Ecotoxicology and Environmental Safety 86 (2012) 188–197

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

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

Functional and structural parameters to assess the ecological status of a metal contaminated area in the tropics

J.C. Niemeyer [a,b,*], M.A. Nogueira [c,1], G.M. Carvalho [d], S.J. Cohin-De-Pinho [b], U.S. Outeiro [b], G.G. Rodrigues [e], E.M. da Silva [b], J.P. Sousa [a]

[a] IMAR–CMA, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal
[b] Institute of Biology, Federal University of Bahia, 40170-115 Salvador, Bahia, Brazil
[c] State University of Londrina (UEL), Department of Microbiology, Londrina, Paraná, Brazil
[d] State University of Santa Cruz (UESC), Department of Biological Sciences, Ilhéus, Bahia, Brazil
[e] Department of Zoology, Center of Biological Sciences, Federal University of Pernambuco (UFPE), Recife, Pernambuco, Brazil

ARTICLE INFO

Article history:
Received 27 June 2012
Received in revised form
11 September 2012
Accepted 14 September 2012
Available online 16 October 2012

Keywords:
Bait lamina
Litter decomposition
Soil invertebrate communities
Vegetation
Microbial activity
Risk assessment

ABSTRACT

Ecological parameters (soil invertebrates, microbial activity, and plant community) were assessed in a metal contaminated site in an abandoned lead smelter and non-contaminated reference sites, as part of an ecological risk assessment (ERA). Vegetation cover inside the smelter area was lower and presented a more homogenous species composition than outside. A more simplified and less abundant vegetation community within the smelter area also simplified the habitat conditions, which in addition to metal toxicity, impaired the soil microbial and faunal communities. A significant reduction in the feeding activity was observed within the smelter area. Also a significant change in community composition of surface dwelling invertebrates was observed at those sites when compared to sites outside the smelter area. Moreover, basal respiration, microbial biomass C, dehydrogenase and phosphatase activity also decreased in several of these points under the smelter area. As a result, a significant impairment of organic material decomposition in the most contaminated sites was observed. Metal contamination affected the ecological status of the site, leading to a risk for ecosystem functioning and provisioning of ecosystem services like organic matter decomposition and nutrient cycling, even 17 years after the end of smelting activities. Regarding the sensitivity of the ecological parameters assessed, most were able to distinguish sites within the smelter area boundaries from those outside. However, only bait lamina (feeding activity), basal respiration and microbial biomass carbon presented high capacity to distinguish the level of soil contamination, since they were significantly correlated with metal loadings, and thus are promising candidates to be integrated in the Ecological Line of Evidence of an ERA.

1. Introduction

Ecological parameters have been recommended to be used in ecological risk assessment (ERA) of contaminated sites (Sprenger and Charters, 1997; Jensen and Mesman, 2006; Rutgers and Jensen, 2011). Ecological parameters are integrative indicators of adverse impacts resulting from contaminant exposure and they can be used either independently or integrated in a TRIAD approach (Jensen and Mesman, 2006; Semenzin et al., 2008) as one line of evidence in ERA.

Ecological effects of contaminants in soil can be assessed both from a structural and a functional perspective (Rutgers, 2008; Semenzin et al., 2009; Van Straalen, 2002). However, data on how biological processes are impaired due to soil pollution is still scarce since most of assessed ecological parameters are structural (e.g., vegetation surveys, soil faunal density and taxonomic composition). Therefore, links between pollutant effects on soil organisms and on soil functions should be investigated (Cortet et al., 1999) by simultaneously assessing structural and functional ecological parameters in a site evaluation.

In ERA schemes for contaminated soils, ecological parameters can be measured at different tiers and include different groups of organisms (i.e., from microorganisms to soil macrofauna) and different biological levels (e.g., populations, communities). Soil fauna is a key component of soil environments, involved in many aspects of organic matter decomposition and nutrient cycling (acting mainly as regulators of microbial activity), and contributing to the maintenance of soil structure (Lavelle, 1996). Different studies have demonstrated that soil fauna comprise groups of

* Corresponding author at: IMAR–CMA, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal. Fax: +351 239 855789.
E-mail address: juliacarina@yahoo.com.br (J.C. Niemeyer).
[1] Present address: Embrapa Soybean, Londrina, Paraná, Brazil.

0147-6513/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2012.09.013

organisms that are suitable indicators of soil ecological status and can be used to compare areas with different levels of contamination (Gongalsky, 2003; Creamer et al., 2008). In addition to the evaluation of soil faunal communities (usually done on a later phase of the assessment) the use of bait-lamina sticks has been proposed as a relevant tool for ecological assessments and was successfully tested in temperate (e.g. Hamel et al., 2007) and tropical soils (e.g., Römbke et al., 2003).

Vegetation surveys are one of the most used tools to evaluate habitat quality in terrestrial ecosystems (Godínez-Alvarez et al., 2009) because plants as primary producers are the key structural habitat component for many soil inhabitants. Measurements of vegetation cover and composition are important measures of habitat quality that can change in response to pollution stress. Besides, some advantages such as their immobility and easy sampling make the vegetation assessment a suitable tool to be used in ERA (Suter et al., 2000).

Microbial endpoints are frequently used to assess functional processes of ecosystems. Soil microorganisms are crucial to carbon and nutrient cycling (Nannipieri et al., 2002) and on decontamination processes. Indices related to microbial diversity, biomass and activity can provide important information about the functional impairment or improvement in the soil (Giller et al., 1998; Nogueira et al., 2006). These functional attributes make microbial parameters suitable for assessing risk associated with impacted soils (Moreno et al., 2009; Jiang et al., 2010). Organic matter decomposition integrates several processes occurring in soil and, as such, the rate of organic matter decomposition can be used to indicate negative effects on both the soil microbial community and/or soil fauna. Although some studies reported a low sensitivity of this parameter when assessing risks of contaminants, showing either none or transient effects (Dinter et al., 2008; Van Gestel et al., 2009), studies involving mainly metal contaminated sites have shown significant effects (Creamer et al., 2008).

The aim of this study was to investigate the ecological status of a former smelting area with a long-term history of metal contamination located at Santo Amaro (Bahia, Brazil). Moreover, by comparing the sensitivities of the different parameters used to assess metal contamination, we propose to identify the most appropriate methods for the different metal contamination scenarios. We hypothesized that each ecological endpoint responds to a metal contamination gradient, indicating a decrease in ecological status in the highly contaminated sites as compared to the reference sites.

2. Materials and methods

2.1. Study area

The study was carried out on land with an abandoned lead smelter that operated between 1960 and 1993, in the neighborhood of Santo Amaro city, BA, Brazil (12° 32′ 49″ S, 38° 42′ 43″ W) (Fig. 1). Tailings and airborne dust containing metals were spread in the region, leading to soil contamination (Costa, 2001; Carvalho et al., 2003). Despite an attempt in 1995 to encapsulate the residues with soil rich in organic matter, signs of habitat degradation due to contamination were still visible in 2011. A detailed description of the study area is given in Niemeyer et al. (2010).

Based on the total metal (Pb, Cd, Cu, Zn) concentrations in soil derived a priori from six radial transects (unpublished data), two 1-km transects (T1 and T3) were selected to represent two major gradients of contamination which samples of soil were collected. The transects shared a central starting point (P0—located next to the smelter) and sampling points were established on each transect at 20, 50, 150, 400, and 1000 m (P20T1–P1000T1 and P20T3–P1000T3; see Fig. 1). Three reference sites were selected at 3 km (Ref. 2 and 3) and 9 km (Ref. 1) away from P0 (Fig. 1). The soils at these three reference sites had properties similar to those of the soils along the transects but they were without metal contamination. Points P400T1, P1000T1, P1000T3, and the three reference sites were located outside the smelter area boundaries. Additional details concerning sampling methodology and selection of the reference sites are given in Niemeyer et al. (2010).

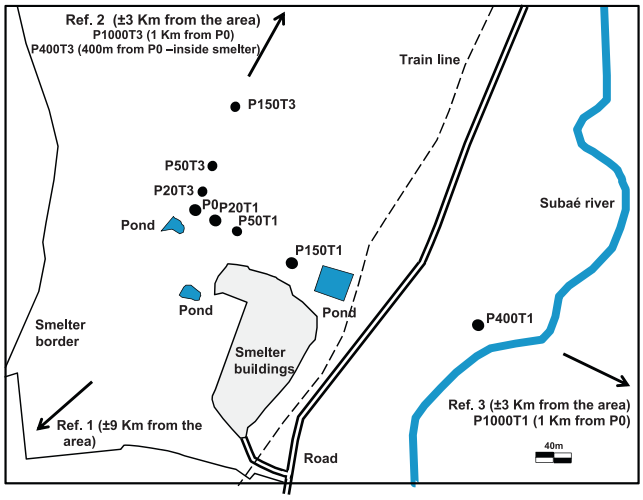

Fig. 1. Schematic representation of the study area (an abandoned lead smelter, Santo Amaro, BA, Brazil) showing the location of 11 sampling sites along two transects and three reference sites. (Extracted from Niemeyer et al. (2010)).

