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Characterization of bacterial communities at heavy-metal-contaminated sites

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ABSTRACT

The microbial community in soil samples from two long-term contaminated sites was characterized by using culture-dependent and culture-independent methods. The two sites investigated contained high amounts of heavy metals and were located in the upper Silesia Industrial Region in southern Poland. The evaluation of the aerobic soil microbial population clearly demonstrated the presence of considerable numbers of viable, culturable bacteria at both sites. A high fraction of the bacterial population was able to grow in the presence of high amounts of metals, i.e. up to 10 mM Zn^{2+}, 3 mM Pb^{2+} or 1 mM Cu^{2+}. Site 1 contained significantly ($P < 0.05$) lower bacterial numbers growing in the presence of 10 mM Zn^{2+} than site 2, while the opposite was observed for bacteria tolerating 1 mM Cu^{2+}. This coincided with the contents of these two metals at the two sites. Ecophysiological (EP) indices for copiotrophs (r-strategists) and oligotrophs (K-strategists) pointed to high bacterial diversity at both sites. Fluorescence in situ hybridization (FISH) analysis indicated that *Actinobacteria* and *Proteobacteria* represent the physiologically active fraction of bacteria at the two sites. Shannon diversity (H') indices for FISH-detected bacterial phylogenetic groups were not significantly different at the two sites.

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1. Introduction

Soil contamination with heavy metals is often a result of anthropogenic activities. Contrary to organic pollutants, heavy metals cannot be degraded and can thus constitute a persistent environmental hazard. Long-term impacts of heavy metals on soil microorganisms result in adverse effects on microbial activities and abundance, and altered microbial community structures (Tsezos, 2009).

Soil biological investigations give information on the the impact of environmental conditions on the metabolic activity of soil (Kiss, 2001; Margesin and Schinner, 2005). For the microbiological characterization of soils contaminated with heavy metals, the evaluation of soil microbial activities (e.g. enzyme activities and respiration rates), the catabolic versatility of soil microorganisms with regard to the utilization of carbon sources as well as ecotoxicological parameters have been used (Castaldi et al., 2004; Kizilkaya et al., 2004; Oliveira and Pampulha, 2006; Yuangen et al., 2006; Leitgib et al., 2007; Liao and Xie, 2007; Płaza et al., 2010). In general, significant negative relationships were observed between total heavy metal contents and these soil microbial characteristics.

To evaluate microbial community composition, culture-dependent and culture-independent methods have been used. Microbial abundance is often based on culture-dependent methods (Ellis et al., 2003; Oliveira and Pampulha, 2006). Microbial diversity indices, based on culturable microorganisms, have been developed to describe structures and dynamics of soil microbial communities (De Leij et al., 1993; Kotsou et al., 2004). Since culturable cells may only represent ⩽0.1–1% of the total microbial community (Alexander, 1997; Rappé and Giovannoni, 2003), culture-independent, molecular assays, such as profiling soil DNA, rRNA, or phospholipid fatty acids, are increasingly used in environmental microbiology (Muyzer et al., 1993; Zelles, 1999; Gremion et al., 2003; Sorensen et al., 2009). Fluorescence in situ hybridization (FISH) is based on the detection of rRNA. Since the rRNA content is associated with the metabolic state of microbial cells, FISH is a valuable tool to describe the composition of the more active, ecologically relevant part of the microbial community (Amann et al., 1995, 2001; Motor and Göbel, 2000; Wagner et al., 2003) even in complex matrices, such as soil (Sandaa et al., 1999; Barra Caracciolo et al., 2005a,b).

In an earlier study we characterized soils from industrial sites, including two heavy-metal-contaminated sites, by combining physico-chemical, microbiological and ecotoxicological parameters, and we demonstrated the impact of total and water-soluble contents of heavy metals on toxicity (Płaza et al., 2010). It was the objective of this study to survey the microbial community inhabiting these two long-term contaminated sites containing high amounts of heavy metals by using culture-dependent methods and culture-independent FISH analysis.

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2. Materials and methods

2.1. Study sites

The two metal-contaminated sites were located in the Upper Silesia Industrial Region in southern Poland. Briefly, site 1, the Tarnowskie Góry megasite, belongs to Poland's most serious sources of soil and groundwater contamination (Malina, 2004), and has been recognized as the top priority of land management. For centuries, this area was well known for extraction of silver, zinc and lead ores and the development of chemical industry. In 1997 the liquidation process of the chemical plant was initiated. Site 2, the Piekary Site, is a former mine and smelter area, situated between the towns of Bytom and Piekary Śląskie, at Upper Silesia Industrial Region. The primary minerals of concern were zinc, lead and cadmium ore, dolomite, silt and grave. In 1989 production was closed down and activities towards the revitalisation of 460 ha property were started (Kucharski et al., 2005).

