

## Metal and metalloid bioaccumulation in three centipedes (Chilopoda)

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Received: May 14, 2022; Revised: June 11, 2022; Accepted: June 12, 2022; Published online: June 16, 2022

**Abstract:** Three centipede species (*Clinopodes flavidus*, *Cryptops anomalans* and *Eupolybothrus transsylvanicus*) were used as bioindicators of trace metal and metalloid pollution in Belgrade, Serbia. The concentrations of 13 elements (the metals Mn, Co, Ni, Cu, Zn, Rb, Sr, Cd, Tl, Pb and U and metalloids As, Se) in whole animals and soil were measured by inductively coupled plasma mass spectrometry (ICP-MS). Differences in the concentrations of some elements in the analyzed species were observed, both in response to the sites and between species. In most cases, the trace element concentrations were higher in centipedes from a polluted site (an industrial area near a busy street with heavy traffic) but *C. anomalans* and *E. transsylvanicus* had higher Mn concentrations at an unpolluted site (a deciduous woodland on Mt. Avala). *C. flavidus* was a good bioindicator for detecting differences between Zn, Se and Cd. *C. flavidus* and *C. anomalans* were more efficient in accumulating Zn than *E. transsylvanicus*. It appears that *C. anomalans* poorly accumulated Cd, unlike *C. flavidus* and *E. transsylvanicus*, which accumulated Cd according to the high bioaccumulation factor (BAF) values. We conclude that the centipedes *C. flavidus*, *C. anomalans* and *E. transsylvanicus* can be used as suitable bioindicators of trace element exposure. Their ability to accumulate trace elements was different and depends on their physiology and lifestyle as well as the route of exposure.

**Keywords:** centipedes; trace metals; trace elements; bioaccumulation factor (BAF)

## INTRODUCTION

Due to its negative impact on health, the environment in urban areas has become the subject of intense interest. Elements, such as copper (Cu) and zinc (Zn), are nutritionally essential elements at low levels but toxic at higher levels, while other elements, such as lead (Pb), cadmium (Cd) and mercury (Hg), have no known biological functions. They originate from many different sources, including vehicle emissions, industrial discharges and other activities [1-4].

Besides human exposure, the accumulation of trace elements in urban soils can also affect soil fauna and the animals that feed on these organisms. Soil

invertebrates are often used as indicators of pollutant levels [5-20]. They exhibit differences in the response to trace element exposure due to their physiology, feeding habits, mobility and microhabitat preferences. For example, earthworms are less mobile and consume a mixture of soil and detritus while isopods and millipedes are more active and feed mainly on plant detritus. An additional effect of elements in soil fauna is the potential for the bioconcentration of elements up the food chain. Soil organisms are a primary food source for many invertebrate and vertebrate predators, and thus in heavily contaminated areas, there is an increased risk of secondary poisoning [4].

The bioaccumulation of trace elements in animals has often been expressed by the bioaccumulation factor (BAF), which is the ratio between the concentration of elements in the organism to their concentration in the soil. This factor is a good measure of bioaccumulation in ecosystems in which an organism lives from the beginning of its life to the moment of investigation [21].

Centipedes (Chilopoda) are predatory soil inhabitants that avoid light and show a distinct preference for moist microhabitats. Generally, they spend the day under stones, bark and leaf litter or inside the soil, coming out to hunt at night. Although frequently occurring in woodlands, some centipedes are common in gardens in suburban and even urban localities [22-26]. As predators, Chilopoda feed predominantly on living animals. However, there are numerous references that vegetable food is not disdained (indeed it can even be essential). Centipedes can endure long periods of hunger (up to 6 months). Differences in food spectra exist between different species as well as different stadia within one species, depending on the body size. Geophilomorpha inhabit deeper soil layers, preferring slow but relatively large prey (e.g., Lumbricidae, larvae of Diptera), whereas the more surface-active Lithobiomorpha feed on small, more active forms (e.g., Collembola) [27].

In the present study, we compared the content of thirteen elements (Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Tl, Pb and U) in the whole bodies of three centipede species, *Clinopodes flavidus*, *Cryptops anomalans* and *Eupolybothrus transsylvanicus*, and in soil collected from urban and rural sites in Belgrade and calculated the bioaccumulation factors (BAF) to determine which elements accumulate in the body of these myriapods.

## MATERIALS AND METHODS

### Study area and sampling of centipedes and substrate

Belgrade, the capital of the Republic of Serbia, has a population of about 1.6 million. Adults of three centipede species, *C. flavidus*, *C. anomalans* and *E. transsylvanicus*, were sampled by hand at two

sites (Supplementary Fig. S1) as follows: the Old Industrial Area (OIA) near a busy street with heavy traffic (44°49'3.183"N; 20°29'1.029"E), and a deciduous woodland on Mountain Avala (MA), 18 km southeast of the center of Belgrade (44°41'37.547"N; 20°30'24.097"E). Most of the animals were found to inhabit moist places under stones, decaying leaf litter, or woodpiles. To exclude seasonal factors, the sampling period was restricted to several days in October 2019. At both sites, specified number of individuals of each species (*C. flavidus*: n=8 at MA, n=16 at OIA; *C. anomalans*: n=7 at MA, n=9 at OIA; *E. transsylvanicus*: n=11 at MA, n=7 at OIA) were placed in numbered Petri dishes with a small amount of soil (one animal per dish). The body lengths of *C. flavidus*, *C. anomalans* and *E. transsylvanicus* were 50-65 mm, 25-50 mm and 28-50 mm, respectively. The soil was collected from the top 10 cm layer after removing surface vegetation from several holes made in the ground near the place where the animals were found.

