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Terrestrial Biota as Bioindicators for Microplastics and Potentially Toxic Elements

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Abstract: Plastic products used in our daily life remain in the environment for a long time. Plastics decompose gradually into smaller fragments (<5 mm) known as microplastics. There are different sources of microplastics contamination, including plastic bags, masks, synthetic textiles, and various coatings. Microplastics’ smaller size enhances toxic pollutants’ adsorption, through which they are easily digested by small biota and finally accumulated along the food chain. Many studies are found concerning marine microplastic distribution and pollution; however, rarely do they address terrestrial contamination. The terrestrial species Eobania vermiculata, Rumina decollata, Porcellio, Armadillo, Lumbricus terrestris, and Scolopendra were evaluated as bioindicators for soil pollution by microplastics and some potentially toxic metallic elements. Microplastics were isolated with the help of caustic potash. The particles were characterized by infrared spectroscopy (FTIR); some associated potentially toxic metals were assessed in the filtrate by inductively coupled plasma spectrometry (ICP). The following polymers were present in all studied samples: copolyamide, nylon, high- and low-density polyethylene, polyamide, and polyester. In addition, the metallic elements antimony, iron, aluminum, selenium, and zinc were determined with different concentrations. Thus, terrestrial biota can serve as bioindicators for microplastic pollution of soil, which could act as a vector for potentially toxic elements.

Keywords: microplastics; toxic elements; terrestrial biota; bioindicator; contamination; KSA

1. Introduction

Plastics have various characteristics that implicate their increased usage in daily lives; this has also developed into a significant environmental threat that needs complete awareness [1,2]. For example, it was reported that 40% of all plastic production was used in the food and drink packaging industry [3]. As a result, plastic contamination becomes omnipresent worldwide. The volume of plastic waste has expanded due to fast industrialization and the increased use of plastic materials [4]. In 2017, about 348 million tons of plastics were produced worldwide, of which roughly 42% were utilized once [5,6]. In the Arabian Gulf region, the Kingdom of Saudi Arabia (KSA) accounts for about 64% of the total plastic production, followed by the United Arab Emirates with 20% [7]. Rapid population growth, industrialization, and urbanization in the last few decades in the KSA resulted in large amounts of solid waste release, including plastics [8]. In addition, the many pilgrims and their massive use of disposable items is the primary source of plastic waste [9]. A previous study reported an average solid waste production in the KSA and its main cities of 15,300 × 103 tons, including the high-altitude Taif Governorate. Its annual waste production is about 540 × 103 tons. Plastic wastes represent the second most significant proportion of municipal solid waste in the KSA [10]. A severe problem of plastics is their degradation to small-sized microplastics.

Microplastics range at several micrometers, which have properties different from larger plastic pieces that contaminate the environment. The main problem of microplastics...
is their small size, leading to easy ingestion by small organisms and accumulation along the food chain. Moreover, their smaller sizes increase their surface areas, leading to the resorption of other pollutants, including potentially toxic elements [11]. Different primary and secondary sources of microplastics found their pathway to the terrestrial and marine environment. Primary microplastics are found in paints, personal care products, synthetic clothing, and car tires. On the other hand, the degradation of large plastics to microplastics represents the secondary source [12]. Coating substances consist of polymers combined with other additives are often deliberately overlooked in the pool of microplastics, including architectural, marine, automotive coatings, and road-marking paint [13].

There are many research concerns about marine microplastic distribution and pollution. Still, there is scarce information related to terrestrial microplastics contamination [14,15], even though there is frequent documentation of the presence of microplastic particles in the soil. For example, Zubris and Richards [16] used polarized light microscopy to detect synthetic fibers in the soil of the USA; others quantified microplastics in soils from an industrial area in Australia using pressurized fluid extraction combined with Fourier transform infrared spectroscopy (FTIR) [17]. In addition, it has been estimated that about 700,000 tons of microplastics could enter farmland through manure application in Europe and North America annually, which is more than the burden of marine surface waters [18]. Microplastics that arrived at the soil surface could have potential adverse effects on soil biota, including annelids (earthworms) [19], Chironomus leperi larvae [20], polychaetes (Perinereis aibuhitensis), and nematodes (Caenorhabditis elegans) [21].

