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Research review paper

Biotechnological strategies applied to the decontamination of soils polluted with heavy metals

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ABSTRACT

Soils have been submitted to several contaminants that vary in concentration and composition. Heavy metals can be widely spread and accumulated in those environments due to some inappropriate actions. In this present review some remediation techniques to remediate soils are presented, focusing on the use of plants that are capable of surviving in soils with heavy metals along with the function of some microorganisms in the restoration process.

Contents

1. Soil contamination

Soils in the same way as aquatic environments are the target of thousands of contaminants that vary in composition and in concentration. These contaminants enter the system as a result of a wide range of actions such as intentional applications, inadequate residue disposal, accidental wastes and inappropriate use (Knaebel et al., 1994). The pollution by inorganic compounds as nitrates, phosphates and perchlorates is due to and inadequate disposal of manufacture residues of fireworks and matches (Nozawa-Inoue et al., 2005); explosives such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) from their manufacture and tests (Kitts et al., 1994); monoaromatic hydrocarbons like benzene, toluene, ethylbenzene and xylene (known as BTEX) from oil spills and of storage tanks leaking (Rooney-Varga et al., 1999); polycyclic aromatic hydrocarbons from accidental spills (Wang et al., 1990); a range of herbicides such as diuron, linuron and chlorotoluron used in weed control (Fantroussi et al., 1999) and by heavy metals (Glick, 2003).

2. Heavy metal contamination

According to Glick (2003), heavy metal contamination can be a consequence of industrial activities that eliminate residues in the soil

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that in long terms, promote their accumulation. The majority of the sources are originated by human actions like metal manufacture and mining industries with storage, disposal and transportation problems (Çelo et al., 1999; Zhang et al., 2005). Among the metals found more frequently there are cadmium, lead, cobalt, copper, mercury, nickel, selenium and zinc. For cadmium, lead, copper and zinc, their toxicity increases as follows: lead < zinc < copper < cadmium (Bååth, 1989), depending on countless abiotic and biotic factors.

3. Soil microorganisms — structure and analysis tools

The soil, as any other ecosystem, is an important habitat to thousands of organisms associated. Among the microscopic ones, there is a wide variety of fungi, actinobacteria, algae, protozoa and different types of bacteria that vary in physiology, in temperature and in the use of oxygen (Pelczar et al., 1981). The microorganisms can occur in association to clay particles or organic matter, in the rhizosphere of plants and in small colonies in the pores among the particles. In quantitative terms, the microorganisms vary with soil depth, becoming more diverse as the depth decreases (Alexander, 1977). Kuske et al. (2002) have done a counting of bacteria from an arid soil, noticing that in the outer layers the count of bacteria was significantly higher than in the inner ones and the quantity of DNA extracted was reduced with the increased depth (Sait et al., 2002). Torsvik et al. (1990) achieved a conclusion that the bacterial genetic diversity in the soil of a deciduous forest is extremely high, existing about 4000 different genotypes, excluding those of unculturable bacteria. Recently it has been discussed by Gewin (2006) the emergence of a new era in microbial ecology based on genomics, where there is a hope of sequencing the soil genome because over 99% of soil microorganisms remain unculturable. They also discuss that it is an important tool to help understand the interactions among the organisms that live underground and their role in the ecological processes (Gewin, 2006).

The techniques to gather information concerning the soil microbial communities can be based on either culturable methods or unculturable ones. However the techniques based on culturable methods are known for their selectivity and do not represent the real diversity (Amann et al., 1995). So, due to these limitations, it is necessary to use complementary molecular tools of identification.

In this way, the complexity of the interactions in different habitat can be studied by the use of molecular approaches based on PCR techniques, for example. Among these techniques, there are: a) DGGE (*Denaturing Gradient Gel Electrophoresis*); b) TGGE (*Temperature Gradient Gel Electrophoresis*); c) RFLP (*Restriction Fragment Length Polymorphism*); d) ARDRA (*Amplified Ribosomal DNA Restriction Analysis*); e) T-RFLP (*Terminal Restriction Fragment Length Polymorphism*); f) RISA (*Ribosomal Intergenic Spacer Analysis*) (Kirk et al., 2004). Besides, the availability of a large number of sequences of 16S rDNA in a data bank — RDP (*Ribosomal Database Project*) has allowed us to do the phylogenetical analysis. The association of community structure with its function and activity is the main focus of microbial ecology studies.