### Preparation of samples and element analysis

In the laboratory, all samples were thoroughly rinsed with distilled water and placed in Petri dishes with one Whatman No. 1 filter paper and a few drops of distilled water to maintain moisture. Specimens were kept at 14°C for 7 days and the filter paper was changed daily to allow for the complete evacuation of the gut contents. The centipedes were then killed by freezing. Element concentrations in the body were determined after purging of their guts to ensure that the actual concentrations in their tissues were measured. The whole animal (with an approximate body length of 50 mm and weight of 1 g) was dissected and then separated from the macerated mass to measure the concentration of elements.

The concentrations of 13 elements (Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Tl, Pb and U) were determined by inductively coupled plasma mass spectrometry, ICP-MS (ICAP Qc; Thermo Scientific X series 2, Waltham, MA, USA). After collection, the lengths and weights of the animals were measured. Tissue samples were transferred into microwave cuvettes and decomposition was performed using the ETHOS 1 Microwave System (Milestone, Italy). Four mL of high-grade 65% nitric acid and 1 mL of 30% hydrogen

peroxide (Merck, Darmstadt, Germany) were added to each cuvette and microwave digestion was performed as follows: warm-up for 3 min to 85°C, 5 min to 135°C and 15 min to 180°C. After cooling, the samples were quantitatively transferred into a volumetric flask (25 mL) and diluted with ultrapure water (Milli Q plus system, Merck, Germany).

After removing pebbles and grass, soil was sampled at a depth of roughly 4 cm using plastic spoons. All soil samples were transported in plastic containers to the laboratory where they were homogenized and dried. About 0.5 g of soil per sample was measured and digested in aqua regia (concentrated HCl and HNO<sub>3</sub> in a 1:3 milliliter ratio). Following 12 h of digestion at 90°C, the samples were filtered through Whatman paper (no. 42), then through HPLC filters (pore size 0.45 µmL), and finally diluted to 500 mL with ultrapure water. For method validation, certified reference material of soil (SRM 2710a, NIST) was used. All element contents were checked by applying the standard addition recovery (R) experiment. The R-value ranged from 82-126%. The optimal conditions for ICP-MS are provided in Supplementary Table S1.

### Bioaccumulation factor (BAF)

BAFs are considered a simple tool to estimate the bioaccumulation of contaminants in a particular organism in a specific medium. The most used formula for calculating the BAF is as follows:

$$\text{BAF} = \frac{\text{C}_{\text{substance in the organism}}}{\text{C}_{\text{substance in the soil}}} \quad (1)$$

where C is the concentration and can be expressed in mass unit per mass or volume unit [21].

### Statistical analysis

The data are expressed as the mean±SE (standard error). Before testing, all data were checked for normality and homogeneity using Shapiro-Wilk's statistic test to meet statistical demands. One-way analysis of variance (ANOVA) was performed to determine all interactive effects between the species and localities. When an interactive effect was observed, Tukey's honest significant difference (HSD) post-hoc test was used to obtain significant differences among the means. A

minimum significance level of P<0.05 was accepted for all cases. Additionally, a discriminant function analysis was run to detect the variables that significantly contributed to differences in element concentrations in the examined species at the two sites. Statistical analysis was performed using STATISTICA 10.0 software.

## RESULTS

Table 1 presents the concentrations of selected elements in the whole bodies of three centipede species *C. flavidus*, *C. anomalans* and *E. transsylvanicus* from MA and OIA (Table 1A), and in the soil at the same sites (Table 1B). Statistically significant differences in trace element concentrations in *C. flavidus* between MA and OIA were obtained for the following elements: Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Tl, Pb and U. The results show lower concentrations of all elements in *C. flavidus* from MA than from OIA (P<0.05). In *C. anomalans*, significantly higher concentrations of Ni, As, Se Rb, Sr, Cd, Pb and U were observed at OIA. In *E. transsylvanicus*, we detected increased concentrations of Co, Ni, Cu, Zn, Se, Sr, Cd, Tl, Pb and U at OIA. If the concentration of a given element in the body of an organism was higher than in the soil (BAF>1), then this signifies bioaccumulation. We observed that the concentrations of trace elements in centipedes were higher at OIA, the locality that is under great anthropogenic pressure because of heavy traffic and high air pollution. Soil from OIA contained significantly higher Mn, Ni, Cu, Zn, Sr, Cd and Pb concentrations than soil from MA, while the concentrations of other elements did not differ significantly (Table 1B).

Differences in element concentrations between these three species from the same site (Table 1A) were detected. The differences are represented as follows by letters: a – *C. flavidus* vs. *C. anomalans*; b – *C. flavidus* vs. *E. transsylvanicus*; c – *C. anomalans* vs. *E. transsylvanicus*. Thus, there were significant differences in the concentrations of Mn, Cu, Zn, As, Sr, Cd and Tl in *C. flavidus* compared to *C. anomalans* at MA. At the same site, the trace element concentrations of *E. transsylvanicus* show significant differences compared to the other two species for Co, Zn, Rb, Sr, Cd and U.

**Table 1.** Concentrations ( $\mu\text{g/g}$ ) of selected elements (A) in three centipede species (*C. flavidus*, *C. anomalans*, and *E. transsylvanicus*) and soil (B) at sites MA and OIA.

