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Seasonal variation in the abundance, biomass and biodiversity of earthworms in soils contaminated with metal emissions from a primary smelting works

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Summary

1. Earthworms (Annelida: Oligochaeta) were sampled on four occasions (spring, summer, autumn and winter) at 14 sites along two transects from a primary lead/zinc/cadmium smelting works at Avonmouth, UK.
2. Total abundance and biomass of earthworms decreased with proximity to the smelter. No worms were collected from the two sites closest to the factory ($<0{\cdot}6$ km) and catches were significantly lower than controls at a further five sites (<3 km).
3. Seasonal composition of sampled communities differed only for summer with lower numbers of individuals and species collected at all sites. Reduced catches in the summer sample is a response to drought.
4. Species richness was lowest at sites close to the factory. For example, worms such as *Aporrectodea caliginosa* (Savigny) and *Allolobophora chlorotica* (Savigny) that were dominant at relatively clean sites further from the smelter are absent from the most contaminated soils.
5. Reduced species richness resulted in lower Shannon–Weiner diversity and higher Berger–Parker dominance. Multivariate cluster analysis for spring, summer and winter indicated that sites could be split into three groups based upon relative species composition. In autumn, two clusters were identified.
6. The absence of sensitive species from sites close to the smelting works supports the inclusion of earthworms as a key group in a terrestrial prediction and classification scheme for quantifying the effects of pollutants on soil biodiversity. However, sampling should be carried out in spring or autumn to obtain an accurate picture of community structure.

Key-words: community monitoring, metal pollution, size classes, species richness, zinc.

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Introduction

Recent developments that have lead to an increased understanding of the structure and function of soil ecosystems have raised the possibility of using community parameters to measure the impact of pollutants. Of the groups available for monitoring, particular attention has been paid to the macroinvertebrates, because these communities frequently consist of a large number of species that differ in their niche preferences, life-histories and sensitivity to pollutants (Siepel 1994). Additionally, soil macroinvertebrates are present over a range of diverse habitats, are relatively easy to sample, can be simple to identify (when good keys are available) and frequently have low mobility, which means they are representative of the habitat being sampled (Van Straalen 1997).

The suitability of macroinvertebrates for monitoring has resulted in the development of procedures for assessing the impact of environmental stresses from changes in community structure. The most sim-

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ple form of monitoring is to use abundance data for single species. Such data can be used as an input for diversity indices, or can be used in more complex analysis using multivariate statistics (Van Straalen 1997). A further method that has proved useful in freshwater is a probabilistic approach, comparing actual fauna to the theoretical community predicted for the site. The predicted community structure is determined by comparing previous data on the distribution, abundance and ecophysiological preferences of species to the location and environmental characteristics of the site to determine the probability that a given species will occur. In the UK, the River Invertebrate Prediction and Classification System (RIVPACS) developed by Wright, Armitage & Furse (1989) has been adopted as a standard monitoring technique by regulatory authorities. Spurgeon, Sandifer & Hopkin (1996) have suggested that a Soil Invertebrate Prediction and Classification Scheme (SOILPACS) would represent a significant advance in the identification, quantification and monitoring of contaminated land.

Any system developed to monitor pollutants from their impact on community structure must account for temporal variations in abundance. Clearly, large changes in population size would be anticipated for semelparous and iteroparous groups that show strong seasonal trends in fecundity. However, variations may also occur for species with a more continuous pattern of reproduction. Thus, in the current paper, the effects of pollutants on the community composition of a non-seasonal iteroparous invertebrate group have been measured over four seasons at sites along a gradient of contamination from a smelting works. The group selected for this work were the earthworms, because a preliminary study of their distribution has shown that communities are influenced by high soil metal concentrations (Spurgeon & Hopkin 1996a). For the present study, the impact of metals was assessed using a number of potential monitoring techniques including measurement of mean abundance, biomass, population and age structure, species richness, diversity and multivariate statistics.

Materials and methods

ASSESSING THE ABUNDANCE, DIVERSITY AND BIOMASS OF EARTHWORM COMMUNITIES

To assess the effects of metals on earthworm communities, 14 sites were surveyed. Of these, 13 were situated along two transects to the north-east (direction of prevailing wind) of a smelting works situated at Avonmouth in south-west England. A 'control' site was situated on the University of Reading campus, 100 km from the factory. Due to limited access to land in the Avonmouth area, samples were taken from unmanaged grassland adjacent to minor roads. Sites were always at least 2 m from the kerb.

Each site was visited on four occasions over a 1-year period: spring (22–30 April 1996), summer (29 July–6 August 1996), autumn (21–26 October 1996) and winter (17–26 February 1997). For each site on each sample occasion, six 25×25-cm ($= 0{\cdot}0625\,m^2$) quadrats were marked on the soil surface. The number of samples taken at each site was a compromise between statistical considerations for the correct prediction of earthworm population size and practical considerations of time and manpower. Daniel *et al.* (1992) studied the impact of sample number on the accuracy of earthworm population size predictions. From this work it was concluded that at a density of 20 individuals per $0{\cdot}25\,m^2$, which is below the values found at the least contaminated sites in this survey, a sample size of six is sufficient to allow good prediction reliability. Thus, the number of replicates used in the current study should be sufficient to allow comparisons of earthworm populations, particularly as the contamination gradient in the Avonmouth region is relatively clear.

