Microbial function after assisted natural remediation of a trace element polluted soil

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We studied the effect of different amendments and/or a plant cover on soil microbial properties of a trace element polluted soil. The experiment lasted 30 months and was carried out in containers filled with ca. 150 kg of contaminated soil. The remediation measures consisted of the application of different amendments and/or development of a plant cover (*Agrostis stolonifera L.*). Seven treatments were established: four with organic amendments (leonardite LEO, litter LIT, municipal waste compost MWC and biosolid compost BC) and one inorganic amendment (sugarbeet lime SL), where agrostis was sown, and two controls without amendment addition (with *Agrostis* CTRP or without *Agrostis* CTR). Microbial function was analysed by means of microbial biomass C (MBC), microbial biomass C to total organic C ratio (MBC/TOC), enzyme activities (dehydrogenase, aryl-sulphatase, β-glucosidase, acid-phophatase and protease) and microbial heterotrophic potential (MHP) to MBC ratio (MHP/MBC). The MWC and BC treatments were the most effective in raising MBC, MBC/TOC, dehydrogenase and aryl-sulphatase activities and reducing the MPH/MBC ratio. Whereas β-glucosidase was higher in the amended treatments, acid-phosphatase and protease activities showed no significant differences between the control and the amended treatments. Assisted natural remediation can be a useful and reliable technique to improve soil microbial function in the mid-term. Further monitoring is necessary to evaluate the potential of this technique in long-term experiments.

Keywords: *Agrostis*; amendments; assisted natural remediation; enzyme activities; trace elements

1 Introduction

Trace elements are toxic to most living organisms at excessive concentrations [1]. Unlike organic contaminants, trace elements cannot be degraded, therefore remediation of trace element polluted soils is either based on the extraction or the stabilization of the contaminants. Assisted natural remediation (ANR) is an "in situ" stabilization technique based on the use of amendments to accelerate those processes (sorption, precipitation and complexation reactions) that take place naturally in soils to reduce mobility and bioavailability of toxic elements [2]. Due to their restricted nature, natural attenuation processes alone may not be sufficient in mitigating risks from trace elements. Moreover, ANR may enhance microbial activity, plant colonisation and development, and thus re-start the nutrient cycling in the affected soils.

Trace elements can exhibit negative effects towards soil biota affecting both microbial key processes and the number and activity of soil microbial populations [3, 4]. Microorganisms respond quickly to changes and can rapidly adapt to environmental conditions. Changes in soil microbial populations and activities can be sensitive and early indicators of both natural and anthropogenic disturbances [1].

This work aims to evaluate the effects of mid-term ANR on microbial function of a trace element polluted soil.

2 Materials and methods

Soil (pH= 3.41; As=120 mg kg^{-1}; Cd=2.43 mg kg^{-1}; Cu=78.3 mg kg^{-1}; Pb=201 mg kg^{-1}; Zn=226 mg kg^{-1}) was sampled in an area affected by the Aznalcóllar mine accident named "El Vicario", where the only remediation work carried out by the authorities was the removal of the sludge layer together with the first 15 cm of the top soil. The experiment was carried out for 30 months in containers, filled with polluted soil. Four organic amendments (MWC, BC, LEO and a LIT) and an inorganic amendment (SL) were mixed with the top soil twice at the rates of 100 Mg ha^{-1} (beginning of the experiment) and 50 Mg ha^{-1} (12 months later). Characteristics of the amendments are shown in Table 1. *Agrostis stolonifera* L., a trace element tolerant plant, was sown in the containers one month after the beginning of the experiment. Two control treatments were also established: CTRP soil without amendment addition but sown with *Agrostis* and CTR soil without amendment addition and without *Agrostis*. A soil sampling was carried out at the end of the experiment. Microbial biomass C (MBC) content was determined by the chloroform fumigation-extraction method modified by [5]. Enzyme activities were determined as described in [6]. Microbial heterotrophic potential (MHP) was estimated as detailed in [4].

