnology) is the field of research within the biogeosciences which deals with the adoption and optimization of remediation strategies such as bioclogging (sealing of soil pores by microbial means) and biocementation (soil particle consolidation and precipitation due to microbial metabolism). These microbiological soil installations could improve the mechanical properties of soil in situ. Thus, they can replace the more energy demanding and eco-unfriendly mechanical and chemical methods (Ivanov and Chu 2008).

In contrast to microbial soil technologies for metal immobilization, microbial metabolites are investigated on their influence on metal mobility in soil. The release of microbial chelators can lead to a significant metal solubilization. Siderophores are secondary metabolites produced by many very different microorganisms under iron depleting conditions for a specific sequestration and uptake of ferric iron. Structurally they comprise at least three groups: hydroxamates, catecholates and carboxylates. The biosynthesis of these compounds is considered as part of the secondary metabolism, which means they are not indispensable to life. Nevertheless, iron deficiency stimulates synthesis and release of siderophores in many bacteria and also fungi. It has been shown that some siderophores can sequester other metals than Fe^{3+} as well. If for instance the soil actinomycete *Streptomyces pilosus*, the yeast *Rhodotorula mucilaginosa* or the fungus *Ustilago sphaerogena* release their siderophores, lead becomes mobilized from a mineral absorbent (Dubbin and Ander 2003). Siderophores of streptomycetes have also been shown to bind Ni and to promote plant growth in Cd contaminated soil (Dimkpa et al. 2008, 2009). The beneficial role siderophores can play in plant growth promotion and protection from metal toxicity in combination with enhanced metal accumulation during phytoremediation has been shown in a few studies. Nevertheless, an important but currently still unanswered question that remains is how inoculated siderophore producing microorganisms colonize plant roots and survive at metal contaminated sites. The detailed mechanisms by which these bacteria contribute to plant metal acquisition is yet unsolved (Rajkumar et al. 2010). Another group of soil microorganisms fulfills an ecological function opposed to mobilizing reactions. These bacteria and fungi are prone to degrade organic compounds that bind metals and keep them soluble; unbound metals often precipitate. Degradation of chelators that keep metals in solution has been shown, for example, for phytosiderophores (Zhang 1993). Gathering together all these often antagonizing biological and geological processes in soil biogeosciences could be described as an attempt to develop an overall understanding of matter cycle at different scales from micro-habitat, soil layer, cropland to globally influenced landscapes.

2.5 Plant–Microbe Interactions

The best illustration for the perfectly tuned interplay of plant and microbe is the mucilage layer of the root hair. 20–40% of the photosynthetically derived assimilates leave the plant as exudates through the root. This corresponds approximately to a release of 5–21% of the fixed carbon (Marschner 1995). Root exudates provide microorganisms with essential nutrients. Hiltner coined the term "rhizosphere effect" in 1904 therewith expressing the phenomenon of a largely increased microbial colonization on or in close vicinity of the root hair. Cell numbers can be 10–1,000 times higher compared to bulk soil (Hiltner 1904; Lugtenberg and Kamilova 2009). Between 60 and 80% of the exudates are taken up by the microflora of the rhizosphere as carbon and nitrogen source. The microflora in turn supports and protects plants by a huge number of growth promoting effects. This sum of interactions between microbial cells and plant roots in a hostile environment such as metal contaminated soils of mining areas may demonstrate the complexity of biogeosciences.

There seems to be a strict separation in phytostabilization and phytoextraction for most of the publications dealing with bioremediation of metal stressed soils. Chaney first described the potential that lies in the use of metal accumulating plants for the extraction of metals from agrable soils (Chaney 1983). It is an average calculation of 2–5% of accumulated toxic elements in the dry weight of plants exposed to the contaminated soil to make the plant suitable for a phytoremediation approach (Brown 1995). However, there is still ongoing discussion on the reasonable use of criteria to select the plants most suitable for phytoremediation.

Why does the combination of microbially supported phytostabilization and phytoextraction appear such appealing to some soil scientists? Soils of metal rich postmining areas are commonly scarce in nutrients, poor in carbon and infertile concerning basic plant growth conditions. Thus, before starting with a large-scale phytoextraction the basis of vegetation needs to be established. Phytostabilization means progressing soil formation and improvement of plant growth conditions while metal seepage with the water path is prevented. Phytostabilization mechanisms comprise precipitation of metals by bacterial and root surfaces, precipitation of metals by bacterial and root exudates, bacterial uptake and sequestration of metals, and root uptake of metals (Mendez and Maier 2008). The extrusion of immobilized metals can be prevented during nonvegetation period in well-rooted soils. Plants for phytostabilization are not chosen according to the potential of metal accumulation but rather referring to fast and huge biomass production. The well-developed plant cover results in minimization of the metal efflux thus being comparable to plants used for rhizofiltration. Pedogenesis directly depends on the functional interplay of soil microorganisms and vegetation. Subsequently, if stabilization has reached an advanced stage, a modified set of plants (accumulator plants) can be used to extract metals from soil. Plant growth promoting bacteria and metal mobilizing microorganisms are essential to accomplish metal removal from the ground. For a functional metal extraction it is evidently the "rhizo-environment" which needs to be developed. The ideal phytoremediation should combine phytostabilization and phytoextraction even if this concept seems conflictive since the first corresponds to accumulation and the latter rather to exclusion.

