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THE ROLE OF BIOMONITORING IN THE RISK ASSESSMENT OF HEAVY METALS AND METALLOIDS IN MINING AND URBAN ENVIRONMENTS

The application of monitoring techniques, to assess levels of heavy metals and metalloids, is possible using a variety of approaches. In particular the analysis of biological species as indicators of contamination offers opportunity for immediate assessment of wide regions. This study allows investigating the potential of different biological indicators (terrestrial animals, plants, animal faces) for identifying the spatial distribution of a range of heavy metals and arsenic in contaminated and relatively pristine soil systems in Central and Southern Scotland.

Correlation of selected analytes gave good linear relations but varied from element to element. The results obtained established woodlice, fern and sheep faces as suitable objects for further long-term monitoring research, which can provide quantitative information on different pollutants in soils.

1. INTRODUCTION

Determining concentrations of heavy metals and metalloids in the environment is an important part of understanding biogeochemical processes and gauging ecosystem health [1]. Concentrations of pollutants in terrestrial environments have increased significantly as a direct result of human activities through emissions from industrial plants, thermal power stations, waste disposal, soil amendments and vehicle traffic/road infrastructures. Heavy metals reaching the soil remain present in the pedosphere for many years even after the removal of the pollution sources [2]. A combination of physical, chemical and environmental conditions determine total metal

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deposition, the extent of biological uptake, and the subsequent effects of these elements on biological communities [3].

In urban environments, contamination can be widespread and diffuse, but is of most concern when concentration hot spots are located close to sensitive parts of the urban ecosystem – receptors, such as the human population or natural resources [4]. These urban soils have until fairly recently received little attention as a functional medium in the urban ecosystem, beyond the identification of contaminated sites and specific programmes to mitigate risks posed by the contaminants contained within [5]. Chemical contamination has been also reported in areas where mining and smelting were carried out in the past, and where significant amounts of various elements were mobilized by weathering and leaching from abandoned mining wastes. The impact on ecosystems due to wastes from mining and processing activities may appear in groundwater, surface water, and soil. Flora, fauna, and human beings are all examples of living organisms susceptible to contamination. Key pathways to humans are transfer through the food chain via weathering, erosion, hydrology, soil use and plant uptake [6]. Methods to assess the function and chemical characteristics of heavy metals are needed to provide indicators of general quality and to assist long-term soil management.

Biomonitors have been widely studied in order to assess terrestrial and aquatic ecosystems, particularly for heavy metal contamination [7], [8]. The biomonitoring capability of terrestrial animals [9] and plants [10] has been recognised and they are promising indicators to assess metal pollution on contaminated sites. Woodlice and ferns have a wide tolerance for certain contaminants, can accumulate metals at levels higher than any other soil animals and plants [11]–[13] and provide sufficient material for analysis [9], [12]. But also other biological materials like animal faeces can be considered to be significant indicators of heavy metals and metalloids [14]. Although not all regulated elements (e.g., arsenic and selenium) are heavy metals, in this study the term *heavy metal* is used for all regulated elements.

This study contains an evaluation of the relationship between selected heavy metal and metalloid (As, Cu, Cd, Pb and Zn) concentrations in woodlice, ferns, sheep faeces and soils collected from two areas: at 13 locations of urban environment from across the district of Renfrewshire (Central West Scotland, UK) and from an abandoned antimony mine at Glendinning (South West Scotland, UK).

Woodlice and soil samples were collected from public open spaces – forest and grassy areas in parks, ornamental gardens and recreational footpaths. The district has been described in general detail previously [15] and sites were selected to represent examples of general urban soil rather than being known contaminated locations. Renfrewshire is situated 7 miles (11 km) west of Glasgow, and 53 miles (85 km) west of Edinburgh.

One former Scottish mining and smelting site has been chosen for the collection of fern and sheep faeces. This site is located in a zone of lead mineralization, with elevated levels of contaminations such as copper and arsenic. Glendinning is located next to Jamestown, 13 km northwest of Langholm and 26 km southwest of Hawick in southern Scotland.

