

# Article



# Revealing the Combined Effects of Microplastics, Zn, and Cd on Soil Properties and Metal Accumulation by Leafy Vegetables: A Preliminary Investigation by a Laboratory Experiment

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Abstract: A pot experiment was carried out to investigate the effects of polyethylene (PE), a broadly utilized polymer type, on soil properties and lettuce growth. Two Zn- and Cd-contaminated soil samples were obtained from urban and rural areas of Greece, respectively. PE fragments (<5 mm) were added at different concentrations (2.5%, 5% w/w). Lettuce seeds were then planted in the pots in a completely randomized experiment. Plant growth patterns and tissue metal accumulation were investigated. The presence of PE in soils resulted in a reduction in pH, significantly enhanced the organic matter content, and increased the cation-exchange capacity. The availability of both metals was also increased. Metal migration from soil to plant was determined using appropriate tools and indexes. A higher metal concentration was detected in lettuce roots compared with that in the edible leaves. The presence of PE MPs (2.5% w/w) increased the amount of available Zn more than that of Cd in highly contaminated soils. When PE MPs were added to agricultural soil, Zn concentrations increased in the plant leaves by 9.1% (2.5% *w/w*) and 21.1% (5% *w/w*). Considering that both metals and microplastics cannot be easily and quickly degraded, the fact that the less toxic metal is more available to plants is encouraging. Taking into account the physicochemical soil features, decision makers may be able to limit the risks to human health from the coexistence of heavy metals and microplastics in soils.

Keywords: contamination factor; lettuce; uptake; agricultural and urban soils

# 1. Introduction

In recent years, plastics have been used in many applications in our daily lives, as they are an easy and economical solution for everyday issues in our home and workplace. Given their massive global production and indiscriminate use, the amount of plastic entering the human environment, particularly the soil, is exceptionally high [1]. Plastic waste accumulates in most parts of the world, taking many years to decompose, as it is corrosion-resistant, chemically stable, and difficult to degrade [2]. When plastics reach the soil, they are frequently broken down into smaller (<5 mm) particles known as microplastics (MPs) due to physical and chemical erosion as well as the impact of UV light [3]. The term microplastics (MPs) covers a large group of plastic materials that include a wide range of polymers with varying chemical compositions, sizes, and dimensions [4]. Microplastics refer to plastic particles, fragments, films, or fibers with a diameter less than 5 mm [5]. MPs are divided into two types: primary MPs, which are intentionally made in sizes less than 5 mm, and secondary MPs, which are formed through the fragmentation of larger

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plastics or primary MPs [6]. MPs have been proven to influence both physical and chemical soil attributes, as well as the soil's microbial composition and health. The chemical composition of MPs, together with their size and shape, all have a significant impact on the variability of soils' physicochemical properties [7]. Multiple studies have been carried out regarding the effects of MPs on soil environmental variables, yielding often opposing results [8]. Previous studies have shown that the addition of polyethylene, polypropylene, and polystyrene reduces the bulk density of soil [9]. Furthermore, alterations of soil acidity owing to the presence of MPs or even smaller nanoplastics (NPs) have been repeatedly reported. The acidity of soils is a crucial parameter that defines the distinct properties and functionality of soil systems. Numerous studies have shown that the application of MPs or NPs has the potential to increase soil pH; however, a decrease in soil acidity or even a moderate effect has been observed in other instances. The researchers Wang et al. [10] showed that the presence of polylactic acid (PLA) and high-density polyethylene could increase soil pH, while Boots et al. [11] found that the long-term persistence of high-density polyethylene (HDPE) decreased soil pH values. It is widely recognized that in acidic soils, there is reduced adsorption and increased metal mobility. This phenomenon can be attributed to the increased competition between hydrogen cations and dissolved metals for negatively charged surfaces, and this could lead to an increase in the availability of both toxic and trace elements in soils and plants. On the other hand, in alkaline soils, the formation of strong organometallic complexes is favored, resulting in a reduction in the mobility and therefore the availability of these elements, which could lead to severe nutrient deficiencies for plants [11]. The chemical reactions occurring between metals and plastic particles appear to exhibit similarities to those observed between metals and soil organic matter [3]. By altering soil acidity, MPs and NPs can affect the mobility and adsorption of metals and metalloids, the formation and stability of organometallic complexes, bond strength, and the chemical selectivity of soil components [7]. MPs and NPs also have variable effects on soil organic matter, which is essential for both soil fertility and plant nutrition [8]. Studies have shown that the addition of polyethylene decreased soil organic carbon (OC) content [12], while the addition of 2% (w/w) PLA significantly increased soil OC [13]. These contradictory results suggest that the effects of MPs on the soil environment may be regulated by several factors, such as the MP properties (e.g., polymer type, concentration, particle size, and shape), as well as different soil and local climatic conditions [14].

# 1.1. Identity and Distinguishing Features of the Most Common Microplastics in Soil Environments

Shi and his colleagues in their study [15] explored the impact of three types of microplastics-polyethylene (PE), polystyrene (PS), and polypropylene (PP)-on the early growth of tomato seeds using a combined approach of oxidative stress and nutritional quality analysis. The outcomes indicated that all the different MPs exhibited negative impacts on the tomato seedlings' physiological and metabolic functions, root development, seed germination percentage, and germination index. PE was proven to be the most toxic, while PP was the least toxic. Considering the two most common plastics in soils, PE and PP, it has been documented that polyethylene is carcinogenic at the class 3 level, according to the World Health Organization [16]. Both PE and PP are frequently and widely dispersed in the agricultural environment and in diverse types of microplastics, as claimed by Hu et al. [17]. In their study, Qi et al. [17] investigated the effects of low-density polyethylene (LDPE) and biodegradable starch-based microplastic films of different sizes on potted wheat. It was found that biodegradable-plastic soil-cover films had a particularly significant effect on wheat growth compared with PE. In another study conducted by Machado et al. [18], it was found that polyester fibers and polyamide beads had a positive influence on the growth of spring onions compared with other polymer types (polyethylene, polyester terephthalate, polypropylene, and polystyrene). The environmental pathway of microplastics in soils was also revealed by Gan et al. [19] in a study about their classification, sources, and fate, as well as their effects on cultivated plants.