The governmental organization "Environment Canada" is involved in many programs for environmental protection and has developed the database PHYTOREM to collect knowledge on plants with capabilities to accumulate or hyperaccumulate metals. Until now the database comprises 775 plants from 76 families (McIntyre 2003). Only for a fractional part of these plants the synergistic interplay with PGPB in a potentially powerful bioremediation operation was studied. But in various reports it was shown how and to what extent PGPB can enhance the capacity of plants to extract metals from soils and sediments. Studies on nickel uptake by different *Brassica* species showed an increase in the mobile metal fraction and metal accumulation in the plant without yield loss after treatment with PGPB (Ma et al. 2009, Rajkumar and Freitas 2008a). Comparable observations were reported for the uptake and accumulation of copper and zinc by *Ricinus communis* after soil inoculation with different positively plant growth promoting *Pseudomonas* strains (Rajkumar and Freitas 2008b). Often the accumulated amount of metals reaches the multiple of the non-PGPB treated plant. This small selection of examples should illustrate: (1) The interaction of PGPB and plant can be very effective for metal removal from the ground, (2) The dependency of the extraction yield from the chosen plant and applied microorganisms and (3) There is no overall uptake for all metals present in the contaminated soil.

The interest in phytoremediation as alternative remediation technique grew tremendously during the last two decades of research on phytoextraction. Nevertheless, obviously much more is known on the plant part in the microbe–plant interaction during plant growth in contaminated land. Databases such as PHYTOREM deliver an indispensable basis for a systematic research on the potential microbial boost of metal mobilization and removal at concurrent support of plant nutrition and stress reduction. The plant physiological potential of metal extraction and accumulation in terms of element specificity and concentration are not only species dependent as has been shown by Lai and colleagues in a screening of more than 30 plants but also very much ecotype related and even highly variable among different clones derived from the same mother plant (Lai et al. 2010; Lombi et al. 2000; Nehnevajova et al. 2007).

Phytoremediation does not only benefit from the application of plant growth promoting bacteria the usage of mycorrhiza in the remediation of metal contaminated land is also valuable. Mycorrhiza plays a crucial role in plant nutrition especially phosphorous supply and protection from metal toxification. Some mycorrhiza fungi such as *Gigaspora margarita* help to keep the metal concentration in the above ground plant material low (Andrade et al. 2010). This would support the erection of a pioneering vegetation cover in hostile postmining land. Other fungal partners in this symbiosis seem to "feed" the plant associate with an extra portion of the metal load of the soil. Some strains of several *Glomus* species increase the metal content in plant tissue (Citterio et al. 2005).

2.6 Characteristics of Plant Growth Promotion in the Context of Biogeosciences

Plant growth is often limited by phosphate availability. With up to 80% of the total soil phosphorous the main resources are organic (Condron et al. 1985). Nevertheless, for accessing both the organic and the inorganic phosphorous pool microbial mineralization is a prerequisite. After the continuous application of inorganic P fertilizer for decades, many soils contain comparably high but inaccessible P sources. P immobilization in soils or washout into the waterpath is a rapid process resulting in both an ecological threat and an increase in fertilizer expenses. P mobilizing bacteria seem to afford the necessary alternative for the remobilization of the unavailable phosphorous pool of soils. The microbiological P fertilization becomes an important issue especially when realizing resources of rock phosphate which delivers the basis for P fertilization are declining. The global reserves would be exhausted within the next 80–250 years after estimation based on the current extraction rate (Smil 2000). Globally, there is no complete cycling of phosphorous as for sulfur or nitrogen.

Nitrogen fixing bacteria are other important associates in biofertilization. Costs for nitrogen fertilizers can be lowered by exploiting the potential of bacteria such as *Azotobacter*, *Azospirillum* and *Rhizobium* to fix nitrogen from air. The main advantage of this "biological nitrogen fertilization" in the rhizosphere is the supply of nitrogen sources at exactly the right place. Sixty-five percentage of the synthetically derived mineral nitrogen is lost from the plant–soil system by degassing and leaching (Bhattacharjee et al. 2008). Thus, application of nitrogen fixing bacteria helps to reduce costs for fertilizers and keeps the N supply of plants at a balanced level.