At each site, soil was dug from each quadrat to a depth of 40 cm, hand sorted on site and all earthworms present removed to the laboratory for identification. The sampling technique used was considered an efficient way to sample epigeic (litter-dwelling) and endogeic (soil-dwelling) worms in favourable soil conditions. However, it was possible that some anecic species (deep-burrowing) (and epigeic and endogeic species during unfavourable conditions) may have been missed. Such worms could be collected by formalin extraction techniques. However, it was not possible to use these techniques in the current study, due to the inaccessibility of some of the sites selected and the unwillingness of landowners to allow formalin application to soil.

Adult worms were identified from the UK fauna key of Sims & Gerard (1985) whilst for juveniles, characteristics such as behaviour, pigmentation, prostomium form, setae pattern and size were used. Some juveniles could not be classified due to similarities between worms, e.g. *Lumbricus terrestris* (L.), *Lumbricus rubellus* (Hoffmeister), *Lumbricus castaneus* (Hofmeister) and *Aporrectodea caliginosa*, *Aporrectodea rosea* (Savigny). Thus, these individuals were recorded as *Lumbricus* sp. and *Aporrectodea* sp., respectively. Any animals damaged during sampling were recorded as 'unidentified'. After separation to species, worms were recorded as adults or juveniles based on the presence or absence of a fully developed clitellum, and weighed. All weights were measured for fresh unstarved animals.

The key of Sims & Gerard (1985) recognizes a number of species as complexes of at least two distinct morphs. For example, *Aporrectodea caliginosa* is represented by four principal forms: the small endogeic morph *Aporrectodea caliginosa caliginosa*; two medium-sized endogeic morphs *Aporrectodea caliginosa tuberculata* and *Aporrectodea caliginosa trapazoides* and a large anecic morph *Aporrectodea caliginosa nocturna*, while the epigeic species *Allolobophora chlorotica* has a green form *Allolobophora chlorotica chlorotica* and a red form *Allolobophora chlorotica virescens*. Although in the current study these different phenotypes have been considered as single species and not separated as proposed by Bouché (1977) and Lee (1985), the distribution of the different morphs was recorded and will be discussed as appropriate for explaining distribution patterns.

CHEMICAL ANALYSIS OF SOILS

In addition to examining earthworm communities at each site, soil samples were also collected. Samples were taken from the top 2 cm of the soil profile (below the litter horizon). This layer contains the highest metal concentrations to which earthworms are exposed due to the depth stratification of metals found in soils surrounding the smelter (Martin & Bullock 1994; Sandifer 1997). After sampling, using a clean trowel, collected soils were returned to our laboratory for analysis of water content, pH(H_2O), percentage loss-on-ignition (%LOI) and cadmium, copper, lead and zinc concentrations. Soil pH, %LOI and metal contents were all measured for the autumn sample only, while water content was measured in all four seasons to allow changes in moisture levels to be monitored throughout the year.

To measure soil water content, collected soils were sieved through a 4-mm mesh. Samples were dried to constant weight and the moisture content calculated. The soil %LOI was determined after heating dried soil for 12 h at 500 °C. For the measurement of pH, 10 g of dried soil was weighed into a container with 25 mL of deionized water. Suspensions were shaken for 2 min and acidity measured after a further 5 min. To analyse soil metal levels, $\approx$1 g was placed into a conical flask with 10 mL of concentrated nitric acid. Flasks were heated until all organic matter was digested and then diluted with distilled water to 100 mL. Solutions were analysed for cadmium, copper, lead and zinc content by flame atomic absorption spectrometry. During soil analysis, standard reference materials (tomato leaf and bovine liver from the National Bureau of Standards, Washington; lobster hepatopancreas from the National Research Council, Canada; and calcareous loam soil from the Community Bureau of Reference, Brussels) were used as recommended by Hopkin (1989). In all cases measured values were within 10% of certified values.

Results

CHEMICAL ANALYSIS OF SOILS

No relationship was found between the moisture levels of collected soils and distance from the smelter. However, a clear seasonal trend was found. Levels in summer were lower than for the autumn, spring and winter, which were comparable (Table 1). Median soil pHs were within a narrow range. No relationship was found between soil acidity and distance from the factory. Measurement of %LOI indicated a high organic matter content, with values greater than 9·4% in all cases. No relationship was found between %LOI and distance from the factory. Highest metal concentrations were found at Site 1, while lowest values were found for the control soil. For each metal, a linear relationship was found between log soil concentration and distance from the smelter. This pattern of metal distribution indicates an exponential decline with distance from the factory.

EARTHWORM COMMUNITY COMPOSITION AT THE SAMPLED SITES

Abundance

Large differences in the number of earthworms were found in the different seasons (Fig. 1a–d). Highest catches were in spring and winter, with lower numbers in autumn and a large reduction in summer. Comparisons of mean earthworm catches at each site in the four seasons, using Tukey's test for the multiple comparison of means, indicated reduced numbers at seven sites in spring and autumn, 11 in winter and 12 in summer. Mean catches were lower than controls at Sites 1–7 in all four seasons. No worms were collected from the two most heavily contaminated sites.

To assess the relationship between earthworm abundance and soil metal levels, linear regression parameters were determined for the comparison of cube root mean earthworm numbers and log soil concentration for each season sample. Cube root transformation were used for abundance data, since Boag *et al.* (1994) found this transformation to be most appropriate for use prior to analysis of earthworm population data. Regression calculations indicated a significant negative association between abundance and soil metal concentrations in each season, with the exception of zinc in the summer sample. Relationships with the highest correlation coefficients for each metal ($R^2 \geqslant 0{\cdot}61$) were for the spring sample, whilst the weakest values were in summer ($R^2 \geqslant 0{\cdot}35$). Similar comparisons of cube root mean earthworm abundance and soil median pH and mean %LOI indicated no significant associations.

