Long-term effects of mine soil reclamation using different amendments on microbial and biochemical properties in Southeast Spain

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Abstract
Microbial biomass carbon, soil respiration and enzyme activities were measured five years after reclamation of a polluted soil affected by former mining activities in SE Spain. This abandoned mine site contains materials of high Fe-oxyhydroxides, sulphates, and elevated contents of potentially leachable heavy metals due to extreme acidic conditions. Thus, soils have scarce or null vegetation due to very poor properties including low soil organic matter. A field trial was established in 2004in which different experimental plots were designed, using marble wastes, pig manure and sewage sludge as amendments to recover soil properties. Results show that all biochemical properties were higher in treated soils than in control, despite soil organic matter being similar amongst the treatments. As general pattern, biochemical properties showed higher values after application of pig manure than after application of sewage sludge. The different doses used did not have a great effect, being only significant for β-glucosidase, phosphodiesterase and arylsulfatase. This study confirms the high sensitivity of biochemical properties in the evaluation of soil quality and reactivation of nutrients cycles.

Key Words
Metal pollution, in situ remediation, pig slurry, sewage sludge, microbial biomass, hydrolase activities

Introduction
The environmental impacts of the long- time mining activities in southeast Spain include large areas of soils being affected by strong acidification processes, high salinity and accumulation of metals. These mining activities have generated high amounts of sterile materials for many years; the wastes are accumulated in pyramidal structures called tailing ponds. Mine sites contain materials of high Fe-oxyhydroxides, sulphates, and potentially leachable elevated contents of heavy metals (mainly Cd, Pb, Cu and Zn) due to extreme acidic conditions. As a consequence, these mine soils have scarce or null vegetation due to very poor properties, including extremely low soil organic matter. Since the polishing of metals from a mining area is a difficult task, the transformation of metals into harmless species or their removal in a suitable recycled mineral form such as carbonates using marble wastes or lime (Geebelen *et al.* 2003) is a possible solution for the remediation of a mining area. In addition, incorporation of organic amendments into contaminated mine soils has been proposed as feasible, inexpensive and environmentally sound disposal practice, as generally such wastes can improve soil physical and chemical properties and contain nutrients beneficial to microorganisms and plants (Barker 1997), favouring the reactivation of biogeochemical cycles and the natural establishment of vegetation. Although there is a general consensus that efficiency of soil remediation also depends on the presence and activity of microorganisms, the long-term ecological consequences of inorganic and organic amendments for these features have received little attention (Mench *et al.* 2006). The goal of this work was the evaluation of the long-term effectiveness of the remediation of contaminated mine soils by means of different inorganic and organic amendments using soil microbial and biochemical properties as indicators.

Methods
Study site and experimental design
The study was conducted in the province of Murcia (SE Spain), in the Cartagena-La Unión Mining District, where great mining activity has been carried out for more than 2500 years, the activity being stopped only in the nineties. The climate of the area is semiarid Mediterranean with mean annual temperature of 18ºC and mean annual rainfall of 275 mm. One tailing pond generated by mining activities was selected (El Lirio), representative of the rest of existing ponds in Cartagena-La Unión Mining District.

The field trial was established in 2004. Plots (2 m x 2 m) were randomised and replicated 3 times. Two different organic amendments were used to reclaim the soils, pig manure (P) and sewage sludge (S). In addition, 3 different doses per amendment were applied. Thus, the treatments were: Untreated contaminated soil (Control: C), soil treated with pig manure at dose 1 (P1), dose 2 (P2) and dose 3 (P3); and soil treated with sewage sludge at dose 1 (S1), dose 2 (S2) and dose 3 (S3). For pig manure, dose 1, 2 and 3 where 2.5, 5 and 10 kg per plot, respectively. For sewage sludge, doses were 1.99, 3.98 and 7.97 kg per plot, respectively. Doses were established by thresholds imposed by legislation regarding the addition of N to soil (Council Directive 91/676/EEC, 1991). With the purpose of increasing soil pH to immobilise metals and create better conditions for microbial and plant development, marble mud was applied in all plots except for control, at the rate of 22 kg per plot.

