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THE POTENTIAL OF SOME INDIGENOUS MICROORGANISMS AND PLANTS FOR THE REMOVAL OF HEAVY METALS FROM SOIL

POTENŢIALUL UNOR MICROORGANISME ŞI PLANTE INDIGENE DE ELIMINARE A METALELOR GRELE DIN SOL

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Abstract. *Heavy metals found in soils from different industrial sources or mining activities are persistent inorganic pollutants able to bioaccumulate along the food chain and cause negative effects in the environment and for human health. Different physical, chemical and biological processes are applied for their removal from soil environments. Biological processes become more and more preferred, since bioremediation strategies have often proved to be more advantageous than the conventional remediation tools, mainly because these processes can be implemented directly onto the contaminated sites (in situ). In this context, the present paper examines the ability of microorganisms and plants to remove heavy metals from soil, in terms of tolerance and bioaccumulation. A particular interest is given to the bioaccumulation processes of metals by proteobacteria, bacilli and actinobacteria, alone or in synergism with indigenous plants. Also, some advances in the biosorption of highly toxic heavy metal ions as Cr(VI) and Cd(II) are just discussed, together with various strategies and practices to explore the synergism between microorganisms and plants as valuable biological resource for increasing tolerance against heavy metals and strengthening the bioremediation processes.*
Key words: bioaccumulation, microorganisms, plants, soil pollution, synergism, tolerance

Rezumat. *Metalele grele provenite din diferite surse industriale sau din activităţile miniere sunt poluanţi anorganici persistenţi ai solurilor, capabili să se bioacumuleze de-a lungul lanţului trofic şi să genereze efecte negative pentru mediu şi sănătatea umană. Pentru îndepărtarea metalelor grele din sol se pot aplica procese fizice, chimice şi biologice. Procesele biologice sunt preferate din ce în ce mai mult, deoarece strategiile de bioremediere s-au dovedit adesea mai avantajoase decât instrumentele de remediere convenţionale şi pot fi implementate direct pe siturile contaminate (in situ). În acest context, lucrarea analizează capacitatea unor microorganisme şi plante indigene de a elimina metalele grele din sol, în relaţie cu aspecte ce privesc toleranţa şi bioacumularea metalelor grele. O atenţie aparte se acordă proceselor de bioacumulare a metalelor în proteobacterii, bacili şi actinobacterii, singure sau*

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în sinergism cu unele plante indigene. De asemenea sunt evidenţiate progresele în biosorbţia ionilor unor metale grele foarte toxice, precum Cr(VI) şi Cd(II) şi sunt discutate pe scurt diferite strategii şi practici destinate explorării sinergismului dintre microorganisme şi plante ca o resursă biologică valoroasă pentru creşterea toleranţei la metale grele şi progresului în cunoaşterea proceselor de bioremediere.

Cuvinte cheie: bioacumulare, microorganisme, plante, poluarea solului, sinergism, toleranţă

INTRODUCTION

Nowadays numerous sites worldwide are contaminated with various organic and inorganic pollutants having different toxic and persistence characteristics. The presence of inorganic toxic pollutants in soils is especially given by heavy metals ions (Gavrilescu, 2014; Pavel *et al.*, 2012; Sobariu *et al.,* 2017; Tóth *et al.*, 2016a). This type of pollution can cause negative impacts in the environment and for ecological systems (e.g. inhibition of cytoplasmic enzymes, damage to cell structures, inhibition of protein synthesis, negative effects in animals by disturbing the central and peripheral nervous and circulatory systems, decrease of plants growth, performance and crop yield etc.) (Cozma and Gavrilescu, 2014; Hlihor *et al.*, 2017; Siminciuc *et al.*, 2015; Sobariu *et al.*, 2017). As a consequence, human health can suffer numerous injuries (e.g. on the functions of brain, lungs, kidney, liver, blood composition; physical, muscular, and neurological degenerative diseases; immune deficiency disorders, heart complications, digestion problems, cancer etc.) (Hlihor *et al.*, 2017; Iordache *et al.*, 2016; Rosca *et al.*, 2015; Sobariu *et al.*, 2017).

