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Uptake of Potentially Toxic Elements in Microplastic-Contaminated Soils: A Controlled Laboratory Study Using *Eisenia Fetida*

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A B S T R A C T

Background: Earthworms are known to respond quickly to various environmental stressors. The present study aimed to investigate the relationship between varying levels of tire-derived microplastic (TMP) exposure and the accumulation of specific heavy metals in the earthworm *Eisenia fetida*.

Methods: We assessed the accumulation of arsenic (As), cadmium (Cd), chromium (Cr), tin (Sn), aluminum (Al), lead (Pb), zinc (Zn), and molybdenum (Mo) in *Eisenia fetida* through a 14-day controlled exposure to TMP concentrations of 0, 10, 50, 100, and 200 mg/g dry artificial soil, with three replications.

Results: The accumulation of Cr, Sn, Al, Pb, Zn, and Mo increased significantly with TMP concentrations exceeding 100 mg/g. The concentration of Cd remained statistically similar in TMP concentrations above 50 mg/g, while a significant increase in Zn concentration was observed at TMP levels higher than 100 mg/g. The correlation coefficients between Potentially Toxic Elements (PTEs) concentration in the species and inhabited soil were negative and statistically significant for Cd (*p-value* < 0.05), Al (*p-value* < 0.01), and Mo (*p-value* < 0.05), suggesting a depletion of PTEs from the soil as the earthworms accumulated higher concentrations. Maximum levels reached 130.44 ± 4.43 μg/L for Sn, 2.46 ± 1.74 mg/L for Zn, and 0.057 ± 0.006 mg/L for Cr in the 200 mg TMP exposures.

Conclusion: This study used artificial soil samples, and the impact of soil physicochemical characteristics on the mobility and bioavailability of MP-associated PTEs remains an area open for future investigation.

1. Introduction

Earthworms are vital organisms in soil ecosystems, playing a crucial role as both indicators of soil quality and facilitators of contaminant transfer between abiotic and biotic components (Yadav et al., 2023). They have a widespread presence and a remarkable ability to respond rapidly to various environmental stressors (He et al., 2021). Moreover, earthworms exhibit heightened sensitivity to elevated concentrations of Potentially Toxic Elements (PTEs) in soils, making them valuable for assessing the impact of human-induced disturbances on terrestrial ecosystems and species (Yan et al., 2021; Yuvaraj et al., 2021). Extensive research has shown that prolonged exposure of earthworms to PTEs leads to a range of acute or chronic impairments at structural, molecular, and functional levels (Yadav et al., 2023). These impairments encompass growth impediments, biomass reduction, delayed sexual maturation, and the inhibition or

