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Relationship Between Testate Amoeba (Protist) Communities and Atmospheric Heavy Metals Accumulated in *Barbula indica* (Bryophyta) in Vietnam

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Abstract

We studied the relationships between testate amoeba communities and heavy metal (Pb, Cd, Zn, Ni, Cu, Mn, and Fe) concentrations in the moss *Barbula indica* sampled at 29 sites in and around the city of Hanoi (Vietnam). Our first approach was to compare the heavy metal concentrations and testate amoeba variables between the city (zone 1) and the surrounding (zone 2). Mean moss concentrations of Pb, Cd, Zn, Ni, and Cu were significantly higher and testate amoeba species richness and abundance were significantly lower in zone 1 and the abundance of eight taxa differed significantly between the two zones. We then studied the correlation between heavy metals and testate amoebae. Species richness and abundance were correlated negatively to Pb concentration. Shannon H' was negatively correlated to both Pb and Cd. The abundance of several species was negatively correlated with Pb, Cd, Zn, and Ni; however, at the community level, Pb emerged as the only significant variable in a redundancy analysis. Our results suggest that testate amoebae are sensitive to and may be good bioindicators for heavy metal pollution, especially lead. Further research is needed to understand the causal relationships underlying the observed patterns.

Introduction

Atmospheric pollution has important consequences on the environment, economy, human health, and society. The types of atmospheric pollutants and the pollution levels depend on many factors, such as emission sources, physical conditions, and meteorological parameters. The monitoring and surveillance of air quality have been carried out mostly in urban areas. Chemical analyses with automatic analyzers continuously measuring the pollution level and mathematical models evaluating the diffusion of atmospheric pollutants are commonly used.

The biomonitoring approach, based on the sensitivity of organisms to pollution, is complementary to physicochemical methods for the evaluation of air pollution. This approach presents several advantages in comparison with physicochemical methods [58]. One of these is that it integrates the pollution level over a long period of time and therefore provides data on an average pollution level for a given place. This aspect is well known and has been used to monitor the quality of aquatic environments. For example, indexes of freshwater invertebrates, such as the invertebrate community index or the Ephemeroptera, Plecoptera, Trichoptera index, have been used to assess water pollution [34]. The biomonitoring approach may also allow the detection of extreme or catastrophic events that may not be recorded by noncontinuous pollution monitoring.

Several groups of organisms have been used as bioindicators of atmospheric pollution. The most commonly used are lichens [7, 24] and mosses [43, 45]. The latter is well known for the monitoring of heavy metal atmospheric deposition in terrestrial ecosystem [48] and *Sphagnum* mosses have also been used to reconstruct metal atmospheric deposition in peatlands [20, 59]. Heavy metals are accumulated in the mosses because of their anatomical and physiological characteristics. Mosses are deprived of cuticle and epidermis and their leaves are therefore highly permeable to water and solutes, such as

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ions of trace elements. In addition, the lack of roots and vascular system makes mosses well suited for studies of atmospheric deposition. Indeed, mineral salts and ions present in mosses come from atmospheric precipitation and dry deposition through simple processes of ion exchange, whereas the uptake from substratum is negligible [50].

Although the effects of heavy metal pollution on different groups of higher organisms have been quite extensively studied, data concerning microorganisms is much scarcer. Few studies have focused on the effect on microorganisms of heavy metal pollution in soil and water pollution [25, 26, 38] or of atmospheric pollution [16, 27, 28, 49].

Testate amoebae living in terrestrial mosses may be interesting candidates for monitoring air pollution for several reasons: (1) they live in a subaerial environment where they are directly exposed to atmospheric pollutants; (2) they are very abundant, diverse (about 100 potential species in mosses alone), and most of these species appear to be cosmopolitan [4]; (3) their identification is relatively easy based on the morphology of their test (shell) that remains even after the death of the organism; and (4) they are good integrators of disturbances because of their trophic position at the end of the microbial food webs [18, 19].

The value of testate amoebae as bioindicators in organic and mineral soils is well established [14, 17, 52]. In addition to responding to natural ecological gradients, testate amoebae may be affected by pollution both directly, through direct contact, and indirectly, through the contamination of their prey or modifications of the structure of bacteria, fungi, or protist communities on which they prey. However, to our knowledge, only three studies have focused on the relationship between these protists and atmospheric pollution [2, 32, 41]. Studying the effect of air pollution from vehicle traffic on testate amoebae in Warsaw (Poland), Balik [2] reported a decrease in abundance, richness of species, diversity of species (Shannon Weaver index), and the index of equitability of soil testate amoebae. Lüftenegger and Foissner [32] also observed the density, biomass, and species richness of testate amoebae along two 100-m transects on both sides of a high-traffic road. In this latter study, the lowest density, biomass, and species richness of testate amoebae was observed nearest to the road, but were not correlated with the highest concentrations of the measured pollutants, most of which peaked 50 m from the road. In a previous study, we examined the impact of atmospheric pollution characterized by nitrogen dioxide (NO_2) pollution on testate amoeba abundance, diversity, and community structure in and around the city of Besançon (France). The number of testate amoeba species was found to be significantly lower in the city center than in the less polluted surrounding areas but the total abundance did not vary between the two zones and the density of only one species, *Paraquadrula irregularis*, differed significantly between the two zones [41].

Our general aims were as follows: (1) to investigate the accumulation of heavy metals (Pb, Cd, Zn, Ni, Cu, Mn, and Fe) in the moss *Barbula indica* (Hook.) in and around the city of Hanoi, Vietnam, and (2) to investigate the relationship between testate amoeba communities living in *B. indica* and heavy metal concentration in the moss. We hypothesized that (1) the heavy metal concentration would be higher and the abundance and diversity of testate amoebae would be lower in the city; (2) the structure of communities would differ between city and surroundings; and (3) the abundance, diversity, and community structure of testate amoebae would be correlated with concentrations of heavy metal in mosses. This research contributes to a more general goal of assessing the potential for testate amoebae to be used as biomonitors of atmospheric pollution.

Methods

Site Selection and Sampling Protocol

Selection of Study Sites. This study was conducted from April to June of 2002 in Hanoi, the capital city of Vietnam (21.02°N, 105.85°E). The study zone of 912 km^2 was divided into two subareas corresponding to urban (82 km^2, i.e., 9%) and rural (830 km^2, i.e., 91%) areas. This division was based on the administrative division of Hanoi region and our underlying assumption was that pollution levels would differ between the city center (urban area, zone 1) and the surroundings (rural area, zone 2). Indeed, the urban area of Hanoi, composed of seven inner city districts at the moment of this study, shows a high population density (i.e., 53% of the 3.5 million habitants), heavy traffic, and some medium-sized industrial zones [39]. In contrast, the rural area is composed of four surrounding districts where agriculture is the principal activity with some small preexisting industrial zones and also a few larger ones that are currently being built. In these surrounding districts, agricultural activities can be considered as the main pollution source via pesticides and chemical fertilization [1, 39].

