Ecotoxicology and Environmental Safety 72 (2009) 115–119

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Microbial biomass, respiration and enzyme activities after in situ aided phytostabilization of a Pb- and Cu-contaminated soil

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ARTICLE INFO

Article history:
Received 21 March 2008
Received in revised form
1 July 2008
Accepted 2 July 2008
Available online 8 August 2008

Keywords:
Trace element contaminated soils
Phytostabilization
Soil microbial biomass
Soil enzyme activity

ABSTRACT

We conducted a pilot-scale experiment to study the effects of an aided phytostabilisation on soil microbial and biological endpoints in an ore dust-contaminated soil. Soil was amended with alkaline fly ashes plus peat to reduce mobility of trace elements and vegetated with a proprietary grass/herb mixture. Results indicated that the proposed aided phytostabilization approach of Cu–Pb contaminaed soil significantly increased microbial biomass and respiration, reduced microbial stress and increased key soil enzyme activities. Further research is needed to unambiguously determine whether the soil biochemical endpoints that were studied responded more to decreased metal mobility or to general soil amelioration.

1. Introduction

Trace element contaminated soils (TECS) represent a permanent threat to soil ecosystems, hampering plant growth and reducing soil functionality by two distinct mechanisms. Firstly, by reducing the soil microbial biomass and microbial activity as a result of selection of trace element resistant or tolerant organisms that are less metabolically efficient (Giller et al., 1998), and secondly by inhibiting the activity of hydrolase enzymes that are important in nutrient cycling and inhibiting hydrolase activities involved in nutrient cycling (Tyler et al., 1989).

It is generally accepted that the risk factor associated with TECS is related to mobile elemental pools rather than their total concentrations. Leaching tests and chemical extraction protocols have been devised to predict risks related to TECS (Peijnenburg et al., 2007). Successful long-term phytostabilization of TECS, based on trace element stabilization with various amendments such as alkaline wastes, organic matter Fe (hydr)-oxides rich industrial by-products, followed by revegetation of soil have been reported (Adriano et al., 2004; Brown et al., 2005).

Efficient phytostabilization of TECS may have beneficial effects on soil functionality due to reduction of labile elemental pools in TECS, but the dynamics and restoration of soil-based ecosystem processes have received little systematic investigation. Lombi et al. (2002a, b) reported that amendment of As-contaminated soils with various Fe oxyhydroxides rich products reduced the labile As pool and had positive effects on both soil microbial biomass and activity and growth of lettuce and ryegrass. Kumpiene et al. (2006) and Mench et al. (2006) reported recovery of soil functionality after aided phytostabilization of Cu- Cr- As-contaminated soils. Demonstration of recovery of soil functionality and increasing complexity of microbial communities in long-term stabilized TECS might be a key issue for the acceptance of such a remediation option. Kumpiene et al. (2007) reported that incorporation of coal fly ashes and peat into a Cu- and Pb-contaminated soil from an ore transhipment site in North Sweden, steadly decreased copper and lead (Cu and Pb) leaching and soil toxicity to plants and bacteria and allowed revegetation in a pilot-scale experiment, showing that this stabilisation approach could be a sustainable remediation technique for trace element contaminated soils. This paper reports the effects of the aided phytostabilization on soil microbial biomass, microbial respiration and key enzyme activities.

2. Materials and methods

2.1. Soil and amendments

Non vegetated soil samples were collected from a Cu ore dust-contaminated site in northern Sweden from the surface to a depth of 20 cm.

Fly ash (FA), a by-product from wood and coal combustion, was collected at a power and heating plant in Öresundskraft (Sweden). Fibrous moss peat was obtained (OM) was obtained from a private provider of soil and substrates (Norrlandsjord och Miljö AB, Luleå, Sweden). The main properties of the soil

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doi:10.1016/j.ecoenv.2008.07.002

Table 1
Main properties of the untreated and treated soil and used amendments

	Unit	Soil		Amendment	
		UNT	TR	Peat	Fly ashes
pH (1:2 H_2O)	–	4.1	6.8	3.9	12.4
Electrical conductivity (EC)	mS cm^{-1}	0.08	0.9	0.1	20
Loss on ignition (LOI)	%	1.4	ND	94.9	6
Total carbon (TC)	%	0.4	2.1	ND	ND
Organic carbon (OC)	"	0.03	0.35	74.8	ND
Cation exchange capacity (CEC)	cmol kg^{-1}	2.9	ND	ND	ND
Water holding capacity (WHC)	%	28	45	ND	ND
Texture	%				
Sand	"	85.5	ND	ND	ND
Silt	"	12.5	ND	ND	ND
Clay	"	2.0	ND	ND	ND
Trace elements:	mg kg^{-1} dw	$\pm$SD ($n=3$)			
Cu	"	248 ± 8	248 ± 97	17 ± 1	71 ± 3
Pb	"	2557 ± 366	2692 ± 55	8 ± 0	32 ± 3

