

Effects of Heavy Metal Contamination and Remediation on Soil Microbial Communities in the Vicinity of a Zinc Smelter

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ABSTRACT

Heavy metal contamination can impact soil ecosystems sufficiently to result in significant losses in soil quality. The negative impact of heavy metals results from their toxicity to biological processes, including processes catalyzed by soil microorganisms. Therefore, it is postulated that the soil microbial community could serve as an indicator of losses in soil quality due to heavy metal contamination and of changes in soil quality resulting from reclamation. In this study, the size, activity, and structure of microbial communities from remediated and unremediated soils in the vicinity of a Zn smelter were evaluated. Both total and soluble metal loadings in these soils increased with proximity to the smelter. Indicators of microbial activity (dehydrogenase activity) and viable population size (plate counts) were negatively affected by the elevated metal levels. Microbial community structure also varied with increasing contamination, as indicated by cluster analysis and principal component analysis of BIOLOG community metabolic profiles. Remediated soils at this site were treated by surface application of a mixture of sewage sludge and fly ash. Remediation resulted in a decrease in soluble metals and an increase in indicators of biological activity and viable population size. Remediated soils also showed metabolic profiles that were more similar to the least contaminated site, suggesting recovery of the microbial populations. These data suggest that the microbial community may be a useful indicator of changes in soil quality with management of these highly contaminated soils.

METAL CONTAMINATION of soil ecosystems negatively impacts a number of soil microbiological properties that could be potential indicators of soil quality and thus could be used as measures of reclamation progress and/or success. Historically, soil chemical and physical parameters have been used as indicators of soil quality (Janke and Papendick, 1994), but due to the role of the microbial community in total ecosystem function and the sensitivity of soil microbial communities to disturbance, biological indicators may also be useful as measures of soil quality. The soil microbial community is an integral component of soil quality due, for example, to the critical role it plays in the cycling of nutrients and the formation of soil structure (Turco et al., 1994). Soil microorganisms are also highly sensitive to disturbances in the soil ecosystem, and thus changes in soil microbial communities may be effective early signals of degradation or improvement of soil (Turco et al., 1994).

Biological processes are particularly sensitive to soil heavy metal loadings. Field studies of metal-contaminated soils have demonstrated that elevated metal loadings can result in diminished microbial biomass (Brookes and McGrath, 1984; Chander and Brookes, 1991a), reduced viable bacterial population densities (Jordan and LeChevalier, 1975), inhibition of organic

matter mineralization (Chander and Brookes, 1991a), as well as decreased leaf litter decomposition (Strojan, 1978), symbiotic N₂ fixation (Heckman et al., 1987), and mycorrhizal infection of clover (*Trifolium* sp.) roots (Koomen et al., 1990). These examples of the effects of elevated metal loadings on microbial activity in soil indicate the potential for use of selected essential processes as indicators of soil quality.

In addition to effects on specific soil microbial processes, effects of metal contamination on the structure of soil microbial communities have been shown by the phospholipid fatty acid method (Pennanen et al., 1996) and by isolation and identification of bacterial strains (Barkay et al., 1985). A relatively new procedure that may be useful in evaluating changes in microbial community structure is the determination of the *metabolic profile* of a particular system via the BIOLOG procedure. BIOLOG is a redox system that allows characterization of entire microbial communities based on the pattern of utilization of 95 different C substrates, i.e., the metabolic profile. The BIOLOG procedure offers an interesting opportunity to study microbial community structure because it is based on community function, and it does not require isolation of individual bacterial strains. BIOLOG profiles have been found to be reproducible for model bacterial communities with consistent community structure, and BIOLOG profiles have been shown to change for model bacterial communities with different compositions (Haack et al., 1995). BIOLOG has been shown to be capable of separating microbial communities from different soil systems (Garland and Mills, 1991), soil microbial communities from the same site under different plant types (Zak et al., 1994), and microbial communities from cultivated and noncultivated soils (Windig, 1994). BIOLOG has also been used to examine changes in microbial community structure related to different C inputs and different moisture levels in agricultural plots (Bossio and Scow, 1995). Recently, an effect of metals on the metabolic diversity of communities was demonstrated by a study in which the BIOLOG assay was applied to soils that had been amended in the laboratory with solutions of metal salts (Knight et al., 1997). To date, the BIOLOG assay has not been applied to metal-contaminated or remediated field soils.

Remediation of metal-contaminated soils offers unique challenges. The fact that metals cannot be degraded limits remediation to two main options, removal of the metals or sequestering of the metals in nonbioavailable forms. Metals can be removed either by removal and disposal of contaminated soil or by leaching metals out of the soil and capturing the metals by some

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Abbreviations: OD, optical density; PCA, principal component analysis; AWCD, average well color development; PC, principal component; CEC, cation exchange capacity.

mechanism such as an artificial wetland system. Metal bioavailability can be decreased by reducing metal solubility. The remediation method used at the study site attempted to reduce the bioavailability of the metals by lowering their solubility. Because the goal of the remediation strategy was a reduction in bioavailability of the metals, microbial indicators should be especially useful in assessing its effectiveness.

The primary objective of this study is the evaluation of soil biological properties as indicators of the impact of heavy metal contamination and subsequent remediation at a field site. Specific objectives of this study were (i) to assess the relationship between metal loadings and microbial indicators by examining soils at increasing distances from a Zn smelter, and (ii) to determine the effects of remediation on the microbial indicators. In selecting specific microbial indicators for this study, measurements of microbial activity, population size, and community structure were all included, since combinations of microbial parameters have been shown to be more useful in monitoring soil metal pollution than any single parameter (Brookes, 1995).