A grid of 2 × 2 km was used in zone 1 and a 10 × 10 km grid was used for zone 2. The sampling locations were based on these grids. *B. indica* mosses were sampled in each grid gap. However, this moss was absent at several sites and because of field constraints, we could not sample in all gaps of the grids and the sampling positions sometimes were not respected as expected. In total, moss samples were taken at 29 different sites, comprising 19 sites in zone 1 and 10 sites in zone 2 (Fig. 1). Coordinates of sampling sites registered and collected by Global Positioning System allowed the location of sampling sites on the maps.

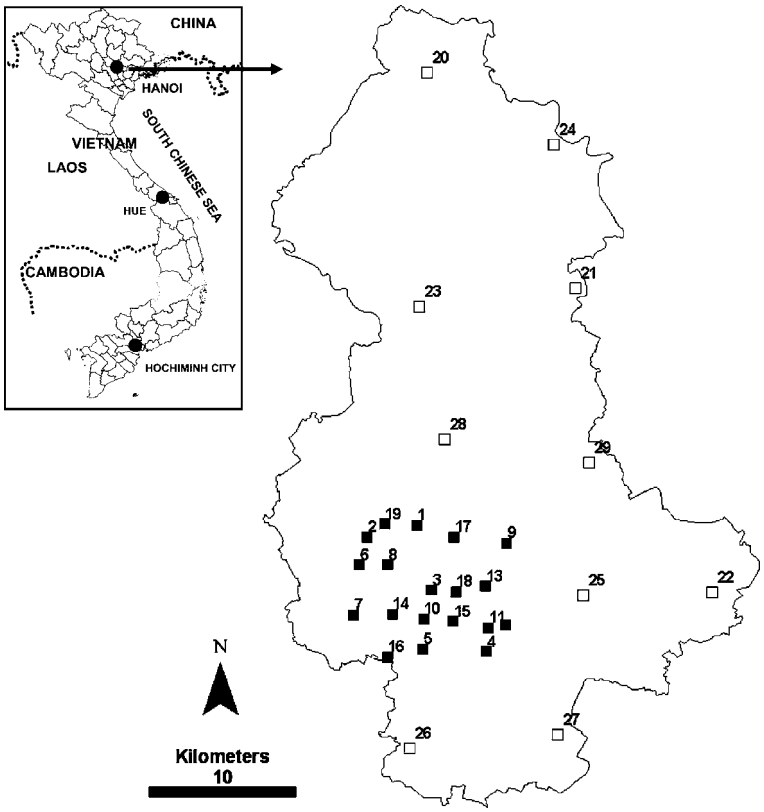


Figure 1. Map showing the location of sampling sites in zone 1 (inner city districts, *19 black squares*) and in zone 2 (surrounding districts, *10 open squares*) in Hanoi, Vietnam. *Numbers* are the name of sampling sites.

Moss Sampling. In this study, the moss *B. indica* was chosen as a bioaccumulator of heavy metals and a habitat for testate amoebae for several reasons. *B. indica* is a small bryophyte, green or yellowish, forming low tufts with evenly distributed stems seldom exceeding 1 cm in height [12]. This moss is widespread in Vietnam and abundant throughout tropical Asia [12]. It usually grows on soils, rocks, and artificial substrates such as pavement, especially on moist and calcareous substrates. Preliminary observations and analyses of several moss species showed that *B. indica* constitutes a favorable habitat for the development of testate amoebae.

Moss sampling was carried out according to the protocol defined in the guidelines of the program "European survey of atmospheric heavy metal deposition" [47], which states that sampling sites should be selected at least 300 m away from main roads and at least 100 m away from small roads. Moss samples were collected in a 50 × 50 m area around each sampling site. However, in some cases of our study, *B. indica* was not very abundant at sampling sites and/or the existing mosses were not far enough from the road, in particular in the city center. In these cases, mosses were sampled in an area, which was bigger than 50 × 50 m and as far from the road as possible. For each site, between 5 and 10 subsamples were collected on hard substrate and in nontrampled places (walls and roofs) and then combined in a composite sample. In the laboratory, soil particles and dust were removed from the moss samples without using water. The top part of the mosses (living, green) was separated from the lower part (brown, dead), and then only the top part was used for heavy metal and testate amoeba analyzes. The sampling height (height of the moss sampling position above the ground) was recorded. The sampling height was taken into account because it seemed to be related to the exposure of mosses and moss testate amoebae to different environmental conditions. Consequently, it may be related to the variation of metal concentrations in the moss, as well as moss testate amoeba communities.

Meteorological Data. The climate in Hanoi during summer is characterized by elevated temperature and high precipitation [57]. During the sampling period from April to June of 2002, the minimal temperature varied from 16.8 to 25.0°C, the maximal from 33.6 to 38.6°C, and the average temperature was 27.6 ± 2.1°C. The humidity varied from 80.0 to 82.0% (mean 81.0 ± 1.0%). The rain during this period was from 59.0 to 240.0 mm of water (mean 171.0 ± 97.9 mm of water). The total evaporation in this period was from 79.0 to 86.0 mm of water (mean 83.3 ± 3.8 mm of water) [57].

Laboratory Analyses

Heavy Metal Analysis. For heavy metal analysis, the mosses were dried at 40°C to constant weight. About 150 mg of dry mass was ground and then digested in a mixture of 3.4 mL of concentrated nitric acid (HNO_3, 65%) and 0.6 mL of H_2O_2 at 65°C during 48 h. The resulting solution was diluted in 16 mL of deionized water and filtered through 0.2-μm mesh. The heavy metal concentrations in the moss were determined using furnace (for Pb, Cd, Ni, and Cu) and flame (for Zn, Mn, and Fe) atomic absorption spectrometer. Concentration measurement of these seven elements in the reference material ray grass (CRM 281, No. 766 of the community bureau of reference, Commission of the European Community) was also performed to check the accuracy of this analysis. Analytical replication was used for every analysis; the same material reference was used after every 10th analysis and during the whole period.

