Environmental Engineering and Management Journal
June 2012, Vol.11, No. 6, 1159-1169
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

DIVERSITY OF ACARI AND COLLEMBOLA ALONG A POLLUTION GRADIENT IN SOILS OF A PRE-PYRENEAN FOREST ECOSYSTEM

Jesús Miguel Santamaría[1*], María Lourdes Moraza[2], David Elustondo[1], Enrique Baquero[2], Rafael Jordana[2], Esther Lasheras[1], Raúl Bermejo[1], Arturo Hugo Ariño[2]

[1]University of Navarra, Department of Chemistry and Soil Science, Irunlarrea No.1, 31008 Pamplona (Navarra), Spain
[2]University of Navarra, Department of Zoology and Ecology, Irunlarrea No. 1, 31008 Pamplona (Navarra), Spain

Abstract

Mites and springtails are important members of soil mesofauna and have been proven to be good bioindicators of airborne pollutants. We studied the surrounding area of a steel mill located in a mountain valley of North Spain. Previous studies had documented the existence of a pollution gradient in this area due to the emissions of the factory, thus providing an interesting site to investigate the potential effects of pollutants (heavy metals and nitrogen) on soil biodiversity.
The density of Acari and Collembola significantly decreased with the increase in concentration of Cr, Mn, Zn, Cd and Pb. Mites appeared to be more sensitive to heavy metal pollution than springtails. Likewise, the density of these microarthropoda was lower in those soils exhibiting higher nitrogen content.
The species composition of the community of Acari and Collembola changed according to heavy metal pollution. Significant differences in abundance, species richness and diversity were observed between the communities of the sampling sites. Some species were exclusive of the less polluted sites, while other appeared in the most contaminated ones. This different response of soil mesofauna to pollutants suggests that some mite or springtail species could be used as bioindicators of heavy metal pollution.

Key words: bioindicators, diversity, heavy metals, nitrogen, soil mesofauna

Received: December, 2011; Revised final: May, 2012; Accepted: June, 2012

1. Introduction

Heavy metals tend to accumulate in high concentrations in the surface horizons near stationary sources (Khalil et al., 2009; Migliorini et al., 2005; Seniczak et al., 2002). In consequence, in the litter and humus layers of soils, where decomposition of dead organic matter and nutrient mineralization through soil organisms occurs, heavy metal concentrations are maximal. This can induce direct toxicity to soil decomposers, creating modified habitats which may affect the abundance, diversity and species richness of soil fauna, even when the critical load of heavy metals for individual species is not reached by individual species (Gillet and Ponge, 2003; Russell and Alberti, 1998; Spurgeon et al., 2009; Syrek et al., 2006; Zaitsev, 2009).

Within the soil biota springtail and mite communities constitute two of the most species-rich components, accounting for up to 95% of the total number of microarthropods (Norton, 1990; Stanton, 1979; Van Straalen, 1998). These groups play an important role in soil functions, being especially vulnerable to alterations of environmental conditions, such as heavy metal (Caruso et al., 2009; Fountain and Hopkin, 2004; Gongalsky et al., 2010; Hopkin, 2004; Skubala and Kafel, 2004), nitrogen (Xiankai et al., 2008; Xu et al., 2009) and hydrocarbon contamination (Blakely et al., 2002). Because of their abundance and species richness, and their almost ubiquitous presence in soils, mites and springtails have been proposed as soil quality indicators (Black et al., 2003; Bokhorst et al., 2008; Parisi et al., 2005). However, the knowledge about the effects of

* Author to whom all correspondence should be addressed: E-mail: chusmi@unav.es; Phone: +34 948425600

contaminants on these groups is still limited. The lack of a standardized methodology to assess biodiversity, the steady decline in the number of taxonomic experts, and the extremely high time consumption of the classification procedures have been posited as drawbacks that hinder these types of studies (Breure, 2004; Brussaard et al., 1997; Freckman, 1994). However, one of these drawbacks has been addressed here by developing a standardized method for collecting and extracting soil mesofauna (Ariño et al., 2007, 2008; Bermejo et al., 2009; Jordana et al., 2000).

The aim of this research was to investigate and quantify the effects of soil pollution on Acari and Collembola communities in a forest area of the pre-Pyrenean mountain range located in the surroundings of a steel plant. Previous studies by González-Miqueo et al. (2010) had shown a markedly decreasing gradient of heavy metals in the same area as a function of the distance to the plant, thus providing an interesting place where to investigate shifts in the density and species composition of mites and springtails.

2. Materials and methods

2.1. Area of study

The study was carried out in the surroundings of a steel mill located in the village of Zumarraga (Basque Country), in the North of Spain. This region has one of the highest densities of heavy industry in Spain, with a large number of steel factories which emit large amounts of pollutants to the atmosphere (PRTR-España 2006, http://www.en.prtr-es.es/).

Zumarraga (43° 5' 37" N - 2° 18' 51" W, 357 m.a.s.l.) has a temperate-humid climate of the western maritime variety (Cfb in the Köppen system) (AEMET/IMP, 2011). The average annual temperature is 12.6°C, with maximal temperatures in summer (24.7ºC) and minima in winter (2.8ºC). The prevailing North and Northwest winds contribute to abundant rainfall, with an annual precipitation of 1408 mm.

The area is included in the domain of Upper Cretaceous materials: marlaceous lime, marls and argillaceous limestones of calcareous Flysch interbedded with volcanic rocks (Garrote Ruiz et al., 1992). The town of Zumarraga lies in the valley of the river Urola, one of the rivers crossing transversally the pre-Pyrenean mountain range, deeply wedged between the resistant basaltic materials. The main types of soils in this valley are luvisols, with an acid pH and a low degree of base saturation, linked to relatively cool and humid conditions.

The steel mill is placed at the bottom of the valley and next to the village, emitting large quantities of airborne pollutants, namely NO_x, SO_x, and heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn).

2.2. Sample collection and analysis

Soil samples were taken during November 2010 after a rainy period along a gradient established in a *Pinus radiata* forest on an upwards slope southwest of the steel facility, following the prevalent wind direction (Fig. 1).

Fig. 1. Location of the steel plant of Zumarraga and sampling sites

Six plots were selected 50 m apart along a straight line starting close to the factory. In each plot, five soil cores were taken with a 20.25-cm^2 low disturbance soil corer (Jordana et al., 2000).

