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SOIL POLLUTION WITH HEAVY METALS AND BIOREMEDIATION METHODS

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Abstract

The soil is a living and dynamic ecosystem permanently subject to anthropogenic pollution. Heavy metal pollution is a major factor of microbiota imbalances, and thus of severe soil degradation. The microbiota is represented by a wide range of microbial species that maintain and improve soil fertility by performing biogeochemical cycles in nature. Heavy metals are found naturally in the soil and play a key role in supporting microbial processes, but excess waste stored and neutralized improperly can be harmful, due to bioaccumulation along the trophic chain. Among the heavy metals commonly found in the soil are: lead (Pb), chromium (Cr), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). The mobility of heavy metals in the environment, in general, and in the soil, in particular, is reduced, and therefore their contact with microorganisms is long and influences the structure of the microbial community. Existing studies emphasize the high bioaccumulation capacity of cadmium but also increased mobility affecting an entire trophic chain, and lead exerts an inhibitory effect on telluric microorganisms, even at very low concentrations. The overall activity of the soil, denitrifiers, and enzymes, is deeply affected by the presence of heavy metals. Their effect depends on the concentration and pH of the soil. Pedoclimatic factors can also contribute to these changes. Even in this situation, microorganisms can contribute to bioremediation and rendering the soil in the agricultural circuit. Among the bacterial genera that have developed mechanisms of resistance to certain heavy metals are Bacillus, Arthrobacter, Corynebacterium, Pseudomonas, Alcaligenes.

***Key words**: bioremediation, coliforms, denitrifiers, heavy metals, soil.*

INTRODUCTION

Soil pollution is a global problem that should attract the attention of all scientists in various fields, but especially those in agriculture, because it is the substrate that provides food for humans and animals, and its quality influences the quality of food (Savu, 2002; Petcu et al., 2019; Sandu, 2016), which has an impact directly on our health. As soil pollutants are very diverse, a unitary classification could not be made using a single criterion and therefore the sources of contamination and their effects on soil quality, as well as for bioremediation methods, need to be analyzed separately. For the most part, soil pollutants are residues of human activities - biological, household, and industrial waste, improperly stored waste, or manure, which will affect the earth's metabolism. Researchers' interest in soil microbial imbalances is growing in the context of increased soil degradation due to heavy metal pollution from anthropogenic actions. Soil is an important reservoir for many contaminants (Sandu et al., 2018). Anthropogenic activities generate soil biological pollution with pathogenic microorganisms that can migrate from soil to water or contaminate cultivated plants, can affect animals by eating contaminated plants and can reach food through processing (Petcu, 2013; Ghimpeteanu, 2015; Petcu et al., 2020), affecting the health of the human consumer. Along with pathogenic microorganisms, from the contaminated soil, can be isolated and various parasites, in different evolutionary stages, which could also pose a danger to human and animal health. Other soil contaminants come from household or industrial activities - chemicals, wastewater, hydrocarbons, heavy metals, radioactive substances. Due to the very wide range of pollutants, we focused on the study of heavy metals that pollute soils in western and southwestern Romania, their impact on terrestrial microbial populations, and the possibilities of soil bioremediation, to restore their natural environment.

BIODIVERSITY OF SOIL MICROORGANISMS

Soil is a complex habitat populated by a diverse microbiota represented by bacteria, fungi, algae, protozoa, and viruses (Tate, 2000; Lenart-Boroń and Boroń, 2014) (Table 1).

Table 1. Number and biomass of the main groups of microorganisms in the soil layer of 0-15 cm (Zarnea, 1994)

The group of microorganisms	Number/g soil/dry weight	The biomass (g/m^3)
Eubacteriae	10^8	160
Actinomicetae	10^5-10^8	160
Fungi	10^5	200
Algae	10^4-10^5	32
Protozoa	10^4	38

In addition to the biotic component, the soil also includes an abiotic component between the two there are trophic and energetic connections (Zarnea, 1994) (Figure 1).