All surveys were carried out in July 2008, with the exception of the litter bag test which was conducted between October 2009 and February 2010.

2.2. Vegetation cover and successional stage

Vegetation cover assessment was carried out according to Veiga and Wildner (1993). Further details are given in Niemeyer et al. (2012). Simultaneously, an inventory of the plant species was made within a 10 m radius considering the center of each sampling point; all species present within the area were identified. Classification of successional plant community stage followed the Brazilian criteria established by the Environmental National Council (Brazil, 1994; see Supplementary Material).

2.3. Surface dwelling invertebrates

Surface dwelling invertebrates were sampled using pitfall traps (plastic cups, 8 cm diameter, 11 cm depth) containing 50 percent ethanol and some drops of neutral detergent. Three traps were set up at each sampling point; they were placed 5 m apart in a triangular configuration for a one week period in July 2008. The collection was carried out at the 7th day. Specimens were preserved in 70 percent ethanol until identification at Order and morphospecies level, with the support of specialized bibliography (e.g., Triplehorn and Jonnson, 2011).

The total number of individuals of each morphospecies at each location was obtained by pooling the results from the three traps. Number of species, abundance, species richness (Margalef index; Margalef, 1957), species diversity (Shannon index; Shannon, 1948), evenness (Pielou index; Pielou, 1966) and dominance (Berger–Parker index; Magurran, 1988) were calculated.

2.4. Soil fauna feeding activity (bait lamina test—BLT)

The BLT (developed by Von Törne (1990)) was prepared using a 1:5:14 ratio of finely ground oat (0.106 mm sieved), activated charcoal powder (Panreac, ref. 211237.0914) and cellulose powder (Fluka, ref. 22197), respectively. Five groups (samples) of five bait-lamina strips were exposed in each sampling point for 14 days, in July 2008. Bait strips were inserted vertically into the soil; each group occupied an area of 15 cm × 15 cm. The soil moisture content at each location was determined according to ISO (1993). After the exposure period, the bait lamina strips were removed from the soil and taken to the laboratory. After carefully dislodging soil adhering to the strips with tap water, each bait lamina strip was assessed visually by holding the strips against a light and counting the number of pierced (i.e., eaten) holes. No distinction was made between partially or fully pierced holes. The feeding activity per sample (group of five strips) at each sampling point was expressed in percentage of pierced holes.

2.5. Microbial parameters

Soil samples assessed for microbial parameters were collected from three parallel transects (2 m apart, and 10 m long), defined at each sampling point. Along each transect, 15 subsamples of soil (0–10 cm depth) were collected and

pooled for form a composite sample that was sieved (< 5 mm), stored at 4 °C and processed within 72 h.

Basal respiration was determined according to Alef (1995) every two days, for 8 days, using 1 M NaOH as CO_2 trap. Microbial biomass carbon (MBC) was estimated by fumigation-extraction method and C determination in the extracts according to Anderson and Ingram (1993), using a k_C=0.33, used to correct the extraction effectiveness of the soluble C (Vance et al., 1987). Dehydrogenase activity (DHA), was assessed in field-moist samples incubated with 1.5 percent triphenyl tetrazolium chlorine (TTC) for 24 h at 37 °C (Casida et al., 1964). Acid phosphatase activity was determined using 0.05 M sodium p-nitrophenyl phosphate as substrate (Tabatabai and Bremner, 1969). For nitrification rate, samples either received 125 μg g^{-1} of NH_4^+–N as ammonium sulfate or had no N added and were incubated at 28 °C for 21 days in the dark. After determinations of NO_3^-–N and NH_4^+–N concentrations before and after incubation (Keeney and Nelson, 1982), the nitrification rate was calculated following procedures described by Schuster and Schroder (1990).

2.6. Litter decomposition

Litter bags were used to measure litter decomposition. Nylon bags (30 cm × 20 cm) with a relatively large mesh size (1.0 cm × 0.2 cm) were used to allow activity both by macro- and microorganisms (Cortez, 1998). Leaves of *Schinus terebinthifolius* Raddi (Anacardiaceae), a native tree species, were collected in a non-contaminated area and used as substrate in the litter bags (4 g dry wt. in each bag). This species is commonly found at the site and is palatable to the soil macrofauna (Podgaiski and Rodrigues, 2010). Litter bags were placed onto the soil surface. At each sampling point, four small areas of approximately 1 m^2 (4 m apart on a quadrangular shape) were defined and four bags were placed in each area (total of sixteen bags per sampling site). One litter bag of each small area was selected randomly for collection at each point in time (15, 43, 83 and 131 days) and processed immediately. Any visible plant material from other species, organisms, or soil were removed manually. The material was dried at 60 °C and its weight was recorded. Afterwards, the ash-free dry weight (AFDW) was calculated by subtracting the mass of the residue ignited at 600 °C for 1 h. Litter mass loss was calculated by subtracting the AFDW of the remaining litter from the AFDW of the initial input, and using soil and litter correction factor according to EPFES protocol (Römbke et al., 2003). Results were expressed as percent of mass lost. The monthly decay rate constant was calculated by using the single negative exponential decay model $m_t/m_0=e^{-kt}$, where m_t/m_0 is the proportion of mass remaining at time t, and t is the time elapsed in days (months), and k is the derived daily (monthly) decay constant. Further details are given in OECD (2006) and Römbke et al. (2003).

2.7. Data analysis

The Widianarko's pollution index (WPI) was calculated for each sampling point (Widianarko et al., 2000); the index pooled the information from all metals. Initially developed for sediment samples, the W index can be used to synthesize the metal loading of any environmental sample in comparison to metal background levels, including soils (Timmermans et al., 2007; Janssens et al., 2008). The geometric mean of each metal concentration in the three reference sites was used as background level for each metal. This index can take negative values where metal loadings are below the background levels, while positive values increase with the metal loading above the background levels.

For BLT, arthropod abundance and richness, vegetation cover, soil microbial respiration, MBC, DHA, phosphatase activity and nitrification rate, differences between sampling points were assessed by applying one-way ANOVA (analysis of variance) procedures to the data followed by Dunnett's test to compare with the overall reference value (the average of the reference sites). Soil moisture and organic matter contents were used as covariables in the ANOVA for basal respiration and phosphatase activity. For MBC, DHA and nitrification rate, mineral N was also included as a covariable. Differences in abundance and morphospecies richness of surface dwelling arthropods from sites inside vs. sites outside the smelter area were assessed using a t-test for independent samples. Additionally, the ecological parameters assumptions of normality and homogeneity of variances were assessed with Kolmogorov–Smirnov and Bartlett's tests when ANOVA procedures were applied and F-ratio (ratio between the two variances) when t-test procedures were applied, respectively. Transformations were applied whenever necessary and according to the type of data. Differences between decomposition rates were tested with ANCOVA (analysis of covariance). Additionally, the relationship between the ecological parameters and the WPI was examined using correlation analyses to determine which parameters best responded to the contamination gradient. For microbial parameters, partial correlations were run using soil moisture, organic matter and mineral nitrogen contents as covariables, as a way to exclude the possible influence of these covariables in the analysis, evaluating only the effects of metals. Analyses were carried out using the Statistica 7.0 software (Statsoft, Tulsa, OK, USA).

Principal component analysis (PCA) was used to display similarities in vegetation composition, surface dwelling arthropod community, and microbial parameters among sites. Soil moisture, organic matter and mineral nitrogen contents were used as covariables in the microbial PCA, using Canoco for Windows® v.4 (Ter Braak and Smilauer, 1998). To check whether the differences for microbial parameters, surface dwelling arthropods and vegetation were different between sites inside and outside the smelter area, an Analysis of Similarity (ANOSIM) followed by a Similarity Percentage Analysis (SIMPER) were run for each matrix, based on a Bray–Curtis similarity matrix using the Primer v.5 software (Clarke and Gorley, 2001).