Testate Amoeba Analyses. Each sample, approximately 0.5 g (fresh weight) of the top part of the moss, was fixed in formaldehyde solution (4% final concentration) in a small polyvinyl chloride bottle. To extract testate amoebae, moss samples were shaken with a vortex mixer, filtered through a 100-μm mesh, and washed with deionized water. The remaining fraction on the filter was dried at 80°C for 48 h and weighed. The filtrate containing the testate amoebae was placed in a plankton-settling chamber for 24-h sedimentation. The slides were then analyzed at a magnification of ×200 and ×400 with an inverted microscope following Uthermöhl's method [54]. The whole slide was analyzed for testate amoebae. All whole (living, encysted, and dead) amoeba tests were counted. The total number of tests counted varied between 182 and 3169 tests per sample, except for two samples in which only 37 and 57 tests were observed. Books and monographs on testate amoeba identification were used to determine species and species groups [5, 8–11, 42]. The same person (first author) performed all counts.

Numerical Analyses. Taxa occurring in only one sample and always having few individuals (e.g., two for *Corythion dubium*) were removed from the data set for further analyses. To evaluate the diversity of testate amoeba communities, the Shannon index was calculated using the following formula:

$$\text{Shannon index } H' = \sum n_i/N \times \log_2 (n_i/N) \quad (1)$$

where n_i is the number of individuals of testate amoeba species i in the community and N is the total number of individuals in the community.

Testate amoeba abundance data were transformed [with $x' = \ln(x + 1)$] before the analyses to reduce the weight of dominant species in the analyses. The normality of the data was assessed using Kolmogorov-Smirnov test. Nonnormal data were analyzed using Mann-Whitney tests. To assess the correlations between testate amoebae (species richness, total abundance, and abundance of each species) and environmental variables (moss heavy metal concentrations and sampling height), simple regression or Spearman rank correlation analyzes were performed depending on the data normality. Finally, to assess the relationship between the composition of testate amoeba communities and the heavy metal concentrations in the mosses, a redundancy analysis (RDA) was carried out using the program CANOCO 4 [51]. Eight quantitative variables ([Pb], [Cd], [Zn], [Ni], [Cu], [Mn], [Fe], and the sampling height) were used as explanatory variables to perform the RDA.

Details of this method can be found in Ter Braak and Smilauer's study [51]. Briefly, the importance of the environmental variables was determined by stepwise forward selection. In each step, the "extra fit" was determined for each variable. The variable with the largest extra fit, if significant (Monte Carlo permutation test, 999 permutations), was then included, and the process was repeated until no variables remained that could significantly improve the fit. Taxa contributions on the axis are considered to be significant when their contribution is equal or higher than a threshold S (‰) calculated by the following empirical formula: S_n (‰) = $1/n \times 1000 \times 2$, where S_n is the contribution to axis 1 or axis 2 of the biplot; n is the total number of species in the community (A. Renaud, Ph.D. thesis, Université de Droit, d'Economie et des Sciences d'Aix-Marseille, France, 2003). Biplot of relationship between testate amoeba species and environmental variables were visualized for interpretation. Moss heavy metal concentrations were presented on the maps using software MapInfo Professional Version 6.0.

Results

Heavy Metal Concentrations in B. indica. The spatial distribution of metal concentrations in *B. indica* moss is shown by Fig. 2 and heavy metal descriptive results in two zones are presented in Table 1. [Pb] varied between 5.9 and 56.5 μg g^{-1} dry weight (d.w.) of moss (mean ± SD = 25.6 ± 14.5 μg g^{-1} d.w. of moss) and was significantly higher in zone 1 than in zone 2 (Table 1). However, one value observed in zone 2 showed high-level concentration (39.6 μg g^{-1}, sample 22; Fig. 2) and another in zone 1 remained very low (9.9 μg g^{-1}, sample 18; Fig. 2). [Zn], [Cd], [Ni], and [Cu] were significantly higher in zone 1 than in zone 2 (Table 1). [Mn] and [Fe] were slightly but not significantly higher ($p = 0.35$ and 0.68, respectively) in zone 1 than in zone 2 (Table 1). [Pb] was significantly correlated with [Cd] ($r = 0.54$, $p = 0.002$, linear correlation) and with [Ni] ($r = 0.40$, $p = 0.03$, linear correla-

