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Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil

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ABSTRACT

Microbial and biochemical indicators of soil health were used to assess the ecological conditions and biological activity of soils contaminated with metals at a lead smelter plant and surrounding area in northeast Brazil. Soil respiration, microbial biomass of C and N, acid phosphatase, asparaginase, and density of ammonifying and ammonium-oxidizing microorganisms were positively correlated with soil organic carbon and/or water content, but showed negative correlations with metal contents in soil. Nitrification rate and metabolic quotient (qCO_2) were positively correlated with metal contamination, suggesting favorable conditions for N loss and microbial stress, respectively. No significant correlations were found between metal concentrations in soil and dehydrogenase activity or ammonification rate, considering water content and soil organic carbon as covariables. Soil respiration, microbial biomasses of C and N, dehydrogenase, acid phosphatase, asparaginase activities, and ammonifying microorganisms were positively correlated with percentage vegetation cover, while nitrification and ammonification rates were negatively correlated with this parameter. In general, soil respiration, microbial biomass of C and N, acid phosphatase, asparaginase, density of ammonifying and ammonium oxidizing microorganisms, nitrification rate and qCO_2 indicated high ecological risk for soil functions mediated by microorganisms (concerning to C and nutrient cycling) due to deposition of tailing contaminated with metals, even 17 years after the smelter activities had stopped. Besides direct effect of metal toxicity on microbial biomass and activity, there are indirect effects related to changes in vegetation cover, soil organic carbon, pH, and nutrient availability, and consequently changes in the soil microclimate and physical–chemical properties that may lead to losses of habitat function for soil microorganisms and the key processes they play. However, a multivariate decomposition of variance indicated that vegetation cover explained only 3.1%, whereas metals explained 26.9% of the variation associated to the microbial/biochemical indicators, showing a stronger effect of metals.

1. Introduction

Contaminants may affect a variety of microbial processes in soil, thereby affecting the nutrient cycling and the capacity to perform key ecological functions, such as mineralization of organic compounds and synthesis of organic matter (Giller et al., 1998, 2009; Moreno et al., 2009). Microbial biomass, soil basal respiration, enzyme activities, and nutrient transformations are important attributes related to soil fertility (Edwards, 2002) and can be used as indicators of soil health to monitor soil contamination (Castaldi et al., 2004; Smejkalova et al., 2003), agricultural use (Araújo et al., 2003; Tu et al., 2006), suitable management or success of restoration practices (Balota et al., 2004; Nogueira et al., 2006; Clemente et al., 2007). These biological indicators have the advantage of being easy and relatively fast to measure, thus being cost-effective tools for monitoring (Alkorta et al., 2003). Moreover, they provide an integrative biological assessment of soil health (Alkorta et al., 2003; Epelde et al., 2006).

Microbiologically mediated processes, catalyzed by enzymes, are essential for soil functioning, providing the basis of carbon, nitrogen, phosphorus, and sulfur cycling in soil (Alkorta et al., 2003). Microbial biomass is an important constituent of the soil biological fertility, involved in the biogeochemical cycle of nutrients and

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carbon. In addition, it is an important reservoir of nutrients in ecosystems. Soil microorganisms immobilize carbon and nitrogen by forming new biomass using the energy they obtain from oxidation of carbon sources through respiration, or inorganic chemical reactions (Chen et al., 2003). Therefore, more microbial biomass can stock and cycle more nutrients (Gregorich et al., 1994), improving the sustainability of an ecosystem (Kaschuk et al., 2010). Soil enzymes can be used as indicative of biological activity on a given biochemical processes in soil, being sensitive to alteration of soil health as a consequence of use and management (Balota et al., 2004; Bastida et al., 2006; Nayak et al., 2007). They also have been used as responsive indicators of contamination with metals on soil biochemical properties involved in carbon and nutrient cycling (Kuperman and Carreiro, 1997; Dias-Junior et al., 1998; Gülser and Erdogan, 2008).

Effects derived from a long-term exposure of microbial communities to metals cannot be predicted by recent addition of metal salts into the soil because microbial communities respond differently to chronic or acute exposures (Giller et al., 1998; Renella et al., 2002). In addition, metal bioavailability may change according to aging after contamination as a consequence of physical–chemical interactions with the soil matrix (McGrath, 2002; Vig et al., 2003). Moreover, the microbial community may adapt to the novel condition (Sobolev and Begonia, 2008) overcoming the negative effect of contamination (Lejon et al., 2010). Thus, long-term metal contaminated sites represent good conditions for studying chronic exposition of microbial communities. In general, there have been observed negative effects of metal contamination on soil microbial community size and diversity (Kelly et al., 2003), enzymatic activities (Begonia et al., 2004; Zeng et al., 2007), microbial biomass (Yuangen et al., 2004), N mineralization, and microbial respiration (Rost et al., 2001).

Microorganisms in soil under stress may be metabolically less effective because they need to invest more energy for cell maintenance, resulting in increased C—CO_2 release per unit of microbial biomass (Epelde et al., 2006), a ratio also known as microbial metabolic quotient, qCO_2 (Anderson and Domsch, 1993). qCO_2 has been used as indicator of microbial stress caused by metal contamination in soil (Zhang et al., 2008), where the higher values, the higher stress.

This study is integrated in a broader project dealing with assessing the ecological risk of a metal multicontaminated area at Santo Amaro, Bahia, Brazil. Time- and cost-effective microbial and biochemical indicators of soil health were assessed aiming at investigating the extent to which a long-term contamination changed the ecological status of a former lead smelting area. We hypothesized that each microbial or biochemical indicators respond to the metal contamination gradient, being impaired in highly contaminated sites as compared to sites at greater distances from the source of contamination.