Table 1. Characterization of the amendments.

	pH	EC		N		K	As		Cu	Mn	Pb	Zn
		dS m^{-1}		%		%				mg kg^{-1}		
MW	7.36	6.16	18.	1.04	0.44	0.43	8.37	1.49	362	252	385	396
BC	6.93	2.91	19.	1.31	1.24	0.93	5.63	0.73	121	257	137	258
LEO	6.08	17.4	28.	1.17	0.04	3.97	34.9	0.83	28.2	66.2	22.0	64.5
LIT	4.49	0.92	54.	0.90	0.04	0.19	1.90	nd	6.45	676	9.36	27.0
SL	9.04	-	6.7	0.98	0.51	0.53	1.63	0.43	51.0	297	39.2	138

EC electrical conductivity, TOC total organic carbon.

A normality test was carried out for all variables prior to analysis of the variance. The data was analysed by ANOVA, considering the treatment as the independent variable. Significant statistical differences of all variables between treatments were established by Tukey's test when there was homogeneity of the variance and by Games-Howell's test in the opposite case.

3 Results and discussion

Mid-term and long-term studies are necessary to understand the effects of remediation techniques on soil restoration. Evaluating soil functionality is of utmost importance since soil microorganisms are essential for nutrient cycling and plant colonization and development. Soil quality assessment in remediated soils requires monitorization of both chemical and microbiological properties. Criteria for choosing the microbiological characteristics analysed were based on previous experience with their sensitivity to soil disturbance and attenuation processes.

In general, MBC was significantly higher in the amended treatments compared with the control treatments (Figure 1a). Highest values were found in the MWC and BC treatments, which can be attributed to both the addition of microorganisms with the amendments and the incorporation of easily degradable organic matter and other nutrients, which stimulate the growth of the autochthonous microorganisms of the soil.

The MBC/TOC ratio is usually comprised between 10-40 mg g^{-1}. Values below 10 mg g^{-1} can be regarded as a sign of stress such as that produced by trace elements. After 30 months, the MWC, BC and SL treatments were the only ones that showed mean values above this level (Figure 1b). These treatments were therefore more efficient to raise the active soil organic matter.

Dehydrogenase activity has been used to assess heavy metal toxicity in soils and microbial activity in semiarid Mediterranean areas. This activity was significantly higher in the MWC, BC, LIT and SL treatments compared with the control treatments (Figure 1c). These results seem to be related with higher pH values and lower soluble trace element concentrations in these treatments [4]. Aryl-sulphatase has been suggested as the most sensitive in tracing trace element effects. At the end of the experiment, mean values of this activity were higher in the MWC, BC and SL treatments (Figure 1d).

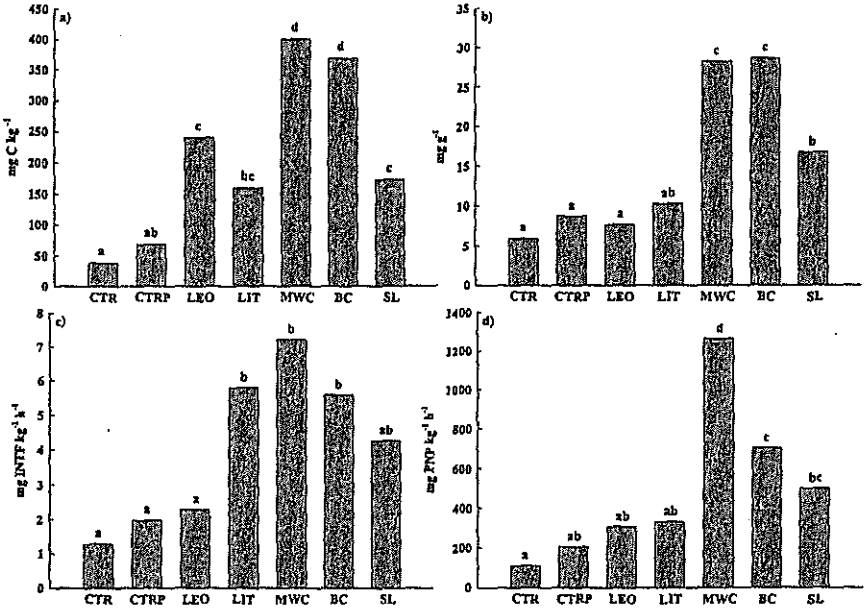

Figure 1. Mean values of a) MBC b) MBC/TOC c) dehydrogenase and d) aryl-sulphatase activities. Bars with the same letter do not differ significantly ($p<0.05$).

