

# First Insights Into Trace Element Accumulation by *Philoscia affinis* (Crustacea, Isopoda): a Novel Tracer to Assess Soil Contamination in Lowland Plains?

Paolo Pastorino<sup>1</sup>  · Marco Bertoli<sup>2</sup> · Paola Brizio<sup>1</sup> · Maria Cesarina Abete<sup>1</sup> · Vittoria Dalla Nora<sup>2</sup> · Marino Prearo<sup>1</sup> · Elisabetta Pizzul<sup>2</sup>

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## Abstract

Isopods are terrestrial invertebrates that accumulate trace elements in large quantities, thus providing information on levels of soil contamination. However, the accumulation pattern seems to be species dependent. For this study, specimens of *Philoscia affinis* (Isopoda, Oniscidea) and soil samples were collected from both a protected area (site 1) and urban roadside (site 2) in the low plain of Friuli-Venezia Giulia (northeast Italy) to determine whether *P. affinis* could serve as a potential candidate for monitoring soil contamination. To do this, the following objectives were achieved: a) the level of trace elements (Al, Cd, Cu, Fe, Hg, Mn, Pb, Zn) were detected in soils and isopods; 2) the difference in trace elements accumulation was compared in the two sampling sites; 3) the bioaccumulation factor (BAF) was calculated for each element. With some exceptions, trace element concentrations were higher in both isopods and soil samples from the urban roadside compared to the protected area. Furthermore, except for Cd, Cu, and Zn, trace element levels were higher in the soil than in the isopod samples. The higher mean BAF values were recorded for Cd (6.169 and 6.974 for site 1 and 2, respectively), Cu (10.324 and 11.452 for site 1 and 2, respectively), and Zn (1.836 and 2: 1.943 for site 1 and 2, respectively), whereas BAF values <1 were recorded for the other elements. *Philoscia affinis* was found to be a potential candidate to monitor soil contamination as a macro-concentrator of Cu and Cd and a micro-concentrator of Zn.

**Keywords** Bioaccumulation factor · Cadmium · Copper · Environmental monitoring · Isopoda · Zinc

## Introduction

Although most trace elements occur naturally in the Earth's crust, rapid industrialization has accelerated their flux into the environment through a variety of human activities [1]. The main sources of trace element contamination are industrial (e.g., mining, tanning, combustion, and smelting), civil (e.g., vehicular traffic), agricultural (e.g., fertilizers, fungicides, insecticides), and wastewater activities [2]. Trace elements can occur in municipal waste landfill sites in considerable concentrations, causing soil contamination via the dispersion of particulate matter by wind and leachate release [3]. Also, waste

incineration processes are often the causes of concern due to trace element release from combustion ash [3]. Thus, trace element contamination in soil and the potential risk of contamination in the food chain are attracting interest [1].

Macroinvertebrates are often used as bioindicators of contaminants and to assess the effects of trace elements in terrestrial [4–6] and aquatic ecosystems [7, 8]. Diverse soil invertebrate taxa can serve as bioindicators: oligochaetes [9], nematodes [10, 11], insects [12], and terrestrial isopods [13, 14].

Arthropods make up the majority of soil fauna. They occupy a wide variety of microhabitats and niches where they have a central ecological role [15]. For instance, oniscidean isopods play a key part in soil ecology by helping decompose organic matter and recycle nutrients [16]. An estimated 10% of annual litter undergoes fragmentation by isopods [17], which are highly sensitive to changes in their habitats [18]. Furthermore, they are known to accumulate significant quantities of heavy metals and to survive even in areas of high concentrations of trace elements [19].

In oniscidean isopods, the main organ where heavy metals accumulate is the hepatopancreas; although it accounts for

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✉ Paolo Pastorino  
paolo.pastorino@izsto.it

<sup>1</sup> The Veterinary Medical Research Institute for Piemonte, and Valle d'Aosta, via Bologna 148, 10154 Torino, Liguria, Italy

<sup>2</sup> Department of Life Sciences, University of Trieste, via L. Giorgieri 10, 34127 Trieste, Italy

only 5% of body weight, it can contain 75–95% of absorbed heavy metals [20]. Studies on *Porcellio scaber* (Isopoda, Oniscidea) showed that ultrastructural modifications of the hepatopancreatic epithelium induced by heavy metals accumulation can be used as biomarkers to evaluate soil concentration and biotoxicity [5, 21]. In their study, van Straalen et al. [22] found that terrestrial isopods are among the macroinvertebrates most efficient at bioaccumulating heavy metals owing to physiological mechanisms that regulate heavy metal content that allow the isopods to survive despite high concentrations in both the litter and the soil. The ability of oniscidean isopods to accumulate metals is species dependent [23]; the selection of species for use in monitoring programs relies on their ability to accumulate a given element and their biological responses to it. Furthermore, some isopod species can discriminate food containing certain concentrations of heavy metals, while avoiding the most contaminated materials and not accumulate harmful elements [24, 25]. By virtue of these characteristics, the body burden of metals in isopods can yield information about the degree of soil contamination [19].

Among the oniscidean isopods, *Philoscia affinis*, a member of the Philosciidae family, is widely distributed in Europe (Spain, France, Italy, Austria, Germany, Croatia) [26], Northern Africa (northern Algeria) [27], Slovenia [28], and Hungary [29], where it can be found in forests, forested river banks, and wooded terrains [30]. Its body size makes it easy to collect and use for detecting trace elements.

