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The impact of the Almalyk Industrial Complex on soil chemical and biological properties

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Soil nematodes and microbes are suitable biomonitors for metals in soils.

Abstract

The effect of heavy metals on soil free-living nematodes, microbial biomass (C_{mic}) and basal respiration (BR) was studied along a 15 km downwind deposition gradient, originating at the Almalyk Industrial Complex. Soil samples from $0-10$ and $10-20$ cm layers were collected at 5 km intervals. A significant decrease in heavy metal deposition was found going from the source in the downwind direction and with depth. The soil microbial biomass, basal respiration and derived microbial indices for soil samples from the Almalyk industrial area were analysed. The lowest soil microbial biomass and total number of free-living nematodes were found in soil samples near the industrial complex, with a high heavy metal and weak total organic carbon (C_{org}) content. The highest C_{mic} was found in the soil samples collected 15 km from the pollution source. BR displayed similar results. The derived indices, metabolic quotient (qCO₂) and microbial ratio ($C_{\text{mic}}/C_{\text{orge}}$), revealed significant differences with distance, confirming environmental stress in the first and second locations. The present study elucidates the importance of soil nematode and microbial populations as suitable tools for bio-monitoring the effect of heavy metals on soil systems.

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Keywords: Heavy metals; Soil; Contamination; Bioindicators

1. Introduction

Metal mining and smelting activities are important sources of heavy metal air pollution, and result in considerable soil contamination ([Davies, 1983; Alloway,](#page-8-0) [1990](#page-8-0)). The main pollutants, including Pb, Zn, Cu, Cd, and As, are usually dispersed in relatively higher concentrations in the vicinity of an industrial complex, with a gradual depletion as distance from the source increases [\(Thornton et al., 1980; Kabata-Pendias and](#page-9-0) [Pendias, 1989; Verner et al., 1996; Wilcke et al., 1999\)](#page-9-0). According to [Steinnes et al. \(1997\); Loska et al. \(2003\),](#page-9-0)

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long-range atmospheric transport is one of the main factors for dispersion and deposition of a wide range of pollutants.

The long-standing Almalyk mining and smelting industrial facility in Uzbekistan is one of the heavy metal industrial complexes which was found to contaminate the soil in the surrounding area with increasing levels of Pb, Cu, and Zn [\(Talipov et al., 1997; National](#page-9-0) [Report on State of the Environment and Use of Natural](#page-9-0) [Resources, 1998; State Committee for Nature Protection](#page-9-0) [of the Republic of Uzbekistan, 2000](#page-9-0)). [Shukurov \(1999\)](#page-9-0) studied heavy metal emission effect and reduction of negative impacts on a 20 km transect starting at the industrial complex, and showed that the levels of pollutants in the upper layer $(0-20 \text{ cm})$ of soil decreased gradually.

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[Suter et al. \(2000\)](#page-9-0) emphasised the importance of using bioindicators in assessing ecological risks and not only to relay additional information on the soil physicalchemical properties in soil surveys.

Properly chosen parameters allow both the identification of current changes in the soil and the prediction of future changes. For these purposes, the use of microbial parameters seems to be very promising, because of their well-accepted importance as indicators of soil quality [\(Tate, 2000](#page-9-0)). Many studies have been published in which changes in soil microbial parameters gave early warning of decreasing soil quality (e.g., [Powlson et al., 1987; Kandeler et al., 1999\)](#page-9-0). Parameters describing the amount, activity, and diversity of soil microorganisms are also used as biological indicators of soil quality and health ([Doran and Parkin, 1996;](#page-8-0) [Sparling, 1997; Stenberg, 1999](#page-8-0)), integrating the chemical and physical properties of the ecosystem ([Oberholzer](#page-9-0) [et al., 1999](#page-9-0)).