Biomass, size class distribution and age structure

Analysis of the mean earthworm biomass using Tukey's test indicated a significant reduction compared to controls at 5 sites in spring, 12 in summer, 8 in autumn and 10 in winter. Biomass was closely linked to mean earthworm catch; thus, as for abundance, mean biomass was lower at Sites 1–7 in all seasons, with the exception of Sites 4 and 7 in spring.

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Table 1. Ordnance Survey Grid Reference (OSGR) of the sites in the vicinity of the Avonmouth smelter and the % water content, pH, percentage loss on ignition (% LOI) and concentration of metals in soils (top 2 cm) collected from each site. Values for water content are for each season sample, values for pH, % LOI and metal concentrations are for the autumn sample only (all values are mean (median for pH) of six replicates ± SE)

	Site location		Soil characteristics									
Site No.	OSGR	Distance to smelter (km)	Water content (spring)	Water content (summer)	Water content (autumn)	Water content (winter)	pH (median)	% LOI	Cadmium (μg Cd g^{-1})	Copper (μg Cu g^{-1})	Lead (μg Pb g^{-1})	Zinc (μg Zn g^{-1})
14	737714	110	23·6 ± 0·6	13·9 ± 1·5	20·8 ± 6·7	18·7 ± 0·6	5·82	20·1 ± 3·4	< 0·2 –	12·4 ± 0·7	17·2 ± 4·1	47·3 ± 2·3
13	562881	9·7	25·2 ± 0·2	19·6 ± 0·6	19·1 ± 0·5	30·5 ± 2·2	7·33	12·4 ± 0·5	0·3 ± 0·1	18·5 ± 0·4	18·5 ± 0·4	167 ± 3
12	594826	7·7	24·6 ± 0·7	15·4 ± 0·4	24·5 ± 0·5	24·4 ± 1·2	5·79	15·2 ± 0·4	1·0 ± 0·1	25·7 ± 0·4	146 ± 3	499 ± 11
11	553854	6·5	27·2 ± 0·5	17·2 ± 1·5	26·3 ± 0·9	28·8 ± 2·7	7·14	12·9 ± 1·2	0·3 ± 0·1	23·1 ± 0·3	114 ± 2	282 ± 8
10	570822	5·4	27·6 ± 0·3	17·3 ± 2	31·7 ± 0·3	31·7 ± 0·5	6·43	19·4 ± 0·3	3·1 ± 0·2	30·1 ± 1·5	143 ± 2	418 ± 10
9	554835	5·1	28·5 ± 0·5	19·6 ± 1·6	31·7 ± 0·9	34·5 ± 1·5	7·2	25·2 ± 5·3	3·5 ± 0·2	38·8 ± 2·4	153 ± 3	519 ± 8
8	540816	3·2	29·3 ± 0·8	12·6 ± 1·8	23 ± 1·2	27·6 ± 2	6·57	13·7 ± 1·3	6·1 ± 0·3	55 ± 0·6	324 ± 11	814 ± 53
7	552800	2·8	30·7 ± 1·5	16·6 ± 0·5	25·5 ± 1·2	33·8 ± 0·9	7·07	22·7 ± 4·6	13·8 ± 0·4	148 ± 1	746 ± 12	1290 ± 79
6	549807	2·4	26·5 ± 0·9	21 ± 0·6	27·6 ± 1·13	26·9 ± 0·7	7·36	20·7 ± 2·6	18 ± 0·2	80·5 ± 2·1	411 ± 16	1530 ± 29
5	543802	1·9	27·3 ± 1·2	19 ± 1·4	30·7 ± 1·4	34·1 ± 1·3	5·42	23·8 ± 2·5	31·6 ± 0·8	113 ± 3	1130 ± 39	2110 ± 43
4	540793	1·4	30·5 ± 1·8	18·2 ± 1·2	30·6 ± 1·6	36·7 ± 2·9	6·68	23·4 ± 1·9	66·6 ± 1·1	326 ± 10	2780 ± 95	4990 ± 111
3	529806	1·3	25·1 ± 1·1	16·8 ± 0·8	32·3 ± 5·7	36 ± 3·3	6·68	30·4 ± 1	51·6 ± 2	169 ± 9	1900 ± 165	2750 ± 72
2	532791	0·6	23·4 ± 1·2	14·8 ± 0·6	30·2 ± 1·8	26·3 ± 0·9	6·83	27·8 ± 2·8	177 ± 11	275 ± 62	5962 ± 499	11400 ± 855
1	528795	0·5	26·5 ± 1	16·6 ± 0·8	34·2 ± 1·9	30 ± 1·4	7·09	13·2 ± 1·3	275 ± 61	2310 ± 147	20700 ± 3210	37300 ± 4520