2. Materials and methods

2.1. Study area

This study was carried out within and around an abandoned lead smelter that operated between 1960 and 1993, near to the urban area of Santo Amaro, BA, Brazil (12°32′49″S, 38°42′43″W). The site presents a high health risk for humans (Costa, 2001; Carvalho et al., 2003) due to high levels of metals in soil and water. A total of 500,000 t of tailings were deposited inside and in the surroundings of the smelter, and buried under roads and house's backyards (approx. 55,000 m^3) (Machado et al., 2004). In addition, airborne contaminated dust from atmospheric deposition through chimney emissions reached up to 3 km away from the industrial area during the period the smelter operated (Anjos, 2003; Machado et al., 2004). In 1995, the Bahia State environmental agency recommended the encapsulation of tailings with soil rich in organic matter to mitigate contamination. However, the process failed and currently, in some areas, tailings are still exposed and the aerial dispersion by dust is still occurring within and outside the smelter area (Anjos, 2003; Machado et al., 2004), leading to risks of soil and water contamination.

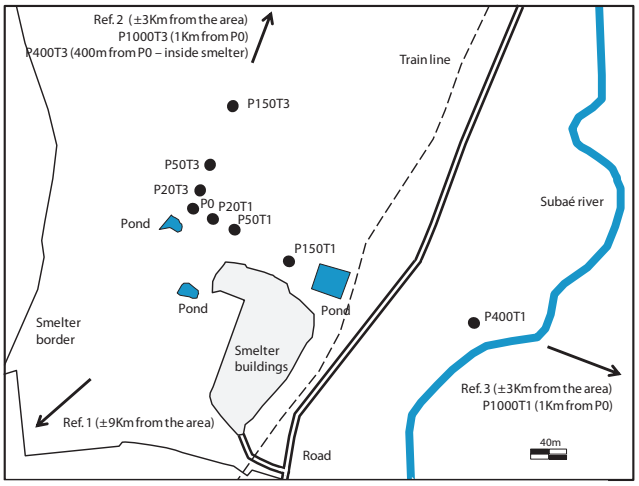

Fig. 1. Schematic representation of the study area (an abandoned lead smelter at Santo Amaro, BA, Brazil) indicating the location of 11 sampling sites along the two transects and three reference sites. Extracted from Niemeyer et al. (2010). Ref.: reference point; P0: central point (start of both transects). The code from the other points contains the distance from the central point and the transect code (Ex.: P20T1 – 20 m from P0 at transect 1).

The soils at the sampling sites were classified as Vertisols and Inceptisols (Soil Taxonomy, USDA) originated from carbonaceous shale, rich in expansive clay (montmorillonite), with generally low porosity and consequently low permeability (Machado et al., 2002).

2.2. Soil sampling and estimation of vegetation cover

Two 1-km transects (T1 and T3) were defined along the two major detected gradients of contamination (Fig. 1). The two transects shared a central point (P0 – located close to the smelter plant) and comprised 5 sampling points each (at 20, 50, 150, 400, and 1000 m from P0).

Soil sampling was done in April 2009 at 0–10 cm topsoil layer. At each point, three parallel transects of 10 m long were defined 2 m apart. Along each parallel transect 10 subsamples were collected and pooled to form a composite sample. After mixing, the samples were sieved (<5 mm), stored at 4 °C, and processed within 72 h.

Sites in the surrounding area were screened, analyzed for metals, soil properties and vegetation. Three reference sites were selected at 3 km (Ref. 2 and 3) and 9 km (Ref. 1) away from the central sampling site (P0) (Fig. 1) aiming not only to represent the diversity of habitat composition of the area surrounding the smelter, but also to match the properties of soils and vegetation from sites inside the smelter area.

Assessment of vegetation cover was carried out according to Veiga and Wildner (1993). Briefly, a plastic grid with 50 cm × 50 cm size, subdivided in 100 small squares of 5 cm × 5 cm, was randomly released four times on each sampling site. The sum of the intersections of small squares over vegetation in each grid represents the percentage of vegetation cover.

2.3. Soil metals concentration and physical–chemical analyses

Soil samples were analyzed for the main four metals causing contamination in the smelter area and proximities (Pb, Cd, Cu, and Zn) and also for Cr, Ni, Fe, Co, and Mn. Metals were quantified in the bulk soil and in 0.01 M $CaCl_2$ extracts by inductively coupled plasma-atomic spectroscopy. Extractions using 0.01 M $CaCl_2$ have been proposed as a suitable technique for determination of available fraction of metals in soil (Houba et al., 1996). The extracts were obtained by shaking 15 g of soil (dry weight) for 2.5 h at 200 rpm with 150 mL of a 0.01 M $CaCl_2$ solution. The slurry was then centrifuged for 5 min at 3000 rpm and extracts (supernatants) were filtered through a Schleicher & Schuell filter paper (Dassel, Germany, Reference no. 595).

Other soil physical–chemical parameters measured were pH (1 M KCl) (ISO, 1994a), soil moisture after oven drying at 105 °C overnight, water holding capacity (ISO, 1998), cation exchange capacity (ISO, 1994b), organic matter content (mass loss on ignition at 500 °C for 6 h), and soil texture (LNEC, 1970). Mineral N (NO_3^-—N and NH_4^+—N) was quantified in aqueous extracts by titration with 0.01 N sulfuric acid (Chapman and Pratt, 1978).

2.4. Soil microbial and biochemical analyses

For nitrogen transformation rates (nitrification and ammonification) each sample was divided into three aliquots. One was used to measure the initial NO_3^-—N and NH_4^+—N concentrations as described above. The second aliquot received 125 μg g^{-1} of NH_4^+—N as ammonium sulfate, while the third one was left with no N addition before being both incubated at 28 °C for 21 days in the dark. NO_3^-—N and NH_4^+—N concentrations were again determined and values obtained before and after incubation were used to calculate the nitrification and ammonification rates (Schuster and Schroder, 1990).