Mining at the Luisa mine at Glendinning began on this site in 1793 and approximately 100 t of antimony was refined in its first 6 years. Work ceased in the early 1800s except for brief periods sat at the end of the 19^{th} century and during the First World War [16].

2. MATERIAL AND METHODS

Samples were collected during May/June and October 2003. The isopods were found under stones, wood and rotten wood stumps. The individuals were identified to be either *Oniscus asellus* or *Porcellio scaber*, the most common species in Great Britain [9]. The ferns (*Athyrium flexile*) and the sheep faeces together were collected from spoil heaps in the surrounding area of the mine. Bulk soil samples from urban and mining environments were taken from the top 0–10 cm of soil at each location. Each soil sample was placed in a separate plastic bag and stored at ambient temperature prior to treatment. Biological samples were kept in plastic containers.

2.1. SAMPLE PREPARATION AND ELEMENT ANALYSES

Within approximately 30 minutes after collection, woodlouce samples were washed in UHP (ultra-high-pure) water, dried, identified and separated into species groups before being starved in plastic containers for 2 days to defecate [17]. Woodlice and containers were cleaned each day before individuals were finally freeze dried at –50 °C and their weight recorded (Sartorius RD500). Individuals of each species from each site were pooled and ground by hand using a pestle and mortar. The fern samples were washed carefully with tap water, dried and ground in an agate mortar. Soil and faecal samples were homogenised by manual mixing, air-dried for 24 hours and disaggregated to pass through a 2 mm mesh sieve.

All samples were digested in a CEM MARS X microwave system, in closed PTFE containers in order to reduce the risk of loss of volatile elements, using Method 3051a [18]. All reagents and standards were of analytical grade (AnalaR or Spectrosol) and ultra-high purity water (18MΩ, Elgastat UHP) was used for dilutions.

Aliquots of 0.200 g of soil were digested using aqua-regia method (3 cm^3 of HNO_3 + 9 cm^3 of HCl). For biological materials, an aliquot of 0.100 g of sample was digested for 10 min with 5 cm^3 of 65% nitric acid at 175 °C [17]. After cooling, the samples were filtered (Whatman 42 ashless, paper filters), diluted to 25 cm^3 with UHP water and stored in small PE-tubes before analysis. Subsamples for each species were analysed in triplicate.

Analyses of all samples for As, Cd, Cu, Pb and Zn were carried out by Inductively Coupled Plasma-Atomic Emission Spectrometry (Perkin-Elmer Optima 3000).

In order to check analytical quality, two certified reference materials were digested as described above. For soil, a Metals in Soil Certified Reference Material (Resource Technology Corporation No. CRM020-050) and for biological samples, lobster hepatopancreas

(NRC Canada, TORT) were chosen. The natural soil matrix showed very good recovery between 86.29 and 99.85%. The recovery of the nitric acid-extracted elements in lobster lepatopancreas was also high and amounted to above 90% for all elements.

2.2. STATISTICAL DESCRIPTION OF EXPERIMENTS

A linear analysis was carried out to find an accurate description of the correlation-taking place. The parameters of linear function, which the best approximated the experimental data, were calculated using Microcal Origin 5.0. Programme. For built-in functions, all the derivatives were computed using analytic expressions. The statistical parameters such as mean, median, correlation coefficient (R) and probability/significance (P) were calculated.

3. RESULTS AND DISCUSSION

pH is one of the major factors controlling the availability of heavy metals in soils [19], [20]. Background pH values of the urban soil types were approximately between 5.29 and 7.95, while the pH for the soils from Glendinning mining site varied between 2.66 and 7.83. The results obtained for pH of urban soil fall well within the range for UK soils, with lower values typical of local glacially-derived parent materials and higher values reflecting the potential influence of human material inputs to the surface soils. Meanwhile the pH results for the mining soil showed a very acidic environment.

The concentrations of heavy metals, especially lead (41.945 mg/kg) and arsenic (19.360 mg/kg), in the soils collected from Glendinning, were far above the background levels and generally exceeded those given in the International Guidelines for metals in soil [21]. The concentrations of metals in biota served to indicate the status of the metal contamination of the site and also revealed the abilities of various species to take up and accumulate the metals from the soil.

