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Soil macroinvertebrates as indicators of pollution by heavy metals

Les macroinvertébrés du sol en tant qu'indicateurs de pollution du sol par les métaux lourds

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Abstract

A broad range of soil pollutants were found to decrease with distance from a zinc smelter from 35 000 to 77, 8270 to 40 and from 190 to less than 1 ppm for zinc, lead and cadmium, respectively. Along this gradient, observed species richness of soil macro-organisms seemed to be more affected by the land-use type than by soil pollution – minimum in crops (21), maximum in woody sites (126). IndVal index allowed isolation of 21 indicator species from the 339 morphospecies identified. Most of these indicator species were characteristic of the unpolluted sites: only two diplopods and one gastropod from polluted poplar plantations, and none from the most polluted site. Since soil invertebrates respond to different environmental factors, including direct effect of heavy metals, we suggest there may be some confounding factors generating spurious relationships between the values of species as bioindicators and the pollution status they are supposed to indicate. ***To cite this article: J. Nahmani, J.-P. Rossi, C. R. Biologies 326 (2003).***

Résumé

Le long d'un gradient de pollution, où les teneurs totales en zinc, plomb et cadmium varient de 35 000 à 77, de 8270 à 40 et de 190 à moins d'un ppm, la richesse spécifique des macroorganismes du sol semble être plus fortement influencée par le type d'utilisation des sols que par la pollution de ceux-ci, avec une richesse spécifique minimale rencontrée dans les champs cultivés (21) et maximale dans les sites boisés (126). L'indice IndVal a permis d'isoler 21 morphoespèces parmi les 339 récoltées, considérées comme des indicateurs fiables. La plupart de ces espèces sont indicatrices des sites non pollués ; seules deux espèces de diplopodes et une espèce de gastéropodes sont caractéristiques des peupleraies sur sols pollués. Aucune espèce n'est apparue comme indicatrice du site le plus pollué. Sachant que de nombreux facteurs environnementaux affectent le patron de distribution des invertébrés du sol – incluant l'effet direct des métaux lourds –, nous suggérons que la combinaison de ces facteurs puisse limiter l'identification d'espèces indicatrices de la pollution. ***Pour citer cet article : J. Nahmani, J.-P. Rossi, C. R. Biologies 326 (2003).***

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Mots-clés : pollution ; métaux lourds ; invertébrés du sol ; espèces bioindicatrices

1. Introduction

In France, 7% of polluted sites (so-called *black-points*) come from non-ferrous metal industry [1]. These industrial complexes are often responsible for local depositions and diffuse contamination of surrounding agricultural lands [2]. Although less contaminated than soils of industrial complexes, the situation of pollution in agro-ecosystem is a matter of concern [3]. Nowadays, the regulation on heavy metal pollution only accounts for total content of trace elements [4] but estimation of the environmental risk requires more than an evaluation of the stock of pollutants. A comprehensive analysis of the response of different taxa that live directly in contact with polluted wastes is a way to evaluate the real risk. Considering the functional importance of the soil fauna [5], it is important to understand the effect of pollution on their community structure [6–8].

The aim of our study was to assess the effect of heavy metal pollution (Cd, Pb, and Zn) on soil macroinvertebrate species. We used a single species approach to identify which species displayed a clear response to pollution. In so doing, we aimed to find indicator species of either direct or indirect effects of heavy metal pollution. Beyond the simple segregation between sensitive and non-sensitive species, indicator species may allow us to identify the effects of pollutants, even when metal concentrations remain close to the background.

2. Material and methods

2.1. Site

The study was carried out in October 1999, at Mortagne-du-Nord (Nord-Pas-de-Calais, France), where a zinc smelter complex operated from 1901 to 1962 [9,10]. Sampling was carried out at five sites located within the metallurgical zone and nine sites at different distances away from this source of pollution, in the direction of the prevailing winds. Among the five sites within the metallurgical complex, two were located on a metallophyte grassland, and three on a poplar plantation. Among the nine sites of the agricultural perimeter potentially concerned by metal pollution, three grasslands, two fields, two poplar plantations and two forests were surveyed (Table 1).