3. Results

3.1. Soil characterization and metal concentrations in soil

A full characterization of sites is given in Niemeyer et al. (2010). Soils from the study sites showed low (< 2 percent) to medium (2–6 percent) organic matter content, a cation exchange capacity (CEC) mostly between 30 and 40 meq/100 g, and pH ~7, with the exception of sites P1000T1 and Ref. 2, which soils are acidic and slightly acidic, respectively (Table 1).

For at least one metal (Pb, Cd, Cu, or Zn), sandy soils presented levels exceeding the Dutch $HC50_{cor}$ benchmarks (Table 2), indicating high ecological risk (Rutgers et al., 2008). P0 had a high Zn concentration exceeding the corresponding $HC50_{cor}$ value by a factor of three, whereas P150T1 and P50T3 had critical levels of Zn that exceeded the screening levels by 1.6 and 73.5 times, respectively. The corresponding WPI corroborate these data, with slightly positive (or even negative) values in the reference sites or sites outside the smelter areas, in contrast to highly positive values in most of the contaminated sites.

3.2. Vegetation cover and successional stage

The highest values of vegetation cover were found outside the smelter area (Ref. 1, Ref. 2, P400T3, P1000T3, P1000T1 and P400T1; 67.5–100 percent, average=86.0 percent, SD=16.1 percent), whereas the lowest values were found on sites close to the smelter (P0, P20T1, P50T1, P150T1, P20T3, and P50T3, 20–100 percent, average=43.4 percent, SD=26.9 percent) (Table 3). Vegetation cover correlated negatively with metal contamination (represented by Widinarko's index; $r=-0.59$, $p<0.05$). No survey was possible in Ref. 3 due to logistical constrains.

A total of 53 plant species were identified; the full list for each site is shown in supplementary material (Table S1). Sites P1000T3 and Ref. 2 were in a more advanced successional stage than the other sites, with arboreal cover predominating over herbaceous, with occurrence of climbing plants, litter and mean diameter at breast high (DBH) of 8–18 cm. The other sites showed secondary vegetation in an initial stage of succession (please see Supplementary Material 1 for details about stages of succession).

The first axis of the PCA diagram represents a clear gradient of contamination, showing a separation of the sites outside of the study area (including reference sites—right side of the vertical axis) from those located inside (left side of the vertical axis) of the study area; groupings showed a relatively high similarity in plant composition (Fig. 2). The dissimilarity between plant community composition in sites inside vs. outside the smelter area was 63.98 percent (SIMPER analysis) with both communities differing significantly from each other (ANOSIM—global $R=0.36$, $p<0.01$). The homogeneity of vegetation composition inside the smelter was much higher (77.9 percent similarity) than outside (20.1 percent similarity).

3.3. Surface dwelling invertebrates

A total of 1277 individuals, grouped into 72 morphospecies, were collected in the pitfall traps. Hymenoptera, Coleoptera and Orthoptera occurred at all sites, Araneae and Dermaptera at 92.3

Table 1
Physical–chemical characteristics of sampling points and reference soils.

Soil group	Coarse sand (percent)	Fine sand (percent)	Sand (total) (percent)	Silt (percent)	Clay (percent)	Texture (USDA)	CEC (meq 100g)	pH (KCl 1:5 v-v)	P (mg/kg)	Organic matter (percent)	Mineral N (mg/kg)	Water content (percent)[a]	WHC (%)
Ref. 1	2.3	8.5	10.9	42.1	47.0	Silty clay	34.16	7.1	72	1.1	42	19.5	53.8
Ref. 2	50.9	38.5	89.4	2.8	7.7	Loamy sand	37.60	4.9	1	1.0	42	13.2	27.5
Ref. 3	22.2	15.0	37.2	11.1	51.7	Clay	36.48	6.1	52	3.9	56	47.2	60.8
P0	43.2	31.3	74.5	11.9	13.6	Sandy loam	38.56	6.7	47	0.3	70	31.0	44.1
P20T1	48.0	13.8	61.8	19.0	19.3	Sandy loam	37.28	7.1	58	0.2	42	32.7	46.4
P20T3	11.4	30.0	41.4	22.3	36.3	Clay loam	42.16	6.8	106	1.9	42	35.0	67.7
P50T1	25.2	13.4	38.6	29.0	32.4	Clay loam	38.16	6.7	63	1.1	56	28.6	54.5
P50T3	69.2	9.1	78.3	10.4	11.3	Sandy loam	16.56	7.2	> 200	2.8	56	39.5	22.1
P150T1	56.2	21.1	77.4	12.3	10.3	Sandy loam	21.28	6.7	> 200	2.1	42	29.4	28.6
P150T3	8.4	15.2	23.5	21.4	55.1	Clay	49.20	6.8	16	2.5	42	40.7	61.8
P400T1	19.6	23.9	43.5	20.2	36.3	Clay loam	37.44	6.8	> 200	5.1	56	24.4	58.9
P400T3	6.5	8.6	15.1	52.4	32.5	Silt clay loam	35.84	7.1	1	1.9	70	45.5	56.7
P1000T1	2.5	21.8	24.3	19.9	55.8	Clay	43.20	3.7	35	2.0	56	28.7	60.0
P1000T3	10.3	19.5	29.8	29.8	40.4	Clay loam	42.72	7.0	> 200	5.7	42	n.d.	57.6

USDA—United States Department of Agriculture; CEC—Cation Exchange Capacity; WHC—Water Holding Capacity.

[a] Soil moisture in the samples used for microbial assessments.

Table 2
Total metal concentrations (mg/kg) and the pollution index for sampling points and reference soils.

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

Numbers in superscript indicate an exceedance 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).

percent, and Opiliones in 69 percent of the sites (Table 4). Isopoda (30.8 percent of sites), Diplopoda and Hemiptera (15.4 percent), and Mantodea (one site) were observed less frequently. Coleoptera and Araneae presented the highest morphospecies richness (twenty and nineteen, respectively), followed by Hymenoptera with sixteen morphospecies. Hymenoptera was the most abundant group in terms of individuals ($n=459$), followed by Coleoptera and Araneae ($n=265$ each).

Differences in abundance, morphospecies richness or biodiversity descriptors were only found when comparing sites inside and outside the smelter area boundaries. Sites outside the area presented more morphospecies than sites inside ($t=2.48$, $p<0.05$). However, no significant differences were detected for abundance or any other descriptor of biodiversity (Table 4). Analyzing each major faunal group individually, the aforementioned trend was found for abundance and richness of spiders ($t=2.51$, $p<0.05$ and $t=2.63$, $p<0.05$, respectively), and abundance of opilionids ($t=2.61$, $p<0.05$). In contrast, hymenopterans followed the inverse trend for number of individuals ($t=2.72$, $p<0.05$). No significant correlation was found between any of the invertebrate parameters and WPI.

The PCA showed marked dissimilarity between sites inside and outside the smelter area (Fig. 3), with sites outside (400 m or more beyond P0) located in the positive side of the vertical axis 1. The ANOSIM revealed significant differences between these two groups (Global $R=0.239$, $p<0.01$). This separation pattern was mainly attributed to 40 morphospecies (SIMPER analysis, average dissimilarity 74.6 percent), with the highest contributions (up to 50 percent) attributed to morphospecies of Hymenopeta (2), Coleoptera (3), Orthoptera (2), Dermaptera (1) and Opilionidae (1).

Table 3
Ecological parameters (average values ± standard deviation) for the sampling point soils and combined (overall) reference soils. The values for the three reference soils were geometrically averaged to give an overall reference value. Asterisks indicate significant differences for a one-tailed hypothesis of a Dunnet's test between each sampling point and the overall reference value (assuming that Ref. value higher than sampling point value and lower for Potential Nitrification). In the ANOVAs for soil microbial parameters, soil moisture, soil organic matter and soil nitrogen contents were used as co-variables (see text for more details). *n*—number of replicates.

