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Microbial biomass and activity in urban soils contaminated with Zn and Pb

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Summary. The effects of heavy metals on microbial biomass and activity were investigated in 30 urban soils, contaminated mainly with Zn and Pb to different extents, in terms of the physicochemical and biological characteristics of the soils. Evaluated by simple and multiple regression analyses, the microbial biomass was not affected significantly by easily soluble Zn+Pb (extractable with 0.1 *N* HCl). The biomass was accounted for as a function of cation exchange capacity (CEC), total organic C and the numbers of fungal colonies present ($R^2 = 0.692$). Carbon dioxide evolution from soils, which reflected microbial activity, was studied on soils incubated with microbial-promoting substrates (glucose and ammonium sulfate) or without. Carbon dioxide evolution was negatively related to Zn+Pb, and this inhibitory effect of the metals was greater in the soils incubated with substrates. Carbon dioxide evolution in soils with substrates was closely related to Zn+Pb, bacterial numbers and the numbers of fungal colonies ($R^2 = 0.718$). Carbon dioxide evolution in soils without substrates was accounted for as a function of Zn+Pb, biomass and the C/N ratio ($R^2 = 0.511$). Using these relationships, the effects of heavy metals on soil microorganisms are discussed in terms of metabolically activated and dormant populations.

Key words: Soil respiration – ATP – Heavy metal effects – Dormant population – Microbial biomass – Urban soils

Most heavy metals are essential micronutrients for microorganisms, plants and animals, including humans. However, in industrialized countries, soil contamination with heavy metals is a serious problem. Heavy metals readily accumulate in the soil during many industrial and agricultural processes, but only small amounts are leached from the soils. The effects of heavy metals on plants and the behavior of the metals in soils have been much studied. Domsch (1984) has reviewed the effects of heavy metals on biological processes and discussed procedures for an ecological assessment of the effects. Microbiological studies of these effects are limited, especially from the ecophysiological point of view.

Heavy metals released through human activities may become immediately available to microorganisms more readily than those occurring naturally. Komai (1981 a) reported heavy metal contamination in urban soils from various parts of Japan. In southern Osaka, there are high concentrations of Zn and Pb in city parks and urban arable lands (Komai 1981 b); we have studied the effects of Zn on soil microorganisms under experimental conditions (Ohya et al. 1985, 1986). We found that carbon dioxide evolution from soil incubated with glucose and ammonium sulfate decreased when Zn was added. In contrast, basal respiration was little affected. The external nutrient input into field soils is generally very limited. Consequently, from an ecological point of view, the finding that Zn does not greatly affect basal respiration is interesting. However, we did not study field soils with a known history of metal contamination.

In addition to microbial activity, microbial biomass and populations are important soil biological parameters in any assessment of the impact of heavy metals. The magnitude of the impact on these parameters can be affected by physicochemical characteristics in soils (Lighthart et al. 1983; Williams and Wollum 1981). To assess the effects of heavy metals on soil microorganisms, it is necessary to take into account all such characteristics.

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Our aim in the present study was to determine the effects of Zn and Pb on biological characteristics, especially microbial biomass and activity, in urban soils contaminated with these metals under field conditions, while taking into consideration the physicochemical characteristics of the soils. We evaluated the effects from an ecophysiological point of view.

Materials and methods

Sampling sites of soils. Thirty soil samples (0–5 cm depth) were collected from arable lands, city parks and near roadsides in Sakai, Osaka. Sakai City is in the southern part of the Hanshin Industrial Megalopolis and faces Osaka Bay on the west. The downtown area of Sakai has been built in alluvial lowland along the bay, and industrial, commercial and residential areas are intermingled. Some areas of the city, especially the northern parts, have been contaminated with heavy metals through atmospheric pollution by industrial and various other activities, including traffic (Komai and Yamamoto 1982).