MATERIALS AND METHODS

Soil Remediation Management. The remediation management procedure used at the site was a one time application of a 2:1 (wet wt.) mixture of municipal sewage sludge and power plant fly ash. The sludge-fly ash mixture was surface-applied at a rate of approximately 448 Mg ha⁻¹, which corresponds to a layer of approximately 2.5 to 4 cm. For sites treated before 1992, limestone was also added to the mixture in an amount equal to 22 Mg ha⁻¹.

Soils. Soil samples, selected for degree of contamination (distance from contamination source) and time since initiation of reclamation, were collected from a site that is downwind from a Zn smelter in a Mid-Atlantic state. Early data on the level of biological activity in relationship to the smelter has been reported (Jordan and LeChevalier, 1975). The soils were from the DeKalb (loamy-skeletal, mixed, mesic Typic Dystrichrepts), Klinsville (loamy-skeletal, mixed, mesic Lithic Dystrichrepts), and Holly (fine-loamy, mixed, nonacid, mesic Typic Fluvaquents) soil series.

Composited samples consisting of three subsamples were collected (with a minimum distance between samples of 3 m) from the top 15 cm of soil. For remediated sites, any remaining sludge layer was not included in the soil sample. Soil samples were maintained at 4°C by storage in an ice chest during transport to the laboratory. All soil samples were sieved to pass through a 2-mm sieve and maintained at 4°C until analysis. Soils from unremediated sites were collected in the Fall of 1995 and soils from both remediated and unremediated sites were collected in the Summer of 1996. Detailed site descriptions are provided in Table 1.

Soil particle size and texture were determined using the hydrometer method (Bouyoucos, 1953). Soil pH was measured in a 1:1 distilled water slurry (McLean, 1986). For soils with an organic matter content <10%, the organic matter content was determined with the Walkley-Black method (Walkley, 1947). For soils with an organic matter content >10%, the organic matter content was determined by loss on ignition (Bear, 1955). Soil CEC was determined by saturation with ammonium acetate at pH 7 (Chapman, 1965). Total metals were determined by the USEPA 3050 soil digestion method (USEPA, 1986). Following extraction, the metal concentra-

tions were determined with a multielement direct current plasma spectrometer (Applied Research Laboratories, Valencia, CA; Spectraspan model 7) at a wavelength of 2025 nm. Soluble metals were extracted by shaking for 18 h with 0.01 M CaCl₂ (Aten and Gupta, 1996; Sauerback and Styperek, 1985). For the fall 1995 sampling, soluble Zn was measured by atomic absorption (Perkin Elmer, Co., Norwalk, CT; model 3030) at a wavelength of 213.5 nm. For the summer 1996 sampling, soluble metals were measured with a direct current plasma arc furnace as described above.

Microbial Assays. For determination of soil metabolic profiles, the BIOLOG method, adjusted for use in metal-contaminated soils (Kelly and Tate, 1998), was used. Soil samples were extracted by shaking 20 g of soil with 100 mL 0.1 M Tris buffer (pH 7.5) for 10 min. Three BIOLOG extractions were conducted for each sampling site. After shaking, samples were centrifuged for 10 min. at 2600 × g, and the supernatant liquid was inoculated onto BIOLOG plates. Three replicate BIOLOG plates were inoculated from each extraction. The plates were incubated at 30°C, and color development on BIOLOG plates was read using the BIOLOG microstation and microplate reader (BIOLOG, Inc., Hayward, CA). The BIOLOG microstation records the optical density (OD) values for each of the 96 wells on each plate. Plates were read beginning at the first sign of color development and read at 4-h intervals up to approximately 52 h. If a plate showed color formation in all of the substrate wells before 52 h, no further readings were taken for that plate.

The BIOLOG data were analyzed in three ways. First, the rate of color development on the BIOLOG plates over time was determined. Then, BIOLOG profiles for different sites were compared by cluster analysis using BIOLOG software and by principal component analysis (PCA) using SAS statistical software.

To determine the rate of color development on the BIOLOG plates over time, the average level of color on each plate at each reading time was calculated. The net OD for each substrate well was calculated by subtracting the control well OD from the substrate well OD. If this subtraction gave a negative number, 0 was used in the subsequent analyses. An average well color development (AWCD) for each plate at each reading time was calculated by taking the mean of the net OD values for all of the 95 substrate wells (Garland, 1996). The AWCD for each set of triplicate plates was averaged, and the level of AWCD at each reading time was plotted. For further analysis of the BIOLOG data, comparisons were made between sets of triplicate plates that showed a similar level of overall response. An AWCD of 0.25 was used as the standard reference point for profile comparisons (Garland, 1996).

Cluster analysis was performed using the BIOLOG soft-

Table 1. Description of soil sites.

Site	Soil series	Distance from source	Remediation	Remediation date
Fall 1995				
A	Dekalb	6.5 km east	None	None
B	Klinsville	4.8 km east	None	None
C	Dekalb	1.6 km west	None	None
D	Holly	1.6 km east	None	None
Summer 1996				
A	Dekalb	6.5 km east	None	None
D	Holly	1.6 km east	None	None
E	Klinsville	0.8-1.2 km east	None	None
F	Dekalb	0.6-0.8 km east	Ecotoam	1986
G	Klinsville	0.5-0.6 km east	Ecoto	1991
H	Dekalb	1.6-2.4 km east	Ecotoam	1993
I	Dekalb	3.2-4.0 km east	Ecotoam	1995

ware. For each set of triplicate plates, the reading with an AWCD closest to 0.25 was used for the analysis. The BIOLOG software converts the OD reading for each substrate well to a percent change value using the following formula:

$$\frac{[(\text{Substrate well OD} - \text{Control well OD}) / \text{Control well OD}] \times 100$$

If the percent change value is negative, 0 is used for the remaining analysis. The percent change value for each well is coded as either a positive or a negative reaction using a proprietary threshold algorithm, generating a pattern of positive and negative reactions for each plate. The pattern of positive and negative reactions for each set of triplicate plates is compared and a dendrogram is produced based on the Un-weighted Pair Group Method. The dendrogram shows degree of similarity between the patterns on each set of triplicate plates. Each unit of distance on the dendrogram is equivalent to a difference of 100% on a single substrate well.

