Biological indicators of soil quality in area of lead mining and metallurgy, Paraná State, Brazil.

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Abstract
This work aimed to determine heavy metal contents in soil and native plants and to evaluate microbial and fauna activities in soils of the Pb mining and metallurgy area, to establish the biological indicators of these soils qualities. The collections were made at five different locations (sites 1, 2, 3, 5, 6), in four seasons. Fertility and physical analysis and total (HF, HNO_3, H_2O_2 microwave extraction) and bioavailable (HNO_3 0.5 mol/L boiling extraction) Pb, Cu, Ni and Zn contents were measured for soil samples. Samples of native plants were collected and after nitric-perchloric digestion, total heavy metal contents were determined. The microbial and mesofauna studies in the soil were done for the depth of 0 to 5 cm. Total heavy metal contents were determined in Formicidae group (HNO_3 microwave digestion). The maximum total and bioavailable soil Pb contents were at site 3 (25,930 and 15,370 mg/kg, respectively). The alteration of organisms of the soil's mesofauna were more evident than for microbiology activity: site 1 (reference - low and natural soil Pb levels) presented the highest mesofauna diversity. The Pb levels in the Formicidade group and in plants were consistent with the contents of Pb in the soils.

Key Words
Heavy metals, soil bacteria and fungi, soil microbiology metabolism, soil mesofauna diversity, plant toxicity.

Introduction
In Adrianópolis, Paraná State, Brazil, after mining and Pb metallurgy for almost 60 years, about 177,000 t of rejects were left on the soil. In previous studies, the dissemination of contamination was observed through the high metal levels in the blood of people that lived around the area (Cunha, 2003). These Pb mining and metallurgy activities caused visible impacts on the environment, such as intense erosion and great volume of rejects dispersed on the soil.
Microorganisms are the most numerous organisms in the soil biological fraction and are dependent on changes in the quantity contaminants and metabolism relations due to environmental changes, such as pollutant transportation to the soil (Doelman *et al.* 1994). The influence of soil management or contaminant addition to the soil, usually induce a quicker response from soil mesofauna than for other pedogenic attributes, making these organisms good environmental quality indicators (Lanno *et al.* 2004).

Methods
Physical and chemical assessments
Heavy metal content and some physical and chemical characteristics (fertility, field capacity and texture) were determined (Lim and Jackson, 1986, with adaptations), to assess soil quality, through the chemiometric Principal Component Analysis (PCA). Soils (0 to 5 and 5 to 10 cm) from 5 locations, in 4 seasons of the year, were sampled in Adrianópolis, Paraná State, Brazil, with the following characteristics in regard to the contamination forms (Figure 1): site 1 – reference (native vegetation); site 2 – incorporated residue in the profile; site 3 – next to one of the factory's chimneys, with potential transport of the particulate matter; site 5 – greater reject volume on the soil; site 6 – similar conditions to site 3, but with sandy textured soil. The total Pb, Cu, Ni and Zn contents were determined through ICP-AES, after digestion of the soil samples with concentrated HNO_3, HF and H_2O_2 in microwave. To extract the heavy metals in bioavailable forms, a boiling HNO_3 0.5 mol/L solution was used.

Microbiological assessments
The following soil microbiology parameters were estimated (Wollum II, 1982): Total Bacteria (TB); Sporulating Bacteria (SB); percentage of Sporulating Bacteria comparing to TB (SB%); Fungi (FG); ratio between FG/TB; Microbiology Respiration in the time 1 (5 days) (MR1) and 2 (10 days) (MR2); Microbiology Carbon Biomass (MCB); Microbiology Carbon Biomass percentage comparing to the soil total

organic carbon (MCB%); Metabolic Quotient in 5 days (qCO_2a) and in 10 days (qCO_2b). Three methods were used in statistical analysis i) determination of an Environmental Quality Index regarding to microbiological parameters (EMQI); ii) differentiation of the sites through Principal Component Analysis (PCA); and iii) simple correlation analysis.

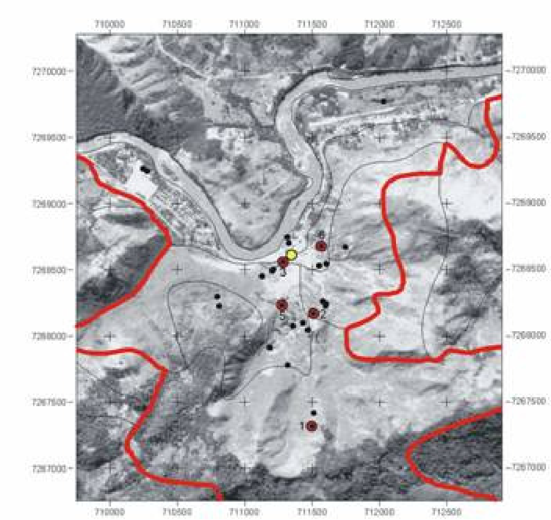

Figure 1. Aerial photo with the sampled points (1, 2, 3, 5 and 6 - red) and waste (yellow) locations (the red line delimits the mining influence area). Note that the Ribeira River is close to the wastes.