2.2. Sampling protocol

At each site, eight soil cores ($25 \times 25 \times 15$ cm) distributed every 2 m on two transects, were sampled, air-dried, sieved and mixed thoroughly to form a composite sample. The resulting soil samples were analysed to quantify soil pollution by Zn, Cd, Pb (NF X31-151, [11]). In addition, the following supplementary variables were measured: C and N contents, CEC, pH and granulometry (Table 1). Soil macro-organisms were sampled on the same transects. Soil macrofauna was extracted using a modified Tropical Soil Biology and Fertility protocol, which consists of an application of a 0.2% formaldehyde solution to the upper 10 cm of soil, followed by soil hand sorting. Individuals collected were immediately fixed in 4% formaldehyde for further identification. The macro-organisms were identified at the level of morphospecies. The identification of most adult specimens was done at the level of species, while the remaining adults and larvae were identified to genus, family or class on the basis of the morphological differences between individuals.

2.3. Data analysis

2.3.1. Species diversity and species richness

We have chosen to gather the 14 investigated sites into six groups, in function of our knowledge of pollution status and land use [12] (Table 1, Fig. 1). This a priori classification was very close to the site clustering obtained using the K-means clustering method applied to the three first axes of the PCoA of species abundance [13]. In each case, the observed species richness was recorded and the Shannon diversity index computed. The evenness was also determined as the ratio of the Shannon index to its maximum value, i.e.

Table 1
Vegetation cover and edaphic parameters of the 14 investigated sites. Letters from A to N represent the site name. Numbers, from 1 to 6, represent the six groups of sites obtained by an a priori classification

Site N°	Group of site N°	Vegetation cover of the 14 sites	Pollution status of the 14 sites	Total content (ppm) Zn	Pb	Cd	Litter status	Particularity
A	1	Metallophyte grassland *Armeria maritima ssp halleri* *Cardaminopsis halleri*	Highly polluted	17 956	4720	79	High quantity Undecomposed (6 cm depth)	C/N = 23 Alluvial soil Metallurgical zone
B	1	Metallophyte grassland *Armeria maritima ssp halleri* *Cardaminopsis halleri*	Highly polluted	35 116	8271	190	High quantity Undecomposed (6 cm depth)	Alluvial soil
C	2	Poplar plantation *Arrhenaterum elatius* (pseudometallophyte)	Polluted	1112	616	12	High quantity Undecomposed (3 cm depth)	Alluvial soil
D	2	Poplar plantation *Urtica dïoica*	Polluted	3499	401	26	High quantity Undecomposed (3 cm depth)	Alluvial soil Metallurgical zone Liming
E	2	Poplar plantation *Urtica dïoica*	Polluted	>1000	>400	>10	High quantity Undecomposed (2 cm)	Alluvial soil
F	3	Poplar plantation	Unpolluted	286	73	2	Abundant	C = 64.5 mg g^{-1} hydromorphy
G	3	Poplar plantation	Unpolluted	104	63	<1	Abundant	
H	4	Forest	Unpolluted	44	75	<1	Abundant	pH = 3.7
I	4	Forest	Unpolluted	101	115	2	Abundant	
J	5	Field	Unpolluted	241	58	1.8	Absent	pH = 6.9 Tillage/pesticids
K	5	Field	Unpolluted	241	58	1.78	Absent	pH = 6.3 Tillage/pesticids
L	6	Grassland	Unpolluted				Absent	
M	6	Grassland	Unpolluted	300	85	<1	Absent	C/N = 12
N	6	Grassland	Unpolluted	77	40	<1	Absent	

ln 2(species richness) [14]. The estimator of species richness and its associated standard deviation proposed by Chao [15] were computed to compare with observed values. Computations were realised using the software EstimateS [16].

2.3.2. *The indicator value*

In order to identify indicator taxa, we used a method proposed by Dufrêne and Legendre [13]: the indicator value (IndVal). Sites are first gathered into groups, e.g. various levels of perturbation or different habitat types using either a hierarchical or a non-hierarchical method to form a site typology. A given indicator species is defined as a species mostly present in a single group of sites and present in the majority of the sites belonging to that group. There are thus two components interfering in the computation of the IndVal index: one accounting for the specificity of the species, and the second accounting for the fidelity of that species to the groups of sites.