**A**

Element concentration (mean $\pm$ SE) ( $\mu\text{g/g}$ )	<i>C. flavidus</i>		<i>C. anomalans</i>		<i>E. transsylvanicus</i>	
	MA	OIA	MA	OIA	MA	OIA
Mn	7.33 $\pm$ 0.54	9.11 $\pm$ 1.66	6.98 $\pm$ 1.84 <sup>a</sup>	6.49 $\pm$ 0.96	8.80 $\pm$ 0.86 <sup>c</sup>	8.77 $\pm$ 1.51
Co	0.71 $\pm$ 0.31	0.85 $\pm$ 0.12 <sup>*</sup>	0.28 $\pm$ 0.06	0.45 $\pm$ 0.07 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>bc</sup>	0.28 $\pm$ 0.04 <sup>bc</sup>
Ni	0.31 $\pm$ 0.05	1.19 $\pm$ 0.19 <sup>*</sup>	0.63 $\pm$ 0.18	0.82 $\pm$ 0.18 <sup>*</sup>	0.50 $\pm$ 0.07 <sup>b</sup>	0.81 $\pm$ 0.25 <sup>*</sup>
Cu	12.01 $\pm$ 2.83	28.01 $\pm$ 5.40 <sup>*</sup>	38.56 $\pm$ 6.85 <sup>a</sup>	42.81 $\pm$ 8.69	19.95 $\pm$ 3.62 <sup>c</sup>	35.23 $\pm$ 8.63 <sup>*</sup>
Zn	140.29 $\pm$ 7.69	223.42 $\pm$ 22.02 <sup>*</sup>	262.35 $\pm$ 55.86 <sup>a</sup>	375.25 $\pm$ 61.63 <sup>a</sup>	63.54 $\pm$ 2.41 <sup>bc</sup>	169.21 $\pm$ 36.97 <sup>bc</sup>
As	1.21 $\pm$ 0.20	1.93 $\pm$ 0.20 <sup>*</sup>	0.78 $\pm$ 0.06 <sup>a</sup>	1.32 $\pm$ 0.27 <sup>*</sup>	1.00 $\pm$ 0.18 <sup>c</sup>	1.20 $\pm$ 0.43
Se	0.82 $\pm$ 0.24	2.83 $\pm$ 0.96 <sup>*</sup>	0.47 $\pm$ 0.03	1.05 $\pm$ 0.15 <sup>a</sup>	0.40 $\pm$ 0.05	0.79 $\pm$ 0.12 <sup>bc</sup>
Rb	0.74 $\pm$ 0.06	2.44 $\pm$ 0.31 <sup>*</sup>	0.64 $\pm$ 0.03	1.52 $\pm$ 0.15 <sup>a</sup>	1.35 $\pm$ 0.06 <sup>bc</sup>	1.38 $\pm$ 0.15 <sup>b</sup>
Sr	1.09 $\pm$ 0.09	4.57 $\pm$ 0.74 <sup>*</sup>	0.65 $\pm$ 0.04 <sup>a</sup>	1.86 $\pm$ 0.20 <sup>*</sup>	1.29 $\pm$ 0.13 <sup>bc</sup>	2.26 $\pm$ 0.37 <sup>*</sup>
Cd	1.34 $\pm$ 0.16	5.74 $\pm$ 0.80 <sup>*</sup>	0.28 $\pm$ 0.03 <sup>a</sup>	0.34 $\pm$ 0.08 <sup>a</sup>	0.53 $\pm$ 0.20 <sup>bc</sup>	3.31 $\pm$ 1.19 <sup>bc</sup>
Tl	0.02 $\pm$ 0.002	0.04 $\pm$ 0.006 <sup>*</sup>	0.004 $\pm$ 0.0002 <sup>a</sup>	0.004 $\pm$ 0.0005 <sup>a</sup>	0.002 $\pm$ 0.0002 <sup>b</sup>	0.02 $\pm$ 0.006 <sup>bc</sup>
Pb	0.19 $\pm$ 0.03	0.86 $\pm$ 0.17 <sup>*</sup>	0.70 $\pm$ 0.05	0.80 $\pm$ 0.06 <sup>*</sup>	0.16 $\pm$ 0.02	0.48 $\pm$ 0.09 <sup>bc</sup>
U	0.003 $\pm$ 0.0003	0.02 $\pm$ 0.001 <sup>*</sup>	0.002 $\pm$ 0.0006	0.005 $\pm$ 0.0006 <sup>a</sup>	0.0007 $\pm$ 0.0001 <sup>bc</sup>	0.003 $\pm$ 0.0007 <sup>bc</sup>

MA – Mountain Avala; OIA – Old Industrial Area; the values are given as the mean $\pm$ SE, the level of statistical significance was defined as  $P < 0.05$ . A statistically significant difference between the element concentration in one centipede species at two different sites is marked with an asterisk (\*), and significant differences between three centipede species at the same site: a – *C. flavidus* vs. *C. anomalans*; b – *C. flavidus* vs. *E. transsylvanicus*; c – *C. anomalans* vs. *E. transsylvanicus*.

**B**

Element concentration in soil (mean $\pm$ SE) ( $\mu\text{g/g}$ )	MA	OIA
Mn	811.97 $\pm$ 40.22	2730.81 $\pm$ 88.33 <sup>*</sup>
Co	11.32 $\pm$ 0.44	13.49 $\pm$ 0.56
Ni	40.80 $\pm$ 9.32	72.28 $\pm$ 13.70 <sup>*</sup>
Cu	22.10 $\pm$ 5.41	56.58 $\pm$ 10.70 <sup>*</sup>
Zn	85.53 $\pm$ 18.33	372.01 $\pm$ 62.23 <sup>*</sup>
As	23.37 $\pm$ 6.80	23.45 $\pm$ 5.74
Se	0.66 $\pm$ 0.04	0.64 $\pm$ 0.05
Rb	22.69 $\pm$ 4.42	30.88 $\pm$ 7.42
Sr	29.13 $\pm$ 6.72	76.86 $\pm$ 16.34 <sup>*</sup>
Cd	0.47 $\pm$ 0.02	0.66 $\pm$ 0.03 <sup>*</sup>
Tl	0.11 $\pm$ 0.03	0.11 $\pm$ 0.02
Pb	41.55 $\pm$ 7.23	108.66 $\pm$ 8.54 <sup>*</sup>
U	0.51 $\pm$ 0.06	0.57 $\pm$ 0.04