Soil analysis. The collected soils were ground to pass a 2-mm stainless steel sieve and stored at 5 °C until needed. The textures of the samples were sand (4), loamy sand (10), sandy loam (8), sandy clay loam (4) and loam (4). We measured the pH (H_2O), CEC, total organic C, total N, moisture content and amounts of easily soluble heavy-metals (extractable with 0.1 *N* HCl). The CEC was measured by saturation with NH_4^+, using ammonium acetate and subsequent displacement with Na^+. Total organic C and total N were analysed by combustion on a Yanagimoto CN Corder (NT-500). The moisture content was shown as the percentage of the maximum water-holding capacity of the soil. The extractable heavy metal concentrations were measured by atomic absorption spectrometry. Bacterial numbers and numbers of fungal colonies of the soils were determined with an albumin agar and a rose bengal medium, respectively, by the dilution-plate counting technique described elsewhere (Ohya et al. 1985). The albumin medium contained 0.25 g egg albumin, 1.0 g glucose, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, a trace of $Fe_2(SO_4)_3$ and 15 g agar per liter of distilled water (pH 6.8). The rose bengal medium had 5.0 g peptone, 10 g glucose, 1.0 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.033 g rose bengal and 20 g agar per liter of distilled water (pH 6.8). Bacterial and fungal colonies were counted after 7 and 4 days, respectively.

Measurement of microbial biomass. Microbial biomass was calculated by the adenosine 5′-triphosphate (ATP) content of the soils. The ATP content was measured by a modified version (Tate and Jenkinson 1982) of the Jenkinson and Oades method (1979). A constant factor (5.85 mg ATP/g biomass C) was used to convert the ATP content to biomass C. The soils were kept at 25 °C with the same moisture content as that when the samples were taken 6 days before the ATP measurement, because the constant factor used is applicable only to soils stored in this way (Tate and Jenkinson 1982). The ATP was extracted by a trichloracetic acid-phosphate-paraquat reagent. The extract was diluted with the reagent to eliminate inhibition by co-extracted heavy metals and allowed to react with a mixture of luciferase-luciferin (Sigma Chem. Co.). Light produced during the reaction was measured with a liquid scintillation spectrometer.

Measurement of carbon dioxide evolution. Carbon dioxide evolved from the soils was trapped by NaOH and determined by titration 24 h after the soil incubation, with or without substrates, as described in Ohya et al. (1985). Glucose (5 mg C/g soil) and ammonium sulfate (0.5 mg N/g soil) were added as incubation substrates, and the moisture content of the soils was adjusted to 60% of the water-holding capacity. The soils were incubated at 28 °C in the dark. Carbon dioxide evolution from the soils incubated with substrates was shown as the increment of that without substrates.

Statistical analysis. The physicochemical and biological soil characteristics were used for simple and multiple regression analyses. In the selection of predictor variables in the multiple regression analysis, forward selection, backward elimination and stepwise-forward regression methods (Kawabata 1984) were used, taking 2.00 as the *F* value. The most suitable form for the multiple regression was chosen on the basis of the adjusted *R*-square, the Mallows *Cp* statistics (Mallows 1973), and the Akaike information criterion (Tanaka et al. 1985).

Results and discussion

Effects of heavy metals on biological characteristics evaluated by simple regression

The ranges, mean values and coefficients of variation of the physicochemical and biological characteristics of the soil samples are reported in Tables 1 and 2. The mean values of easily soluble Zn and Pb were 860 and 265 μg/g, respectively. In Japan, the mean values of natural occurrence of Zn and Pb in arable soils are 86 and 29 μg/g, respectively (Iimura 1981). Easily soluble Zn and Pb were significantly correlated with each other at $\alpha = 0.001$ ($r = 0.813$). The analysed characteristics and the sum of easily soluble Zn and Pb were used as soil parameters for simple and multiple regression analyses.

Table 1. Range, mean, and coefficient of variation (CV) of physicochemical characteristics of 30 urban soils

	pH (H_2O)	CEC[a] (meq/100 g soil)	Total organic C (%)	Total N (%)	C/N	Moisture content (%)[b]	Easily soluble[c] (μg/g)	
							Zn	Pb
Range	5.3–8.5	2.4–13.5	1.79–12.5	0.13–1.15	4.9–20	12–35.2	49–4680	3–1510
Mean	7.0	7.2	5.65	0.55	11	25.7	860	265
CV (%)	10.0	39.3	49.2	51.7	28.9	22.8	139	144

[a] Cation exchange capacity
[b] Percentage of water-holding capacity
[c] Extracted with 0.1 *N* HCl

Table 2. Range, mean, and coefficient of variation (CV) of biological characteristics

	Bacterial numbers ($\times 10^6$/g soil)	Numbers of fungal colonies ($\times 10^4$/g soil)	Biomass C (μg/g soil)	CO_2 evolution (mg/100 g soil)	
				With substrates[a]	Without substrates
Range	0.33 – 54	1.6 – 37	33 – 439	80.5 – 1080	1.5 – 141
Mean	20	9.3	168	569	42.5
CV	72.7	81.2	58.7	59.5	77.1