ND-not determined

pre- and post-amendment, together with those of the amendments, are given in Table 1. Air dried, non-sieved soil was manually mixed with 5% (w/w) CFA plus 5% peat (w/w). Impact on contaminant stability with separate addition of ash and peat was tested in preliminary experiments, but adding ash or peat resulted in excessively alkaline or acid soils that represented unfavourable environments for plant growth. Moreover, leaching of Cu and Pb was effectively reduced in this soil by the combination of amendments. Therefore, the pilot lysimeter experiments were conducted only with the mixed amendments.

2.2. Lysimeters and leachate sampling and analysis

Lysimeters were designed to quantify the water percolating through a certain depth of soil and function as a transitive stage between laboratory and full-scale field experiments. Four cylindrical polyethylene lysimeters (0.5 m diameter, 0.5 m height, 100 l volume) were filled with soil and placed outdoors in northern Sweden (65.33° N, 22.07° E). Two lysimeters were filled with untreated (UNT) soil and two with treated soil (TR). Initially, one lysimeter with UNT soil and one with TR soil were sowed with a seed mixture generally used for revegetation of nutrient deficient soils, consisting of six grass and thirteen herb species obtained from VegTech AB (Vislanda, Sweden). However, plants in the sowed UNT soil did not survive, whereas the TR soil not previously sowed was colonized by the same plants as those previously sowed in the other TR soil. Therefore, the resulting experimental trial consisted of two lysimeters with steadily vegetated TR soil and two lysimeters containing UNT soil without vegetation (Fig. 1). Lysimeters were inserted into ground to a depth of 0.5 m and exposed to ambient conditions, with an average annual air temperature 1.6 °C and precipitation 506 mm $year^{-1}$ (SMHI, 2001). Leachates were collected from the bottom of the lysimeters on 10 occasions over a 400 day period from the incorporation of amendments, filtered through 0.45 μm syringe filters, acidified with concentrated HNO3 (1:100 v/v) and stored at 4 °C prior to elemental analysis. Leachate pH was measured within 1 h after the sampling. Concentrations of Cu and Pb in leachate were measured by ICP-OES (Perkin Elmer Optima 2000 DV).

2.3. Soil biochemical analyses

Soils for biochemical measurements were sampled 400 d after soil treatment, when the TR soils had supported vegetation for 3 months, with similar plant biomass in the two lysimeters (0.3 and 0.5 kg dw m^{-2}) and trace elements were stabilized. Moist soils were sieved (<2 mm) and preincubated at 25 °C and 50% water holding capacity for 7 d prior to analyses. Basal soil respiration was measured by a gas-chromatograph method (Hewlett-Packard 6890) equipped with a thermal conductivity detector according to Blackmer and Bremner (1977). After measurement of basal respiration, microbial biomass of the soils was determined by measuring ATP content according to Ciardi and Nannipieri (1990). The metabolic quotient (qCO_2) values, an index of microbial stress, were calculated by the CO_2-C-to-ATP ratio.

Hydrolase activities involved in the biogeochemical cycles of C, N, P, and S in soil were measured. Acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969) and phosphodiesterase activity, according to Browman and Tabatabai (1978). The β-glucosidase and β-galactosidase activities were assayed according to Tabatabai (1982). Urease activity was determined as described by Nannipieri et al. (1974) and protease activity was determined by hydrolysis of N-benzoylargininamide (BAA) (Ladd and Butler, 1972). All enzyme activities were measured after 1 h incubation at 37 °C

