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Soil Ergosterol, Dimethyl Sulphide Reduction, and Microbial Biomass Along a Zn Concentrations Gradient in Soils from a Mine Spoil Tip

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Several *in situ* methods provide alternative approaches to estimate the effect of heavy metals on soil microbial biomass and microbial activity in either long-term field experiments or in polluted soils. These methods include microbial biomass measured by Fumigation-Incubation (FI), Fumigation-Extraction (FE), Substrate-induced respiration (SIR) soil ATP, and microbial activity by CO_2, arginine ammonification rate and dehydrogenase activity (Brookes and McGrath 1984; Chander and Brookes 1991a; Bååth et al. 1991; Fritze et al. 1989; Barajas Aceves et al. 1999). However, it is seldom known whether the observed effects are due to changes in species composition or to reduced physiological capacity of the microbial community (Chander and Brookes 1991b). Bacteria and fungi might be differently affected by heavy metals content in soils (Hiroki 1992).

Research efforts currently are underway to develop techniques for determining the structure of the microbial community in soils, such as analysis of fatty acids, ergosterol, DNA, etc. In comparison to other chemical methods, ergosterol has been found to be a more sensitive indicator of changes in fungal population (West et al. 1987). Similarly, Sparling and Searle (1993) reported that dimethyl sulphide reduction rate (DMSO) was more sensitive than the SIR assay to the presence of As andCr.

The first aim of this study was to test and compare soil ergosterol content and microbial activity by DMSO in soils contaminated with heavy metals, predominantly Zn, in a gradient of increasing concentration. The second aim was to determine the relationship between ergosterol, DMSO and microbial biomass measured by different methods including Fumigation-extraction (biomass C) and substrate-induced respiration (SIR).

MATERIALS AND METHODS

The area selected for this study is located near Alditurri Mine in the valley of the river Oyarzun in Gipuzkoa, Spain (Barajas Aceves et al. 1999). Mainly Zn and Pb, the major metals obtained from the mine contaminated the soil of the valley. The soils were characterized by different organic C content, clay content, cultivation and topography. Near the edge of the river (5 to 200m distances) more

than 6500 µg Zn g^{-1} soil has been found and decrease up to 286 µg Zn g^{-1} soil (200m distance). A full description of the site is given in Barajas Aceves et al. (1999).

Eleven soil samples (0-23cm depth) were collected along a pollution gradient perpendicular to River Oyarzun during two sampling periods. Five soils samples were collected (5, 20, 40, 60 and 105 m distance) in November 1992 (transect 1A). In other to obtain more information, another six samples were obtained (15, 30, 50, 70, 105 and 200 m distance) at the beginning of March 1993 (transect 1B). The temperature was similar at both sampling times (10°C) but the weather was drier in March than in November. The cores from each sampling point were bulked, plant material, stones and visible fauna discarded and then stored at 4°C for about 4 weeks prior to analysis of biomass C, SIR and DMSO in transect 1A and 1B and 10 weeks for ergosterol analysis in transect 1A and 5 weeks in transect 1B. The soil samples were divided into two fractions, one of which was sieved to <2mm and the other was air-dried and ground to 160 pm in a Tema mill for soil analysis.

The sieved soils from each sampling point of the transect were adjusted to 40% of water holding capacity (WHC). Sub-samples, each equivalent to 50 g oven-dry soil (105°C, 24 hr) were placed in 60 mL glass bottles which were then put into 1 L jar each containing 10mL water, to avoid desiccation. The jars were sealed and incubated at 25°C in the dark for 10d prior to microbial biomass measurements. Microbial biomass C was determined by the fumigation-extraction (FE) method (Vance et al. 1987; Wu et al. 1990).

Biomass C was also measured by SIR (Anderson and Domsch 1978) as outlined by Lin (1994). The analyses of CO_2 by GC (Ai 93 chromatogram, Ai Cambridge, UK). Carbon dioxide was determined on a thermal conductivity detector, using the method of Hall and Dowdell (198 1).

Ergosterol was determined by the procedure of Grant and West (1986) with minor modifications (Hart and Brookes 1996). In order to calculate the recovery of ergosterol by the extraction procedure, 1 ml of a 145 µg mL^{-1} solution of re-crystallised ergosterol (Aldrich, Gillingham, UK) in HPLC-grade methanol, was added to an additional portion of soil at the same time as the methanol and extracted exactly as described the procedure (Hart and Brookes 1996). Ergosterol in the methanol solutions, ergosterol recovery and ergosterol standards (2 -10 µg mL^{-1} in HPLC grade methanol) were measured in a LDC Analyst series 7800 HPLC using methanol: water (HPLC grade) (98:2 v/v) as the mobile phase with a flow rate of 1.5 min^{-1} and uv detection at 282 nm.