The ARDRA technique is the digestion with specific restriction enzymes, followed by the separation of the fragments in agarose gel electrophoresis (Ranjard et al., 2000). This technique has been used in the study of genetic changes in microbial communities submitted to alterations in environmental conditions or exposure to toxic exogenous compounds (Smit et al., 1997). Another technique commonly used is the DGGE technique based on PCR amplification, however there is no need to use a clone library. This method allows us to identify differences in the behavior of the double-strand DNA that when submitted to a urea-formamide gradient, form a complex band pattern. It has been widely used when a comparison of the genetic structure of the communities in different types of soil and submitted to environmental disturbance is needed (Ranjard et al., 2000).

So, some of these methods used made possible the identification of strains belonging to *Acidobacteria*, *Actinobacteria* e *Proteobacteria* (Janssen et al., 2002; Joseph et al., 2003; Sait et al., 2002), *Verrucomicrobia* (Janssen et al., 2002; Joseph et al., 2003) and *Gemmatimonadetes* (Joseph et al., 2003). In heavy metal contaminated soils using DGGE it was found bacteria belonging to the genus: *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Brochothrix*, *Comamonas*, *Cytophaga*, *Deinococcus*, *Enterobacter*, *Hafnia*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Rathayibacter*, *Rhodococcus*, *Salmonella*, *Serratia*, *Staphylococcus*, *Variovorax* e *Xanthomonas* (Ellis et al., 2003). However, the existence of these microorganisms can be influenced by several factors such as the presence of nutrients, pH and especially the humidity and temperature (Zilli et al., 2003). Alexander (1977) also states that aeration, cultivation, the season and the depth are relevant factors. Besides, the soil bacterial composition of tropical regions differ from temperate ones (Giller et al., 1997), being higher in areas with warmer climate (Alexander, 1977). Øvreas and Torsvik (1998) have demonstrated that bacterial diversity is higher in organic soils than in sandy ones. In a study done by Silva and Nahas (2002), it was also proved that bacterial diversity was increased in the samples submitted to liming, different crops and unfertilized soils.

The size of soil particles can also alter the structure of microbial communities, the richness tend to be higher in soils with smaller particles (Sessitsch et al., 2001). The soil microbiota plays an important role in the biogeochemical cycles and the high diversity is responsible for exerting a positive effect in the efficiency of nutrient recycling, contributing in the increase of ecological processes, promoting, in this way, the maintenance of good soil quality (Loreau, 2001).

4. Microorganisms and the contamination by heavy metals

Heavy metals exert some important roles in some biochemical reactions, being essential to the growth and development of microorganisms, plant and animals (Higgins and Burns, 1975). However, in high concentrations they can form unspecific compounds, creating cytotoxic effects (Nies, 1999). Besides, metals have some differences in relation to their biological importance, metals such as cerium, tin, gallium, thorium and zirconium do not have biological influence. Iron, manganese and molybdenium are important trace elements and present low toxicity. Cobalt, copper, chromium, nickel, tungsten, vanadium and zinc are toxic with high importance as trace elements. Antimony, arsenic, cadmium, lead, mercury, silver and uranium are highly toxic with limited biological function. Meargeay et al., 1985 tested the minimal inhibitory concentration (MIC) of some metallic ions to *Escherichia coli*, observing that Mn^{2+} had a MIC index of 20 mM being the least toxic and Hg^{2+} as the most toxic with concentration of 0.01 mM.

It is known that the high concentration of heavy metals can affect the microbiota directly through the modification of the population size, diversity and activity. However, it has been proved that fungi and bacteria are affected differentially (Rajapaksha et al., 2004). In a study done by Kandeler et al. (1996), it was noticed that an elevation in the concentration of heavy metals in the soil exerted a negative influence over microbial activity, resulting in a decrease in the functional diversity of soil microorganisms. In another study that corroborates this affirmation, it has been observed a significative reduction of some enzyme activities when submitted to high concentrations of metals in the soil (Kandeler et al., 2000) like dehydrogenase, acid phosphatase and β-glucosidase (Lee et al., 2002). Valsecchi et al. (1995) noticed and adverse effect of metals in the soil microbiota, because as the concentration of metals was elevated, there was an increase in the accumulation of organic matter due to the indigenous microbiota be less effective in the organic matter mineralization. Nordgren et al. (1983) studied the activity of some fungi in soils with high rates of Cu and Zn, concluding that exist a reduction in the biomass, respiration