Soil sampling and analytical methods

The soil sampling was carried out in May 2009 (5 years after application of amendments). One sample (0-15 cm depth) was collected for each plot, taken to the lab, air-dried for 7 days, passed through a 2-mm sieve and stored at room temperature prior to laboratory analyses. The physico-chemical characterization of the plots is shown in Table 1.

Microbial biomass carbon (MBC) was determined using the fumigation-extraction procedure (Vance *et al.* 1987); basal soil respiration (BSR) determined according to Anderson (1982); β-glucosidase activity according to Tabatabai (1982); arylesterase activity according to Zornoza *et al.* (2009); acid and alkaline phosphatase according to Tabatabai and Bremmer (1969); phosphodiesterase following the method of Browman and Tabatabai (1978); and arylsulphatase measured by the method of Tabatabai and Bremner (1970).

Table 1. Main physico-chemical properties of control and amended plots from El Lirio pond.

Treatment	pH	EC (dS/m)	SOC (g/kg)	Nt (g/kg)	WSC (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Control	7.1	2.5	2.13	0.05	33	5.6	1.9	177	495
P1	7.6	2.4	2.96	0.12	25	4.2	2.5	320	418
P2	7.5	2.5	2.92	0.15	29	3.3	2.2	333	352
P3	7.3	2.4	2.14	0.06	29	2.9	0.8	309	446
S1	7.5	2.4	2.66	0.05	26	4.0	1.7	223	428
S2	7.6	2.3	2.29	0.06	33	3.9	1.7	295	433
S3	7.4	2.5	2.33	0.06	33	3.7	1.4	288	406

EC: Electrical conductivity, SOC: soil organic carbon, Nt: total nitrogen, WSC: water soluble carbon. Cd, Cu, Pb and Zn have been extracted with DTPA (Bioavailable metals).

Results and discussion

Results of the different biochemical properties determined in the plots 5 years after the application of the amendments are shown in Figure 1. According to the general trends, all biochemical properties were higher in treated soils than in control, despite the fact that soil organic carbon, total nitrogen or water soluble carbon were similar amongst the treatments after 5 years of the application (Table 1). The highest increases with respect to control were for MBC in P plots, (100%), β-glucosidase in P plots (250%), phosphodiesterase in P plots (210%) and arylsulfatase in P3 (4000%). This confirms the high sensitivity of biochemical properties to evaluate soil quality (Nannipieri *et al.* 1990), as undetected shifts occurred with other chemical properties. In addition, also as general pattern, biochemical properties showed higher values after application of pig manure than after application of sewage sludge. These results are promising in an area like Murcia province where more than 10% of pig production in Spain is located. Annually, Murcia province generates an estimated 8 millions m^3 of waste residues from the pork industry (CAAMA, 2003). This generation of large volume of pig slurry continuously increases with high demands for pork, and consequently creates disposal problem for many pig producers. However, doses did not have a great effect, being only significant for β-glucosidase, phosphodiesterase and arylsulfatase. The fact that SOC remains similar in all plots indicate a mineralization or leaching of the organic amendments, since treated plots had initially significantly higher values of SOC (Zanuzzi, 2007). Nonetheless, this initial incorporation of organic matter has triggered the activation of microbial populations which has increased their activity, favouring the recovery of soils and the establishment of vegetation, since spontaneous colonization of vegetation has taken place in treated plots (data not shown). However, the values of microbial biomass, respiration and enzyme activities are still low comparing with non contaminated soils from other zones from SE Spain with the same climatic conditions

(Zornoza *et al.* 2006; 2007; Bastida *et al.* 2008). This can be explained by the still extreme edaphic conditions, like the already moderate levels of heavy metals, low organic matter and high salinity.