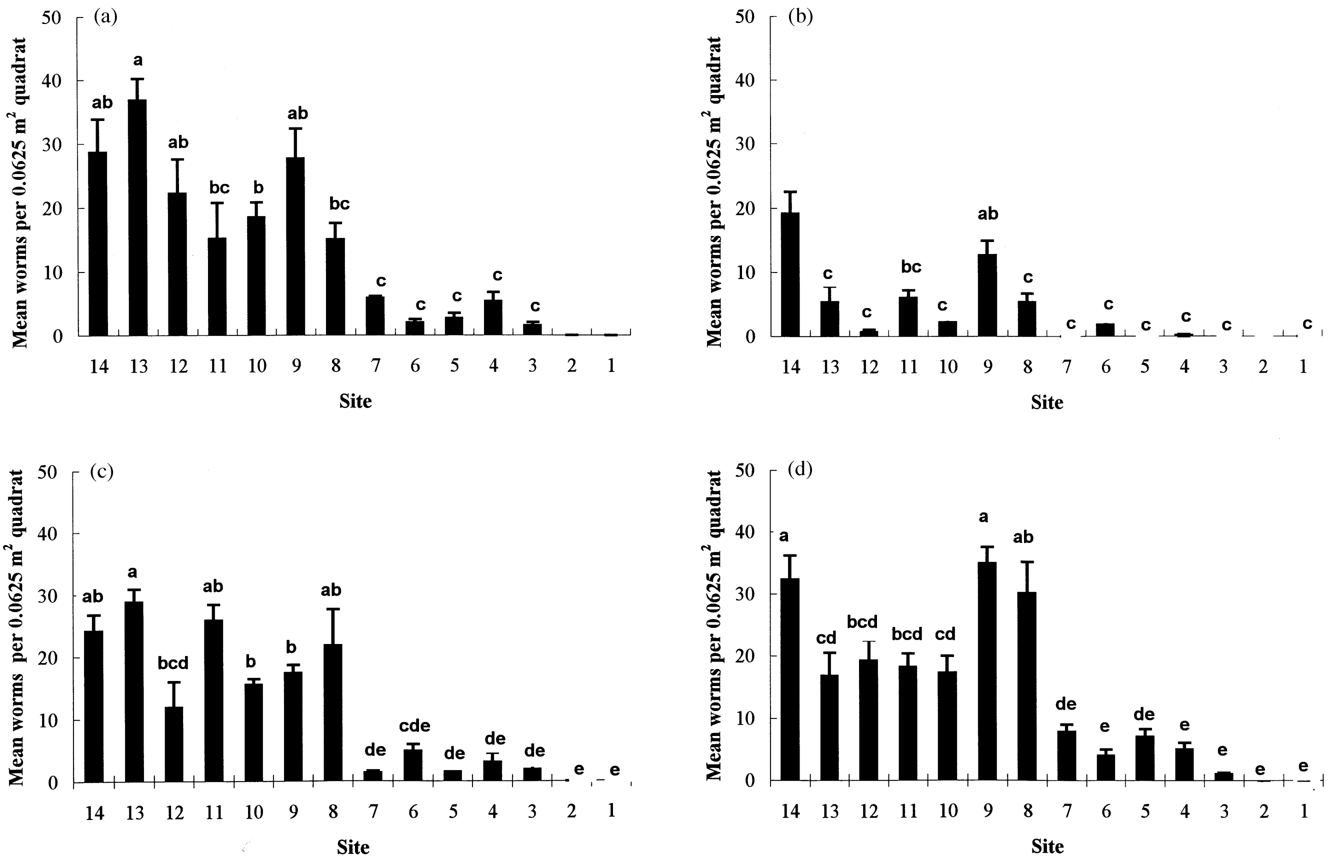

Fig. 1. Mean abundance of worms collected from six 25 × 25-cm quadrats taken at 13 sites in the Avonmouth area and a control (Site 14) 100 km from the factory (error bars indicate SE values) in (a) spring, (b) summer, (c) autumn, and (d) winter. Sites sharing the same letter indicate no significant differences at $P > 0{\cdot}05$ as given by Tukey's test for the multiple comparison of means.

In addition to measuring the impact of metals on mean collected biomass, effects on the relative abundance of different earthworm size classes were also studied. Only data for *Allolobophora chlorotica* were used for these comparisons, due to similarities in the appearance of newly emerged juvenile *Lumbricus* sp. and *Aporrectodea* sp.

For size analysis, the number of *Allolobophora chlorotica* in six classes (<0·05, 0·05–0·1, 0·1–0·15, 0·15–0·2, 0·2–0·25, >0·25 g fresh weight) caught at Sites 8, 9, 13 and 14 were counted. These sites were selected because they represent the range of distribution for this species at Avonmouth. Site 8 is the closest location to the factory at which substantial numbers of *Allolobophora chlorotica* were collected (eight worms were found at Site 6 in the summer sample). Highest abundance was usually in the 0·5–0·1 and 0·1–0·15 g classes, with lower numbers of small individuals and relatively few larger worms. No clear differences in the abundance of different size classes were found at the selected sites, due in part to the low catches of *Allolobophora chlorotica* in some samples; however, seasonal differences were found. Few <0·05 g worms were found in summer and autumn, compared to winter and spring, suggesting that *Allolobophora chlorotica* hatch during winter and spring and grow to adulthood over the summer and autumn months.

In addition to assessing the impact of metals on the size structure of *Allolobophora chlorotica* populations, effects on the relative abundance of different age classes were also studied. For these comparisons, numbers of adults and juveniles present at the four sites used for the size class analysis were calculated as a ratio (Table 2). At Site 14, juveniles were more numerous than adults in spring and summer. However, subsequent to this, adult numbers increased and ratios fell to below one in the autumn and winter samples. A similar pattern of juvenile:adult ratios was also found at Site 9. For Site 13, the proportion of adults collected increased throughout the year. Thus, highest juvenile:adult ratios were found in spring, with lowest values in the winter sample. Ratios for Site 8 did not follow the trends found at any of the three less contaminated sites. In this soil, numbers of juveniles exceeded adults in spring (although only six worms were collected), whilst in the remaining seasons in which sufficient worms were collected the abundance of the two life stages was approximately equal.