Although microorganisms represent less than 0.5% of the soil mass, they have an intense activity that influences the properties and functions of the soil (Tate, 2000). It is also estimated that 60-80% of the total metabolism of this habitat is due to the microbiota (Balasubramanian, 2017).

Soil microorganisms are located mainly in the upper layers. Their number decreases with depth.

Also, the microbial community has a major role in all areas of human activity, agriculture, industry, biotechnology, and health. All biogeochemical cycles support the life of the planet and are based on the activity of microorganisms (Haney et al., 2008; García-Orenes et al., 2016; Sheth et al., 2016). Microorganisms are considered the effectors of a soil's fertility (Johns, 2017).

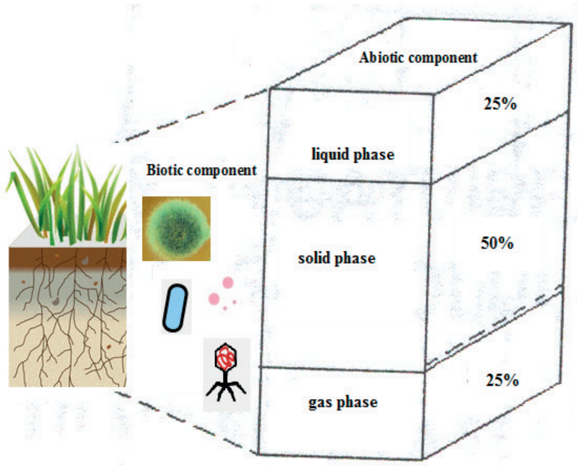

Figure 1. Soil components (processed image) (Zarnea, 1994; Sheth et al., 2016)

Soil algae belong to the phylum: *Chlorophyta*, *Diatomeae* and *Xanthophyta*. The number of algae can vary from a few hundred to 50,000 or even a few million when they grow on the surface of moist soil. Green algae are more common in acidic soils: *Chlorella, Cladhofora, Pleurococcus, Protococcus, Chlorococcus humicola* and are soil specific. The most important genera present in the soil are *Chlamydomonas, Chlorella, Cladophora, Dictyosphaerium, Pleurococcus, Protococcus, Scenedesmus* (*Chlorophyta*); *Cymbella, Fragilaria, Navicula, Nitzchia, Pinnularia, Synedra,* (*Bacillariophyta*); *Botrydium, Heterococcus, Vaucheria* (*Xanhophyta*).

The intense development of algae on the soil surface is the consequence of an intense photosynthetic process and the synthesis of organic matter. Soil fungi belong to 600 species, of which 200 *Phcomcomycetes*, 32 *Ascomycetes,* and 385 Imperfect Fungi. The class of Imperfect Fungi is the most numerous and is represented by *Alternaria* sp., *Aspergillus* sp., *Botrytis* sp., *Fusarium* sp., *Penicillium* sp., *Trichoderma.* From a quantitative point of view, but also the point of view of the biochemical activity carried out in the soil, the most important fungal genera are *Mucor* sp., *Rhizopus* sp., *Pythium* sp. - class *Zigomycetes*; *Chaetomium* sp., *Morchella* sp. - class *Ascomycetes*; *Alternaria* sp., *Aspergillus* sp., *Botrytis* sp., *Cladosporium* sp., *Fusarium* sp., *Geotrichum* sp., *Penicillium* sp., *Trichoderma* sp. *and Verticillium* sp. - class *Deuteromycetes*. Microscopic fungi are organisms with wide adaptability, predominantly aerobic, preferring well-aerated and free soils (Borozan, 2006). Compared to other microbial groups, the dominant bacteria in the soil are the most generous biomass, being followed in descending order by actinomycetes, fungi, algae, and protozoa. Their number is estimated to range from a few hundred million to 1 billion/g soil (Roselló-Mora and Amann, 2001). Bacteria with a high frequency in the soil belong to the bacterial orders: *Eubacteriales, Pseudomonadales, and Myxobacteriales*. Thus, following the studies, it was concluded that the dominant bacteria in the soil, in terms of quantity, belong to the following genera: *Bacillus* sp. (7-67%), *Arthrobacter* sp. (5-60%), *Agrobacterium* sp. (1-20%) and *Pseudomonas* sp. (1-20%). Other bacterial genera with a significant frequency in the soil are: *Achromobacter* sp., *Agrobacterium* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Bacillus* sp., *Brevibacterium* sp., *Cellulomonas* sp., *Clostridium* sp., *Chromobacterium* sp., *Corynebacterium* sp., *Flavobacterium* sp., *Micrococcus* sp., *Pseudomonas* sp., *Mycobacterium* spp., *Sarcina* sp., *Staphylococcus* sp. and *Streptococcus* sp. (Borozan, 2006).