2.7 Tool Box Strategy: Optimizing the Microbiologically Supported Remediation of Heavy Metal Contaminated Soils

Fifty-two million hectares land in the European Union was estimated to be affected by soil degradation (Peuke and Rennenberg 2005). This is more than 16% of the total land area. The costs to remediate contaminated sites within the EU have been calculated to be between €59 and €109 billion (ib.). These numbers emphasize the demand for soil preserving clean-up technologies. The development of tool boxes could be a big step forward in the development of powerful phytoremediation strategies.

The tool box in bioremediation operates similar to the letter case in a printing press. The basic principle is the combination of three collections: (1) site description of postmining areas with a major consideration of pedologic characteristics, (2) collection of metal resistant and plant growth promoting microbial strains, including their metal resistance patterns and (3) seed collection of metal accumulating or excluding plants. Microorganisms as well as plants are adapted to their specific habitats or niches. This adaptation together with particular traits of the "metal metabolism" of microorganisms is exploited to shift metalliferous habitats into nonmetal-determined ones. By the strength of the microbe–plant interplay postmining land could be changed to fertile ground (again) which is attractive to agriculture or any other soil use regime. The selection of plants depends on the factors climate, soil type, extent and kind of contamination. The plants in turn determine (in conjunction with above-mentioned factors) the choice of microorganisms. The innovation is the usage of the many possibilities microorganisms possess to solubilize/mobilize metals for phytoextraction and contrary to prevent mobilization for the phytostabilization approach. The tool box system would offer a huge choice of combinations from a pool of accumulator or excluder plants and plant growth promoting or metal mobilizing microorganisms for the realization of a remediation plan. Chapter 11 within this volume reflects on the development and usage of such tool box systems. The basis for any screening of soil strain collections on PGP properties is their metal tolerance or resistance. This characteristic is considered to be most important to keep the ecological fitness after reintroduction for remediation purposes. To cover a broad spectrum of PGP properties, the screening should comprise assays on (1) nitrogen fixation, (2) phosphate mobilization, (3) siderophore release and (4) phytohormone production. The cumulative effect of the various PGP traits which almost every PGPB strain exhibits makes the evaluation of the contribution of single biochemical properties difficult. Nevertheless, the above-mentioned BIOLOG system provides an option for biochemical activity estimation in pure and mixed cultures. For the caskets of the tool box to be filled with plants active in the remediation process one should consider (1) genera and species known and characterized as hyperaccumulators or excluders, (2) ecotype adaptation of single species, (3) metal enrichment specificity, (4) transfer factors and (5) biomass production.

From an ecological point of view, it is not very clear if the application of a strain multitude as initial bioinoculum leads to a more successful and persistent colonization of the plant root compared to a minimal bioaugmentation consisting of only few strains with the same PGP properties. Nevertheless, following results of Kloepper and Schroth it seems advantageous to inoculate the rhizosphere with a high number of PGPB strains since only about 2–5% of the reintroduced rhizobacteria survive and exert beneficial activities when soil already contains a particularly adapted microflora which easily outcompetes new intruders (Kloepper and Schroth 1978).

2.8 Conclusions

Biogeosciences delivers the fundamental for the analysis of both natural processes and anthropogenically induced imbalances in the environment from the vantage point of the bio-geo interphase. Examples for the interphase of inanimate matter and living, metabolically active organisms are microbially colonized mineral surfaces (e.g., pyrite oxidation by *Acidithiobacillus ferrooxidans*), root hairs in sediment (e.g., rhizofiltration of arsenic by *Phragmites* rhizofiltration) or mineral formation due to CO_2 reduction (e.g., calcareous shale of coralline algae). The main focus in biogeosciences is laid on the cycle of matter. When different elements are investigated in the cycle of matter microorganisms as destruents, mineralizers, sorbents and mobilizers are of special interest with respect to remediation. Most of the reactions within the cycle can be performed by bacteria and fungi but no other organism. Especially for the state-of-the-art means of environmental protection, the metabolism of microorganisms is of great importance. The increase of worldwide occurring environmental disasters is likely to be one reason of the ascent of the biogeosciences we observe.

Biogeosciences in metal contaminated soil gives a paramount example for the interdisciplinary of research. The knowledge gained thanks to the pronounced interdisciplinary was implemented into many bioremediation projects for the decontamination of soils. Phytoremediation nicely demonstrates the exploitable interplay of soil, microorganism and plant for metal removal. The development of tool box systems indicates a new and much stronger habitat-oriented access in remediation practices. The tripartite remediation tool box system consists of (1) A hydrogeochemical site description, (2) A set of metal excluder or accumulator plants and (3) Metal-resistant and well-adapted plant growth promoting bacteria. The combination of plants and microorganisms is thought to result in an optimized phytoremediation of postmining sites greatly varying in soil parameters. Therewith the tool box strategy is an application of the accumulated knowledge from biogeosciences and could ideally be used for soil resource protection.

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