Based on the information provided by LUCAS database, Tóth *et al.* (2016a) found that about 1.2 million km^2 (28.3%) of the total surface area of the European Union are affected by pollution with one or more heavy metals ions (e.g. As, Cd, Cr, Cu, Pb, Zn, Sb, Co, Ni), whose concentration exceedsthe maximum admissible limits. The largest areas affected by this type of pollution are situated in the West and Central of Europe, center of Italy, Greece and South-East of Ireland. The highest concentrations of Pb in soils were found in the center of Italy, France, Germany, and United Kingdom. Ni is mostly abundant in Mediterranean region of Europe, especially in Greece, while pollution with chromium affects largest areas of Piemonte, Lorraine-Alsace, Western-Macedonia and center of Greece. High concentrations ofcadmium can be found in Ireland and Greece, whereas the concentration of Hg ions exceeds the legal limits especially in the center of Italy, North-West of England and East of Slovakia (Tóth *et al.*, 2016a, 2016b). In Romania, approximately 6,639 ha are affected by heavy metals pollution, noting that for 5,773 ha the concentration of some heavy metals found in soil exceeds the maximum admissible limits. The most affected areas are in the West of Romania (23.2%), North - East (20.5%), North - West (19.7%), Center (12.3%) and South - West Oltenia (2%) (Dumitru *et al.*, 2011).

Various physical, chemical and biological processes can be applied for the decontamination of soils polluted with heavy metals, but a special importance is given today to bioremediation techniques (fig. 1). Biological processes become more and more preferred, since the bioremediation strategies have often proved to be more advantageous than the conventional remediation tools, mainly because they are economic and ecological friendly and can be implemented directly onto the contaminated sites (*in situ*). Heavy metals can be eliminated by their bioaccumulation in specific microorganisms and plants. In this context, the present paper examines the abilities of some microorganisms and plants to eliminate heavy metals from polluted soils, considering also their tolerance and bioaccumulation capacity for heavy metals.

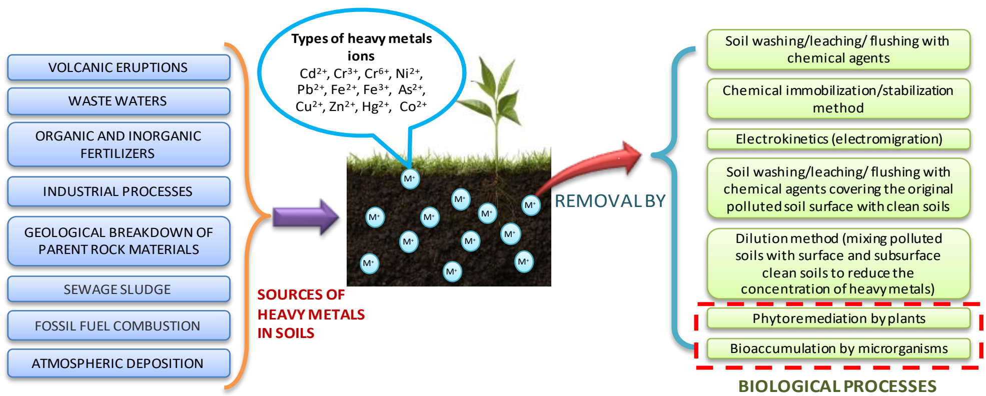

Fig. 1 Sources of heavy metals in soils and the processes used for remediation

BIOREMEDIATION OF SOILS

Recent studies have shown that biological methods based on phytoremediation and bioaccumulation are two *in situ* eco-friendly and economically feasible methods appliedfor an efficient soil remediation. These processes are based on the potential of plants (native, woody, herbaceous, ornamental, perennial, flowering, wild etc.) or microorganisms (bacteria, fungi, yeasts) to remove heavy metal ions from soil by extracting, transferring and accumulating them in biomass (Colin *et al.*, 2012; Kumari *et al.*, 2016; Rosca *et al.*, 2015; Sobariu *et al.*, 2017). The results of some studies on the potential of plants (tab. 1) and microorganisms (tab. 2) to eliminate heavy metals from soils by their bioaccumulation have demonstrated that various plants and microorganisms possess ahigh capacity to tolerate and absorb heavy metals ions, playing also an important role in reducing the contamination risk of food with toxic metals (Hlihor *et al.*, 2017; Sobariu *et al.*, 2017).