Each core was sliced into two 2-cm thick soil layers, obtaining a total of 60 sub-samples. Once in the laboratory, mites and springtails of all sub-samples were separately and simultaneously extracted with a modified high-gradient MacFayden system (Ariño et al., 2007) and species were identified by taxonomic experts. After fauna extraction, soil sub-samples were dried to a constant weight, ground and sieved (0.125 mm mesh size) for the analysis of heavy metals and total nitrogen.

Heavy metal concentrations were determined by means of inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500a). Mercury levels were analyzed by cold-vapor atomic absorption after dry combustion at 850ºC in a Mercury Analyzer (MA 2000 Series, Nippon) and the total nitrogen content of soils was estimated by means of the Kjeldahl method. Besides this, soil pH was measured potentiometrically in H_2O with a soil/extractant ratio of 1:2.5.

In all cases quality control procedures were followed by analyzing certified reference material. More information about the protocols of analysis can be found in González-Miqueo et al. (2010).

2.3. Enrichment factor

The enrichment factor is the relative abundance of a chemical element in a soil compared to the bedrock.

Due to its universal formula, it is a relatively simple and easy tool for assessing enrichment degree and comparing the contamination of different environmental sites (Blaser et al., 2000; Manta et al., 2002; Singh et al., 2010).

To determine the relative degree of heavy metal contamination along the gradient, comparisons were made to background concentrations in the Earth's crust (using cesium as reference element), following the assumption that its content in the crust has not been disturbed by anthropogenic activities. EFs were calculated according Eq. (1).

$$EF_M = \frac{[M]_{soil} / [Cs]_{soil}}{[M]_{crust} / [Cs]_{crust}} \quad (1)$$

where [M] is the concentration of any element, [Cs] is the concentration of cesium, and the subscripts ''soil" and ''crust" indicate which medium the concentration refers to.

On the basis of the enrichment factor five contamination categories (Table 1) are recognized (Sutherland, 2000).

Table 1. Contamination categories of soils on the basis of enrichment factor

Enrichment factor	*Category*
EF < 2	Deficiency to minimal enrichment
EF > 2-5	Moderate enrichment
EF > 5-20	Significant enrichment
EF > 20-40	Very high enrichment
EF > 40	Extremely high enrichment

2.4. Statistical analysis

Metal contents of samples were compared by one-way ANOVA between sampling sites, testing significance with a Tukey post-hoc test. The microarthropod communities of four groups (Acari: *Prostigmata, Mesostigmata and Oribatida*; and *Collembola*) were characterized by their species composition. Abundance (N) and species richness (S) were measured for each subsample. Shannon's diversity index (H') and equitability (J') were calculated for each site by combining data from all five replicas; differences of H' between adjacent sites were calculated by a t-test according to Poole (1974). Total species richness (R) was calculated for each sampling site by the jackknife procedure on the five replicas.

As most variables showed non-normality on Shapiro-Wilks tests, correlations between variables were determined by Spearman rank correlation. Total metal load was calculated on the sum of their levels standardized by $(x\text{-}x_{min})/(x_{max}\text{-}x_{min})$. The same standardization was applied to the abundances of the four microarthropod groups for comparison with the metal load.

A correspondence analysis (CA) was performed to examine the relationship between species and sites. OTUs classified to species level totaling 90% of the abundance were retained for the analysis. Analyses were performed with SPSS, Excel and PAST.

3. Results and discussion

3.1. Soil analysis

Table 2 shows the mean concentrations of the different elements analyzed in soil samples. As it was expected according to previous studies (González-Miqueo et al., 2010), a clear gradient of pollution was observed in the vicinity of the steel factory, with a decreasing trend of heavy metal concentrations (between 50.1% and 74.8% reduction) for almost all elements within a distance of 500 m.

In this context, the Spearman correlation analysis showed significant ($P < 0.01$) and negative associations between the distance to the plant and the concentrations of the elements Cr, Mn, Cu, Zn, Cd, Co, Pb and Hg (Table 3).

The differences in heavy metal concentrations between sampling sites were significant (one-way ANOVA) for all elements except for As, that exhibited a regular distribution pattern throughout the transect.

The different behavior of As, and to a lesser extent Hg, not showing a clear distribution pattern throughout the transect, may be linked to the elevated mobility and volatility of both elements in gaseous form, as well as to their high persistence in the atmosphere (Dang-Yu et al., 2011; Schroeder and Munthe, 1998; Zhang et al., 2009).

The most abundant heavy metal in soils was Zn, reaching 2,808.4 mg kg^{-1} at site B, which is located just in front of the slag deposits coming from the steel plant, decreasing steadily with the distance to the industry. On the contrary, Hg showed the lowest concentrations, with a maximum level of 0.9 mg kg^{-1} at the closest site to the factory.

The elements Cd, Cr, Pb and Zn presented concentrations clearly above the mean values established for soils in Europe (Rademacher, 2001) and in the Basque Country (IHOBE, 1994), thus indicating an anthropogenic pollution for these elements (Table 2). Moreover, the upper toxicity limits set for soil micro and mesofauna were exceeded in several sites for Cd, Cr, Cu, Pb and Zn (Rademacher, 2001).

Heavy metal concentrations recorded at Zumarraga were higher than those reported in smelting areas (Stone et al., 2001) and comparable to those detected in areas heavily contaminated by industrial activities (Acosta et al., 2011; Roca-Pérez et al., 2010; Schulin et al., 2007; Yaylalı-Abanuz, 2011).

Table 2. Mean pH, nitrogen content and heavy metal concentrations (mg kg^{-1}) in soil samples by forest site (A-F). The table includes the mean values of heavy metals for soils in Europe, the toxicity limits for micro and mesofauna of soils (Rademacher, 2001), and the reference concentrations calculated for the Basque Country (IHOBE, 1994). Values with the same letter do not differ significantly (analysis of variance, $P < 0.05$) between sampling sites