Data on β-glucosidase activity and trace elements is contradictory and both high and low sensitivity has been reported. In our study, β-glucosidase was higher in all amended treatments compared with the control treatments (figure 2a). Highest values were found in the LIT treatment probably due to its higher content in cellulose, the substrate of this enzyme. Acid-phosphatase was highest in the LEO treatment, and mean values in the control treatments were similar to those of the amended treatments (Figure 2b). These results seem to be related with feedback inhibition of the enzyme due to the available-P rather than with soil attenuation. The lowest activity was found in the SL, BC and MWC treatments, which were amended with P-rich materials (Table 1). Generally, protease

activity did not show significant differences between amended and control treatments, although mean values were lowest in the CTR treatment (Figure 2c). These results could imply a recovery of the enzyme with time and thus a lower sensitivity to low pH and higher soluble trace element concentrations than other properties studied.
The MHP was estimated as the maximum rate of glucose mineralized by the soil microbial biomass after addition of glucose in excess to soil samples. The ratio MHP/MBC can be representative of stress conditions. Mean values of this ratio were, in fact, higher in the two control treatments compared with the amended treatments (Figure 2d). Significant differences with respect to the control treatments were observed for the MWC and BC treatments.

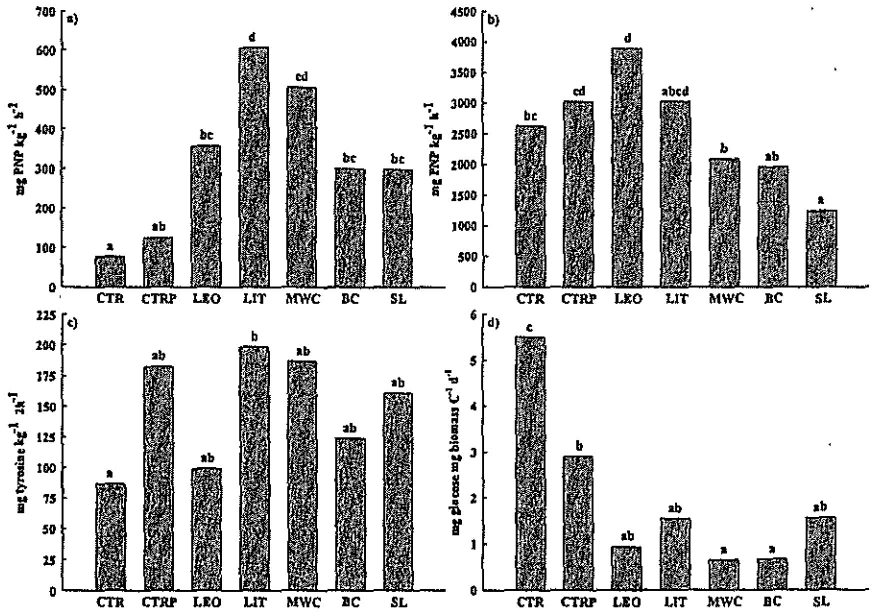

Figure 2. Mean values of a) β-glucosidase b) acid-phosphatase c) protease activities and d) MHP/MBC ratio. Bars followed by the same letter do not differ significantly ($p<0.05$).

4 Conclusions

Assisted natural remediation proved to be a reliable and effective technique to restore soil microbial function of a trace element polluted soil in the mid-term. In general, the two compost treatments gave the best results and were more efficient in enhancing soil microbial properties. Monitoring in the long-term should be encouraged, since mineralization of the organic matter could increase soluble trace element concentrations thereby reversing soil restoration and affecting negatively soil functionality.

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