Soil microorganisms are usually studied and monitored at the process and biomass levels. The process level includes overall activities of the soil microorganisms, especially respiration ([Sparling, 1997; Alon and](#page-9-0) [Steinberger, 1999; Sarig et al., 1999\)](#page-9-0). At the biomass level, the entire microbial community is evaluated as a single mass of microbial matter, without specification of its structure. It is well known that heavy metal contamination reduces soil respiration [\(Hattori, 1992\)](#page-8-0), microbial biomass ([Brookes et al., 1984; Brookes and](#page-8-0) [McGrath, 1984; Chander and Brookes, 1991; Klumpp](#page-8-0) [et al., 2003\)](#page-8-0), nematode density and that it affects trophic interactions [\(Vig et al., 2003; Caussy et al., 2003\)](#page-9-0).

[Baath \(1989\)](#page-8-0) studied the effect of heavy metals on microbial biomass and nematode density and showed that Cd, Cu, Zn, Pb, and As significantly affect the size and dynamics of the above populations.

A number of soil microbiological parameters, notably microbial biomass carbon and basal respiration ([Doran](#page-8-0) [and Parkin, 1996; Sparling, 1997\)](#page-8-0), have been suggested as possible indicators of soil environmental quality, and are employed in national and international monitoring programs. Soil microbial biomass, which plays an important role in nutrient cycling and ecosystem sustainability, has been found to be sensitive to increased heavy metal concentrations in soils [\(Giller et al.,](#page-8-0) [1998\)](#page-8-0). Furthermore, the soil free-living nematode population has been used as a bioindicator because of its ubiquity, known response to chemical and physical perturbations to soil and water, and current consideration in regional and national monitoring programs ([Elliott, 1994; Gupta and Yeates, 1997; Neher, 2001;](#page-8-0) [Heinz, 2002\)](#page-8-0).

The aim of this study was to examine heavy metal impact on the soil microbial and nematode population along the emission gradient of the Almalyk Industrial Complex, Uzbekistan.

2. Materials and methods

2.1. Study site and locations

The study site is a semi-arid area located between the mountain ranges of Qurama in the south and Chatkal in the north (Tien-Shan), extending into the southeast part of the Tashkent region of the Republic of Uzbekistan. The industrial complex includes a mining processing enterprise, metallurgical and chemical plants located in the flat bottom of the Akhangaran River valley near the city of Almalyk $(40°85' - 69°69'E)$ [\(Fig. 1\)](#page-2-0).

The Almalyk Mining and Metallurgical Combine (AMMC) is the main source of environmental pollution in this area. As one of the largest mining companies in Uzbekistan, it produces refined copper, gold, silver, lead, metallic zinc and other products, and has a capacity for mining and processing approximately 25 million tons per year). This plant has an annual metal-producing capacity of Cu=130,000, Zn=40,000, and Pb=80,000 tons per year [\(Levine, 1999](#page-9-0)). Due to a lack of efficient air-treatment facilities for copper smelting, the complex is also a major source of air pollution. According to an environmental report that was prepared in 2000, the Almalyk mining and smelting complex was found to emit about 100,000 tons of toxic substances (sulphur dioxide, carbons, nitrogen oxides, sulphuric acid, heavy metals, arsenic, etc.) per year. This is approximately 13% of all of Uzbekistan's air emissions from stationary sources ([UNESCO, 2001](#page-8-0)).

The research area represents a mountain-valley area with a large variability of seasonal and daily air temperature and wind direction. Thermal inversions provide cyclic circulation of air masses and cause pendulum distributions of dust and gas-smoke emissions from the industrial enterprises. The prevalent wind at the study site is in a western and southwestern direction.

The climate is continental; minimal temperature measured between -25 and -30 °C in January and maximal temperature reached $42-47$ °C in July. Annual rainfall at the study site ranged between 320 and 550 mm. Most of the precipitation fell in spring and winter ([Information Agency Jahon of the Ministry of Foreign](#page-8-0) [Affairs of the Republic of Uzbekistan, 2003\)](#page-8-0).

The vegetation cover along the study site is dominated by annual and perennial plants, where the most common are: Astragalus, Stipa, Medicago and Artemisa genera. The soils at the study area belong to the lithosols $(FAO, 2003)$ $(FAO, 2003)$, with high levels of $CaCO₃$ contributing to a stable accumulation of heavy metals on top of the soil layer.