In addition, few studies report the adsorption of trace elements to microplastics suspended in the marine environment [22–24] and, in turn, become bioavailable to animals upon ingestion [25–27]. Until now, mechanisms of the adsorption of potentially toxic elements to microplastics surfaces remain relatively unexplored. Although those processes are likely to be varied and of complex mechanisms, weathering seems to be more important for adsorption capacity than the type of plastic [23]. A serious problem is the transfer of microplastics and adsorbed pollutants through food chains and their bioaccumulation ability in higher trophic levels, including humans [28].

Terrestrial biota has significant potential in terrestrial ecosystems maintenance [29]. Earthworms (Lumbricus terrestris) have a key role in soil structure by transferring organic material from the soil surface into depth, producing humus, making biopores, and increasing plant growth; they are considered ecosystem engineers [30]. Terrestrial isopods (including Porcellio and Armadillo) are saprophagous; they have a role in litter fragmentation and, in turn, tolerate environmental contaminants at high concentrations [31]. Terrestrial gastropods such as Eobania vermiculata and Rumina decollata serve as food for various soil arthropods, birds, and small mammals, and have a role in litter decomposition and nutrient cycling. Scolopendra, one of the top carnivorous invertebrates, are generalist feeders used commercially in traditional medicine [32]. Different terrestrial biota have been used to assess the ecological risk of contaminants on soil health and are referred to as bio-indicators, complementary to the traditional chemical and physical analyses [29,33]. Bio-indicators are highly responsive to environmental conditions and could be effective tools for environmental contamination assessment [34].

Therefore, microplastics contamination in the terrestrial environment could be reported by small terrestrial animals. Moreover, we suggest that microplastics contamination in terrestrial biota could have a transporting role of metallic elements. The present study aims to assess microplastics and related potentially toxic elements contamination in different terrestrial biota (gastropods: Eobania vermiculata (E. vermiculata) and Rumina decollata (R. decollata); isopods: Porcellio and Armadillo; annelids: Lumbricus terrestris; arthropods: Scolopendra) as pollution bioindicators in the high-altitude Taif Governorate of KSA.
2. Materials and Methods

2.1. Chemicals, Instrumentation, and Software

Chemicals used in the present study were KOH and NaI (Sigma Aldrich, Darmstadt, Germany). Instruments that were used in the investigation included an inverted microscope (Nikon Eclipse Ti-U, Melville, NY, USA), FTIR microscope (Cary 620, Agilent, Santa Clara, CA, USA), and inductively coupled plasma–mass spectrometry (ICP, PerkinElmer Sciex Elan 5000, Beijing Kechuang Haiguang Instrument Company, Beijing, China). Software used for data analysis were OriginLab 2021 software (Northampton, MA, USA), siMPle 2020 software (systematic identification of microplastics in the environment, Helgoland, Germany), and GraphPad software (GraphPad®® 2017, San Diego, CA, USA).

2.2. Collection of Animals

The terrestrial organisms were collected by hand sorting from two different areas (area 1: 21°26′25.1″ N 40°26′38.8″ E and area 2: 21°26′01.0″ N 40°29′30.8″ E according to Google Maps) of the high-altitude Taif Governorate, KSA (Figure 1). Taif Governorate is rich in plant and animal biodiversity due to its climate, high altitude, seasonal rain in summer, and continental rain [35]. The terrestrial species for the present study were the gastropods E. vermiculata (Helix) (n = 16, average body weight (b.w.) 5.9 ± 3.4 g) and R. decollata (n = 20, 1.19 ± 0.26 g); the isopods Porcellio (woodlice) (n = 9, 0.10 ± 0.03 g) and Armadillo (pill woodlouse) (n = 9, 0.07 ± 0.06 g); the annelid L. terrestris “earthworm” (n = 14, 0.94 ± 0.29 g); the arthropod Scolopendra (n = 3, 0.04 ± 0.02 g). All studied animals were collected from area 1, except R. decollata from the second area. Figure 2 shows external features of different collected terrestrial samples.

2.3. Minimization of Contamination

Cotton lab coats and gloves were worn during the experiment. The use of any plastic tools was avoided. All used glasses and dissecting tools were washed with deionized water and ethanol prior to use. Any prepared solutions and incubated samples were kept in glass containers and capped with aluminum foil to prevent plastics contamination.