Summary results of metal content of bulk soil, biological samples and comparison to previously published data are given in tables 1 and 2. According to the Netherlands Soil Quality Criteria [21] none of urban soils could be considered as heavily polluted, requiring remedial investigations and cleanup. As far as mining soils are concerned, the obtained levels for the majority of analysed heavy metals are thousands times higher (table 1) than the allowed limits for good soil quality [21].

There are five native British families of woodlice but only two species, i.e. *Oniscus asellus* (*OA*) and *Porcellio scaber* (*PS*), were found in sampling sites in this study. *O. asellus* was found at all thirteen locations and *P. scaber* at only eight sites. Interpretation of this observation cannot be made in terms of abundance of species as an intrusive sampling approach was used rather than pitfall traps common in ecological investigations [11], [17].

Table 1

Metal contents of urban and mining soil materials and their comparison to typical soil values. All values are reported as mg kg^{-1} dry weight

Urban Soil	As	Cd	Cu	Pb	Zn
Min	<0.5	0.83	14.67	21.30	53.75
Max	<0.5	4.00	135.07	261.79	511.55
Mean	N.A.	2.55	63.88	126.45	246.48
Median	N.A.	2.42	57.97	95.02	183.76
N	13	13	13	13	13
Mining Soil	**As**	**Cd**	**Cu**	**Pb**	**Zn**
Min	270	N.A.	20	63	7
Max	19.367	N.A.	520	41.945	3.558
Mean	5.304	N.A.	91	17.156	769
Median	1.908	N.A.	57	17.978	382
N	11	N.A.	11	11	11
Previous studies	**As**	**Cd**	**Cu**	**Pb**	**Zn**
Dutch A [21]	20	8	36	85	140
Dutch C [21]	50	12	190	530	720
Rural-urban soil UK range [31]	N.A.	0.1–2	22.068	2–500	10–300
Soils average [19]	6 (0.1–40)	0.35 (0.01–2)	30 (2–250)	35 (2–300)	90 (1–900)
Central lowlands (Scotland) [32]	N.A.	1.28 (0.62–2.48)	12 (9.1–17)	1.39 (76–230)	67 (50–83)

Vascular plants like ferns absorb most of their nutrients from the soil, but can also take up ions through their leaves. Ferns are well known to hyperaccumulate arsenic [13], [22], although some orders can accumulate more arsenic than others. The *Pteridaceae* (an arsenic hyperaccumulator family) are closely related to other families of ferns, which do not exhibit hyperaccumulation, but with only approximately 1/1000 of all fern species having been studied in this context. Similarly, the search for a nonaccumulating species is underway [13]. The Glendinning fern samples belong to the *Dryopteridaceae* family, species *Athyrium flexile*, which is wholly or predominantly (more than 75%) found in Scotland [23]. It was previously proved that ferns besides being arsenic hyperaccumulators can also accumulate zinc and cadmium [13].

Figure 1 presents a comparison of the mean contents of copper, cadmium, lead and zinc in *Porcellio scaber* (*PS*), *Oniscus asellus* (*OA*), sheep faeces and fern (*Athyrium flexile*). It is noticeable that different bioindicators contain different levels of heavy metals, e.g. the highest values for copper and zinc concentrations were found in *P. scaber*, and those of cadmium – in *O. asellus*. High concentration of zinc was also detected in fern. On a dry matter basis the maximum lead

Table 2

Metal contents of woodlouce species (*Oniscus asellus* and *Porcellio scaber*), fern (*Athyrium flexile*) and sheep faeces and their comparison to previously published data. All values are reported as mg kg^{-1} dry weight