#### 1.2. Effects of Microplastics on Metal Uptake by Plants

To prevent potential risks, the first thought is to remove microplastic residues from green vegetables using appropriate washing techniques so that they cannot be passed on to the human body [20]. Further efforts are underway to understand the mechanisms of the coexistence of microplastics, toxic elements, and trace elements in the soil [9]. It has been revealed that the presence of PEs together with Cd in soil is able to cause synergistic toxicity with regard to plant growth, such as the suppression of photosynthesis and increased oxidative damage [21]. Various MP polymer types can significantly increase Cd accumulation in plant shoots and roots, while PE appears to have a higher promotive effect. That is mainly explained by the fact that PEs cause a decrease in pH followed by an increase in Cd bioavailability [22]. Huang et al. [23] noticed a significant increase in Cd availability by adding various quantities of PEs to soils. Furthermore, it was observed that the addition of MPs modified the physicochemical properties of the soils, causing an increase in Cd bioavailability and uptake by the plants [7]. In their experiments, Wang et al. [24] examined the effect of HDPE (high-density PE) and PS on maize plants and found that PS caused greater phytotoxicity to the plants. Both HDPE and PS increased the DTPAextractable Cd content in the soil. Compared with HDPE, PS appears to have more pronounced effects on Cd bioavailability and plant growth inhibition, indicating a higher risk in soil-plant systems [19]. The effects of various MPs with different chemical compositions on soil properties and the availability of Cu, Zn, Cd, and Pb were investigated by Wen et al. [25]. Linear low-density polyethylene (LLDPE), polyamide (PA), polyurethane (PU), polystyrene (PS), and low-density polyethylene (LDPE) MPs caused a decrease in soil pH, while they increased soil organic matter and cation-exchange capacity. These alterations in both soil parameters and the metal adsorption and desorption on the solid surface of MPs determine their availability [26]. It is well known that the addition of certain materials, organic or inorganic, can modulate the mobility and metal availability to plants [27– 29]. At the same time, the concentration of MPs in the soil also plays a decisive role in metal mobility. The absorption of harmful metals or beneficial trace elements by plants cultivated in MP-contaminated soils in varying quantities and chemical compositions has been at the epicenter of recent research [30-33].

In the present study, laboratory experiments were conducted to investigate the effects of polyethylene on the metal intake and accumulation by lettuce plants. The primary objectives and aims of the present study were to detect the impact of PE MPs on the physicochemical soil properties and to reveal their effects on metal availability and concentration in moderately and heavily contaminated soils in Mediterranean urban and rural areas.

#### 2. Materials and Methods

#### 2.1. Sampling Areas and Soil Sample Preparation

Two different alkaline soil types from rural and urban areas of central and northern Greece, respectively, were used for our study. Urban soil samples were collected from Thessaloniki city's metropolitan area, whereas rural samples were taken from Almyros town [34] in the region of Thessaly [35].

Specific handling techniques were used during the soil sampling to measure the content of heavy metals. More specifically, a wooden shovel and a two-meter radius were used to obtain six sub-samples from each main sample. The samples were then transferred to the Soil Science Laboratory at the Aristotle University of Thessaloniki, where they were air-dried and prepared for further physical and chemical analysis.

# 2.2. Preparation of Microplastics

Polyethylene (PE), a common polymer type present in the environment, was selected as a plastic contaminant for the purpose of this research. Plastic particles were obtained by manually cutting commercially available transparent polyethylene bags into smaller pieces [8]. These pieces were then ground and separated by sieving using a 0.5 mm sieve to obtain microplastics of appropriate dimensions (<5 mm). The MPs were then washed with NaClO 0.01 M solution in order to remove any impurities and to inhibit potential microbial activity. The MPs were subsequently incorporated into the soil samples and left to incubate for several days.

#### 2.3. Pot Experiments

Equal quantities of the two metal-contaminated soil samples were placed in plastic pots with a base area of 26 cm<sup>2</sup> and a height of 16 cm. To achieve the best possible homogeneity, microplastics were added at two different concentrations (2.5% and 5% w/w) and mechanically mixed into the soil. Irrigation (up to 70% saturation) was carried out to maintain an appropriate level of moisture content, and the pots were left to incubate. Ten days later, lettuce seedlings that had been cultivated in the same soil were transferred and planted in the pots. A completely randomized experiment was set up, consisting of 2 soils (urban and rural) × 3 MP treatments (0%, 2.5%, and 5% w/w) × 4 replicates for a total of 24 pots. Experiments were carried out in February 2023 at the Aristotle University of Thessaloniki's Soil Science Laboratory and the University of Thessaly's Analytical Chemistry Laboratory. After 45 days, the lettuce plants were harvested, dried in an oven, and subjected to chemical analysis.

#### 2.4. Analyses of Soil

After air drying, soil samples were ground and passed through a 2 mm sieve. They were subjected to the following soil analyses [36,37]: The soil reaction (pH) and electrical conductivity (EC) values were determined using a mixture fixed by soil and distilled water at a ratio of 1:1. The soil mechanical composition was evaluated by the Bougioukos method, and the percentage (%) of CaCO<sub>3</sub> by the Bernard method. The modified Walkley–Black method was used to calculate the percentage of organic matter in the soils. After extraction with 1 N ammonium acetate solution (pH 7.0), a Sherwood's flame photometer was used to determine the percentage of exchangeable cations, while the sum of them was used for calculating the soil Cation-Exchange Capacity value. For the evaluation of Zn and Cd, water-soluble concentrations of a dilute CaCl<sub>2</sub> solution were used [29]. The available and total metal concentrations were determined using soil-extraction methods with DTPA and HCl:HNO<sub>3</sub> in a 3:1 ratio (Aqua Regia) solution [38]. Metals were quantified using a Perkin Elmer Analyst 700 atomic absorption spectrometer (AAS). The CRM 141R soil standard was used for the method validation. Metal analysis accuracy ranged from 9.1% to 11%, with detection limits of 0.01 and 0.85 mg/kg for Cd and Zn, respectively.