2.2. Soil sampling and extraction methods

Soil samples were collected along a 15 km transect, in a downwind direction from the industrial complex

Fig. 1. Location of the study sites along the transect from the Almalyk Industrial Complex (A, 0 km; B, 5 km; C, 10 km; D, 15 km from the complex).

emission sources. The soil samples were collected from the $0-10$ and $10-20$ cm layers at 0 (location A, at the industrial complex), 5 (location B), 10 (location C) and 15 (location D) km along the transect. Five random soil samples were collected at each location, placed in individual plastic bags and transported to the laboratory for chemical and biological component determination. The soils were kept in cold storage at $4 \degree C$ and were sieved though a 2-mm mesh sieve before biological and chemical analyses.

2.3. Laboratory analysis

1. Total concentration of heavy metals was determined using the atomic absorption spectrometry (AAS) method. Subsamples from each sample were airdried and pounded into powder using an agate

mortar. Metals were extracted by digestion with 3 parts concentrated $HNO₃$ and 1 part concentrated $HCIO₃$ and the concentration was determined using AAS.

- 2. Soil moisture (SM) of the subsamples was measured gravimetrically as percentage of dry mass by drying the samples to a constant weight at 105 $^{\circ}$ C.
- 3. Total organic carbon (C_{org}) was determined using a modified method of [Rowell \(1994\)](#page-9-0). The method is based on organic matter oxidation by K-dichromate.
- 4. Total soluble nitrogen (TSN) in soil was determined by using the method of [Houba et al. \(1987\).](#page-8-0) The amounts of TSN in the soil extracts were determined using a Skalar Autoanalyzer unit [\(SFAS, 1995](#page-9-0)).
- 5. Soil pH was determined in H_2O (soil solution ratio 1:2.0) with a potentiometric glass electrode.
- 6. Soil salinity was determined in soil extracts and expressed as electrical conductivity (EC). Soluble

cations (Ca^{2+}, K^+, Na^+) were determined by a flame photometer [\(Rhoades, 1982](#page-9-0)).

- 7. Nematode population was determined by extraction from 100 g soil samples using the Baermann funnel procedure [\(Cairns, 1960\)](#page-8-0). The recovered organisms were counted using a compound microscope and preserved in formalin ([Steinberger and Sarig, 1993\)](#page-9-0).
- 8. Soil microbial biomass (C_{mic}) was determined using a chloroform fumigation incubation (CFI) assay, according to [Jenkinson and Powlson \(1976\)](#page-8-0). Fivegram soil samples were adjusted to 40% water-holding capacity and fumigated in a CHCl₃-saturated atmosphere in a desiccator for 24 h. The fumigated and corresponding non-fumigated (control) samples were then transferred to 0.5-L glass jars and incubated for 10 days at 25 °C in the dark. $CO₂$ concentration was measured in the head space of the glass jars using a gas chromatograph (GC) and C_{mic} was calculated as: $C_{\text{mic}} = [(CO_2-C \text{ from fumigated soil})-(CO_2-C$ from control sample)]/ k_c . A k_c of 0.41 was used, as proposed by [Anderson and Domsch \(1990\).](#page-8-0)
- 9. Soil basal respiration (BR) as $CO₂$ evolution, was determined by GC [\(Sparling and West, 1990\)](#page-9-0).
- 10. Metabolic quotient $(qCO₂)$ was calculated as the ratio between basal respiration (BR) and microbial biomass (C_{mic}) ([Anderson and Domsch, 1990\)](#page-8-0). The $qCO₂$ is a specific parameter for evaluating the effects of environmental conditions on the soil microbial biomass.
- 11. Microbial coefficient, known as substrate availability, was determined as the $C_{\text{mic}}/C_{\text{org}}$ ratio.