Parameter	*A*	*B*	*C*	*D*	*E*	*F*	*Mean values*	*Toxicity limit*	*Basque Country*
As	9.9^{ab}	10.2^{ab}	10.8^{b}	8.6^{a}	10.9^{ab}	10.4^{ab}	0.8-1.5	-	23
Cd	7.2^{a}	7.8^{ab}	7.7^{b}	5.4^{c}	3.5^{d}	2.9^{d}	0.1-0.2	0.3-2.0	0.8
Cr	178.2^{a}	211.5^{b}	178.6^{a}	132.9^{c}	105.0^{d}	108.5^{d}	50-100	20-130	-
Cu	69.7^{a}	83.2^{b}	74.6^{a}	75.7c	42.8^{d}	36.7^{e}	30-60	30-70	10
Hg	0.9^{a}	0.3^{b}	0.3^{cd}	0.1^{e}	0.2^{d}	0.3^{bc}	0.07	0.1-1	-
Mn	1451.3^{a}	2345.6^{b}	1922.7^{c}	1218.4^{d}	786.5^{e}	591.5^{f}	-	-	-
Ni	34.2^{ac}	42.0^{b}	33.7^{c}	$27.1a^{d}$	23.4^{d}	58.9^{e}	30-60	10-85	-
Pb	648.7^{a}	865.4^{b}	769.8^{c}	575.3^{d}	406.8^{e}	344.9^{f}	50-100	70-150	16
Zn	2187.3^{a}	2808.4^{b}	2345.4^{c}	1718.9^{d}	1097.5^{e}	899.2^{f}	100-200	70-200	50
N	0.5	0.7	0.7	0.5	0.6	0.4	-	-	-
pH	6.6	6.9	6.5	6.3	5.7	5.3	-	-	-

Table 3. Spearman correlation coefficients between abundance of Acari (N_A) and Collembola (N_C), number of species (S_A and S_C), distance to the factory (d), heavy metals, nitrogen and pH. Underlined: Non-significant correlations

	N_c	N_a	S_c	S_a	*d*	*Cr*	*Mn*	*Co*	*Ni*	*Cu*	*Zn*	*As*	*Cd*	*Pb*	*Hg*	*N*
N_c																
N_a	0.93															
S_c	0.49	0.38														
S_a	0.75	0.84	0.59													
d	0.71	0.79	0.54	0.58												
Cr	-0.78	-0.81	-0.38	-0.65	-0.81											
Mn	-0.82	-0.84	-0.34	-0.63	-0.82	0.90										
Co	-0.67	-0.69	-0.48	-0.64	-0.63	0.83	0.82									
Ni	-0.08	-0.10	0.08	-0.07	-0.04	0.37	0.09	0.26								
Cu	-0.65	-0.70	-0.43	-0.63	-0.61	0.77	0.86	0.84	-0.04							
Zn	-0.83	-0.86	-0.35	-0.63	-0.82	0.87	0.97	0.83	0.10	0.84						
As	0.09	0.07	0.53	0.31	0.12	-0.06	-0.02	-0.18	0.07	-0.22	-0.02					
Cd	-0.77	-0.77	-0.39	-0.59	-0.81	0.85	0.91	0.79	0.04	0.77	0.94	0.10				
Pb	-0.82	-0.85	-0.33	-0.64	-0.82	0.90	0.98	0.82	0.09	0.84	0.98	-0.04	0.93			
Hg	-0.43	-0.33	-0.05	-0.14	-0.53	0.48	0.30	0.15	0.65	-0.04	0.28	0.06	0.24	0.29		
N	-0.45	-0.55	0.17	-0.22	-0.35	0.50	0.71	0.49	-0.05	0.59	0.72	0.28	0.63	0.72	-0.04	
pH	-0.82	-0.83	-0.42	-0.61	-0.90	0.91	0.93	0.77	0.11	0.81	0.93	-0.08	0.87	0.92	0.39	0.60

All these metals showed very high and significant correlations among them (Table 3), suggesting a common origin, probably related to the steel slag deposits facing the forest. The decrease of soil pH along the transect confirms that slag, with an alkaline composition, is the main source of heavy metals in the surrounding forests (Caruso et al., 2009; Dutra et al., 2006; Lind et al., 2001; Rai et al., 2002), which are mainly transported as particulate matter by prevailing winds.

Concerning the soil enrichment factor, the following sequence was found in the studied soils: Cd, Zn, Pb, Hg > As, Cu, Cr > Mn, Ni (Fig. 2). In all sites, excluding the farthest one, EF was above 5 for all metals, indicating a significant enrichment. Among these, an extremely high enrichment was detected for Cd, Zn, Pb and Hg (EF > 40).

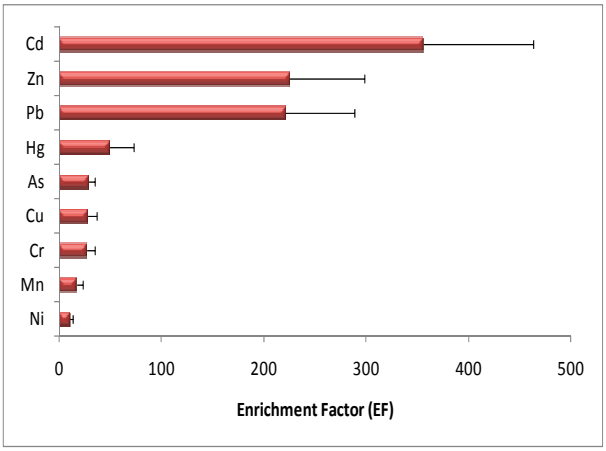

Fig. 2. Heavy metal enrichment factors (error bars are standard deviations

Soils from site B showed the highest EF values for all elements, with the exception of mercury, that reached the highest values at the site A. The site F, located 500 m away from the factory, presented the lowest EF values (Table 4).

However, even in this case, soils exhibited symptoms of enrichment in the content of heavy metals with respect to the background concentrations of the Earth's crust, except in the case of Mn.

Table 4. Enrichment factors of heavy metals in soils from the sampling sites

Parameter	*A*	*B*	*C*	*D*	*E*	*F*
As	31.1	40.1	33.3	35.0	26.9	4.0
Cd	439.3	600.5	463.9	434.3	170.7	21.7
Cr	31.9	47.3	31.4	31.0	14.8	2.4
Cu	30.5	45.5	32.1	43.2	14.7	2.0
Hg	139.6	57.5	39.5	24.0	28.4	5.3
Mn	17.2	34.8	22.4	18.9	7.3	0.9
Ni	11.5	17.7	11.2	11.9	6.2	2.4
Pb	238.8	398.5	278.4	276.5	117.7	15.5
Zn	263.2	422.8	277.3	270.0	103.8	13.2

3.2. Acari and Collembola communities

A total of 2,012 specimens of mites and springtails were collected in the forest sites. The Acari were represented by 1,687 individuals (belonging to 107 species), oribatid mites being the most abundant group (868 individuals and 51 species). The total density of Acari was 27,430 ± 5,199 individuals m^{-2}. With respect to Collembola, a total of 325 individuals were found (5,284 ± 1,649 individuals m^{-2}), distributed in 33 species (Table 5).