Soil groups	Bait lamina (percent pierced holes) $n=5$	Vegetation cover (percent) $n=4$	Respiration (μg CO_2/g soil/day) $n=3$	MBC (μg/g) $n=3$	Dehydrogenase (μg PNP/g/d) $n=3$	Acid phosphatase (ug PNF/g/h) $n=3$	Nitrification (percent) $n=3$	Decomposition rate[a] k (monthly) $n=4$
Overall reference	48.6 ± 13.9	81.3 ± 21.0	139.4 ± 106.4	642.4 ± 416.1	7.2 ± 2.3	617.1 ± 233.2	3.9 ± 2.2	0.266 ± 0.144
P0	18.4 ± 14.3***	22.5 ± 22.2***	34.9 ± 7.8***	178.1 ± 55.1***	0.7 ± 0.4**	269.0 ± 22.1**	15.2 ± 9.6**	0.047***
P20T1	17.8 ± 10.2***	30.0 ± 16.3***	35.1 ± 7.1***	252.4 ± 142.3**	1.3 ± 1.9**	196.4 ± 33.9***	12.4 ± 2.1*	n.d.
P20T3	30.4 ± 15.4	32.5 ± 12.6***	82.6 ± 15.8	170.3 ± 174.1***	1.4 ± 1.0**	443.4 ± 9.3	13.3 ± 3.5*	n.d.
P50T1	19.8 ± 6.8***	57.5 ± 12.6	41.4 ± 2.4**	412.9 ± 31.4	1.2 ± 2.0**	235.7 ± 50.3**	17.1 ± 4.9***	0.063***
P50T3	11.8 ± 5.7***	20.0 ± 14.1***	52.2 ± 12.6**	461.7 ± 20.1	2.1 ± 0.5*	450.3 ± 45.4	13.5 ± 4.5*	0.041***
P150T1	7.3 ± 8.1***	30.0 ± 42.4***	49.2 ± 6.6**	115.5 ± 87.0***	3.3 ± 0.5	355.3 ± 166.0*	8.7 ± 2.8	0.044***
P150T3	5.5 ± 6.9***	57.5 ± 9.6	60.5 ± 9.2*	543.6 ± 160.8	2.1 ± 1.1*	651.2 ± 150.7	10.2 ± 0.5	0.025***
P400T1	61.5 ± 23.8	100.0 ± 0.0	234.9 ± 83.3	797.3 ± 193.3	16.8 ± 3.7	573.1 ± 133.3	−0.2 ± 5.9	0.166*
P400T3	10.3 ± 6.7***	97.5 ± 5.0	165.2 ± 41.3	805.3 ± 216.2	1.5 ± 1.1**	792.0 ± 34.5	−3.3 ± 3.17	0.042***
P1000T1	45.3 ± 16.1	67.5 ± 15.0	164.0 ± 79.1	1098.1 ± 184.1	4.8 ± 6.2	515.6 ± 353.5	1.9 ± 7.4	0.452
P1000T3	26.3 ± 17.5*	100.0 ± 0.0	n.d.	n.d.	n.d.	n.d.	n.d.	0.383

MBC—Microbial Biomass Carbon.
n.d.—not determined.

[a] After log of percentages of mass lost.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

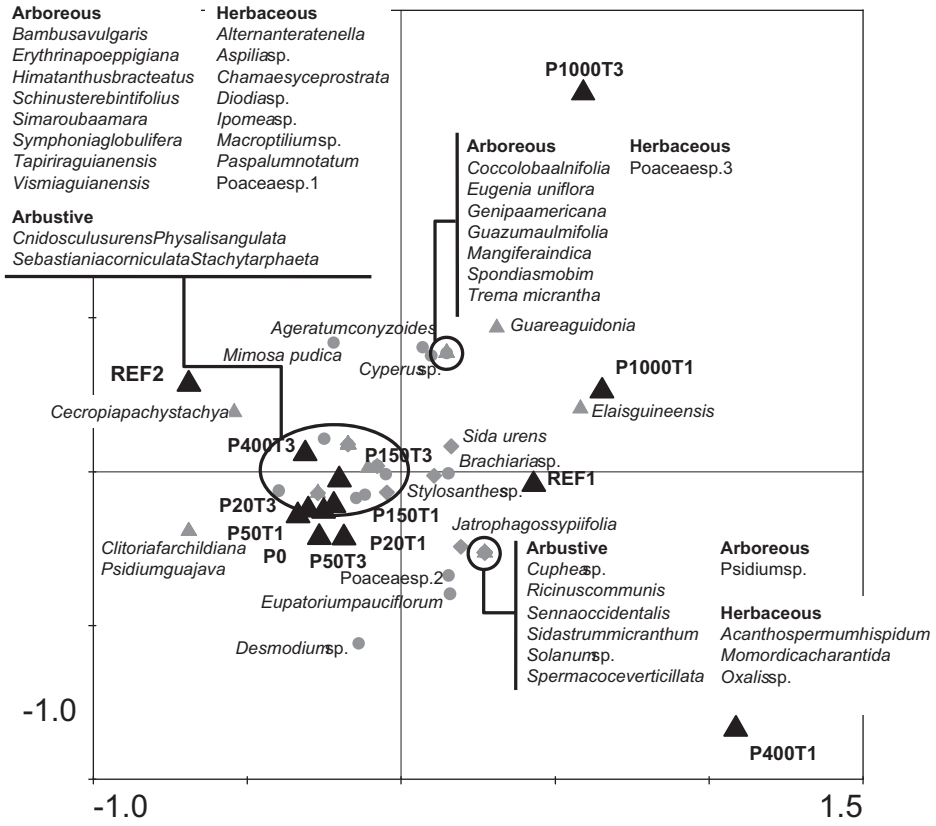

Fig. 2. Principal component analysis (PCA) diagram of plant community composition. Legend: grey upper triangles—arboreous species; grey diamonds—shrub species; grey circles—herbaceous species; black upper triangles—sampling points (no survey was possible in Ref. 3 due to logistical constrains). Variance explained: Axis 1–24.4 percent; Axis 2–19.4 percent. Circles grouping show the main vegetation composition.

3.4. Soil fauna feeding activity (bait lamina test)

Lower feeding activity as reflected by the BLT was observed at sites within the smelter area usually associated with contamination (P0, P150T1, P50T3 and P150T3) or low organic matter content (P20T1 and P50T1) (Table 3). Point P400T3 also presented low feeding activity, however other soil or habitat parameters (not assessed) must explain these results. A significant negative

Table 4
Number of individuals and morphospecies (shown in brackets) of each Order of surface dwelling invertebrates caught in pitfall traps ($n=3$) at each site, and the biodiversity descriptors for each site, considering all groups together.

	Ref. 1	Ref. 2	Sites											Sites inside smelter	Sites outside smelter
			P0	P20T1	P20T3	P50T1	P50T3	P150T1	P150T3	P400T1	P400T3	P1000T1	P1000T3		
Orders															
Araneae (Ar)	73 (5)	9 (4)	4 (3)	2 (2)	7 (4)	2 (1)	3 (1)	5 (4)		14 (4)	5 (5)	2 (2)	139 (7)	23 (8)	242 (16)
Hymenoptera (Hy)	15 (5)	15 (5)	31 (3)	26 (3)	48 (4)	76 (3)	57 (5)	33 (2)	52 (4)	15 (3)	16 (6)	45 (5)	30 (3)	323 (10)	136 (14)
Coleoptera (Co)	11 (4)	13 (6)	8 (4)	2 (2)	28 (7)	16 (5)	32 (7)	2 (1)	14 (5)	88 (4)	13 (3)	7 (4)	31 (4)	102 (17)	163 (11)
Orthoptera (Ort)	14 (3)	10 (3)	16 (4)	8 (3)	21 (3)	13 (2)	21 (6)	4 (2)	9 (2)	10 (2)	16 (2)	24 (2)	23 (4)	92 (7)	97 (5)
Isopoda (Iso)			1 (1)							5 (2)	11 (1)	1 (1)		1 (1)	17 (3)
Opiliones (Opil)		4 (2)		1 (1)	2 (2)	2 (1)			1 (1)	6 (1)	7 (3)	10 (1)	2 (2)	6 (3)	29 (3)
Dermaptera (Derm)	3 (1)	2 (1)		1 (1)	9 (1)	4 (1)	4 (1)	1 (1)	6 (1)	2 (1)	5 (1)	2 (1)	1 (1)	25 (1)	15 (1)
Hemiptera (Hem)		1 (1)								1 (1)					2 (1)
Diplopoda (Dipl)				1 (1)								2 (1)		1 (1)	2 (1)
Mantodea (Mant)		1 (1)													1 (1)
Biodiversity descriptors															
ABUNDANCE (total)	116	55	60	41	115	113	117	45	82	141	73	93	226		
TAXA (total)	18	23	15	13	21	13	20	10	13	18	21	17	21		
SHANNON	2.59	4.06	2.83	2.85	3.67	2.48	3.38	1.86	2.32	3.05	3.71	3.33	2.24		
PIELOU	0.62	0.90	0.72	0.77	0.84	0.67	0.78	0.56	0.63	0.73	0.85	0.81	0.51		
MARGALEF	3.58	5.49	3.42	3.23	4.22	2.54	3.99	2.36	2.72	3.44	4.66	3.53	3.69		
BERGER–PARKER	0.58	0.18	0.45	0.37	0.22	0.52	0.33	0.69	0.56	0.40	0.15	0.19	0.58		

SHANNON—diversity index.
PIELOU—eveness index.
MARGALEF—richness index.
BERGER—PARKER—dominance index.

correlation ($r=-0.72$, $p<0.01$) was found between feeding activity and metal loading, given by WPI.