Given the structural and functional characteristics of bacteria - unicellular, sporogenic, and non-sporogenic organisms, Gram-positive and Gram-negative - it can be said that their metabolic functions strongly affect the structure of the soil. At the time of imbalances in the soil, sporogenic bacteria have resistance compared to non-sporogenic ones. The most populated area is considered the rhizosphere of the soil. The microbial activity in this area is intense. The ecological and nutritional connections between microorganisms and plants are strong. In general, the dominant bacteria in the rhizosphere are unsporulated and Gram-negative.

The distribution and diversity of the microbiota are conditioned by edaphic factors, especially the requirements and availability of nutrients, water content, and pH (Sessitsch et al., 2001), but it is also influenced by plants (Dennis et al., 2010). Bacteria play a major role in agriculture, being involved in the circuit of elements in nature, in important processes for plant nutrition and production, and, in general, for the life of the planet (Johns, 2017). Mention should be made of symbiotic and free microorganisms fixing aerobic and anaerobic nitrogen, as well as nitrifying bacteria or those involved in the process of photosynthesis. Bacteria also help maintain and improve soil quality and health, improve soil aeration and structure. The fact that bacteria ensure soil stability proves their importance in revitalizing degraded soils. Cyanobacteria are ubiquitous, belong to the group of bacteria, the first to appear in terrestrial ecosystems, and are autotrophic. They are involved in the biogeochemical cycles of carbon and nitrogen. They improve the structure of the soil, increasing its fertility (Chamizo et al., 2018).

Actinomycetes belong to a large group of bacteria, they are filamentous, saprophytic, and parasitic. They actively participate in the mineralization of organic compounds, resulting in beneficial associations with the roots of some plants. The genus *Streptomyces* synthesizes antibiotics that help maintain a certain balance of soil microflora. Their number is from a few hundred thousand to a few million/gram of soil.

The genera of actinomycetes common to the soil are *Streptomyces* sp., *Nocardia* sp., *Micromonospora* sp., *Actinoplanes* sp., *Sreptosporangium* sp.

The varied metabolic potential of telluric bacterial populations, recommends them as the main pawns in various degradative processes and the annihilation of natural or anthropogenic pollutants and determines the diversity within the microbial community. Among the best decomposers, we mention actinomycetes (*Actinobacteria*) which are also major producers of antibiotics. All metabolic processes are catalyzed by enzymes.

Among the groups or bacterial species that serve as indicators of environmental quality, Sumampouw and Risjani (2014) mentions coliform bacteria, through the most representative exponent - *Escherichia coli, Streptococcus* sp., *Pseudomonas* sp., *Vibrio* sp., *Clostridia* sp., *Bifidobum* sp., *Arcobacter* sp., and *Thiobacillus* sp.

Many studies recommend the use of bacteria as indicators for soil imbalances. These imbalances can be caused by pollution with household waste, industry, fertilizers, pesticides (Kure et al., 2018), petroleum products, heavy metals (Oksfriani et al., 2014), and many others.