These data on community-level abundance were consistent with those obtained by several authors in different polluted sites (Caruso et al., 2009; Gillet and Ponge, 2003; Khalil et al., 2009; Migliorini et al., 2005; Russell and Alberti, 1998; Skubala and Kafel, 2004), even though the high-gradient extraction method used here is significantly more efficient than traditional Berlesse-Tullgren methods used in other studies and should therefore have yielded significantly higher counts (Ariño et al., 2007). In consequence, we suspected that the high levels of heavy metals recorded in Zumarraga were promoting a reduction of soil fauna.

This suspicion was confirmed by the significant and negative correlations found between heavy metal pollution and the abundance and species richness of Acari and Collembola, showing significant ($P < 0.01$) and negative correlations with Cr, Mn, Cu, Zn, Cd, Pb and pH (Table 3).

In agreement with Migliorini et al. (2005), mites were better correlated with soil parameters than springtails (Table 3). This may be due to the capacity of springtails to withstand moderately heavy metal loads through several strategies (Bengtsson and Rundgren, 1988; Bengtsson et al., 1994; Tranvik and Eijsackers, 1989; Van Straalen et al., 1987).

The total number of microarthropods and species richness differed significantly among sites (Table 6), showing different pollution levels (one-way ANOVA, $P < 0.05$), with an increasing number of individuals and species towards the lowest polluted sites.

In general, both types of microarthropods exhibited a better association with abundance than with the number of species (Figs. 3 and 4).

Although the relation between richness and pollution seems obvious, it is known that pollution affects soil microarthropods as a community not only by toxicity but may also cause indirect effects through changes in the content of soil organic matter and the associated microbial communities. For instance, in areas polluted by heavy metals, accumulation of leaves and thick humus layers are often observed (Khalil et al., 2009), related to a decrease of decomposition rates.

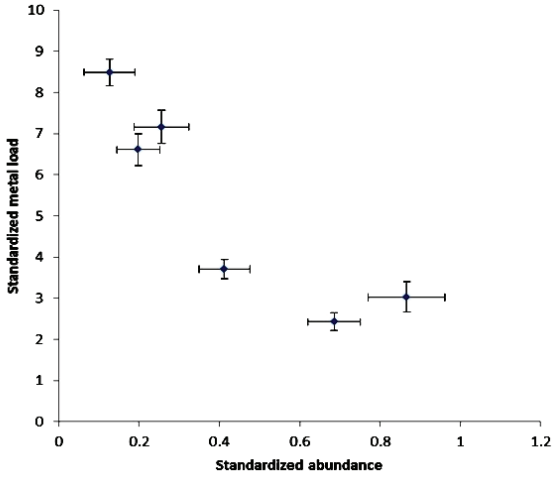

Fig. 3. Correlation between sum of standardized heavy metal concentrations and abundance of microarthropoda in soil (error bars are standard deviations)

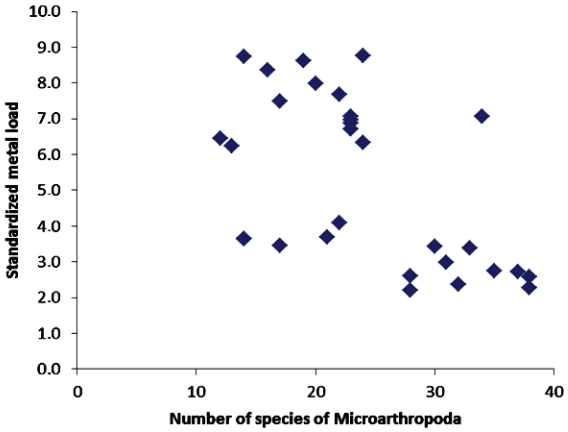

Fig. 4. Correlation between sum of standardized heavy metal concentrations and species richness of microarthropoda in soil samples

The results obtained in this study seemed to confirm the exceedance of toxicity limits as fixed by Rademacher (2001) to protect soil fauna. However, in many cases the reduction of sensitive species to metal

pollution is compensated by the increase of opportunistic/tolerant species and, thus having little effect on total species richness (Khalil et al., 2009; Steiner, 1995). Moreover, some authors have even noted higher density and species richness in polluted areas with respect to the reference sites (Migliorini et al., 2005; Seniczak et al., 1997).

Changes in species composition were related to variations in abundance or in presence/absence of species (Fig. 5).

Table 5. Total number of Acari and Collembola recovered from soil samples taken in the studied area. Total sample size for each site was 101.25 cm^2 at a depth of 4 cm (five 20.25 cm^2 cores with two 2-cm slices from each core)

Species	*A*	*B*	*C*	*D*	*E*	*F*	*Total*
Prostigmata							
Alicorhagia sp.	0	1	7	1	8	23	40
Bimichaelia sp.	0	2	2	0	1	8	13
Brotereunetes sp.	1	0	0	4	13	14	32
Cocceupodes sp.	0	0	0	0	3	3	6
Cocorhagidia sp. 1	0	0	0	0	0	3	3
Cocorhgidia sp. 2	0	0	0	0	0	6	6
Cyta latirostris	0	0	0	0	1	0	1
Ereynetes sp.	0	0	0	2	0	1	3
Eupodes ereneytoides	1	0	0	12	27	40	80
Eupodes sp. 1	2	0	0	0	0	1	3
Eupodes sp. 2	1	5	0	0	10	8	24
Eupodes sp. 3	0	0	2	2	2	1	7
Eupodes sp. 4	0	1	1	0	8	5	15
Eupodidae sp.	0	0	2	0	0	0	2
Eustigmaeus plumiger	0	2	3	0	0	0	5
Labidostoma sp.	1	2	2	0	0	1	6
Ledermulleria segnis	1	0	0	5	3	0	9
Linopodes sp.	0	0	0	1	2	1	4
Lorryia polita	0	0	0	0	10	0	10
Mediolata similans	0	1	0	0	0	0	1
Nanorches pulvinar	0	4	14	5	8	15	46
Prostigmata sp.	0	0	0	1	3	0	4
Rhagididae sp.	1	2	3	5	9	5	25
Stigmaeus pilatus	0	2	4	9	6	3	24
Stigmaeus sphagneti	1	0	0	0	0	0	1
Tarsonemus sp.	0	0	0	0	0	2	2
Tetranychidae sp.	0	0	1	0	0	0	1
Tydeidae sp.	0	0	0	0	1	0	1
Oribatida							
Achipteria coleoptrata	20	1	0	0	0	0	21
Banksinoma lanceolata	0	0	0	0	9	8	17
Brachychochthonius gracilis	0	0	0	0	2	0	2
Brachychochthonius meridionalis	0	0	0	1	0	0	1
Cerachipteria jugata	1	2	0	40	17	36	96
Ceratozetes mediocris	1	0	0	0	0	0	1
Ceratozetes simulator	1	0	0	0	0	0	1
Connoppia palmicinta	0	1	9	1	0	2	13
Chamobates borealis	10	6	3	4	5	6	34
Chamobates pussillus c.f.	1	0	0	0	0	0	1
Damaeus sp.	2	3	2	1	1	6	15
Dissorhina ornata ornata	1	0	0	0	2	0	3
Dorycranosus dickersoni	2	0	0	0	0	0	2
Epidamaeus pyrenaicus	0	0	3	0	2	0	5
Eremaeus cordiformis	5	0	0	7	3	0	15
Eremaeus travei	0	0	0	1	0	0	1
Eueremaeus granulatus	0	0	0	1	0	0	1
Eupelops sp.	0	0	3	2	8	9	22
Eupelops torulosus	0	1	8	10	3	1	23
Galumna sp.	0	0	1	0	1	0	2
Gustavia longirrostris	1	0	0	0	0	0	1
Hemileius initialis	0	0	0	0	5	9	14
Heminothrus grandjeani	0	0	0	1	0	0	1
Liochthinius simplex	0	0	1	15	2	0	18
Liochthonius hystricinus	0	0	0	0	0	1	1