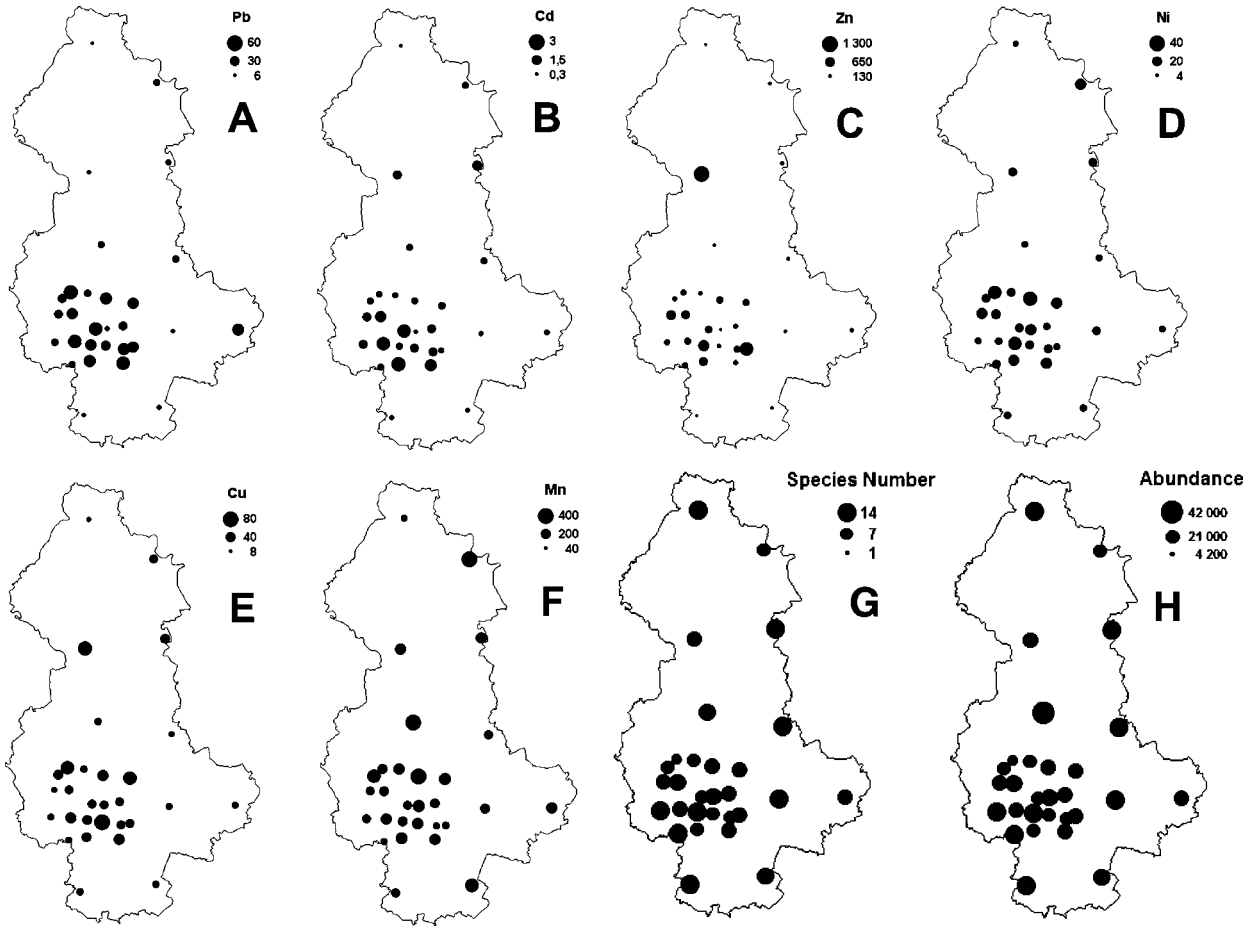


Figure 2. Spot map of Pb (A), Cd (B), Zn (C), Ni (D), Cu (E), and Mn (F) concentrations (μg g^{-1} d.w.) and testate amoeba species richness (G) and abundance (H) in the moss *B. indica* in different sampling sites in Hanoi. Metal concentrations, testate amoeba species richness, and abundance are proportional to the size of *symbols*.

tion). [Ni] and [Mn] were significantly correlated with [Fe] ($r = 0.40$, $p = 0.04$ and $r = 0.82$, $p < 0.01$, respectively, linear correlation). The sampling height was significantly and positively correlated to the moss [Ni].

Testate Amoeba Abundance. A total of 23 testate amoeba taxa, belonging to 12 genera, were identified in the moss samples (Table 2). The testate amoeba community was strongly dominated by three species: *Euglypha strigosa*, *Centropyxis platystoma*, and *Phryganella acropodia*, which were found at all 29 moss sample sites and also presented the highest average and relative abundance. By contrast, *Euglypha dilociformis*, *Hyalosphenia minuta*, *Quadrulella symmetrica*, *P. irregularis*, *Nebela*

Table 1. Comparison of moss metal concentrations (μg g^{-1}) in zone 1 (city center) and zone 2 (periphery)

	Zone 1 (n = 19)					*Zone 2 (n = 10)*					
Metal	*Min*	*Max*	*Median*	*Mean*	*SD*	*Min*	*Max*	*Median*	*Mean*	*SD*	p^a
Pb	9.9	56.5	31.1	31.9	12.6	5.9	39.6	10.9	13.7	9.9	<0.001
Zn	86.0	1000	298	363	226	73.2	1294	111	228	374.9	<0.005
Cd	0.4	2.7	1.0	1.2	0.7	0.3	1.5	0.7	0.7	0.3	0.024
Ni	9.4	37.6	16.0	19.1	7.8	8.6	21.5	11.9	13.0	4.1	0.026
Cu	16.8	80.4	30.3	37.0	17.0	12.8	70.0	24.1	27.7	16.1	0.039
Mn	92.5	392	198	207	76.3	97.8	404	214	246	101.7	n.s.
Fe	1873	12,598	6918	6825	2872	2281	13,896	5714	6864	3478	n.s.

SD: standard deviation; n.s.: nonsignificant.
[a]Mann-Whitney test.

Table 2. Overall frequency density and relative density of 23 testate amoeba taxa extracted from the moss *B. indica* in 29 sampling sites in Hanoi

		Average density		*Average relative abundance*	
Species	*Frequency (/29)*	*Tests g^{-1} d.w.*	*SD*	*Percentage (%)*	*SD*
Arcella artocrea Leidy var. *aplanata* Grospietsch[a]	12	67	145	0.8	1.6
Arcella discoides Ehrenberg[a]	22	54	74	0.9	1.3
Assulina muscorum Greeff	2	0	1	0.0	0.0
Centropyxis aculeata Stein	5	3	9	0.0	0.1
Centropyxis aerophyla Deflandre var. *sylvatica*[a]	27	850	1147	10.4	10.4
Centropyxis platystoma Deflandre	29	1330	1271	16.5	11.2
Difflugia pristis Penard type[a]	10	38	82	0.3	0.5
Euglypha ciliata (Ehrenberg) Leidy for. *glabra* Wailes	6	113	515	0.3	1.3
Euglypha ciliata Ehrenberg	19	109	221	1.4	2.8
Euglypha dilociformis Bonnet[a]	4	6	21	0.2	0.5
Euglypha rotunda Wailes type	10	183	584	1.1	2.8
Euglypha rotunda Wailes var. *dorsalis* Decloitre	7	51	132	0.4	1.0
Euglypha rotunda Wailes	27	380	545	3.7	3.6
Euglypha strigosa Ehrenberg	29	2793	2409	30.3	18.2
Heleopera sylvatica Penard	8	14	30	0.2	0.5
Hyalosphenia minuta Cash[a]	4	5	16	0.0	0.1
Nebela scotica Brown type[a]	2	7	31	0.1	0.3
Paraquadrula irregularis[a]	2	7	37	0.1	0.4
Phryganella acropodia Hertwig & Lesser	29	1284	1773	14.4	13.4
Quadrulella symmetrica Wallich (Schulze)[a]	4	4	12	0.1	0.6
Tracheleuglypha dentata Moniez	23	1725	5600	11.3	15.6
Trinema enchelys Ehrenberg	19	243	525	2.1	2.9
Trinema lineare Penard	26	539	772	5.3	6.2

SD: standard deviation.
[a]Taxa previously not recorded in Vietnam.

scotica type and *Assulina muscorum* were rarely present and were the least abundant (Table 2).

