Response of soil microorganisms to organic and inorganic amendments in heavy metal polluted soils

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The effect of different kind of amendments in soils polluted with Pb, Cd and Zn on soil microflora was studied in pot experiment. The farmyard manure extract, natural zeolite, NPK and NPK+straw were involved. The total biological activity and quantity of main group of soil microorganisms were determined. It was established that applied amendments influenced with a different extent the structure and function of microbial communities. The most pronounced positive effect was observed with zeolite and combination of NPK+straw, which depends of soil characteristics. The amendments have different effect on individual group microorganisms.

Key words: soil microorganisms, soil biological activity, amendments, polluted soil.

Introduction

The functioning of the soil as a vital system and the support on its biological productivity depends at a higher extent on the soil microflora activity. The soil is a habitat for a vast, complex and interactive community of soil organisms whose activities largely determine its chemical and physical properties.

The microorganisms can be active destructors of the pollutants, but on the other side the pollutants can destroy the microbial succession, can suppress or kill some varieties of microorganisms and activate the development of others, which will lead to change in the resistance of the soil ecosystem as a whole.

High concentrations of heavy metals have been shown to adversely affect the size, diversity, and activity of microbial populations in soil. The risk of heavy metals presence in soil is related to the fact that they can accumulate everlastingly in toxic doses, and cannot be degraded. The most harmful are Cd, Pb, As, Cu, Ni, etc (McGrath, et al, 1995; Chen, 2000). Significant negative correlation between the influence of the heavy metals on the soil microflora and their available concentration was established. Thus the fixation of heavy metals in a non-available form could be a useful method for soils that are already contaminated by heavy metals.

The aim of this investigation is to study the size of microbial population and its activity in heavy metal polluted soil using different kind of soil amendments.

Materials and methods

The study was carried out on the basis of a pot experiment with two significantly different in pH and humus content soils - Chromic Luvisol , according to FAO classification from the vicinity of Kremikovtzi area near Sofia in Bulgaria and Typical Cambisol from Pribram area (Czech Republic) (FAO-UNESCO,1997). The high content of zinc, lead and cadmium in both soils was established surpassed PLC respectively 3.6, 16.9, and 2.1 times for Kremikovtzi area and 2.3, 19.1 and 5.0 times for Pribram area (table 1).

Table 1. Soil chemical characteristics

Soils	Humus	C	pH	Zn	Pb	Cd
	%	%		$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
Kremikovtsi	3.55	2.06	7.4	1320	1300	5.5
Pribram	2.79	1.62	5.8	270	1150	5.5

The experiment was carried out by the following scheme for each soil:

1. **Control** – Highly polluted soil.
2. **Farmyard manure extract** – Water extract of farmyard manure (in w/w proportion 3:2=water: manure) was applied in rate corresponding to 60 ton farmyard manure per hectare) 30 $ml.kg^{-1}$
3. **Processed zeolite** – Zeolite supplied by Institute of ore and natural substances with nitrogen and organic substances additions for better absorption of heavy metals. The trademark of this product is "Ecolin".
4. **NPK** – 200 mg $N.kg^{-1}$soil, 100 mg $P.kg^{-1}$; 300 mg $K.kg^{-1}$
5. **NPK + Straw** – NPK + well ground straw 10 $g.kg^{-1}$
6. **NPK + Zeolite** – NPK + natural zeolite 10 $g.kg^{-1}$ (**lime** instead of zeolite to obtain neutral pH for Pribram soil).

The experiment was conducted in pots containing 2.0 kg of air-dry soil collected from the plough layer 0-20 cm from Kremikovtsi and 0.8 kg from plough layer 0-20 cm from Pribram. In each pot triticale were grown as test crop in six replications. During the vegetation, soil moisture was maintained at 60% FWC. Three month after triticale sowing soil samples were taken for microbiological analysis. The main groups of soil microorganisms were determined by decimal dilution method and total microbial activity (quantity of CO_2 released) by the method of Alef (1998). Data were processed using analysis of variance ANOVA.

Result and discussion

Results from the experiment show that the soil from Kremikovtsi area is characterized with higher biogenisity than the soil from Pribram area. It can be explained with its higher humus content and pH. As a result the bigger part of the heavy metals is bound in the soil absorption complex, and thus has lower toxicity. In both soils the amendments influenced with a different extent the structure and function of the resident culturable microbial communities as stronger influence was observed on Kremikovtsi soil. For this soil it was established that all the amendments reduced the number of heterotrophic fungi. These results correspond with the data obtained by *Garau (2007).* The increase of number of other studied groups of microorganisms compared with polluted variant was observed. The positive effect was stronger pronounced on heterotrophic bacteria and actinomycetes which quantity is from 1.5 to 3.5 ways higher, following by cellulose decomposing microorganisms and the less pronounced is positive effect of amendments on the bacteria utilizing mineral nitrogen.

