STUDY OF IMMOBILIZED MICROBIAL COMPOSITION ON THE RECLAMATION PROCESS OF HEAVY METAL POLLUTED SOILS

V. Savov[1], K. Chakalov[2], G. Angelova[2] ,T. Popova[2], R. Hristova[3]
[1]Sofia University St Kliment Ohridski, Faculty of Biology, 8"Dragan Tsankov" Str., 1164 Sofia,Bulgaria
[2]ROMB ltd, 1"Aboba" Str. 1606 Sofia, Bulgaria,
[3]Central Laboratory of Ecology, BAS, Sofia, Bulgaria
Correspondence to: Valentin Savov
E-mail: romb_ltd@abv.bg

ABSTRACT

Heavy metal pollutions are in certain cases phytotoxic. Bioremediation can sometimes be realized with the using of microorganisms for "removing" of problematic pollutions from soil and underground water by helping the phytoremediation processes. The purpose ot the study is to determine the influence of biotransformed lignites' derivatives (HA) alone and as an agent for immobilization of micro fungi upon some bioremediation processes in heavy metal polluted soils. The pot vegetative test is realized with Ap horizons of polluted soils from the regions of Lead-Zink Procesing Plant Plovdiv (Alluvial-Meadow, Eutric Fluvisol, FAO) HA are a matrix for immobilization of T.viride and T. harzianum (10^5CFU/ml solution), at a background treatment with Fe-zeolite. Test plants – garlic and grass mixture. A strong influence of the studied meliorating agents - bioproducts - upon bioremediation processes in polluted soil is observed. A good effect of immobilized micro flora on bioremediation processes is determined. The total content of the accumulated heavy metals from the test culture demonstrates the possibility of using HA as an immobilizing factor for T.viride and T. harzianum during rehabilitation of the productivity of heavy metal polluted soils.

Keywords: bioremediation, heavy metal, humic substanses, *Trichoderma sp.*

Introduction

Heavy metal pollutions are in certain cases phytotoxic and influence on the normal physiological or genetic development of plants; some plants, however, seem to be tolerant to the accumulation of toxic substances that leads to phyto-chelatization in their cellular structures. The reason is that these plants contain antioxidants such as ascorbic acid, alfatocoferol, antocyanines, beta-caroten and so on, which assist plant adaptation to toxicants. Bioremediation can sometimes be realized with the using of microorganisms for "removing" of problematic pollutions from soil and underground water by helping the phytoremediation processes (4). It is determined by experiments with isolated humic acids from Canadian sphagnum peat and humin (an extract from semi-degraded peat) that binding of Cu in soils is pH determined. At pH 4,0-5,0 99% binding of copper is achieved with all three used adsorbents, but the sorption on humic acids is the fastest and the most stable one. In our previous work we have proved the effect of natural and modified zeolites on the transformation of organic matter to humic substances depending on the total reactivity of medium, especially in connection with Eh. (3). The different ferritization of the natural zeolite component determines the affinity of clinoptilolite for ionization of amphoteric elements in such organic-zeolite media (2). Ferritized zeolites Fe-Z increase Eh in the medium from 443-449 mV to 471-492 mV. That results in preserving of 6-40% of the total organic carbon in heavy metal polluted soils. Thus bio-productivity of polluted soils increases up to 417 % (2). An increase of free and R_2O_3-bounded humic acids is determined in a pot greenhouse test with polluted soil, meliorated with peat, limestone and different zeolite combinations as a result of the different stage of transformation of peat in the medium (1). The purpose of the study is to determine the influence of *T.viride* and *T. harzianum* on the background of humic acids (HA), ferritized zeolite (F-z) and phosphorite powder (P) on some bioremediation processes in heavy metal polluted soils.

