SCIENCE OF THE TOTAL ENVIRONMENT 396 (2008) 89–99

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scitotenv

Effects of pyrite sludge pollution on soil enzyme activities: Ecological dose–response model

M. Belén Hinojosa[a,b,*], José A. Carreira[a], José M. Rodríguez-Maroto[c], Roberto García-Ruíz[a]

[a]Dpto. Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, 23071 Jaén, Spain
[b]Environment Plant Interactions Programme, Scottish Crop Research Institute, Dundee, DD2 5DA, United Kingdom
[c]Dpto. de Ingeniería Química, Universidad de Málaga, 29071 Málaga, Spain

ARTICLE INFO

Article history:
Received 6 November 2007
Received in revised form
8 February 2008
Accepted 11 February 2008

Keywords:
Ecological dose
Soil pollution
Pyrite sludge
Lime
Enzyme activities

ABSTRACT

A laboratory study was conducted to evaluate the response of soil enzyme activities (acid and alkaline phosphatase, β-glucosidase, arylsulfatase, urease and dehydrogenase) to different levels of trace elements pollution in soils representative of the area affected by the pyrite sludge mining spill of Aznalcóllar (Guadiamar basin, SW Spain). Three uncontaminated soils from the study area were mixed with different loads of pyrite sludge to resemble field conditions and criteria applied for reclamation practices following the pollution incident: 0% ("reference" or background level), 1.3% ("attention level", further monitoring required), 4% ("intervention level", further cleaning and liming required) and 13% (ten times the "attention level"). Enzyme activities were analysed 4, 7, 14, 21, 34 and 92 days after pollutant addition and those measured after 92 days were used to calculate the ecological dose value (ED_{50}). Soil enzyme activities and pH decreased after the pyrite sludge addition with respect to the "reference level" (0% pyrite sludge), whereas soil bioavailable (DTPA-extractable) trace elements concentration increased. Arylsulfatase, β-glucosidase and phosphatase activities were reduced by more than 50% at 1.3% pyrite sludge dose. Arylsulfasate was the most sensitive soil enzyme (in average, ED_{50}=0.99), whereas urease activity showed the lowest inhibition (in average, ED_{50}=7.87) after pyrite sludge addition. Our results showed that the ecological dose concept, applied to enzyme activities, was satisfactory to quantify the effect of a multi-metalic pollutant (pyrite sludge) on soil functionality, and would provide manageable data to establish permissible limits of trace elements in polluted soils.

Additionally, we evaluate the recovery of enzyme activities after addition of sugar-beet lime (calcium carbonate) to each experimentally polluted soil. The amount of lime added to each soil was enough to raise the pH to the original value (equal to control soil), resembling field remediation practices. After lime amendment, soil recovery was still incomplete in terms of bioavailable trace elements. However, the recovery of soil enzyme activities varied widely, ranging from 0 to 100% depending on soil type and the specific enzyme.

* *Corresponding author*. Environment Plant Interactions Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, United Kingdom. Tel.: +44 1382 562731; fax: +44 1382 568502.
E-mail address: belen.hinojosa@scri.ac.uk (M.B. Hinojosa).

0048-9697/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2008.02.014

1. Introduction

Enzyme activities have been considered as indicators of soil quality because: i) they are strongly connected with important soil properties such as organic matter, physical properties and microbial activity or biomass; ii) they respond earlier than other soil properties; and iii) they involve relatively simple methods compared to other soil quality bioindicators (Dick et al., 1996). Soil enzymes have been reported to be highly sensitive to heavy metals (Speir and Ross, 2002) and therefore, have been recommended as standard biochemical indicators for assessing soil quality under heavy metal pollution (Brookes, 2001; Nannipieri, 1995). Soil enzyme activities have been used to assess the ecological status in several monitoring programs of heavy metal polluted soils (Kuperman and Carreiro 1997; Karaca et al., 2002; Hinojosa et al., 2004b; Wang et al., 2007).

On the other hand, soil microcosm experiments have the advantage of being easy to handle and inexpensive, and can be kept under controlled condition with more replication and provide more reproducible results than field tests. Thus, laboratory ecotoxicology studies give detailed information on the relative toxicity of a pollutant. Dose–response curves can be constructed when a range of pollutant application rates is used, and from such response curves the ecological dose (EDx) values can be calculated (Giller et al., 1998). Thus, for example the ED_{50}, the most widely used effective dose, is the toxicant concentration that inhibits a microbe-mediated ecological process by 50% (Babich et al., 1983). In addition, the ED_{50} can be used to establish which microbiological and biochemical properties of soil are most sensitive to heavy metal contamination (Speir et al., 1995).