Physico-chemical properties of the sites have been described in detail by Płaza et al. (2010) and are summarized in Table 1. Large differences between minimum and maximum values were attributed to soil heterogeneity. Both sites are characterized by high total heavy metal contents. The two sites differ in a number of parameters: site 1 is characterized by higher pH values, higher sand fraction and lower P contents than site 2, while site 2 contains significantly higher contents of a number of metals, which results in higher toxicity.

The research areas of our investigation at the two sites were approximately 20 m^2. Sampling included the collection of five composite soil samples (obtained from five subsamples) from each site from the upper 30–40 cm, immediate transport to the laboratory, sieving, and storage at field humidity in polyethylene bags at 4 °C until processing (for details, see Płaza et al. (2010)).

2.2. Total bacterial counts

Total counts of bacteria were determined by non-selective DAPI (4′,6′-diamino-2-phenylindole; 1.5 μg mL^{-1}) staining using CitiDAPI and epifluorescence microscopy as described below (see Section 2.4).

2.3. Enumeration of culturable aerobic soil bacteria

Culturable soil bacteria were enumerated by the plate-count method for viable cells. Soil suspensions were prepared by shaking soil samples (5 g fresh mass) for 30 min at 180 rpm with 45 mL of sodium pyrophosphate solution (0.1% w/v). Appropriate dilutions of soil suspensions were surface spread onto agar plates. Cyclohex-

Table 1
Physico-chemical and ecotoxicological properties of the investigated soil samples from the two contaminated sites (all values are expressed on a soil dry mass basis; data are from Płaza et al., 2010).

	Site 1			Site 2		
Parameter	Mean	Min	Max	Mean	Min	Max
Physico-chemical parameters						
Dry mass (%)	89.7	76.8	97.6	87.0	84.4	89.4
pH ($CaCl_2$)	7.4	7.3	7.5	6.4	6.2	6.7
CEC ($cmol^+ kg^{-1}$)	5.7	2.2	13.7	6.9	4.7	10.6
Conductivity (μs cm^{-1}) [a]	347	154	518	143	121	158
Ntot (%)	0.095	0.010	0.260	0.156	0.110	0.210
Ptot (%) *	0.007	0.003	0.011	0.114	0.026	0.218
CoRG(%)	2.5	0.8	6.4	3.0	1.7	5.8
Loss on ignition (%)	7.2	2.5	17.8	11.2	7.3	15.6
Sand (2–0.05 mm; %) [a]	83.8	68.0	90.0	56.0	52.0	64.0
Silt (0.05–0.002 mm; %) [a]	8.5	2.0	24.0	36.4	28.0	41.0
Clay (<0.002 mm; %)	7.8	7.0	8.0	7.6	7.0	8.0
Total heavy metal contents (mg kg^{-1})						
As[a]	9.0	2.8	21.4	102	66	146
Ba	57 429	883	219 618	146	109	186
B	66	5.5	228	10.6	9.0	11.8
Zn[a]	627	91	1404	11 713	8930	16 231
Pb[a]	81	67	97	9543	6287	15 303
Cd[a]	1.35	n.d.	5.39	361	262	467
Sr	3153	42	11 646	31	17	36
Ni[a]	3.73	1.24	6.59	10	8.82	12
Cr[a]	7.70	6.19	9.65	17	14	25
Co[a]	0.83	n.d.	2.13	6.11	5.42	6.79
Cu	39	9	74	26	19	40
Fe[a]	3825	1545	5274	9012	7915	9883
Water soluble heavy metal contents (mg kg^{-1})						
As	0.31	0.02	0.98	0.17	0.13	0.22
Ba	323	2.94	1267	0.31	0.20	0.49
B	8.41	0.26	29	0.19	0.063	0.32
Zn[a]	2.00	n.d.	3.54	29	14	53
Pb	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cd	n.d.	n.d.	n.d.	0.95	n.d.	2.58
Sr	50	1.16	188	0.02	0.008	0.03
Ni	n.d.	n.d.	n.d.	0.03	n.d.	0.08
Cr	n.d.	n.d.	n.d.	0.01	0.001	0.03
Co	n.d.	n.d.	n.d.	0.08	0.000	0.14
Cu	n.d.	n.d.	n.d.	0.044	0.001	0.09
Fe	8.47	2.08	12	4.87	0.000	7.14
Toxicity tests						
Microtox (TU) [a]	64	3.0	244	501	80	865

[a] Significantly different at the two sites.

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