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Table 2. Ratios of immature : adult *Allolobophora chlorotica* collected from four sites in the Avonmouth region for four seasonal samples taken over a 1-year period. Ratios for each season sample are calculated for all worms collected from six 25 × 25-cm quadrats

Site No.	Spring	Summer	Autumn	Winter
14	1·43 : 1 (17)	1·8 : 1 (9)	0·4 : 1 (14)	0·5 : 1 (21)
13	2·02 : 1 (149)	3 : 1 (4)	1·14 : 1 (75)	0·72 : 1 (19)
9	1·05 : 1 (44)	1·46 : 1 (24)	0·59 : 1 (27)	0·52 : 1 (34)
8	0·2 : 1 (6)	2 : 1 (3)	1·43 : 1 (17)	1 : 1 (34)

Species richness and community composition

Ten species were collected during the survey, with a maximum of nine on a single sample occasion (spring sample, Site 8). Of the worms collected, *Eiseniella tetraedra* and *Murchieona minuscula* were found in only one sample, while *Octolasion cyaneum* was found in three samples. The remaining species *Lumbricus terrestris*, *Lumbricus rubellus*, *Lumbricus castaneus*, *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Aporrectodea longa* (Ude), were collected in sufficient numbers to be considered characteristic of the normal earthworm fauna of the region (Table 3). A comparison of the number of species collected in each season indicated increased richness at sites furthest from the factory. Thus, five or more species were found at Sites 8–14 in spring, autumn and winter, although lower numbers were collected at Sites 10 and 12 in summer. These communities were dominated by *Allolobophora chlorotica* and *Aporrectodea caliginosa*, with the remaining worms present at low densities. Fewer species were collected from the sites closest to the factory, with only 1–3 species found at Sites 3–7 in spring, autumn and winter. Comparisons of catches indicated that *Lumbricus rubellus*, *Lumbricus castaneus* and *Lumbricus terrestris* were dominant, with *Allolobophora chlorotica* and *Aporrectodea caliginosa* reduced. No worms were collected from Sites 1 and 2.

Biodiversity

Differences in earthworm species richness influenced the diversity and dominance of sampled communities as estimated using the Shannon–Weiner index and Berger–Parker statistic. Shannon–Weiner values indicated three diversity levels (Table 4). The highest diversity was found at Sites 8–14 with values greater than one in all cases, except Sites 10 and 12 in summer when total catches were low (Fig. 1c). Diversity at Sites 3–7 was lower than at sites further from the factory, with values below one in all cases except Site 6 in summer and spring. Comparisons of Shannon–Weiner estimates for the four seasons generally gave highest values for spring and winter, with lower values in autumn and a large reduction in summer.

Berger–Parker values, which indicate the proportion of the total catch due to the dominant species, were generally higher at sites closest to the factory. Thus, values at Sites 3–7 were frequently in excess of 0·5, while those for Sites 8–14 rarely exceeded this value. No clear seasonal trends were observed (Table 4).

Multivariate analysis of community composition

The effects of smelter emissions on earthworm communities were also analysed using a multivariate cluster technique. Analysis was performed using Euclidean distance, Pearson's product moment and Ward's minimum variance amalgamation method. Clustering for the spring sample indicated that sites could be split into three groups (Fig. 2a). For the summer sample, sites were again divided into three groups (Fig. 2b). In the autumn sample two groups were identified, while in the winter sample, sites could be divided into three clusters (Fig. 2c,d).

Discussion

POPULATION AND COMMUNITY RESPONSES TO METAL CONTAMINATION

A significant negative linear relationship was found between log soil metal concentrations and distance from the smelter. This pattern of metal distribution, which indicates an exponential decline with distance from the factory, is in agreement with the results of previous studies on the spatial distribution of metals at Avonmouth (Hopkin, Hardisty & Martin 1986; Martin & Bullock 1994; Spurgeon & Hopkin 1995; 1996a,b). Previous analysis of depth profiles for cadmium, copper, lead and zinc at Avonmouth have generally found lower metal concentration in the deeper soil layers compared to surface soils (Martin & Bullock 1994). For example, Sandifer (1997) found that metal concentrations in surface soil exceeded those at 30 cm depth by a factor of between three and 50. The same study also indicated that levels in litter generally exceed those in surface soils. The presence of depth profiles in smelter-contaminated soils suggests that epigeic earthworms, which usually inhabit upper soil and litter layers, may be more exposed than endo-

Table 3. Mean number and species of worms collected from 25 × 25-cm quadrats (six replicates) taken at 14 sites in the Avonmouth area in spring, summer, autumn and winter. 'Others' includes *Eiseniella tetraedra*, *Murchieona minuscula* and *Octolasion cyaneum* and all worms that could not be identified due to similarities of the newly hatched individuals of some species. Values should be multiplied by 16 to obtain earthworm density m^{-2}