The ammonifying and ammonium oxidizing microorganisms were estimated by most probable number (MPN) (Woomer, 1994) after serial dilution of soil samples in sterile 0.85% saline, and inoculation in multiple 5-replication vials containing the respective liquid culture medium. For ammonifying microorganisms, hydrolyzed casein was used as source of organic N (Sarathchandra, 1978), while for ammonium oxidizers, NH_4^+—N was used as energy source in the mineral medium (Schmidt and Belser, 1994). After appropriate incubation time at 28 °C in the dark, the positive vials were counted, confronted to a most probable number table, and results expressed as log MPN g^{-1} dry soil.

Dehydrogenase activity was assessed in field-moist soil samples incubated with 1.5% triphenyl tetrazolium chlorine (TTC) for 24 h at 37 °C in the dark and expressed as microgram of triphenyl tetrazolium formazan (TTF) per gram per day at 37 °C (Casida et al., 1964). Asparaginase activity was estimated by incubation at 37 °C for 2 h in sodium acetate buffer pH 10 and L-asparagine as substrate. The NH_4^+—N produced was quantified by steam distillation in KCl–$AgSO_4$ extracts (Frankenberger and Tabatabai, 1991) and expressed as μg N—NH_4^+ g^{-1} h^{-1} at 37 °C. Acid phosphatase activity was determined using 0.05 M sodium p-nitrophenyl phosphate as substrate in samples incubated in modified universal buffer pH 6.5 at 37 °C for 1 h; the color intensity was measured colorimetrically and the activity expressed as μg of p-nitrophenol (PNP) g^{-1} h^{-1} at 37 °C (Tabatabai and Bremner, 1969).

Microbial biomass of carbon (MBC) and nitrogen (MBN) were estimated by fumigation–extraction method. Two 25 g aliquots of field-moist soil samples were weighed and one of them was fumigated for 24 h at 28 °C with ethanol-free chloroform in the dark. Afterwards, fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 and the organic C was quantified (Anderson and Ingram, 1993). MBC was estimated considering the difference between C concentrations in the fumigated and non-fumigated extracts, by using a $k_C = 0.33$ (Vance et al., 1987). MBN was estimated in the same extracts after sulfuric digestion of an aliquot of the extract and determination of N content by semi-micro Kjeldahl method considering a $k_N = 0.68$ (Brookes et al., 1985). The metabolic quotient (qCO_2) was obtained by the ratio between the C—CO_2 evolved from soil samples (data from Niemeyer et al., 2010) and the respective MBC (Anderson and Domsch, 1993), expressed as milligram C—CO_2 per gram MBC per hour. The qCO_2 values are inversely related to the efficiency to which the microbial biomass uses the substrates, i.e., higher values indicate higher stress and less efficiency.

2.5. Data analysis

Statistical differences on microbial parameters between sites were evaluated using a one-way ANOVA followed by a Dunnet's test. Soil moisture, total organic C and mineral N were used as covariables in all analyses. A partial Principal Component Analysis (pPCA) was used to visualize the major response pattern of microbial indicators, using the aforementioned soil parameters as covariables.

Partial correlations between microbial parameters and total and extractable metal concentrations, and vegetation cover were done using the Pearson's correlation coefficient. Moreover, partial correlations were also performed between the assessed microbial variables and the index of metal pollution (W) proposed by Widianarko et al. (2000). This index is the ratio between the metal concentrations in each site by the corresponding background concentration (reference value or basal level). The result indicates how much the background concentration is exceeded at a given site. The factors for each metal were then multiplied by one another and the logarithm of the product was taken. In this study, the basal level for each metal was calculated as the average from the three reference sites. This index was calculated for each sampling point, aiming at synthesizing the information on metal contamination joining information from all metals analyzed. Initially developed for sediment samples, the W index can be used to synthesize the metal loading of any environmental sample in comparison to metal basal levels, including soils (Timmermans et al., 2007; Janssens et al., 2008). This index can take negative values in sites where metal loadings are below the basal levels, whereas positive values indicate metal loadings higher than the basal levels.

In order to separate the contribution of metals and vegetation cover in explaining the differences for microbial parameters, a multivariate decomposition of variance was performed. This was done via several redundancy analyses using the microbial parameters as response variables, and metals and/or vegetation cover (depending on the analysis) as explanatory variables, in addition to soil moisture, soil organic carbon, and mineral nitrogen contents as covariables. The significance of the percentage of variation explained by metals alone, vegetation cover alone, and the interaction between both factors was assessed by the Monte Carlo's permutation tests. All analyses of variance and correlations were performed on the Statistica 7.0 package. All multivariate analyses were done using CANOCO 4.0 software.

3. Results

3.1. Characterization of the sampling sites

Soils from the study sites showed low to medium organic carbon content (USEPA, 2004), ranging from 0.12 to 3.31%, a Cation Exchange Capacity (CEC) mostly between 30 and 40 meq/100 g, and

pH values near to neutral, except in the sites P1000T1 and Ref. 2 that presented low values (Table 1).

Vegetation cover ranged between 20 and 100% (Table 1). In general, a significant reduction of the vegetation cover in comparison to the reference sites was observed in most of the sampling points within the smelter area (P0, P20T1, P150T1, P20T3, and P50T3). These points, together with P50T1 and P150T3, correspond to sites in which tailings were deposited and where the unsuccessful revegetation can be observed. At these sites, vegetation was dominated by one herbaceous species (*Brachiaria* sp.). In some of these sites, there were evidences of erosion, which could have delayed the natural regeneration process. Further discussion on this issue is given in Niemeyer et al. (2010).

3.2. Soil metal concentrations

Total and 0.01 M $CaCl_2$-extractable metal concentrations are shown in Table 2. For at least one out of four metals (Pb, Cd, Cu, and Zn), soils from three sampling points inside the smelter area (P0, P50T3, and P150T1) presented critical levels of contamination with total metal levels exceeding the Dutch benchmark values for ecological assessment, as defined by Rutgers et al. (2008). Results of Co (not included in Table 2) were below the detection limit in all sites: <24 mg/kg (total) and <10.8 mg/kg (extracts).