Oniscus asellus	As	Cd	Cu	Pb	Zn
Min	<0.5	3.88	150.6	<10	63.97
Max	<0.5	15.57	430.32	275.4	252.86
Mean	N.A.	8.37	267.75	43.41	122.46
Median	N.A.	7.54	246.86	17.21	100.96
N	13	13	13	13	13
Porcellio scaber	As	Cd	Cu	Pb	Zn
Min	<0.5	<1.00	200.8	<10	101.75
Max	<0.5	3.01	491.07	<10	425.25
Mean	N.A.	2.17	320.52	<10	247.42
Median	N.A.	2.29	285.50	<10	209.83
N	8	8	8	8	8
Fern	As	Cd	Cu	Pb	Zn
Min	<0.5	<1.00	2	<10	16
Max	13	0.38	78	160	280
Mean	N.A.	0.24	21	16	169
Median	N.A.	0.24	6.01	1.73	168
N	11	11	11	11	11
Sheep faeces	As	Cd	Cu	Pb	Zn
Min	<0.5	0.22	9	<10	58
Max	217	1.11	25	2.929	197
Mean	24	0.54	16	324	108
Median	5.38	0.49	14	20.37	109
N	11	11	11	11	11
Previous studies	As	Cd	Cu	Pb	Zn
Oniscus asellus [11]	N.A.	16.9	137.6	29	299.6
Porcellio scaber [11]	N.A.	3.6	303.4	18.6	321.6
Fern [19]	1.30	0.13	15.00	2.30	60–400
Sheep faeces [26]	N.A.	N.A.	4.8	N.A.	6.2

content in the analysed sheep faeces was 10 times higher than in *Oniscus asellus* and approximately 20 times higher than in fern. It has been previously shown by MENZI and KESSLER [24] that at average grazing animal density and mean heavy metal contents, heavy metal inputs from manures can exceed plant uptake. Within the studied biological samples, it was found that sheep faeces contained the high-

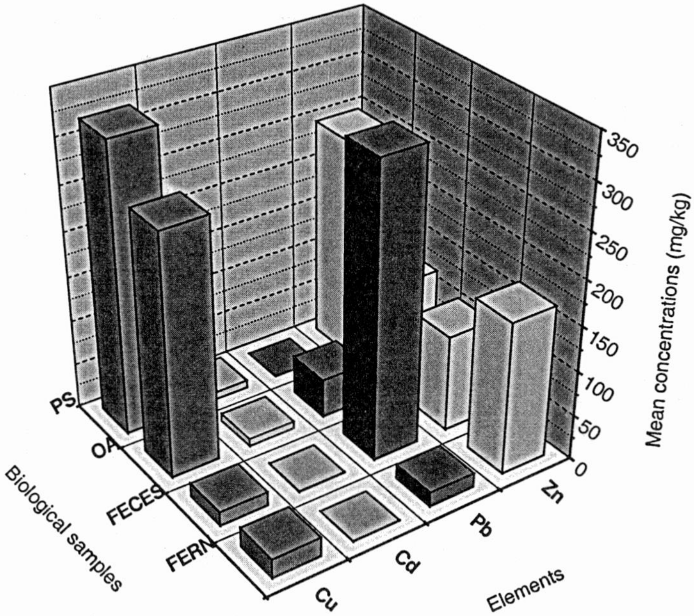

Fig. 1. Mean contents of Cu, Cd, Pb and Zn in *Porcellio scaber* (*PS*), *Oniscus asellus* (*OA*), sheep faeces and fern (*Athyrium flexile*)

Table 3

Pearson correlation coefficient (R) for correlation of heavy metal concentrations in urban (Renfrewshire) and mining (Glendinning) soils and in biological samples ($*P < 0.05$, $**P < 0.01$)

Biological samples	Soil				
	As	Cd	Cu	Pb	Zn
Oniscus asellus (*OA*)	N.A.	0.706*	0.405*	0.31	0.623*
Porcellio scaber (*PS*)	N.A.	0.748*	0.401*	N.A.	0.519
OA and *PS*	N.A.	0.323	0.399*	N.A.	0.349
Fern	N.A.	N.A	–0.140	0.077	0.538*
Sheep faeces	0.108	N.A.	0.698**	0.430	0.206

est arsenic concentrations. A recent study of sheep revealed that the major elimination route of inorganic arsenic was with faeces (78%) and reported that 24.3±5.2% of this faecal excretion was of endogeneous origin [14]. The results of the regression analysis for biological material and soil metal content are given in table 3, and selected examples of correlation are depicted graphically for the separate species in figures 2–4.