Machuela draconis	0	0	1	0	2	0	3
Medioppia subpectinata	11	5	0	4	8	30	58
Metabelba papillipes	1	0	0	0	1	0	2
Microppia minus longisetosa	0	1	0	1	9	0	11
Minunthozetes pseudofusiger	0	0	0	0	4	0	4
Minunthozetes reticulatus	0	3	0	0	0	0	3
Moritzoppia unicarinata	0	0	0	0	4	0	4
Oppiella nova	0	2	4	1	5	45	57
Oribatella berninii	2	0	0	0	0	0	2
Oribatida (inmature instars)	31	22	29	95	84	75	336
Oribatula tibialis	16	15	3	13	8	10	65
Phthiracarus flexisetosus	4	2	0	1	1	0	8
Phthiracarus ligneus	3	1	3	0	4	0	11
Poecilochthonius italicus	0	0	0	0	2	0	2
Porobelba espinosa	0	0	0	0	6	0	6
Quadrioppia maritalis	1	3	2	1	1	0	8
Rhysotritia duplicata	0	1	0	0	0	0	1
Scheloribates latipes	12	4	4	1	0	0	21
Suctobelba regia	0	1	9	1	4	3	18
Suctobelbella sarekensis	0	0	0	0	1	0	1
Suctobelbella secta	0	0	0	0	0	2	2
Suctobelbella subcornigera	0	0	1	0	8	1	10
Suctobelebella alloenasuta	0	1	9	0	0	0	10
Tectocepheus sarekensis	0	5	3	2	0	0	10
Xenillus tegeocranus	0	0	0	0	6	5	11
Mesostigmata							
Arctoseius minutus	0	0	0	1	0	0	1
Asca aphidioides	7	6	4	1	0	1	19
Cilliba cassidea	8	3	4	1	2	1	19
Dendrolaelaps foveolatus	0	0	0	11	0	0	11
Dendrolaelaps rotundus	0	0	0	1	0	0	1
Epicrius johnstoni	2	1	1	0	0	0	4
Geolaelaps aculeifer	0	1	3	0	1	0	5
Macrocheles (M.) dentatus franzi	1	1	3	1	2	0	8
Macrocheles montanus	0	0	0	0	1	3	4
Olodiscus minima	1	0	0	4	0	0	5
Paragamasus robustus	1	0	1	0	0	0	2
Paragamasus sp. 1	7	5	16	0	6	45	79
Paragamasus sp.2	6	0	1	0	0	0	7
Parasitidae sp.	2	1	2	1	9	0	15
Pergamasus sp.	0	1	0	1	3	3	8
Polyaspinus cylindricus	1	0	0	0	4	6	11
Prozercon tellecheai	0	3	8	0	3	1	15
Pseudolaelaps doderoi	4	0	0	0	1	1	6
Rhodacarus aequalis	8	0	0	0	0	0	8
Rhodacarus sp.	0	2	0	0	0	0	2
Veigaia bouveri	2	1	0	0	0	2	5
Veigaia cervus	0	1	6	0	0	1	8
Veigaia exigua	0	1	9	0	0	1	11
Veigaia nemorensis	8	4	1	0	15	24	52
Veigaia perinsolita	0	0	1	0	0	0	1
Veigaia planicola	1	3	4	5	5	3	21
Veigaia sanmamedi	0	0	1	0	1	1	3
Veigaia sp.	0	1	3	3	0	0	7
Collembola							
Ceratophysella armata	0	1	3	0	0	0	4
Ceratophysella tergilobata	3	2	0	0	3	0	8
Entomobrya sp.	0	0	3	0	8	0	11
Folsomia ocellata	0	0	0	0	1	0	1
Folsomia trisetata	1	17	0	0	29	59	106
Friesea albida var. *atipica*	0	1	0	0	0	0	1
Friesea isabellae	0	1	0	0	0	0	1
Friesea sp.	0	2	0	0	2	1	5
Friesea subterranea bioculata	0	0	0	0	0	1	1
Heteromurus major	0	0	0	2	19	9	30
Isotomiella maderiensis	0	0	1	0	1	3	5