The indicator value for the species i, IndVal_i is the largest value of IndVal_{ij} observed across all groups j of the site typology. It is maximum (100%) when all individuals of a species are found in a single group of samples and when the species occurs in all samples of that group.

The statistical significance of each index is evaluated using a standard permutation test. Sites are randomly reallocated among site groups (clusters) and the indicator values computed for each species. For a

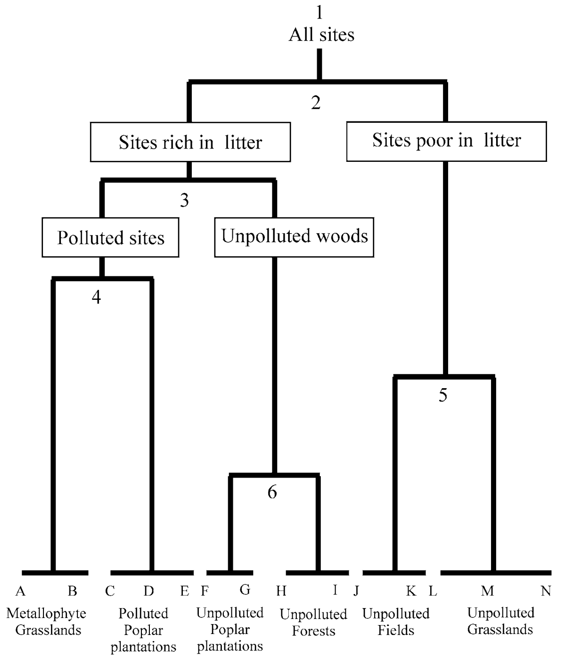

Fig. 1. Dendrogram of sites. Sites (A to N) were distributed into six groups (defined in Table 1) according to their pollution status and the type of vegetation cover. Numbers represent the cluster level.

given species, the rank of the observed value in the randomly generated distributions ordered in decreasing order produces a permutational probability.

The indicator values can be estimated for any given level of clustering, which constitutes a useful property of the approach. Species may have different indicator values according to the clustering level under consideration. Generalist (core) species have decreasing values of the indicator index from high level to lower levels of the typology. Specialised (satellite) species, on the contrary, display increasing indicator values from higher to lower levels of the typology. In addition, the method allows identification of species typical for intermediate levels of site hierarchy. Computations were realised using the software IndVal2 [13].

3. Results

3.1. Site typology

Sites presented in Table 1 were empirically grouped into six units, according to their pollution status and the type of vegetation cover (Fig. 1). The first level of the classification grouped all sites. The second level separated sites according to the litter layer depth and distinguished herbaceous unpolluted sites (low litter quantity) from unpolluted woody and polluted sites (rich in litter). Among sites rich in litter, level 3 distinguished polluted sites from unpolluted wood. Level 4 separated metallophyte grassland from the polluted poplar plantation. Depending on the management practice, herbaceous unpolluted sites were divided at level 5 into grasslands and crops. The last level separated unpolluted natural forest from unpolluted poplar plantation.

3.2. Species density, richness and diversity

A total of 339 morphospecies was found among the 112 sampling units, with a cumulative number of 3465 individuals. Oligochaeta, Hymenoptera, Coleoptera, Diptera larvae, Arachnida and Isopoda were the most abundant groups (Table 2). Oligochaeta [Lumbricidae and Enchytraeidae] had higher density than the other taxa in the majority of sites. Their density decreased principally according to the land use type from 428 ind m^{-2} to 57 ind m^{-2} in unpolluted poplar plantation and unpolluted crops respectively, and according to the level of soil pollution, from 342 ind m^{-2} in unpolluted grassland to 4 ind m^{-2} in polluted metallophyte grassland. Coleoptera, Arachnida and Hymenoptera were the most abundant taxa in this metallophyte grassland. Rare species were generally found in unpolluted sites.