3.3. Soil microbial and biochemical parameters – differences among sites

Soil microbial properties varied among sites, being conditioned mainly by the metal loadings and degree of habitat disturbance, as measured by vegetation cover. The basal respiration and microbial biomass of carbon and nitrogen presented significantly lower values especially at sites near the central point (P0) when compared to the overall reference (Table 3). Contrary to expected, the metabolic quotient qCO_2 only presented high values at P20T3 and P150T1, but with high variability, and no significant differences from the overall control. The activities of dehydrogenase, phosphatase, and asparaginase had significant decreases in the soils inside the smelter.

Some other soil microbial properties related to N cycling, namely ammonification and nitrification rates, presented higher values in sites inside the smelter area. However, significant differences against the overall reference were found only for nitrification rate (Table 3). Regarding the number of ammonifiers, significantly lower numbers were found in sites inside the smelter area, whereas no differences were found among sites for the nitrifiers (ammonium oxidizers).

The partial principal component analysis permitted to see how the attributes were correlated to the sampling sites (Fig. 2). Axis 1 separated the sites with lower metal loadings (the 3 references, P1000T1 and P400T3), and located them in the positive side of the axis. These were mostly located outside the smelter area, and were associated to higher levels of microbial respiration, microbial biomass C and N, ammonifiers, and higher phosphatase and asparaginase activities. Sites in the negative side of the axis 1, presenting higher W index (Table 2) values and located mostly inside the smelter area, were mainly characterized by higher ammonification and nitrification rates.

3.4. Soil microbial and biochemical parameters – relationship with metal contamination and vegetation cover

Most microbial parameters presented significant partial correlations (using soil moisture, soil organic carbon, and mineral nitrogen as covariables) with metal loadings in soil given by the Widianarko's pollution index (W). Negative relations were found for basal respiration, microbial biomass (C and N), phosphatase and

Table 1
Soil physical–chemical characteristics and vegetation cover (%) in the sites within and around the lead smelter area, and in the three reference sites.

Sites	Coarse sand (%)	Fine sand (%)	Sand (total) (%)	Silt (%)	Clay (%)	Texture (USDA)	CEC (meq/100 g)	pH (KCl 1:5 v:v)	P (mg/kg)	Organic carbon (%)	Mineral N (mg/kg)	Water content (%)	WHC (g/100 g)	Vegetation cover (%)
Ref. 1	2.3	8.5	10.9	42.1	47.0	Silty clay	34.16	7.1	72	0.64	42	19.54	53.78	81.3 ± 21.0
Ref. 2	50.9	38.5	89.4	2.8	7.7	Loamy sand	37.60	4.9	1	0.58	42	13.21	27.53	
Ref. 3	22.2	15.0	37.2	11.1	51.7	Clay	36.48	6.1	52	2.26	56	47.20	60.75	
P0	43.2	31.3	74.5	11.9	13.6	Sandy loam	38.56	6.7	47	0.17	70	31.04	44.12	22.5 ± 22.2
P20T1	48.0	13.8	61.8	19.0	19.3	Sandy loam	37.28	7.1	58	0.12	42	32.67	46.40	30.0 ± 16.3
P20T3	11.4	30.0	41.4	22.3	36.3	Clay loam	42.16	6.8	106	1.10	42	35.04	67.73	32.5 ± 12.6
P50T1	25.2	13.4	38.6	29.0	32.4	Clay loam	38.16	6.7	63	0.64	56	28.59	54.51	57.5 ± 12.6
P50T3	69.2	9.1	78.3	10.4	11.3	Sandy loam	16.56	7.2	>200	1.62	56	39.48	22.05	20.0 ± 14.1
P150T1	56.2	21.1	77.4	12.3	10.3	Sandy loam	21.28	6.7	>200	1.22	42	29.41	28.55	30.0 ± 42.4
P150T3	8.4	15.2	23.5	21.4	55.1	Clay	49.20	6.8	16	1.45	42	40.71	61.76	57.5 ± 9.6
P400T1	19.6	23.9	43.5	20.2	36.3	Clay loam	37.44	6.8	>200	2.96	56	24.43	58.93	100.0 ± 0.0
P400T3	6.5	8.6	15.1	52.4	32.5	Silt clay loam	35.84	7.1	1	1.10	70	45.48	56.67	97.5 ± 5.0
P1000T1	2.5	21.8	24.3	19.9	55.8	Clay	43.20	3.7	35	1.16	56	28.74	59.95	67.5 ± 15.0
P1000T3	10.3	19.5	29.8	29.8	40.4	Clay loam	42.72	7.0	>200	3.31	42	n.d.	57.57	n.d.

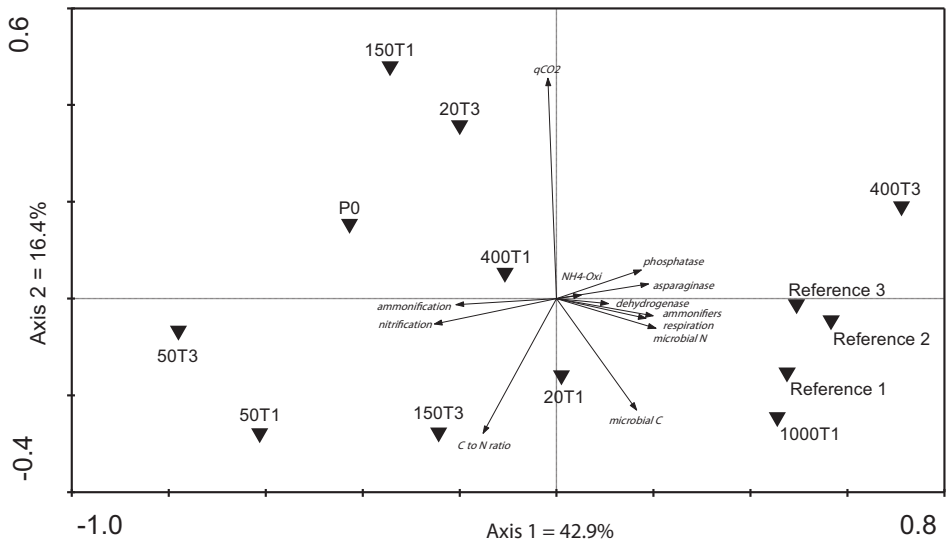

Fig. 2. Partial Principal Component Analysis (pPCA) ordination diagram, using the parameters of Table 3. Soil moisture, soil organic carbon, and mineral nitrogen contents were used as covariables in the analysis.

asparaginase activities, and number of ammonifiers, whereas a significant positive relation was observed for nitrification rate (Table 4). No significant correlations were observed for microbial C/N ratio, qCO_2, dehydrogenase activity, and ammonification rate.