	Spring								**Summer**							
Site No.	*L. terrestris*	*L. rubellus*	*L. castaneus*	*A. chlorotica*	*A. rosea*	*A. caliginosa*	*A. longa*	Others	*L. terrestris*	*L. rubellus*	*L. castaneus*	*A. chlorotica*	*A. rosea*	*A. caliginosa*	*A. longa*	Others
14	4·7	4·8	5·5	2·8	6·7	2·5	1	0·3	2	1·2	0·2	1·5	2·7	8·5	2	1·2
13	0·2	0·3	0·5	23·7	3·8	3·5	3·5	1·3	0	1	0·3	0·7	1·3	0	2	0
12	1·5	4	1·5	4·2	0·8	8·5	0·7	0·7	0	0	0	0	0	0	0·7	0
11	1·7	0·7	0·5	6	1·3	4	0	1	0	0·7	0	3·2	0·3	1·2	0·7	0
10	0·8	2·5	1	8·7	0·8	3·5	0·2	1	0	0	0	0·8	0·2	0	1·2	0
9	1·3	0·7	1·8	7·3	1·8	7·8	5	1·8	0·2	0·8	0	4·5	0·3	2·2	3·8	0·8
8	0·5	0·8	2·3	1	1·3	4	1·8	2·5	0	0·2	0	0·5	1·8	0·8	2	0
7	0·2	4·3	0	0	0	0·2	0	1·2	0	0	0	0	0	0	0	0
6	0·3	0·8	0·5	0	0	0	0	0·3	0	0·3	0	1·3	0	0	0	0
5	0·2	1·7	0·7	0	0	0	0	0·2	0	0	0	0	0	0	0	0
4	2·2	2·8	0	0	0	0·3	0	0	0	0	0·2	0	0	0	0	0
3	0	0·3	0·7	0	0	0	0	0·5	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Autumn								**Winter**							
	L. terrestris	*L. rubellus*	*L. castaneus*	*A. chlorotica*	*A. rosea*	*A. caliginosa*	*A. longa*	Others	*L. terrestris*	*L. rubellus*	*L. castaneus*	*A. chlorotica*	*A. rosea*	*A. caliginosa*	*A. longa*	Others
14	1·5	1·2	0·3	2·3	0·2	10·3	3·2	4·8	1·2	0·5	7	3·5	0·7	12·2	3·5	3·8
13	0·5	1	0	12·5	1·3	3	1·8	8·7	0·5	0·5	1·3	3·2	1·2	2·3	2·7	5·2
12	0	1·3	0	3·2	0·2	3·3	1·8	2	0·2	2	2·8	5·3	0·8	2·3	1·8	4
11	1·5	0·7	1	7	1·2	5·2	0·8	8·5	2·5	0·5	2	6·8	1·3	2·7	1·2	1·3
10	0·2	0·2	0	6	0·7	4	0·5	4	0·5	0·2	1·5	5·3	0·5	3·5	0·8	5
9	0·7	0·2	0	4·5	1·3	6·2	2·2	2·3	1·8	0·5	1·3	6·8	2	12	2·7	7·8
8	1	0·2	0	2·8	5	5	0·8	7	1·3	0·3	1·3	5·7	2·2	9·5	0·8	9
7	0·3	0·5	0	0	0	0	0	0·5	0·5	1·7	2·7	0	0	0	0	3
6	2·2	1·7	0·2	0	0	0	0	0·8	2·3	0·2	0	0	0	0	0	1·5
5	0	1·5	0	0	0	0	0	0	0·2	1·5	1·2	0	0	0	0	4·2
4	1·2	0·8	0	0	0	0	0	1	1·7	1	0	0	0	0·2	0	2·2
3	0	0·8	0	0	0	0	0	1	0	0·3	0·7	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Shannon–Weiner diversity and Berger–Parker dominance statistics for earthworm communities sampled from sites along two transects from a primary smelting works. All values are calculated from the total number of worms collected from six 0·25 × 0·25-cm quadrats. Only worms identified to species have been included for biodiversity and dominance calculations. Note the lower diversity and higher dominance values for Sites 3–7 in all seasons. No worms were collected from Sites 1 and 2

Site No.	Shannon–Weiner diversity				Berger–Parker dominance			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
14	1·83	1·55	1·37	1·56	0·24	0·47	0·54	0·42
13	1·1	1·46	1·22	1·76	0·67	0·38	0·62	0·27
12	1·65	0	1·38	1·69	0·40	1	0·34	0·35
11	1·46	1·3	1·56	1·68	0·42	0·53	0·40	0·40
10	1·45	0·9	1·13	1·56	0·50	0·54	0·52	0·42
9	1·66	1·39	1·41	1·53	0·30	0·38	0·41	0·44
8	1·95	1·36	1·44	1·49	0·29	0·38	0·34	0·45
7	0·31	0	0·67	0·93	0·93	–	0·60	0·55
6	1·03	1	0·83	0·24	0·50	0·73	0·54	9·93
5	0·80	0	0	0·87	0·67	–	1	0·53
4	0·88	0	0·68	0·85	0·53	–	0·58	0·59
3	0·64	0	0	0·64	0·67	–	1	0·67