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stimulation of enzyme activities (Kniuipyte et al., 2022; Sharma et al., 2021). In their natural habitat, earthworms prefer to consume fresh organic matter alongside substantial quantities of soil, allowing them to increase their intake of micronutrients and construct and maintain burrows (Akhila & Entoori, 2022). This behavior, however, results in the inadvertent ingestion and accumulation of contaminants such as microplastics in their digestive systems (Sheng et al., 2021). The escalating release of harmful pollutants into the environment further emphasizes the significance of earthworms as effective bioindicators of soil health. Soil contamination by PTEs such as As, Pb, and Cd, has become a widespread global issue rather than an isolated occurrence (Briffa et al., 2020). The large-scale production and disposal of fertilizers, pesticides, municipal and industrial waste, and wastewater contribute to this problem, as these sources contain high levels of non-essential PTEs (Bahiru, 2021). Additionally, plastic production has experienced rapid growth, with estimates indicating that over 25 billion tons of plastics will be produced by 2050, of which approximately 18% is projected to be discarded as environmental waste (Chen et al., 2021). The adverse effects of plastic waste are exacerbated when they fragment into smaller pieces, known as microplastics (MPs) (particle size < 5 mm). It has been observed that the likelihood of earthworms ingesting MPs increases MPs size decreases (Cui et al., 2022; Sheng et al., 2021). In certain cases, the chemical composition, taste, and flexibility of MPs, as well as the nutritional state of the earthworms, influence their ingestion preferences for MP (Cui et al., 2022; Wang et al., 2019). The effects of MPs on soil-dwelling organisms depend on the size and concentration of the MPs as well as the associated pollutants, such as PTEs (Yadav et al., 2023). MPs generally possess large specific surface areas, enabling them to adsorb PTEs such as Cd (Wang et al., 2019), Pb (Shen et al., 2021), and Nickel (Ni) (Kim et al., 2017). MPs can also form microbial ecological niches (biofilms), which further affect the adsorption behavior of PTEs (Tu et al., 2020). Several factors, including MPs size, ionic strength, contact duration, and environmental conditions such as pH and temperature, regulate the complex interactions between MPs and PTEs (Liu et al., 2021). When ingested by earthworms, MPs can obstruct the gut and stomach of earthworms, impeding their regular feeding and hindering their growth (Cui et al., 2022). The coexistence of MPs and PTEs in soil can lead to more severe impacts on earthworms, including oxidative stress that damages normal cell functions and alters enzyme activities (Sheng et al., 2021). However, the understanding of the earthworm growth and health risks resulting from the combined exposure to PTEs and MPs, particularly in the form of PTE-adsorbed MPs remains in its early stages. Car tires are a crucial and widely prevalent source of MPs on a global scale (Tamis et al., 2021). Through mechanical abrasion and wear, they partially break down into MPs and disperse into the environment. The continuous growth of the automotive industry and road network at a global level further contributes to the generation and release of tire-derived MPs (TMPs) (Lackmann et al., 2022). Research by Worek et al. (2022) showed that TMPs can accumulate in sediments flowing from motorways, with concentrations reaching up to 480 mg/g soil. TMPs contain various PTEs such as Zn, Cd, and Pb (Adachi & Tainosho, 2004). In a study, Lackmann et al. (2022) exposed *Eisenia andrei* to TMPs in natural soil for 2 to 28 days to investigate its ecotoxicological effects, finding significant changes in the activity of enzymatic biomarkers but minor impacts on the subcellular level. Sheng et al. (2021) also assessed the effect of TMPs on *E. fetida* at different TMP concentrations, size fractions, and exposure duration. Their results showed that TMP ingestion by *E. fetida* altered the bioaccumulation of Zn, Cd, and Pb, leading to the stimulation of oxidative stress ($p < 0.05$). *E. fetida* is a species of epigeic earthworm that is widely utilized in vermiculture and vermicomposting due to its efficient decomposition of organic matter (Karmegam et al., 2019). It is also used frequently in ecotoxicological studies (Brown et al., 2008). The body of *E. fetida* is cylindrical and consists of closely-paired segments exhibiting varying colors, ranging from purple to red. The typical length of an *E. fetida* falls within the range of 35 to 70 mm, with a composition of 20 to 100 segments. This species has a high reproduction and a relatively short juvenile period (7 to 8 weeks). It exhibits a rapid growth rate, possesses a relatively short juvenile period lasting 7 to 8 weeks, demonstrates a high reproductive capacity and is capable of consuming a wide range of organic materials (Gupta & Garg, 2017). MPs, particularly those derived from tire wear, have emerged as a pressing environmental concern due to their widespread distribution and potential ecological consequences. Soil-dwelling organisms, such as earthworms, which play a crucial role in soil health and nutrient cycling, are particularly vulnerable to the impacts of MPs. *Eisenia fetida*, a common epigeic earthworm species, is frequently employed in ecotoxicological studies to evaluate the environmental risks associated with pollutants, including TMPs. This study seeks to extend the current understanding by investigating how varying concentrations of TMPs affect the bioaccumulation of PTEs in *E. fetida* under controlled experimental conditions. To achieve this, TMPs were incrementally introduced into artificial soil inhabited by earthworms, with concentrations reaching up to 200 mg TMP per gram of soil, to assess PTEs accumulation in both the earthworms and the surrounding soil.

2. Materials and Methods

2.1 Test earthworms

For this study, *E. fetida* earthworms were obtained from the Isfahan Municipal Waste Management Organization (http://pasmand.isfahan.ir). The earthworms selected were healthy, adult specimens aged over two months, with body weights ranging between 0.3 and 0.5 g. Subsequently, the selected earthworms were introduced into the study soil, allowing them to acclimate for 24 h. During this period, each earthworm was provided with a diet of 2 g of 105 °C-dried cow manure per 100 g soil.

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2.2 Preparation of TMPs and artificial soil

The artificial soil in this study was prepared following the guidelines provided by the Organization for Economic Co-operation and Development (OECD) Guideline No. 222 (OECD, 2004). The soil composition consisted of kaolinite clay (20% dry weight), quartz sand (70% dry weight), and sphagnum peat (10% dry weight) as the organic component. Sphagnum peat was sourced from a local gardening store, while the kaolinite clay and quartz sand were purchased from the Tooma Mineral Production Company located in Iran (http://toomaminerals.com). The initial pH of the soil was adjusted to neutral using calcium carbonate, and the pH was monitored with a soil-to-water ratio of 1:2.5 to ensure optimal conditions for the earthworms. Room temperature was maintained to simulate real-world conditions. Organic matter content was restricted to the dried cow manure provided as food to avoid introducing additional organic matter that could be consumed by the earthworms and affect the results. For the production of TMPs, the treads of randomly selected used car tires, made from styrene-butadiene rubber or butadiene rubber, were cut into small pieces. These pieces were then rapidly frozen by immersing them in liquid nitrogen, resulting in the formation of frozen particles. Subsequently, the samples were ground using a mortar and pestle until a fine black powder was obtained. To ensure consistent size, the powder was sieved to obtain fractions smaller than 500 μm, making them entirely ingestible by the earthworms (Ragoobur et al., 2022).