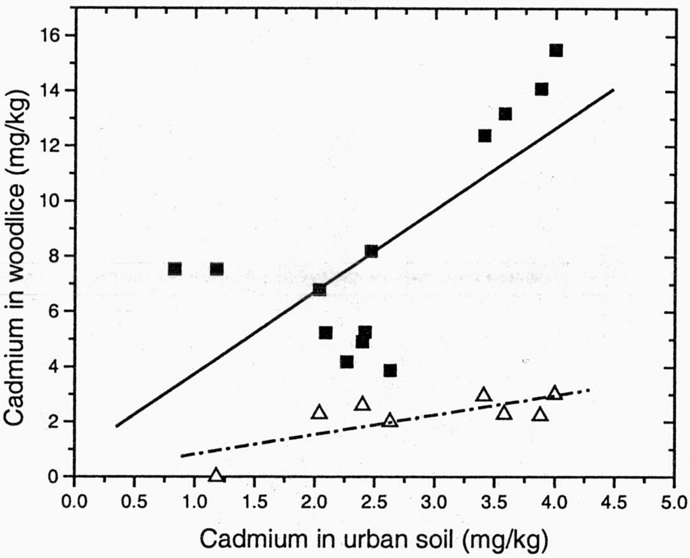

Fig. 2. Scatter plots of cadmium concentration in soil and woodlice with associated best-fit lines: *Oniscus asellus* (■, solid line) and *Porcellio scaber* (△, broken line) from Renfrewshire urban area

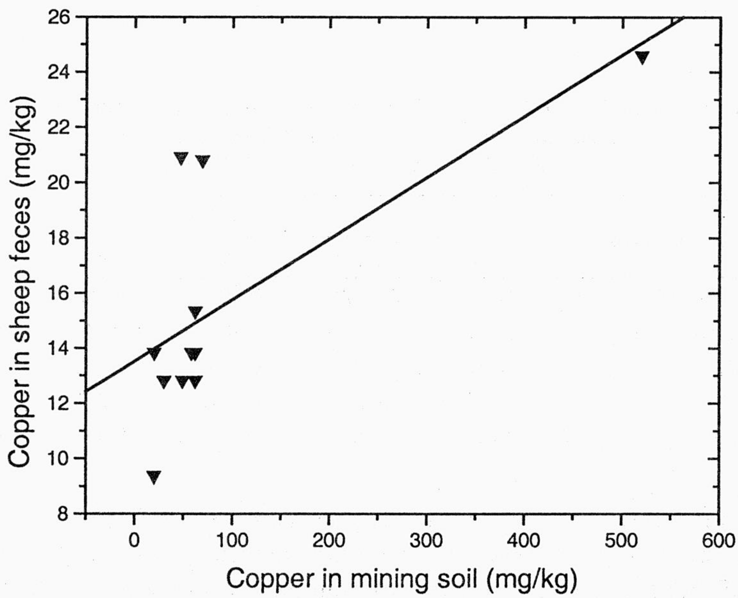

Fig. 3. Scatter plot of copper concentration in soil and sheep faeces with associated best-fit lines from Glendinning mining site