Principal component analysis is a well-defined statistical procedure that is used to analyze data sets that contain large numbers of variables. Principal component analysis represents the large number of variables with a smaller set of derived variables called principal components (PCs). Each sample is assigned a score on each of the PCs. A sample's score for a given PC is a combination of the sample's scores on each of the original variables weighted based on the correlation between each variable and the PC. The correlation between a variable and a PC is represented by that variable's correlation coefficient. Correlation coefficients can vary from -1.00 to +1.00. A variable with a correlation coefficient for a given PC close to +1.00 or -1.00 has a strong positive or negative correlation, respectively, with that PC. A variable with a correlation coefficient close to 0 for a given PC has a weak correlation to that PC. Each sample can be graphed based on its scores on the first two or three PCs, and separation of the samples in PC space indicates differences in the samples with respect to the variables measured. Correlation coefficients can then be used to determine the variables on which the samples differ (Kachigan, 1991).

Principal component analysis was used in this study to analyze the BIOLOG data, because the BIOLOG assay produces a data set containing a large number of variables. For PCA, each of the 95 substrates was treated as a variable, and each set of triplicate plates was treated as a sample. For each set of triplicate plates, the reading with an AWCD closest to 0.25 was used for the analysis, and the score for a substrate was the net OD for that substrate averaged over the three plates.

Principal component analysis was used to produce PCs based on the substrate scores and to plot each of the samples in PC space. Differences observed between samples in PC space indicates differences in the samples with respect to the substrates utilized. Separation of samples along one PC can be related to differences in substrates utilized by looking at those substrates with high positive or negative correlations to that PC.

Supernatant liquid from each BIOLOG extraction was also used to obtain counts of viable organisms on soil extract agar plates. The percentage of organisms resistant to Zn was determined by plating the supernatant on soil extract agar plates amended with 1mM ZnSO₄ (Jordan and LeChevalier, 1975).

Microbial biomass was determined for each soil by the chloroform fumigation method (Jenkinson, 1966). Dehydrogenase activity was determined by a modification of the method of Casida (Tate and Terry, 1980).

RESULTS

Nonremediated Soils. Although the soils that were selected to evaluate the impact of metal loadings on soil biological indicators without reclamation management did vary somewhat in physical and chemical properties (Table 2), they were probably as similar as is possible in a field study of this type. Soil texture and soil pH varied slightly between sites. These differences in texture and pH likely represent differences in local soil genesis rather than the impact of the smelting operation. Greater differences were observed in soil organic matter content and cation exchange capacity. Organic matter content ranged from approximately 2.2% at the site most distant from the smelter (A) to nearly 30% at the site 1.6 km west (C). The inhibitory effect of the metals on microbial activity is the most probable explanation for elevated organic matter in the more highly contaminated sites. The high levels of metals would be anticipated to slow microbial degradation of organic matter entering the system, thereby resulting in the observed accumulation (Chander and Brookes, 1991a; Valsecchi et al., 1996). Greater cation exchange capacity (CEC) with proximity to the smelter parallels the organic matter content of the soils.

Both total metal loadings and soluble Zn loadings of soils reflected proximity to the smelter (Table 3).

Table 2. Description of soils.

Site	Particle-size distribution			Soil texture	pH	Organic matter %	CEC [†] cmol kg ⁻¹
	Sand	Silt	Clay				
	%						
Fall 1995							
A	60.0	30.0	10.0	Sandy loam	5.7	2.2d*	3.66b
B	66.0	22.0	12.0	Sandy loam	4.8	17.9b	19.30a
C	52.0	40.0	8.0	Sandy loam	4.5	29.8a	26.04a
D	25.0	51.0	24.0	Silt loam	5.4	11.7c	13.58ab
Summer 1996							
A	49.0	38.6	12.4	Loam	5.7	2.9f	4.55d
D	38.0	44.4	17.6	Loam	5.5	4.8e	10.1c
E	51.8	33.0	15.2	Loam/sandy loam	6.0	6.4d	10.56c
F	52.8	34.0	13.2	Sandy loam	6.2	16.4b	17.27a
G	59.8	31.4	8.8	Sandy loam	6.6	20.8a	15.99a
H	60.4	22.4	17.2	Sandy loam	6.8	15.0c	13.72b
I	51.8	31.8	16.2	Loam/sandy loam	6.9	3.9ef	9.32c

* Values in same column for same testing period followed by a different letter are significantly different ($\alpha = 0.05$), Duncan's new multiple range test.

† CEC, cation exchange capacity.

Table 3. Metal content of soils.