2.3 Sample exposures

Sample exposures were prepared by mixing TMPs with artificial soil at four different concentrations: 10, 50, 100, and 200 mg TMP per g of dry artificial soil. These TMP concentrations were selected to reflect the elevated levels often observed in soils adjacent to roads, a consequence of the increasing global expansion of road networks (Rødland et al., 2022; Worek et al., 2022). To maintain consistent soil moisture, the experimental beakers were monitored daily, with moisture levels adjusted to 25% of the field capacity. Each beaker was then inoculated with 10 earthworms. The earthworms were placed on the moist soil surface, assuming that healthy individuals would burrow into the substrate within 15 min. If an earthworm failed to do so within this time frame, it was deemed unhealthy and replaced with a new one. A Blank group consisting of "soil + 10 earthworms" and a Control group consisting of "soil + TMP (at the abovementioned four levels)" were created. All tests were conducted in triplicates to ensure accuracy and reliability. Throughout the experiment, samples were handled in isolation to prevent potential contamination. The light-dark cycle was artificially regulated to provide 16 h of light and 8 h of darkness using 800 lux lighting. The air temperature was maintained within the range of 20 to 22 °C.

2.4 Heavy metal measurement

The earthworms and artificial soil samples were carefully prepared in accordance with established guidelines to ensure compliance. The concentration of PTEs in the soil samples containing different levels of TMPs and species was compared with the control groups after a 14-day period of exposure. Following this exposure, a single living earthworm was selected from each beaker. The selected earthworms were washed with distilled water, air-dried, and frozen using liquid nitrogen before being homogenized into a fine powder to estimate PTEs concentrations. To analyze PTE concentrations in the soil samples, the earthworms were removed, and the remaining soil samples were ground using an agate mortar and sieved (63 μm). 1 g of each sample was digested in 20 mL of 65% nitric acid solution (Merck) and 5 mL of hydrogen peroxide solution. After 48 h under a fume hood, the samples were heated for 5 h at 70 °C. The air-cooled samples were then distilled with distilled water twice, filtered using Whatman Grade 42, and reached a volume of 25 mL. The concentration of PTEs was determined by inductively coupled plasma-mass spectroscopy (ICP-MS). The studied PTEs were As, Cd, Cr, Al, Pb, Zn, tin Sn, and Mo which showed to be a common element in similar studies or changed significantly in the studied species and soil. The concentration of PTEs in the soil and species was reported in mg/kg and mg/L (and μg/L), respectively. The detection limits for Cr, Sn, Al, Pb, Zn, and Mo in soil were 1, 0.1, 100, 1, 1, and 0.5 mg/kg, respectively. For earthworm samples, detection limits were 0.1, 0.001, 0.01, 0.001, 0.01, and 0.001 mg/L, respectively.

2.5 Data analysis

The data were checked for variance equality (Odoi et al., 2022) and normality (Tutorials, 2021). We assessed the statistical difference in the concentration of PTEs between exposed species, between exposed and Blank species, and between different earthworm-affected and Control soils using the one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test. Differences were considered statistically significant at $p < 0.05$. This analysis compared the PTE levels between the exposed earthworm species, between the exposed species and the control (Blank) species, and among different soil samples affected by earthworm activity versus control soils. The correlation analysis (at $p < 0.05$ and $p < 0.01$) was also used to analyze the relationship between the accumulation of different PTEs in the body of earthworms, between the earthworm-affected soil samples as well as between the soil and earthworms. Furthermore, we utilized Fourier-Transform Infrared Spectroscopy (FTIR) to investigate the chemical composition and molecular structure of TMPs across various exposure treatments. This technique provided insights into the specific interactions between TMPs and the soil environment, enhancing our understanding of how these elements contribute to PTE accumulation in earthworms and the subsequent effects on soil health.

3. Results and Discussion

The concentrations of PTEs in earthworms from the Blank and TMP-contaminated soils were measured and compared

(Figure 1). As was the only PTE whose mean concentration ranged between 0.047 and 0.063 mg/Kg, with no significant variation between the Blank and TMP-treated soil samples. However, a statistically significant increase (*p-value* < 0.05) in the concentrations of Cd, Cr, and Sn was observed in earthworms exposed to TMP-containing soils to the Blank. The mean concentration of Cd in the Blank was 7.09 ± 0.69 µg/L while it was 7.13 ± 2.67 µg/L in the T-10 exposed species, and remained within a statistically similar range of 9.75-10.08 in the T-50, T-100, and T-200 treatments. On the other hand, the mean concentrations of Cr and Sn showed significant differences among every treatment, exhibiting a steady increase from the Blank to the T-200 treatment. The mean concentration of Cr increased from 0.010 ± 0.001 mg/L in the Blank to 0.057 ± 0.006 mg/L in the T-200 group and the accumulation of Sn ranged from 14.07 ± 1.09 µg/L (in the Blank) to 130.44 ± 4.43 µg/L (in the T-200). The concentrations of Al and Mo remained statistically similar between the Blank and T-10 treatments, ranging from 1.48-1.99 mg/L and 7.80-8.63 µg/L, respectively. The accumulation of Zn in the species' body was lower than 0.360 mg/L and statistically similar in the Blank, T-50, and T-100 treatments. However, a sharp increase in Zn concentration was observed in the T-100 and T-200 treatments, reaching 2.46 ± 1.74 mg/L and 3.96 ± 3.17 mg/L, respectively. Based on the results, the accumulation of the majority of PTEs, including Cr, Sn, Al, Pb, Zn, and Mo, significantly increased with TMP concentrations exceeding 100 mg TMP/g soil.