#### 2.5. Chemical Analyses of Lettuce Tissues

Lettuce samples were weighed to determine the fresh weight and then put in paper bags to dry. Following an extraction using the Aqua Regia method in a closed digestion system for 4.5 h, Zn and Cd were determined for each sample's root and aboveground portion [27,29]. Using an atomic absorption spectrophotometer, the metals were quantified after being diluted in 25 mL volumetric flasks. The analytical procedure was validated using a NIST-certified standard tomato sample.

#### 2.6. Statistical Analysis, Soil Pollution Indices and Metal Mobility Indicators

To assess and characterize the contamination level of both soils used in the present study, three typical soil pollution indices were used to classify the soil samples, namely, Contamination Factor (CF), Geo-accumulation Index (Igeo), and Bioavailability Factor (BF) [29]. Furthermore, to reveal the metal behavior and distribution between the soilplant systems under exposure to MPs, three appropriate indicators were calculated: Transfer Coefficient (TC), Bioaccumulation Factor (BAF), and Translocation Factor (TF) [30]. Microsoft Office Excel statistical packages (Microsoft, Redmond, WA, USA) and SPSS statistical software (IBM, Armonk, NY, United States) were used for data management and processing. For each data group, the mean, median, minimum, and maximum values, as well as the standard deviation, were calculated. Identifying statistically significant differences at the 0.01 and 0.05 levels was accomplished using the ANOVA method. Additionally, the data were analyzed using the *t*-test by repeatedly comparing value pairs.

## 3. Results and Discussion

## 3.1. Influence of Microplastics on Soil Chemical Properties

The physical and chemical properties of the study's soil samples are shown in Table 1. Urban and agricultural soils both have an alkaline pH and comparable levels of electrical conductivity. The application of fertilizers during agricultural activities and crop cultivation is likely responsible for the agricultural soil's comparatively high electrical conductivity rating.

Table 1. Physicochemical properties of the soil samples.

| pH                                       | EC (Electric<br>Conductivity)<br>(µS/cm) | OM (Organio<br>Matter)<br>(%) | c CEC (Cation-Exchange<br>Capacity)<br>(cmold/kg) | Clay<br>(%)    | Texture           | Cd Zn<br>(mg/kg)(mg/kg) |
|--|--|-------------------------------|---|----------------|-------------------|-------------------------|
| Soil 1 (Agri-<br>cultural) $7.4 \pm 0.3$ | $2234 \pm 54$                            | $2.8 \pm 0.2$                 | 30.4  | $47 \pm 1.1$   | CL (Clay<br>Loam) | $0.9 \pm 0.174 \pm 1.2$ |
| Soil 2 (Urban) 8.1 ± 0.5                 | $2093 \pm 49$                            | $2.1 \pm 0.4$                 | 26.5  | $46.5 \pm 2.1$ | CL                | $1.1 \pm 0.379 \pm 0.6$ |

However, urban soil is usually affected by a variety of anthropogenic activities, including horticultural decorative landscaping in city flowerbeds [34,36]. The presence of microplastics in the soil samples was the decisive factor in altering the values of their chemical properties. Zhao et al. [37] investigated the effect of different polymers with different shapes on soil pH by conducting a 21-day incubation experiment. A decrease in the pH value was initially observed when polymers were incorporated into the soil samples; however, the pH value increased over time. In the current investigation, the addition of both amounts of PE MPs to soil samples resulted in a decrease in soil reactivity. The pH value of agricultural soil 1 was reduced by 5.4% and 2.9%, respectively, after 2.5% and 5% (w/w) PE MPs were applied, as determined immediately before planting the lettuce seedlings. The corresponding percentages for the urban soil sample were 6.2% and 2.6%, respectively.

Figure 1 depicts the changes in the soils' properties after the addition of PE MPs. The pH of both soils was checked again at the completion of the experiment, and an increase was detected. This is consistent with reports from other studies [37]. Gharahi and Zamani-Ahmadmahmoodi [8] observed that after a 30-day exposure of two soil samples to PET, the pH value decreased. In another study, it was found that soil pH was not significantly affected by MPs when these were added at a low dose; however, the acidity decreased with the addition of high doses of PE and PS MPs and increased with high doses of PLA and PHB MPs [13]. When PE MPs were applied at a lower dose, the pH value decreased more in both soils. The soil pH value is a key factor for achieving metal mobility and for forecasting heavy metal pollution [34].







Figure 1. Effect of polyethylene microplastic (PE MP) levels on the physicochemical properties of the study's soil samples.

During the soil organic matter evaluation, a comparable outcome was obtained. When lower concentrations of PE MPs were added to the soils, a considerable increase of 17.9% and 23.8% was detected. However, one would anticipate that as the amount of microplastics in the soil increased, so would its organic content. The soil organic matter content is increased by adding sludge or wheat straw residues [27,35], which is desirable as it enhances soil fertility. The Cation-Exchange Capacity (CEC) value increased significantly in the first and second soil samples: by 8.6% and 9.8%, respectively.