SOIL POLLUTION

Sources of periurban pollution of soil

Waste resulted from anthropogenic activities can be harmful to both the environment (terrestrial and aquatic ecosystems), and human health (Gutberlet and Uddin, 2017; Virsta and Sandu, 2020). The amount of garbage has increased with the increasing population, urbanization, and industrialization. For example, in the EU member states, every year around 3 billion tons of solid waste are thrown away (Panagos et al., 2013).

For efficient waste management, each European state has a national waste management plan. However, uncontrolled disposal, especially in peri-urban and rural areas, may result in severe heavy metal pollution occurring in water, soil, and plants (Ferronato and Toretta, 2019).

The main sources of waste in peri-urban areas are those from construction and demolition (C&D), especially because of the poor availability of land in growing urban areas for landfill sites. C&D waste represents more than 25% of the total municipal solid waste disposed of in landfills.

Increasing the volume of C&D waste has long-term negative effects on the environment, as well as harmful economic and social consequences (Yeheyis et al., 2013).

C&D waste management is still an unsolved issue in some European countries (Romania, Poland, Croatia).

The illegal dumping of C&D waste consisting of abandonment or improper storage on farming soil reflects C&D waste mismanagement (Mihai, 2019).

Soil pollution with construction and demolition waste

The composition of C&D waste is complex, the most commonly identified compounds being heavy metals, organic compounds, carbon, methane, sulfide, and hydrogen sulphide (Table 2).

The most important consequences of improper disposal of these wastes are soil pollution by heavy metals, which results in the degradation of the quality of the soil. Soils contaminated with heavy metals limit the development of plants due to their toxicity. Also, heavy metal toxicity affects microbial population size, diversity, and activity, as well as their genetic structure (Ayangbenro and Babalola, 2016). Moreover, long-term studies show that some compounds emit gases (e.g. hydrogen sulphide), which pollute the areas where they are stored (Chung, 2019; Wu et al., 2019).

Except for some materials (e.g. wood, gypsum, some plastics, and some paints), most of the components from C&D waste are not biodegradable (Yeheyis et al., 2013). Often, the concentrations of Cr, Cu, Ni, and Zn exceed maximum admitted concentrations for environmental matrices (Bianchini et al., 2020; Gao et al., 2015).

PAHs are widely present as contaminants in the environment, including soil, leading to widespread and accumulation in vegetation and ultimately in the food chain, with serious consequences for the public health (toxic, mutagenic, carcinogenic potential) (Ghosal et al., 2016).

The most important sources of soil PAH pollution are considered different industries, waste, and human activities (Lawal and Fantke, 2014).

The highest concentrations of PAH were found on the bituminous surfaces, chimney soot, and asphalt and in the case of the demolition waste category (Butera et al., 2014; Roussat, 2008).

Table 2. Waste composition in peri-urban soil pollution

Type of waste	Composition	Pollutant composition	Common adversely effects	Sources
Construction and demolition waste	concrete, bricks, cement, ceramic, bituminous mixtures, card, gypsum, insulation, asphalt, glass, steel, aluminum, plastics, paint, solvents, wood, asbestos, earth, and sand construction waste	heavy metals (Co; Cr; Cu; Ni; Pb; V; Zn), organic matter (polycyclic aromatic hydrocrack), carbon, methane, sulfuret, hydrogen sulphide	quality of soil, crop production, public health	Bianchini et al. (2020); Chung et al. (2019); Ferronato and Torretta (2019); Ojuederie and Babalola (2017); Yeheyis et al. (2013); Wu et al. (2019)
Domestic waste	organic waste (food, drinks, and beverages), packaging waste (paper, card, plastics, metals, glass), chemical products (cleaning solvents, paints, pesticide), textile, wood	microorganisms (*Bacillus, Clostridium*, lactic acid bacteria, *Enterococcus, Listeria, Pseudomonas*, Enterobacteriaceae) heavy metals		Burnley (2007); Chung et al. (2019); Gutberlet and Uddin (2017)

Soil pollution with household waste

The main constituents of household waste are paper and card, kitchen and garden waste, plastics, and glass, out of which, about 60% is biodegradable waste (Burnley, 2007). Research conducted by Bräutigam et al. (2014) showed that, in EU countries, the specific amount of food waste was between 174.8 kg (Slovakia) and 434 kg (Greece) per capita, with an average of 268.3 kg per capita, of which the group of fruits and vegetables represents on average, about 32%.