Dimethyl sulphoxide reduction rate (DMSO) was measured by the method of Alef and Kleiner (1989). The dimethyl sulphide (DMS) produced was estimated in a Perkin Elmer 3 gas fitted with a flame ionization detector and using a column (2m) Haye Sep R. The temperature of the detector, injector and column were 220, 200 and 160°C respectively. The concentration of DMS in soils was obtained by a DMS calibration curve (50-200 ng mL^{-1}).

Organic C was determined by dichromate digestion (Kalembasa and Jenkinson 1973) and metal concentrations were determined using inductively coupled plasma optical emission spectrometry after digestion of the soils using Aqua Regia (McGrath and Cunliffe 1985). The clay contents of the soils were determined by the pipette method (Tanner and Jackson 1947). 'High-metal' soils were defined as those containin more than 400 µg Zn g^{-1} soil and 'low-metal' soils those below 400 µg Zn g^{-1}. The value was at current permitted soil metal concentrations (currently 300 µg Zn g^{-1} soil) in agricultural soils (Commission of the European Communities, 1986).

All measurements are the mean analysis of triplicate determinations and are given on an oven-dry basis (105°C, 24 hr). Statistical analyses were performed using SAS statistical analysis package (SAS, 1988).

RESULTS AND DISCUSSION

Soil ergosterol was significantly correlated with soil organic C (r=0.82, P<0.5) but not with biomass C, percentage of clay or Zn concentration (Table 1). However microbial biomass measured by FE (biomass C) and SIR and microbial activity by DMSO were positively correlated with clay content (r=0.92, 0.95 and 0.77 respectively, P<0.001). While the paired correlations between biomass C, SIR or DMSO and clay plus Zn gave slightly higher correlations (r=0.97, 0.98 and 0.92 respectively, P<0.001) (Table 1). Ergosterol was positively correlated with soil organic carbon (r=82, P<0.001) and was similar to the paired correlation with soil organic C plus Zn (r= 0.81, P<0.001).

Figure 1 show ergosterol content (a) and dimethyl sulphoxide reduction rate (b) along a gradient of Zn concentration. Soil ergosterol content (Fig la) accounted for 0.67 of the coefficient of correlation with Zn concentration in a second-degree polynomial correlation. The changes of ergosterol content decreased slowly at low levels of Zn, slower at intermediate levels (2000-4000 µg g^{-1} soil) and increased positively at high levels of Zn concentrations (>6000 µg g^{-1} soil). The results of high content of ergosterol (Fig. la) and percentage of soil organic C in the high concentration of Zn in sandy soils (see Barajas Aceves et al. 1999) and the high correlation coefficient of ergosterol and percentage of soil organic matter (Table 1) indicate that not only organic matter decomposition was affected by the high concentration of heavy metals but also soil ergosterol content.

Although the ergosterol recovery from soil transect 1A was very similar to that of 1B (average 89.5%) the values of soil ergosterol were different on these two sampling periods; the results have not been pooled, but treated separately (data not shown). Thus, the ergosterol ratio (ergosterol in transect 1A/ ergosterol in transect 1B) in every soil sample with similar Zn concentration and soil texture ranged from 3.13 to 6.45 (average 4.9) while biomass C, SIR and DMSO ratios were remarkably similar with the mean of 1.32, 1.12 and 1.52 respectively. This significant difference in ergosterol content in soils from transects 1A and 1B might be due to the rain increasing the fungal population in transect 1A.

Table 1. Correlation matrix and the best pair of multiple linear regression between microbial biomass and microbial activity with soil parameters compared by linear regression.

	Organic C (%)	Clay (%)	Zn (μg g^{-1} soil)
Clay			-0.88**[c]
Organic C		0.49ns	0.060ns
Biomass C	0.58*	0.92**	-0.73**
SIR[a]	0.47ns	0.95**	-074**
Ergosterol	0.82**	0.20ns	0.10ns
DMSO[b]	0.72**	0.77**	-0.60*

Biomass C = -11.77 + 34.10(organic C) + 17.48 (clay)	0.97**
Biomass C = 22.03 + 200.24(organic C) – 0.057(Zn)	0.94**
Biomass C = -5.00 + 21.17 (clay) + 0.007(Zn)	0.97**
SIR[a] = -0.98 + 17.78(organic C) + 12.23(clay)	0.98**
SIR = 19.76 + 126.26(organic C) – 0.032(Zn)	0.90**
SIR = -3.10 + 13.86(clay) + 0.007(Zn)	0.98**
Ergosterol = -0.23 + 1.23(organic C) –0.044(clay)	0.82**
Ergosterol = 0.217 + 0.597 (organic C) + 0.0002(Zn)	0.81**
Ergosterol = 0.53 + 0.03(clay) + 0.0004(Zn)	0.70**
DMSO = 15.92 + 61.16(organic C) + 4.71(clay)	0.94**
DMSO = 7.22 + 129.06(organic C) –0.031(Zn)	0.96**
DMSO = -2.13 + 12.93(clay) + 0.012(Zn)	0.92**