Species diversity analyses were performed using all the morphospecies (339). Results showed differences in species richness and diversity between the six groups (Table 3). Observed species richness was mainly affected by the type of land use and, to a lesser extent, by soil pollution. Cultivated plots had much lower species richness than grasslands and woody sites (21, 76–80 and 105–126, respectively). Polluted grassland had higher species richness than unpolluted grassland, even if the differences were not important. The same trend appeared for polluted and unpolluted poplar plantations. The Shannon diversity index was higher in the polluted poplar plantation than in the unpolluted forest or poplar plantation. In grasslands, a similar trend was observed, with 50% higher values in the polluted site than in the unpolluted grasslands. Patterns of evenness and the Shannon index were

Table 2
Macroinvertebrate density (ind m^{-2}) in each group of sites and total number of morphospecies. Letters from A to N represent the site name

	Total number of morphospecies	Unpolluted grassland (L, M, N)	Unpolluted poplar plantation (F, G)	Unpolluted forest (H, I)	Unpolluted crops (J, K)	Metallophyte grassland (A, B)	Polluted poplar plantation (C, D, E)
Oligochaeta	18	342.7	468	279	57	4	76
Hymenoptera	10	62.7	0	1	0	28	0.7
Coleoptera	131	60.7	29	115	28	75	40
Diptera	58	10	49	611	2	14	74.7
Arachnida	57	14.7	28	33	2	24	22
Isopoda	3	7.3	113	26	0	0	23.3
Gastropoda	20	2	56	3	0	2	60
Lepidoptera larvae	11	2	2	0	0	8	3.3
Myriapoda	18	1.3	37	57	0	13	37.3
Thysanoptera	2	0.7	0	0	0	0	0
Trichoptera larvae	2	0	16	9	0	0	0
Hemiptera	4	0	2	2	0	0	1.3
Dermaptera	2	0	0	5	0	0	0
Homoptera	2	0	2	0	0	0	0
Neuroptera	1	0	0	0	1	0	0

Table 3
Observed species richness and indices of diversity in the six groups of the site. Letters from A to N represent the site name

Sites groups	Site N^{o}	Sample number	Observed species richness	Shannon index	Evenness	Chao estimator
Polluted metallophyte grassland	A, B	16	80	4.05	0.64	130
Polluted poplar plantation	C, D, E	24	126	4.15	0.59	203
Unpolluted field	J, K	16	21	2.38	0.54	26
Unpolluted grassland	L, M, N	24	76	2.52	0.4	138
Unpolluted poplar plantation	F, G	16	105	3.09	0.46	156
Unpolluted forest	H, I	16	117	2.22	0.32	190

similar across sites, except for the unpolluted crop that had a high evenness index, but a low Shannon index. This means that this site had a low diversity, because of the very low species richness and rare or very abundant species were less frequent than in the other sites. The Chao index was linearly correlated to the observed richness and thus accounting for it did not change the between-site comparison. This species richness estimation accounts for occurrences of both singletons and doubletons, i.e. those species present in only one and two samples respectively [15]. Seemingly, the proportion of these species was of no effect upon the pattern of species richness across our sites.

3.3. *The indicator value index*

To determine if a species was an indicator, we first examined the significance of the index and arbitrarily retained a threshold of 25%. This means that a characteristic species is present in at least 50% of one site group and that its relative abundance in that group reaches 50% ([13] (p. 356)). Two hundred fifty-seven species were encountered less than five times and were removed from indicator values analysis (i.e., 75% of the total). Among the 82 species examined, only 21 were indicator species at least at one level of the hierarchy, i.e. five Coleoptera, two Diplopoda, three Chilopoda, three Gastropoda, six Oligochaeta, one Diptera, and one Crustacea (Table 4). Among the 21 indicator species, three were generalists, i.e. the IndVal index was maximum for the lower hierarchy level. An example of such a species (*Lithobius crassipes*) is provided in Fig. 2A. Nine species were found to be specialised species (satellite) like the centipede (scolopendridae) *Cryptops savignyi* (Fig. 2B) or the diplopod *Polydesmus complanatus* (Fig. 2C). Nine species like the geophilid *Haplophilus subterraneus*

Table 4
List of the indicator morphospecies and some characteristics of their ecology