Biological treatment of food waste has multiple advantages, including the recovery of plant nutrients and replacement of conventional fertilizers and peat, sequestration of carbon in the soil and mitigation of greenhouse gases, biogas production, and substitution of fossil fuels (Edjabou, 2016).

Along with general household waste, household hazardous waste (HHW), which includes chemical fertilizers residues, pesticides, herbicides, cleaning products is disposed of in landfills.

Residues potentially dangerous to the environment and public health can reach 4% of the total municipal solid waste (Adamcová, 2016).

The effect of heavy metals on soil microorganisms

Metals are considered major pollutants for terrestrial and aquatic ecosystems, as a consequence of improper handling, treatment, or storage of waste (Valdman et al., 2001). Aljerf and AlMasri in 2018, mentioned that heavy metals tend to bioaccumulate and represent a real danger for the food chain (Aljerf and AlMasri, 2018).

Metals are found in the earth's crust (Sarubbo et al., 2015) and are essential for the proper development of physiological processes (Pete et al., 2017), but anthropogenic surplus disrupts the activity of microorganisms (Förstner and Wittmann, 2012).

Merchant and Helmann, in 2012, pointed out that processes, such as photosynthesis, nitrogen fixation, and respiration are dependent on metal ions. On the other hand, other studies have shown that the absence of too little metal can prevent the growth of bacteria (Hood and Skaar, 2012) and an excess amount exerts a bactericidal effect (Djoko et al., 2015).

The effect of metals on the diversity of soil microorganisms

Soils normally contain low levels of heavy metals, except in areas where agricultural, industrial, and municipal waste is found. The heavy metals commonly found in soil are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). They occur in soil naturally, as a result of pedogenetic processes (conc < 1000 mg kg^{-1}), anthropogenic (pedogenetic processes (conc < 1000 mg kg^{-1}), and anthropogenic

(Kure et al., 2018). Some studies have highlighted metals in soil (lead, chromium, cadmium, copper, zinc, and selenium) stating that they do not exceed the critical threshold (WHO/FAO, 2001; UNEP 2013; Toth et al., 2016). Heavy metals have low mobility in the environment, which means that the soil microbiota can be exposed to the harmful effect for a long time, regardless of concentration. Data from the literature show that microorganisms are quantitatively reduced in metal-contaminated ecosystems compared to uncontaminated environments (Kandeler et al., 2000). Stress caused by metals differs depending on soil pH, organic matter content, cation exchange capacity, temperature, and moisture content (Bafiafith, 1989; Giller et al., 1998). Heavy metals present in soils affect microorganisms and plants. It is also a danger to human and animal health, although most organisms play an important role in detoxification, especially bacteria that help the sustainable functioning of the biosphere (Díaz, 2004). Research has shown that they can also have an inhibitory effect on bacteria, fungi, and actinomycetes (Wyszkowska et al., 2008; Lugauskas et al., 2005).