At site OIA, *C. flavidus* differed from *C. anomalans* in the following trace elements: Co, Zn, Se, Rb, Cd, Tl and U, while in relation to *E. transsylvanicus*, significant differences were observed for the concentrations of Co, Tl, Pb and U. In organisms collected at this site, there were also significant differences in the concentrations of Se, Rb and Cd between *C. flavidus* and *E. transsylvanicus*. There were significant intraspecific differences in the two localities, but also interspecific differences within one locality, so these centipedes accumulated different amounts of trace elements from their environment. According to our

results, it seems that *C. flavidus* is suitable for detecting differences in Zn, Se and Cd. *C. flavidus* and *C. anomalans* are more efficient in accumulating Zn than *E. transsylvanicus*. On the other hand, *C. anomalans* seems to be poor in accumulating Cd, unlike *C. flavidus* and *E. transsylvanicus* which accumulate Cd in high concentrations (high BAF values). According to this study, *C. anomalans* seems to be suitable for determining differences in Cu, Zn and Se. This scolopendromorph also accumulated higher concentrations of Zn than other centipede species at the other site. It seems that *C. anomalans* are either inefficient in accumulating Cd or efficient in eliminating it.

In the present study, the BAF values of measured elements were determined and compared in these species (Table 2). Of the 13 elements, only four had BAF values higher than one, as follows:  $\text{Cd} > \text{Se} > \text{Zn} > \text{Cu}$ . The results of the BAF values calculated for Cd in *C. flavidus* and *E. transsylvanicus* were greater than one, which indicates that bioaccumulation occurred. Only in *C. anomalans* was the BAF less than one, indicating that there was no bioaccumulation in this instance. When the BAF was between  $0 < \text{BAF} < 1$  there was no bioaccumulation. In other words, the concentration of a given element in the body of an organism was lower than in the soil. The results of BAF estimation for Cd (Table 2) indicated bioaccumulation at both sites in

*C. flavidus* (the highest BAF value was 8.7 at OIA). In *E. transsylvanicus*, Cd bioaccumulation was recorded at OIA, while bioindication or a BAF of about 1 was present in the tested specimens from MA.

**Table 2.** Bioaccumulation factor (BAF) calculations in three centipede species (*C. flavidus*, *C. anomalans*, and *E. transsylvanicus*) at Mountain Avala (MA) and the Old Industrial Area (OIA).

BAF	<i>C. flavidus</i>		<i>C. anomalans</i>		<i>E. transsylvanicus</i>	
	MA	OIA	MA	OIA	MA	OIA
Mn	0.009	0.003	0.009	0.002	0.01	0.003
Co	0.06	0.06	0.02	0.03	0.01	0.02
Ni	0.007	0.02	0.02	0.01	0.01	0.01
Cu	0.54	0.5	1.74	0.8	0.9	0.62
Zn	<b>1.64</b>	0.6	<b>3.07</b>	<b>1.00</b>	0.7	0.45
As	0.05	0.08	0.03	0.06	0.04	0.05
Se	<b>1.24</b>	<b>4.42</b>	0.7	<b>1.64</b>	0.6	<b>1.23</b>
Rb	0.03	0.08	0.03	0.05	0.06	0.04
Sr	0.04	0.06	0.02	0.02	0.04	0.03
Cd	<b>2.85</b>	<b>8.70</b>	0.6	0.51	<b>1.13</b>	<b>5.02</b>
Tl	0.2	0.4	0.04	0.04	0.02	0.2
Pb	0.005	0.008	0.02	0.007	0.004	0.004
U	0.005	0.04	0.002	0.1	0.001	0.005

BAF values greater than one are presented in bold. BAF values were calculated according to equation (1).

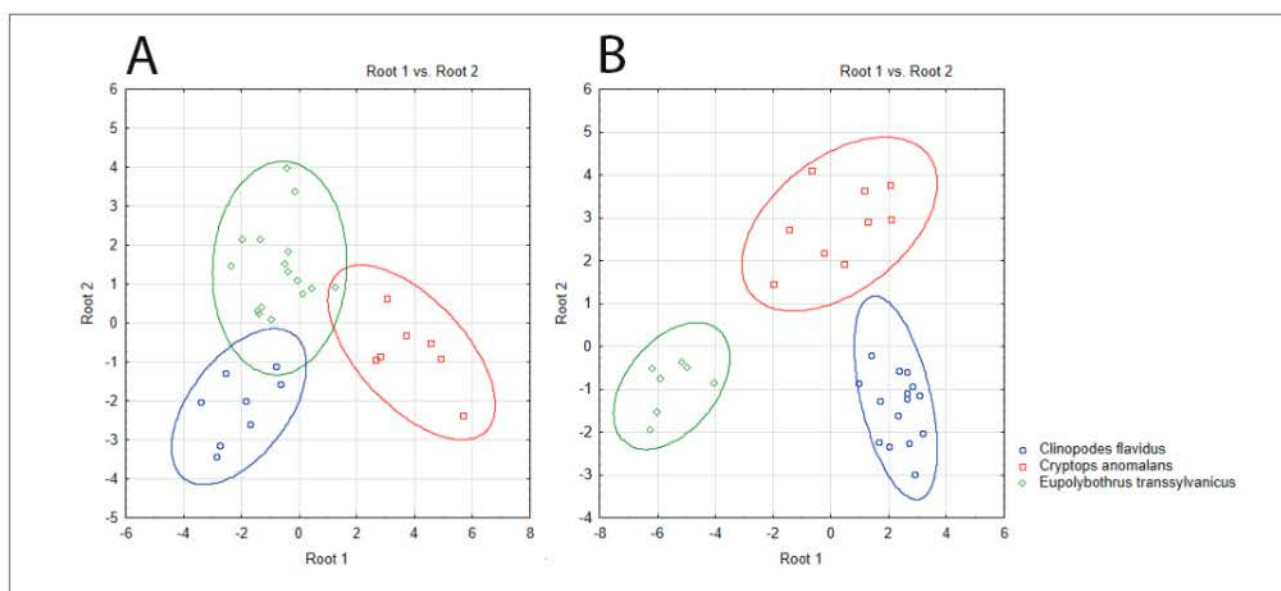
The BAF results for Zn displayed the highest values in *C. anomalans* at MA (BAF=3.07). At the site OIA, BAF=1 indicated that this species is a bioindicator for this element. The concentration of a given element in the body of an organism was equal to the