#### 3.2. Influence of Microplastics on Metal Availability

Figure 2 depicts the variations in water-soluble, available, and pseudo-total Zn and Cd content in the soil samples studied. In both soils, the water-soluble content of Zn increased in excess compared with Cd. When 2.5% w/w PE MPs were added to the first soil, the increase in water-soluble Zn content reached 57.3%, while the Cd concentration increased by more than 33.3%. When 2.5% w/w PE MPs were introduced to soil 2, the water-soluble concentrations of Zn and Cd increased by 38% and 16.6%, respectively. In a relevant study, it was found that the methods using pure water were highly correlated with each other and showed the strongest correlation with agronomic effectiveness [38–40].





**Figure 2.** Effect of polyethylene microplastic (PE MP) levels on Zn and Cd water-soluble, DTPAextractable (available) and Aqua Regia-extractable (total) concentrations in the studied soil samples.

As a result, given the current study's slightly alkaline soils, the water-soluble concentration of metals may indicate the concentration that plants can absorb. The metals' watersoluble concentrations increased; however, it is promising that Zn increased at a significantly higher rate compared to the more hazardous Cd. According to other studies, the addition of microplastics, particularly PE, to the soil at high mixing ratios increased the availability of bivalent lead because it rendered the big aggregates unstable and reduced the rate at which Pb adsorbs onto them [26,41].

Figure 2 also demonstrates the impact of PE MP addition on the extractable quantities of Zn and Cd using the DTPA solution and Aqua Regia. As the total amount of microplastics in both soils rises, so do the amounts of both metals. This is consistent with prior research. In a relevant study, the Cd availability in clay- and sand-based soils was examined following the addition of PU and PP microplastics [42]. In clay soils, accessible Cd was considerably negatively correlated with dissolved organic carbon and pH, whereas in sandy soils, available Cd was strongly negatively correlated with Fe (II). The synergistic toxicity generated by the presence of PE MPs and metals in the soil samples was out of proportion to the amount of MPs supplied [13]. In both rural and urban soil, the addition of microplastics does not seem to have an impact on the pseudo-total concentration.

# 3.3. Effects of Microplastics on Zn and Cd Levels in Lettuce Plants

Figure 3 depicts the impact of various polyethylene microplastic concentrations on the levels of Zn and Cd in lettuce plants grown in the studied soils. The level of both hazardous and nutritious (trace elements) metals absorbed by the cultivated plants is critical [31,43].





**Figure 3.** Effect of different polyethylene microplastic (PE MP) amounts on the levels of Zn and Cd in lettuce plants grown in the two soil samples.

In agricultural soil 1, the Zn concentration in lettuce roots was higher than that in the leaves. When PE MPs were added to the soil, Zn concentrations increased in the plant's roots and leaves by 11.5% and 9.1% (2.5% w/w) and 26.6% and 21.1% (5% w/w), respectively. Furthermore, in the trials with the second (urban) soil sample, which was less Zn and Cd-contaminated, metals accumulated more in the lettuce roots than in the leaves. Following the addition of PE MPs, the Zn concentration in the roots and leaves rose by

4.1% and 3.4% (2.5% *w/w*) and by 0.2% and 6.8% (5% *w/w*), respectively. It is well known that the amount, chemical composition, shape, and size of microplastics in soils determine their impacts and, consequently, influence the absorption of hazardous or nutritious compounds by cultivated plants [8,44]. It is widely known that the addition of several materials, as well as the modification of certain soil parameters, can affect the plants' uptake of metals [29,45,46].

The effects of microplastics on heavy metal or trace element uptake by plants are of great concern in the scientific community. MPs may increase the accumulation of heavy metals by altering the rhizosphere microorganisms in lettuce plants [47]. Additionally, in research conducted on strawberry plants, it was found that the increased bioavailability of Cd caused by the presence of microplastics was responsible for the observed negative effects on soil properties and plant performance [48].

In the current investigation, MP addition to the first soil sample at a ratio of 2.5% (w/w) resulted in an increase in the Cd content by 11.1% and 7.3% in the roots and leaves, respectively. At a higher ratio of 5% (w/w), the corresponding increase was 11.2% and 10%. The variation of Cd content in the lettuce roots and leaves in the second soil sample was 7.5% and 2.8% (2.5% w/w) and 11.3% and 5.6% (5% w/w), respectively.

Numerous research investigations have demonstrated that metals accumulate at varying levels in different parts of the plant [28,30,49,50].

It is important to note that, in both the rural and urban soils examined, the increase in the total amount of metal accumulation imposed by the presence of microplastics was greater in the roots. However, the leaves, which are consumable plant parts, are also impacted but to a lesser degree.

Although the precise mechanisms that define the behavior of microplastics in soils or plants are not well understood, preliminary data suggest that they might enhance metal mobility and translocation [11]. Although several metals are hazardous, there are essential minerals for the growth of plants, i.e., trace elements. Therefore, the presence of microplastics could enhance nutrient absorption, resulting in increased plant growth [13]. However, high microplastic concentrations may induce toxicity to plants, which could be further aggravated by synergistic effects caused by the coexistence of microplastics and heavy metals [18,26].

## 3.4. The Impact of Polyethylene Microplastics on Soil Pollution Indices

Figure 4 depicts the variations in the soil pollution indices as well as the indices related to the metal content between the soil and the plants.









Figure 4. Effect of different polyethylene microplastic (PE MP) amounts on Zn and Cd soil contamination indices.

The CF index values do not appear to change statistically significantly after microplastic addition, since the values used in the estimation do not change. It is well known that the value of the total metal concentration is taken into account when calculating the value of the contamination factor. The first level of polyethylene microplastics, or 2.5% w/w concentration, had no effect on the CF value of zinc in either of the study's examined soil samples. The values of the index place both rural and urban samples in group II, or moderately contaminated soils, based on the index categorization. It was found by Yu et al. [12] that a possible decrease in the bioavailability of soil heavy metals caused by the presence of microplastics varies across aggregate levels, leading to the conclusion that conflicting factors define the outcome of nutrient and pollutant intake.