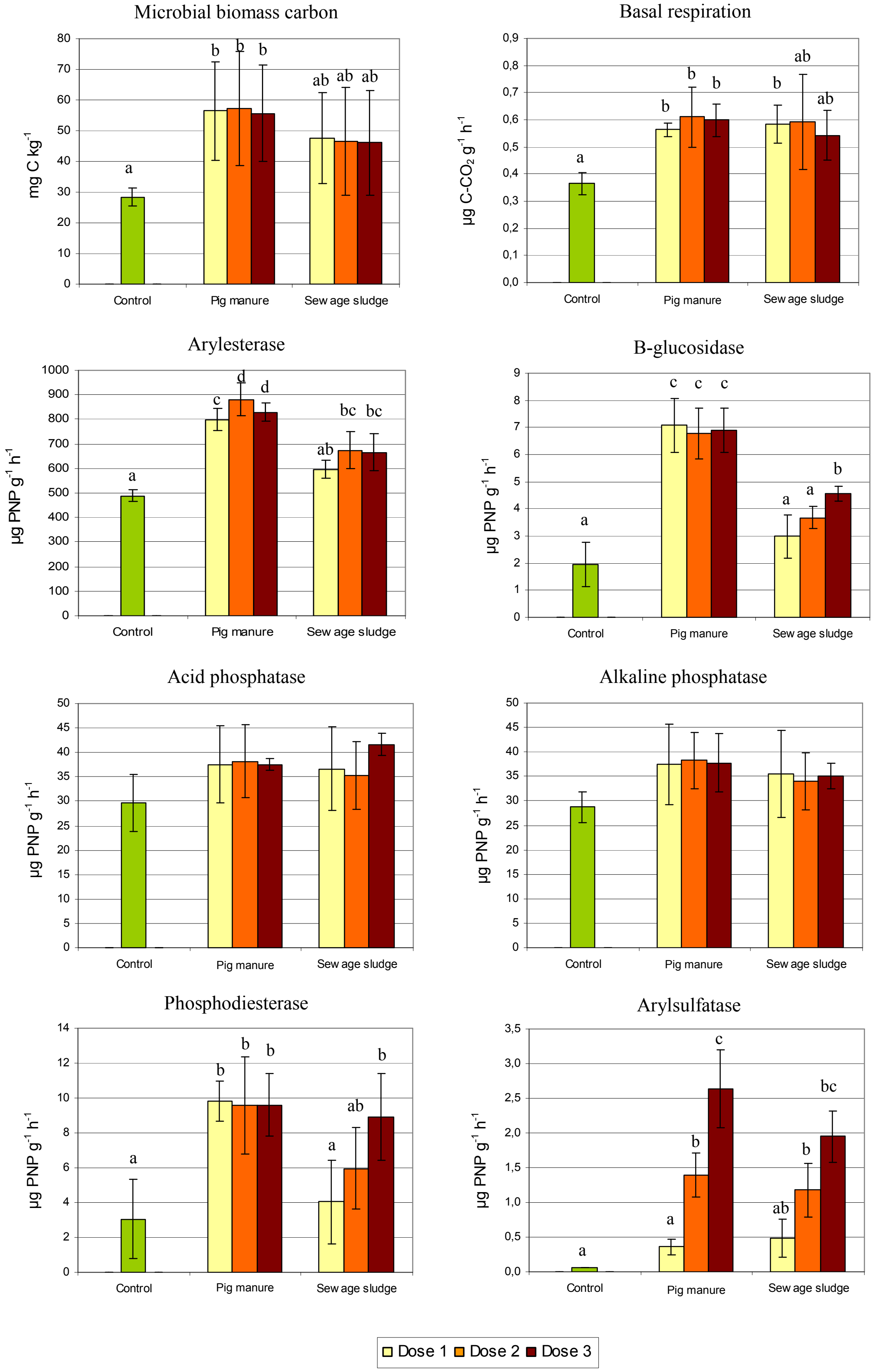

Figure 1. Microbial biomass carbon, soil respiration and enzyme activities of the control soil and remediated plots with different organic amendments at three different doses. Different letters indicate mean values significantly different after Tukey's honestly significant difference at *P*<0.05.