concentration in the soil, and such organisms can be treated as bioindicators of the element in similar environments. In the case of *C. flavidus*, the BAF was about 1 (BAF=1.24) for Se at MA, and for *E. transsylvanicus* at OIA, BAF=1.23).

Canonical discriminant analysis (Fig. 1) clearly shows that all the studied centipede groups can be used as indicators of trace element pollution. In addition, element concentrations can be used to classify centipedes into groups. Using discriminant analysis, we showed that the centipede groups are well discriminated. We first showed how well the first two canonical discriminant functions separate the centipede groups at MA (Fig. 1A) and OIA (Fig. 1B). The variables that separated the examined centipedes were Se, Rb, Sr, Tl and Pb at MA (Fig. 1A), and Cu, Zn, Pb and U at OIA (Fig. 1B). It can be seen that discriminant functions separate the centipedes into groups almost perfectly in a more polluted locality such as OIA, in contrast to MA, where we found greater similarities between the tested element concentrations, with overlaps between the three centipede species.

## DISCUSSION

During movement through the soil, centipedes and other soil-dwelling invertebrates encounter and interact with only a specific portion of an environmentally



**Fig. 1.** Canonical discriminant analysis of trace element concentrations in three centipede species (*C. flavidus*, *C. anomalans* and *E. transsylvanicus*) at Mountain Avala (A) and the Old Industrial Area (B).

available chemical. Interaction could be through either direct dermal contact with chemicals in the soil solution or ingestion of food (living animals and sometimes vegetable food), and it is dependent on the physiology and behavior of these species as well as the route of exposure [28]. The bioavailable fraction of the chemical may be absorbed across the external membranes of the centipedes (e.g., the gastrointestinal tract). After uptake, a portion of elements can be excreted, while other fractions can be distributed and accumulated in different subcellular compartments within the body of animals [29]. Detoxification systems such as metallothioneins and antioxidant enzymes can detoxify a certain fraction of trace elements and thus reduce their toxic effects. There are several methods for measuring element exposure. Bioaccumulation is a direct biological measure of the bioavailability of elements because it measures the actual amount of element taken in by centipedes.

Element bioaccumulation can be defined as the net accumulation of an element in a whole organism or a tissue of interest that results from exposure to all relevant element sources (e.g., water, food and sediment). A wider definition of bioaccumulation was also proposed; namely, that bioaccumulation is any case where the concentration of an element/substance increases in an organism during its growth [30-31]. When the concentration of an element in an organism is higher than in the natural matrix, the BAF assumes values greater than 1, which means that bioaccumulation has occurred. The concentrations of Cd in the bodies of *C. flavidus* and *E. transsylvanicus* (high BAF values) show that this element has accumulated from the soil, especially at the more polluted site (Table 2).

It was shown that the centipede *Lithobius variegatus* accumulates heavy elements such as Zn, Cd, Pb and Cu [5-7]. Specimens from both the uncontaminated deciduous woodlands and the two sites close to smelting works contained large amounts of Zn, which were stored primarily in the fat body and tissues associated with the exoskeleton. Cd, Pb and Cu were present in much smaller amounts than Zn and were found mostly in the midgut. Concentrations of Cu were much higher in the midguts of centipedes near the smelting works than in animals from the isolated rural woodlands. In contrast, the levels of Zn, Cd and Pb in *L. variegatus* showed little correlation with the

degree of contamination of the site from which they were collected [5].

If the BAF is less than one, it indicates that accumulation in the organism is lower than that of the medium from which the xenobiotic was taken, and the organism can be considered an excluder. A special case is where BAF=1. In this case, the concentration of an element in the organism corresponds to the concentration in its environment. This situation can be referred to as bioindication, and organisms that show this tendency can be called bioindicators [32-33]. For the species *C. anomalans* at the OIA site, BAF=1 for Zn, which revealed that this species is a bioindicator for the mentioned element. In the case of *C. flavidus*, the BAF is about 1 (BAF=1.24) for Se at MA and for *E. transsylvanicus* at OIA (BAF=1.23), and for Cd (BAF=1.13) at MA. All analyzed centipedes showed the ability to bioaccumulate Se (especially *C. flavidus* at OIA; BAF=4.42) and Zn (*C. flavidus* and *C. anomalans* at MA). This phenomenon is unusual and significant because it is known that in Serbia, the soil and food are poor in these two elements [34-35].