2.4. Sample Preparation and Microplastics Extraction

In the beginning, animals were anesthetized by alcohol before further steps. Each animal was subjected to caustic digestion separately. The soft body of gastropods of the helix and R. decollata snails were removed from the shells and weighed again before microplastics extraction. The soft tissue of helix and R. decollata (without snail shell), earthworms, Scolopendra, Porcellio (woodlice), and pill woodlouse (Armadillo) were separately treated according to Rochman et al. [36], with few modifications. Samples were incubated with 3 volumes (relative to body weight) of aqueous 10% KOH for 6 days at 60 °C (with agitation every 48 h) for complete digestion. The rigid bodies of Porcellio, Armadillo, and Scolopendra were carefully ground by a tissue grinder to help digestion. An aqueous solution of NaI (10 mL, 4.4 mol/L) was added to help the microplastics floating for easy filtration. Shells of Eobania and R. decollata snails were separately treated with aqueous solutions of KOH (10%) and NaI (4.4 mol/L) under the same conditions to assess any microplastics or/and potentially toxic elements adsorbed on their surfaces from the surrounding living area.

Toward the end of incubation, the colorless KOH digest turned yellow, with the whole sample being successfully digested without residues. Each sample was filtered through Whatman®® Grade 2 cellulose filters (8 µm); filters were rinsed with 5 mL deionized water into Petri dishes, dried overnight at 60 °C, and reweighed at room temperature to determine the recovery of microplastics per sample. Dry filter samples were pooled for each species separately. Filtrates were used later for elements’ determination. Figure 3 shows, in brief, the steps of treatment and microplastics extraction.
2. Materials and Methods

2.1. Chemicals, Instrumentation, and Sample Preparation

The terrestrial organisms were collected by hand sorting from two different areas (area 1: 21°26'25.1" N 40°26'38.8" E and area 2: 21°26'01.0" N 40°29'30.8" E) in Taif (Google Map 2020). Representative photos of the collected terrestrial samples: (A) E. vermiculata (helix), (B) Lumbricus terrestris (earthworm), (C) R. decollata (decollate), and (D) Porcellio (woodlice).

2.2. Collection of Animals

In the beginning, animals were anesthetized by alcohol before further steps. Each sample was filtered through Whatman® Grade 2 cellulose filters (8 μm) and ethanol prior to use. Any prepared solutions and incubated samples were kept in glass containers and capped with aluminum foil to prevent plastics contamination.

2.3. Minimization of Contamination

Shells of NaI (10 mL, 4.4 mol/L) was added to help the microplastics floating for easy filtration. Cotton lab coats and gloves were worn during the experiment. The use of any plastic ware used for data analysis were Origin Lab 2021 software (Northampton, MA, USA), Clara, CA, USA), and inductively coupled plasma–mass spectrometry (ICP, PerkinElmer, Germany). Instruments that were used in the investigation included an inverted microscope (Nikon Eclipse Ti-U, Melville, NY, USA) to help differentiate plastic particles from others to be further analyzed by FTIR (FTIR microscope Cary 620, Agilent, Santa Clara, CA, USA).

2.4. Sample Preparation and Microplastics Extraction

For each species, three replicates of the digested filtrate were transferred to labeled, acid-washed glass bottles with stoppers, and kept for elemental analysis. Inductively coupled plasma–mass spectrometry (ICP) was used for the quantitative determination of Ag, Al, As, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Sb, Se, Sr, Ti, V, and Zn. The parameters for ICP-MS were forward power 1000 W, and nebulizer gas flow rate 0.8 L/min. The concentrations of elements were calculated as the means in mg/L in the digested filtrate volume of each animal tissue. Validation of the analytical method...

![Figure 1. Map of Kingdom of Saudi Arabia showing high-altitude Taif Governorate (A) and sample collecting areas (21°26'25.1" N 40°26'38.8" E and 21°26'01.0" N 40°29'30.8" E) in Taif (B) (Google Map 2020).](image)

![Figure 2. Representative photos of the collected terrestrial samples: (A) E. vermiculata (helix), (B) Lumbricus terrestris (earthworm), (C) R. decollata (decollate), and (D) Porcellio (woodlice).](image)
including instrumental detection limits, limits of quantification, precision, and accuracy were constructed according to previous studies [37,38].