With this study, we detected for the first time eight trace elements (Al, Cd, Cu, Fe, Hg, Mn, Pb, Zn) in the whole body of *P. affinis* and in soil samples from a protected area and an urbanized roadside located in the low plain of Friuli-Venezia Giulia (northeast Italy) to determine whether it could be a potential candidate for monitoring soil contamination. The objectives were to (1) measure the trace element level in soils and isopods; (2) compare the difference in trace element levels at the two sampling sites; (3) define the ratio of trace element concentration in the isopods and in the soil (bioaccumulation factor, BAF) and categorize the species as macro-concentrator, micro-concentrator, or deconcentrator for each trace element [19].

## Material and Methods

### Study Area

The sampling sites (Fig. 1) were a protected area and an urbanized area in the lowland plain of Friuli-Venezia Giulia (northeast Italy). The plain has a complex water network (main rivers: Livenza, Noncello, Meduna, Sile, Fiume, Lemene, Tagliamento, Stella, Torsa, Turgnano, Corno, Ausa, Natissa, Torre, Isonzo, Timavo). The entire area is important for the fertility of the soils with the presence of several

farmlands. Also, it hosts several protected areas including the “Riserva naturale della Foce dell’Isonzo,” “Riserva naturale Foci dello Stella,” and “Boschi di Muzzana.”

Site 1 (45° 47' 20.03" N; 13° 07' 01.42" E) is located inside the Special Area of Conservation “Boschi di Muzzana” (SAC IT3320034), in the municipality of Muzzana del Turgnano (Udine Province). The site is not affected by direct human pressure, being a lowland residual forest composed mainly of white hornbeam (*Carpinus betulus*), ash (*Fraxinus* spp.), hazel (*Corylus avellana*), and lesser quantities of English oak (*Quercus pedunculata*). The herbaceous component includes wild garlic (*Allium ursinum*). The soil texture is mainly composed of sand and silt and in smaller amounts by clay and fine gravel (soil skeleton: 12%) [31]. Site 2 (45° 50' 01.557" N; 13° 12' 50.277" E) is inside the inter-municipal park of the Corno River, just outside the town of San Giorgio di Nogaro (Udine Province), where there are also residues of plain woodlands. The sampling site is located alongside the main route with heavy traffic connecting the towns in the plain. The soil texture is mainly composed of sand and silt and in smaller amounts by clay and fine gravel (soil skeleton: 7%) [31].

### Soil Sampling and Processing

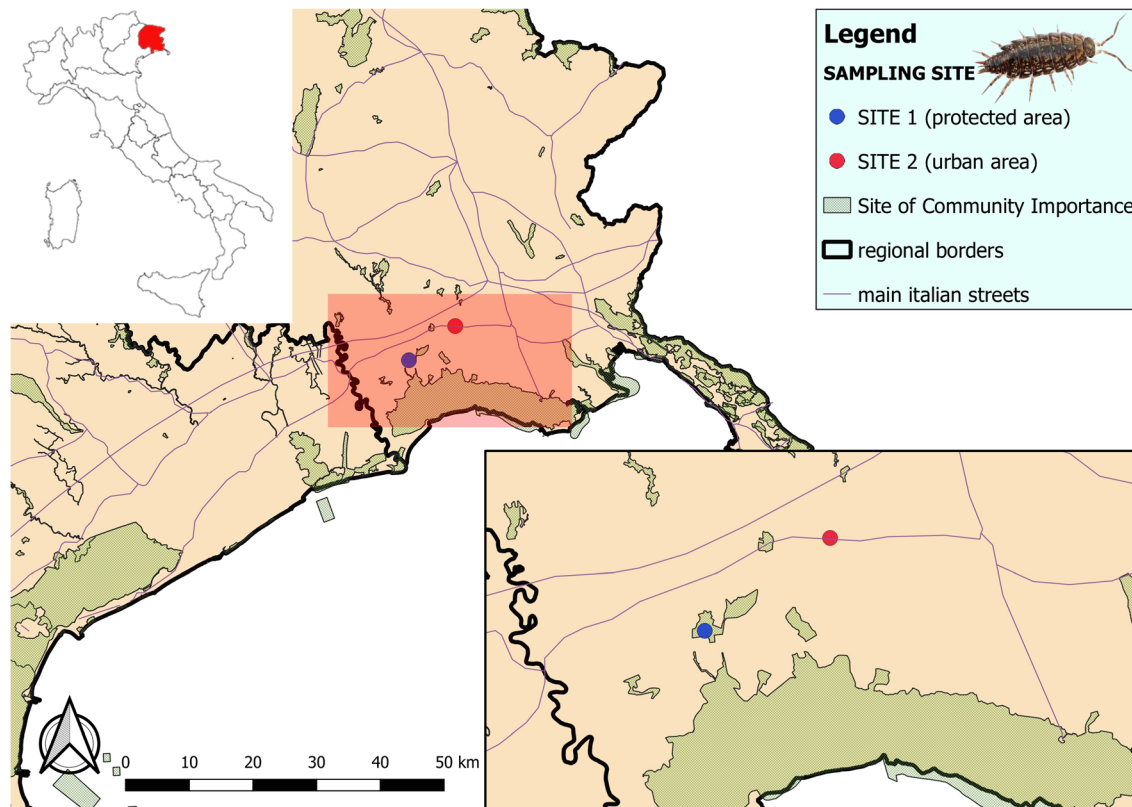
Sampling was performed in April 2018. Soil samples were collected according to the protocol proposed by Nannoni et al. [32]. Briefly, the first 10 cm of the soil profile was collected. At each site, four subsamples were collected a few meters apart and mixed to create a single representative sample. The samples were then transported to the laboratory in glass containers, dried in a ventilated oven at 30 °C, and sieved (2 mm mesh). Only the fraction < 2 mm was used for trace element analysis [33]. Samples from both sites were subdivided into four subsamples for trace element detection.