The data presented in our study are reported as ovendried weights. All data were subjected to statistical analysis of variance using the SAS model (ANOVA, Duncan's multiple range tests and Pearson correlation coefficient) and were used to evaluate differences between separate means. Differences obtained at levels of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Heavy metals

The concentration of heavy metals in soil samples collected along the transect showed a gradual decrease ([Fig. 2](#page-4-0)), while no significant differences were observed between the upper $(0-10 \text{ cm})$ and deeper layer $(10-20$ cm) soil layers. However, significant effect of distance was obtained [\(Table 1](#page-4-0)) for all the heavy metals detected in this study. The rates of decrease of heavy metals along the transect were found to be elucidated by two main patterns. In the first, As, Zn, and Cd were found to decrease gradually along the 15 km transect from mean value levels of 38.9, 56.2, and 5.5 mg kg^{-1} to levels of

11.9, 13.8, and 0.3 mg kg^{-1} , respectively. A decrease of 75% , 80% , and 95% along the 15-km transect was found to be significantly ($p < 0.05$) affected by distance from the pollution source. In the second pattern, Pb and Cu were found to reach mean values of 303.8 and 1605 mg kg^{-1} near the source and decreased to levels of 78.2 and 192.5 mg kg^{-1} at a distance of 5 km, representing values 3 and 8 times lower, respectively. The changes in the Pb and Cu values between the 5 km and the 15 km distances were not found to be statistically significant.

3.2. Soil moisture and organic carbon

Significant increases were found in soil moisture (SM) and organic carbon (C_{org}) contents along the transect downwind from the industrial complex ([Fig. 3](#page-5-0)). Mean SM levels were found to increase from values of 0.6% to values ranging between 1.9 and 2.0%, indicating a tripling of these values along the 15 km transect, showing a location and sampling layer effect [\(Table 2](#page-5-0)). C_{org} exhibited a sharp increase at the second sampling location, reaching a mean value of 1.0%, with a significant sampling layer (depth) effect [\(Fig. 3; Table 2](#page-5-0)).

3.3. Total soluble nitrogen

TSN values were found to increase from a mean value of 2.0 mg kg^{-1} near the industrial complex to values of 4.0 mg kg^{-1} at locations B and C (5 and 10 km, respectively). However, toward location D (15 km away from the pollution source), TSN levels decreased by almost 40% [\(Fig. 3\)](#page-5-0). The changes in TSN values along the transect were found to be significant ($p < 0.01$), with no differences between soil layers ([Table 2](#page-5-0)).

3.4. pH

pH levels in soil samples taken along the 15 km transect showed a significant decrease ([Fig. 4\)](#page-5-0) from an alkaline nature (8.2) to a neutral pH (7.5) ([Table 2](#page-5-0)). No significant differences between the two soil sampling $(0-10$ and $10-20$ cm) layers were found along the transect.

3.5. Soil electrical conductivity, Ca^{2+} , K^+ and Na^+

The electrical conductivity (EC) of soil along the transect reached its highest level at locations B and C, with mean values of 0.76 and 0.71 mS g^{-1} , respectively, while near the industrial complex and at location D, a value of 0.6 mS g^{-1} was found [\(Fig. 4\)](#page-5-0). A significant sampling location [\(Table 2](#page-5-0)) effect was obtained without any differences between the two depths.

A spatial comparison of the cation content of both soil layers [\(Fig. 4\)](#page-5-0) revealed that the concentration of K^+ and $Na⁺$ in soil near the industrial complex was

Fig. 2. Distribution of heavy metal concentrations and total number of nematodes along the deposition gradient at two soil layers (\mathbb{Z}) 0-10 cm; $10-20$ cm) at the Almalyk industrial area.

Table 1

Heavy metal concentrations and soil respiration, microbial biomass, metabolic quotient and $C_{\text{mic}}/C_{\text{org}}$ ratio in two soil depths at the sampling locations