Lepidocyrtus sp.	0	0	1	0	0	0	1
Megalothorax minimus	1	2	16	1	4	4	28
Mesaphorura macrochaeta	3	0	4	0	0	1	8
Mucrella acuminatta	0	0	1	1	5	0	7
Paratullbergia callipygos	0	1	3	0	0	20	24
Parisotoma notabilis	1	2	7	0	11	1	22
Protaphorura armata	0	0	0	0	1	0	1
Protaphorura fimata	0	0	7	0	0	0	7
Protaphorura florae	0	0	0	0	1	0	1
Protaphorura quadriocellata	0	0	0	0	1	1	2
Protaphorura sp.	0	2	0	0	0	0	2
Pseudisotoma monochaeta	0	0	1	0	0	0	1
Pseudisotoma sensibilis	0	0	0	0	3	0	3
Pseudosinella bidenticulata	0	0	0	0	0	1	1
Pseudosinella sp.	2	0	1	2	1	3	9
Sminthurides schoetti	0	2	2	2	0	0	6
Sphaeridia pumillis	0	0	0	1	0	2	3
Symphypleona sp.	0	0	0	0	0	7	7
Tomocerus minor	0	1	1	0	2	0	4
Vertagopus sp.	0	0	0	0	1	0	1
Willemia denisi	1	0	0	0	0	2	3
Xenylla tullbergi	0	0	0	11	0	0	11

Table 6. Total number of individuals (N) and of species (S), Shannon diversity (H'), equitability (J'), Simpson dominance (D), total richness (R) and sum of standardized metal load (L) in soil samples by forest site (A-F). Values with the same letter do not differ significantly (analysis of variance for N, S and SL; t-test for H'; $P < 0.05$) between sampling sites

Parameter	***A***	***B***	***C***	***D***	***E***	***F***
N	175^{a}	149^{a}	225^{a}	204^{ab}	393^{bc}	521^{c}
S	49^{a}	57^{a}	58^{ab}	49^{a}	76^{cd}	62^{bc}
R	69.8	81.8	74.8	73.8	99.2	80.4
H'	3.39^{a}	3.66^{b}	3.71^{b}	3.23^{c}	3.91^{d}	3.37^{e}
J'	0.87	0.91	0.91	0.83	0.90	0.82
D	0.05	0.04	0.03	0.07	0.03	0.05
SL	6.61^{a}	8.49^{b}	7.16^{a}	3.71^{c}	2.43^{d}	3.03^{d}

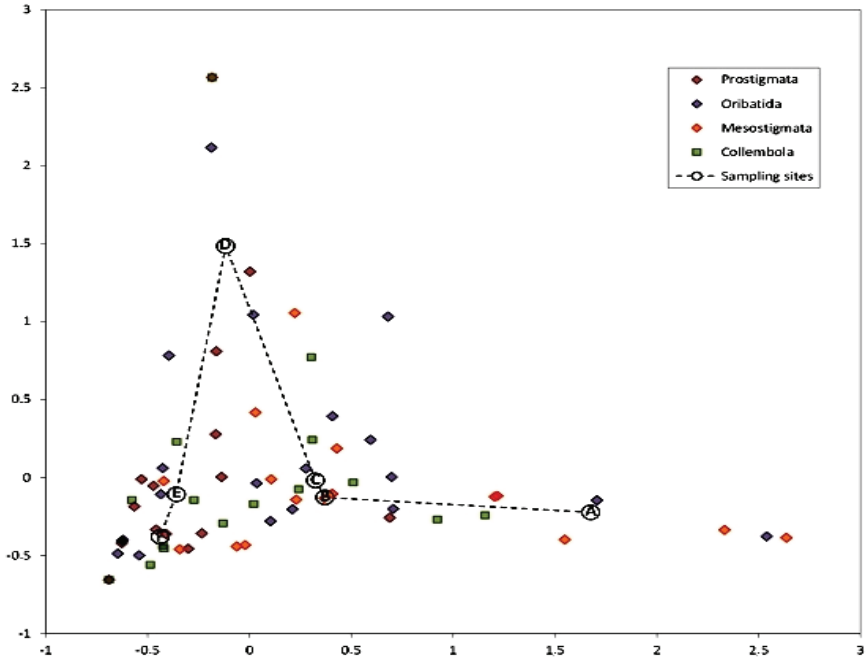

Fig. 5. Axis 1 and 2 of correspondence analysis of the contingency table (percent eigenvalues for axis 1 to 5 were respectively 31%, 26%, 23%, 13%, 7%)

The main source of variance in the correspondence analysis (first axis, accounting for one third of variance) can be associated to the distance to the source. All six sites ordered themselves by distance from the factory along the axis. Some genera of mites and springtails were restricted to sites with different degree of pollution, while others were found, in a greater or lesser extent, throughout all forest sites.

The species most closely associated to the most contaminated sites closer to the factory were the mites *Achipteria coleoptrata*, *Rhodacarus aequalis*, *Paragamasus* sp. 2*, Pseudolaelaps doderoi, Phthiracarus flexisetosus* and *Schleroribates latipes*, along the Collembolan *Mesaphorura macrochaeta,* were mostly associated to the sites closer to the Factory, while *Cocceupodes* sp., *Cocorhagidia* sp., *Tarsonemus sp.*, *Macrocheles montanus, Banksinoma lanceolata*, *Hemileius initialis*, *Suctobelbella secta* and *Xenillus tegeocranus*, and the Collembolan *Friesea subterranea* and *Pseudosinella bidenticulata* were only present in those soils with lower heavy metal content.

Axis 2 seems to be related to the particular composition of D site, having comparatively high abundance but low richness. The Acari *Liochthonius simplex, Dendrolaelaps foveolatus, Olodiscus minima, Ledermulleris segnis* and *Eremaeus cordiformis* and the Collembolan *Xenylla tullbergi* seem to characterize this site.

Several authors (Gackowski et al., 1997; Skubala and Kafel, 2004) have found that small concentrations of heavy metals are positively correlated with the development of mite communities and with the stimulation on the rate of reproduction (hormesis). This could explain the highest abundance and richness of Acari in the lowest polluted sites. According to our results it seems clear that some species of Acari and Collembola could be considered as bioindicators of heavy metal pollution.

Finally, and with respect to nitrogen (N), it is known that atmospheric deposition of N has become a serious concern for nature conservation, altering species composition, species richness and soil chemistry in a range of habitats.

However, very few studies concerning soil fauna have been performed to select appropriate bioindicators sensitive to this element. In this work, abundance of springtails and mites exhibited a significant ($P < 0.05$) and negative correlation with N, although the correlation coefficient was not very high ($r = - 0.45$ and $r = – 0.55$ respectively). Seniczak et al. (2002) found similar results in soils of young pine forests, where a high concentration of nitrogen pollution slightly reduced the density of mites. Other studies (Xu et al., 2009) have also demonstrated that *Collembola* are negatively affected by N-soil pollution, but in this case a relation between springtail richness or abundance and nitrogen was not detected.

4. Conclusions

The high heavy metal concentrations (and nitrogen to a lesser extent) detected in the soils near a steel factory induced significant changes in species richness and abundance of soil mesofauna.