### Isopod Sampling

Mature individuals of *Philoscia affinis* ( $n = 80$  from each site) around the soil sampling sites were captured by hand either from under stones or by digging with plastic forceps. The specimens were then put in plastic bags and transported to the laboratory where they were sexed and divided into four subsamples ( $n = 20$  individuals each; 50:50 sex ratio) per sampling site and euthanized at  $- 80$  °C for 24 h. Each subsample was then oven-dried at 105 °C overnight until they reached a constant dry weight (dw) to obtain an aliquot of 1.2–1.5 g and then homogenized for trace element detection [14].

### Trace Element Analysis

For trace element analysis of soil, the samples ( $n = 4$  subsamples per site) were further divided into two subgroups: one for



**Fig. 1** Sampling sites (site 1: 45° 47' 20.03" N, 13° 07' 01.42" E; site 2: 45° 50' 01.557" N, 13° 12' 50.277" E), Friuli-Venezia Giulia, northeast Italy. The red rectangle denotes the inset of the study area

the quantification of mercury and the other for the detection of the other trace elements. To measure the mercury content, quantities of 0.1–0.2 g of sample were processed and analyzed without pre-treatment using a DMA-80 (Direct Mercury Analyzer, Milestone, Shelton, CT, USA) as reported by Maggi et al. [34]. Determination of the other elements (Al, Mn, Fe, Cu, Zn, Cd, Pb) was performed after wet digestion. A mixture of 2 mL of HNO<sub>3</sub>, 1 mL of HF, 2 mL of HCl, and 1 mL of HClO<sub>4</sub> was added to 200 mg of soil. Digestion was performed in a microwave oven (ETHOS 900, Milestone). The solution was filtered, and ultrapure water was added to a final volume of 100 mL [32].

The isopod samples (four subsamples per site; each subsample: 20 specimens) were divided into two subgroups: one for total mercury was directly analyzed by DMA-80 and the other for detection of the other elements. Quantities between 1.0 and 1.5 g of dried isopods underwent digestion in a microwave oven (ETHOS 1, Milestone) with 7 mL of HNO<sub>3</sub> and 1.5 mL of H<sub>2</sub>O<sub>2</sub>. Ultrapure water was added to a final weight of 50 mL.

Trace element detection was performed using inductively coupled plasma-mass spectrometry (ICP-MS Xseries II, Thermo Scientific, Bremen, Germany). Analytical performance was verified by processing certified reference materials-CRM (1566b and NIST 2710 from the National Institute of Standard and Technology) along with blank

reagents in each analytical session. Recovery rates of elements did not differ by more than 10% from the CRM. Limit of detection (LOD), limit of quantification (LOQ), and precision were calculated according to Commission Regulation (EU) No. 2016/582 amending Regulation (EC) No. 333/2007. The LOQ was set at 0.010 mg kg<sup>-1</sup> for all elements to facilitate data interpretation. Concentrations are expressed as milligrams per kilogram dry weight.

### Statistical Analysis

The assumption of normality distribution was verified with the Kolmogorov–Smirnov test. Differences in trace element concentration between the isopods, soil, and BAF from the two sites were checked using the Mann–Whitney *U* test since the null hypothesis for normal distribution could not be rejected. Spearman’s rank correlation coefficient ( $\rho_s$ ) was calculated to determine the relationship between trace element concentration in the isopods and in the soils. Principal component analysis (PCA) was performed to check for trends in trace element concentrations in isopods and soils. To evaluate the patterns of trace element accumulation, the BAF was calculated as follows [32]:

$$\text{BAF} = \frac{C_i}{C_s}$$

where  $C_i$  is the trace element concentration in the tissue of isopods and  $C_s$  is the trace element concentration in the soil. As proposed by Dallinger [19], based on the value obtained, *Philoscia affinis* was divided into macro-concentrator ( $BAF > 2$ ), micro-concentrator ( $1 < BAF \leq 2$ ), and deconcentrator ( $BAF \leq 1$ ) according to the level of each element detected.

Trace elements concentration measured in soil samples were compared with the contamination threshold values defined by the Italian Legislative Decree no. 152/2006 [35].

Results were considered statistically significant at a  $p$  value  $< 0.05$ . Statistical analysis was performed using open-source data analysis software RStudio® version 1.1.463 (RStudio, Inc.).

## Results

### Trace Elements in Soil

A downward trend in the average trace element concentration was noted for both sites:  $Al > Fe > Mn > Zn > Cu > Pb > Hg > Cd$  (Table 1). Generally, the mean concentration was higher at site 2 for Mn, Fe, Cd, Cu, Pb, and Hg, whereas the mean concentration of Al and Zn was slightly higher at site 1. A statistically significant difference in concentration between the soil samples was recorded for all elements (Mann–Whitney  $U$  test;  $p < 0.03$ ), except for Al and Zn (Mann–Whitney  $U$  test;  $p > 0.05$ ) (Fig. 2). Trace elements concentration in all soil samples were always below the respective contamination threshold (Table 1).