The total testate amoeba abundance ranged between 296 and 41,425 tests g^{-1} d.w. of moss (mean 9806 ± 8615 tests g^{-1} d.w. of moss). The highest abundance was observed in samples 28 and 23 from zone 2, whereas the lowest was in samples 19, 8, and 5 from zone 1. The total abundance of testate amoebae was significantly lower in zone 1 than in zone 2 (Fig. 3). In addition, the abundance of eight taxa differed significantly between the two zones: *Euglypha ciliata* for. *glabra*, *Centropyxis aerophila* var. *sylvatica*, *Euglypha rotunda* var. *dorsalis*, *E. rotunda*, *Arcella discoides*, *E. rotunda* type, *Trinema lineare*, and *Difflugia pristis* type (p values varied between <0.001 and

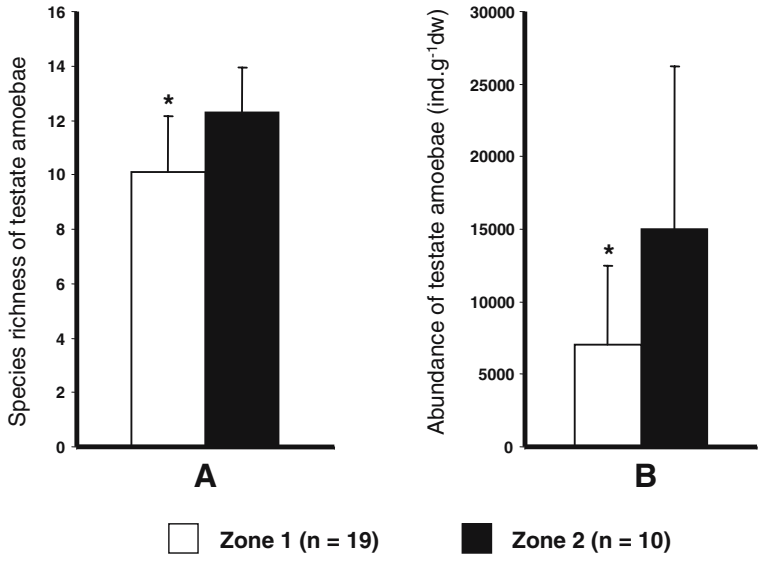


Figure 3. Species richness (A) and abundance (B) of testate amoebae (mean ± standard deviation) in the moss *B. indica* in Hanoi (zone 1: *white bars*) and in the surroundings (zone 2: *black bars*). *Asterisk* indicates that the values are significantly different between the two zones; test Mann-Whitney, $p < 0.05$).

Table 3. Comparison of testate amoeba frequency (*n*) and abundance in zone 1 (city center, 19 samples) and zone 2 (periphery, 10 samples); contribution of testate amoeba species to the first axes of the RDA

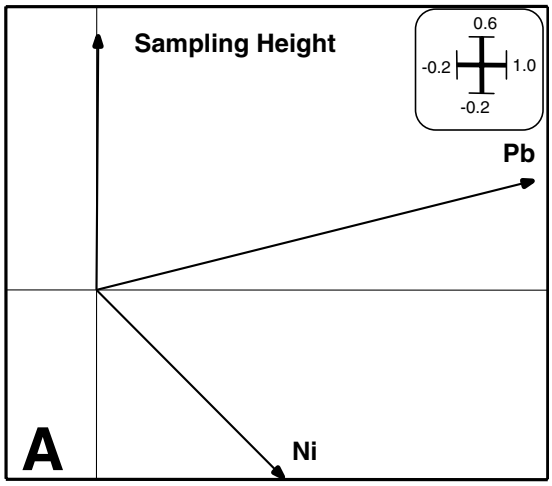
		Zone 1			*Zone 2*				
	n total (/29)	*n (/19)*	*Mean (individuals g^{-1})*	*SD (individuals g^{-1})*	*n (/10)*	*Mean (individuals g^{-1})*	*SD (individuals g^{-1})*	*p*[a]	*RDA, axis 1 score*
Euglypha ciliata for. *glabra*	6	0	0.0	0.0	6	548.3	1093.8	<0.001	–0.44
Centropyxis aerophyla var. *sylvatica*	27	17	424.5	497.4	10	1742.3	1506.2	<0.001	–0.50
Euglypha rotunda var. *dorsalis*	7	1	62.2	0.0	6	234.7	212.2	0.00	–0.57
Euglypha rotunda	29	19	185.9	271.4	10	748.3	738.6	0.00	–0.48
Arcella discoides	22	13	40.7	43.1	9	115.2	97.7	0.01	–0.11
Euglypha rotunda type	10	4	191.1	180.4	6	758.8	1169.4	0.03	–0.33
Trinema lineare	26	16	469.5	809.1	10	811.3	759.6	0.04	–0.74
Difflugia pristis type	10	4	139.0	152.7	6	92.4	80.7	0.05	–0.42
Phryganella acropodia	29	19	899.5	1310.2	10	2013.6	2333.3	n.s.	–0.26
Hyalosphenia minuta	4	4	35.3	32.7	0	0.0	0.0	n.s.	0.06
Quadrulella symmetrica	4	4	26.5	25.3	0	0.0	0.0	n.s.	0.21
Euglypha dilociformis	4	4	44.4	42.1	0	0.0	0.0	n.s.	0.23
Trinema enchelys	19	10	278.3	220.1	9	474.0	880.7	n.s.	–0.53
Heleopera sylvatica	8	7	55.6	39.6	1	30.3	0.0	n.s.	–0.01
Tracheleuglypha dentata	23	14	836.3	801.4	9	4256.7	9893.4	n.s.	–0.30
Paraquadrula irregularis	2	2	102.6	137.3	0	0.0	0.0	n.s.	–0.02
Assulina muscorum	2	2	4.4	1.6	0	0.0	0.0	n.s.	0.16
Centropyxis aculeata	5	4	16.1	15.4	1	24.0	0.0	n.s.	–0.29
Nebela scotica type	2	1	166.9	0.0	1	30.3	0.0	n.s.	0.16
Euglypha strigosa	29	19	2736.6	2417.5	10	2900.0	2518.6	n.s.	–0.47
Euglypha ciliata	19	14	99.3	133.1	5	354.9	421.8	n.s.	0.02
Centropyxis platystoma	29	19	1385.8	1469.2	10	1222.5	832.5	n.s.	–0.27
Arcella artocrea var. *aplanata*	12	8	168.2	223.6	4	146.9	138.5	n.s.	–0.05

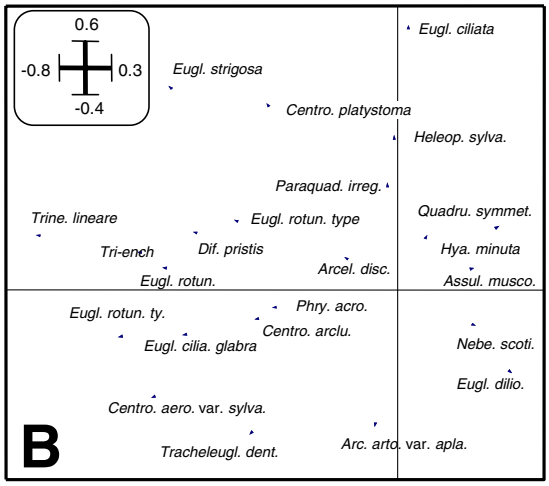
[a]Mann-Whitney test.