The statistical analysis of data revealed the existence of sets of species associated to the most contaminated sites, while others were only present in the cleanest soils. This differential response of soil mesofauna to soil contamination highlights the importance of such components as potential bioindicators of altered ecosystems.

References

Acosta J.A., Faz A., Martínez-Martínez S., Zornoza R., Carmona D.M., Kabas S., (2011), Multivariate statistical and GIS-based approach to evaluate heavy metals behavior in mine sites for future reclamation, *Journal of Geochemical Exploration* **109**, 8–17.

AEMET/IMP, (2011), Iberian Climate Atlas, State Meteorological Agency, Ministry of Environment and Rural and Marine Affairs (Spain) and Portuguese Metorological Institute.

Ariño A.H., Baquero E., Jordana R., Santamaría J.M., (2007), Forest biodiversity and air pollution: a standard method for soil bio-monitoring, *Writings from the Forestry Faculty of the University of Goettingen and the Northwest German Forest Research Station* **142**, 218-224.

Ariño A.H., Belascoáin C., Jordana R., (2008), *Optimal Sampling for Complexity in Soil Ecosystems*, In: *Unifying Themes in Complex Systems IV*, Minai A., Bar-Yam Y. (Eds.), Springer Verlag, 222-230.

Bengtsson G., Rundgren S., (1988), The Gusum case: A brass mill and the distribution of soil *Collembola*, *Canadian Journal of Zoology*, **66**, 1518-1526.

Bengtsson G., Rundgren S., Sjogren M., (1994), Modeling dispersal distances in a soil gradient: The influence of metal resistance, competition, and experience, *Oikos,* **71**, 13-23.

Bermejo R., Garrigó J., Gazol A., Ibañez R., Ederra A., Ariño A.H., Santamaría J.M., (2009), Report on national ICP IM activities in Spain, *The Finnish Environment*, **28**, 106-108.

Black H.I.J., Parekh N.R., Chaplow J.S., Monson F., Watkins J., Creamer R., Potter E.D., Poskitt J.M., Rowland P., Ainsworth G., Hornung M., (2003), Assessing soil biodiversity across Great Britain: national trends in the occurrence of heterotrophic bacteria and invertebrates in soil, *Journal of Environmental Management,* **67**, 255-266.

Blakely J.K., Neher D.A., Spongberg A.L., (2002), Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination, *Applied Soil Ecology,* **21**, 71–88.

Blaser P., Zimmermann S., Luster J., Shotyk W., (2000), Critical examination of trace element enrichments and depletions in soils: As, Cr, Cu, Ni, Pb, and Zn in Swiss forest soils, *Science of the Total Environment,* **249**, 257–280.

Bokhorst S., Huiskes A., Convey P., van Bodegom P.M., Aerts R., (2008), Climate change effects on soil arthropod communities from the Falkland Islands and

the Maritime Antarctic, *Soil Biology and Biochemistry*, **40**, 1547-1556.

Breure A.M., (2004), *Soil Biodiversity: Measurements, indicators, threats and soil functions*, First International Conference Soil and Compost Eco-Biology, León, 83-96.

Brussaard L., Behan-Pelletier V.M., Bignell D.E., Brown V.K., Didden W.A.M., Folgarait P.J., Fragoso C., Freckman D.W., Hattori T., (1997), Biodiversity and ecosystem functioning in soil, *Ambio,* **26**, 563-570.

Caruso T., Migliorini M., Bucci C, Bargagli R., (2009), Spatial patterns and autocorrelation in the response of microarthropods to soil pollutants: The example of oribatid mites in an abandoned mining and smelting area, *Environmental Pollution*, **157**, 2939-2948.

Dang-Yu S., Yin-Juan MA, Yong Q., Wen-Feng W., Chu-Guang Z., (2011), Volatility and mobility of some trace elements in coal from Shizuishan Power Plant, *Journal of Chemistry and Technology,* **39**, 328-332.

Dutra A.J.B., Paiva P.R.P., Tavares L.M., (2006), Alkaline leaching of zinc from electric arc furnace steel dust, *Minerals Engineering,* **19**, 478–485.

Fountain M.T., Hopkin S.P., (2004), A comparative study of the effects of metal contamination on *Collembola* in the field and in the laboratory, *Ecotoxicological*, **13,** 573-587.

Freckman D.W., (1994), *Life in the soil. Soil biodiversity: Its importance to ecosystem processes*, Report on a Workshop at the Natural History Museum, London, 30 August-1 September 1994, London: Natural History Museum.

Gackowski G., Seniczak S., Klimek A., Zalewski W., (1997), Soil mites (Acari) of young Scots pine forests in the region polluted by a copper smelting works at G1ogów (in Polish), *Zeszyty Naukowe ATR, Bydgoszcz, Ochrona Srodowiska,* **208**, 27-35.

Garrote Ruiz A., Muñoz Jiménez L., Zapata Sola M., Cerezo Arasti A., (1992), Geological Map of the Basque Country at 1:25.000 scale. Zumarraga 88 (II). Ed Basque Energy Board (EVE).

Gillet S., Ponge J.F., (2003), Changes in species assemblages and diets of Collembola along a gradient of metal pollution, *Applied Soil Ecology*, **22**, 127-138.

Gongalsky K.B., Filimonova Zh.V., Zaitsev A.S., (2010), Relationship between soil invertebrate abundance and soil heavy metal contents in the environs of the Kosogorsky Metallurgical Plant, Tula oblast, *Russian Journal of Ecology*, **41,** 67-70.

González-Miqueo L., Elustondo D., Lasheras E., Santamaría J.M., (2010), Use of native mosses as biomonitors of heavy metals and nitrogen deposition in the surroundings of two steel works, *Chemosphere,* **78**, 965-971.

IHOBE, (1994), Public Society of Environmental Management, On line at: http://www.ihobe.net.

Jordana R., Arpin P., Vinciguerra M.T., González S., Aramburu M.P., Ariño A.H., Armendáriz I., Belascoáin C., Cifuentes P., Clausi M., Escribano R., García Abril A., García-Mina J.M., Hernández M., Imaz A., Moraza M.L., Ponge J.F., Puig J., Ramos A., (2000), *Biodiversity Across Ecotones in Desertificable Mediterranean Areas*, In: *Mediterranean Desertification Research Results and Policy Implications,* Balabanis P., Peter D., Ghazi A., Tsogas M., (Eds.), Vol. 2, European Commission EUR 19303, 497-505.