### Trace Elements in Isopods

The average trace element concentration showed a downward trend for samples from both sites:  $Al > Fe > Cu > Zn > Mn > Cd > Pb > Hg$  (Table 1). Generally, the mean trace element level was higher in the samples from site 2 (urban area), except for the slightly higher Zn concentration at site 1 (protected area). Statistically significant differences in trace

element levels between isopod samples from the two sites were recorded for Al, Mn, Fe, Cu, Cd, Pb, and Hg (Mann–Whitney  $U$  test;  $p < 0.03$ ), whereas no difference in Zn was found between the isopod samples from the two sites (Mann–Whitney  $U$  test;  $p > 0.05$ ) (Fig. 3).

### Bioaccumulation Factors

Table 2 presents the BAF. A higher mean BAF was recorded for Cd, Cu, and Zn from both sites, whereas a  $BAF < 1$  was recorded for the other elements (Table 2). BAF values at site 2 were significantly higher for all elements (Mann–Whitney  $U$  test;  $p < 0.05$ ), except for Hg, which did not differ significantly between the two sites (Mann–Whitney  $U$  test;  $p > 0.05$ ).

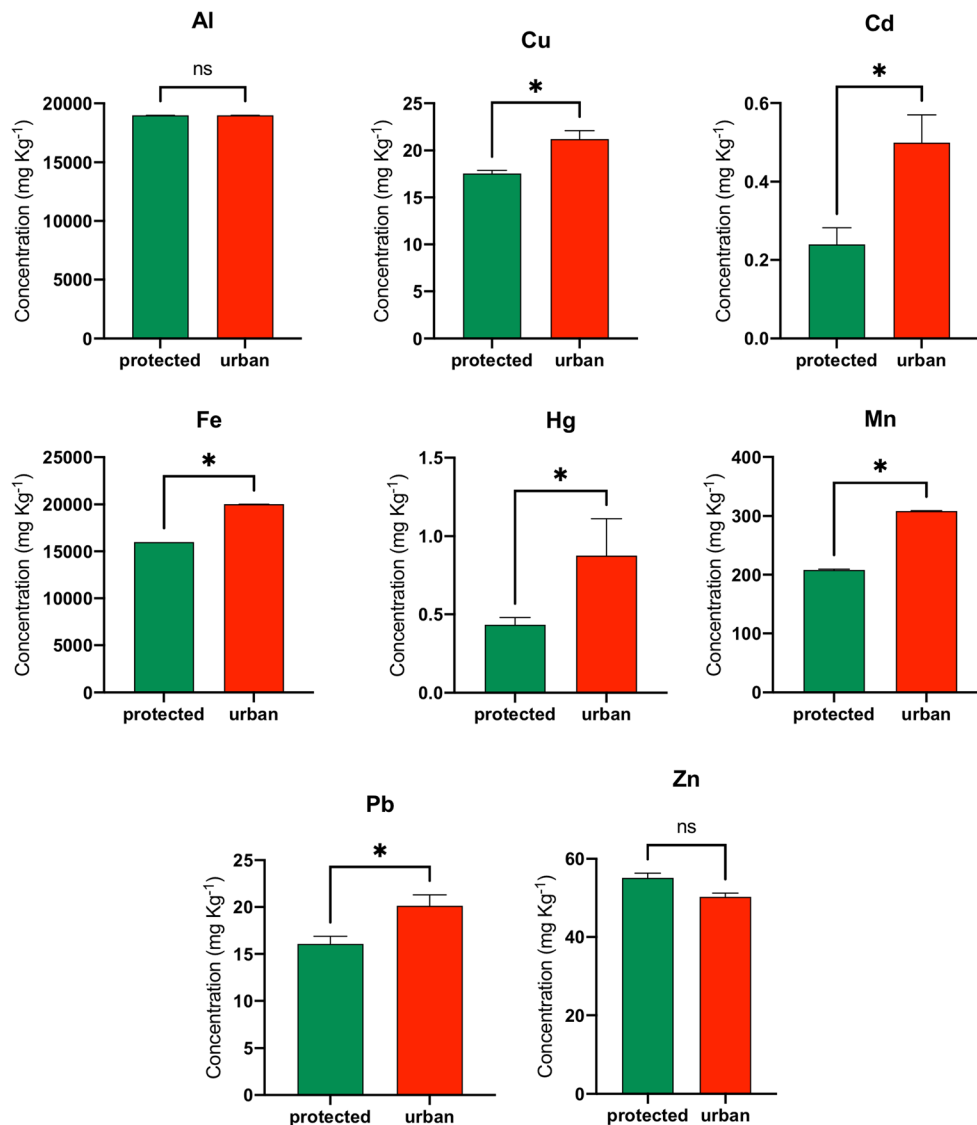
### Correlation Between Trace Element in Soils and Isopods and PCA Analysis