[a] Substrate induced respiration
[b] Dimethyl sulphoxide reduction rate. ns = not significant.
[c] values marked with ** or * are significant at 0.001 and 0.05 probability levels respectively

This suggestion also agrees with Söderström (1979) who reported that the amount of metabolically active biomass measured by fluoresceine diacetate (FDA) was correlated with soil moisture content. These data imply that fungal ergosterol is rapidly degraded in soil after fungal death in air-dried soils.

Because of concern that the slight difference storage conditions and storage time may themselves have caused changes in soil ergosterol contents, sample number 5 transect 1B was also analyzed with the soils of transect 1A after 4 weeks from the first analysis of ergosterol. The ergosterol content was 2.9 μg g^{-1} soil at the first analysis and 2.7μg g^{-1} soil at the second analysis. These similar results suggest that soil ergosterol was not affected by the storage and time conditions. These findings are consistent with West et al. (1987) who reported moderate losses after four weeks storage of hydrated soils (ranged from 5.5 to 19 % losses).

There is currently little information on the relationships between soil ergosterol and microbial biomass content and whether the ratio (ergosterol)/(biomass) is constant enough for ergosterol to be a useful biomarker. The fate of ergosterol either added or from dead cells (Lin 1994, Hart and Brookes, 1996) is not yet

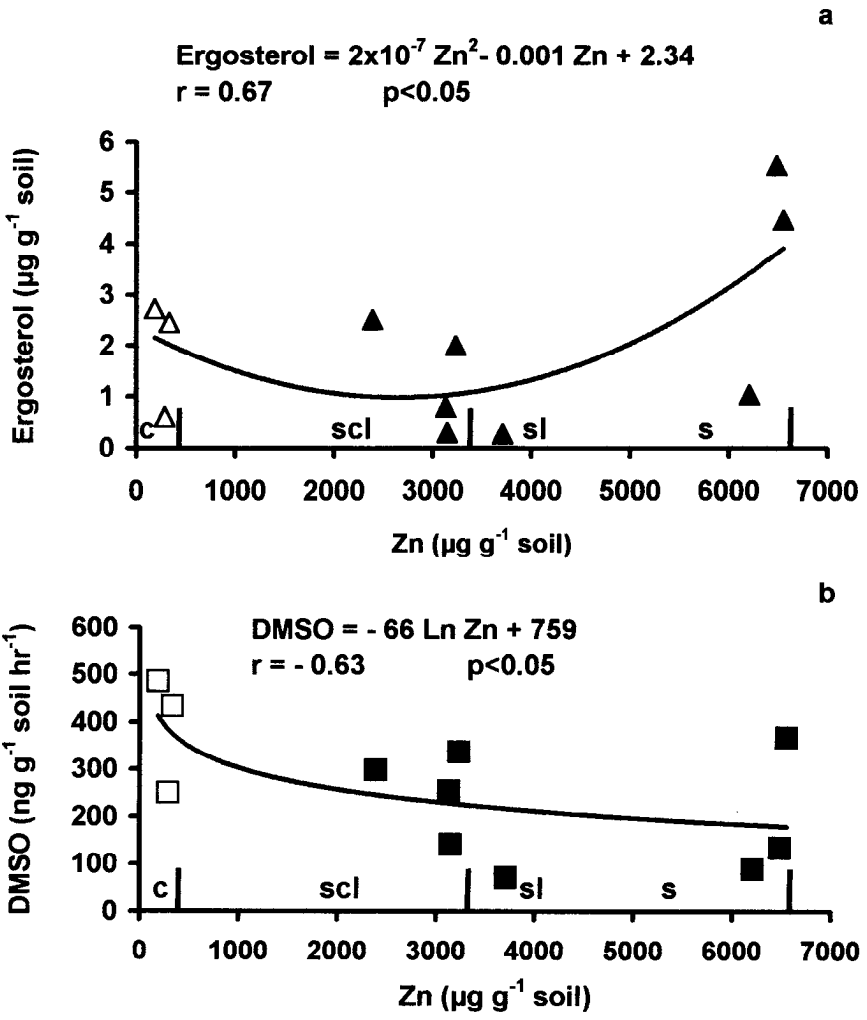

Figure 1. Soil ergosterol content (a), dimethyl sulphoxide reduction rate (DMSO) (b) and soil texture (s=sandy, sl=sandy loam, scl=sandy clay loam, c=clay) in low- (open) and high-metal (closed) soils from Gipuzkoa, Spain.