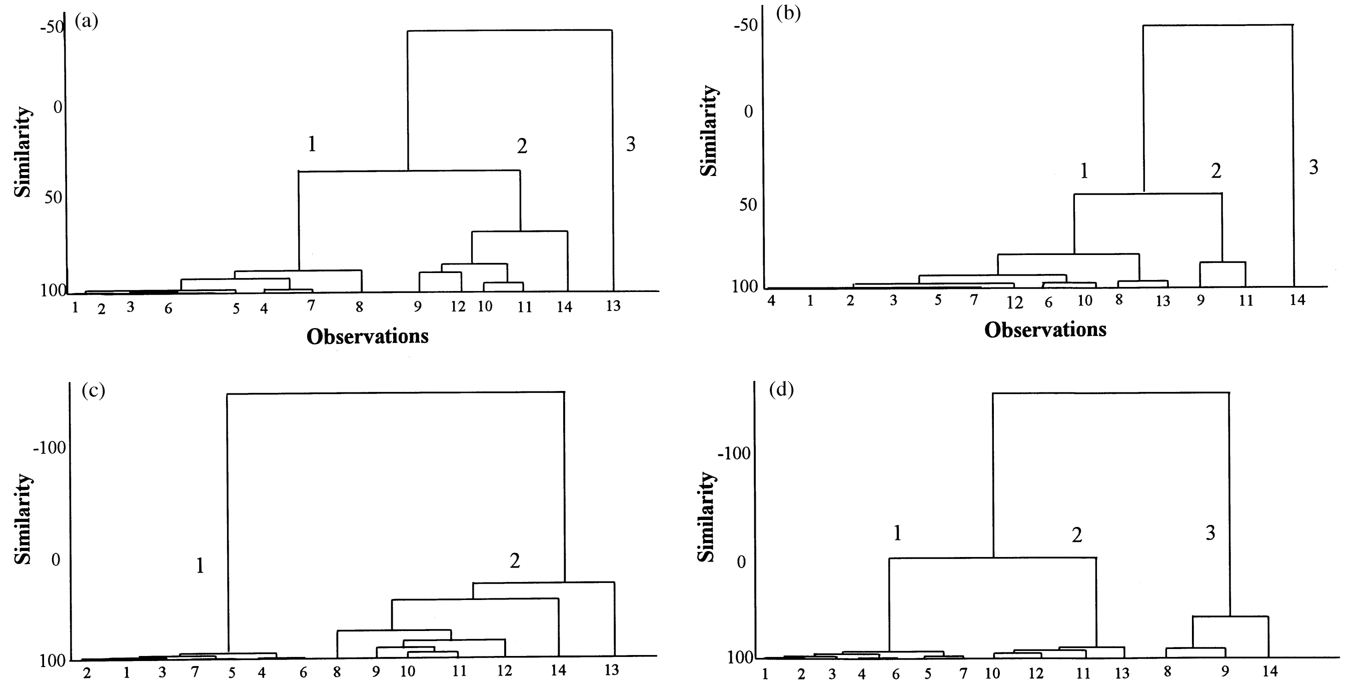

Fig. 2. Dendrogram of earthworms communities sampled from 14 sites at Avonmouth ordered by cluster analysis using Euclidean distance and Ward's minimum variance method in (a) spring, (b) summer, (c) autumn, and (d) winter.

geic and anecic species that live primarily in subsurface soil.

Earthworm abundance was reduced at the sites closest to the factory (Fig. 1a–d). Linear comparisons of mean earthworm catch with soil metal concentrations after appropriate transformations indicated a negative correlation for all metals. No relationships were found between soil pH or %LOI and mean catch. Thus, it is almost certain that the high concentration of metals in soils close to the factory results in a reduction in earthworm numbers at these sites, with zinc likely to be the most important toxic metal (Spurgeon & Hopkin 1995). Previous studies of earthworm distributions in smelter-contaminated soils also found lower earthworm numbers in heavily contaminated soils. Spurgeon & Hopkin (1996a) found a significant reduction in earthworm abundance at sites within 2 km of the Avonmouth smelter, while Bengtsson, Nordstr'm & Rundgren (1983) found lower total numbers and biomass in soils around a brass mill. Bisessar (1982) and Hunter, Johnson & Thompson (1987) found decreased abundance of worms in soils near a lead smelter and copper refinery, respectively.

High cadmium, copper, lead and zinc concentrations have been found to affect the growth and sexual development of earthworms in laboratory tests (Bengtsson, Gunnarsson & Rundgren 1986; Van Gestel, Dirven-Van Breemen & Baerselman 1993; Spurgeon & Hopkin 1996c; Khalil *et al.* 1997; Svendsen & Weeks 1997). If effects on these life-history traits also occur under field conditions, it would be anticipated that the size class and age structure of populations at contaminated sites would be altered (Baveco & DeRoos 1996; Klok & DeRoos 1996). Such effects have been observed for isopod and carabid beetle populations at Avonmouth (Read, Wheater & Martin 1987; Jones & Hopkin 1994, 1996, 1998). Comparisons of *Allolobophora chlorotica* populations at sites along the contamination gradient did not indicate any differences in size class structure. However, relative abundance of life stages did differ between sites. In the less-contaminated soils, the proportion of adults was lowest in spring and highest in winter. This was not the case at the most contaminated site where worms persist, although due to the low number collected in some samples, it is not clear if these differences represent significant variations between populations.

In addition to variations in the density of earthworms along transects from the factory, temporal changes were also found. Catches were lower in summer than in spring, autumn and winter, for which values were similar (Fig. 1a–d). The lower mean catches in summer can be attributed to a response to drought by some species, together with the sampling technique used. For sampling, soil within quadrats was dug to 40 cm and hand sorted. This technique is suitable for collection of most worms in spring, autumn and winter, except some larger anecic species inhabiting deep burrows. However, in summer, when the water content of surface layers is low (Table 1), some species such as *Lumbricus terrestris*, *Lumbricus rubellus* and *Lumbricus castaneus* retreat to deeper soil and will not be captured by the sampling technique used (Morgan & Morgan 1993). In contrast, species such as *Aporrectodea longa*, *Aporrectodea caliginosa* and *Aporrectodea chlorotica* escape drought by aestivating in surface soils and will still be encountered during summer sampling (Lee 1985; Garnsey 1994; Edwards & Bohlen 1996). As a result of summer drought conditions, earthworm sampling for monitoring purposes should not be conducted during this season. Similarly, winter sampling should also be avoided, since frost may limit both ease of sampling and the capture of some species (Røzen 1982).