Spearman’s correlation matrix revealed only significant negative correlations ( $p < 0.05$ ) between Pb in soils and Pb in isopods ( $\rho_s = 0.818$ ); Al in soils and Al in isopods ( $\rho_s = 0.745$ ); and Fe in soils and Fe in isopods ( $\rho_s = 0.823$ ). PCA (Fig. 4) showed that the first (PC1) and the second (PC2) components accounted for meaningful amounts of the total variance (97.5%): PC1 explained 92.4% of the total variance and was positively correlated with Al, Mn, Fe, Pb, and Hg and negatively correlated with Cu, Zn, and Cd. PC2 explained 6.6% of the total variance and was positively correlated with Mn, Fe, Cu, Cd, Pb, and Hg and negatively correlated with Al and Zn. When the environmental matrices (soil and isopod) were compared by trace element concentration, a clear separation between soil and isopod samples could be seen: the isopod samples from site 2 (green triangle) are located in the upper left quadrant of the plot in relation to the higher concentration of Cd and Cu concentration; the soil samples from site 2 (purple plus sign) are located in the upper right quadrant in relation to the higher concentration of Fe, Hg, Mn, and Pb, while the soil (light blue square) and the isopod (red circle) samples from site 1 are located in the lower quadrant of the

**Table 1** Trace element concentration (mean  $\pm$  standard deviation;  $mg\ kg^{-1}\ dw$ ) in isopod and soil samples from the protected (site 1) and the urban (site 2) sampling area. The contamination threshold ( $mg\ kg^{-1}\ dw$ ) defined by Italian Legislative Decree no. 152/2006 is also reported

Site	Matrix	Al	Mn	Fe	Cu	Zn	Cd	Pb	Hg
1	Soil	19005 $\pm$ 7.66	208.33 $\pm$ 1.52	15995.67 $\pm$ 5.86	17.52 $\pm$ 0.93	54.83 $\pm$ 1.26	0.24 $\pm$ 0.05	16 $\pm$ 0.98	0.43 $\pm$ 0.15
	Isopod	324.87 $\pm$ 2.30	38.16 $\pm$ 1.66	226.04 $\pm$ 0.81	180.89 $\pm$ 2.19	100.02 $\pm$ 1.64	1.47 $\pm$ 0.08	0.75 $\pm$ 0.02	0.04 $\pm$ 0.008
2	Soil	19000 $\pm$ 2.01	308.17 $\pm$ 0.76	20000.50 $\pm$ 3.80	22.04 $\pm$ 0.95	50.02 $\pm$ 1.03	0.48 $\pm$ 0.08	19.83 $\pm$ 1.26	0.93 $\pm$ 0.25
	Isopod	599.84 $\pm$ 2.43	48.22 $\pm$ 1.60	444.83 $\pm$ 3.90	240.50 $\pm$ 2.19	96.67 $\pm$ 2.48	3.46 $\pm$ 0.41	1.52 $\pm$ 0.59	0.07 $\pm$ 0.005
Contamination threshold in soil (Italian Legislative Decree no. 152/2006)									
Commercial/industrial use		-	-	-	600	1500	15	1000	5
Residential use		-	-	-	120	150	2	100	1

**Fig. 2** Bars of trace elements (mg kg<sup>-1</sup> dw) detected in soil samples (*n* = 4 for each site) from site 1 (protected area) and site 2 (urban roadside)



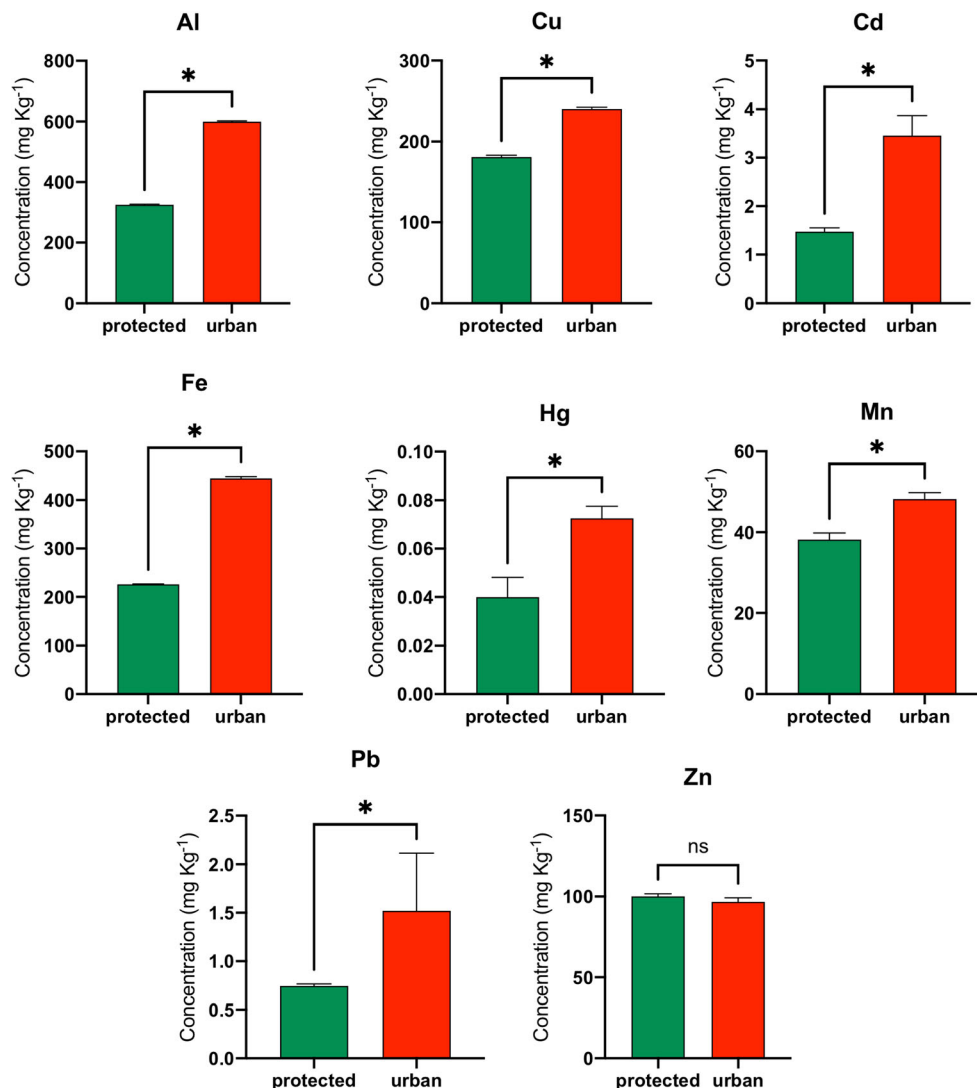
plot based on the lower element concentrations, except for Al and Zn, which were slightly higher in the soil and isopod samples, respectively.