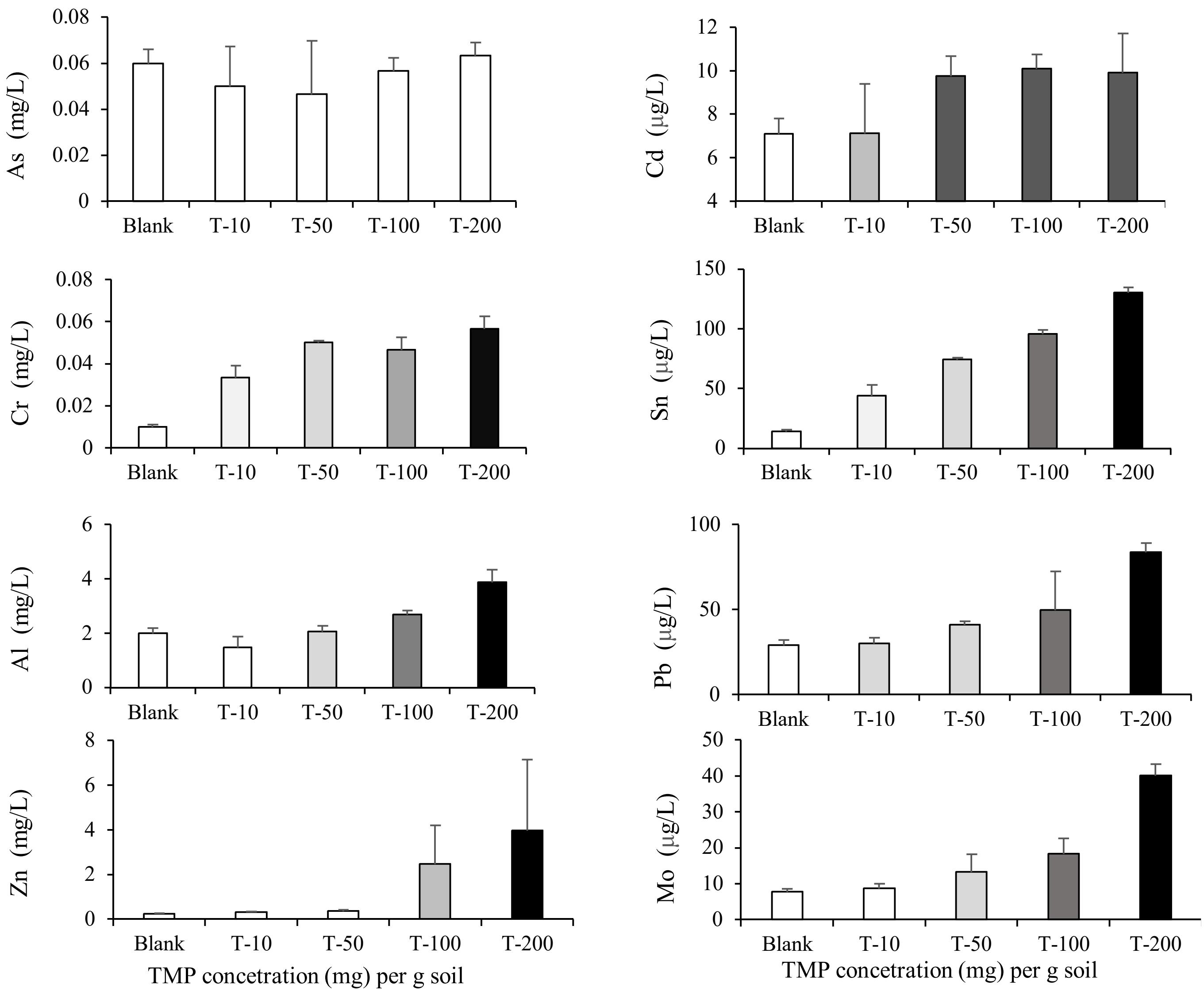

Figure 1. The concentration of PTEs in the species after 14 exposures to different levels of TMPs from 0 to 200 mg/g soil. Different colors indicate significant differences at 0.05