In a comparable manner, as no alteration in the total concentration was observed alongside the addition of 2.5% w/w PE MPs, no change in the corresponding soil indicators was observed during the resulting calculation of CF indicators for Cd in both soil samples. In terms of Cd pollution, both soils were classified as class I, or virgin, soils.

A slight, but not statistically significant, decrease in the total concentration and, consequently, in the CF values for Zn and Cd in both soil samples was observed by the addition of increased amounts of microplastics, i.e., at a rate of 5% w/w. The samples are nonetheless still categorized in the relevant pollution categories to which they formerly adhered.

The Igeo indices of Zn and Cd revealed the same accomplishments, as the addition of both doses of polyethylene microplastics had no effect on their values.

The availability index (BF) of both metals increased substantially with the addition of microplastics at their highest ratio (5% w/w). A greater increase is observed in agricultural soils (soil 1), whereas urban soils exhibit a modest increase in metal availability.

When the higher quantity of polyethylene microplastics is added, the value of the availability index, or the concentration of Zn extracted with the DTPA solution relative to the total, shows the least increase and equals 9.25% in urban soils.

In general, the incorporation of microplastics in soils increased the availability of both the hazardous Cd and the less toxic Zn, in accordance with other studies [6]. It is crucial, however, that the DTPA-extractable concentration be greater rather than the water-soluble concentration. This might be due to a chemical interaction between polyethylene and the DTPA solution, i.e., diethylenetriaminepentaacetic acid. It is well known that metals are able to bind to soil organic compounds as they form chemical complexes along them [27,35]. This should be further investigated, as the increase in the values of the DTPAextractable amounts of Zn and Cd does not correspond to the increase in the levels of the metals in the plants. On the contrary, the water-soluble concentrations of the metals in the soil samples appear to have a better correlation. It has been indicated by Dioses-Salinas et al. [30] that the heterogeneity presented in the described methods in the literature sometimes makes the results uncomparable. Furthermore, novel methods need to overcome important frontiers and challenges.

#### 3.5. The Impact of Polyethylene Microplastics on Soil-to-Plant System Indices

Important inferences about the possible risk of metals to the environment and human health may be derived from the study of indices that represent the metal mobility within the soil–plant system.

Figure 5 depicts three indicators that were examined with respect to their responses to change when the two amounts of polyethylene microplastics were added. Sun and his colleagues [51] studied the foliar uptake and leaf-to-root translocation of plastics with different coating charges in maize plants.





Figure 5. Effect of different polyethylene microplastic amounts on Zn & Cd soil-to-lettuce indices.

In the present study, microplastics appear to dramatically enhance the Transfer Coefficient, which measures the concentration of metals in the aerial portion of the soil sample in relation to the overall concentration in the matching soil sample. Hu et al. [52] also investigated the distribution of micro- and mesoplastics in agricultural soils across China, resulting in alterations to their environmental impacts via soils. When PE MPs are added to both soil types, the BAF index, which reflects the percentage of concentration in the roots compared to the amount of DTPA extractable with the solution, decreases. The TF index, which measures the proportion of concentration in the plant's aboveground vs. subterranean portions, appears to be rising. To explore and assess the phenomenon of microplastic interactions with soil-based metals, it is helpful and essential to apply all three indices. However, it is essential to concentrate our scientific attention on the necessary criteria to meet. Gharahi and Zamani-Ahmadmahmoodi [8] and Zhou et al. [33] found that microplastics appear to have conflicting effects on three distinct crops in the field. In other words, MPs under specific circumstances may improve some growth characteristics of plants.

According to Figure 4, the greatest decrease in the BAF index is observed in the case of Cd in rural soil, followed by Cd in urban soil. A decrease is also observed in the BAF value of Zn in urban soil. The value of BAF in agricultural soil increases with a greater input of microplastics. According to Huang et al. [44] microplastics may influence soil nutrient cycling by affecting the dominant bacteria phyla or genes and enzymes involved in the carbon, nitrogen, and phosphorus cycles. Considering that for the hazardous Cd, the value of BAF decreases overall, for the less toxic Zn, the presence of microplastics seems to contribute to its absorption by plants [22]. Future research in this area must be focused and take into account how time affects the changes in the chemical behavior of plastics in soils. Given that knowledge in this field is still in its infancy, the incubation duration of microplastics in soil and the presence of more metals or other ions should improve the efficacy of this study and provide useful findings.

## 4. Conclusions

The current study was an initial attempt to assess the impact of microplastics on plants cultivated in soils containing Zn and Cd. Polyethylene, a plastic that is commonly found in high quantities in both agricultural and urban soils, was chosen to obtain microplastics of dimensions less than 5 mm. Lettuce, the most well-known and highly consumed vegetable globally, was chosen as an experimental plant to assess the potential risks. Metal pollution was severe in agricultural soils, while moderate contamination with Zn and Cd was detected in urban soils. Microplastics were added to soil samples in two distinct quantities of 2.5% and 5% w/w. The effects of polyethylene microplastics on the soil's physicochemical characteristics and metal concentrations were investigated. Furthermore, the impact of MPs on the Zn and Cd availability and distribution in the soil–plant system was assessed.

The addition of PE MPs resulted in a decrease in soil pH. On the contrary, an increase in both organic matter and soil cation-exchange capacity was detected. Furthermore, MPs enhanced the available concentrations of both metals. In the heavily polluted agricultural soil, the addition of microplastics at 2.5% w/w increased the readily available concentration of Zn more than that of the hazardous Cd. The presence of PE MPs in both the rural and urban soil samples resulted in a higher metal accumulation in lettuce roots than in the edible above-ground parts of the plants. The coexistence of metals and microplastics in soils may pose risks to soil, plants, and even human health. However, with careful study and understanding of the mechanisms that catalyze synergistic toxicity and appropriate management, it is possible to reduce such risks.