![Figure 3](image)

**Figure 3.** Steps of treatment: (A) soft tissues treatment with 10% KOH and incubation at 60 °C for 6 days; (B) filtration after complete digestion; (C) dryness of filters in Petri dishes for further analyses. Red circle refers to a spiral shape that remained undigested after the whole digestion of *R. decollata* soft body.

### 2.8. Statistical Analysis

Data were expressed as means ± standard deviations (M ± SD), and statistical analysis was conducted to differentiate between studied groups: *E. vermiculata* and *R. decollata*; their shells, *Porcellio* and *Armadillo*, and between earthworm and *Scolopendra* by using Student’s *t*-test, and one-way ANOVA, followed by Tukey’s multiple comparisons test using GraphPad software. *** indicates *p* ≤ 0.001, ** indicates *p* ≤ 0.01, * indicates *p* ≤ 0.05, and ns (nonsignificant) means *p* > 0.05.

### 3. Results

#### 3.1. Microplastics Detection

First, visual examination of dried, filtered particles under a 10× lens in an inverted microscope was conducted, in which colored particles, fibers with the same thickness, and dull particles (data not shown) were selected for FTIR examination to determine the type and chemical composition of the selected particles. FTIR spectra of most selected particles were reported as polymers: copolyamide (cPA) in *E. vermiculata* and *R. decollata* soft tissue; nylon 6, and high-density polyethylene (HDPE) were adsorbed on their shell surfaces; nylon 6, polyamide, and low-density polyethylene (LDPE) were present in *Armadillo, Porcellio*, and *Scolopendra*, respectively, and polyester (PS) in earthworm (Figures 4 and 5). Therefore, different polymers were found in the body of terrestrial biota and adsorbed on the external shell surface of gastropods.
Coatings 2021, 11, x FOR PEER REVIEW 6 of 12

Therefore, different polymers were found in the body of terrestrial biota and adsorbed on their shell surfaces; nylon 6, polyamide, and low-density polyethylene (LDPE) were present in the body of terrestrial biota and adsorbed on their shell surfaces; copolyamide (cPA) in Scolopendra, and high-density polyethylene (HDPE) were reported as polymers: copolyamide (cPA) in Scolopendra, and HDPE were adsorbed on their shell surfaces; dull particles (data not shown) were selected for FTIR examination to determine the type of polymer. FTIR spectra of most selected particles were conducted to differentiate between studied groups: polyamide, LDPE, nylon 6, and HDPE present in samples. The FTIR spectra of polyamide, LDPE, nylon 6, and HDPE are shown in Figure 4. The FTIR spectra of copolyamide (cPA) and polyamide (PA) are shown in Figure 5. The FTIR spectra of polyamide (PA), LDPE (B), nylon 6 (C), and HDPE (D) present in samples are shown in Figure 4.