Table 4. Correlation between moss heavy metal concentrations and testate amoeba species richness, overall abundance, Shannon *H′*, and individual species abundance in the moss *B. indica*

	r value						
Variable	*Pb*	*Cu*	*Cd*	*Mn*	*Zn*	*Ni*	*Fe*
Testate amoeba species richness[a]	–0.59[b]	–0.46[c]	n.s.	n.s.	n.s.	n.s.	n.s.
Total testate amoeba abundance[a]	–0.40[c]	n.s.	n.s.	0.4[c]	n.s.	n.s.	n.s.
Shannon H′[a]	–0.45[c]	n.s.	–0.42[c]	n.s.	n.s.	n.s.	n.s.
Species abundance[d]							
Trinema lineare	–0.65[b]	n.s.	–0.46[c]	n.s.	n.s.	n.s.	n.s.
Centropyxis aerophyla var. *sylvatica*	–0.63[b]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Euglypha rotunda	–0.50[b]	n.s.	–0.41[c]	n.s.	n.s.	n.s.	n.s.
Tracheleuglypha dentata	–0.45[c]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Trinema enchelys	–0.44[c]	n.s.	n.s.	n.s.	–0.35[c]	n.s.	n.s.
Euglypha rotunda var. *dorsalis*	–0.26[b]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Euglypha ciliata for. *glabra*	–0.15[b]	n.s.	n.s.	n.s.	–0.15[c]	–0.03[c]	n.s.
Euglypha ciliata	n.s.	n.s.	n.s.	n.s.	n.s.	–0.42[c]	n.s.

[a]Simple regression.
[b]$p < 0.01$.
[c]$p < 0.05$.
[d]Spearman correlation.





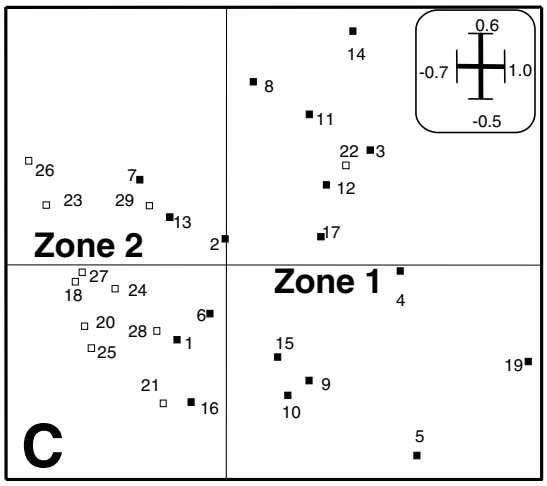


Table 5. Summary results of stepwise selection of variables using RDA with eight quantitative explanatory variables

Variable	*Extra fit (%)*	*Cumulative fit (%)*	p^a
Pb	15	15	0.001
Ni	6	21	0.039
Sampling height	5	26	0.044
Fe	3	29	n.s.
Mn	4	33	n.s.
Zn	3	36	n.s.
Cd	2	38	n.s.
Cu	2	40	n.s.

[a]Significance was determined by Monte Carlo permutation using 999 random permutations.

0.045, Mann-Whitney test). These species were all more abundant in zone 2 than in zone 1 except for *D. pristis* type (Table 3). Furthermore, the total abundance of testate amoebae was negatively correlated to Pb concentration ($r = -0.40$; $p = 0.03$; linear correlation) and positively correlated to Mn concentration ($r = 0.39$; $p = 0.03$; linear correlation; Table 4). No significant correlation was recorded between the total abundance and Cd, Zn, Ni, Cu, and Fe concentrations (Table 4). The abundance of seven taxa was negatively correlated to Pb concentrations in the mosses: *T. lineare*, *C. aerophila* var. *sylvatica*, *E. rotunda*, *Tracheleuglypha dentata*, *Trinema enchelys*, *E. rotunda* var. *dorsalis*, and *E. ciliata* for. *glabra* ($p < 0.01$ for all species except for *T. enchelys* and *T. dentata*, $p < 0.05$; Table 4). Among these seven taxa, *T. lineare* and *E. rotunda* abundance was also significantly correlated negatively to Cd concentration ($p < 0.05$ for two species), whereas the abundance *T. enchelys* and *E. ciliata* for. *glabra* was significantly correlated negatively to Zn concentration ($p = 0.002$ and 0.047, respectively). The abundance of *E. ciliata* and *E. ciliata* for. *glabra* was also negatively correlated to Ni concentration ($p = 0.02$ and 0.04, respectively; Table 4).

Testate Amoeba Species Richness and Diversity. The taxa richness varied between 5 and 14 species, was significantly higher in zone 2 than in zone 1 (Fig. 3), and

Figure 4. Redundancy analysis biplots (axes 1 and 2) of testate amoeba data with the projection of variables (A), species (B), and samples (C). Three environmental variables included in the model explain 26% of the variation in the species data (A). Twenty-three testate amoeba taxa (*black triangles*, B; see Table 1 for complete names). Sample names are expressed by *numbers* (C), from *1* to *19* for zone 1 (*black squares*) and from 20 to 29 for *zone 2* (*open squares*).

Table 6. Heavy metal mean concentrations (μg g^{-1}) in *B. indica* in Hanoi and in different moss species in other studies

Metal	*Vietnam* ($n = 29$)[a]	*France* ($n = 512$)[b]	*Spain* ($n = 175$)[c]	*China* ($n = 16$)[d]
Pb	25.6	11.7	5.6	–
Cd	1.1	0.3	–	3.0
Zn	316.3	37.0	59.2	162.0
Ni	17.0	2.7	1.8	20.8
Cu	33.8	6.1	6.2	46.5
Mn	220.9	291.0	236.4	1114.8
Fe	6838.4	744.0	476.0	182.8

[a]The present study: Hanoi, Vietnam.
[b]Gombert *et al.* [23].
[c]Fernández and Carballeira [13].
[d]Lee *et al.* [29].

was positively correlated with total abundance ($r = 0.45$, $p = 0.02$, linear correlation). Testate amoeba taxa richness and the Shannon index were negatively correlated with Pb and Cu concentrations ($p < 0.001$, $r = -0.64$ and $p = 0.012$, $r = -0.46$, respectively, for taxa richness and $r = -0.41$, $p = 0.03$ and $r = -0.42$, $p = 0.02$, respectively, for Shannon index, linear correlation; Table 4).