Materials and methods

The pot vegetative experiment is realized with Ap horizons of polluted soils from the regions of Lead-Zink Procesing Plant Plovdiv (Alluvial-Meadow, Eutric Fluvisol, FAO). In this experiment pollution is as follows: pH ~6,0 and heavy metal content, mg/kg: Cu-560; Zn-7600; Pb-6700; Cd-100; Mn-1000; As-61. Soil treatment procedure: the total content of mobile heavy metals is calculated as meq/100g. The amendment – humic acids (HA) - is calculated as a proportion (50%; 100%; and 200%) between the total content of mobile heavy metals and SEC of humic acids, Popova et al, 2006. Microbial inoculation is with water suspension of conidia of *Trichoderma viride* (10^5CFU/g soil), islated from Stanyantsy Mine, on the background of treatment with Fe-z and phosphorite powder. Zeolite treatment follows the procedure described in our previous works (Chakalov et al, 2001). The total content of heavy metals is measured with AAS after decomposing by HF and $HClO_4$, plant material is decomposed by $HCLO_4$ and HNO_3. Mobile forms are defined in CH_3 COONa extract, pH 4,8. Test culture – garlic for the first series of tests. This series is realized with polluted soils from the region of Lead-Zink Procesing Plant Plovdiv (Alluvial-Meadow, Eutric Fluvisol, FAO) 0 – Absolute Control; 1. Control – Background of phosphorite powder (B - 1g/kg + ammonia nitrate 0,5 g/kg); 2. Background + leonardite (L 1%); 3. Background + Fe-Z 1% + L 1%; 4. Background + leonardite bio-transformed with *T.harzianum*; 5. Background + Fe-Z 1%; 6 Background + Fe zeolite + HA ; 7. Background-+ Fe-Z 1% + L 1% + HA 10 ml/1dm^3. A test with grass mixtures - *Lolium perene* - 40%, *Festuca rubra* - 60% is realized in the same kind of pots (1l). The sods (4x4x2) are produced on a peat substrate.

Results and Discussion

Practically, the results from the model experiments in pot tests are not always entirely confirmed by the in Situ treatment of polluted soils. Soil sorption complex is capable of traping part of the pollutants; that means in nature part of the toxicants can migrate to the underground waters depending on the mechanical composition of soils, their sorption capacity, and content of organic matter and clay materials and so on. The A-phase of soil absorbs a great amount of metal ions in no exchangeable form.

TABLE 1.

Content of changeable heavy metals mg/kg from Lead-Zink Procesing Plant Plovdiv

Treatment		Pb	Cu	Zn	Cd	Mn	Fresh biomass
1	0 HA	333,33	230,02	2933,72	189,08	348,93	3,16
2	200% HA	1245,28	226,42	2603,77	142,45	669,81	3,39
3	100% HA	1252,11	259,06	3041,11	160,50	564,11	3,59
4	50% HA	1253,62	201,04	2590,37	193,31	383,72	2,93
1	0% Fe zeolite	333,33	230,02	2933,72	189,08	348,93	3,16
5	2% Fe zeolite	654,00	170,00	2220,00	187,00	404,00	4,36
6	1% Fe zeolite	756,00	139,00	2030,00	164,00	342,00	4,93
7	0,50% Fe zeolite	1150,00	210,00	2520,00	199,00	373,00	4,15
6	0 Fe z + HA	756,00	139,00	2030,00	164,00	342,00	4,93
8	200% Fe z + HA	261,09	210,41	2352,72	141,55	482,98	1,76
9	100% Fe z + HA	477,00	197,00	2350,00	219,00	509,00	3,26
10	50% Fe z + HA	348,07	234,26	3205,61	193,47	470,41	3,44
1	Контрола	333,33	230,02	2933,72	189,08	348,93	3,16
11	*T.viride*	324,00	116,00	1900,00	185,00	325,00	3,39
12	1%Fe–z+ *T,viride*	572,05	113,82	1731,99	154,39	321,65	2,68
6	1% Fe-z+*T.viride*	756,00	139,00	2030,00	164,00	342,00	4,93

In this way a big deal of the pollutions are concentrated in the humic-accumulative horizon, thus being easily accessible depending on the exchangeable acidity of media. The tests including micro fungi, HA and Fe-z are realized to determine the potential ability of soils to immobilized phytotoxic amounts of toxicants and therefore to create conditions for phytoremediation.

TABLE 2.