Site	Total metals						Soluble metals					
	Zn	Cd	Cr	Cu	Ni	Pb	Zn	Cd	Cr	Cu	Ni	Pb
	mg kg ⁻¹											
Fall 1995												
A	551d*	8d	61a	42c	25c	85d	104d	nd†	nd	nd	nd	nd
B	2 616c	.63b	42b	169b	31b	414b	1 038b	nd	nd	nd	nd	nd
C	4 032b	.73b	54a	226b	33b	1 096b	951c	nd	nd	nd	nd	nd
D	13 656a	144a	60a	2 390a	59a	2 458a	1 136a	nd	nd	nd	nd	nd
Summer 1996												
A	199c	15c	57c	56c	13d	99c	101e	2.53e	0.00	0.54b	0.00b	0.00
D	5 335b	.70c	44c	594a	32bc	676b	735c	17.66c	0.00	0.69b	0.88a	0.00
E	8 719b	.205b	41c	597a	20cd	1 749a	1 085a	62.76a	0.00	0.07c	0.00b	0.00
F	11 944a	.403a	36c	333b	17d	2 010a	865b	57.43b	0.00	0.01c	0.00b	0.00
G	12 881a	.201b	58c	381b	35b	1 605b	158d	10.40d	0.00	0.04c	1.40a	0.00
H	.807c	.28c	138a	407b	67a	149bc	6f	0.20f	0.00	0.96a	0.00b	0.00
I	.533c	.22c	84b	137c	38b	67c	3f	0.08f	0.00	0.04c	0.00b	0.00

* Values in same column for same testing period followed by a different letter are significantly different ($\alpha = 0.05$), Duncan's new multiple range test.

† nd, not determined.

However, even the site most distant from the smelter (A) had significantly augmented total metal and soluble Zn loadings, indicating the extent of the regional impact of the smelter. For sites closer to the smelter, soluble Zn loadings are elevated in spite of the high CEC of these soils. Low pH of the soils has likely contributed to maintenance of high soluble Zn levels. Note that Site D, which had a higher pH than Sites B and C, had the lowest percentage of total Zn in the soluble pool.

Elevated metal loadings did impact the microbial communities. Indicators of microbial activity (viable population density and dehydrogenase activity) were negatively affected by the high metal levels. Metal-contaminated sites (B, C, and D) all had reduced viable populations and dehydrogenase activity (Table 4). For example, soil collected 1.6 km east of the smelter (D) had 86% fewer colony forming units and 67% less dehydrogenase activity than soil from the most distant site (A).

Total microbial biomass and the proportion of the bacterial population that was Zn-resistant did not appear to be affected by the soluble Zn levels. Other studies have shown a decrease in microbial biomass resulting from metal contamination both in field

Table 4. Soil microbial community and activity parameters.

Site	Biomass	Dehydrogenase	Bacteria g ⁻¹ soil × 10 ⁶	% Zn-resistant bacteria
	mg C g ⁻¹ soil	mg TPF g ⁻¹ soil†		
Fall 1995				
A	0.5660a*	0.143a	1.63 (0.046)‡	11.0
B	0.333a	0.079b	0.60 (0.026)	3.4
C	0.342a	0.080b	0.39 (0.033)	5.4
D	0.543a	0.020c	0.54 (0.043)	18.8
Summer 1996				
A	0.114c	0.043a	0.89 (0.041)	3.9
D	0.317a	0.008c	3.06 (0.326)	13.9
E	0.076c	0.004c	0.15 (0.015)	13.5
F	0.092c	0.007c	1.59 (0.100)	23.0
G	0.273ab	0.027b	8.81 (1.03)	18.2
H	0.288ab	0.031b	40.4 (7.97)	1.9
I	0.156bc	0.05a	52.6 (7.70)	1.8

* Values in same column for same testing period followed by a different letter are significantly different ($\alpha = 0.05$), Duncan's new multiple range test.

† TPF = triphenyl formazan.

‡ Mean value, standard error ($n = 3$) in parentheses.

(Brookes and McGrath, 1984; Chander and Brookes, 1991a) and in laboratory (Leita et al., 1996; Chander and Brookes, 1991b) studies. Failure to observe such a relationship in these soil samples collected in 1995 may have resulted from the fact that the communities have had several decades to adapt to elevated metal loadings (Jordan and LeChevalier, 1975). Site heterogeneity may also have contributed since contrasting data were recorded for comparable soil samples from 1996 (see below).

BIOLOG metabolic profile analysis did differentiate between metal-contaminated and uncontaminated field soils as well as between contaminated soils with varying degrees of metal loadings. Two aspects of the BIOLOG procedure were impacted by metal loading, the rate of color development, and the metabolic profiles. For the most heavily contaminated soil (D), there was a decrease in rate of color development on the BIOLOG plates (Fig. 1). Some researchers have related rate of color development to inoculum size (Haack et al., 1995), but that explanation may not be adequate to explain the delay in color development observed in this study. The same suspension used to inoculate the BIOLOG plates was used to conduct viable plate counts, and samples from site D did not have significantly different viable counts than did samples from sites B or C, even though site D had a slower rate of color development. Therefore, the slower rate of color development in this study may relate to some property of the microbial community other than simply the inoculum size.

There was also another interesting trend in the rate of color development data. There was a significant increase in the rate of color development between 45 and 50 h (Fig. 1). This late increase in rate may be due to a lag in the induction of enzymes necessary to degrade some of the substrates.