Similar trends can also be seen when looking at individual metals, especially when considering the total metal concentrations (Table 4), given that the correlations with extractable metal were weaker. Significant negative correlations were observed for microbial biomass C with Pb, and number of ammonifiers with total Fe, Pb and Ni. Significant positive correlation with extractable concentrations of Cd and Pb were found for qCO_2.

Microbial and biochemical parameters also presented significant correlations with vegetation cover (Table 4). Positive correlations were mostly found with basal respiration, microbial biomass (C and N), enzyme activities (dehydrogenase, phosphatase, and asparaginase), and number of ammonifiers, whereas negative correlations were observed with nitrogen transformation rates (ammonification and nitrification).

As an attempt to decipher the different contribution of metals and vegetation cover in explaining the variation of microbial and biochemical parameters, the multivariate decomposition of variance (partial RDAs using soil moisture, soil organic carbon, and mineral nitrogen contents as covariables) showed that total metal concentrations explained a considerable portion of the variation (49.1%), whereas vegetation cover explained only 25.3% (Table 5). However much of the variation in these values correspond to shared variance between both variables (22.2%), indicating that vegetation alone explains only 3.1% against 26.9% of the variation explained by metals alone (Table 5).

4. Discussion

The high levels of metals in the soil of smelting area have been previously reported (Anjos, 2003; Machado et al., 2004), and resulted both from deposition of residues inside the smelting area and aerial deposition of contaminated particles from the smelter plume during the smelting activity. The plume was also responsible for the extent of contamination outside the smelter area. The heterogeneity of the soil inside the smelter area can be attributed to heterogeneous deposition of tailings and the partially unsuccessful attempt to encapsulate some of the piles by depositing thousands of cubic meters of nearby soil (Anjos, 2003).

This study has revealed significant differences in soil microbial and biochemical attributes among the sampling sites differently affected by the lead smelter activity. This can be attributed to disturbances caused by deposition of tailings in the area and to their unsuccessful encapsulation using soil brought from nearby areas, which affected the soil (Niemeyer et al., 2010), and its microbial and biochemical properties. Negative correlations between the W index with some microbial and biochemical attributes illustrate the negative effects of metal contamination on the soil microbial community and some essential role they play in the biogeochemical cycles. Under such condition, the sustainability of the vegetation in the metal-contaminated sites has not been reached, and may lead to more environmental risks in the future. As key microbial processes on C, N and P cycling have been impaired under such condition, in addition to higher contents of metals, the maintenance of vegetation in these heavily-contaminated sites can be progressively difficult, leading to intensification of erosive processes and dispersion of pollutants (Broos et al., 2005).

There have been some controversial findings regarding the effect of metal contamination on soil respiration, as some works have observed increased respiration rates whereas others a decrease with increasing metal concentrations in soil (Smejkalova et al., 2003; Rajapaksha et al., 2004; Khan and Joergensen, 2006). In the present work, the soil basal respiration rate was lower in the metal-contaminated soils inside the smelter area and correlated negatively with total soil metal concentrations. These results agree with those obtained by Zimakowska-Gnoinska et al. (2000) who observed less oxygen consumption in soils from metal-contaminated sites in comparison to uncontaminated samples, in addition to strong negative correlations between soil respiration and soil pollution levels. Gülser and Erdogan (2008) also observed that soil respiration correlated negatively with contents of several metals in roadside fields near to intensive traffic; soil respiration significantly increased with decreasing the levels of metal contents according to the distance from the roadside. In the present work, the negative correlations between soil respiration and metal concentrations, and the positive correlations between soil respiration and other microbial indicators confirmed that soil respiration can be used for estimations and comparisons between soil ecological conditions and biological activity (Zimakowska-Gnoinska et al., 2000).

Table 2
Total and 0.01 M $CaCl_2$ extractable metal concentrations, and pollution index (W) in the soil within and around the lead smelter area, and in the three reference (Ref.) sites.

Sites	Total (mg/kg)								Pollution index	Extractable (mg/kg)–(0.01 M $CaCl_2$; 1:10 v:v)							
	Pb	Cd	Cu	Zn	Cr	Ni	Fe	Mn	W	Pb	Cd	Cu	Zn	Cr	Ni	Fe	Mn
Ref. 1	16	<0.2	66	94	77	54	45000	840	1.26	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
Ref. 2	13	<0.2	18	24	16	28	2900	34	−0.89	<0.9	<0.09	<7.2	1.8	<7.2	<12.6	<9.9	7.2
Ref. 3	152	<0.2	40	260	59	40	53000	820	−0.37	<0.9	<2.52	<7.2	<1.8	<7.2	<12.6	<9.9	11.7
P0	1264	<0.2	76	3800 (2.8)	72	57	52000	674	5.61	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
P20T1	133	<0.2	56	220	80	56	41000	780	2.44	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
P20T3	308	<0.2	56	420	78	60	49000	672	3.12	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
P50T1	164	<0.2	60	240	80	58	43000	720	2.60	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
P50T3	26074 (7.1)	62	3196 (8.2)	95940 (73.5)	80	40	117000	5880	13.63	<0.9	<0.09	<7.2	9	<7.2	<12.6	<9.9	<4.5
P150T1	37460 (10.4)	771 (9.8)	594 (1.6)	42200 (33.5)	57	70	110000	1720	13.33	19.8	65.7	<7.2	11.7	<7.2	<12.6	<9.9	<4.5
P150T3	2200	12	108	3300	84	58	56000	678	7.83	<0.9	<0.09	<7.2	1.8	<7.2	<12.6	<9.9	<4.5
P400T1	961	8.8	60	840	64	48	35000	540	5.98	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
P400T3	179	0.3	44	90	59	46	34000	760	2.76	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	<4.5
P1000T1	23	<0.2	60	80	62	46	48000	360	0.80	0.9	3.6	<7.2	17.1	<7.2	14.4	<9.9	63.9
P1000T3	99	<0.2	56	156	84	52	49000	568	2.09	<0.9	<0.09	<7.2	<1.8	<7.2	<12.6	<9.9	8.1