Table 1

Removal of heavy metals ions by plants (phytoremediation)

Species of plants	***Removal capacity of heavy metals ions** (mg/kg d.w*.)*								***References***
	Fe^{2+}	***Pb^{2+}***	***Ni^{2+}***	***Cu^{2+}***	***Zn^{2+}***	***Cd^{2+}***	***Cr_{tot}***	***Co^{2+}***	
Brassica napus	-	472	45.7	414	5983	23.5	82.5	-	Marchiol *et al.* (2004)
Raphanus sativus	-	407	51.6	563	4029	59.5	62	-	
Cardaria draba	1452	827	9.5	34.4	1850	2.2	-	-	Chehregani *et al.* (2009)
Amaranthus retroflexus	6230	371.5	7	57.5	233	5.5	-	-	
Boromus sp	520	210	6.5	26	85	10	-	-	
Noea mucronta	1230	1485	18.5	84	1984	14.6	-	-	
Marrubium vulgare	540	78	4	34	58	9	-	-	
Lactuca serriola	-	3	8	18	1030	21	4	-	Porębska and Ostrowska (1999)
Artemisia vulgaris	-	17	6.8	81	398	36	4	-	
Rheum rhabarbarum L.	-	0.015	-	0.03	0.17	-	-	-	Ipătioaie *et al.* (2014)
Herniaria hirsuta	-	34	808	22	29	8	275	63	Shallari *et al.* (1998)
Inula germanica	-	24	211	20	24	5	89	31	
Dittrichia graveolens	-	28	94	1110	849	9	69	34	
Lotus ornithopodioides	-	43	232	14	75	5	63	21	
Alyssum murale	-	23	8463	23	108	3	12	86	
Convolvulus arvensis L.	-	-	-	560	-	1500	800	-	Gardea-Torresdey *et al.* (2004)
Euphorbia cheiradenia	1040	1138	14.2	65	1873	2.35	-	-	Chehregani and Malayeri (2007)
Biebers teiniamultifida	480	23	4	20	-	7	-	-	
Reseda lutea	5490	371	7	57.5	233	5.5	-	-	
Euphorbia macroclada	2261	81.67	13	26	327	3	-	-	
Ceratophyllumd emersum	-	20	-	22.7	104	3.52	-	-	Matache *et al.* (2013)
Potamogeton pectinatus	-	6.63	-	6.24	16.4	0.64	-	-	
Potamogeton lucens	-	1.51	-	9.8	15.6	0.97	-	-	

**d.w. – dry weight*

Phytoremediation and bioaccumulation processes applied in contaminated areas can be influenced by several operational parameters (soil pH, temperature variations, soil morphology, initial concentration of heavy metals etc.) (Pavel *et al.*, 2013). Taking into account these aspects, the selection of the most suitable plants and microorganisms for bioremediation depends on their ability to adapt to the climatic and soil conditions in the polluted area.