Cluster analysis of the BIOLOG data showed a separation of communities from Sites A and B and a clustering together of communities from the two most heavily contaminated sites (C and D) (Fig. 2). Principal component analysis of these data also revealed a separation of all sites due to metal loadings (Fig. 3). Site D, the most contaminated site, showed separation from the

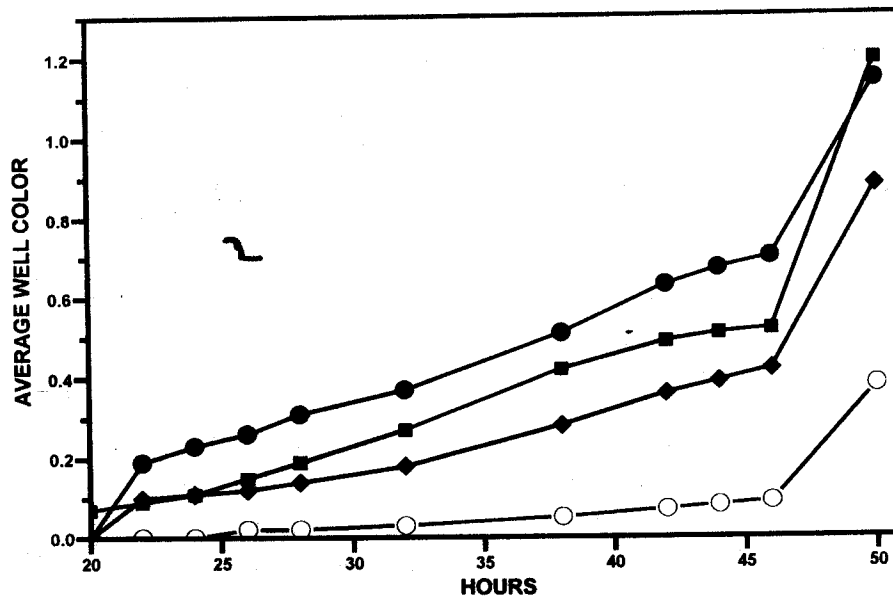


Fig. 1. Comparison of rate of color development on BIOLOG plates for soil samples collected in the Fall of 1995 from four sites in the vicinity of a Zn smelter. Average well color is the mean of the net optical density for all 95 substrate wells on a plate. Each data point represents the mean AWCD value for three BIOLOG extractions from each site (Site A ■, Site B ●, Site C ◇, Site D ○).

other sites along PC 1. Substrates with high correlation coefficients for PC 1 and PC 2 are listed in Table 5. These correlation coefficients, in combination with the PC graph, can be used to determine some of the differences in the metabolic profiles of the sites. For example, succinic acid had a high correlation coefficient (0.875) for PC 1 (Table 5), and based on the graph (Fig. 3) Site D samples tended to have higher scores on PC 1 than Site A samples. Therefore, PCA indicates that Site D samples tended to have higher scores for succinic acid, which means that Site D samples were better able to oxidize succinic acid on the BIOLOG plates. Site D samples also showed a greater ability to oxidize a number of carboxylic acids and amino acids, and a lesser ability to oxidize several carbohydrates, as compared

with the relatively uncontaminated samples from Site A (Table 5).

In addition to the separation of sites, the PCA graph also demonstrates that Site D had the highest degree of variability in its BIOLOG profiles. This suggests the possibility that the metal contamination may be resulting in a community that is more variable and less stable.

To evaluate the reproducibility of the trends observed above, two previously studied unremediated sites plus a new unremediated site were sampled in the summer of 1996 (Table 1) and analyzed by the same methods

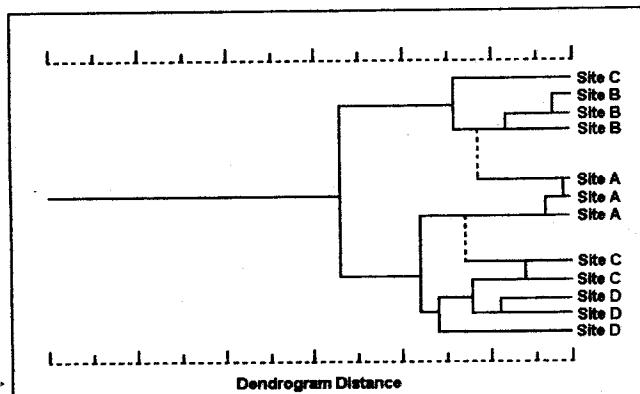


Fig. 2. Cluster analysis of BIOLOG readings for soil samples collected in the Fall of 1995 from four sites in the vicinity of a Zn smelter. Three BIOLOG extractions were conducted per site. Each data point is an average of triplicate plates from each BIOLOG extraction. Sets of triplicate plates with AWCD values closest to 0.25 were compared in this analysis. Each unit of distance on the dendrogram is equivalent to a difference of 100% on a single substrate well.

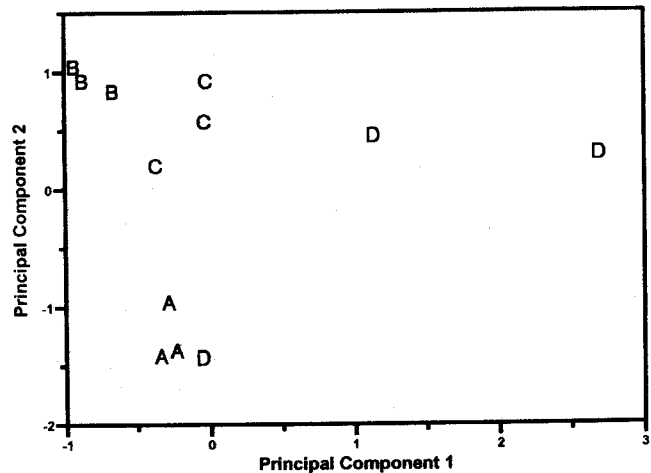


Fig. 3. Principal component analysis of BIOLOG readings for soil samples collected in the Fall of 1995 from four sites in the vicinity of a Zn smelter. Three BIOLOG extractions were conducted per site. Each data point is an average of triplicate plates from each BIOLOG extraction. Sets of triplicate plates with AWCD values closest to 0.25 were compared in this analysis. PC 1 accounted for 31.0% of the variance in the data, and PC 2 accounted for 26.5% of the variance in the data.