Locations ^a	A(0 km)		B(5 km)		$C(10 \text{ km})$		D(15 km)	
	$0 - 10$ cm	$10-20$ cm $0-10$ cm		$10-20$ cm $0-10$ cm		$10-20$ cm $0-10$ cm		$10-20$ cm
Heavy metals (mg kg^{-1}) ^b								
As	$39 + 13$	26 ± 14	$19 + 0.1$	$15 + 0$	11 ± 0.7	$10 + 0.7$	12 ± 1.2	$12 + 2.9$
Zn	$56 + 7$	$48 + 35$	$53 + 3$	$35 + 35$	$24 + 4.3$	$15 + 1.7$	$14 + 2.3$	11 ± 0.5
Pb	$303 + 116$	$194 + 135$	$78 + 31$	$70 + 52$	$73 + 11$	$53 + 16$	91 ± 7.6	$71 + 2$
Cd	$0.2 + 0.1$	5.5 ± 2.7	2.4 ± 2.1	2.5 ± 0.2	2.0 ± 1.8	$0.9 + 0.5$	0.3 ± 0.2	0.2 ± 0.1
Cu	$1605 + 968$	$814 + 801$	$193 + 9.2$	$213 + 133$	$157 + 44$	$107 + 22$	$107 + 17$	81 ± 2
Soil microbial activity ^c								
Basal respiration (BR) [µg CO ₂ -C(g soil·h) ⁻¹]	$172 + 82$	$86 + 35$	$318 + 129$	$207 + 90$	$413 + 228$	$206 + 95$	$394 + 245$	326 ± 142
Microbial biomass (C_{mic}) [µg $C \cdot g^{-1}$ soil]	$83 + 46$	$45 + 24$	186 ± 110	$81 + 44$	$210 + 125$	127 ± 101	$447 + 271$	$365 + 242$
Metabolic quotient (qCO ₂₎ [mg CO ₂ -C (g $C_{\text{mic}} \cdot h$) ⁻¹]	$2.9 + 2.3$	2.4 ± 1.3	2.5 ± 1.9	3.2 ± 1.7	2.3 ± 1.8	2.1 ± 1.5	1.1 ± 0.7	1.1 ± 0.6
Microbial coefficient $(C_{\text{mic}}/C_{\text{orp}})$ (%)	1.1 ± 0.7	$0.9 + 0.5$	1.4 ± 1.1	$0.9 + 0.6$	1.2 ± 0.6	1.0 ± 0.8	3.8 ± 2.8	3.3 ± 2.1

Significant differences between locations (p <0.05).

^a Along the downwind transect.

^b $n=5$

 $n = 5.$
c $n=10.$

Fig. 3. Soil moisture and total organic carbon and total soluble nitrogen contents along the deposition gradient at two soil layers $(\mathbb{Z} \quad 0-10 \text{ cm}; \blacksquare 10-20 \text{ cm})$ at the Almalyk industrial area.

a maximum of 20 and 5 times lower, respectively, than in the other sampling sites along the transect. Ca^{2+} content in the soil samples was 2 and 3 times lower at the end of the transect than in the other three stations (Fig. 4). Data analysis showed significant sampling location effect. Ca^{2+} content was also affected by the soil layer (Table 2).

Table 2

Univariate analysis of variance (ANOVA) for soil conditions along the downwind transect

Index	Location		Depth		
	F-test	p -value	F-test	p -value	
Total nematodes abundance (Tnem)	18.5	< 0.0001	6.1	0.01	
Soil moisture (SM)	49.7	< 0.0001	12.0	< 0.0009	
Total organic carbon (C_{org})	17.0	< 0.0001	14.6	< 0.0003	
Total soluble nitrogen (TSN)	3.8	0.01	0.5	0.4	
pH	22.7	< 0.0001	0.0	1.0	
Soil electrical conductivity (EC)	5.6	0.001	2.0	0.1	
Ca^{2+}	16.8	< 0.0001	4.8	0.03	
$Na+$	26.8	< 0.0001	3.8	0.1	
K^+	33.0	< 0.0001	0.9	0.3	
Basal respiration (BR)	9.1	< 0.0001	12.7	0.001	
Microbial biomass (C_{mic})	20.2	< 0.0001	5.4	0.02	
Metabolic quotient $(qCO2)$	4.4	0.004	0.0	1.0	

Fig. 4. Changes in pH, soil electrical conductivity, Ca^{2+} , K^+ , Na⁺ contents along the deposition transect at $0-10$ (\mathbb{Z}) and $10-20$ cm (\blacksquare) soil layers.