Numbers in brackets indicate an excess of the corrected Dutch $HC50_{EC50}$ values (after Rutgers et al., 2008) (Ex: the [Pb] at P150T1: 37460 (10.4), indicates that [Pb] was 10.4 times higher than the $HC50_{cor}$Pb).

Table 3
Soil microbial parameters (average values ± standard deviation) for the assessed sampling points of the soil within and around the lead smelter area, and three reference sites. The values for the three reference sites were averaged to give an overall reference value. Asterisks indicate significant differences ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$) for a one-tailed hypothesis of a Dunnet's test between each sampling site and the overall reference value (assuming that Ref. – soil value is higher than value at each sampling point for all parameters except for Nitrification Rate, where Ref. value is lower). For ANOVA, considering the soil microbial parameters, the soil moisture, soil organic carbon, and soil nitrogen contents were used as co-variables (see text for more details). $N = 3$.

Sites	Respiration (μg CO_2/g soil/day)	MBC (μg/g)	MBN (μg/g)	C/N	qCO_2 (mg CO_2-C/g biomass C/h)	Dehydrogenase (μg PNP/g/d)	Acid phosphatase (ug PNF/g/h)	Asparaginase (μg N-NH_4^+/g/h)	Ammonification (μg N/g/day)	Ammonifiers (log of MPN/g)	Nitrification rate (%)	Nitrifiers NH_4^+ oxidizers (log MPN/g)
Ref. 1	101.6	431.2 ± 109.7	36.6 ± 7.6	12.10 ± 3.41	2.8	9.6 ± 1.2	383.2 ± 58.1	76.7 ± 5.2	0.4 ± 0.1	6.8 ± 0.5	6.3 ± 2.7	
Ref. 2	50.4	374.3 ± 108.8	33.4 ± 7.5	11.90 ± 5.24	1.6	6.8 ± 2.4	618.5 ± 32.0	36.2 ± 20.2	1.1 ± 0.4	7.0 ± 0.3	3.1 ± 1.6	2.3 ± 0.6
Ref. 3	266.2	1121.7 ± 279.4	80.3 ± 14.3	13.83 ± 1.14	2.7	5.1 ± 2.4	849.7 ± 43.0	141.7 ± 22.8	0.6 ± 0.9	6.8 ± 0.1	2.2 ± 1.6	2.9 ± 0.0
Overall reference	139.4 ± 106.4	642.4 ± 416.1	50.1 ± 16.2	12.61 ± 1.06	2.5 ± 0.6	7.2 ± 2.3	617.1 ± 233.2	84.9 ± 53.2	0.7 ± 0.3	6.9 ± 0.1	3.9 ± 2.2	2.3 ± 0.7
P0	34.9 ± 7.8**	178.1 ± 55.1***	5.4 ± 2.8***	36.12 ± 10.35	2.5 ± 1.3	0.7 ± 0.4**	269.0 ± 22.1**	53.8 ± 29.5	1.7 ± 0.2	4.7 ± 0.9***	15.2 ± 9.6**	2.4 ± 0.1
P20T1	35.1 ± 7.1**	252.4 ± 142.3**	12.8 ± 4.6***	20.37 ± 9.89	1.8 ± 0.6	1.3 ± 1.9**	196.4 ± 33.9***	15.9 ± 18.4***	1.1 ± 0.5	5.3 ± 0.3***	12.4 ± 2.1*	2.5 ±± 0.1
P20T3	82.6 ± 15.8	170.3 ± 174.1***	18.6 ± 3.5***	9.64 ± 10.68	10.5 ± 7.8	1.4 ± 1.0**	443.4 ± 9.3	71.0 ± 12.7	1.5 ± 0.3	6.2 ± 0.2	13.3 ± 3.5*	2.4 ± 0.2
P50T1	41.4 ± 2.4*	412.9 ± 31.4	11.0 ± 4.2***	41.10 ± 15.23	1.1 ± 0.1	1.2 ± 2.0**	235.7 ± 50.3**	11.2 ± 19.5***	1.8 ± 0.3	5.5 ± 0.5***	17.1 ± 4.9***	2.6 ± 0.1
P50T3	52.2 ± 12.6*	461.7 ± 20.1	22.0 ± 4.5*	21.62 ± 4.76	1.3 ± 0.3	2.1 ± 0.5*	450.3 ± 45.4	32.5 ± 35.1**	1.8 ± 0.3	5.6 ± 0.5***	13.5 ± 4.5*	2.2 ± 0.5
P150T1	49.2 ± 6.6*	115.5 ± 87.0***	9.3 ± 1.3***	12.03 ± 9.24	12.6 ± 15.9	3.3 ± 0.5	355.3 ± 166.0*	22.8 ± 11.8***	0.4 ± 0.2	5.6 ± 0.1***	8.7 ± 2.8	2.4 ± 0.3
P150T3	60.5 ± 9.2*	543.6 ± 160.8	26.6 ± 3.1**	20.85 ± 7.62	1.3 ± 0.4	2.1 ± 1.1*	651.2 ± 150.7	37.0 ± 12.4*	1.5 ± 0.3	6.6 ± 0.6	10.2 ± 0.5	2.3 ± 0.0
P400T1	234.9 ± 83.3	797.3 ± 193.3	83.0 ± 21.2	9.62 ± 0.43	3.3 ± 0.4	16.8 ± 3.7	573.1 ± 133.3	91.7 ± 32.9	0.8 ± 0.1	7.0 ± 0.3	−0.2 ± 5.9	2.8 ± 0.0
P400T3	165.2 ± 41.3	805.3 ± 216.2	59.7 ± 26.5	14.18 ± 2.82	2.3 ± 0.3	1.5 ± 1.1**	792.0 ± 34.5	97.8 ± 16.6	0.4 ± 1.0	6.4 ± 0.1	−3.3 ± 3.17	2.9 ± 0.1
P1000T1	164.0 ± 79.1	1098.1 ± 184.1	51.1 ± 22.0	23.79 ± 9.79	1.71 ± 0.7	4.8 ± 6.2	515.6 ± 353.5	71.57 ± 18.8	0.6 ± 1.8	6.4 ± 0.4	1.9 ± 7.4	2.8 ± 0.0
P1000T3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Ref. – Reference soil; MBC – Microbial biomass carbon; MBN – Microbial biomass nitrogen; C/N – C to N ratio of the microbial biomass and n.d. – not determined.