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## References

- Wu, X.; Lu, J.; Du, M.; Xu, X.; Beiyuan, J.; Sarkar, B.; Bolan, N.; Xu, W.; Xu, S.; Chen, X.; et al. Particulate Plastics-Plant Interaction in Soil and Its Implications: A Review. Sci. Total Environ. 2021, 792, 148337.
- Allouzi, M.M.A.; Tang, D.Y.Y.; Chew, K.W.; Rinklebe, J.; Bolan, N.; Allouzi, S.M.A.; Show, P.L. Micro (Nano) Plastic Pollution: The Ecological Influence on Soil-Plant System and Human Health. *Sci. Total Environ.* 2021, 788, 147815. https://doi.org/10.1016/j.scitotenv.2021.147815.
- 3. Yu, H.; Zhang, Y.; Tan, W.; Zhang, Z. Microplastics as an Emerging Environmental Pollutant in Agricultural Soils: Effects on Ecosystems and Human Health. *Front. Environ. Sci.* **2022**, *10*, 217.
- Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in Freshwater and Terrestrial Environments: Evaluating the Current Understanding to Identify the Knowledge Gaps and Future Research Priorities. *Sci. Total Environ.* 2017, 586, 127–141.
- Thompson, R.C.; Olson, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; McGonigle, D.; Russell, A.E. Lost at Sea: Where Is All the Plastic? *Science* 2004, 304, 838. https://doi.org/10.1126/science.1094559.
- Akdogan, Z.; Guven, B. Microplastics in the Environment: A Critical Review of Current Understanding and Identification of Future Research Needs. *Environ. Pollut.* 2019, 254, 113011.
- Wang, F.; Wang, X.; Song, N. Polyethylene Microplastics Increase Cadmium Uptake in Lettuce (*Lactuca sativa* L.) by Altering the Soil Microenvironment. Sci. Total Environ. 2021, 784, 147133. https://doi.org/10.1016/j.scitotenv.2021.147133.
- Gharahi, N.; Zamani-Ahmadmahmoodi, R. Effect of Plastic Pollution in Soil Properties and Growth of Grass Species in Semi-Arid Regions: A Laboratory Experiment. *Environ. Sci. Pollut. Res.* 2022, 29, 59118–59126. https://doi.org/10.1007/s11356-022-19373-x.
- Lozano, Y.M.; Rillig, M.C. Effects of Microplastic Fibers and Drought on Plant Communities. *Environ. Sci. Technol.* 2020, 54, 6166– 6173. https://doi.org/10.1021/acs.est.0c01051.
- Wang, F.; Wang, Q.; Adams, C.A.; Sun, Y.; Zhang, S. Effects of Microplastics on Soil Properties: Current Knowledge and Future Perspectives. J. Hazard. Mater. 2022, 424, 127531.