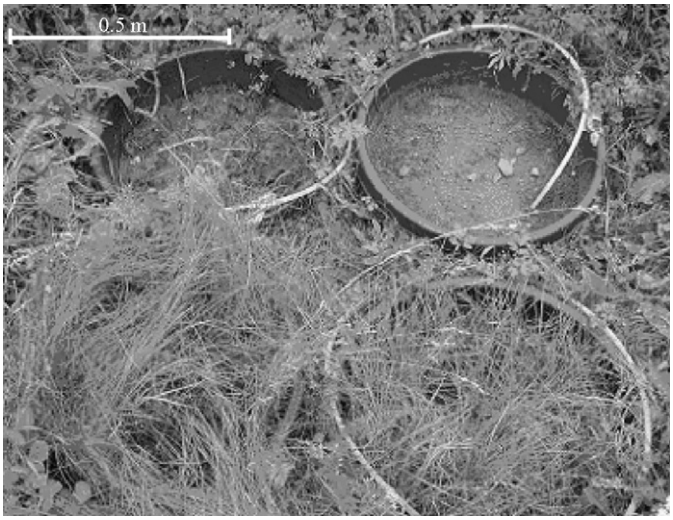

Fig. 1. Lysimeters containing the UNT not vegetated and TR vegetated soils at the stage of soil sampling.

followed by centrifugation of soil slurries at 6000g at 4 °C. Concentrations of *p*-nitrophenol (*p*-NP) in the assays of acid and alkaline phosphomonoesterase, phosphodiesterase, β-glucosidase and β-galactosidase activities were calculated from a *p*-NP calibration curve, after subtraction of the absorbance of the controls at 400 nm wavelength. The NH_4^+–N released by urease and BAA-hydrolysing activities was determined with a flow injection analyzer (FIA Star, Tecator, Sweden). The NH_4^+-fixing capability of both TR and UNT soils were evaluated by shaking them with solutions having NH_4^+ concentrations in the range of those products by urease and protease activities for 1 h at 37 °C and then extracted with 2 M KCl; the recovery was >95% in all cases.

2.4. Statistics

All determinations were carried out in triplicate for each lysimeter. Significance of the differences between mean values was calculated by the unpaired Student *t*-test.

3. Results

3.1. Cu and Pb mobility and soil pH values

Addition of CFA plus peat neutralized soil pH and maintained it within the range of 6.7–7.1 throughout the testing period, whilst soil pH from the UNT soil dropped from 4.3 to 3.0 (Fig. 2). During

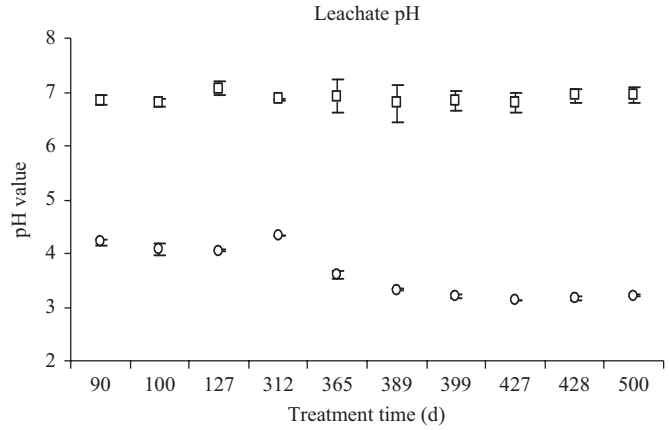

Fig. 2. Leachate pH values from of the UNT and TR soils. The error bars indicate the standard deviation of the means ($n = 2$).

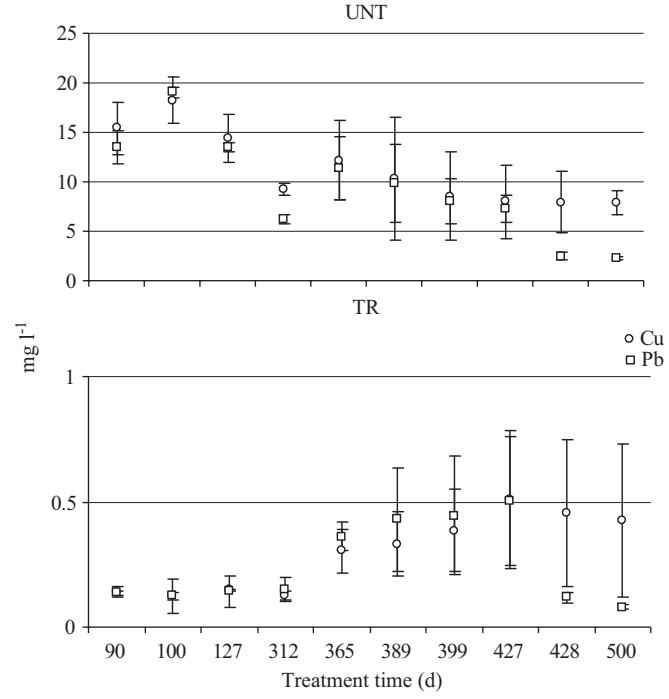

Fig. 3. Leachate Cu and Pb concentrations in leachetes from of the UNT and TR soils. The error bars indicate the standard deviation of the means ($n = 2$).

the experimental period, Cu and Pb leaching were reduced on average by 98% and 97%, respectively, in the TR soils (Fig. 3).