Redundancy Analysis. The summary results of the stepwise forward selection are presented in Table 5. [Pb], [Ni], and the sampling height were significant and together explained 26% of the variance in the testate amoeba data. More than half of this percentage was explained by Pb concentration alone (i.e., 15%). The testate amoeba community data were not significantly correlated to the remaining element concentrations (Fe, Mn, Zn, Cd, and Cu) in this multivariate analysis (Table 5). Figure 4 shows the correlations between environmental variables (Fig. 4A), testate amoebae (Fig. 4B), and samples (Fig. 4C) over the first two canonical (i.e., constrained) axes. Axes 1 and 2 explain 15% and 6% of the variation of testate amoebae, respectively, but only axis 1 is significant ($p = 0.003$; 0.08 for axes 1 and 2, respectively; Monte Carlo permutation test, 999 permutations). Figure 4A also clearly shows that axis 1 is mainly explained by [Pb]. Therefore, the position of the species along this axis reflects their correlation with [Pb]. The species scores on the first axis of the RDA are detailed in Table 3.

Discussion

Moss Trace Element Concentrations in Relation to Atmospheric Pollution in Hanoi. The trace element concentrations in *B. indica* moss in Hanoi were relatively elevated compared with those analyzed in some cities of other countries where industrial emissions are moderate. For example, concentration values are 2.2, 3.8, 8.5, 6.4, 5.5, 0.8, and 9.2 times higher for Pb, Cd, Zn, Ni, Cu, Mn, and Fe, respectively, than those observed in French mosses (data presenting the background pollution in moss in 1996; Table 6) [23], or were 4.6-, 5.3-, 9.5-, 5.5-, 0.9-, and 14.4-fold higher for Pb, Zn, Ni, Cu, Mn, and Fe, respectively, than those analyzed in the moss *Scleropodium purum* in northwest Spain (Table 6) [13]. Conversely, our recorded concentrations in Hanoi are lower than those in the moss *Hypnum plumaeforme* in southern China, except for Zn and Fe (0.2, 0.4, 2.0, 0.8, 0.7, 0.2, and 37.4 times for Pb, Cd, Zn, Ni, Cu, Mn, and Fe, respectively; Table 6) [29]. However, it is difficult to compare heavy metal concentrations in mosses from different studies because the difference in bryophyte species, pollutant emission, time/space scale of the studies, and other environmental conditions (i.e., meteorological) can influence the metal bioaccumulation in mosses.

Although the industrial pollution sources in Hanoi are not extremely high compared with some other cities, air quality has been monitored in the last several years in terms of CO_2 and suspended air particulate matter concentrations [40]. Among well-known air pollution sources in this region, traffic is a major one, characterized in Hanoi by the large number of motorcycles and scooters, which are principally localized in zone 1 representing only a small territory of Hanoi. Furthermore, leaded gasoline was used in Vietnam until July 1, 2001, only 7 months before the moss sampling for this study was done. Thus, the high lead concentration in the mosses was very probably for a large part due to the Pb emissions from leaded gasoline.

However, the pollutant emission from 10 preexisting industrial zones and 7 new industrial zones (L. A. Palladino, research paper, Department of Geography, the Institute for Environmental Studies, University of Toronto, 2001) must also be taken into account. The industries mostly built in the 1960s use fossil fuel, such as coal, fuel oil, and diesel, and lack appropriate waste filtration systems [39]. These industries represent the main source of air emission in terms of industrial pollution in Hanoi. It seems judicious to explain the elevated heavy metal concentration in the moss samples in zone 1 by their proximity to the industrial zones and their position downwind from the emission (dominant winds are from the southeast and northeast) [39]. Therefore, our results logically supported our hypothesis that heavy metal accumulation in mosses was significantly higher in zone 1 than in zone 2. Similarly, a high value of Cd and Zn in zone 2 at sampling site 23 may be explained by its position about 3 km to the northwest of the Noi-Bai airport and 6 km to the southeast Dong-Anh industrial zone.

The distinction between zone 1 and zone 2 in terms of pollution is also demonstrated by both the RDA (Fig. 4C) and the Mann-Whitney test, which both reveal the higher Pb concentration in zone 1 than in zone 2. In the RDA, the separation of sites according to the two zone sites is clear except for sites 22 and 7. Indeed, site 22 was projected in

the right of the RDA biplot with zone 1 sites (Fig. 4C) because it had a high value in Pb, whereas site 7 was rather in the left part with zone 2 sites. This was probably due to the geographical location of site 7, which is near zone 2 and is isolated almost from any dominant wind directions from industrial zones.

Testate Amoebae in B. indica. To our knowledge, this is the first study on moss testate amoebae in Vietnam. Two other investigations dealt with testate amoebae in Vietnam in soil and aquatic environments. Balik [3] observed 126 taxa of testate amoebae in soil and leaf litter from the primary mountain rain forest of Tam Dao, 90 km north of Hanoi. Golemansky [21] identified 21 psammal testate amoeba species in the supralittoral coast of Vietnam. Among 23 testate amoeba species observed in the present study, nine species are new for the fauna of Vietnam (Table 2).

The number of taxa observed (i.e., 23) in this single moss species is relatively low compared with that found in other studies on terrestrial moss testate amoebae. Vincke *et al.* [56] identified 83 taxa of testate amoebae in seven moss species along a moisture gradient on Île de la Possession (Crozet Archipelago, sub-Antarctica). In a study on testate amoebae in Bangkok and in the "Khao Yai" National Park, Thailand, which is relatively close to Hanoi, Golemansky and Todorov [22] identified 77 taxa in 10 different humid soil and epiphytic mosses. In another work, Van Kerckvoorde *et al.* [55] observed 45 taxa in several mosses in northeast Greenland. However, these authors, except for Vincke *et al.*, did not identify moss species, nor present the number of moss species studied. Therefore, the comparison between the species richness of testate amoebae in the present study with that of the others described above remains relative.

We expected the number of testate amoeba taxa to be higher because of the favorable, tropical climate of the study region. It is likely that more species could be found by also analyzing the brown part of the mosses [instead of only the green part], richer in humus, with more constant moisture condition and most likely a higher availability of prey organisms, but this was not our goal. The brown lower parts of the moss cushion were not selected for testate amoeba community analyses because the influence of atmospheric pollution on testate amoebae might be partly masked by other environmental factors (e.g., humidity, abundance of prey organisms, etc.) and therefore the response of testate amoebae to atmospheric pollutants may be less clear. In addition, using only the top living parts can contribute to overcome the microheterogeneity of the distribution of testate amoebae [35, 37].