The concentration of PTEs in the Control group and earthworm-inhabited soil samples were measured and compared (Figure 2). Results showed varying PTE concentrations and changing trends among different treatments. The mean concentration of As and Pb was statistically similar in the control, T-10, and T-50 soils, ranging from 14.33 to 19.80 mg/kg and 21.00 to 26.33 mg/kg, respectively. The mean concentration of Cr was also statistically similar in the Control, T-10, T-50, and T-100 with a mean of 6.58 ± 6.33 mg/kg while that of T-200 was significantly higher (12.00 ± 8.55 mg/kg). The statistical difference between T-200 and other soil samples was also significant for As, Cd, Sn, Zn, and Mo at the 0.05 confidence level. The highest mean concentration of Sn, Cr, and Zn of 0.70 ± 0.01, 12.0 ± 8.55, and 3357.33 ± 1328.66 mg/kg was observed in T-200. The Control soil, however, exhibited the highest mean concentration of As (19.80 mg/kg), Al (22186 mg/kg), and Mo (2.2 mg/kg). The only steady trend of changes in PTE concentration was observed in As and Al whose levels decreased with increasing concentration of TMP from 0 to 200 mg/ g soil. The FTIR analysis revealed significant peaks in specific wavenumber ranges (Figure 3). In the soil and soil + earthworms, strong peaks were observed between 974-1200 cm^{-1}, with distinct peaks at 1089 cm^{-1} and 1107 cm^{-1}. There were also common peaks at 690 cm^{-1} and within the range of 780-793 cm^{-1} between different experiments. Multiple peaks between 1100-1400 cm^{-1} indicated the presence of strengthened double bonds between carbon and oxygen (C = O) and simple carbon-hydrogen bonds (C-H), causing spectral distortion. The presence of TMPs in the soil samples led to weaker peaks in the 1300 cm^{-1}, 1600 cm^{-1}, and 1800 cm^{-1} ranges, signifying a reduction in aliphatic characteristics. Different functional groups were associated with peaks in these regions, such as the bending of the simple carbon-hydrogen bond (C-H) in soil with microplastics (derived from alkanes), hydroxyl functional groups (OH-), and sulfonic groups (S = O). In the 1600-1800 cm^{-1} range, the presence of unsaturated carbon-carbon bonds (C = C) and hydroxyl functional groups increased spectral distortion, also linked to alkanes and alkenes in TMPs. Finally, a broad peak at 3600 cm^{-1} in the TMPs sample indicated tension in bonds related to hydroxyl groups or the presence of primary amine and aliphatic groups (N-H).