3.2. Microbial biomass, respiration and enzyme activities

Microbial biomass and respiration were significantly increased in the TR soils as compared to UNT soils, whereas the qCO_2 values calculated by the ATP-to-CO_2-C ratio were significantly higher in the UNT than in TR soils (Table 2). Soil treatment significantly increased all the measured enzyme activities (Fig. 4).

4. Discussion

Cu and Pb were highly mobile in the acid, UNT soil (Figs. 2 and 3). Treatment with CFA plus OM neutralized soil pH and steadily immobilized metals probably as a result of the sorptive capacity of

Table 2
Soil respiration (CO_2-C), ATP content and qCO_2 values of the untreated and treated soils

Soils	CO_2-C ($mg\,kg^{-1} \times d^{-1}$)	ATP ($ng\,kg^{-1}$)	qCO_2 ($mg\,CO_2$-C $ng\,ATP^{-1} \times d^{-1}$)
UNT	2.35 ± 0.22	90.1 ± 14.2	0.026 ± 0.002
TR	$4.55^{**} \pm 0.32$	$625.9^{***} \pm 100.8$	$0.007^{**} \pm 0.001$

** and *** in each column indicate significant differences at $p < 0.01$ and 0.001, respectively.

weathered fly ashes, aluminosilicates and organic ligands in the peat. Soil treatment decreased soil toxicity to microrganisms and allowed a steady revegetation (Kumpiene et al., 2007). The aim of the reported measurements was to assess the recovery of microbial and biochemical functions in TECS following an aided phytostabilization approach. The results show that effective trace element stabilization and permanent vegetation significantly increased soil microbial biomass and respiration and the activity of key soil enzymes, indicating that the proposed treatment led to a larger and more active microflora in the treated than in the UNT soil (Table 2, Fig. 4). Phosphatase, glycosidase, sulfatase and urease activities are involved in organic matter decomposition and the biogeochemical cycles of macronutrients (Nannipieri, 1994); therefore, their significant increase is indicative of sustainable management of the remediated TECS. Reduction of the qCO_2 further indicated an amelioration of microbial habitat and reduced microbial stress in the treated soils (Brookes, 1995). Increase in soil hydrolase activities depended on the enzyme activity but was always significant (Fig. 4). Inhibition of hydrolase activity by labile pools of trace elements can be caused by ionic competition with metallic enzyme cofactors (Blum and Schwedt, 1998), or by interaction with either the active catalytic enzyme sites or the enzyme substrates (Acosta-Martinez and Tabatabai, 2001). Sensitivity of free and immobilized enzymes to trace elements in contaminated environmental matrices has been reported (Jung et al., 1995). Several studies have demonstrated that root exudation is a major factor controlling microbial activity and community structure in the rhizosphere (Kozdroj and van Elsas, 2000; Falchini et al., 2003; Baudoin et al., 2003). Therefore, in our study the higher microbial biomass and activity in the remediated soils could be also due to the release of root exudates. Analogously, the increase of hydrolase activities in the remediated soils could be related either to release of enzymes by the plant roots, to the enhanced microbial enzyme synthesis in the rhizosphere (Grierson and Adams, 2000; George et al., 2005), and/or to a protective effect on extracellular enzymes by soil organic matter (Burns, 1982). However, the methods used for measuring soil enzyme activity do not allow one to distinguish between the contributions of enzymes located in different soil compartments. Our results do not exclude that overall improved soil functionality could be due to the beneficial properties (e.g. pH neutralization, nutrient addition) of the fly ashes and organic matter themselves. Amelioration of soil fertility due to FA applications has been previously reported (Jala and Goyal, 2006). However, it should be taken into account that on a longer time scale FA may change pH from alkaline to acidic values due to long-term weathering transformations, as reported by Twardowska and Szczepanska (2002), in particular when FA are used as buffering agent. However, reduced labile pools of Cu and Pb in the TR soils (Fig. 2) could be responsible for the observed positive responses of soil functional activity. Reduction of the labile Cu and Pb forms could have reduced the additive negative effects of trace elements on soil microflora and hydrolase activities (Renella et al., 2003; Ranjard et al., 2006; Chaperon