However, the number of taxa identified in this study is comparable with the number identified in several other studies. Mitchell *et al.* [36] identified 25 testate amoeba taxa living in *Hylocomium splendens* moss in a recent work on altitudinal patterns of testate amoeba diversity in Italy. Bonnet [4] recorded a total of 49 species in 13 different moss species growing on a range of substrates (different soils and trees); however, when only a single moss species was considered, the total diversity was of 23 species in 15 samples. Our number of taxa is higher than that found (i.e., nine species) in the moss *Tortula ruralis* in an urban area [41]. The number of testate amoeba species in mosses may vary widely with the moss species analyzed. In addition, the number of testate amoeba species also depends on other factors related to the structure of their habitat, such as the range of moisture, water-holding capacity, or temperature fluctuations to which testate amoebae are likely to be sensitive [6, 30, 31, 53]. Finally, we should keep in mind that such comparison has to be done with caution because of the different taxonomic resolution of the different studies.

Relationship Between Heavy Metals and Testate Amoebae in Moss. In accordance with our working hypothesis, species richness and total abundance of testate amoebae were both significantly higher in zone 2, where moss heavy metal concentrations were lower. The abundance of eight taxa was also higher in zone 2: *E. ciliata* for. *glabra*, *C. aerophila* var. *sylvatica*, *E. rotunda* var. *dorsalis*, *E. rotunda*, *A. discoides*, *E. rotunda* type, and *T. lineare* (Table 3). If we attempt to look for pollutants that caused these differences, Pb is the most likely candidate. This is shown by the negative correlations of testate amoeba abundance; species richness; Shannon index; and *T. lineare*, *C. aerophila* var *sylvatica*, *E. rotunda*, *T. dentata*, *T. enchelys*, *E. rotunda* var. *dorsalis*, and *E. ciliata* for. *glabra* abundance with Pb (Table 4). Our results agree with those of Lüftenegger and Foissner [32] regarding the relationship between lead concentration and testate amoeba abundance and species richness. Cd concentrations also were negatively correlated with *T. lineare* and *E. rotunda* abundance, Zn with *T. enchelys* and *E.* rotunda var. *dorsalis*, and Ni with *E. ciliata* for. *glabra* and *E. ciliata* (Table 2).

At the community level, the structure of the testate amoeba community differed along the pollution gradient characterized by moss Pb concentrations, which significantly explained a large part of the variation in testate amoeba composition (Fig. 4A and B). This is in accordance with the findings of Balik [2] on the effect of road pollution on testate amoebae. However, Balik did not measure atmospheric pollution, but instead, the pollution gradient was inferred from the distance to the road and thus, his recorded results remained qualitative.

The Shannon index values, ranging from 1.43 to 3.3 (mean 2.4 ± 0.5), represent an average diversity of the testate amoeba community [15]. Despite the lack of significant difference in Shannon index between the two zones, this diversity index was significantly and negative-

ly correlated to moss Pb and Cd concentrations in our study (Table 4). Thus, the moderate Shannon index and its negative correlations to Pb and Cd concentrations suggest that the heavy metal atmospheric pollution could cause a decrease in testate amoeba diversity.

We identified taxa that appeared to be the most sensitive to Pb pollution and those that seemed to tolerate the Pb pollution (Fig. 4). In general, species sensitive to the high moss lead concentrations were present only in zone 2 sites (e.g., *E. ciliata* for. *glabra*) or occurred also in both zones; however, their average abundance was higher in zone 2 than in zone 1 (ratio of average abundance zone 2/zone 1 varies from 1.1 to 5.1) except for *D. pristis* type (0.7; Table 3). Species tolerant to Pb pollution occurred only at sites in zone 1 and were absent in all sites of zone 2 (*Q. symmetrica* and *E. diliociformis*; Table 3). We tentatively interpret the absence of *Q. symmetrica* and *E. diliociformis* in the less polluted zone as due to the suppression of competition from more sensitive species rather than to an affinity for lead. In addition, Fig. 4B shows a heterogeneous distribution of testate amoeba species along the first axis, which presents the effect of lead on testate amoeba community. This suggests that the taxa or groups of taxa mentioned above can be of interest to the biomonitoring of air pollution by Pb.

To our knowledge, neither the impact of atmospheric heavy metals on testate amoebae nor the mode of toxicity to these organisms has been studied. However, two studies were conducted on the effects of these pollutants on testate amoebae in aquatic environments. Patterson *et al.* [44] and Reinhardt *et al.* [46] showed that in some lakes heavily polluted by mine tailings in Canada, *Centropyxis aculeata*, *Centropyxis constricta*, and *Arcella vulgaris* are good indicators of arsenic and mercury contamination. Regarding the mode of heavy metal toxicity to microbes, it is now widely accepted that the toxic species in aquatic systems is the free metal ion and, by analogy, it is assumed that the toxic species to soil microorganisms is also the free metal ion [33]. Moss heavy metals are likely to be available to testate amoebae as dissolved ions in the case of either wet depositions by precipitation or dry deposition diluted by moss water. Furthermore, as mentioned in the Introduction, testate amoebae may be indirectly affected by feeding on other microorganisms if they have been previously contaminated by heavy metals. They may theoretically also be indirectly affected by shifts in the prey community, for example, by a decrease in the abundance of a favored prey organism; however, such a degree of prey specificity has not yet been demonstrated in testate amoebae. Among the seven heavy metals considered in this study, Pb and Cd are nonessential metals well known to be highly toxic for organisms and the environment; the remaining trace elements (Zn, Ni, Cu, Mn, and Fe) also affect organisms in cases of excessive concentration [58].

This study showed correlations between heavy metals accumulated in mosses and species number, abundance, as well as some individual species abundance of testate amoebae. Despite the significance of the correlations, our results do not yet permit the establishment of a causal relationship between testate amoebae and the accumulation of heavy metal in the mosses. Indeed, other unmeasured factors may be responsible for some of the observed differences. This is a common limitation of all correlative studies. Our results nevertheless suggest that testate amoebae may be useful as biomonitors of atmospheric pollution from heavy metals, in particular from Pb. However, before this can be carried out, further work should be undertaken, such as detailed studies on the impact of heavy metals on testate amoebae, at different levels of biological organization under controlled conditions and addressing the question of their toxicity mechanisms on testate amoebae. One involuntary large-scale experiment is currently taking place: The switch to unleaded gasoline in Vietnam can be considered as a large-scale experiment and it would be interesting to follow the evolution of Pb concentration in mosses and how these changes correlate to testate amoeba community structure and we plan to do exactly this in a follow-up study.

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