Table 5. Substrates with high correlation coefficients for PC 1 and PC 2 in principal component analysis of BIOLOG profiles for sites sampled in the fall of 1995.

PC 1 substrate	Correlation coefficient	PC 2 substrate	Correlation coefficient
Positive correlation			
Carboxylic acids			
Succinic acid	0.875	Propionic acid	0.955
Citric acid	0.860	Acetic acid	0.882
Saccharic acid	0.857	α -Ketoglutaric acid	0.856
p-Hydroxyphenyl-acetic acid	0.811	D-glucosaminic acid	0.825
Cis-Aconitic acid	0.774	α -Ketobutyric acid	0.822
		Malonic acid	0.781
		Bromosuccinic acid	0.768
		Itaconic acid	0.717
		α -Hydroxybutyric acid	0.708
		Sebacic acid	0.701
Amino acids			
γ -aminobutyric	0.956	Amino acids	
L-histidine	0.921	L-leucine	0.850
L-asparagine	0.896	glycyl-L-aspartic acid	0.847
hydroxy-L-proline	0.856	L-threonine	0.789
L-alanylglycine	0.765	L-phenylalanine	0.742
L-glutamic acid	0.731	glycyl-L-glutamic acid	0.704
Carbohydrates			
D-mannitol	0.818	D,L-carnitine	0.703
		Carbohydrates	
		L-fucose	0.925
		Xylitol	0.876
		D-arabitol	0.730
		i-Erythritol	0.706
Polymers			
Tween-80	0.844	Polymers	
Tween-40	0.806	α -Cyclodextrin	0.880
Esters			
Methyl pyruvate	0.934	Amines/amides	
		2-Aminoethanol	0.830
		Succinamic acid	0.746
Aromatic chemicals			
Urocanic acid	0.853		
Negative correlation			
Carbohydrates			
Lactulose	-0.788		
α -D-lactose	-0.786		
D-raffinose	-0.785		
Carboxylic acids			
Formic acid	-0.754		
Polymers			
Glycogen	-0.713		
Amines/amides			
Alaninamide	-0.737		
Alcohols			
2,3-Butanediol	-0.736		
Aromatic chemicals			
Uridine	-0.736		

as the soils described above. Soil organic matter and CEC increased with proximity to the smelter (Table 2), as they did for the soils sampled in 1995. Total and soluble metals again increased with proximity to the Zn smelter (Table 3), and dehydrogenase activity varied with distance from the smelter (Table 4), as was noted previously. However, for the 1996 samples there was an increase in Zn resistance in the more contaminated sites (D, E) compared with the cleaner soil Site (A). This

trend in Zn resistance had not been observed in the soils sampled in 1995, indicating the variability of this parameter in field samples.

The BIOLOG results for the 1996 samples were similar to the results for the 1995 samples. Again, as was noted with the most contaminated site sampled in 1995 (D), color development was much slower for the most contaminated site collected in 1996 (E) (Fig. 4). Differences in community structure were noted by separation of Sites D and E from the less contaminated site (A) on cluster analysis (Fig. 5). Principal component analysis showed some separation of Sites D and E (Fig. 6), although the separation was not as strong as for the 1995 samples. Substrates with high correlation coefficients for PC 1 and PC 2 are listed in Table 6. Site D samples showed a greater ability to oxidize several carboxylic acids and a number of amino acids, and a lesser ability to oxidize several carbohydrates, as compared with the relatively uncontaminated samples from Site A (Table 6). In addition, as was observed for the 1995 samples, the most contaminated site sampled in 1996 (E) showed the highest degree of variability in its BIOLOG profiles (Fig. 6). Samples from Site E showed a high degree of variability in their ability to oxidize several carboxylic acids and one amino acid, L-alanyl-glycine (Table 6).

The data collected for the unremediated sites sampled in 1996 demonstrate that the trends revealed by the early sampling (1995) were consistent upon resampling of the same sites after approximately 1 yr.

Remediated Sites. Concurrent with the repeat sampling of unremediated metal-contaminated sites in the summer of 1996, four remediated sites were sampled and analyzed along with the unremediated soil samples. Two of the remediated sites (F and G) showed elevated total levels for several metals (Table 3). Basic soil properties were affected by addition of the sludge-fly ash remediation material. Amendment of the soils with the remediation material increased the pH of all treated soils to values above 6.0 (Table 2). Note that for sites treated before 1992, limestone was included in the mixture in an amount equal to approximately 22 Mg ha⁻¹. Also, as would be expected with an organic matter-based soil amendment, the organic matter contents of the remediated soils were increased. Only the most recently treated site (I) did not show an increase in organic matter content. The low organic content for Site I would be anticipated because the remediation material was surface-applied and during soil collection any remaining surface organic material was excluded. In contrast, the organic matter content of soils remediated 3 to 10 yr before sampling was elevated due to stimulation of internal organic matter production by washing of soluble organic products of surface organic amendment decomposition into the surface soil as well as leaching of colloidal fractions of the surface organic matter into the soil horizons.