Table 4
Pearson's partial correlations between microbial parameters and metal concentration (total and extractable), overall metal loading (Widianarko's index – W) and vegetation cover – VEG.

Microbial parameters	Total metal concentrations							Extractable metal concentrations			W index	VEG
	Cu	Fe	Mn	Zn	Cd	Cr	Pb	Ni	Mn	Cd	Pb	Ni		
Basal respiration	−0.54***	−0.53**	−0.53**	−0.57**	n.s.	n.s.	−0.50**	n.s.	n.s.	n.s.	n.s.	n.s.	−0.70***	0.61***
MBC	n.s.	−0.44**	−0.35*	−0.40*	−0.38*	n.s.	−0.47**	−0.45**	0.60***	n.s.	−0.36*	0.60***	−0.64***	0.58***
MBN	−0.47**	−0.64***	−0.48**	−0.54**	−0.39*	−0.39*	−0.55***	−0.45**	n.s.	n.s.	−0.35*	n.s.	−0.72***	0.73***
C/N	n.s.	n.s.	n.s.	n.s.	n.s.	0.39*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
qCO_2	n.s.	n.s.	n.s.	n.s.	0.44**	n.s.	n.s.	0.36*	n.s.	0.45**	0.45**	n.s.	n.s.	n.s.
Dehydrogenase	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.42*
Acid phosphatase	n.s.	−0.51**	−0.34*	n.s.	n.s.	−0.65***	n.s.	−0.56***	n.s.	n.s.	n.s.	n.s.	−0.47**	0.56***
Asparaginase	−0.47**	−0.48**	−0.46**	−0.51**	n.s.	n.s.	−0.48**	n.s.	n.s.	n.s.	n.s.	n.s.	−0.65***	0.55**
Ammonification rate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.42*
Nitrification rate	0.35*	0.43**	0.38**	0.35*	n.s.	0.54**	n.s.	0.35*	n.s.	n.s.	n.s.	n.s.	0.43**	−0.62***
Ammonifiers	−0.37*	−0.61***	−0.41*	−0.45**	−0.40*	−0.40*	−0.51**	−0.47**	n.s.	−0.36*	−0.37*	n.s.	−0.61***	0.73***
NH_4^+ oxidizers	n.s.	n.s.	−0.33*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

MBC – Microbial biomass carbon; MBN – Microbial biomass nitrogen; C/N – C to N ratio of the microbial biomass; NH_4^+ oxidizers – Nitrifiers ammonium oxidizers and n.s. – not significant ($p > 0.05$).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Variance partitioning of microbial and biochemical data according to total metal content and vegetation cover. Values expressed in percentage of total variation excluding covariables (soil moisture, soil organic carbon, and mineral nitrogen contents).

Variables	Variation explained (%)	*p*
Variation of microbial and biochemical parameters (excluding covariables)	66.8[a]	
Covariables	33.2	
Metals and Vegetation (total)	52.2	0.002
Metals	49.1	0.002
Vegetation	25.3	0.002
Metals (pure)	26.9	0.002
Vegetation (pure)	3.1	0.002
Shared	22.2	

[a] Expressed as % of total variation.

Microbial biomass is involved in the control of soil organic matter decomposition and synthesis, besides acting as easy-release storage of nutrient in ecosystems. Therefore, sites with high microbial biomass can stock and recycle more nutrients (Gregorich et al., 1994; Kaschuk et al., 2010) to be used for plant nutrition and thus improving the sustainability of a particular ecosystem. Consequently, sites with low microbial biomass can have these functions impaired, as observed in some sites inside the smelter area around the tail deposits. These sites also showed a low vegetation cover, indicating that the soil functions have still not been reestablished and that further actions are needed for reclamation of the degraded sites.

MBC, MBN, and basal respiration were positively correlated with soil organic C, while MBC was also positively correlated to soil moisture and mineral N (supplementary material), showing that microbial indicators can be impacted due to changes in carbon and nitrogen in soil as consequence of soil pollution or management (Monokrousos et al., 2006; Nogueira et al., 2006; Jiang et al., 2010). Given that most of the soil microbial community is composed by chemorganotrophic microorganisms, improvement of soil organic carbon usually stimulates microbial activity and biomass (Kaschuk et al., 2010). In addition, soil organic matter brings indirect beneficial effects to the soil microbial community by improving the soil capacity for water retention and metal complexation (Giller et al., 2009; Moreno et al., 2009).