3.6. Nematode population

Nematode population densities (TNem) were found to change significantly ($p < 0.0001$) (Table 2) on a spatial (along the sampling gradient) as well as vertical basis $(p<0.01)$ $(0-10$ and $10-20$ cm soil layers). The population size of nematodes in the soil was found to increase from mean values of 70 to 590 and from 34 to 420 individuals per 100 g soil in the upper $(0-10 \text{ cm})$ and in the deeper $(10-20 \text{ cm})$ soil layers, respectively ([Fig. 2](#page-4-0)). This increase yielded a significant correlation $(r^2=0.7$ and $r^2=0.8$ for both depths, respectively) between the total number of nematodes at the two layers and the distance from the pollution source.

A similar trend was found between the total nematode population and the heavy metal concentration in the soil, reflecting its dispersion and yielding significant correlations between the different pollutants (Cd, $r = -0.51$, $n = 16$, $p < 0.04$; Cu, $r = -0.60$, $n = 16$, $p < 0.01$; Pb, $r = -0.58$, $n = 16$, $p < 0.01$; Zn, $r = -0.52$, $n=16, p<0.03;$ As, $r=-0.67, n=16, p<0.004$). A positive significant correlation was also found between the total number of nematodes and the total organic carbon (C_{org}) $(r=0.46, n=80, p<0.0001)$, and soil moisture (SM) at the deeper layer $(r=0.47, n=40,$ $p < 0.0001$).

3.7. Microbial biomass (C_{mic})

Soil microbial biomass (C_{mic}) reached a maximal value of 447 and 365 µg C g^{-1} soil in samples taken at location D from the $0-10$ and $10-20$ cm soil layers, respectively (Fig. 5). The gradual increase in microbial biomass from minimal values of 83 and 45 μ g C g⁻¹soil measured at location A for the $0-10$ and $10-20$ cm soil layers, respectively, toward the end of the transect, were found to be similar for both soil layers. Moreover, statistical analysis elucidated the importance of the sampling location, vertical distribution [\(Table 2](#page-5-0)) and soil moisture content $(r^2=0.66)$ on the soil microbial biomass (Fig. 5).

Soil basal respiration (BR) was found to follow the C_{mic} pattern, with relatively lower values at location A, with 171.5 μ g CO₂-C (g soil h)⁻¹ at the upper 0–10 cm soil layer and $86.5 \text{ CO}_2\text{-}C_{\text{mic}} \text{ g}^{-1}$ at the deeper 10–20 cm soil layer. Toward location D, 15 km from the pollution source, basal respiration increased 2.3 and 3.8 times at the $0-10$ and $10-20$ cm soil layers, respectively (Fig. 5). yielding a significant difference $(p<0.001)$ between location and depth ([Table 2](#page-5-0)).

As a result of significant changes in microbial biomass and basal respiration, the ecophysiological status $(qCO₂)$ of the soil microbial community was found to decrease from a maximal value of 3 to 1 mg CO_2 -C (g C_{mic} h)⁻¹ along the downwind transect from the pollution source to station D (Fig. 5; [Table 3\)](#page-7-0). The pattern obtained for the microbial coefficient $(C_{\text{mic}}/C_{\text{org}})$ was found to be the opposite to that of the $qCO₂$ (Fig. 5). The microbial coefficient $C_{\rm mic}/C_{\rm org}$ value was found to be relatively similar (0.9%) at locations A, B, and C, while the values obtained at location D increased significantly (over four times, to a value of 3.8 and 3.2% at the $0-10$ and $10-20$ cm soil layers, respectively (Fig. 5).