As was the objective of the remediation management plan, amendment of the metal-contaminated soils with the sludge-fly ash remediation material reduced the percentages of total Zn and Cd in the soluble pool by increasing the soil pH and increasing the amount of

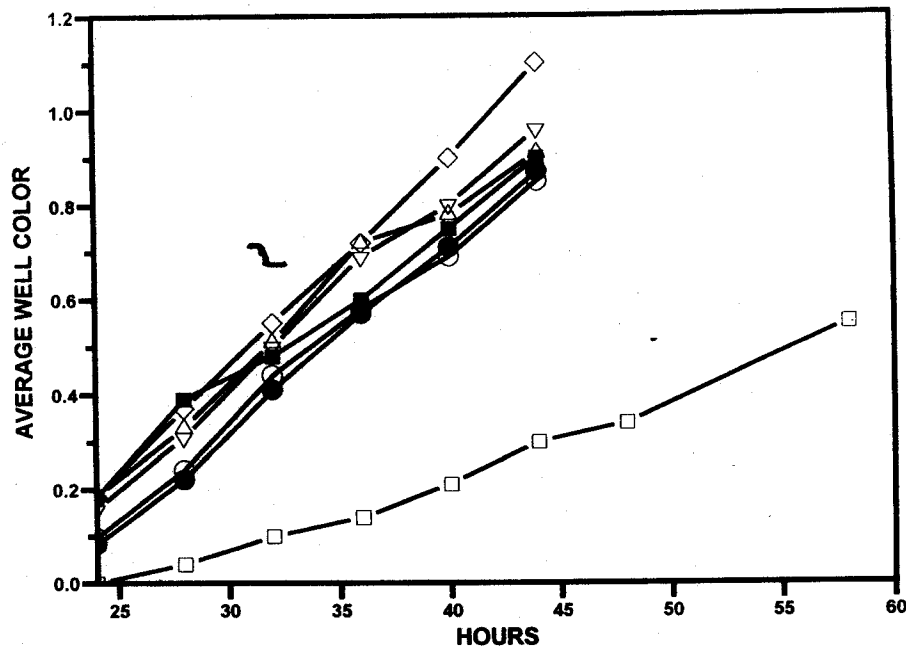


Fig. 4. Comparison of rate of color development on BIOLOG plates for soil samples collected in the Summer of 1996 from seven sites in the vicinity of a Zn smelter. Average Well Color is the mean of the net optical density for all 95 substrate wells on a plate. Each data point represents the mean AWCD value for three BIOLOG extractions from each site (Site A ■, Site D ○, Site E □, Site F ●, Site G △, Site H ▽, Site I ◇)

colloidal organic matter. Although variable with time since remediation, the pH of all remediated soil samples was greater than the unamended soils (Table 2). The total metal levels of the remediated soils were all elevated (Table 3), but with limited exceptions, soluble metal loadings were all reduced, most likely reflecting

the higher pH of the soils. Sites F and G (remediated in 1986 and 1991, respectively), with pH of 6.2 and 6.6, did exhibit the highest total Zn and Cd levels of the remediated soils.

Reduction of soluble metal loadings in remediated soils was reflected by changes in the measures of biological activity. Total viable plate counts and dehydrogenase activity levels were elevated in the remediated soils (Table 4). To provide a more clear test of the capacity of the BIOLOG procedure to differentiate degrees of

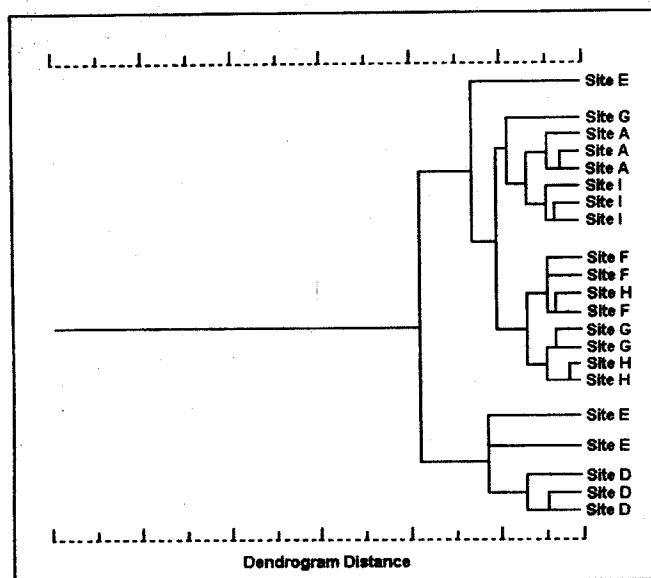


Fig. 5. Cluster analysis of representative BIOLOG readings for soil samples collected in the Summer of 1996 from seven sites in the vicinity of a Zn smelter. Three BIOLOG extractions were conducted per site. Each data point is an average of triplicate plates from each BIOLOG extraction. Sets of triplicate plates with AWCD values closest to 0.25 were compared in this analysis. Each unit of distance on the dendrogram is equivalent to a difference of 100% on a single substrate well.

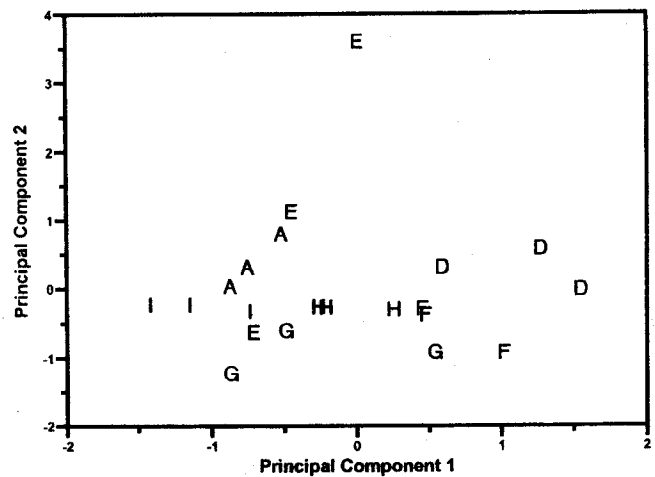


Fig. 6. Principal component analysis of representative BIOLOG readings for soil samples collected in the Summer of 1996 from seven sites in the vicinity of a Zn smelter. Three BIOLOG extractions were conducted per site. Each data point is an average of triplicate plates from each BIOLOG extraction. Sets of triplicate plates with AWCD values closest to 0.25 were compared in this analysis. PC 1 accounted for 17.8% of the variance in the data, and PC 2 accounted for 15.5% of the variance in the data.