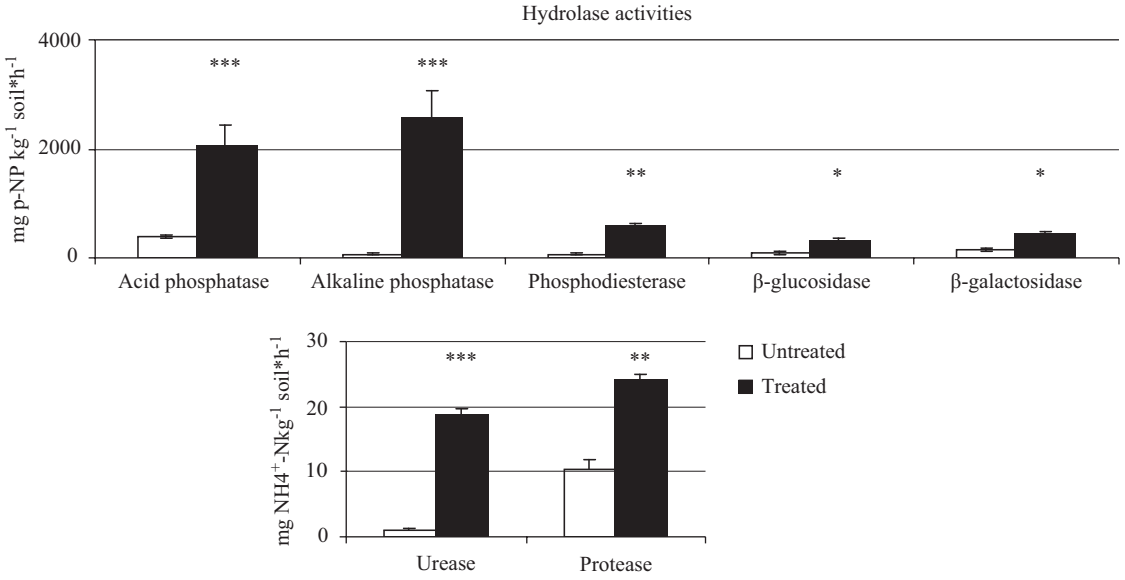

Fig. 4. Hydrolase activity of the UNT and treated soils. The error bars indicate the standard deviation of the means ($n = 3$). Symbols *, **, and *** indicate significant differences at p levels <0.05, 0.01 and 0.001, respectively.

and Sauvé, 2007), whereas Broos et al. (2007) found no relationship between labile metal pools and short term inhibition of microbial nitrification and substrate mineralization in soils artificially contaminated with Cu or Zn. Renella et al. (2002) reported that responses of soil microrganisms in experiments where uncontaminated soils had received a metal spike do not represent long-term effects of trace elements on soil microrganisms, and Wang and Staunton (2006) reported that the behaviour of recently added Cu may differ from that of native metal.

Increase in hydrolase activity due to reduced trace element mobility has been reported in phytostabilized soils (Kumpiene et al., 2006; Mench et al., 2006) and in revegetated mine spoils (Izquierdo et al., 2005; Perez de Mora et al., 2005; Renella et al., 2008). Therefore, neutralization of soil pH and soil enrichment with nutrients obtained by the proposed treatment would be an adjunct to the beneficial trace element sorption capacity of the added fly ashes.

5. Conclusions

The *in situ* aided phytostabilization approach of Cu–Pb contaminated soils proposed in this pilot-scale experiment significantly increased microbial biomass and activity, and reduced microbial stress in the remediated soils. The procedure also significantly increased enzyme activities involved in the biogeochemical cycles of the main nutrients in soil, showing that aided phytostabilization can be a sustainable management option for TECS. Samplings at intermediate times during the soil treatment are needed to better understand the dynamics of temporal change in the soil microbial community. Further research is needed to clarify whether soil microbial biochemical parameters respond to general habitat amelioration or to decreased metal toxicity, and assess the eventual soil acidification during the FA weathering on a longer time scale.

Acknowledgments

This work was financially supported by European Union Structural Funds and New Objective 1, North Sweden Soil Remediation Center, contract no 113-12534-00 and by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

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