## Discussion

The European Union has long recognized the importance of environmental monitoring and biomonitoring for recording the combined exposure of the environment and humans to contaminants, and the unique role these instruments play in identifying exposure to substances problematic for human health and the environment [36]. The widespread environmental problem of trace element contamination could be addressed by the monitoring of organisms that accumulate trace elements and as such reflect the rate and degree of environmental pollution [37].

In this study, trace element concentration in the soil samples was compared with the relative threshold values provided by Italian Legislative Decree no. 152/2006 [35] for residential use and for commercial and industrial use. The levels of the elements mentioned in the legislation (Cu, Zn, Cd, Pb, Hg) were below the expected limits. Nonetheless, the levels in the soil samples were generally higher in site 2 (urban) compared to site 1 (protected area), as clearly highlighted by the biplot obtained from the principal component analysis. Indeed, soil samples from site 2 are located in the order of increasing values of trace elements. This difference stems from the contamination due to the presence of the main route with heavy traffic near the sampling site. The Cd values were < 1 mg kg<sup>-1</sup>, in line with the range reported by Alloway [38] and shared by other authors [32, 39]. Cd is a highly toxic metal, and the main sources were derived from anthropogenic activities (industrial, agricultural, vehicular traffic, fossil fuel combustion) [40]. Alloway [38] reported an average Cu level of

**Fig. 3** Bars of trace elements (mg kg<sup>-1</sup> dw) detected in isopod samples (*n* = 4 for each site) from site 1 (protected area) and site 2 (urban roadside)



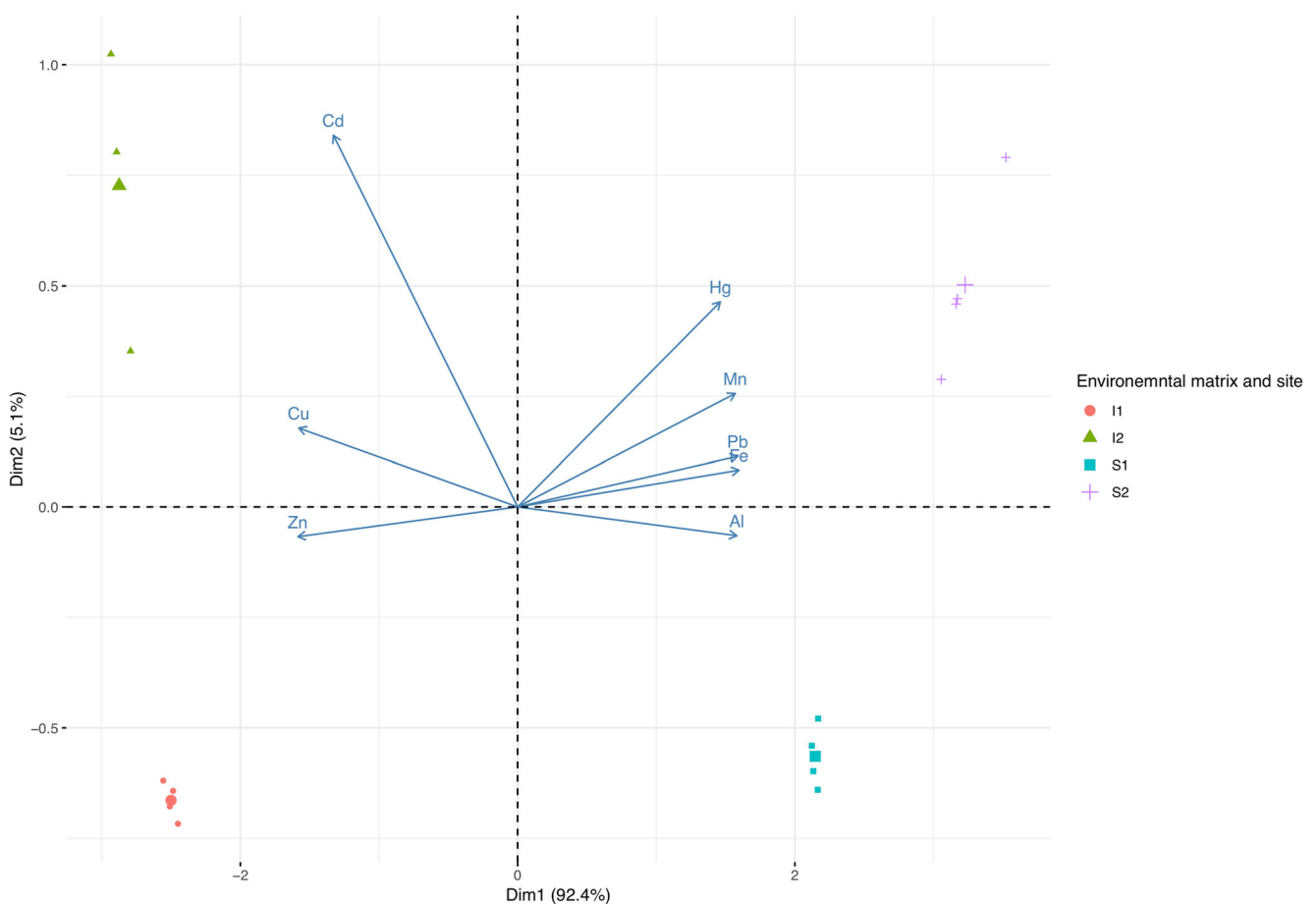
about 30 mg kg<sup>-1</sup>, with lower levels measured in sandy soils and higher levels in clayey soils, which are higher compared to those we found. As regards Cu, the primary sources are agricultural and industrial activities [41]: levels > 100 mg kg<sup>-1</sup> are considered alarming [42].