For the soil from Pribram area the positive influence ($P<0.05$) of zeolite and NPK+straw on fungi, actinomycetes, heterotrophic utilizing mineral nitrogen bacteria were found.

The addition of NPK+straw and zeolite improved in both soils metabolite activity of soil microorganisms. In the case of straw it can be explain with the high C:N ratio which stimulate microbial growth and in zeolite amendment with bounding of available forms of heavy metals. The addition of lime is beneficial for the Pribram soil which adsorption capacity is improved in more significant extent with a view to its physicochemical characteristics. The total biological activity of two soils under different amendments was increased. There is correlation between the quantity of heterotrophic microorganisms and released CO_2 ($r=0.856$ for Kremikovtsi soil and 0,888 for Pribram soil ($P<0.05$), that indicate increased both growth of microorganisms and their mineralization activity.

Table 2. The Influence of supplements on microbiological characteristics of soils

Treatments	CFU.10^6.g^{-1}					Total biological activity mg CO_2/ 100g
	Heterotrofic bacteria	Fungi	Actinomycetes	Cellulose decomposing microorganisms	Bacteria growing on SAA	
Kremikovtsi soil						
1	18,2000	0,0610	4,266	0,0020	34,7300	17,60
σ^2	1,1241	0,0026	0,444	0,0004	2,9352	0,12
2	30,5331	0,1446	8,600	0,0038	38,4000	20,56
σ^2	1,3752	0,0067	0,692	0,0003	8,7557	2,20
3	39,8667	0,0346	7,866	0,0041	43,1673	19,80
σ^2	4,3341	0,0029	1,392	0.0002	7,2825	0,12
4	35,8662	0,0100	16,400	0,0042	39,3334	17,00
σ^2	2,0060	0,0030	0,944	0,0006	6,7672	0,30
5	64,8000	0,0886	13,532	0,0025	52,9332	33,00
σ^2	5,0200	0,0052	1,732	0,0001	7,3443	0,67
6	29,3323	0,0160	4,800	0,0048	45,6000	19,78
σ^2	1,9667	0,0034	0,502	0,0006	13,1112	0,67
LSD	7,5160	0,0242	2,380	0,0005	7,7584	2,84
Pribram soil						
1	10,0606	0,0113	0,800	0,0030	18,2016	11,00
σ^2	4,8000	0,0033	0,400	0,0006	1,9400	1,27
2	10,1034	0,0106	0,732	0,0059	22,0632	13,20
σ^2	1,6182	0,0006	0,132	0,0003	1,2112	1,27
3	13,6000	0,0266	0,666	0,0057	76,8012	13,20
σ^2	4,1248	0,0043	0,132	0,0004	1,8400	0,16
4	12,4066	0,0113	0,866	0,0030	26,7330	14,30
σ^2	1,6050	0,0026	0,132	0,0008	4,9302	0,63
5	26,4000	0,0213	2,666	0,0033	59,7312	22,00
σ^2	6.1064	0,0026	0,480	0,0002	2,4203	0,67
6	12,0066	0,0140	1,334	0,0033	55,2004	20,53
σ^2	4,1072	0,0030	0,346	0,0004	1,0910	0,73
LSD	30,4842	0,0037	0,009	0,0007	11,3809	2,17

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Our results confirmed the findings of *Zorpas, 2000, Huang, 2006, Chande, 2002* which indicated that the bioavailability of heavy metals in contaminated with heavy metal soils amended with zeolite was reduced so that the potential stress of heavy metals was alleviated, and also showed that the soil microbial effects and the metabolic capacity of microbial community were improved.

Conclusion

The amendment of soils from Kremikovtsi and Pribram soils with zeolite and combination of NPK+straw have positive effect on soil microbial community and its activity, which depends on soil characteristics. The amendments have different effect on individual group microorganisms.

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Acnowledgemnets

This research was supported by Research Support Scheme in the frames of Project 1729/2000 : ALTERNATIVE APPROACHES FOR REMEDIATION OF CHEMICALLY DEGRADED SOILS