[a] Glucose and ammonium sulfate added as substrates

Table 3. Correlation coefficients by simple regression analysis of biological characteristics

	Bacterial numbers	Numbers of fungal colonies	Biomass C	CO_2 evolution	
				With substrates	Without substrates
pH (H_2O)	−0.257	−0.262	−0.037	−0.103	0.177
Cation exchange capacity	0.042	−0.028	0.732[b]	0.346	0.432
Total organic C	0.205	0.459[a]	0.636[b]	−0.105	0.337
Total N	0.075	0.401	0.676[b]	−0.056	0.216
C/N	0.201	−0.071	−0.405	−0.304	−0.049
Moisture content	0.282	0.304	0.113	−0.440	−0.029
Easily soluble Zn + Pb	0.127	0.221	−0.356	−0.735[b]	−0.569[b]
Bacterial numbers	–	0.245	0.127	−0.486[a]	0.047
No. of fungal colonies	0.245	–	0.321	−0.110	0.065
Biomass C	0.127	0.321	–	0.194	0.541[a]

[a] Significant at $\alpha = 0.01$
[b] Significant at $\alpha = 0.001$

Coefficients obtained by simple regression analysis indicate the extent and direction of the effects of physicochemical characteristics, in addition to the heavy metal content, on the biological characteristics of urban soils (Table 3). Bacterial numbers were not significantly related to any parameters, including the heavy metals. The numbers of fungal colonies were positively related to total organic C, but not to the heavy metals. These results suggest that the inhibitory effects of heavy metals cannot be evaluated in terms of total microbial populations in urban soils, which agrees with the results of other studies (Pancholy et al. 1975; Olson and Thornton 1982). Nevertheless, the proportions of metal-tolerant bacteria to the total population are quantitatively related to the soil metal content (Olson and Thornton 1982; Ohya et al. unpublished data 1986). According to Yamamoto et al. (1981, 1985) the numbers of fungal colonies are positively related to the soil metal content, and the metal-tolerant proportion of the population increases with the metal content. In contrast, Pancholy et al. (1975) found a significantly larger fungal population in a control compared with a metal-polluted site. These conflicting results probably arise because the metal-tolerant microbial populations measured were developed to different extents. Total microbial populations may be used as an index of the inhibitory effects of metals when compared with a non-contaminated sample of the same soil.

Microbial biomass C was positively related to CEC, total organic C and total N, but not significantly to the heavy metals. Carbon dioxide evolution in soils incubated with microbial-promoting substrates was negatively related to easily soluble metals and bacterial numbers. Carbon dioxide evolution in soils without these substrates was also related to the metals, but the coefficient was lower than for soils with the substrates (significant at $P = 0.01$). Basal respiration was positively related to biomass C as was expected.

Effects of heavy metals on biomass and activity evaluated by multiple regression

Inhibitory effects of heavy metals on soil microorganisms are dependent not on the total metal content in the soils but on the biologically effective amount, which is largely controlled by the physicochemical characteristics of the soils. Since the effects of heavy metals on biological characteristics of soils cannot be simply elucidated, multiple regression analysis was used to determine the effects on microbial biomass and activity. In the analysis, if soil parameters are significantly related to each other by simple regression, one must be eliminated from the variables. Total

Table 4. Correlation coefficients by multiple regression analysis[a] of microbial biomass and activity

	Biomass C	CO_2 evolution	
		With substrates	Without substrates
Multiple correlation			
R-square	0.692	0.718	0.511
Adjusted *R*-square	0.657	0.685	0.454
AIC[b]	334	406	282
*C*p statistics[c]	3.34	2.75	−0.233
Partial correlation			
Cation exchange capacity	0.695	–	–
Total organic C	0.342	–	–
C/N	–	–	0.320
Easily soluble Zn+Pb	–	−0.794	−0.523
Bacterial numbers	–	−0.619	–
No. of fungal colonies	0.316	0.266	–
Biomass C	–	–	0.517

[a] Parameters in Table 3, except for total N, were used as the full model
[b] Akaike information criterion (Tanaka et al. 1985)
[c] Mallows (1973)

organic C was significantly related to total N at $\alpha = 0.001$ ($r = 0.886$), and we therefore decided to eliminate total N from the variables, because our present interest was in the C cycle. Consequently, nine parameters (pH, CEC, total organic C, C/N, moisture content, bacterial numbers, numbers of fungal colonies, easily soluble Zn+Pb and biomass C) were used to construct a full model of the variables. Then, for the analysis of biomass C, for example, the other eight parameters were used as the full model.