3.5. Microbial parameters

Microbial parameters generally decreased inside the smelter area in relation to the overall reference, except for nitrification, which showed inverse trend (Table 3).

Most of parameters correlated negatively with the metal loadings (WPI), like microbial respiration ($r=-0.70$, $p<0.001$), microbial biomass carbon ($r=-0.64$, $p<0.001$), phosphatase activity ($r=-0.47$, $p<0.05$), while nitrification correlated positively ($r=0.43$, $p<0.05$), and no correlation was found for DHA.

The *p*PCA showed a clear separation of the sites outside the smelter area, except P400T1 (Fig. 4). Such separation was confirmed by an ANOSIM (Global $R=0.091$, $p<0.05$) and by a SIMPER analysis, where microbial respiration, microbial biomass carbon and phosphatase activity contributed to over 90 percent of the dissimilarity between both groups.

3.6. Organic material breakdown

The validity criterion of 60 percent of mass loss in the reference sites at the end of the study (Römbke et al., 2003) was fulfilled. The monthly decay rate in contaminated sites within the smelter area was lower than in the overall reference sites (Table 3). Only sites located 1000 m away from P0 presented higher decay rates than the reference sites. Considering the threshold value proposed by Römbke et al. (2003) of >25 percent difference in mass loss between contaminated and the reference sites at the end of the study, all sites within the smelter area showed an unacceptable risk, where differences compared to the overall reference ranged between 30.5 percent and 64.1 percent after 131 days of exposure. A significant and negative correlation was found between the monthly decay rate and WPI ($r=-0.61$, $p<0.05$).

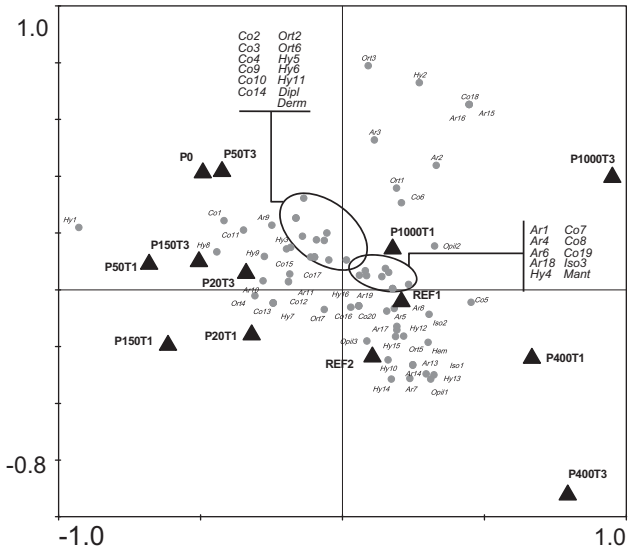

Fig. 3. Principal component analysis (PCA) diagram on surface dwelling arthropods. Legend: grey circles—morphospecies; black upper triangles—centroids of the sampling points (no survey was possible in Ref. 3 due to logistic constrains); Ar—Araneae; Co—Coleoptera; Derm—Dermaptera; Dipl—Diplopoda; Hem—Hemiptera; Hy—Hymenoptera; Iso—Isopoda; Mant—Mantodea; Opil—Opiliones; Ort—Orthoptera.Variance explained: Axis 1–17.7 percent; Axis 2–13.6 percent.

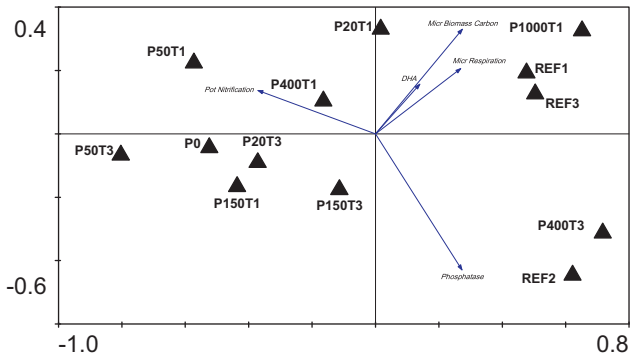

Fig. 4. Partial Principal component analysis (pPCA) ordination of the microbial parameters. Black upper triangles represent centroids of the sampling points (no survey was possible in P1000T3 due to logistical constrains). Pot Nitrification—Potential Nitrification; Micr Biomass Carbon—Microbial Biomass Carbon; Micr Respiration—Soil Basal Respiration; DHA—Dehydrogenase Activity; Phosphatase—Acid Phosphatase Activity. Variance explained: Axis 1–60.1 percent; Axis 2–16.2 percent.

4. Discussion

4.1. Impairment of ecological parameters

The smelting activity resulted in metal contamination of the soils in the study area, which showed moderate to high levels of some metals (namely Pb and Zn) relatively to reference sites mainly due to deposition of tailings and contaminated dust. No clear contamination gradient could be found along the two transects. This spatial heterogeneity of contamination is caused by unequal deposition of tailings and by erosion on the (pseudo) rehabilitated tailings, leaving the pile partially exposed (details are shown in Anjos (2003)).

Metal contamination, physical conditions, and the unsuccessful establishment of vegetation resulted in a loss of suitable habitat, affecting the different ecological parameters assessed. The general trend was towards compromised ecological processes inside the smelter area in comparison with sites outside.

The vegetation cover inside the smelter area decreased and was more homogeneous, with a high frequency of arboreal and invasive herbaceous species in an initial successional stage. This was attributed to metal toxicity, even 17 years after the smelting activity stopped, in addition to the failure in rehabilitating the area. Van Assche and Clijsters (1990) also found potential phytotoxicity in the soil surrounding a zinc smelter more than 20 years after the smelter closure. In addition, nutrient imbalances and a reduced water-holding capacity restrict the plant recolonization on degraded sites (Salemaa et al., 2001; Tordoff et al., 2000); such conditions were observed in the sites where soil encapsulation was not effective. A diverse and structured plant community helps to maintain essential functions in soil like nutrient cycling processes, and providing food and habitat to a highly active and diverse community of decomposing organisms (Jensen and Mesman, 2006; Balvanera et al., 2006). However, contamination mostly impaired plant community and habitat conditions inside the smelter area, affecting other ecological parameters, namely the soil fauna feeding activity and surface dwelling invertebrates. In fact, data from the BLT showed a strong impairment of feeding activity in sites within the smelter area associated with high contaminant levels or to low organic matter content, in addition to a low vegetation cover. Similar results have also been shown in grasslands with a gradient of metal contamination (Filzek et al., 2004) and at an abandoned uranium mine site in Portugal (André et al., 2009), where the abundance and diversity of key detritivore groups were reduced. In fact, the feeding activity measured via BLT seems to be related to the relative abundance of different faunal groups, namely earthworms (Van Gestel et al., 2003) and collembolans (Helling et al., 1998; Birkhofer et al., 2011). Although not assessed in this study, an impact on such organisms would also be expected due to the high metal contamination and the loss of habitat function (Fountain and Hopkin, 2004).

However, a significant decrease in the total morphospecies richness of soil arthropods inside the smelter area and changes in the community composition of surface-dwelling invertebrates corroborated the BLT evidence. Generally, there were more (abundance and richness) morphospecies of spiders and opilionids outside of the study site and a greater abundance of hymenopterans within the impacted areas. Ferreira (2010) reported a decrease in the abundance of spiders as metals in soil increased in concentration at a copper mine site. Different guilds responded differently to contamination and the ground-hunting organisms were the most affected. Read et al. (1998) studied the epigeic macroarthropods along a metal contamination gradient and reported that few species were able to adapt to the contamination and that a larger number of species was found at non-contaminated sites. The decrease in abundance (241 vs. 23 individuals) and species richness (16 vs. 8) inside the smelter area can be attributed to both direct and indirect effects. Depletion of preys for specialist species, and the impoverishment of the habitat structure, may impair the trophic requirements for many species.