Content of heavy metals mg/kgaccumulated in a test culture which is cultivated on a soil from Pb-Zn Procesing Plant Plovdiv

Treatment	Pb	Cu	Zn	Cd	Mn
1 HA	333,3	230,02	2933,72	189,08	348,93
2 HA	1245	226,42	2603,77	142,45	669,81
3 HA	1252	259,06	3041,11	160,50	564,11
4 HA	1253	201,04	2590,37	193,31	383,72
1 Fe zeolite	333,3	230,02	2933,72	189,08	348,93
5 Fe zeolite	654,0	170,00	2220,00	187,00	404,00
6 Fe zeolite	756,0	139,00	2030,00	164,00	342,00
7 Fe zeolite	1150	210,00	2520,00	199,00	373,00
6 Fe z + HA	756,0	139,00	2030,00	164,00	342,00
8 Fe z + HA	261,1	210,41	2352,72	141,55	482,98
9 Fe z + HA	477,0	197,00	2350,00	219,00	509,00
10 Fe z + HA	348,0	234,26	3205,61	193,47	470,41
1 Fa z +*T.viride*	333,3	230,02	2933,72	189,08	348,93
11 Fa z +*T.viride*	324,0	116,00	1900,00	185,00	325,00
12 Fa z +*T.viride*	572,0	113,82	1731,99	154,39	321,65
6 Fa z +*T.viride*	756,0	139,00	2030,00	164,00	342,00

Data presented in **Table 1** prove the role of nitrogen on plant development. Heavy metal accumulation in plants is strongly affected by the introduced mineral nitrogen in the polluted soils from the region of Lead-Zink Procesing Plant Plovdiv (Alluvial-Meadow, Eutric Fluvisol, FAO). Fe-Z improves the growth of garlic: biomass, g = 3, 36252 + 1, 01278*sqrt (Fe-Z); r = 0,817).

This meliorant decrease the assimilation of Cu (r = -0,777), Zn (r = -0,881), but increases the assimilation of Mn (r = 0,725). The total content of accumulated toxicants is relatively high because of the significantly better development of plants. Humic acids, however, have a weak influence on both the general growth of plants and the assimilation of heavy metals.

On the background of Fe-Z humic acids increase the assimilation of the examined elements with the exception of Pb: biomass, g = exp (1,57446 – 0,00491228*Fe-z + HA %; r = -0,982; Pb_1 = 713,575 – 32,4267*sqrt (Fe-Z + HA%); r = -0,894.

The inoculation with *T.viride* leads to a decrease of plant acumulation of heavy metals. Data presented in Table 2 show the strong effect if the examined meliorants – bioproducts – on the bioremediation processes in the treated soil from Lead-Zink Procesing Plant Plovdiv; the tested humic acids strongly support garlic growth in correlation with the reduced plant accumulation of Cu and Cd.

The effect of each toxicant on biomass accumulation as follows: **Cu** – r = -0,771; **Zn** – r = -0,668; **Pb** – r = -0,020; **Mn** – r = -0,604; **Cd** – r = -0,932.

Zn acclimatization is proved by accumulation of Cd and Mn in plant tissue: [Cd = 1/(-0,00577143 + 70,1648/Zn) r = 0,897; Mn = -83,1006 + 0,0949125*Zn; r = 0,991],

Humic acids have a significant influence on the decrease of Pb accumulation depending on the used dose:Pb = 1/(0,00202142 + 0,0000260913*HA); r = 0,665.

A good effect of immobilized micro flora on bioremediation processes is determined. Humic acids decrease the relative portion of changeable ions of heavy metals; this is better shown in the case of Cu. Microfungal inoculation also has some, although weaker effect. The total content of heavy metals accumulated by the test culture demonstrates the possibility of HA using together with other treatments for rehabilitation to acceptable limits the productivity of soils polluted with phytotoxic concentrations of heavy metals. Thereby conditions for phyto-remedition with hyper accumulants are created. Micro fungi have a relatively weak influence in connection with the toxic concentrations of Cu, Zn and Pb. Nevertheless, the inoculation with *Trichoderma viride* has a noticeable effect. The realized second series pot test (Table 3 and 4) – for bioremediation of heavy metal polluted soil from the region of Plovdiv is on the background of phosphorite powder – a well known and popular method for melioration of that type of soils.