In some organisms, this dependence can exist in a wide range of element concentrations and can be used for environmental monitoring. Examples of such organisms used for the biomonitoring of radionuclides, toxic heavy elements and pesticides are freshwater mussels and phytoplankton, bees, earthworms and lichens [33], fungi [36] and small soil invertebrates [37]. Isopods [38], nematodes [39] and collembolans [40] are established models of soil ecotoxicology. Element bioaccumulation in different arthropod groups was also investigated [13]. Diplopoda and Isopoda bioaccumulated Cu and Zn significantly; Pb concentrations were extremely high in Isopoda and Collembola, intermediate in Diplopoda and low in Chilopoda and Coleoptera.

The results of the BAF for Cd pointed to bioaccumulation at both sites in *C. flavidus* (with the highest BAF value of 8.7 at OIA). In *E. transsylvanicus*, the bioaccumulation of Cd was present at OIA. The choice between burrowing- (*C. flavidus*), intermediate- (*C. anomalans*) and running life-forms (*E. transsylvanicus*) could have produced significant differences, especially for Cd bioaccumulation, which is not related to the Cd contamination in the soil. Acclimation of

soil organisms to Cd levels that exceed background concentrations may result in the development of Cd-tolerance mechanisms and allow for the accumulation of Cd with minimum adverse effects. Millipedes have been shown to accumulate Cd [7, 41], which is comparable to the Cd content in *Glomeris hexasticha* and in *Ommatoiulus sabulosus* [42].

The different element accumulation strategies of soil invertebrates are a consequence of diverse detoxification mechanisms [43]. Diplopods possess effective mechanisms to bind and detoxify potentially toxic elements in tissues [44]. It was shown [45] that element pollution is not a dominating factor determining the species richness and densities of the selected detritivore group that consisted of isopods, millipedes and earthworms. According to the authors, an explanation for the absence of negative effects of heavy elements is the combination of low element bioavailability in the soil, development of resistance to element pollution and the presence of confounding factors, such as variation in organic matter content and flooding. Even though the number of diplopod species may be affected by high amounts of element contamination, some species will develop strategies that minimize the effects of these substances on their survival. These strategies may include a decrease in food intake, a decrease in nutrient assimilation, or both [16].

## CONCLUSION

The results of this study showed that differences in the distribution of some trace elements between two sites due to differences in the degree of environmental pollution were clearly reflected by the measured values in *C. flavidus*, *C. anomalans* and *E. transsylvanicus*. The present study is the first report where elements were measured in these three centipede species, as well as their ability to bioaccumulate or bioindicate. The trace element concentrations were higher in centipedes from the polluted site but *C. anomalans* and *E. transsylvanicus* had higher Mn concentrations at the unpolluted site. *C. flavidus* was a good bioindicator for distinguishing between differences in Zn, Se and Cd. *C. flavidus* and *C. anomalans* were more efficient in accumulating Zn than *E. transsylvanicus*. *Cryptops anomalans* poorly accumulated Cd, unlike *C. flavidus* and *E. transsylvanicus*, which exhibited a

propensity for high accumulation of Cd. Their ability to accumulate elements is different, and it depends on their physiology and lifestyle as well as the route of exposure.

**Funding:** This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract Nos. 451-03-68/2022-14/200007, 451-03-68/2022-14/200178, and 451-03-68/2022-14/200288.

**Acknowledgments:** We thank Dr. Dragan Antić for providing photographs of *C. flavidus*, *C. anomalans*, and *E. transsylvanicus*.

**Author contributions:** All listed authors have contributed sufficiently to the work to be included as coauthors. Conceptualization: B.M., S.B.M. and N.R.; field studies: B.M. and D.S.; investigation: A.S. and S.B.M.; data processing: S.B.M. and N.R.; visualization and preparation: B.M. and N.R.; writing of the original draft: B.M., S.B.M., and N.R.; revision and editing: B.M., S.P., S.B.M., L.V., and A.S.

**Conflict of interest disclosure:** The authors declare no conflict of interest.

**Data availability:** All data underlying the reported findings have been provided as part of the submitted article and are available at: [https://www.serbiosoc.org.rs/NewUploads/Uploads/Mitic%20et%20al\\_7783\\_Data%20Report.pdf](https://www.serbiosoc.org.rs/NewUploads/Uploads/Mitic%20et%20al_7783_Data%20Report.pdf)

## REFERENCES

1. Wilcke W, Müller S, Kanchanakool N, Zech W. Urban soil contamination in Bangkok: heavy metal and aluminium partitioning in topsoils. *Geoderma*. 1998;86(3-4):211-28. [https://doi.org/10.1016/S0016-7061\(98\)00045-7](https://doi.org/10.1016/S0016-7061(98)00045-7)
2. Li X, Poon CS, Liu PS. Heavy metal contamination of urban soils and street dusts in Hong Kong. *Appl Geochem*. 2001;16(11-12):1361-8. [https://doi.org/10.1016/S0883-2927\(01\)00045-2](https://doi.org/10.1016/S0883-2927(01)00045-2)
3. Wei B, Yang L. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchem J*. 2010;94(2):99-107. <https://doi.org/10.1016/j.microc.2009.09.014>
4. Pouyat RV, Szlavecz K, Yesilonis ID, Wong CP, Murawski L, Marra P, Casey RE, Lev S. Multi-scale assessment of metal contamination in residential soil and soil fauna: a case study in the Baltimore-Washington metropolitan region, USA. *Landsc Urban Plan*. 2015;142:7-17. <https://doi.org/10.1016/j.landurbplan.2015.05.001>
5. Hopkin SP, Martin MH. Heavy metals in the centipede *Lithobius variegatus* (Chilopoda). *Environ Pollut B*. 1983;6:309-18. [https://doi.org/10.1016/0143-148X\(83\)90016-2](https://doi.org/10.1016/0143-148X(83)90016-2)
6. Hopkin SP, Martin MH. Assimilation of zinc, cadmium, lead and copper by the centipede *Lithobius variegatus* (Chilopoda). *J Appl Ecol*. 1984;21:535-46. <https://doi.org/10.2307/2403427>