Enzyme activities were positively correlated with MBC, MBN, and basal respiration (data not shown), indicating that they are associated with active microorganisms, which are the major source of enzymes in soil. Thus, the probable impact on enzyme activities was caused by direct suppression of microbial growth due to negative conditions in the metal-contaminated sites (Kuperman and Carreiro, 1997). As indicated by the variance partitioning, metal concentrations were the most responsible for changes in these attributes than vegetation cover and the large portion explained by vegetation is shared with metals, showing their strong indirect effect on microbial parameters. The significant correlations between MBC, MBN, and basal respiration with organic C (supplementary material) indicate that higher organic C levels in soil are supporting greater microbial biomass and enzyme activities, not only by acting as C and energy sources for soil microbial community, but also due to a chelating effect protecting microorganisms and soil enzymes from excessive levels of metals in soil (Balota et al., 2004; Moreno et al., 2009; Lejon et al., 2010).

Nitrification and ammonification rates have key roles in the nitrogen cycling in soil. While nitrification is considered one of the most sensitive soil microbial processes regarding to metal stress (Broos et al., 2005), some studies have shown adaptation of nitrifying populations to metal-contaminated sites (Mertens et al., 2006).

In the present work, nitrification rate was positively correlated with metal concentrations, but in this case, part of that behavior can be explained by the high pH in the highly contaminated sites. It is known that nitrification is favored under high pH, which, at the same time, makes metals less available and thus less toxic to the microbial community. Soil moisture, pH and ammonium contents in soil are generally the main factors affecting nitrification (Krave et al., 2002). In fact, soil pH showed the greatest positive correlation with nitrification in the present work ($r = 0.43$, $p < 0.01$, see Table S1), emphasizing the importance of soil pH on this attribute (Sarathchandra, 1978; Sauvé et al., 1999). In addition, in long-term contaminated sites, adaptation or selection of specific microbial groups or (sub) populations resistant or tolerant to metal contamination is likely to occur (Giller et al., 2009). Sobolev and Begonia (2008) suggested that denitrifying microorganisms were adapted to elevated levels of Pb by selecting for metal-resistant enzymes. Adaptation not only of nitrifying populations in contaminated sites but also other microbial communities is also known to occur (Lejon et al., 2010), and this may have occurred in the present work. A long-term exposure to a heavily Zn-contaminated soil induced structural changes and tolerance of the nitrifying microorganisms to Zn, as compared to the nitrifying community in an uncontaminated control soil (Mertens et al., 2006).

Significant correlations were found between qCO_2 and total Cd and Ni, and extractable Pb and Cd. Once this parameter indicates the energetic demand of heterotrophic microorganisms, integrating MBC and basal respiration, these results can indicate metal stress to soil microorganisms, evidencing the need for more C to supply their energetic demand per unit of microbial biomass (Bardgett and Saggar, 1994; Fliessbach et al., 1994; Valsecchi et al., 1995). Soil microbial biomass and activity (*sensu lato*) are closely related to vegetation cover.

In the present work, positive correlations were observed between vegetation cover and MBC, MBN, soil basal respiration, enzyme activities, and ammonifier microorganisms. Vegetation cover can contribute to reduce metal toxicity to microbial community because they offer favorable conditions not only in the rhizosphere region but also in the bulk soil due to inputs of plant residues that will run humification (Tordoff et al., 2000). On the other hand, vegetation cover showed significant negative correlations with individual metal contents and the pollution index (W) (data not shown). These relationships can explain the low percentage of the variation of microbial data explained by vegetation cover alone, and the high percentage explained by the interaction with total metals. This does not mean that vegetation is not important for microbial communities, but that, in this case, the vegetation cover was highly conditioned by the metal loadings. The failed establishment of the vegetation in metal-contaminated sites, conditioning microbial parameters, resulted mainly from direct metal contamination (Tordoff et al., 2000).

Vegetated soils have been reported to have both higher microbial biomass and microbial activity when compared to bare soils (Epelde et al., 2006). Hernández-Allica et al. (2006) and Epelde et al. (2010), in studies on phytoextraction with *Thlaspi caerulescens* in metal polluted soils, the revegetation activated the soil microbial activity and their functionality. This positive response can be attributed to the improvement of soil conditions, such as organic compounds released by the plant roots and the presence of additional surfaces for microbial colonization (Delorme et al., 2001).

Soil metal contamination in Santo Amaro has impaired the vegetation cover in the smelter area and modified the plant species composition and invertebrates, changing and simplifying the ecosystem structure (Niemeyer et al., submitted). Contamination had detrimental effects on soil properties, modifying the microclimatic conditions at the ground level, and the amount and quality of the potential organic inputs into the soil. Our results showed that these changes caused negative impacts to the soil microorganisms and processes inside the smelter area, where worse values were generally observed. The main negative effects seem to be due to limitation of plant reestablishment that results in low amounts of organic matter inputs into the soil to be used as source of C and energy for microbial growth and also on the protection of microbial community against high levels of metals in soil. Nogueira et al. (2006) recommended providing plant covering in degraded areas to prevent the advance of soil degradation by erosion and to increase microbial activity and diversity, which also contribute to decrease the nutrient losses by leaching. This could also be recommended as a management strategy to be applied in the smelter area in Santo Amaro, improving the soil health and preventing the dust dispersion by wind, which is a current problem for human health, once this area is located at the neighborhood of the city.

5. Conclusions

Microbial indicators were positively correlated with soil organic carbon and vegetation cover, while negatively correlated to soil metal levels. In general, microbial indicators showed high ecological risks to soil functions related to tailing deposition even 17 years after the lead smelter have stopped its activities. The main negative effects seem to be due to limitation of plant reestablishment that results in low amounts of organic matter inputs into the soil to be used as source of C and energy for microbial growth and also on the protection of microbial community against high levels of metals in soil.

We can conclude that, besides direct effect of metal toxicity on microbial biomass and activity, there are indirect effects related to changes in the vegetation cover, soil organic carbon, pH, and nutrient availability. These attributes have changed the soil microclimate and physical–chemical properties that may have lead to losses of habitat function for soil microorganisms and the key processes they play, as in carbon and nutrient cycling.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2012.03.019.

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