- Boots, B.; Russell, C.W.; Green, D.S. Effects of Microplastics in Soil Ecosystems: Above and below Ground. *Environ. Sci. Technol.* 2019, 53, 11496–11506. https://doi.org/10.1021/acs.est.9b03304.
- 12. Yu, H.; Hou, J.; Dang, Q.; Cui, D.; Xi, B.; Tan, W. Decrease in Bioavailability of Soil Heavy Metals Caused by the Presence of Microplastics Varies across Aggregate Levels. J. Hazard. Mater. 2020, 395, 122690. https://doi.org/10.1016/j.jhazmat.2020.122690.
- 13. Feng, X.; Wang, Q.; Sun, Y.; Zhang, S.; Wang, F. Microplastics Change Soil Properties, Heavy Metal Availability and Bacterial Community in a Pb-Zn-Contaminated Soil. *J. Hazard. Mater.* **2022**, *424*, 127364. https://doi.org/10.1016/j.jhazmat.2021.127364.
- Zhao, M.; Li, C.; Zhang, C.; Han, B.; Wang, X.; Zhang, J.; Wang, J.; Cao, B.; Zhao, Y.; Chen, Y.; et al. Typical Microplastics in Field and Facility Agriculture Dynamically Affect Available Cadmium in Different Soil Types through Physicochemical Dynamics of Carbon, Iron and Microbes. J. Hazard. Mater. 2022, 440, 129726. https://doi.org/10.1016/j.jhazmat.2022.129726.
- 15. Shi, R.; Liu, W.; Lian, Y.; Wang, Q.; Zeb, A.; Tang, J. Phytotoxicity of Polystyrene, Polyethylene and Polypropylene Microplastics on Tomato (*Lycopersicon esculentum* L.). *J. Environ. Manag.* **2022**, *317*, 115441. https://doi.org/10.1016/j.jenvman.2022.115441.
- Teng, L.; Zhu, Y.; Li, H.; Song, X.; Shi, L. The Phytotoxicity of Microplastics to the Photosynthetic Performance and Transcriptome Profiling of Nicotiana Tabacum Seedlings. *Ecotoxicol. Environ. Saf.* 2022, 231, 113155. https://doi.org/10.1016/j.ecoenv.2021.113155.
- Qi, Y.; Yang, X.; Pelaez, A.M.; Huerta Lwanga, E.; Beriot, N.; Gertsen, H.; Garbeva, P.; Geissen, V. Macro- and Micro- Plastics in Soil-Plant System: Effects of Plastic Mulch Film Residues on Wheat (*Triticum aestivum*) Growth. *Sci. Total Environ.* 2018, 645, 1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229.
- Machado, A.A.D.S.; Lau, C.W.; Kloas, W.; Bergmann, J.; Bachelier, J.B.; Faltin, E.; Becker, R.; Görlich, A.S.; Rillig, M.C. Microplastics Can Change Soil Properties and Affect Plant Performance. *Environ. Sci. Technol.* 2019, 53, 6044–6052. https://doi.org/10.1021/acs.est.9b01339.
- 19. Gan, Q.; Cui, J.; Jin, B. Environmental Microplastics: Classification, Sources, Fates, and Effects on Plants. *Chemosphere* **2023**, *313*, 137559.
- He, D.; Guo, T.; Li, J.; Wang, F. Optimize Lettuce Washing Methods to Reduce the Risk of Microplastics Ingestion: The Evidence from Microplastics Residues on the Surface of Lettuce Leaves and in the Lettuce Washing Wastewater. *Sci. Total Environ.* 2023, 868, 161726. https://doi.org/10.1016/j.scitotenv.2023.161726.
- 21. Huang, F.; Hu, J.; Chen, L.; Wang, Z.; Sun, S.; Zhang, W.; Jiang, H.; Luo, Y.; Wang, L.; Zeng, Y.; et al. Microplastics May Increase the Environmental Risks of Cd via Promoting Cd Uptake by Plants: A Meta-Analysis. *J. Hazard. Mater.* **2023**, *448*, 130887.
- 22. Ding, L.; Huang, D.; Ouyang, Z.; Guo, X. The Effects of Microplastics on Soil Ecosystem: A Review. *Curr. Opin. Environ. Sci. Health* **2022**, *26*, 100344.
- Huang, C.; Ge, Y.; Yue, S.; Zhao, L.; Qiao, Y. Microplastics Aggravate the Joint Toxicity to Earthworm Eisenia Fetida with Cadmium by Altering Its Availability. *Sci. Total Environ.* 2021, 753, 142042. https://doi.org/10.1016/j.scitotenv.2020.142042.
- 24. Wang, F.; Zhang, X.; Zhang, S.; Zhang, S.; Adams, C.A.; Sun, Y. Effects of Co-Contamination of Microplastics and Cd on Plant Growth and Cd Accumulation. *Toxics* 2020, *8*, 36. https://doi.org/10.3390/TOXICS8020036.
- Wen, X.; Yin, L.; Zhou, Z.; Kang, Z.; Sun, Q.; Zhang, Y.; Long, Y.; Nie, X.; Wu, Z.; Jiang, C. Microplastics Can Affect Soil Properties and Chemical Speciation of Metals in Yellow-Brown Soil. *Ecotoxicol. Environ. Saf.* 2022, 243, 113958. https://doi.org/10.1016/j.ecoenv.2022.113958.
- Hurley, R.R.; Nizzetto, L. Fate and Occurrence of Micro(Nano)Plastics in Soils: Knowledge Gaps and Possible Risks. Curr. Opin. Environ. Sci. Health 2018, 1, 6–11.
- Golia, E.E.; Angelaki, A.; Giannoulis, K.D.; Skoufogianni, E.; Bartzialis, D.; Cavalaris, C.; Vleioras, S. Evaluation of Soil Properties, Irrigation and Solid Waste Application Levels on Cu and Zn Uptake by Industrial Hemp. *Agron. Res.* 2021, 19,92-99, https://doi.org/10.15159/AR.21.016.
- Golia, E.E.; Chartodiplomenou, M.A.; Papadimou, S.G.; Kantzou, O.D.; Tsiropoulos, N.G. Influence of Soil Inorganic Amendments on Heavy Metal Accumulation by Leafy Vegetables. *Environ. Sci. Pollut. Res.* 2021, 30, 8617–8632. https://doi.org/10.1007/s11356-021-17420-7.
- Golia, E.E.; Bethanis, J.; Ntinopoulos, N.; Kaffe, G.-G.; Komnou, A.A.; Vasilou, C. Investigating the Potential of Heavy Metal Accumulation from Hemp. The Use of Industrial Hemp (*Cannabis sativa* L.) for Phytoremediation of Heavily and Moderated Polluted Soils. *Sustain. Chem. Pharm.* 2023, *31*, 100961. https://doi.org/10.1016/j.scp.2022.100961.
- Dioses-Salinas, D.C.; Pizarro-Ortega, C.I.; De-la-Torre, G.E. A Methodological Approach of the Current Literature on Microplastic Contamination in Terrestrial Environments: Current Knowledge and Baseline Considerations. *Sci. Total Environ.* 2020, 730, 139164.
- Ren, X.; Tang, J.; Wang, L.; Liu, Q. Microplastics in Soil-Plant System: Effects of Nano/Microplastics on Plant Photosynthesis, Rhizosphere Microbes and Soil Properties in Soil with Different Residues. *Plant Soil.* 2021, 462, 561–576. https://doi.org/10.1007/s11104-021-04869-1.
- 32. Roy, T.; Dey, T.K.; Jamal, M. Microplastic/Nanoplastic Toxicity in Plants: An Imminent Concern. *Environ. Monit. Assess.* 2023, 195, 1–35. https://doi.org/10.1007/S10661-022-10654-Z.
- Zhou, W.; Wang, Q.; Wei, Z.; Jiang, J.; Deng, J. Effects of Microplastic Type on Growth and Physiology of Soil Crops: Implications for Farmland Yield and Food Quality. *Environ. Pollut.* 2023, 326, 121512. https://doi.org/10.1016/j.envpol.2023.121512.
- Golia, E.E.; Diakoloukas, V. Soil Parameters Affecting the Levels of Potentially Harmful Metals in Thessaly Area, Greece: A Robust Quadratic Regression Approach of Soil Pollution Prediction. *Environ. Sci. Pollut. Res.* 2022, 29, 29544–29561. https://doi.org/10.1007/s11356-021-14673-0.