7. Hopkin SP, Watson K, Martin MH, Mould ML. The assimilation of heavy metals by *Lithobius variegatus* and *Glomeris marginata* (Chilopoda; Diplopoda). *Bijdr Dierkd.* 1985;55(1):88-94.
8. van Straalen NM, van Wensem J. Heavy metal content of forest litter arthropods as related to body-size and trophic level. *Environ Pollut.* 1986;42(3):209-21. [https://doi.org/10.1016/0143-1471\(86\)90032-2](https://doi.org/10.1016/0143-1471(86)90032-2)
9. Read HJ, Hopkin SP. A study of myriapod communities in woodlands contaminated with heavy metals. In: Minelli A, editor. *Proceedings of the 7th International Congress of Myriapodology.* Leiden: Brill; 1990. p. 289-98.
10. Dallinger R, Berger A, Birkel S. Terrestrial isopods: useful biological indicators of urban metal pollution. *Oecologia.* 1992;89:32-41. <https://doi.org/10.1007/BF00319012>
11. Dallinger R. Invertebrate organisms as biological indicators of trace metal pollution. *Appl Biochem Biotechnol.* 1994;48(1):27-31. <https://doi.org/10.1007/BF02825356>
12. Grelle C, Fabre M-C, Leprêtre A, Descamps M. Myriapod and isopod communities in soils contaminated by heavy metals in northern France. *Eur J Soil Sci.* 2000;51(3):425-33. <https://doi.org/10.1046/j.1365-2389.2000.00317.x>
13. Heikens A, Peijnenburg WJGM, Hendriks AJ. Bioaccumulation of heavy metals in terrestrial invertebrates. *Environ Pollut.* 2001;113(3):385-93. [https://doi.org/10.1016/S0269-7491\(00\)00179-2](https://doi.org/10.1016/S0269-7491(00)00179-2)
14. Nahmani J, Lavelle P. Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. *Eur J Soil Biol.* 2002;38(3-4):297-300. [https://doi.org/10.1016/S1164-5563\(02\)01169-X](https://doi.org/10.1016/S1164-5563(02)01169-X)
15. Dai J, Becquer T, Rouiller JH, Reversat G, Bernhard-Reversat F, Nahmani J, Lavelle P. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. *Soil Biol Biochem.* 2004;36:91-8. <https://doi.org/10.1016/j.soilbio.2003.09.001>
16. Souza TS, Christofoletti CA, Bozzatto V, Fontanetti CS. The use of diplopods in soil ecotoxicology - a review. *Ecotoxicol Environ Saf.* 2014;103:68-73. <https://doi.org/10.1016/j.ecoenv.2013.10.025>
17. Chrzan A. The impact of heavy metals on the soil fauna of selected habitats in Niepołomice. *Forest. Polish J Soil Sci.* 2017;50(2):291-300. <https://doi.org/10.17951/pjss.2017.50.2.291>
18. Rajoo KS, Ismail A, Karam DS, Zulkifli SZ, Omar H, Lim A. Heavy metal bioaccumulation in soil arthropods at Malaysian sanitary landfill. *J Environ Sci Pollut Res.* 2017;3(1):160-3.
19. Vranković J, Borković-Mitić S, Ilić B, Radulović M, Milošević S, Makarov S, Mitić B. Bioaccumulation of metallic trace metals and antioxidant enzyme activities in *Apfelbeckia insculpta* (L. Koch, 1867) (Diplopoda: Callipodida) from the cave Hadži-Prodanova Pećina (Serbia). *Int J Speleol.* 2017;46(1):99-108. <https://doi.org/10.5038/1827-806X.46.1.1981>
20. Coelho C, Foret C, Bazin C, Leduc L, Hammada M, Inácio M, Bedell JP. Bioavailability and bioaccumulation of heavy metals of several soils and sediments (from industrialized urban areas) for *Eisenia fetida*. *Sci Total Environ.* 2018;635:1317-30. <https://doi.org/10.1016/j.scitotenv.2018.04.213>
21. Proc K, Bulak P, Kaczor M, Bieganski A. A new approach to quantifying bioaccumulation of metals in biological processes. *Biology.* 2021;10(4):345. <https://doi.org/10.3390/biology10040345>
22. Zapparoli M. Centipedes in urban environments: Records from the City of Rome (Italy). *Ber Nat-Med Verein Innsbruck.* 1992;Suppl.10:231-36.
23. Stoev P. Myriapoda (Chilopoda, Diplopoda) in urban environments in the City of Sofia. In: Penev L, Niemelä J, Kotze DJ, Chipev N, editors. *Ecology of the City of Sofia. Species and communities in an urban environment.* Sofia-Moscow: Pensoft; 2004; p. 299-306.
24. Ion M. Centipedes from urban environments. In: Onete M, editor. *Species monitoring in the central parks of Bucharest.* Bucharest: Ars Docendi, Universitatea din București; 2008; p. 79-83.
25. Nefediev PS, Tuf IH, Farzalieva GSh. Centipedes from urban areas in southwestern Siberia, Russia (Chilopoda). Part 1. *Lithobiomorpha. Arthropoda Sel.* 2016;25(3):257-66. <https://doi.org/10.15298/arthsel.25.3.04>
26. Nefediev PS, Tuf IH, Farzalieva GSh. Centipedes from urban areas in southwestern Siberia, Russia (Chilopoda). Part 2. *Geophilomorpha. Arthropoda Sel.* 2017;26(1):8-14. <https://doi.org/10.15298/arthsel.26.1.02>
27. Voigtländer K. Chilopoda - Ecology. In: Minelli A, editor. *Treatise on zoology - anatomy, taxonomy, biology. The Myriapoda Vol 1.* Leiden: Brill; 2011. p. 309-25. [https://doi.org/10.1163/9789004188266\\_016](https://doi.org/10.1163/9789004188266_016)
28. Lanno R, Wells J, Conder J, Bradham K, Basta N. The bioavailability of chemicals in soil for earthworms. *Ecotoxicol Environ Saf.* 2004;57(1):39-47. <https://doi.org/10.1016/j.ecoenv.2003.08.014>
29. Shuo Yu. Bioaccumulation of metals in earthworms. [dissertation]. [Columbus]: Ohio State University. 2009. 173p.
30. Blasco J, Chapman P, Campana O, Hampel M, editors. *Marine ecotoxicology: current knowledge and future issues.* 1st ed. Amsterdam: Elsevier; 2016. 334 p.
31. Yang S, Zhai SW, Shepherd BS, Binkowski FP, Hung SSO, Sealey WM, Deng DF. Determination of optimal feeding rates for juvenile lake sturgeon (*Acipenser fulvescens*) fed a formulated dry diet. *Aquac Nutr.* 2019;25(6):1171-82. <https://doi.org/10.1111/anu.12932>
32. Bulak P, Walkiewicz A, Brzezińska M. Plant growth regulators-assisted phytoextraction. *Biol Plant.* 2014;58:1-8. <https://doi.org/10.1007/s10535-013-0382-5>
33. Asif N, Malik MF, Chaudhry FN. A review of on environmental pollution bioindicators. *Pollution.* 2018;4(1):111-8.
34. Maksimović ZJ, Đujić I, Jović V, Ršumović M. Selenium deficiency in Yugoslavia. *Biol Trace Elem Res.* 1992;33:187-96. <https://doi.org/10.1007/BF02784022>
35. Jagodić J, Rovčanin B, Borković-Mitić S, Vujotić L, Avdin V, Manojlović D, Stojsavljević A. Possible zinc deficiency in the Serbian population: examination of body fluids, whole blood, and solid tissues. *Environ Sci Pollut Res Int.* 2021;28:47439-46. <https://doi.org/10.1007/s11356-021-14013-2>
36. Bai Y, Wang Q, Liao K, Jian Z, Zhao C, Qu J. Fungal community as a bioindicator to reflect anthropogenic activities in a river ecosystem. *Front Microbiol.* 2018;9:3159. <https://doi.org/10.3389/fmicb.2018.03152>