Kabata-Pendias and Pendias [43] reported an average zinc concentration from 17 to 125 mg kg<sup>-1</sup> in soils, which matches the level we detected. Also, Zn levels > 150 mg kg<sup>-1</sup> are often caused by anthropogenic factors [38], and the sources can be linked to mining, agriculture, and livestock [44].

The Hg concentration in soils varies widely and depends on the distance from emission sources and local geology [45]. In addition to local sources of pollution, the Hg levels in soil depend on the type of rocks, the pH, the cation exchange capacity, the presence of water and how it moves in the soil, and biological and erosion processes [46]. Most of the Hg present in uncontaminated soils and sediments is bound to soil organic matter (especially organic, humic, and fulvic acids) [47]. Our results suggest low contamination at the two sites (< 1 mg kg<sup>-1</sup>). Also, Pb concentration was fairly below the legal limit [35]. The main sources of Pb are manure, atmospheric

**Table 2** Bioaccumulation factor (BAF) (mean ± standard deviation) of *Philoscia affinis*. Lowercase letters (a, b) denote differences according to the Mann-Whitney *U* test

Site	Al	Mn	Fe	Cu	Zn	Cd	Pb	Hg
1	0.017 ± 0.01 <sup>a</sup>	0.183 ± 0.01 <sup>a</sup>	0.014 ± 0.01 <sup>a</sup>	10.324 ± 0.90 <sup>a</sup>	1.836 ± 0.06 <sup>a</sup>	6.169 ± 1.09 <sup>a</sup>	0.047 ± 0.01 <sup>a</sup>	0.093 ± 0.18 <sup>a</sup>
2	0.031 ± 0.01 <sup>b</sup>	0.157 ± 0.01 <sup>b</sup>	0.022 ± 0.01 <sup>b</sup>	11.452 ± 0.51 <sup>b</sup>	1.943 ± 0.09 <sup>b</sup>	6.974 ± 1.49 <sup>b</sup>	0.063 ± 0.01 <sup>b</sup>	0.083 ± 0.03 <sup>a</sup>



**Fig. 4** Biplot of score and loadings from the principal component analysis. A color and a symbol (largest symbol = average value) denote the scores of each environmental matrix (I, isopod; S, soil) from the two sites (1, protected area; 2, urban roadside)

deposition, and sludge [48]. Among the other elements not mentioned in Italian legislation, the Al levels were noteworthy. Aluminum is extensively found in the earth's crust and ranked the third most prevalent element in the environment [49]. Generally, agricultural soils can contain high Al levels [50], but the average concentration we recorded was much higher than those found in soil samples collected from sites in Markazi (Iran) ( $13839.169 \pm 348.409 \text{ mg kg}^{-1}$ ) [51]. High Al levels can lead to acidification of soils, posing a serious risk to overall plant productivity [52]. Also, Fe is another relatively abundant element in many cultivated soils (average range, 20.000 to 40.000  $\text{mg kg}^{-1}$ ) [53]. The levels we measured fell within this range. Finally, the Mn levels from the two sites were within the mean background concentration of 330  $\text{mg kg}^{-1}$  reported by Barceloux and Barceloux [54].

The trace element levels were sometimes lower in the isopods than in the soil, except for Cd, Cu, and Zn. This consideration is highlighted by the PCA analysis, in which the projected observations (isopods samples) from site 2 are located in relation to a higher concentration of Cd, Cu, and Zn. Cu and Zn are essential elements for life, which explains their higher concentration in the isopods [14]. The great affinity of isopods for Cu results from its

requirement for hemocyanin synthesis [14, 55], whereas Cd is a non-essential element and its regulation is probably more difficult [19, 55].