Traditionally only single trace element additions to soils have been used in most studies for ED_{50} determination, and scarce information is available on the possible synergic effects of multiple trace elements (Yang et al., 2006). Renella et al. (2003) studied the effects of Cd on the ED_{50} on acid and alkaline phosphatase activities of three contrasting soils with or without Cu and/or Zn. They demonstrated the occurrence of additive effects of Cu and Zn on Cd toxicity to these enzyme activities. This indicates that the calculation of ED_{50} values for enzyme activities in the presence of several trace elements is required to interpret data from field studies in which complex trace elements pollutants are involved.

This is the case of soils affected in 1998 by a spill of pyrite sludge and acidic water with high contents of trace elements (Cd, Zn, Cu, Cr, Fe, As, Mn, Hg, Ni, Pb) in the Guadiamar river basin, as a result of the collapse of a dam in a pond containing mine residues. A general overview of the incident can be found in Grimalt et al. (1999).

Immediately after the spill, the Regional Environmental Authorities began soil-reclamation activities, consisting mainly in the removal of the sludge layer that covered over 4000 ha. Although most of the sludge, together with the surface soil, was removed, high levels of trace elements were still present in some areas. Sulphides from the sludge remaining in the soil could, under adequate moisture and aeration conditions, be oxidised to sulphates and this can drop pH markedly (Förstner and Wittmann, 1983). Thus, the following operational levels of pollution were considered as main criterion for further restoration practices: i) "reference level", that is soil with less than 0.1% of pyrite-S, which it was admissible (i.e. 0% pyrite sludge); ii) "attention level" that is soil with 0.5–1.5% of pyrite-S (equivalent to 1.3–4% of pyrite sludge) which were analysed for bioavailable trace elements and ecotoxicological tests, to consider further reclamation actions; and iii) "intervention level", that is soil with more that 1.5% of pyrite-S levels (equivalent to >4% of pyrite sludge) which needed further cleaning and/or lime addition.

The aim of this study was to investigate the potential toxicological effects of pyrite sludge on soil enzyme activities related with C, N, P and S cycling. We also used the ecological dose approach to evaluate permissible limits for pyrite sludge soil pollution. For this, a laboratory experiment was conducted, in which three different and representative soils from the Guadiamar basin were mixed with several concentration of pyrite sludge (equivalent to 0, 0.5, 1.5 and 5% pyrite-S) and soil enzyme activities (acid and alkaline phosphatase, β-glucosidase, arylsulfatase, urease and dehydrogenase) analysed during three months. In addition, each enzyme activity was monitored after the addition of lime (calcium carbonate from sugar-beet industry) to the polluted soils.

2. Material and methods

2.1. *Experimental set-up and laboratory analyses*

Three unpolluted soils from the Guadiamar basin (SW Spain) with contrasting physical-chemical properties (see Table 1) were collected in April 2001 from the surface layer, (0–5 cm). Fresh soils were sieved (<2 mm) and mixed with pyrite sludge to give a final concentration of 1.3%, 4% and 13% (dry wt/dry wt) of pyrite sludge, equivalent to 0.5, 1.5 and 5% pyrite-S, respectively. Untreated soils (0% pyrite sludge) served as controls. The pyrite sludge used in this study was the same released accidentally into the Guadiamar basin containing on average: As, 2.8 g kg^{-1}; Cd, 0.1 g kg^{-1}; Zn, 38.8 g kg^{-1}; Cu, 9.5 g kg^{-1}; Cr, 0.01 g kg^{-1}; Fe, 234.1 g kg^{-1}; Mn, 0.3 g kg^{-1}; Hg, 0.1 g kg^{-1}; Ni, 0.003 g kg^{-1}; Pb, 39.9 g kg^{-1} (dry weight) at pH 2.5. Soil

Table 1 – Properties of soils used in the experiment

	Upper watershed (sandy loam)	Upper watershed (loam)	Lower watershed (loam)
pH	7.68	7.66	7.72
Sand[1]	78.2	14.8	41.5
Clay[1]	7.1	36.2	11.6
$CaCO_3$[1]	1.0	16.8	10.8
CEC[2]	6.9	12.3	11.3
Ca exchangeable[2]	11.7	29.7	28.3
Mg exchangeable[2]	1.52	3.58	2.03
K exchangeable[2]	0.21	0.74	0.82
Na exchangeable[2]	0.20	1.17	0.31
Organic matter[1]	1.31	2.02	1.58
Total N[1]	0.09	0.15	0.14

[1]Data expressed as %.
[2]Data expressed as meq 100 g^{-1} dry soil.