The toxicity of heavy metals reduces or eliminates certain microorganisms from the environment and contributes to the modification of diversity and the selection of a tolerant microbiota (Moffett et al., 2003; Förstner and Wittmann, 2012). Xie et al. (2016) demonstrated that microbial diversity decreased with the increasing concentration of heavy metals (Xie et al., 2016). Subrahmanyam et al. (2016) mention several bacterial genera that include species that have developed mechanisms of resistance to certain heavy metals. This bacterial group includes both Gram-positive and Gram-negative species. The most representative genera are *Bacillus, Arthrobacter, Corynebacterium, Pseudomonas, Alcaligenes*, s.a (Subrahmanyam, 2016). Many biotic, enzymatic processes and implicitly the soil functions have been influenced by the presence of metals. Microbial activities can be affected by exposure to metals for both a longer and a shorter period time (Doelman, 1979; Hemida et al., 1997). These activities include soil respiration, which shows the global activity of microorganisms. Some authors have stated that it is the process most affected by the presence of heavy metals (Doelman, 1984). We must not forget the enzymatic activity that catalyzes all metabolic processes and which also suffered (Subrahmanyam et al., 2016). Another important activity is that carried out by denitrifying bacteria, which under conditions of anaerobiosis, reduces nitrate to nitrogen gas. According to some authors, this bacterial segment is strongly affected by metals (Holtan-Hartwig et al., 2002). Sobolev and Begonia (2008) state that denitrifying bacteria adapt to higher concentrations of lead (concentrations of 1 ppm, up to 500-2000 ppm), the result being the selection of resistant species. Lead (Pb) is one of the common metals that contaminate the soil and it is potentially toxic to microorganisms even at low concentrations. Pb can persist in the soil for 150 to 5000 years. The species that survive best in lead-contaminated soils are part of the genus *Bacillus* (Bashir Kazaure, 2018). Sobolev and Begonia (2008) showed changes in the diversity of soil microorganisms even at the lowest concentration of lead (1 ppm). Some sources in the literature mention that cadmium is a toxic metal with a high capacity for bioaccumulation. According to studies by Forstner (1995), the biological half-life is about 18 years. This study is also supported by Lin and Aarts (2012) who consider that cadmium has high mobility and can be transferred along the entire food chain. Changing the composition of the microbial community involves profound changes in the environmental biogeochemical processes (Holtan-Hartwig et al., 2002). Zinc is an essential micronutrient for the growth of all organisms, including microorganisms (White et al., 1973). It is a basic element of some enzymes and a factor that ensures the integrity and stability of some cellular structures (ribosomes, cytoplasmic membrane, deoxyribonucleic acid) (Prask and Plocke, 1971). However, some studies have shown that high concentrations affect the survival and growth of microorganisms. Thus, the toxicity of zinc determined the reduction of bacterial diversity by 25% compared to uncontaminated soil. This effect also leads to a reduction in microbial uniformity. The dominant group in both soil types was *Rubrobacter radiotolerans*

(Gram-positive bacteria) (Moffett, 2003). Chander and Brookes (1993) observed that at high zinc concentrations the total microbial biomass was reduced by 41% and the ratio between microbial biomass and organic carbon by 53%. This effect decreased over time as the zinc concentration decreased. Lawlor et al. (2000) found a correlation between total zinc or copper concentration and inhibition of bioluminescence in a strain of *Pseudomonas fluorescens*. Zinc, at a concentration of 10 mM, negatively influenced the bacteria *Escherichia coli*, the fungi *Rhizoctonia solani*, *Fusarium solani*, *Cunninghamella echinulata, Aspergillus niger,* and *Trichoderma viride*, but instead did not greatly influence the species *Pseudomonas aeruginosa, Norcardia ajallina* to the survival of the bacterium *Bacillus cereus*. Chromium is an essential micronutrient in mammalian metabolic processes (Mordenti and Piva, 1997), but it is a non-essential metal for other organisms (plants, microorganisms) (Cervantes et al., 2001). Chromium is present in soils as Cr (III) but can be oxidized to Cr (VI) (Bartlett, 1997), the toxic form for all organisms. It is carcinogenic for mammals, respectively mutagenic for both bacteria and mammals. In general, it spreads in the environment (soil, water) through industrial and agricultural activities. Some studies have shown the resistance of microorganisms to this metal (Bopp et al., 1983; Cervantes, 1991; Turik et al., 1996). Viti and Giovannetti (2001) have shown that high concentrations of chromium affect both oxygen photosynthetic microorganisms and heterotrophic bacteria.