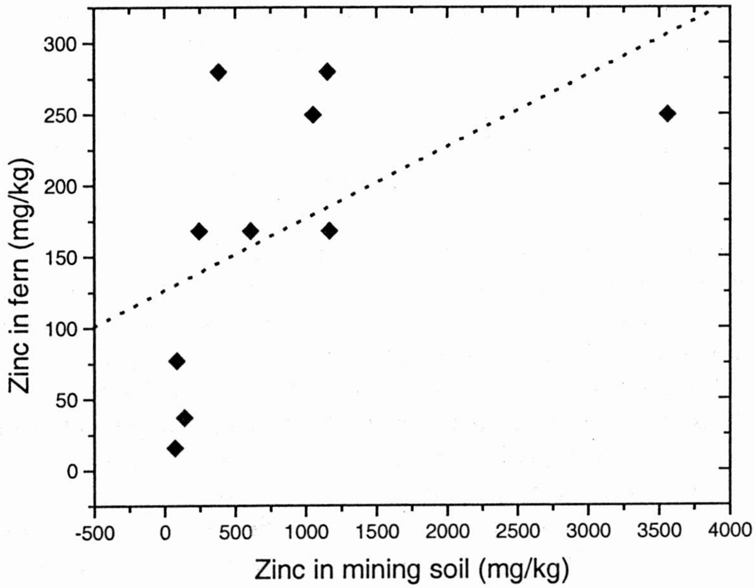

Fig. 4. Scatter plot of zinc concentration in mining soil and fern *Athyrium flexile* with associated best-fit line from Glendinning

The correlations between cadmium in isopods and corresponding soils (figure 2), being 0.706 for *O. asellus*, 0.748 for *P. scaber* and 0.323 for both species combined, were linear and significant. Differences were considered significant if $P < 0.05$ and very significant if $P < 0.01$. These values for copper, also linear and significant, were found to be 0.405, 0.401 and 0.399, respectively. The corresponding correlation for zinc, also linear and significant, was 0.623, 0.519 and 0.349, respectively. Lead was found in *O. asellus* only. BLANUSA et al. [12] have also found linear correlation for cadmium and lead. Arsenic was not detected in neither of woodlouce species. In the case of all measured elements, the correlation between soil content and combined species of woodlice was much lower, so it was concluded that each species should be considered separately.

There was no positive correlation for copper in fern (*Athyrium flexile)* and corresponding soils (–0.140), but the correlation between copper in sheep faeces and the soil was linear (0.698) and very significant (figure 3). In the case of zinc however, the situation was reversed – the correlation between fern and corresponding soils was linear (0.538) and very significant (figure 4) while the correlation between zinc in sheep faeces and the soil (0.206) was not so significant.

The elevation of the arsenic levels in soil causes considerable concern with respect to its uptake by plant and subsequent entry into the food chain. Background arsenic concentrations in terrestrial living organisms are usually <1 mg/kg [25]. Sometimes these levels are higher in biota collected from mine waste sites, arsenic treated areas, near smelters and mining areas. Arsenic was detected in one fern sample (collected in early summer) giving a value of 13 mg/kg, which exceeds the background concentration in terrestrial plants. In this case, *Athyrium flexile* cannot be considered an arsenic hyperaccumulator; to meet this definition, the arsenic levels in the fern should be much higher than in the soil [13]. The sheep faeces showed more varied concentrations with the maximum value reaching 217 mg/kg.

Significant correlation was found between the metals from the fern samples and the sheep faeces. The most significant values were obtained for cadmium (0.901) and zinc (0.706). Such correlations reflect overall concentration similarities and demonstrate the importance of the heavy metal mobility in the food chain.

The availability of heavy metal micronutrients and their distribution in soils and plants as well as their requirements for grazing animals are important when assessing the status of environmental quality for a given area. The availability of such micronutrients occurring in soils to plants and in plants to grazing animals deserves further studies in this region. The copper and zinc contents in the collected faeces of grazing sheep averaged 16 and 108 mg/kg, respectively. In the natural grassland, animal manures, therefore, may be an important source of heavy metals [26].

The correlations between heavy metals can be viewed as a reflection of general metal fluxes at particular plots and as an indication that deposition at discrete locations depends on a number of local complexities, including microenvironmental factors, precipitation variability, and vegetation composition and density. Furthermore, the correlations suggest that bioindication with woodlice, fern and sheep faeces is reflecting these general patterns in local deposition.