Broad groups	Family genus species	Ecology
Coleoptera larvae	Rutelidae hoplinae	Endogeic rhyzophagous
Coleoptera larvae	Elateridae	Endogeic rhyzophagous
Coleoptera larvae	Elateridae	Endogeic rhyzophagous
Coleoptera larvae	Staphilinidae	Endogeic predator
Coleoptera	Staphylinidae *Habrocerus capillaricornis*	Epigeic predator
Adult Crustacea	Oniscidae *Philoscia muscorum*	Epigeic saprophagous
Adult Diplopoda	Polydesmidae *Polydesmus denticulatus* C. Koch, 1847	Epigeic saprophagous
Adult Diplopoda	Polydesmidae *Polydesmus complanatus*	Epigeic saprophagous
Adult Chilopoda	Lithobiidae *Lithobius crassipes* L. Koch, 1862	Epigeic predator
Adult Chilopoda	Geophilidae *Haplophilus subterraneus* Leach, 1817	Epigeic predator
Adult Chilopoda	Scolopendridae *Cryptops savignyi* Leach, 1817	Epigeic predator
Adult Gasteropoda	Snail sp. 1	Phytophagous
Adult Gasteropoda	Snail sp. 2	Phytophagous
Adult Gasteropoda	Slug	Phytophagous
Adult Oligochaeta	Lumbricidae *Aporrectodea caliginosa*	Endogeic geophagous
Oligochaeta	Enchytreidae	Endogeic geophagous
Immature Oligochaeta	Lumbricidae apigmented	Endogeic
Immature Oligochaeta	Lumbricidae pigmented	Epigeic
Adult Oligochaeta	Lumbricidae *Dendrobaena attemsi*	Endogeic acidophile geophagous
Adult Oligochaeta	Lumbricidae *Lumbricus castaneus*	Epigeic, acidotolerant
Diptera larvae		Endogeic geophagous

(Fig. 2D), indicated an intermediate level of the hierarchical typology.

Fig. 3 presents all the species that have a significant indicator value index larger than 25. Two immature pigmented and apigmented lumbricids had their indicator values as maximum for the first level of the hierarchy, i.e. all sites (level 1). At the second level, pigmented and apigmented immature worms and *Aporrectodea caliginosa*, appeared as indicators of the herbaceous unpolluted zone. At the next level, the unpolluted grassland group was characterised by the same three earthworm taxa plus two endogeic Coleoptera larvae (level 5). No indicator species was found for crops (level 5). Four epigeic taxa were indicators of sites with a thick litter system, with a maximum value for the centipede *Lithobius crassipes* (level 2). At level 3, seven taxa were indicators of unpolluted woods: Enchytraeidae (87.25), *Philocia muscorum* (49.29), *Haplophilus subterranus* (34.88), *Lumbricus castaneus* (30.85), Elateridae larvae (30.30), Diptera larvae (28.13) and *Cryptops savignyi* (28.13). Unpolluted woods were further divided into forest and poplar plantations (level 6). Forests were characterised by five specialist indicator species (one Diptera larva, 2 Staphilinidae, an earthworm and a millipede) and by two intermediate indicator species (Elateridae larvae and Enchytraeidae). Two gastropods, an earthworm and an Oniscida, were indicator species of poplar plantations (level 6). At level 4, polluted sites are characterised by three indicator species of the polluted poplar plantation: 2 Polydesmus sp. and a snail. No indicator species was found in metallophyte grassland.

4. Discussion

The effect of soil pollution on macroinvertebrate diversity is rather complex. In this study, even if there were differences between polluted and unpolluted areas, diversity seemed to be more influenced by vegetation type than by pollution. Some studies showed a negative relationship between the Shannon index and heavy metal pollution [17], but for most studies, there is no correlation between these two parameters [18, 19]. Furthermore, diversity indices are often described