The number (CFU) of heterotrophic bacteria was reduced in the contaminated soil (6.4×10^5 g soil^{-1}) compared to the control soil (1.0×10^{-6} g soil^{-1}). Gram-negative bacteria are also more sensitive than Gram-positive bacteria. Among cyanobacteria, species of the genus *Nostoc* dominated in soil with low chromium concentrations, but the number of species of this genus was reduced to high concentrations (Viti and Giovannetti, 2001). Mercury is considered one of the most toxic metals, which is not naturally present in living organisms and disrupts their functions by interacting with other essential elements (copper, zinc, iron, manganese, selenium, and calcium). Agricultural practices (application of herbicides, fungicides, insecticides, sludge) have led to the increased mercury content in the soil (Bryan and Langston, 1992; Azevedo and Rodriguez, 2012).

Muller et al. (2001) observed that the structure and diversity of bacteria and protozoa are reduced by this metal, but no changes in fungal biomass occurred. Salam et al. (2019) observed that mercury causes imbalances in soil microorganisms. Thus, they noticed that in the soil sample treated with 250 mg $HgCl_2$, there is a decrease of over 90% of the bacillus population. Also, of the 28 phylums reported in the untreated soil (*Proteobacteria* 41.55%, *Firmicutes* 31.46%, *Actinobacteria* 15.00%, *Bacteroidetes* 7.64%, and *Candidatus saccharibacteria* 1.84%) only 17 remained in the soil treaty. Some phylums were numerically affected (*Firmicutes* 5.36%, *Actinobacteria* 11.79%), others were stimulated (*Proteobacteria* 56.55%, *Planctomycetes* 14.67%, *Bacteroidetes* 11.46%), and the representatives of the *Archaea* domain were eliminated.

The genera most affected by the presence of mercury were especially *Staphylococcus* (99.71%) and *Brachybacterium* (99.75%). So there is a reduction of taxons, but also individuals inside them. Some literature data have stated that under acidic conditions (Gabriel and Williamson, 2004) soil organic matter and mercury form stable complexes (Wang et al., 1997; Dreher and Follmer, 2004).

The explanation given by some authors regarding the taxa that dominate the soil (even in the presence of metal) is, on the one hand, a large number of individuals within the taxon and, on the other hand, their high capacity to adapt to soil conditions (Goldfarb et al. 2011; Trivedi et al., 2016; Yin et al., 2017). Also, the increase of the representatives of a taxon can be attributed to the pH of the soil which decreased in the presence of the metal and the appearance of resistant strains (Schmidt et al., 1981; Kulichevskaya et al., 2008; Guo et al., 2012). On the other hand, Wagner-Dobler et al. (2000) stated that resistant strains can reduce the concentration of metal in contaminated soil.

Recent data have shown the resistance of *Proteus mirabilis* to heavy metals (Fatimawali et al., 2019; Zhang et al., 2013). Acuña et al. (2013) isolated an *Acidithiobacillus caldus* strain that can detoxify mercury-contaminated ecosystems. Although naturally present in the environment, arsenic in mobile form is an environmentally dangerous metal. Its presence in the environment is also a consequence of anthropogenic actions (Di et al., 2019).

The toxicity of arsenic depends on environmental conditions, but also the metabolic potential and detoxification of microorganisms (Bhattacharya, 2007). The involvement of the microbial community in the biogeochemical cycle of arsenic is already known (Huang, 2014). Also, microorganisms have developed and continue to develop mechanisms of resistance to this metal (Andres and Bertin, 2016), so the number of sensitive microorganisms are difficult to specify.

The resistance of organisms to low concentrations of arsenic (< 7 ppm) has been reported by many authors (Jackson et al., 2005).