37. Arnot JA, Arnot MI, Mackay D, Couillard Y, MacDonald D, Bonnell M, Doyle P. Molecular size cutoff criteria for screening bioaccumulation potential: fact or fiction? *Integr Environ Assess Manag.* 2009;6(2):210-24.
38. Lemos MFL, van Gestel CAM, Soares AMVM. Reproductive toxicity of the endocrine disruptors vinclozolin and bisphenol A in the terrestrial isopod *Porcellio scaber* (Latreille, 1804). *Chemosphere.* 2010;78(7):907-13. <https://doi.org/10.1016/j.chemosphere.2009.10.063>
39. Sochová I, Hofman J, Holoubek I. Using nematodes in soil ecotoxicology. *Environ Int.* 2006;32(3):374-83. <https://doi.org/10.1016/j.envint.2005.08.031>
40. Eom IC, Rast C, Veber AM, Vasseur P. Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. *Ecotoxicol Environ Saf.* 2007;67(2):190-205. <https://doi.org/10.1016/j.ecoenv.2006.12.020>
41. Hunter BA, Johnson MS, Thompson DJ. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. II. Invertebrates. *J Appl Ecol.* 1987;24(2):587-99. <https://doi.org/10.2307/2403895>
42. Kania G. Metals content in the millipede *Ommatoiulus sabulosus* (Linnaeus 1758); Arthropoda: Diplopoda. *Och Środ Zas Nat.* 2010;43:17-25.
43. Gräff S, Berkus M, Alberti G, Köhler HR. Metal accumulation strategies in saprophagous and phytophagous soil invertebrates: a quantitative comparison. *Biometals.* 1997;10:45-53. <https://doi.org/10.1023/A:1018366703974>
44. Köhler HR, Körtje KH, Alberti G. Content, absorption quantities and intracellular storage sites of heavy metals in Diplopoda (Arthropoda). *Biometals.* 1995;8:37-46. <https://doi.org/10.1007/BF00156156>
45. Hobbelen PHF, van den Brink PJ, Hobbelen JF, van Gestel CAM. Effects of heavy metals on the structure and functioning of detritivore communities in a contaminated floodplain area. *Soil Biol Biochem.* 2006;38(7):1596-1607. <https://doi.org/10.1016/j.soilbio.2005.11.010>

### Supplementary Data

The Supplementary Material is available at: [https://www.serbiosoc.org.rs/NewUploads/Uploads/Mitic%20et%20al\\_7783\\_Supplementary%20Material.pdf](https://www.serbiosoc.org.rs/NewUploads/Uploads/Mitic%20et%20al_7783_Supplementary%20Material.pdf)