3.1. Microplastics Detection

3.2. Elemental Analysis

Table 1 and Figure 6 show the concentrations of Sb, As, Fe, Al, Se, and Zn (mg/L) in the samples’ filtrate, while the other analyzed elements were undetectable. Figure 6 shows a highly significant differences between all the studied groups within the same element concentration: for Al (F (7, 16) = 4702; p < 0.001; R square = 0.9995), Se (F (7, 16) = 1075; p < 0.001; R square = 0.9979), and Zn (F (5, 12) = 773.9; p < 0.001; R square = 0.9969) by using one-way ANOVA. Tukey’s test reports a highly significant difference between each group and the others within the same element concentration at p < 0.001, except for Zn concentration of Eobania vs. decollata, and decollata vs. Porcillo at p < 0.01. In addition, Student’s t-test assessed
the significant difference between each related studied groups at \( p < 0.001 \). It was reported that aluminum concentration (Al) in earthworm \((107.028 \pm 20.577 \text{ mg/L})\) was the highest in comparison to the other samples. For gastropods, Al and Se concentrations in the soft tissue of \( E. \text{vermiculata} \) (Al = 45.7460 ± 8.95 mg/L, Se = 1.48 ± 0.25 mg/L) are significantly higher than that of \( R. \text{decollata} \) (Al = 22.4040 ± 7.45, Se = 0.219 ± 0.072 mg/L). However, Al and Se adsorbed on the shell of \( R. \text{decollata} \) (Al = 95.6002 ± 10.00, Se = 1.117 ± 0.089 mg/L) are significantly higher than that of \( E. \text{bombyx} \) (Al = 6.0843 ± 0.980 mg/L, Se = 0.773 ± 0.107 mg/L). Moreover, shells of \( R. \text{decollata} \) (Al = 95.6002 ± 10.00 mg/L, 1.117 ± 0.089 mg/L) significantly have more Al and Se adsorbed on their shells, in comparison to their soft tissue (Al = 22.4040 ± 7.45 mg/L, 0.219 ± 0.072 mg/L), but this was reversed for \( E. \text{vermiculata} \) soft tissue (Al = 45.7460 ± 8.95 mg/L, 1.48 ± 0.25 mg/L) and their shells (Al = 6.0843 ± 0.05 mg/L, 0.77 ± 0.11 mg/L). In addition, it was detected that earthworm (Al = 107.028 ± 20.577 mg/L) significantly have higher Al concentration in comparison to \( Scolopendra \) (Al = 74.472 ± 12.650 mg/L). On the other hand, \( Scolopendra \) (1.78 ± 0.40 mg/L) have significantly higher Se in comparison to earthworm (1.60 ± 0.36 mg/L). For isopods, \( Porcillo \) (Al = 90.5487 ± 17.768 mg/L) significantly have Al concentration higher than that of \( Armadillia \) (Al = 27.03 ± 0.58 mg/L), while only \( Armadillia \) record Se (0.47 ± 0.13 mg/L), but it was absent in \( Porcillo \).

Table 1. Concentration of other metallic elements present in the filtrate of different terrestrial biota in mg/L.

| Element (mg/L) | R. decollata | R. decollata Shell | Scolopendra | Porcillo | Armadillia |
|---------------|-------------|-------------------|-------------|----------|-----------|
| Sb            | 0.625 ± 0.155 | 0.00              | 0.651 ± 0.076 | 0.782 ± 0.106 | 0.00     |
| As            | 0.834 ± 0.100 | 0.00              | 0.857 ± 0.108 | 0.00      | 0.138 ± 0.025 |
| Fe            | 0.00         | 0.911 ± 0.087     | 0.00         | 0.00      | 0.00      |

Figure 6. Assessment of aluminum (A), selenium (B), and zinc (C) present in the filtrate of different terrestrial biota in mg/L. *** indicates \( p \leq 0.001 \) and ** indicates \( p < 0.01 \) by comparing related samples with each other.

Zinc was recorded in a few samples only, in which it was found in higher concentration in \( R. \text{decollata} \) soft tissue \((0.41 ± 0.06 \text{ mg/L})\) in comparison to \( E. \) \((0.29 ± 0.08 \text{ mg/L})\). Antimony was found with the highest concentration in earthworm \((1.03 ± 0.03 \text{ mg/L})\). Arsenic was found in low concentration in \( R. \text{decollata} \) \((0.624 ± 0.155 \text{ mg/L})\), \( Scolopendra \) \((0.651 ± 0.076 \text{ mg/L})\), and \( Porcillo \) \((0.782 ± 0.106 \text{ mg/L})\). Arsenic was found also in low concentration in \( R. \text{decollata} \) \((0.83 ± 0.10 \text{ mg/L})\), \( Scolopendra \) \((0.857 ± 0.108 \text{ mg/L})\), and \( Armadillia \) \((0.138 ± 0.025 \text{ mg/L})\), and Fe was found only adsorbed on the shell of \( R. \text{decollata} \) with concentration 0.911 ± 0.087 mg/L (Table 1 and Figure 6).
4. Discussion

In the present study, we aimed to assess microplastics contamination in the body of small animals from different species: *E. vermiculata*, *R. decollata*, *Porcellio*, *Armadillo*, *Lumbricus terrestris*, and *Scolopendra*. In addition, we aimed to evaluate any metallic elements that could be accompanied by microplastics contamination. We chose various species that have different feeding habitats. Soil biota represent the biological engine of the earth [39]. Therefore, any change due to toxicity could affect many of the critical processes within soils and affect the food web/chain causing ecological imbalances. Most of the selected species were completely digested in 10% KOH at the end of treatment time, except for a spiral body of the *R. decollata* snails (Figure 3C, red circle). This spiral body represents the formation of a new apex (spiral calcareous septum) inside the soft body, separated from the snail shell [40]. Filtration of all samples was easily carried out except for *E. vermiculata*, due to high mucus secretion according to chemical stress [41].