TECHNIQUES, EFFECT PARAMETERS AND COMMUNITY MONITORING

Effects of metals on the earthworm communities were analysed using a range of monitoring parameters. A number of these techniques proved useful for the identification of contaminated sites, while other were clearly unsuitable for this purpose. Changes in mean abundance and biomass are the most simple measurements of the effects of metals on earthworm communities, because taxonomic expertise is not required to determine a value for each site. Comparisons of the mean abundance and biomass at Avonmouth with control values, indicated significant reductions in the seven soils closest to the factory in all seasons. However, significant reductions in abundance and biomass were also found in summer and winter at less-contaminated sites for which no effects were found using any other measurement parameter (Fig. 1b,d). Although these differences may reflect the spatial heterogeneity of earthworm populations and may be removed by the use of additional sample replicates (Daniel *et al.* 1992), it is known that earthworm densities can vary by local factors even in related soils (Boag *et al.* 1994; Ponge & Delhaye 1995). This variability will hamper the use of simple measurements of abundance and biomass for pollution monitoring.

Species richness, species dominance, biodiversity and dominance data all indicated that the selected sites can be allocated to three contamination levels. The first level contains Sites 8–14, at which soil metal concentrations were relatively low. At least five species were always collected from these sites in spring, autumn and winter, with *Aporrectodea caliginosa* or *Allolobophora chlorotica* the dominant species. Shannon–Weiner values were greater than 1, with Berger–Parker dominance usually below 0·5. The second level contained Sites 3–7. Metal concentrations at these sites exceed those for sites further from the factory. At these sites, only 1–3 species were present, with *Lumbricus rubellus* and *Lumbricus castaneus* dominant and *Aporrectodea caliginosa* and *Aporrectodea rosea* usually absent. Shannon–Weiner diversities were less than 1, with dominances greater than 0·5. The third level consisted of Sites 1 and 2 at which all worms were absent.

Multivariate cluster analysis for the four season samples showed close relationships for Sites 1–7, indicating a higher level of similarity for the communities in these soils (Fig. 2a–d). The close clustering of these sites, at which sensitive earthworm species were frequently absent, demonstrates that multivariate techniques can be used to identify communities affected by high soil metal concentrations. For the sites further from the factory, a high similarity in community composition was frequently found. Furthermore, communities at these sites were closely related to those at the control site in most cases, although Site 13 frequently showed lower similarity, probably because this site is subject to additional stress resulting from exposure to seawater spray during high spring tides. The relatively close clustering of communities at Sites 8–13 to controls, suggests no effects on assemblages in these soils due to the metal concentrations present.

Results from this study demonstrate that it is possible to relate reductions in earthworm abundance and diversity directly to metal contamination. Of the monitoring techniques, species richness, diversity and cluster analysis all highlighted the detrimental effect of metals on communities at Sites 1–7 (within 3 km from the factory) and could be used to classify selected sites to one of three contamination levels. From these results, it is clear that earthworms have a role to play in a terrestrial monitoring systems. However, experience gained during the development of RIVPACS indicates that is difficult to identify disturbed ecosystems by considering effects on individual groups in isolation. Instead, impact across a range of faunal groups should be used. Indeed, studies at Avonmouth, which have found changes in springtail, carabid beetle, isopod, ant and spider communities, have indicated that a SOILPACS system would have the capability to identify metal-contaminated sites (Spurgeon, Sandifer & Hopkin 1996; Sandifer 1997).

If earthworms are to form a key group within a SOILPACS system (as their usefulness in monitoring studies, key role in ecosystems and importance as a food source demands) a number of practical questions need to be addressed. These include the selection of a suitable measurement parameter, how to deal with between-year variations, and the choice of a sampling protocol. The parameter used in a potential SOILPACS method is governed by the design of prediction and classification systems. In RIVPACS, pollution effects are quantified by predicting which species should be present at a site based on ecological surveys of uncontaminated areas and then surveying the contaminated region to see which species are absent. Such assessments are only as good as the data upon which they are based. Thus, SOILPACS development would require quantitative soil invertebrate surveys to be undertaken at many different types of clean habitat. Such detailed studies would allow between-year and habitat variations to be quantified, allowing these to be rationalized at potentially impacted sites.

The choice of sampling technique for earthworm population studies is the focus of a considerable debate that is outside the scope of this paper (see Edwards & Bohlen 1996 for references). However, when selecting a sampling technique for a potential SOILPACS system, consideration such as statistical robustness, replicability, equipment portability, time and manpower requirement are all important considerations. The sampling technique used in the current study was specifically designed to suit the requirement of the Avonmouth region. The use of on-site digging and hand-sorting (and not formalin extraction, which requires large volumes of liquid to be transported in the field) meant that relatively inaccessible sites could be included in the survey. The techniques gave good results in spring, autumn and winter, although catches were low in summer when some earthworm species withdraw to the lower soil layers. Despite these problems, it is our opinion that digging and hand-sorting are appropriate techniques for obtaining the presence/absence and abundance data required for a SOILPACS system.

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