The inverse trend observed for abundance of ants is in agreement with Grzes (2009), who found an increase in species richness along a metal contamination gradient. An explanation could be that ants have the ability to regulate and resist in metal contaminated sites (Grzes, 2009, 2010). Furthermore, indirect effects on ant population may have occurred, since ants may have benefited from the decrease in abundance or richness of spiders and coleopterans, as these groups are known to be predators or competitors of ants. In addition, metal pollution affected the habitat structure, creating patches of low vegetation cover, resulting in increase in soil temperature and decrease in moisture content, which may have favored thermophilic species that may exist in the area (Grzes, 2009).

Metal contamination highly impaired the microbial community and processes, as all parameters inside the smelter area significantly differed from those from the reference sites. Moreover, significant negative correlations with metal loadings were found. Kapustka (1999) advised against the inclusion of microbial parameters for ecological assessments due to their strong functional redundancy, rapid change across a small spatial scale, and high influence of confounding factors (e.g., moisture, nutrients). However, several other authors have reported a decrease in enzyme activity, microbial biomass carbon and basal respiration in impacted soils (Gülser and Erdogan, 2008; Zimakowska-Gnoinska et al., 2000; Jiang et al., 2010), pointing out the usefulness of microbial endpoints for assessing metal effects on ecological processes in contaminated areas. Soil microbial communities and the key processes they mediate are closely related to vegetation and land use (Fagotti et al., 2012; Nogueira et al., 2006; Zak et al., 2003). Vegetation contribute to reduce metal toxicity by offering favorable conditions in the rhizosphere (Dias-Junior et al., 1998) and in the bulk soil due to inputs of organic residues that act as carbon and energy sources, in addition to a protective effect against metals by chelation. The failure in vegetation to establish in soils inside the smelter area, especially in sites the failed attempt to encapsulate the tailings was visible, contributed to the decrease of microbial activity and biomass in these soils.

Nitrification was the only microbial parameter that increased significantly within the most contaminated soils inside the smelter area. Although considered sensitive to metal stress (Broos et al., 2005), adaptation of nitrifying populations to metal-contaminated

sites has been shown (Mertens et al., 2006). High nitrification rates may indicate an imbalance in the N-cycling, leading to N losses by leaching or denitrification. The nitrification rate tends to decrease along the successional stages in a forest (Singh et al., 2001), and thus recently disturbed ecological systems show higher nitrification rates, which decrease along the advance of the successional status (Montagnini et al., 1989).

Decomposition of litter was significantly impaired within the smelter area, and the decay rate was negatively correlated with metal loadings. Although transient or no effects have been observed in response to some environmental stressors (e.g., Dinter et al., 2008; Van Gestel et al., 2009; Podgaiski and Rodrigues, 2010), impairment on litter decomposition due to metal (e.g., Creamer et al., 2008) or pesticide contamination (Förster et al., 2006) have been reported.

The litter bag study showed low decay rates in soils at the most contaminated sites. The reduced microbial activity, faunal feeding activity and density of detritivores, allied to a low moisture and high temperature in the more exposed sites due to the low vegetation cover, may have contributed to reduce the litter decomposition in these sites. Thus, the effects on litter decomposition were attributed not only to a direct effect of metals on microbial and faunal communities, but also to indirect effects resulting in sub-optimal conditions for soil fauna and microbial communities as mentioned earlier.

4.2. Sensitivity of ecological parameters for risk assessment

The sensitivity analysis took into account not only the ability of each parameter to detect differences between contaminated and non-contaminated sites, but also the ability to detect a gradient of contamination and time required to measure or assess the parameter (Table 5).

All microbial parameters were able to differentiate the contaminated from the reference sites. With the exception of DHA, the microbial parameters provided similar information and there were significant correlations among the parameters. However, only basal respiration and microbial biomass carbon had the capacity to distinguish the level of soil contamination. Since these two parameters were highly correlated ($r=0.82$, $p<0.001$), measuring either one is sufficient for assessing microbial activity.

Conversely to microbial parameters, soil fauna structural parameters were not able to detect contamination gradients when considering each site individually. However, abundance and taxonomic richness were able to differentiate the sites inside and outside the smelter area. These results were expected because we sampled highly mobile surface dwelling organisms which are influenced more by the features of the area around the site than by the properties of the site itself. Semenzin et al. (2008) found that soil dwelling invertebrate abundance, taxonomic richness and the QBS index (a measure of soil quality based on microarthropods morphotypes) were sensitive parameters for assessing effects of soil contamination with metals and PAHs. Similar to our findings, diversity indices were not sensitive to contamination. More elaborated conclusions could be taken, namely in terms of effects to particular functional groups and to find better cause–effect relationships, if identification would be done to the species level. However, the separation into morphospecies seems to be sensitive enough for a first evaluation of contamination or habitat disruption, being able to detect changes in community composition between contaminated and non-contaminated sites. Therefore, even if taxonomic expertise is

Table 5
Summary of the sensitivity of each ecological parameter assessed at the smelter area. Light grey—parameters derived from multivariate analysis based on individual parameters from the corresponding organism group; Dark grey—parameters selected based on sensitivity criteria (see text for details).

Organism group	Category	Parameter	Significant response in contaminated sites (1)	Ability to differentiate the level of contamination (2)	Days needed to obtain the parameter (estimated number of working days)	Use in ERA (Tier)
Microrganisms	Community activity	Microbial respiration	Low to High	High	8 (4)	1-2
	Community structure	Microbial biomass C	Low to High	High	2 (2)	1-2
	Community activity	Dehydrogenase activity	Low to Medium	No	1 (1)	1-2
	Community activity	Acid phosphatase activity	Low to High	Medium	1 (1)	1-2
	Biological process	Nitrification rate	Low to High	Medium	21 (3)	1-2
		Multivariate analysis with all microbial parameters	Medium			1-2
Invertebrates	Community activity	Feeding activity (bait lamina)	High	Medium	14 (4)	1
	Community structure	Abundance	Group dependent; low	No	37 (30)	2
	Community structure	(Morpho) Species richness (3)	Group dependent; low	No		2
	Community structure	Shannon diversity index (3)	No differences	No		2
	Community structure	Pielou evenness index (3)	No differences	No		2
	Community structure	Margalef richness index (3)	No differences	No		2
	Community structure	Berger-Parker index (3)	No differences	No		2
	Community structure	Changes in species composition (3)	High			2
Plants	Community structure	% of vegetation cover	High	Low	1 (1)	1
	Community structure	Species richness (3)	No differences	No	2 (2)	1-2
	Community structure	Changes in species composition (3)	High			2
Microrganisms and invertebrates	Ecosystem function	Litter breakdown (decay rate)	High	Low	140 (25)	2

[a] For individual parameters, information based on observed significant differences against reference points (ANOVA); for soil fauna abundance and taxonomic richness and for integrated multivariate analysis (ANOSIM), based on significant differences between sampling points outside the area (no or low contamination) from points inside the area (high contamination); high ($p<0.001$); medium ($p<0.01$); low ($p<0.05$).
[b] Information based on significant correlations with metal loadings (Widianarko index): high ($p<0.001$); medium ($p<0.01$); low ($p<0.05$).
[c] Parameters that require specific taxonomic knowledge.

lacking in similar studies, this level of identification should be tried and incorporated in the ecological line of evidence when soil fauna is an ecological receptor under potential risk.

Contrary to the structural parameters of fauna, the feeding activity as reflected by the BLT was sensitive to soil metal contamination. This relative sensitivity agreed with the results of several studies that showed a relationship between BLT and abundance of microarthropods and lumbricids (Birkhofer et al., 2011). Its sensitivity, allied to the possibility to have data from a large number of sampling points over a short period of time, makes the BLT a highly suitable parameter to be included in the Ecological LoE in site specific assessments (particularly in Tier 1).

Despite differences observed for vegetation cover and composition between sites inside and outside of the smelter area, these parameters were not able to detect gradients of contamination. Critto et al. (2007) presented a low rank for vegetation related parameters in Tier 1, mainly due to their cost. However, for higher tiers (2 or 3) such parameters were considered important because of their site-specific relevance. In this study, we considered vegetative parameters important because they reflected direct effects (e.g., habitat disruption) of contamination. Therefore, both assessed parameters (vegetation cover and species composition) should be incorporated into the Ecological LoE. Plant litter decomposition was highly sensitive to metal contamination and habitat disruption but had a low capacity to differentiate the levels of contamination. Despite being sensitive, it provided information similar to that of the BLT, thus we concluded that the litter bag decomposition study was not a priority parameter to measure as part of a tiered scheme. The BLT is faster and easier to implement than the litter bag test.