Table 4 shows the multiple and partial correlation coefficients of biomass C and carbon dioxide evolution with or without substrates in the most suitable model, the selection of which was based on the adjusted *R*-square, the Akaike information criterion and *C*p statistics. Generally, for the best model, it is preferable to adopt the variables that enlarge the adjusted *R*-square and diminish the Akaike information-criterion and the Cp statistics, or both. Biomass C was defined as a function of CEC, total organic C and numbers of fungal colonies in the best model, and these variables accounted for about 70% of the biomass C. The regression equation for the relationship is as follows:

$$\text{Biomass C} = 21.7(\text{CEC}) + 9.35\ (\text{total organic C}) + 63.6(\text{numbers of fungal colonies}) - 349$$

The standard partial regression coefficients for CEC, total organic C and numbers of fungal colonies were 0.622, 0.263 and 0.217, respectively, and CEC was the most significantly related to biomass C ($r = 0.695$). These results indicate that microbial biomass is primarily and positively influenced by CEC but little affected by easily soluble Zn+Pb.

Carbon dioxide evolution in soils incubated with substrates was related to Zn+Pb, bacterial numbers and numbers of fungal colonies; their partial correlation coefficients were −0.794, −0.619 and 0.266, respectively. The following regression equation accounted for 72% of the carbon dioxide evolution in soils incubated with substrates:

Carbon dioxide evolution in soils with substrates = −391 (easily soluble Zn + Pb) − 333 (bacterial numbers) + 154 (numbers of fungal colonies) + 3240

The standard partial regression-coefficients for Zn+Pb, bacterial numbers and numbers of fungal colonies were −0.714, −0.433 and 0.154, respectively. These results mean that most of the reduction in carbon dioxide evolution in soils incubated with substrates is explained by easily soluble Zn+Pb, and that the evolution is not closely related to biomass C.

In contrast, carbon dioxide evolution in soils incubated without substrates was predictable by the following equation:

Carbon dioxide evolution in soils without substrates = −24.5 (easily soluble Zn + Pb) + 0.160 (biomass C) + 2.66 (C/N) + 51.0

The standard partial regression coefficients of easily soluble Zn+Pb, biomass C and C/N were −0.462, 0.482 and 0.260, and the partial correlation coefficients were −0.523, 0.517 and 0.320, respectively. These results suggest that easily soluble Zn+Pb and also biomass C affect carbon dioxide evolution to a similar extent in soils without substrates but in different directions. However, only 51% of the carbon dioxide evolution in soils without substrates could be accounted for by the above variables.

Ecophysiological aspects of the heavy metal effects on microorganisms

In soil ecosystems, microbial biomass and activity are particularly important parameters in an evaluation of the effects of heavy metals on soil microorganisms. From the ecophysiological point of view, microbial biomass comprises "metabolically active" and "dormant" populations. The dynamic equilibrium of microorganisms between the metabolically activated and dormant states seems to play a key role in the soil ecosystem. Microorganisms in the dormant state are vegetative cells with reduced metabolic activity, and under field conditions, most of the total biomass is thought to be dormant for most of the year (Gray and

Williams 1971). Biomass C is probably associated mostly with the dormant population. We found that carbon dioxide evolution without an added C source, the so-called basal respiration, was related to the biomass (Tables 3 and 4), as we expected. This indicates that the major parts of the biomass and of the basal respiration were correlated with the dormant population. In contrast, carbon dioxide evolution in soils with substrates is generally attributed mostly to the metabolically activated population. Our results showed that microbial biomass and basal respiration were only slightly affected by the easily soluble metals, although carbon dioxide evolution in soils with substrates was considerably influenced by the amount of heavy metals. From an ecophysiological viewpoint, these results suggest that the dormant population is less sensitive to inhibition by the metals than the metabolically activated one. It is likely that the amount of heavy metals taken up determines the toxicity to the microorganisms (Sterritt and Lester 1980), since heavy metal uptake by dormant microbial cells is doubtless slight compared with the metabolically active cells. For organisms that cannot tolerate metals, in particular the dormant state may have survival value in helping microorganisms to avoid heavy metal toxicity.

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