The statistical processing of data from the biometrical measurements shows that all of the additionally introduced amendments increased soil bioproductivity. However, there are proven differences between the variants in the end of the test only in comparison with the melioration with lignite.

TABLE 3.

Bioremediation of heavy metal polluted soil - dry biomass, g/pot

Variant	Variant	P	L	Fe-z+BL	BL	Fe-z	Fe-Z+BL
Swath 1	0,35	0,57	1,22	0,94	1,06	0,79	0,72
Swath 2	0,30	0,31	0,23	0,24	0,41	0,44	0,41
Swath 3	0,54	0,39	0,61	0,63	0,67	0,74	0,89
Swath 4	0,24	0,17	0,40	0,29	0,25	0,33	0,38
Sum	1,43	1,45	2,46	2,10	2,39	2,31	2,40
%	100	101,40	172,43	146,96	167,52	162,15	168,46

TABLE 4.

Heavy metals content in dry biomass of the grass, mg/kg

№	Cu	Pb	Zn	Cd	Mn
1.	31,45	179,72	613,05	16,47	286,55
2.	49,00	590,50	650,44	25,00	339,50
3.	34,47	242,31	611,04	17,98	308,76
4.	43,47	404,23	620,59	18,98	170,38
5.	45,45	503,99	640,85	22,47	339,66
6.	44,46	417,68	643,27	22,48	311,26
7.	46,41	542,96	643,58	21,45	326,38

The positive influence of Fe-Z that has been determined in our previous studies, now is confirmed (variant 5). As a confirmation, a good correlation is found between the formed biomass and the quantities of iron assimilated by plants. The modified Fe-Z is obviously also responsible for the assimilation of heavy metals by plants – this is a direct result from the improved general functional state of plants. Iron assimilation influences proportionally the mineral nutrition with nitrogen and potassium and increases Ca assimilation. Probably that is the reason why an increase of Pb and Zn accumulation is observed on the background of the assimilation of more biogenic elements. Actually, the ability of plants to grow is restored and on the background of the normal mineral nutrition the grass assimilates bigger amount of heavy metals without having problems with the biomass accumulation. The restoration of nitrogen nutrition has the great influence on the possibility plants to be cultivated in the tested soil: (Pb = 1/(-0,0066766 + 36,0431/N); r = 0,516). The influence of Mg^{2+} accumulated by plants on Zn and Pb accumulation is significant.

Conclusions

Combining of Fe-Z with biotransformed leonardite and hydrolisates of them increases soil productivity, but reduces the content of heavy metals in plant tissues. However, the percentage extraction of manganese and lead from soil increases. The results from this preliminary pot test showe the strong effects of the used meliorants on the potential of tested soils for bioremediation, in particular - for their use in conditions of phytoremediation with hyper accumulants for the removal of heavy metal pollution from them.

Bioremediation depends on the introduced quantities of mineral nitrogen and humic acids. Biotransformed leonardite is better than the natural but its influence is comparable with that of Fe-Z. Biotransformed leonardite gives a relatively equivalent result on bio productivity, but on a relatively more polluted medium, thus it can be said that the meliorant has good qualities during bioremediation of heavy metal polluted soils. Combining of Fe-Z and biotransformed leonardite slightly increases the effectiveness of both two meliorants.

REFERENCES

1. **Benkova M., Filcheva E. and Raychev T**. (2005) *In Proc.*: National Conf. With Int, Partcipation : MANAGEMENT, USE AND PROTECTION OF SOIL RESOURCES. 15-19 May. Sofia, Bulgaria, p. 432-436,
2. **Chakalov K.,, Popova T., Filcheva E., Mitov K.** (2002) *In Proc.*: 6th International Conference on the Occurrence.Properties and Utilization of Natural Zeolites. Thessaloniki.GREECE. June 3-7.
3. **Filcheva E., Popova T., Chakalov K, and Mitov K.** (2002) *In Proc.:* 6th International Conference on the Occurrence, Properties and Utilization of Natural Zeolites. Thessaloniki. GREECE. June 3-7.
4. **Ghosh S.K., and Guhasarkar C.K..** (2001) Bioremediation to clean up environment, .www.thehindubusinessline.com