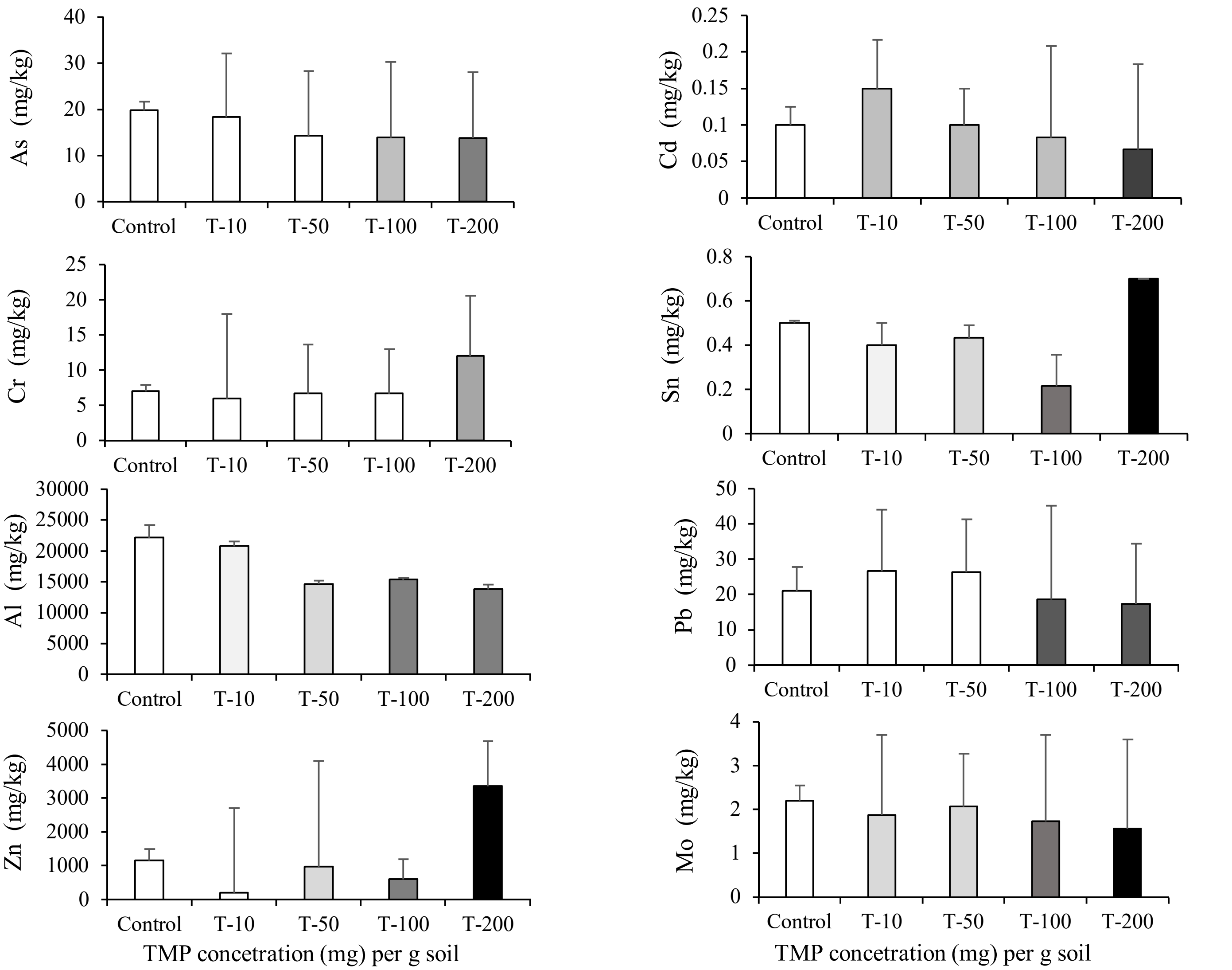

Figure 2. Concentration of PTEs in the TMP-containing soil samples (from 0 to 200 mg TMP/g soil) after rearing earthworms for 14 days. Different colors indicate significant differences at 0.05

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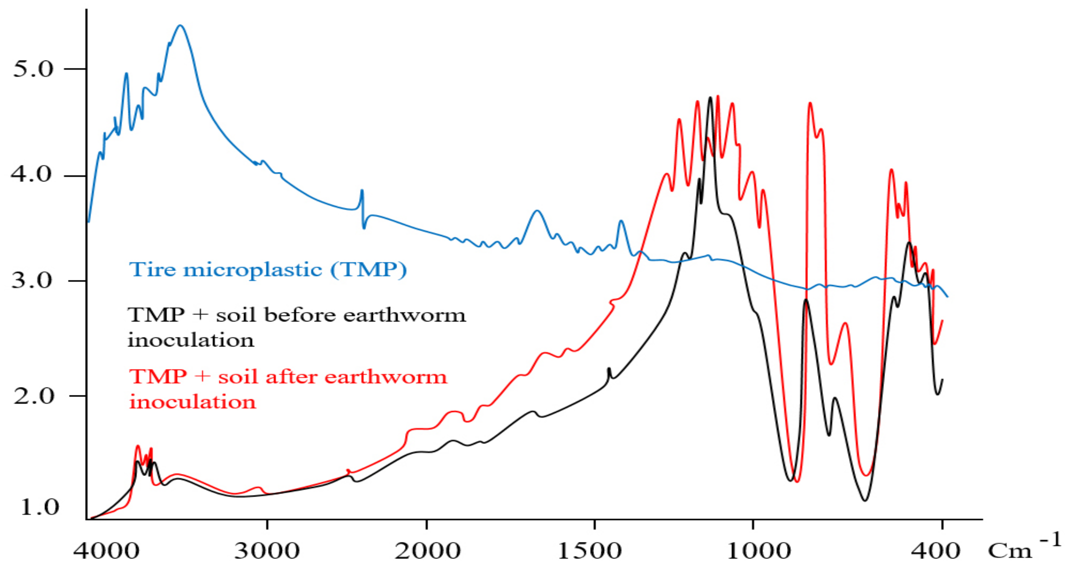

Figure 3. Results of the Fourier-Transform Infrared Spectroscopy (FTIR) performed on different combinations of TMP, soil, and earthworm