5. Conclusion

In general, the ecological parameters indicated a clear distinction between sites inside and outside of the smelter area, indicating an ecological risk to soil ecosystems even 17 years after cessation of smelting activities, associated with exposure of ecological receptors to site soils. Metal-rich tailings within the area and the failed attempt to encapsulate them have impaired the establishment of vegetation, leading to a simplification of the habitat structure. The organic matter input into the soil, which acts as a source of carbon and energy for microbial growth, failed to protect the microbial community. Moreover, these changes in habitat structure negatively impacted microbial activity and soil animals (feeding activity and species composition of surface dwelling invertebrates), consequently affecting the ecosystem processes that they mediate.

The encapsulation of the smelter residues and concomitant re-establishment of a vegetation cover is essential for improvement of the conditions at this site to prevent further loss of soil and to improve soils quality.

Acknowledgments

The study was supported by two Ph.D. grants to Júlia C. Niemeyer, from Program Alban (E05D058118BR, from Fev/06 to Fev/07) and Fundação para a Ciência e a Tecnologia (FCT, Portugal; SFRH/BD/28796/2006). E.M. da Silva and M.A. Nogueira are scholars from the Brazilian Research Council (CNPq). The authors would like to thank Alexandre Wegner for his help with the field work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2012.09.013.

References

Alef, K., 1995. Soil respiration. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, UK, pp. 214–219.
Anderson, J.M., Ingram, J.S.I., 1993. Tropical Soil Biology and Fertility: A Handbook of Methods. CAB international, Wallingford, Connecticut 171 pp.
André, A., Antunes, S.C., Gonçalves, F., Pereira, R., 2009. Bait-lamina assay as a tool to assess the effects of metal contamination in the feeding activity of soil invertebrates within a uranium mine area. Environ. Pollut. 157, 2368–2377.
Anjos, J.S.A., 2003. Avaliação da eficiência de uma zona alagadiça (wetland) no controle da poluição por metais pesados: o caso da Plumbum em Santo Amaro da Purificação/BA. Thesis, University of São Paulo, São Paulo, Brazil.
Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.S., Nakashizuka, T., Raffaelli, D., Schmid, B., 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. 9, 1146–1156.
Birkhofer, K., Diekötter, T., Boch, S., Fischer, M., Müller, J., Socher, S., Wolters, V., 2011. Soil fauna feeding activity in temperate grassland soils increases with legume and grass species richness. Soil Biol. Biochem. 43, 2200–2207.
Brazil,1994. Biomas - Estágios sucessionais da vegetação da Mata Atlântica. Resolução CONAMA n° 5, de 18 de Março de 1994. Conselho Nacional do Meio Ambiente, Brasil.
Broos, K., Mertens, J., Smolders, E., 2005. Toxicity of heavy metals in soil assessed with various soil microbial and plant growth assays: a comparative study. Environ. Toxicol. Chem. 24, 634–640.
Carvalho, F.M., Neto, A.M.S., Tavares, T.M., Costa, A.C.A., Chaves, C.R., Nascimento, L.D., Reis, M.A., 2003. Chumbo no sangue de crianças e passivo ambiental de uma fundição de chumbo no Brasil. Pan Am. J. Public Health 13, 19–23.
Casida Jr., L.E., Klein, D.A., Santoro, T., 1964. Soil dehydrogenase activity. Soil Sci. 98, 371–376.
Clarke, K.R., Gorley, R.N., 2001. PRIMER v5: User Manual/Tutorial. Primer-E, Plymouth, UK, 91 pp.
Cortet, J., Vauflery, A.G.D., Poinsot-Balaguer, N., Gomot, L., Texier, C., Cluzeau, D., 1999. The use of invertebrate soil fauna in monitoring pollutant effects. Eur. J. Soil Biol. 35, 115–134.
Cortez, J., 1998. Field decomposition of leaf litters: relationships between decomposition rates and soil moisture, soil temperature and earthworm activity. Soil Biol. Biochem. 30, 783–793.
Costa, A.C.A., 2001. Avaliação de alguns efeitos do passivo ambiental de uma metalurgia de chumbo em Santo Amaro da Purificação, Bahia. MSc. Thesis, Federal University of Bahia, Salvador, Bahia, Brazil.
Creamer, R.E., Rimmer, D.L., Black, H.I.J., 2008. Do elevated soil concentrations of metals affect the diversity and activity of soil invertebrates in the long-term? Soil Use Manage. 24, 37–46.
Critto, A., Torresan, S., Semenzin, E., Giove, S., Mesman, M., Schouten, A.J., Rutgers, M., Marcomini, A., 2007. Development of a site-specific ecological risk assessment for contaminated sites: Part I. A multi-criteria based system for the selection of ecotoxicological tests and ecological observations. Sci. Total Environ. 379, 16–33.
Dias-Junior, H.E., Moreira, F.M.S., Siqueira, J.O., Silva, R., 1998. Metais pesados, densidade e atividade microbiana em solo contaminado por rejeitos de indústria de zinco. Rev. Bras. Ciênc. Solo 22, 631–640.
Dinter, A., Coulson, M., Heimbach, F., Keppler, J., Krieg, W., Kölzer, U., 2008. Technical experiences made with the litter bag test as required for the risk assessment of plant protection products in soil. J. Soils Sediments 8, 333–339.
Fagotti, D.S.L., Miyauchi, M.Y.H., Oliveira, A.G., Santinoni, I.A., Eberhardt, D.N., Nimtz, A., Ribeiro, R.A., Paula, A.M., Queiroz, C.A.S., Andrade, G., Zangaro, W., Nogueira, M.A., 2012. Gradients in N-cycling attributes along forestry and agricultural land-use systems are indicative of soil capacity for N supply. Soil Use Manage., online first.
Ferreira, J.G., 2010. Impact of the Mine Pollution on the Abundance and Community Structure of Ground-Dwelling Spiders (Araneae): Potential Use as Bioindicators. Dissertation. University of Lisbon, Lisbon, Portugal.
Filzek, P.D.B., Spurgeon, D.J., Broll, G., Svendsen, C., Hankard, P.K., Parekh, N., Stubberud, H.E., Weeks, J.M., 2004. Metal effects on soil invertebrate feeding: measurements using the bait lamina method. Ecotoxicology 13, 807–816.
Förster, B., Garcia, M., Francimari, O., Römbke, J., 2006. Effects of carbendazim and lambda-cyhalothrin on soil invertebrates and leaf litter decomposition in semi-field and field tests under tropical conditions (Amazônia, Brazil). Eur. J. Soil Biol. 42, 171–17**9**.
Fountain, M.T., Hopkin, S.P., 2004. A comparative study of the effects of metal contamination on collembola in the field and in the laboratory. Ecotoxicology 13, 573–587.
Giller, K.E., Witter, E., McGrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30, 1389–1414.
Godínez-Alvarez, H., Herrick, J.E., Mattocks, M., Toledo, D., Van Zee, J., 2009. Comparison of three vegetation monitoring methods: their relative utility for ecological assessment and monitoring. Ecol. Ind. 9, 1001–1008.
Gongalsky, K.B., 2003. Impact of pollution caused by uranium production on soil macrofauna. Environ. Monit. Assess. 89, 197–219.
Grzes, I.M., 2009. Ant species richness and evenness increase along a metal pollution gradient in the Bolesław zinc smelter area. Pedobiologia 25, 65–73.
Grzes, I.M., 2010. Zinc and cadmium regulation efficiency in three ant species originating from a metal. Bull. Environ. Contam. Toxicol. 84, 61–65.