Table 2

Removal of heavy metals ions by microorganisms through bioaccumulation process

Microorganisms species	***Initial conditions***	***Ions***	***Removal efficiency (%) or uptake (mg/g)***	***References***
Arthrobacter phenanthrenivorans	$Ci_{Pb(II)}$=180 mg/L, $Ci_{Cd(II)}$=178 mg/L, $Ci_{Ni(II)}$=85 mg/L, 72 hours contact time	Pb^{2+}	79.9 %	Banerjee *et al.* (2016)
		Ni^{2+}	47.62%	
		Cd^{2+}	34.05%	
Pseudomonas putida	$Ci_{Pb(II)}$=1.53 mg/L, $Ci_{Co(II)}$=8.82 mg/L, $Ci_{Ni(II)}$=11.48 mg/L, $Ci_{Cu(II)}$=5.9 mg/L, $Ci_{Cd(II)}$=50 mg/L, 5 days contact time	Pb^{2+}	96%	Nanganuru and Korrapati (2012)
		Ni^{2+}	57%	
		Cu^{2+}	49 %	
		Co^{2+}	71%	
		Cd^{2+}	93%	
Enterobacter sp. J1	$Ci_{heavymetal}$=100 mg/L, 24 hours contact time	Pb^{2+}	50.9 mg/g	Lu *et al.* (2006)
		Cu^{2+}	32.5 mg/g	
		Cd^{2+}	46.6 mg/g	
Bacillus cereus	$Ci_{heavymetal}$=0.2-0.6 mmol/mL, 24 hours contact time	Pb^{2+}	36 mg/g	Banerjee *et al.* (2015)
		Ni^{2+}	28 mg/g	
		Cu^{2+}	33 mg/g	
		Mn^{2+}	38 mg/g	
		Hg^{2+}	35 mg/g	
		Co^{2+}	31 mg/g	
Saccharomyces cerevisiae	$Ci_{Hg(II), Co(II)}$=500 mg/L, 21 days contact time	Hg^{2+}	90.48 %	Imam *et al.* (2016)
		Cd^{2+}	92.68 %	
	$Ci_{Pb(II)}$= 100 mg/L $Ci_{Cd(II)}$= 300 mg/L 30 days contact time	Pb^{2+}	67 %	Damodaran *et al*. (2011)
		Cd^{2+}	73 %	
Bacillus subtilis B38	$Ci_{heavymetal}$=0.1-1.25 mmol/L 24 hours contact time	Pb^{2+}	2.48 mmol/g	Wang *et al.* (2014)
		Hg^{2+}	4.09 mmol/g	
		Cd^{2+}	3.04 mmol/g	
		Cr_{total}	1.83 mmol/g	
Mix of proteobacteria: O .intermedium, A. ebreus, A.caviae, B. diminuta, B. vietnamiensis, P. mendocina, P. alcaligenes, S.marcescens, S. acidaminiphilia, D. tsuruhatensis	$Ci_{As(II)}$=141 mg/kg soil, 20 mincontact time	As^{2+}	71%	Fauziah *et al.* (2017)
	$Ci_{Zn(II)}$=49 mg/kg soil $Ci_{Ni(II)}$=21 mg/kg soil 10 min contact time	Ni^{2+}	50.8%	
		Zn^{2+}	47.6%	
Thiobacillus ferrooxida	C_i= 2199 mg/kg soil	Zn^{2+}	90 %	Sur *et al.* (2012)
	C_i= 27660 mg/kg soil	Pb^{2+}	0.53 %	
	C_i= 132 mg/kg soil	Cr_{total}	25 %	
	C_i= 99500 mg/kg soil	Fe^{2+}	100 %	
	C_i= 7520 mg/kg soil	Cu^{2+}	100 %	
	C_i= 11.68 mg/kg soil	Cd^{2+}	60 %	
	C_i= 7360 mg/kg soil 72 hours contact time	Mn^{2+}	65 %	

In this case, the best choices for soil bioremediation is through plants that normally grow in the contaminated area, as well as indigenous microorganisms. Plants and microorganisms age and the synergistic or antagonistic effects of elements/compounds on going in soil against plants and microorganisms are also important factors that can influence process efficiency (Elekes, 2014). Barbeş and

Bărbulescu (2017) analyzed the accumulation of Cu, Zn, Ni, Pb, Cr, Cd and Co in the leaves and bark of *Populus nigra* L., a species of plant grown in Navodari area, in the region of Romanian Black Sea Littoral. The results have shown that the plant possesses a high potential in the removal of heavy metals ions from polluted area (the effectiveness of the process was higher than 70%). Elekes (2014) studied the potential of some plants, which normally grow on the industrial platform of Targoviste city (*Lolium perenne*, *Festuca pratensis*, *Stipa capillata*, *Agrostis alba*, *Cynodon dactylon*, *Agrostis tenuis* and *Luzula campestris*). The samples taken were analyzed and the results have shown that the plants studied are able to remove the heavy metals (Cu, Zn, Sn, Pb, Co, Ni, Mn, Cr) (e.g. *Lolium perenne* has removed 921.67 mg/kg Zn, 201.23 mg/kg Pb, 114.19 mg/kg Cr, 61.95 mg/kg Cu).

CONCLUSIONS

When comparing the data available from literature, we can notice that biological *in situ* processes can be applied with high potential for the removal of heavy metals from polluted soils, considering different plants and microorganisms. Some species of plants such as: *Amaranthus retroflexus*, *Noea mucronta*, *Alyssum murale*, *Convolvulus arvensis* L., *Brassica napus*, *Raphanus sativus* and *Cardaria draba* can be considered as hyperaccumulators for some heavy metals ions. Also, it has been observed that a wide variety of indigenous microorganisms from proteobacteria, actinobacteria and bacilli class can be used with high effectiveness for the bioremediation of soils polluted with heavy metals (e.g.: *S. cerevisiae*, *Streptomyces*, *A. phenanthrenivorans*, *Pseudomonas putida*, *Enterobacter* sp. etc.). In Romania, the use of these processes for the remediation of soils polluted with various heavy metal ions can be successfully applied, as confirmed by the studies carried out in Navadori and Targoviste regions, as well as in other areas of the country. In order to increase the efficiency of the process, it is necessary to select plants and microorganisms adapted or easily adaptable to climatic conditions in the area, structure and parameters of the polluted soil.

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