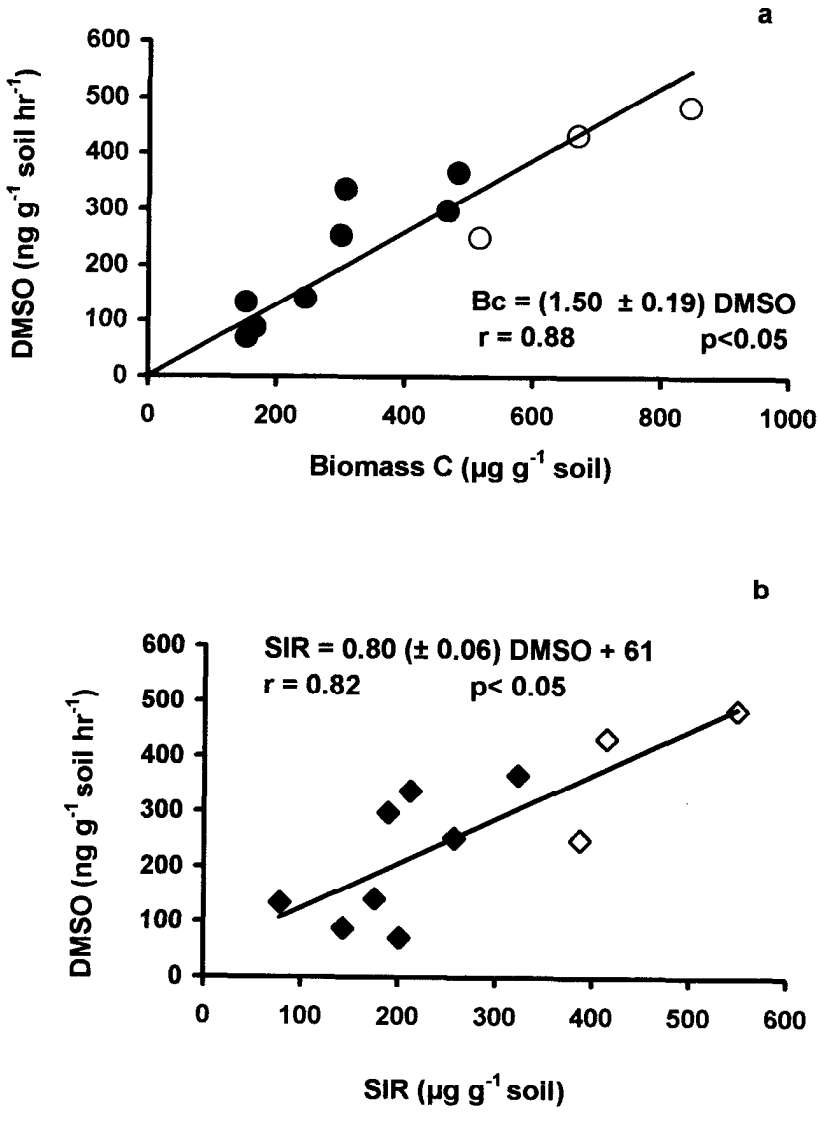

Figure 2. Correlations between microbial biomass C and dimethyl sulphoxide reduction rate (DMSO) (a) and SIR (b) in low- (open) and high-metal (closed) soils from Gipuzkoa, Spain

clear in soils. The results from this work indicate that further work is required to determine if ergosterol correlates with biomass C in long-term experimental soils contaminated with heavy metals, where soil heterogeneity is less than in soil from natural environments.

The amount of DMSO in soil (Fig lb) was highly significant and negatively logarithmic correlated with soil Zn concentration ($r=-0.63$, $P<0.05$) and gave only a slightly higher correlation coefficient than that for linear correlation (Table 1). Comparable trends of DMSO were found for biomass measured by Fumigation-extraction and SIR methods (data not shown). There was a highly significant linear correlation between biomass C and DMSO (Fig. 2a). There was also a linear correlation between SIR and DMSO (SIB = 0.80 DMSO + 60, $r=0.82$, $p<0.001$) (Fig. 2b), similar to that reported by Sparling and Searle (1993) (SIR = 0.72 DMSO + 220, $r=0.81$) obtained from 45 soils representing a variety of soil type and land uses.

In this work, the correlations between DMSO and biomass C by FE and SIR were consistent and linear over different Zn concentrations and soil types. This suggests that DMSO can provide a rapid measure of microbial activity in low- and high-metal soils from the natural environment and that microbial biomass has the same ratio DMSO/biomass C in uncontaminated and contaminated soils.

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