Mesofauna and heavy metals in native plants assessments

Soil samples were collected with Berlese funnel the depth 0 to 5 cm (20 funnels x 5 sites x 1 depth x 4 seasons = 400 samples). After the mesofauna separation, the selection and identification of the organisms (21 distinct groups) were accomplished. Individuals of the Formicidae group were digested with concentrated HNO_3 in microwave and the Pb, Cu, Ni and Zn contents were determined through ICP-AES. Plants from *Poaceae* family were collected in all sites and after digestion by nitric-perchloric method, the Pb, Cu, Ni and Zn contents were also determined. The same statistical treatments were employed.

Results

The maximum total and bioavailable soil Pb contents were, respectively, 25,930 and 15,370 mg/kg. The reject incorporation to the soil profile elevated Pb solubility, causing a lesser difference between the total and the bioavailable content in site 2 than in site 5. The sites 3 and 5 showed higher contamination risk: site 5 - samples with higher total Pb contents, associated to the pronounced area declivity and high Ribeira river water contamination risk by erosive process; site 2 - samples with higher bioavailable Pb contents, associated with the proximity to the hydrostatic level and leaching contamination risk. Site 5 present plants growing restrictions, where the soil samples were grouped (PCA) due to the low fertility and clay content, that can potentially cause erosion in this area. The high capacity of exchangeable of soil capacity and pH in water (above 7.5) and the high clay content reduced the solubility Pb forms in site 3.

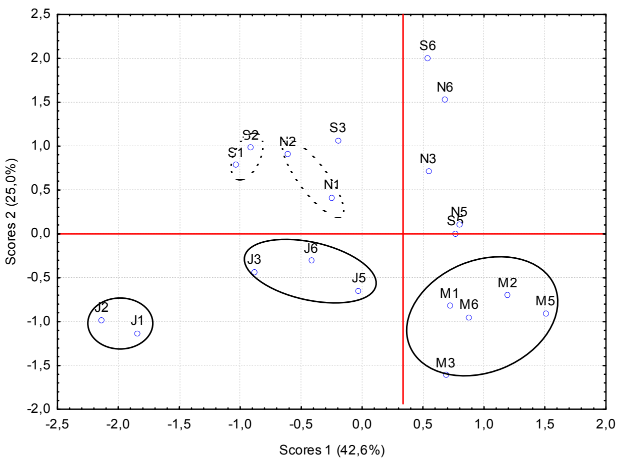

Figure 2. Principal Component Analysis (PCA) of the soil samples (0 - 5 cm), based on the microbiological characteristics, indicating similar sample groups. The notation represents the sampling month and the site number.

The most favorable temperature and humidity conditions, associated with the high soil fertility, caused the heavy metals prejudicial effects to have less effect on the microorganisms. However, in the month with the highest climatic stress to the organisms (May – lowest temperatures), the soils with the highest Pb content showed reduced populations and microbiology activity (Table 1), meaning that in these conditions the bacteria and fungi were good soil quality indicators. The highest Sporulation Bacteria (SB) proportion in the most Pb contaminated soils in the coldest month (0.95* correlation for May) can be interpreted as a resistance mechanism of these organisms. The Environment Microbial Quality Index (EMQI) in the 0 to 5 cm soil layer (Table 2) was more efficient than PCA in distinguishing the locations with heavy metal contamination. The climatic conditions (sampling month) were more important in soil grouping in the PCA graphic (Figure 2). Excepting site 6 (low soil fertility and clay content), the EMQI values decreased in the opposite sense to the increasing soil Pb content. However, in the 5 to 10 cm layer, the PCA was more efficient to this purpose. The total organism number from the 21 identified groups and the Environmental Mesofauna Quality Index were not good indicators of the soil heavy metal contamination level. The quantity and distribution of isolated species were more efficient to this purpose. The best environmental quality from site 1 was evidenced by the major diversity of organism groups and occurrence of the Pseudoscorpiones, Mollusca and Isopoda groups only in this soil. The Arachinida and Psocoptera groups were also considered good environmental indicators, with an increase of their populations in sites with higher heavy metals content (sites 2, 3 and 5), possibly because of the lesser occurrence of competitors/predators organisms. The heavy metal content in the individuals from the Formicidae group (site 1 - 11.5 mg/kg; site 2 - 70.5 mg/kg; site 3 - 84.6 mg/kg; site 6 - 13.4 mg/kg) had a direct relation to the soil's bioavailable Pb content. In regard to the accumulation of heavy metals in native species, with the exception of site 1 (Table 3), all plants showed the phytotoxic effect of Pb, which suggests the prohibition of pasture as a land use in the area. The higher Pb levels were detected in the roots, especially in location 3, which presented the higher bioavailable Pb contents in the soil.