FTIR represents a fingerprinting technique extensively used to identify and differentiate between organic and inorganic particles by their unique spectra [42]. We detected different microplastics in the present studied animals such as nylon 6, LDPE, HDPE, polyamide, and PS. Different studies were conducted concerning the microplastic contamination in aquatic biota, while few studies concerning terrestrial ones. Due to the excessive use of plastics everywhere, recently, it was clear that particulate plastics contaminate the environment was associated with many impacts upon organisms. Upon reaching the terrestrial ecosystem, many synthetic polymers take a long time to degrade through physical, biological, or chemical processes [43], and thus, they remain in the soil for a long time, leading to more toxicity due to low oxygen and sunlight conditions [15,44]. Microplastics might interact with various terrestrial fauna by changing their biophysical environment [19,45,46]. Once entering the environment, microplastics find their way up the food chain, as recorded in earthworms [45], freshwater invertebrates [47], oysters [48], fish [49], and birds [50,51].

Our findings were in agreement with very few other studies that record microplastics in terrestrial microorganisms. Maass et al. [52] and Rillig et al. [53] have reported microplastics in springtails and earthworms. Therefore, those animals could share in microplastics’ transportation within the soil vertically and horizontally. Microplastics were reported in the alimentary tract of dead terrestrial birds (94%) with different foraging behavior [51]. In addition, Lwanga et al. [54] have recorded the quantitative trophic transfer of microplastic from soils to earthworm casts that end up in chicken feces. In addition, in our view, sequential layers of chitin-protein fibers in *Armadillo* and *Porcellio* [55] could help in microplastics accumulation with any adsorbed pollutants in their cuticle.

Recently, it was determined that microplastic could act as a vector to other inorganic pollutants in the environment, such as metallic elements [56]. Therefore, the present study aimed to assess different potentially toxic elements accompanied by microplastics accumulation in terrestrial biota.

In the present study, some metallic elements were determined that could be associated with microplastics uptake, in agreement with Wijesekara et al. [57], who determined different potentially toxic elements in biosolids derived microplastics. The utilization of recycled organic waste (biosolids) and plastic film mulching have been reported to contaminate agroecosystems with microplastics and adsorbed trace metals and other agrochemicals [58,59].

Al is the most concentrated element in the studied biota. However, Al is naturally recorded as the most abundant metal in the earth’s crust and is present in building, construction, packaging, electrical equipment, and transportation [60]. In the present study, we recorded different concentrations of the same potentially toxic element in different species due to feeding habits having a great influence on elements deposition in their tissue. In addition, elements toxicity varies depending on its concentration, exposure, speciation, the position of the animal in the food chain, and interaction with other solutes. As an example,
Zn and Cu are needed in small amounts by organisms. However, they become toxic at higher concentrations [61].

Pollutant's adsorption and desorption associated with microplastics in the soil environment (especially agricultural practices and landfill leachate) are affected by different factors, including weathering, surface area, microbial activity, and dissolved organic matter [23]. In addition, the presence of potentially toxic pollutants with microplastics could be attributed to reasons other than adsorption. However, in plastics manufacturing, additives could be used to enhance plastic’s properties that contain trace elements such as Zn, Pb, and Cd [62,63]. Therefore, microplastics could increase chemical and trace element exposure for different biota, leading to bioaccumulation in different environments (aquatic and terrestrial) and impacting human and environmental health [59,64]. Finally, different terrestrial biota could be used as bioindicators for microplastics and toxic elements pollution according to different human and industrial activities.

5. Conclusions

For the first time, to the best of our knowledge, different terrestrial biota *Eobania vermiculata*, *Rumina decollata*, *Porcellio*, *Armadillo*, *Lumbricus terrestris*, and *Scolopendra* were used as bioindicators for microplastics pollution. Different microplastics particles were determined in all samples including cPA, nylon 6, LDPE, HDPE, polyamide, and PS. In addition, potentially toxic elements such as Sb, As, Fe, Al, Se, and Zn were found in the studied samples with different concentrations that could be accompanied by microplastics toxicity. More other studies must be conducted to report the interaction between those two pollutant groups that severely affect terrestrial ecosystems.

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