Gülser, F., Erdogan, E., 2008. The effects of heavy metal pollution on enzyme activities and basal soil respiration of roadside soils. Environ. Monit. Assess. 145, 127–133.
Hamel, C., Schellenberg, M.P., Hanson, K., Wang, H., 2007. Evaluation of the ''bait-lamina test'' to assess soil microfauna feeding activity in mixed grassland. Appl. Soil Ecol. 36, 199–204.
Helling, B., Pfeiff, G., Larink, O., 1998. A comparison of feeding activity of collembolan and enchytraeid in laboratory studies using the bait-lamina test. Appl. Soil Ecol. 7, 207–212.
ISO, 1993. Soil Quality—Determination of Dry Matter and Water Content on a Mass Basis—Gravimetric Method. ISO 11465, International Organization for Standardization, Geneva, Switzerland.
Janssens, T.K., Lopéz, R.del R., Mariën, J., Timmermans, M.J., Montagne-Wajer, K., Van Straalen, N.M., Roelofs, D., 2008. Comparative population analysis of metallothionein promoter alleles suggests stress-induced microevolution in the field. Environ. Sci. Technol. 42, 3873–3878.
Jensen, J., Mesman, M. (Eds.), 2006. Ecological Risk Assessment of Contaminated Land—Decision Support for Site Specific Investigations. Report 711701047, RIVM, Bilthoven, The Netherlands.
Jiang, J., Wu, L., Li, N., Luo, Y., Liu, L., Zhao, Q., Zhang, L., Christie, P., 2010. Effects of multiple heavy metal contamination and repeated phytoextraction by *Sedum plumbizincicola* on soil microbial properties. Eur. J. Soil Biol. 46, 18–26.
Kapustka, L., 1999. Microbial endpoints: the rationale for their exclusion as ecological assessment endpoints. Hum. Ecol. Risk Assess. 5, 691–696.
Keeney, D.R., Nelson, D.W., 1982. Nitrogen inorganic forms. In: Page, A.L., Miller, R.H. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Am. Soc. Agron. Soil Sci. Soc. Am., Madison, pp. 643–698.
Lavelle, P., 1996. Diversity of soil fauna and ecosystem function. Biol. Int. 33, 3–16.
Margalef, R., 1957. La Teria de la informacion en las communidades de organisms. Invest. Pesq. 3, 99–100.
Magurran, A.E., 1988. Ecological Diversity and its Measurement. Princeton, University Press, Princeton, New Jersey, US 192 pp.
Mertens, J., Springael, D., De Troyer, I., Cheyns, K., Wattiau, P., Smolders, E., 2006. Long-term exposure to elevated zinc concentrations induced structural changes and zinc tolerance of the nitrifying community in soil. Environ. Microbiol. 8, 2170–2178.
Montagnini, F., Haines, B., Swank, W., 1989. Factors controlling nitrification in soils of early successional and oak/hickory forests in the southern Appalachians. Forest Ecol. Manage. 26, 77–94.
Moreno, J.L., Bastida, F., Ros, M., Hernández, T., García, C., 2009. Soil organic carbon buffers heavy metal contamination on semiarid soils: effects of different metal threshold levels on soil microbial activity. Eur. J. Soil Biol. 45, 220–228.
Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment: Activity, Ecology and Applications. Marcel Dekker, New York, USA, pp. 1–33.
Niemeyer, J.C., Moreira-Santos, M., Ribeiro, R., da Silva, E.M., Sousa, J.P., 2010. Environmental risk assessment of a metal contaminated area in the tropics. Tier I: screening phase. J. Soils Sediments 10, 1557–1571.
Niemeyer, J.C., Lolata, G.B., Carvalho, G.M., da Silva, E.M., Sousa, J.P., Nogueira, M.A., 2012. Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil. Appl. Soil Ecol. 59, 96–105.
Nogueira, M.A., Albino, U.B., Brandão-Júnior, O., Braun, G., Cruz, M.F., Dias, B.A., Duarte, R.T.D., Gioppo, N.M.R., Menna, P., Orlandi, J.M., Raiman, M.P., Rampazo, L.G.L., Santos, M.A., Silva, M.E.Z., Vieira, F.P., Torezan, J.M.D., Hungria, M., Andrade, G., 2006. Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil. Agric. Ecosyst. Environ. 115, 237–247.
OECD, 2006. Breakdown of Organic Matter in Litter Bags. OECD—Organization for Economic Cooperation and Development, Paris.
Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13, 131–144.
Podgaiski, L.R., Rodrigues, G.G., 2010. Leaf-litter decomposition of pioneer plants and detritivore macrofauna assemblages on coal ash disposals in southern Brazil. Eur. J. Soil Biol. 46, 394–400.
Read, H.J., Martin, M.H., Rayner, J.M.V., 1998. Invertebrates in woodlands polluted by heavy metals—an evaluation using canonical correspondence analysis. Water Air Soil Pollut. 106, 17–42.
Römbke, J., Heimbach, F., Hoy, S., Kula, C., Scott-Fordsmand, J., Sousa, J.P., Stephenson, G., Weeks, J. (Eds.), 2003. SETAC Press, Pensacola.
Rutgers, M., Tuinstra, J., Spijker, J., Mesman, M., Wintersen, A., Posthuma, L., 2008. Ecological Risks of Soil Contamination in the Second Step of the Remediation Criterion. Report 711701072, RIVM, Bilthoven, the Netherlands (In Dutch with English Summary).
Rutgers, M., 2008. Field effects of pollutants at the community level—experimental challenges and significance of community shifts for ecosystem functioning. Sci. Total Environ. 406, 469–478.
Rutgers, M., Jensen, J., 2011. Site-specific ecological risk assessment. In: Swartjes, F.A. (Ed.), Dealing with Contaminated Sites: From Theory Towards Practical Application. Springer, Dordrecht, The Netherlands, pp. 1114.
Salemaa, M., Vahan-Majamaa, I., Deromeu, J., 2001. Understory vegetation along a heavy-metal pollution gradient in SW Finland. Env. Poll. 112, 339–350.
Schuster, E., Schroder, D., 1990. Side effects of sequentially-applied pesticides on target soil microorganisms: field experiments. Soil Biol. Biochem. 22, 67–373.
Semenzin, E., Critto, A., Marcomini, A., 2009. Ecosystem impairment evaluation on biodiversity and functional diversity for contaminated assessment. IEAM 5, 399–413.
Semenzin, E., Critto, A., Rutgers, M., Marcomini, A., 2008. Integration of bioavailability, ecology and ecotoxicology by three lines of evidence into ecological risk indexes for contaminated soil assessment. Sci. Total Environ. 389, 71–86.
Shannon, C.E., 1948. The mathematical theory of communication. In: Shannon, C.E., Weaver, W. (Eds.), The Mathematical Theory of Communication. University of Illinois, Urbana, US.
Singh, K.P., Mandal, T.N., Tripathi, S.K., 2001. Patterns of restoration of soil physicochemical properties and microbial biomass in different landslide sites in the sal forest ecosystem of Nepal Himalaya. Ecol. Eng. 17, 385–401.
Sprenger, M.D., Charters, D.W., 1997. Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessment, Interim Final. US Environmental Protection Agency, Environmental Response Team, Edison, NJ.
Suter II, G.W., Efroymson, R.A., Sample, B.E., Jones, D.S., 2000. Ecological risk assessment for contaminated sites. Lewis, Boca Raton, Florida.
Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrofenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.
Ter Braak, C.J.F., Smilauer, P., 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power, Ithaca, NY, USA.
Timmermans, M.J.T.N., Ellers, J., Van Straalen, N.M., 2007. Allelic diversity of metallothionein in *Orchesella cincta* (L.): traces of natural selection by environmental pollution. Heredity 98, 311–319.
Tordoff, G.M., Baker, A.J., Willis, A.J., 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41, 219–228.
Triplehorn, C.A., Jonnson, N.F., 2011. Estudo dos insetos. Cengage Learning, São Paulo, SP, Brazil.
Van Assche, F., Clijsters, H., 1990. A biological test system for the evaluation of the phytotoxicity of metal-contaminated soils. Environ. Pollut. 66, 157–172.
Van Gestel, C.A.M., Koolhaas, J.E., Hamersb, T., Hoppea, M., Roovert, M., Korsmana, C., Reinecke, S.A., 2009. Effects of metal pollution on earthworm communities in a contaminated floodplain area: linking biomarker, community and functional responses. Environ. Pollut. 157, 895–903.
Van Gestel, C.A.M., Kruidenier, M., Berg, M.P., 2003. Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. Biol. Fertil. Soils 37 386–386.
Van Straalen, N.M., 2002. Assessment of soil contamination—a functional perspective. Biodegradation 13, 41–52.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Veiga, M.D., Wildner, L.D.P., 1993. Manual para la instalación y conducción de experimentos de perdida de suelos. EPAGRI/FAO, Santiago, Chile 34 pp.
Von Törne, E., 1990. Assessing feeding activities of soil living animals. I. Bait lamina test. Pedobiologia 34, 89–101.
Widianarko, B., Verweij, R., Van Gestel, C., Van Straalen, N.M., 2000. Spatial distribution of trace metals in sediments from urban streams of Semarang, Central Java, Indonesia. Ecotoxcol. Environ. Saf. 46, 96–100.
Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050.
Zimakowska-Gnoinska, D., Bech, J., Tobias, F.J., 2000. Assessment of the heavy metal pollution effects on the soil respiration in the Baix LLobregat (Catalonia, NE Spain). Environ. Monit. Assess. 61, 301–313.