BIOREMEDIATION

Some heavy metals, namely Cu, Zn, and Fe, are essential for the normal growth of microorganisms but can become toxic at high concentrations. It has been shown that heavy metals affect the microbial population, with negative influences on morphology, growth, metabolic activities, leading to decreased biomass and soil microbial diversity.

The tolerance of soil microorganisms to heavy metal contamination varies widely, and the proportion of resistant cultivable microorganisms can range from 10% to almost 100% (Abdu et al., 2017; Kamal et al., 2010).

Bioremediation is a modern technique using particularly microorganisms or their products for the removal of heavy metals in polluted environments, to restore the polluted environment to its original condition. Since microbial processes mediate bioremediation, two important ways are used to accelerate microbial activity in polluted areas, namely: biostimulation and bioaugmentation. Biostimulation involves the addition of nutrients or substrates to contaminated soil to stimulate the autochthonous microorganisms, and bioaugmentation refers to the introduction or increase of the microbial population with degradation properties of polluting compounds (Azubuike et al., 2016).

Microbial remediation uses various microorganisms to inactivate heavy metals in the soil, through various mechanisms, including absorption, precipitation, oxidation, and reduction. Various factors influence this microbial activity, such as metal bioavailability to the microbe, pollutant concentration, and soil characteristics (moisture content, nutrients, osmotic pressure, oxygen, pH, redox potential, temperature, soil structure, etc.). Although the toxicity of heavy metals is influenced by some soil characteristics (organic matter, divalent cation concentrations, and pH), the relative toxicity of some metals, namely Cd, Cu, Zn, and Pb, appear to be the same, regardless of soil type (Ayangbenro and Babalola, 2016).

The most common mechanisms by which microorganisms uptake heavy metals from the soil are biosorption (passive uptake) and bioaccumulation (active uptake).

Metal-binding metabolites, extracellular polymeric substances, including microbial biofilm structures, can bind significant amounts of heavy metals. It seems that fungi are the most resistant to the action of heavy metals, followed by Gram-negative and Gram-positive bacteria (Enuneku et al., 2020). However, the structure of the cell wall influences the number of heavy metals absorbed. Thus, in Gram-positive bacteria, the process of biosorption is more efficient, due to the presence of glycoproteins, compared to the Gram-negative bacteria, which contain phospholipids and LPS in their wall cell.

Several bacterial species, including *Pseudomonas, Enterobacter, Streptomyces, Rhodococcus, Amycolatopsis, Escherichia, Bacillus*, and *Micrococcus*, have proven special biosorption capacity (Ayangbenro and Babalola, 2016; Sharma et al., 2018; Wang and Chen, 2008).

Fungi (*Trichoderma*, *Aspergillus, Mucor, Rhizopus, Pleuritus, Penicillium*), and algae (*Asparagopsis, Codium, Padina, Cystoseira,* etc.) are also used to remedy areas contaminated with heavy metals, due to their ability to accumulate toxic metals (Ayangbenro and Babalola, 2016; Tiwari and Lata, 2018; Sharma et al., 2018).

CONCLUSIONS

Bioremediation is the phenomenon by which microorganisms, capable of degrading organic or inorganic pollutants in the lithosphere, bring the soil back into the agricultural circuit. Thus, among the microorganisms with the highest biodegradation capacity, the following bacterial genera can be listed - *Arthrobacter* sp., *Bacillus* sp., *Flavobacterium* sp., *Micrococcus* sp., *Pseudomonas* sp., *Thiobacillus* sp. or fungal - *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp.

The efficiency of bioremediation is influenced by certain physicochemical factors of the substrate subjected to depollution: environmental pH, temperature, humidity, texture, permeability, and soil bioventilation, but also by the quality and quantity of the contaminant.

Micro-organisms intended for the soil decontamination process must adapt to existing environmental conditions and competition with other micro-organisms. Thus, for the bioremediation process to be efficient, the environment must be favorable for the multiplication of depolluting microorganisms.

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