A correlation analysis of PTE concentration was conducted among species, soil samples, and species with soil samples after a 14-day exposure period (Table 1). Except for the species' mean concentration of As, which remained statistically constant across treatments, and the correlation between Cd and Zn, all other correlation coefficients were positive and statistically significant at both the 0.01 and 0.05 confidence levels. This indicates an increasing accumulation of PTEs in the species' bodies as the concentration of PTE-containing TMP in the soil rises to 200 mg TMP/g soil. In contrast to the species, the associations between PTE concentrations in soil samples were mostly insignificant and, in some cases, contradictory. For example, the relationship between Al and As (r = 0.955, *p-value* < 0.01) and Zn-Sn (r = 0.632, *p-value* < 0.01) was significantly positive, while the associations of Zn-As (r = -0.527, *p-value* < 0.05) and Al-Cr (r = -0.432, *p-value* < 0.05) were significantly negative (Table 1). The correlation coefficients for PTE concentrations between species and soil samples are provided along the diagonal axis of Table 1. The results showed that the increasing accumulation of Al, Cd, and Mo in the species' bodies resulted in their depletion in the soil samples, as indicated by the significant negative associations (−0.566 < r < −0.670, *p-value* < 0.05 and 0.01). The concentration of Zn, however, was found to increase in both the species and soil samples across different treatments (r = 0.567, *p-value* < 0.05). Additionally, there were no significant associations observed between Pb, Sn, Cr, and As concentrations in the species and soil samples. The increasing MP presence in the environment is one of the serious hazards of the present era, which can severely affect the functioning and resilience of terrestrial and marine ecosystems and their species. Earthworms play a crucial role in soil health through processes such as aeration, nutrient cycling, and organic matter decomposition. However, when earthworms accumulate high levels of PTEs, their health can decline, which may lead to reduced soil functionality, including diminished nutrient availability and compromised soil structure. This accumulation can also pose serious health risks due to bioaccumulation and biomagnification up the food chain (He et al., 2021). Studies have shown that the accumulation of PTEs in the body of earthworms occurs more easily when they are in contact with MPs compared to when they are in combination with soil (Zhou et al., 2020). This study also demonstrated significant differences in the accumulation of PTEs in *E. fetida* immediately upon the addition of MPs from tire sources which is in line with those of previous studies (Lackmann et al., 2022; Sheng et al., 2021) which confirmed the TMP contribution to increasing PTE uptake by soil-dwelling earthworms like *E. fetida*. This result could be due to the high capacity of TMPs to adsorb PTEs through electrostatic and physical attractions as well as cation-π bonding interaction. Moreover, this study classifies TMPs as a type of MP that are easily preferred and ingested by soil-dwelling earthworms because their flexibility, taste, and stability affect their ingestion preference (Wang et al., 2022). This conclusion can be also observed in Sheng et al. (2021) which identified the easy ingestion of TMP by *E. fetida*. Accordingly, the presence of TMPs, whose production volume is increasing in recent years, can be recognized as a threat to terrestrial ecosystems and food chains that begin with soil-dwelling earthworms. The findings of this study confirm many previous studies. Our results showed insignificant changes in the absorption and accumulation of As in *E. fetida* with increasing concentrations of TMPs. The Wang et al. (2019) study also confirmed this finding, showing that ingestion of PVC MPs by the earthworm *Metaphire californica* will not increase the body accumulation of As nor impose significant toxic effects. However, the species accumulation of Cd increased significantly after the addition of the initial level of 10 mg TMP/g soil. Huang et al. (2021) and Zhou et al. (2020) also achieved similar results, suggesting that the absorption of Cd attached to MPs (at the minimum concentration of 2 mg/kg) is much higher than the presence of this metal alone in the soil, leading to multiple oxidative stress symptoms in earthworms. Fourie et al. (2007) also determined the sensitivity of epigeic species such as *E. fetida.* is significantly higher to Cd contamination than other earthworm species. Up to a concentration of 100 mg TMP/g soil, no significant changes were observed in the accumulation of Zn in *E. fetida*, which is similar to studies like Hodson et al. (2017) who showed that Zn-containing high-density polyethylene MPs (up to a concentration of 236-4505 mg/kg) do not affect the accumulation and toxicity of Zn on the earthworm *Lumbricus terrestris*. However, the accumulation of Zn in the body of *E. fetida* became statistically significant at concentrations higher than 100 mg TMP/g soil. Although this TMP concentration is not commonly found in nature, studies like Rødland et al. (2022) have shown that with the increasing development of road networks and vehicle production, these levels will not be unexpected as Zn is an essential activator element in tire construction. It is important to note that at low MP concentrations, the adsorption of PTEs may be limited, resulting in lower uptake by earthworms. Conversely, as MP concentrations increase, they can act as vectors for PTEs, leading to higher accumulation of these elements in earthworms. It is also possible that the ingestion of MPs causes gut blockage, oxidative stress, and alterations in enzyme activity in earthworms, further exacerbating metal uptake at higher MP levels. Moreover, the flexibility, taste, and stability of TMPs make them more susceptible to ingestion by earthworms, contributing to the increased accumulation of metals at elevated TMP concentrations. Pb imposes significant adverse impacts on *E. fetida* (Yadav et al., 2023) and is also widely used in tire production as a pigment and heat stabilizer (Sheng et al., 2021). The results of this study showed that concentrations of TMP exceeding 10 mg TMP/g soil significantly increased the accumulation of Pb in *E. fetida*. The same finding was also reported by Pandey et al. (2016) on *Metaphire posthuma* who observed a significant increase in Pb accumulation immediately after its introduction to the soil compared to the control sample. The statistical increase in the accumulation of Cr was observed following the addition of 10 mg/g TMP. In uncontaminated soils, *E. fetida* accumulated less than 0.01 mg/L Cr, which then increased significantly to 0.033 and 0.056 in the T-10 and T-200 experiments, respectively. These findings suggest that TMPs can serve as vectors for enhancing the body accumulation of Cr in *E. fetida*. Another earthworm species, *E. foetida*, also displayed a high potential for uptake and accumulation of Cr from MP, leading to increased mortality in the species (Mai et al., 2023). Sn and Mo are among the elements that have not been investigated regarding their absorption and accumulation in earthworms in similar studies. However, the present study showed that with an increase in TMP concentrations, there was a significant steady increase in their accumulation in *E. fetida*. Regarding the Mo accumulation in soil-dwelling earthworms, Van Gestel et al. (2011) demonstrated a linear relationship between increasing Mo concentration in the soil and its accumulation in the body of *E. andrei,* indicating that this species cannot regulate the body concentration of Mo. Considering the levels of TMPs and PTE accumulation found in this study, disturbances in reproduction, growth, and survival rates of *E. fetida* are expected as observed even in lower concentrations of Cd (Lapinski & Rosciszewska, 2008), Pb (Žaltauskaitė et al., 2020), and Zn (Domínguez-Crespo et al., 2012). However, when interpreting these results, it should be noted that changes in soil parameters may alter the ingestion of each of these PTEs in the earthworm body.