contamination and the effect of remediation, untreated and remediated summer 1996 soil samples were analyzed as a group. As noted above, the greatest effect on the rate of color development was detected with the most contaminated, unremediated site (E) (Fig. 4). Note that although remediated Sites F and G had total metal loadings similar to Site E, they did not exhibit the same decreased rate of color development that was observed for Site E. Instead, the rates of color development for remediated Sites F and G were similar to the rates for the less contaminated sites. This result indicates that the remediation may have had a positive effect on the soil microbial communities.

The effect of remediation on BIOLOG metabolic profiles was reflected by clustering of remediated sites closer to the least contaminated site (A) in the cluster analysis (Fig. 5). The most recently treated site (I) clustered most closely to the relatively clean site (A). Principal component analysis of BIOLOG profiles also showed a clustering of remediated sites closer to the

relatively clean site (A). As with the cluster analysis, the most recently treated site (I) clustered most closely to the relatively clean site (A). Substrates with high correlation coefficients for PC 1 and PC 2 are listed in Table 6.

DISCUSSION

The data presented in these studies demonstrate that elevated metal loadings in soils in the vicinity of a Zn smelter have negatively affected soil microbial communities. The combination of soil biological properties evaluated herein was sufficient to reveal this negative impact. However, the data also demonstrate that no single biological property would have been sufficient to assess soil quality. Indeed, evaluation of the variation of individual properties with soil metal loadings could lead to conflicting conclusions, whereas the complete battery of biological parameters suggested in this study has provided a more complete profile of the microbial communities.

As part of this battery of parameters, the BIOLOG assay proved to be a useful tool for examining differences in the structure of microbial communities based on their ability to oxidize 95 different C substrates. Principal component analysis was an effective method for analyzing differences in BIOLOG metabolic profiles. In this study, PCA of the BIOLOG data demonstrated that elevated metal loadings have resulted in changes in the structure of the soil microbial communities, as indicated by the changes in their metabolic profiles.

In addition to the differences in communities with metal contamination, it is perhaps equally interesting to note that even in the most highly metal-contaminated soils, a viable microbial community remains. Although total metal loadings were several thousands of micrograms per gram of soil with generally elevated soluble metal levels, total plate counts were only reduced by approximately two-thirds and total microbial biomass was not reduced significantly in the Fall 1995 soil samples. In Summer 1996, the microbial biomass values paralleled the plate count data. Thus, the data suggest that even in the most severely metal-stressed sites, adequate populations exist to support remediation efforts. Adjustment of the soil physical and chemical parameters into a range more conducive to microbial activity should be sufficient to support recovery of the soil ecosystem at these sites. This hypothesis was supported by analysis of the soil sites remediated at various times.

The remediation method used at the site resulted in increases in indicators of biological activity and viable population size. Remediated soils also showed metabolic profiles that were more similar to the least contaminated site, suggesting recovery of the microbial populations. These improvements in the microbial community with remediation most likely have resulted from decreased solubility of the metals, which resulted in a decrease in the bioavailability and biological toxicity of the metals. The remediation method achieved this decrease in the solubility of the metals by increasing the pH of the soils.

Table 6. Substrates with high correlation coefficients for PC 1 and PC 2 in principal component analysis of BIOLOG profiles for sites samples in the summer of 1996.

PC 1 substrate	Correlation coefficient	PC 2 substrate	Correlation coefficient
Positive correlation			
Carboxylic acids			
α -Ketoglutaric acid	0.737	D-glucosaminic acid	0.861
Quinic acid	0.676	Itaconic acid	0.841
Propionic acid	0.672	Formic acid	0.669
α -Hydroxybutyric acid	0.655	Succinic acid	0.625
Amino acids			
L-aspartic acid	0.812	Amino acids	0.625
L-pyroglutamic acid	0.768	L-alanyl-glycine	
L-leucine	0.748		
Hydroxy-L-proline	0.734		
L-threonine	0.721		
D,L-carnitine	0.719		
L-asparagine	0.704		
L-glutamic acid	0.684		
D-serine	0.673		
γ -aminobutyric acid	0.665		
L-proline	0.639		
L-serine	0.616		
Carbohydrates			
Sucrose	0.714		
Polymers			
α -cyclodextrin	0.627		
Amines/amides			
2-Aminoethanol	0.742		
Phenyl-ethylamine	0.704		
Aromatic chemicals			
Urocanic acid	0.617		
Negative correlation			
Carbohydrates			
Maltose	-0.620		
N-acetyl-D-glucosamine	-0.602		
Carboxylic acids			
D-gluconic acid	-0.717		
Phosphorylated chemicals			
Glucose-6-phosphate	-0.652		
Glucose-1-phosphate	-0.628		

In conclusion, these studies have demonstrated that the combination of traditional measures of soil microbial population size and activity (viable plate counts, biomass, and dehydrogenase) combined with an assessment of community structure (BIOLOG) provided an indication of the status of the biological communities in highly contaminated Zn smelter soils and remediated soils. The data suggest that these microbial parameters may be useful in demonstrating reclamation progress and stability of the developing microbial community.

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