Table 1. Microbiology analysis of 0 - 5 cm soil samples (the terms are explained in the Methods section. FCU - Formation Colonies Units, C - carbon. nd - noun determined parameter by analytical problems)

Sampling month	Site	Microbiology count			SB%	FG/TB	Microbiology respiration		MCB	MCB%	qCO_2a	qCO_2b
		TB	SB	FG			MR1	MR2				
		FCU/g of soil					mg $C\text{-}CO_2$/kg/h		mg C/kg			
May	1	9.52	2.78	0.89	2.92	0.009	0.79	0.58	nd	nd	nd	Nd
	2	0.80	0.74	0.05	9.25	0.006	0.71	0.46	nd	nd	nd	Nd
	3	2.70	8.94	0.24	33.11	0.009	1.31	0.78	nd	nd	nd	Nd
	5	0.76	2.16	0.24	28.42	0.032	0.13	0.14	nd	nd	nd	Nd
	6	6.66	4.64	1.70	6.97	0.026	0.51	0.34	nd	nd	nd	Nd
Sep.	1	15.86	1.17	0.69	0.74	0.004	1.49	1.19	571.6	0.98	2.60	2.08
	2	6.79	1.06	1.26	1.56	0.019	1.29	1.05	426.6	0.83	3.03	2.46
	3	3.58	0.20	1.53	0.56	0.043	0.71	0.55	366.6	0.73	1.94	1.49
	5	0.91	1.60	0.29	17.58	0.032	0.17	0.14	257.2	0.97	0.67	0.53
	6	0.42	0.02	1.30	0.48	0.310	0.16	0.13	86.9	0.44	1.84	1.54
Nov.	1	1.97	1.07	0.73	5.43	0.037	1.11	0.84	873.0	1.63	1.27	0.96
	2	2.07	0.66	2.91	3.18	0.141	1.37	1.06	803.0	1.60	1.70	1.32
	3	0.27	0.34	0.34	12.39	0.124	0.37	0.31	296.5	0.60	1.25	1.06
	5	1.51	1.08	0.52	7.16	0.034	0.07	0.07	383.0	2.77	0.18	0.17
	6	0.88	0.06	2.75	0.65	0.314	0.12	0.12	181.2	1.12	0.67	0.67
Jan.	1	34.53	64.94	23.54	18.81	0.068	1.67	1.44	1158.4	1.42	1.44	1.24
	2	56.72	77.05	10.70	13.58	0.019	1.84	1.64	877.1	2.08	2.10	1.87
	3	37.37	15.08	9.52	4.03	0.025	0.75	0.61	690.4	1.20	1.08	0.88
	5	10.30	14.60	1.15	14.17	0.011	0.29	0.25	903.2	4.65	0.32	0.28
	6	23.96	9.54	8.72	3.98	0.036	0.36	0.31	521.6	1.95	0.69	0.59

Table 2. Environmental Quality Index based on microbiological parameters (EMQI) (Numbers in parenthesis in the last column represent the median soil total Pb contents (mg/kg) of the 4 months)

Local	May	September	November	January	Media
1	3.3	26.9	32.0	55.4	29.4 (654.4)
2	1.5	21.0	30.8	52.3	26.4 (6,889.3
3	3.8	16.0	11.9	32.3	16.0 (15,437.5)
5	0.7	9.7	13.1	33.3	14.2 (20,949.3)
6	2.5	5.4	7.1	23.0	9.5 (845.8)

Table 3. Pb contents (mg/kg) of the native plants (sites 1 and 3 - *Panicum maximum*; sites 2 and 5 - *Paspalum notatum*; site 6 - *Pennisetum purpureum*)

Sampling month	Site				
	1	2	3	5	6
	Shoot				
September	16.89	182.04	171.39	575.53	47.15
November	19.86	90.02	126.05	512.16	102.55
January	nd	3.06	13.28	211.35	nd
	Root				
September	22.65	99.80	939.96	376.07	164.90
November	12.67	122.90	733.68	420.88	129.96
January	24.14	74.04	414.72	436.45	168.17

Conclusion

In the months with the highest climatic stress to the organisms (May and September – lowest temperatures), the soils with the highest Pb content showed lesser population and microbial activity, meaning that in these conditions, the bacteria and fungi were good soil quality indicators. The highest sporulating bacteria proportion in the most Pb contaminated soils in May is attributed to the resistance mechanism of these organisms. The best environmental quality from site 1 (natural Pb levels - reference soil) was evidenced by the major diversity of organism groups and the occurrence of the Pseudoscorpiones, Mollusca and Isopoda. The first group is recognized in the literature as sensitive to heavy metal in soil.

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