Conclusion

The application of pig manure and sewage sludge together with marble wastes has proved to be effective to activate microbial populations and activity even after 5 years of application, pig manure being the organic amendment with the best effects on microbial biomass and activity. However, the values of the biochemical properties are still low even for a semiarid environment. Thus, future research is needed to monitor the evolution of soil properties to guarantee long-term rehabilitation of polluted mine soils in SE Spain, and assess if phytostablization or more applications of organic amendments could help to facilitate reclamation.

References

Anderson JPE (1982) Soil respiration. In 'Methods of soil analysis, part 2, 2nd edition'. (Eds. Page AL, Miller RH, and Keeney DR), pp. 837-871. (Amercian Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, USA).

Barker AV (19997) Composition and uses of compost. In 'Agricultural uses of by-products and wastes'. (Eds JE Rechling, HC Mackimon), pp. 140-162. (ACS Symposium Series No. 668, Vol 10. American Chemcial Society, Washington DC).

Bastida F, Barberá GG, García C, Hernández T (2008) Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. *Soil Biology & Biochemistry* **38**, 3463-3473.

Browman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. *Soil Science Society of America Journal* **42**, 284-290.

CAAMA (2003) Consejería de Agricultura, Agua y Medio Ambiente de la Comunidad Autónoma de la Región de Murcia. Regsitro de explotaciones Porcinas de la Región de Murcia.

Council Directive 91/676/EEC. Protection of waters against pollution caused by nitrates from agricultural sources. Official Jorunal L 375, pp. 1-8, 31-12-1991

Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected biovailability assays to test the effect of amendment-induced immobilization of lead in soils. *Plant and Soil* **249**, 217-228.

Mench M, Renella G, Gelsomino A, Landi L, Nannipieri P (2006) Biochemical parameters and bacterial species richness in soils contaminated by sludge-borne metals and remediated with inorganic soil amendments. *Enrivonmental Pollution* **144**, 24-31. Methods of Soil Analysis, Part 2, 2nd ed.

Nannipieri P, Greco S, Ceccanti B (1990) Ecological significance of the biological activity in soil. In: 'Soil Biochemistry. Volume 6'(Eds JM Bollag, G. Stotzky). pp. 293-355. (Marcel Dekker Inc., New York).

Tabatabai MA (1982) Soil enzimes. In: 'Methods of soil analysis, part 2, 2nd edition'. (Eds. Page AL, Miller RH, Keeney DR), pp. 501-538. (Amercian Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, USA).

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate in assay of soil phosphatase activity. *Soil Biology & Biochemistry* **1**, 301-307.

Tabatabai MA, Bremner JM 1(970). Arylsulphatase activity of soils. *Soil Science Society of America Journal* **34**, 225-229.

Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* **19**,703-707.

Zanuzzi A (2007) Reducción de impactos ambientales asociados a suelos contaminados por metales pesados mediante el uso de residuos antropogénicos. PhD Dissertation. Departamento de Ciencia y Tecnología Agraria. Universidad Politécnica de Cartagena.

Zornoza R, Guerrero C, Mataix-Solera J, Arcenegui V, García-Orenes F, Mataix-Beneyto J (2006) Assessing air-drying and rewetting pretreatment effect on some soil enzyme activities under semiarid Mediterranean conditions. *Soil Biology and Biochemistry* **38**, 2125-2134.

Zornoza R, Guerrero C, Mataix-Solera J, Arcenegui V, García-Orenes F, Mataix-Beneyto J (2007) Assessing the effects of air-drying and rewetting pre-treatment on soil microbial biomass, basal respiration, metabolic quotient and soluble carbon under Mediterranean conditions. *European Journal of Soil Biology* **43**, 120-129.

Zornoza R, Landi L, Nannipieri P, Renella G (2009) A protocol for the assay of arylestease activity in soil. *Soil Biology & Biochemistry* **41**, 659-662.