Khalil M.A., Janssens T.K.S., Bergb M.P., van Straalen N.M., (2009), Identification of metal-responsive oribatid mites in a comparative survey of polluted soils, *Pedobiologia,* **52**, 207-221.

Lind B.B., Fällman A.-M., Larsson L.B., (2001), Environmental impact of ferrochrome slag in road construction, *Waste Management*, **21**, 255–264.

Manta D.S., Angelone M., Bellanca A., Neri R., Sprovieri M., (2002), Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy, *Science of the Total Environment,* **300**, 229–243.

Migliorini M., Pigino G., Caruso T., Fanciulli P.P., Leonzio C., Bernini, F., (2005), Soil communities (*Acari Oribatida, Hexapoda Collembola*) in a clay pigeon shooting range, *Pedobiologia,* **49**, 1-13.

Norton R.A., (1990), *Acarina: Oribatida*, In: *Soil Biology Guide*, Dindal D.L. (Ed.), Wiley, NewYork, 779–803.

Parisi V., Menta C., Gardi C., Jacomini C., Mozzanica E., (2005), Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy, *Agriculture, Ecosystems and Environment,* **105**, 323-333.

Poole R.W., (1974), *An Introduction to Quantitative Ecology*, McGraw-Hill, New York.

Rademacher P., (2001), Atmospheric heavy metals and forest ecosystems, Federal Research Centre for Forestry and Forest Products (BFH), CLRTAP.

Rai A., Prabakar J., Raju C.B., Morchalleb R.K., (2002), Metallurgical slag as a component in blended cement, *Construction and Building Materials,* **16**, 489–494.

Roca-Pérez L., Gil C., Cervera M.L., Gonzálvez A., Ramos-Miras J., Pons V., Bech J., Boluda R., (2010), Selenium and heavy metals content in some Mediterranean soils, *Journal of Geochemical Exploration,* **107**, 110–116.

Russell D.J., Alberti G., (1998), Effects of long-term, geogenic heavy metal contamination on soil organic matter and microarthropod communities, in particular *Collembola*, *Applied Soil Ecology,* **9**, 483-488.

Schroeder W., Munthe J., (1998), Atmospheric mercury e an overview, *Atmospheric Environment,* **32**, 809-822.

Schulin R., Curchod F., Mondeshka M., Daskalova A., Keller A., (2007), Heavy metal contamination along a soil transect in the vicinity of the iron smelter of Kremikovtzi (Bulgaria), *Geoderma,* **140**, 52–61.

Seniczak S., Klimek A., Gackowski G., Kacmarek S., Zalewski W. (1997). Effect of copper smelting air pollution on the mites (Acari) associated with young Scots pine forests polluted by a copper smelting works at Glogow, Poland. II: Soil mites, *Water Air Soil Pollution,* 97, 287–302.

Seniczak S., Kaczmarek S., Klimek A., Seniczak A., (2002), The effect of some air pollutants on the vertical distribution of mites (Acari) in soils of young Scots pine forests in Poland, *European Journal of Soil Biology,* **38**, 311−314.

Singh R., Singh D.P., Kumar N., Bhargava S.K., Barman S.C., (2010), Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area, *Journal of Environmental Biology,* **31**, 421–430.

Skubala P., Kafel A., (2004), Oribatid mite communities and metal bioaccumulation in oribatid species (*Acari, Oribatida*) along the heavy metal gradient in forest ecosystems, *Environmental Pollution,* **132**, 51-60.

Spurgeon D.J., Rowland P., Ainsworth G., Rothery P., Long S., Black H.I.J., (2008), Geographical and pedological drivers of distribution and risks to soil fauna of seven metals (Cd, Cu, Cr, Ni, Pb, V and Zn) in British soils, *Environmental Pollution,* **153**, 273-283.

Stanton N.L., (1979), Patterns of species diversity in temperate and tropical litter mites, *Ecology,* **60**, 295–304.

Steiner W.A., (1995), Influence of air pollution on mossdwelling animals. Terrestrial fauna, with emphasis on *Oribatida and Collembola*, *Acarologia,* **36**, 149–173.

Stone D., Jepson P., Kramarz P., Laskowski R., (2001), Time to death response in carabid beetles to multiple stressors along a gradient of heavy metal pollution. *Environmental Pollution,* **113**, 239–244.

Sutherland R.A., (2000), Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii, *Environmental Geology,* **39**, 611 – 637.

Syrek D., Weiner W.M., Wojtylak M., Olszowska G., Kwapis Z., (2006), Species abundance distribution of collembolan communities in forest soils polluted with heavy metals, *Applied Soil Ecology*, **31**, 239–250.

Tranvik L., Eijsackers H., (1989), On the advantage of Folsomia fimetaroides over Isotomiella minor (Collembola) in a metal polluted soil, *Oecologia,* **80**, 195-200.

Van Straalen N.M., (1998), Evaluation of bioindicators systems derived from soil arthropod communities, *Applied Soil Ecology,* **9**, 429–437.

Van Straalen N.M., Burghouts T.B.A., Doornhof M.J., Groot G.M., Janssen M.P.M., Joosse E.N.G., van Meerendonk J.H., Theeuwen J.P., Verhoef H.A., Zoomer H.R., (1987), Efficiency of lead and cadmium excretion in populations of *Orchesella cinta* (Collembola) from various contaminated forest soils, *Journal of Applied Ecology*, **24**, 953-968.

Xiankai L., Jiangming M., Shaofeng D., (2008), Effects of nitrogen deposition on forest biodiversity, *Acta Ecologica Sinica*, **28**, 5532-5548.

Xu G.L., Schleppi P., Li M.H., Fu S.L., (2009), Negative responses of *Collembola* in a forest soil (Alptal, Switzerland) under experimentally increased N deposition, *Environmental Pollution,* **157**, 2030-2036.

Yaylalı-Abanuz G., (2011), Heavy metal contamination of surface soil around Gebze industrial area, Turkey, *Microchemical Journal,* **99**, 82–92.

Zaitsev A.S., van Straalen N.M., (2001), Species diversity and metal accumulation in oribatid mites (*Acari, Oribatida*) of forests affected by a metallurgical plant, *Pedobiologia*, **45**, 467-479.

Zhang L., Blanchard P., (2009), A review of current knowledge concerning dry deposition of atmospheric Mercury, *Atmospheric Environment,* **43**, 5853–5864.