The bioconcentration factors (BCF) and the ratio between element concentrations measured in isopods, ferns and sheep faeces and in soils samples, coming from the same sites, also were calculated (table 4). In the case of woodlice, the average BCF for cadmium was found to be 3.28 (*OA*) and 0.26 (*PS*). Similar average values for both species were found in the case of copper (4.19 (*OA*) and 4.79 (*PS*)). For zinc the bioconcentration factor took the values of 0.50 (*OA*) and 1.01 (*PS*), and for lead it was 0.34 (*OA*). Other literature data reported similar factors, less than 1, for cadmium, lead and zinc in bulk woodlice. BCF for copper was found to be in the range from 1 to 12 [12], [27]. Zinc was retained over twice better by *P. scaber* than by *O. asellus*, which explains the fact that *O. asellus* can excrete zinc, while *P. scaber* is not able to lose this element once it was accumulated in its body [28]. In contrast, cadmium was accumulated to a much greater extent in *O. asellus*, and lead was below detection limit in *P. scaber*. The same phenomenon was observed previously by HOPKIN [28], who found that the lead concentration in *O. asellus* was much higher than in *P. scaber*. In this study, copper was accumulated by both species [28] but slightly better by *P. scaber,* confirming the results from other studies [28], [29]. According to the literature data, the differences in the concentrations of elements between both species from the same microhabitat seem to be due to ecological and physiological factors controlling metal pollutant assimilation, storage and excretion [28], [30].

Table 4

Biological material: pooled soil bioconcentration factor (BCF), ratio between element concentrations (mg/kg, dry) in biological material and in soil samples (range of site observations)

Biological material	As	Cd	Cu	Pb	Zn
Oniscus asellus	N.A.	3.28 (1.47–9.10)	4.19 (2.22–18.86)	0.34 (0.04–4.70)	0.5 (0.2–2.58)
Porcellio scaber	N.A.	0.75 (0.58–1.13)	4.79 (1.85–13.69)	N.A.	1.01 (0.32–7.45)
Fern	N.A.	N.A.	0.43 (0.01–1.64)	0.007 (3.4E-5–0.067)	0.34202 (4.9E-6–0.89)
Sheep faeces	0.009 (6.2E-6–0.065)	N.A.	0.32 (0.05–0.70)	0.036 (3.4E-5–0.036)	1.45 (0.03–12.18)

The mean BCF of arsenic in sheep faeces was 0.009. In the case of copper, the mean factors for fern and sheep faeces were 0.43 and 0.32, respectively. BCFs, for both lead and zinc, were much higher in sheep faeces (0.036; 1.45) than in fern (0.007; 0.34). Sheep faeces showed the highest BCF for zinc from all the samples studied (see table 4).

4. CONCLUSIONS

The experimental approach outlined here can serve as a baseline for finding the interactions of important, selected heavy metals and arsenic with biological materials such as woodlice (Isopods), fern and sheep faeces. Chemical composition of biological samples provided quantitative information on the contamination of the environment and on the pollutant occurrence. The data presented confirmed the previous literature data that isopods, ferns and sheep faeces might be reliable bioindicators of soil contamination with heavy metals. The correlation between the metal concentrations in soil samples and biota was linear and significant and varied from element to element.

In order to confirm the results obtained in this study, in our further work more samples from additional locations should be analyzed. A larger number of sites/samples will enable the more accurate and precise results.

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ROLA BIOINDYKATORÓW W OCENIE RYZYKA ZANIECZYSZCZENIA TERENÓW MIEJSKICH I KOPALNIANYCH METALAMI CIĘŻKIMI

Aby ocenić zanieczyszczenie środowiska metalami ciężkimi i metaloidami, stosuje się różne techniki monitoringu. Analiza niektórych gatunków organizmów, tzw. bioindykatorów (wskaźników) zanieczyszczeń, daje możliwość szybkiej oceny stanu bardzo dużych obszarów. Zbadano możliwość wykorzystania wybranych indykatorów (organizmów żyjących w ziemi, roślin i odchodów zwierzęcych) do ustalenia, jak rozprzestrzeniają się metale ciężkie i arsen w glebach na terenach przemysłowych (kopalnianych) i terenach relatywnie nieskażonych w środkowej i południowej Szkocji.

Korelacje pomiędzy wybranymi bioindykatorami a glebą były liniowe, chociaż różniły się w przypadku poszczególnych analizowanych zanieczyszczeń. Uzyskane wyniki umożliwiają zastosowanie stonogi (*Oniscus asellus*), paproci czy też owczych odchodów jako odpowiednich bioindykatorów w przyszłym długoterminowym monitoringu środowiska.