Bioaccumulation in terrestrial organisms is usually based on a sum of the amount of metal adsorbed into the body wall and absorbed into the body [56]. However, the isopod exoskeleton has a low binding affinity for metals [56]. The isopod *Porcellio scaber* takes up metals mainly via the alimentary tract [56]. The main organ for the storage of Cd, Cu, Zn, and Pb is the hepatopancreas (midgut gland) packed with different types of granules consisting of blind-ending tubes protruding from the intestine at the border between the foregut and the stomach [57]. According to Witzel [58], Cd accumulates because terrestrial isopods are unable to expel it from the body; studies have shown that Cd bioaccumulation may also depend on the presence of other metals (i.e., Zn) and their concentration in the soil/litter [14, 58, 59]. Studies on *Porcellio scaber* and *Porcellio laevis* (Isopoda, Oniscidea) showed how the excretion rate of Cd can be influenced by the Zn concentration in the environment and/or in the food they ingest [58, 60]. It follows then that the presence of Zn and Cd in food affects the

assimilation potential of both metals in *P. scaber* [61]. Also, Godet et al. [59] suggested that high Zn concentrations in litter allow *P. scaber* to excrete Cd or to limit its uptake, whereas lower Zn concentrations in food do not allow isopods to eliminate Cd or to limit its uptake from food. The BAF we calculated identifies *P. affinis* as a micro-concentrator for Zn and as a macro-concentrator for Cu and Cd. Our data are the first to report on the bioaccumulation capacity of *P. affinis*.

Site 1 is located within a natural area in a special area of conservation; it is surrounded by extensive farmlands where elements such as Zn and Cu are heavily used in pesticides and fertilizers [38], while high Cd concentrations may result from the use of mineral fertilizers [62]. Site 2 is located alongside a busy route. This and the wear of mechanical parts of road vehicles are the main anthropogenic sources of Cu, Cd, and Zn [63, 64]. In their study in uncontaminated subtropical locations (Assiut, Egypt), Hussein et al. [55] reported a BAF for Zn and Cu in *Porcellio laevis* (Isopoda, Oniscidea) higher than ours, while their BAF for Cd was in line with ours. Furthermore, the BAF we calculated for Zn and Cd was higher than the range reported by Udovic et al. [65] in *P. scaber* collected in two non-polluted managed gardens in Slovenia. Ghemari et al. [14] measured Cd, Pb, Zn, and Cu concentration in *P. laevis* from Tunisian industrialized areas and found a BAF of almost  $> 1$  for Cd, Cu, and Zn, suggesting a role of bioaccumulator for the genus *Porcellio*. Also, *Porcellionides pruinosus* (Isopoda, Oniscidea) could be defined as a macro-concentrator of Cd, Zn, and Cu (BAF  $> 2$ ).

On the other hand, *Armadillidium vulgare* (Isopoda, Oniscidea) has the ability to accumulate Cu and Zn, but not Cd [5].

Mazzei et al. [5] reported a BAF of 0.03 in *P. laevis* exposed to various Pb concentrations for 21 days. Also, Ghemari et al. [66] found a BAF for Pb  $< 1$ , indicating *P. laevis* as a deconcentrator for Pb, an observation shared by the present study. This was confirmed by the significant negative correlation between Pb concentrations in the isopod and the soil samples. Heikens et al. [4] also found a negative correlation between Pb concentration in Isopoda and soil concentration, suggesting regulation of Pb by this taxonomic group. There is scarce literature about the BAF for other trace elements. Isopods sampled from the industrialized areas of Sfax (south-eastern Tunisia) resulted in deconcentrators of Fe [67], as we also noted. Unfortunately, no data for Al, Mn, and Hg are available for comparison. However, the BAF here calculated indicates a role for *P. affinis* as a deconcentrator also for Al, Fe, Hg, and Mn. These new findings suggest that *P. affinis* can excrete and/or regulate certain elements (e.g., Al, Fe, Pb) with the lowest BAF. Moreover, trace element levels may exceed an organism's physiological tolerance; in response, terrestrial

isopods have developed not only mechanisms for uptake and storage of essential elements but also the mechanisms for handling excess uptake of metals, especially those not essential for life [57].

## Conclusion

In this study, trace elements were measured in both soils and isopods from two sites that differed in the degree of anthropization. As discussed above, the majority of bioaccumulation studies on oniscidean isopods has been conducted on *Porcellio laevis*, *P. scaber*, and *Porcellionides pruinosus*, which are the most common and widespread species of the temperate zone [27]. These organisms can accumulate Cd, Cu, and Zn in large amounts. The trace element levels detected in the isopods analyzed in the present study suggests that *Philoscia affinis* can also cope with high accumulation levels of Cd, Cu, and Zn. In particular, it was found how *Philoscia affinis* can be considered as a macro-concentrator of Cu and Cd and a micro-concentrator of Zn, representing a potential candidate for monitoring soil contamination being widely distributed. Trace element accumulation kinetics and the factors that influence their uptake and loss have a key role in ecotoxicology. Thus, future studies are needed to predict the physiological fate of trace elements in this species. Furthermore, the role of trace elements in the ecology of this species and in other terrestrial invertebrates should be investigated in the near future.

**Authors' Contributions** Conceptualization: P.P. and E.P.; data curation: P.P. and M.B.; investigation: P.P., M.B., P.B., M.C.A., V.D.N., M.P., and E.P.; methodology: P.P., M.B., P.B., and V.D.N.; supervision: M.P. and E.P.; writing—original draft: P.P.; writing—review and editing: P.P., M.C.A., and E.P.

**Data Availability** The dataset analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Ethics Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent for Publication** All authors have read and agreed to the published version of the manuscript.

**Code Availability (Software Application or Custom Code)** Not applicable.



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