4. Discussion

The location of the study sites in relation to the main wind direction at Almalyk determined the degree of

Fig. 5. Micrbial biomass, basal respiration, metabolic quotient and microbial coefficients values obtained in the soil samples along the deposition transect at $0-10$ (\oslash) and $10-20$ cm (\Box) soil layers.

heavy metal accumulation in the soil to a large extent, as did the distance to the emission source. Change in total heavy metal concentration in the two soil layers along a downwind transect of only 15 km was found to decrease the total concentration levels by 3 to 20 times. The distribution of heavy metals in this study was found to be strongly correlated to heavy metal physicochemical properties, as reported in other studies ([Alloway, 1990; Talipov et al., 1997](#page-8-0)). This distribution yielded two different distribution patterns: (1) a gradual, continuous decrease from the pollution source along the

downwind transect; and (2) a relatively low level of contamination at the source with a gradual increase moving away from the source (in some cases a gradual decrease occurs at a certain distance from the source). Both distribution patterns are defined by both the abiotic components and the physico-chemical properties of the pollutant. Because of the alkaline nature of our study site, pH and soil organic carbon values were found to exhibit a different pattern than those reported for the Camacary region of Brasilia ([Klumpp et al., 2003\)](#page-9-0). A high level of carbonate (CO_3^{2-}) content in the studied soils might reduce heavy metal mobility in soils by increasing chemisorptions ([Pinsky, 1997](#page-9-0)), causing a significant difference in heavy metal contamination between soil layers, as obtained in our study. However, only Cd exhibited a different pattern, perhaps due to its different chemo-physical properties.

With the exception of Cd in the first location, all the heavy metals studied, As, Cu, Pb, Zn, accumulated in the upper $(0-10 \text{ cm})$ soil layer at values twice as high as in the deeper $(10-20 \text{ cm})$ soil layer. These values were found to decrease to values more than 10 times lower along the 15 km transect, similar to results reported by [Klumpp et al. \(2003\)](#page-9-0) for Cu. Earlier studies by [Hutchinson and Whitby \(1974\); Kandeler et al. \(1999\)](#page-8-0) support our findings that copper contamination accumulates mainly in the top soil layer and that a rapid and gradual decrease occurs with increasing distance from the smelter.

The decrease in heavy metal concentration along the transect was found to be an opposite trend to the changes in soil moisture availability, organic carbon and total soluble nitrogen, which may act as a source and sink of nutrients in the soil, triggering the soil biota community. In our study, the studied biotic components and their indices showed a clear relationship with the pollutant concentration in soil. Such detrimental effects of heavy metals and other soil contaminants have repeatedly been reported in the literature [\(Hattori,](#page-8-0) [1992; Bardgett et al., 1994; Kandeler et al., 1996; Wilcke](#page-8-0) [et al., 1999; Klumpp et al., 2003](#page-8-0)).

Because of its biological function as a well-known bioindicator, the soil free-living nematode population ([Wardle et al., 1995; Yeates and Williams, 2001](#page-9-0)) exhibited a significant positive response to a decrease in soil heavy metal contamination.

In this study microbial biomass as well as $CO₂$ evolution patterns were found to increase gradually in response to the decrease in heavy metal concentration at the two soil layers, yielding a clear inverse significant relationship with the pollutant concentration in the soil. These results are supported by similar studies [\(Kandeler](#page-8-0) [et al., 1996; Kandeler et al., 1999\)](#page-8-0). Moreover, the ecophysiological quotient $qCO₂$, which represents a specific physiological status evaluating the environmental effect on the soil microbial community, revealed a small

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difference between the two soil layers, with a significant change toward the end of the transect. Such differences were mainly affected by heavy metal distribution. Results from our study on microbial coefficients were found to be within the range of values reported by Hofman et al. (2003), working on a monitoring microbial biomass and respiration program aimed to determine the relationship between soil properties and contamination. The microbial coefficient was found to be an important aid for gaining more complete understanding of the heavy metal, abiotic, soil chemical composition and soil microbial interaction. Since the microbial coefficient interpreted as substrate availability exhibited a significant increase at station D, which is complementary to the significant decrease in ecophysiological status, it seems to elucidate the sensitivity of the microbial community as one of the most sensitive indicators of soil contamination.

Studies on the relationship between soil biota and pollution levels have raised the question regarding the importance of natural soil abiotic properties, stressing the importance of background data of environmental conditions, and elucidating the importance of further studies on this subject.

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