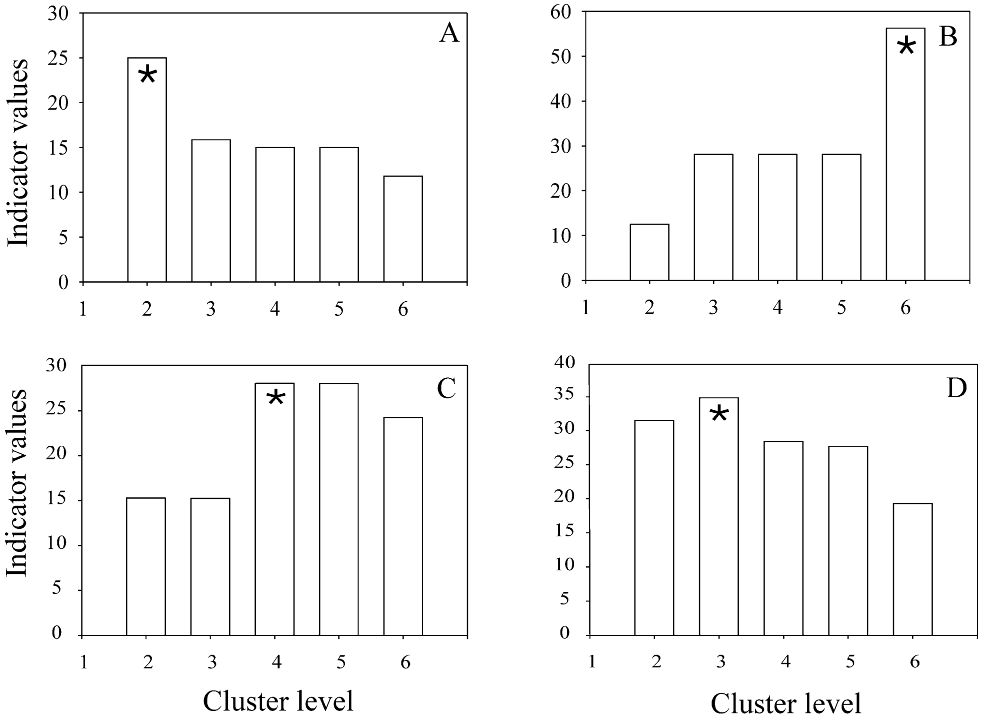

Fig. 2. Changes in the IndVal index value from level 2 to level 6 of the cluster. (A) *Lithobius crassipes*. (B) *Cryptops savignyi*. (C) *Polydesmus complanatus*. (D) *Haplophilus subterraneus*. Star (∗) indicates the higher value of the IndVal index.

as very sensitive to various factors, e.g., the size of sampling unit, and are thus of limited predictive capabilities [20]. Cortet et al. demonstrated that diversity indices should be used carefully and only in extremely polluted sites [21].

The IndVal index [13] allows an evaluation of the indicator value of each species independently. Some problems may arise when considering the case of social insects like ants. Because these organisms are highly aggregated in space, their fidelity may be low, even in case of high specificity. This problem is obviously related to the standard sampling protocols that perform poorly for social insects.

We found three indicator species associated with polluted sites. These species were all epigeic (two Diplopoda and a snail) and this may be explained by the lower exposure of epigeic species compared to endogeic species [22]. However, both specificity and fidelity may also be favoured by the typical excess in litter observed in polluted sites due to reduction in decomposition rates [9,23]. As a consequence, distinguishing between direct and indirect effects of pollution is not straightforward.

However, direct effect of pollutant and the corresponding adaptation by species is well documented in some cases. For instance, snails are known to be able to develop heavy metal adaptation [24]. Some of them can survive high levels of metal in their food by modifying metal uptake and excretory rates [24]. Response of Diplopoda to metal pollution differs among species, as some species can be more abundant in polluted areas than in unpolluted sites [17,25]. Nevertheless, a recent study in comparable sites [6] showed the absence of correlation between Diplopoda density and soil metal content. This lucifugous group was probably favoured by the important quantity of litter and by moisture condition.

The results reported here suggest that in some cases species primarily respond to certain soil attributes rather than to the pollutant concentration, which is in accordance to the results reported by Grelle [6]. As an example in forest, soil pH was about 3.7 and