Table 1. Correlation results of PTE concentrations between soil samples with different TMP loads, between species reared in different TMP-containing soils and between two groups

	As	Cd	Cr	Sn	Al	Pb	Zn	Mo
As	-0.104	0.343	-0.281	-0.090	0.955**	0.402*	-0.527*	0.142
Cd	0.170	-0.566*	-0.209	0.027	0.365	0.064	-0.482	0.540*
Cr	-0.109	0.513*	0.344	0.443*	-0.432*	-0.258	0.512**	-0.038
Sn	0.177	0.637**	0.890**	0.196	-0.259	-0.269	0.632**	0.041
Al	0.339	0.525**	0.592*	0.816**	-0.670**	0.447*	-0.562*	0.029
Pb	0.182	0.445*	0.716**	0.840**	0.862**	-0.381	-0.175	-0.317
Zn	0.263	0.117	0.533*	0.645**	0.578**	0.700**	0.567*	-0.557
Mo	0.324	0.456*	0.671**	0.882**	0.934**	0.859**	0.681**	-0.651**

During the exposure process, the amount of the soil PTE content might change due to PTE accumulation in the earthworm and adsorption/desorption cycles attributed to the MP breakdown and size change (Sheng et al., 2021). In most of the PTEs studied in the present research, the amount of PTE decreased with increasing concentration of TMP, suggesting that they are accumulated in the earthworm body and removed from the soil. For instance, the mean concentration of Cd in the soil decreased to the lowest value of 0.066 mg/kg in T-200 while it accumulated up to 10 μg/L in the species of this experimental group. Similarly, due to the accumulation in the species body, the lowest Al, As, Mo, and Pb concentrations were observed in the T-200 which were assumed to have higher PTE concentrations than the lower TMP-content groups. According to Table 1, this negative trend was statistically significant for Cd (r = -0.566, *p-value* < 0.05), Al (r = -0.670, *p-value* < 0.01), and Mo (r = -0.651, *p-value* < 0.05). Except for these metals, the soil mean concentration of Sn, Cr, and Zn exhibited a sharp increase after the addition of 200 mg/g TMP, indicating that the

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concentration of these metals increased statistically significantly (*p-value* < 0.05) in both the species and soil with the increasing addition of TMP up to 200 mg/g. This finding should be due to the isolated sampling of the earthworm-inhabited soil from areas where the species excrete their casts. Hence, it can be concluded that earthworm activity such as burrowing and formation of casts might create PTE high-concentration hotspots. However, it should be noted that the mobility and bioavailability of MP-associated PTEs can be also a function of highly variable physicochemical characteristics of the soil.

4. Conclusion

This study assessed the interaction between TMP and PTE uptake in *E. fetida*. The results revealed a significant accumulation of Cr, Sn, and Pb (*p-value* < 0.05) in earthworms exposed to increasing TMP concentrations (0 to 200 mg/ g) during a 14-day experiment. The accumulation of Zn occurred at TMP concentrations above 100 mg/g, while Cd levels remained statistically constant after 50 mg/g TMP exposure. Except for Al, most PTEs showed positive and significant correlation coefficients between soil TMP content and PTE accumulation in the earthworms, indicating a high potential for *E. fetida* PTEs uptake. The inability to metabolize these elements presents risks to the species' growth and survival and, potentially affects other organisms in the food web. Changes in PTE concentration in earthworm-inhabited soils yielded contradictory findings, with most showing significant negative correlations. This suggests that PTEs were progressively removed from the soil as they accumulated in the earthworms. This study utilized an artificial soil sample and the effects of soil physico-chemical characteristics on the mobility and bioavailability of MP-associated PTEs remain open for future studies. Importantly, the demonstrated ability of *E. fetida* to accumulate high concentrations of heavy metals like Cr, Pb, and Sn, especially in the presence of TMPs, highlights the critical role earthworms can play in soil bioremediation efforts. Incorporating earthworms into contaminated environments could enhance the removal of heavy metals from soils, particularly in areas impacted by MP pollution. This study recognizes that the use of artificial soil may limit the applicability of our findings to natural environments. Therefore, the effects of soil physicochemical characteristics on the mobility and bioavailability of MP-associated PTEs remain open for future studies, highlighting the complexity of soil ecosystems and interactions with other soil organisms.

Authors' Contributions

Najla Hamidianfar: Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing-original draft. Atefeh Chamani: Conceptualization; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Validation; Writing-review & editing. Mitra Ataabadi: Conceptualization; Resources; Supervision; Writing-review & editing. Rasool Zamani-Ahmadmahmoodi: Software; Writing-review & editing.

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Conflicts of Interest

No potential conflict of interest was reported by the author(s).

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Ethical considerations

The proposal for the present study was reviewed and approved by the Research Committee of Isfahan (Khorasgan) Branch, Islamic Azad University (Research code: 162530188).

Using artificial intelligence

This research does not utilize any artificial intelligence (AI) techniques.

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