- Golia, E.E. The Impact of Heavy Metal Contamination on Soil Quality and Plant Nutrition. Sustainable Management of Moderate Contaminated Agricultural and Urban Soils, Using Low Cost Materials and Promoting Circular Economy. *Sustain. Chem. Pharm.* 2023, 33, 101046. https://doi.org/10.1016/j.scp.2023.101046.
- Golia, E.E.; Papadimou, S.G.; Cavalaris, C.; Tsiropoulos, N.G. Level of Contamination Assessment of Potentially Toxic Elements in the Urban Soils of Volos City (Central Greece). *Sustainability* 2021, *13*, 2029. https://doi.org/10.3390/SU13042029.
- Zhao, T.; Lozano, Y.M.; Rillig, M.C. Microplastics Increase Soil PH and Decrease Microbial Activities as a Function of Microplastic Shape, Polymer Type, and Exposure Time. *Front. Environ. Sci.* 2021, 9.101-114 https://doi.org/10.3389/fenvs.2021.675803.
- Degryse, F.; da Silva, R.C.; Baird, R.; Cakmak, I.; Yazici, M.A.; McLaughlin, M.J. Comparison and Modelling of Extraction Methods to Assess Agronomic Effectiveness of Fertilizer Zinc. *J. Plant Nutr. Soil Sci.* 2020, 183, 248–259. https://doi.org/10.1002/jpln.201900340.
- Alexakis, D.E.; Bathrellos, G.D.; Skilodimou, H.D.; Gamvroula, D.E. Land Suitability Mapping Using Geochemical and Spatial Analysis Methods. *Appl. Sci.* 2021, *11*, 5404. https://doi.org/10.3390/app11125404.
- Alexakis, D.E.; Bathrellos, G.D.; Skilodimou, H.D.; Gamvroula, D.E. Spatial Distribution and Evaluation of Arsenic and Zinc Content in the Soil of a Karst Landscape. *Sustainability* 2021, *13*, 6976. https://doi.org/10.3390/su13126976.
- Chen, L.; Han, L.; Feng, Y.; He, J.; Xing, B. Soil Structures and Immobilization of Typical Contaminants in Soils in Response to Diverse Microplastics. J. Hazard. Mater. 2022, 438, 129555. https://doi.org/10.1016/j.jhazmat.2022.129555.
- Zhao, M.; Liu, R.; Wang, X.; Zhang, J.; Wang, J.; Cao, B.; Zhao, Y.; Xu, L.; Chen, Y.; Zou, G. How Do Controlled-Release Fertilizer Coated Microplastics Dynamically Affect Cd Availability by Regulating Fe Species and DOC Content in Soil? *Sci. Total Environ.* 2022, *850*, 157886. https://doi.org/10.1016/j.scitotenv.2022.157886.
- 43. Rillig, M.C.; Lehmann, A.; de Souza Machado, A.A.; Yang, G. Microplastic Effects on Plants. New Phytol. 2019, 223, 1066–1070.
- 44. Huang, D.; Wang, X.; Yin, L.; Chen, S.; Tao, J.; Zhou, W.; Chen, H.; Zhang, G.; Xiao, R. Research Progress of Microplastics in Soil-Plant System: Ecological Effects and Potential Risks. *Sci. Total Environ.* **2022**, *812*, 151487.
- Su, R.; Ou, Q.; Wang, H.; Dai, X.; Chen, Y.; Luo, Y.; Yao, H.; Ouyang, D.; Li, Z.; Wang, Z. Organic–Inorganic Composite Modifiers Enhance Restoration Potential of *Nerium oleander* L. to Lead–Zinc Tailing: Application of Phytoremediation. *Environ. Sci. Pollut. Res.* 2023, 30, 56569–56579. https://doi.org/10.1007/s11356-023-26359-w.
- 46. Esposito, M.P.; Domingos, M. Establishing the Redox Potential of *Tibouchina pulchra* (Cham.) Cogn., a Native Tree Species from the Atlantic Rain Forest, in the Vicinity of an Oil Refinery in SE Brazil. *Environ. Sci. Pollut. Res.* **2014**, *21*, 5484–5495. https://doi.org/10.1007/s11356-013-2453-8.
- Xu, G.; Lin, X.; Yu, Y. Different Effects and Mechanisms of Polystyrene Micro- and Nano-Plastics on the Uptake of Heavy Metals (Cu, Zn, Pb and Cd) by Lettuce (*Lactuca sativa* L.). *Environ. Pollut.* 2023, *316*, 120656. https://doi.org/10.1016/j.envpol.2022.120656.
- Pinto-Poblete, A.; Retamal-Salgado, J.; López, M.D.; Zapata, N.; Sierra-Almeida, A.; Schoebitz, M. Combined Effect of Microplastics and Cd Alters the Enzymatic Activity of Soil and the Productivity of Strawberry Plants. *Plants* 2022, 11, 536. https://doi.org/10.3390/plants11040536.
- Su, R.; Xie, T.; Yao, H.; Chen, Y.; Wang, H.; Dai, X.; Wang, Y.; Shi, L.; Luo, Y. Lead Responses and Tolerance Mechanisms of Koelreuteria Paniculata: A Newly Potential Plant for Sustainable Phytoremediation of Pb-Contaminated Soil. *Int. J. Environ. Res. Public Health* 2022, *19*, 14968. https://doi.org/10.3390/ijerph192214968.
- Dou, C.-M.; Fu, X.-P.; Chen, X.-C.; Shi, J.-Y.; Chen, Y.-X. Accumulation and Detoxification of Manganese in Hyperaccumulator Phytolacca americana. Plant. Biol. 2009, 11, 664–670. https://doi.org/10.1111/j.1438-8677.2008.00163.x.
- Sun, H.; Lei, C.; Xu, J.; Li, R. Foliar Uptake and Leaf-to-Root Translocation of Nanoplastics with Different Coating Charge in Maize Plants. J. Hazard. Mater. 2021, 416, 125854. https://doi.org/10.1016/j.jhazmat.2021.125854.
- Hu, J.; He, D.; Zhang, X.; Li, X.; Chen, Y.; Wei, G.; Zhang, Y.; Ok, Y.S.; Luo, Y. National-Scale Distribution of Micro(Meso)Plastics in Farmland Soils across China: Implications for Environmental Impacts. *J. Hazard. Mater.* 2022, 424, 127283. https://doi.org/10.1016/j.jhazmat.2021.127283.

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