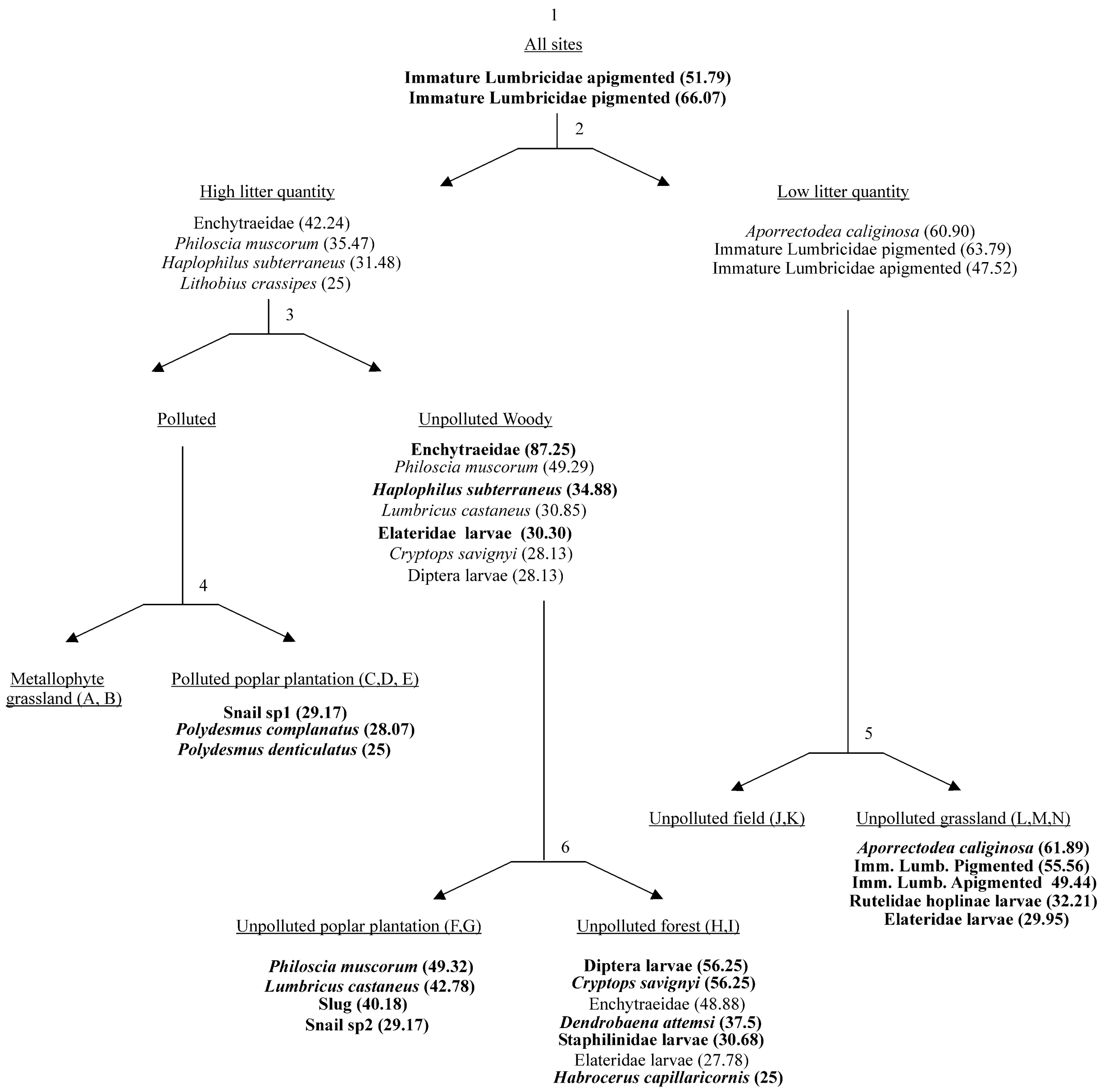

Fig. 3. Site typology and associated indicator species with indicator values in parentheses. Bold characters identify the highest IndVal index observed among clustering levels. Letters from A to N represent the site number (details in Table 1).

the indicator value of *Dendrobaena attemsi* reached its maximum significant value. The species seems to be indicator of soil acidity rather than any other factor, in accordance with the description of the ecology of this endogeic worm proposed by Bouché [26]. Another earthworm species, *Aporrectodea caliginosa*, appeared to be an indicator of unpolluted grassland. Since this species is absent in cultivated plots, it may be affected by tillage and pesticide, as most earthworm species are [27]. On the other hand, this species is considered as very sensitive to soil pollution by heavy metals [28] and was absent from our polluted sites. This leads to a very important point regarding use of soil macroinvertebrates as bioindicators of soil perturbations. Since these species are very likely to respond to different environmental factors including direct effect of heavy metals there may be some confounding factors generating spurious relationships between the values of species as bioindicators and the pollution status that they are supposed to indicate. Extreme caution is therefore needed and field experimental testing should be promoted as often as possible.

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