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ORIGINAL PAPER

Stimulation of corn zinc efficiency in the soil polluted with petroleum hydrocarbons and lead using *Arbuscular mycorrhizal* fungi and zinc oxide nanoparticles

Amir Hossein Baghaie[1,✉], Mehran Keshavarzi[2]

1. Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran
2. Department of Agronomy and Plant Breeding, Isfahan University of Technology, Isfahan, Iran

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ABSTRACT

Zinc (Zn) deficiency is among the main concerns regarding human nutrition. The present study aimed to evaluate the effects of zinc oxide (ZnO) nanoparticles and *Arbuscular mycorrhizal* fungi (AMF) on the changes of Zn concentration in the corn plant grown in the soil contaminated with petroleum hydrocarbons and lead (Pb). Treatments consisted of applying 0% (Zn_0) and 1% (w/w) Zn oxide nanoparticles (Zn_1), soil pollution with zero (Pb_0), 400 (Pb_{400}), and 600 (Pb_{600}) mg Pb/kg soil and 0% (P_0), 1.5% ($P_{1.5}$), and 3% (W/W) of crude oil (P_3) in the presence (AMF^+) and absence of AMF (AMF^-). After the corn harvesting, plant Zn and Pb concentrations were measured using atomic absorption spectroscopy (AAS). Also, total petroleum hydrocarbons (TPHS) degradation in the soil was measured via gas chromatography. The least significant difference (LSD) test was used to determine the statistical differences between the mean values. According to the findings, the presence of AMF in soil containing 1.5% (W/W) crude oil and polluted with 400 mg Pb/kg soil caused a significant increase in plant Zn concentration by 52%. However, increasing soil pollution to petroleum hydrocarbon or Pb caused a significant decreasing in the plant Zn concentration. The highest TPHS degradation has belonged to the soil treated with 1% (W/W) ZnO nanoparticles nanoparticle in the presence of AMF that containing 1.5 % (W/W) crude oil. The results of this study showed that regardless of pollution type, application of AMF in soil treated with ZnO nanoparticles nanoparticle can increase plant Zn concentration.

Keywords: Environmental pollution, Lead, Zinc, Soil

Introduction

Soils in arid and semi-arid regions are often deficient in micronutrients such as zinc (Zn), and this is considered to be an environmental issue across the globe.[1, 2] Use of chemical fertilizers has been reported to significantly increase crop yield, while the harvesting of plants could significantly decrease the levels of soil micronutrients, which requires special attention.[3]

In arid and semi-arid areas, Zn availability is extremely low due to its high pH and soil deficiency in organic matters.[4] Cereal crops are among the main sources of the nutrients that are essential for human health. Today, many countries have reported that the levels of soil nutrients are inadequate for health, and half the world's population are faced with nutritional deficiency, which is also referred to as 'hidden hunger'.[5] Zn is considered to be an essential element for human health[6, 7] which has numerous biochemical functions in the metabolism. For instance, Zn is involved in the activation of several enzymes (e.g., carbohydrase, alkaline phosphatases, and nucleic acid synthase), while it also affects metabolic hormones. Zn deficiency in humans leads to several disorders, such as slow growth rate, diarrhea, brain disorders, decreased immune function, skin ulcers, and eye damage.[8]

✉ *Amir Hossein Baghaie*
a-baghaie@iau-arak.ac.ir

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Zn deficiency could be effectively overcome through selecting the plant genotypes with high Zn efficiency,[9, 10] However, it is preferred that strategies be adopted to increase the soil nutritional availability. Notably, the total concentrations of soil nutrients such as Zn are not low in arid and semi-arid areas, while they have low availability due to the physicochemical properties of soil. Application of Zn chelates[3] or organic amendments[11] (e.g., cow manure) could increase Zn availability. However, special attention should be paid to the effects of heavy metals on these compounds.

Fungal symbiosis with plant roots may affect the chemical properties of the rhizosphere (e.g., pH reduction), which in turn affects Zn availability. Moreover, root exudate (phytosiderophore) positively influences Zn availability. *Arbuscular mycorrhizal* fungus (AMF) plays a key role in Zn deficiency in plants.[12] AMF could form a mutualistic relationship with several species, such as corn. In addition, AMF stimulates the production of the hormones that promote plant growth and tolerance against biotic and abiotic stress factors, such as heavy metals.[13] On the other hand, AMF could accumulate heavy metals in the roots of plants, preventing their translocation to the aerial parts. In a study, Konieczny *et al.* assessed the role of AMF in Zn uptake by the lettuce grown in the soil amended with phosphorous, concluding that AMF could significantly increase the Zn concentration in lettuce. However, the effects of the soil physicochemical properties (e.g., heavy metals concentration) on the Zn availability of soil were not investigated in the mentioned research.[14]

Heavy metals adversely affect the Zn concentration in plants, which is considered to be a major problem. Thus, decreasing heavy metal concentration can play an important role in increasing nutritional elements.[15] In a research in this regard, Baghaie *et al.* evaluated the phytoavailability of lead (Pb) in the corn cultivated in the soil treated with Pb-enriched sewage sludge and cow manure, reporting that the application of organic amendments could decrease heavy metal availability in soil.[16] However, organic matter decomposition may lead to the redistribution of heavy metals in soil, which was not denoted in the mentioned study.

In several industrial areas, the simultaneous contamination of petroleum hydrocarbon and heavy metal has been reported, urging the reduction of such pollution using proper approaches. Petroleum products contain more than 1,200 hydrocarbons, which consist of 230 components with 3-12 carbon atoms (e.g., aliphatic, aromatic, and cyclic hydrocarbons) that decrease soil quality.[17]

The use of nano-particle such as zinc oxide (ZnO) nanoparticle can be a suitable proposition for reducing the soil heavy metals availability due to their high surface area. Thus, this research was conducted to evaluate the effect of ZnO nanoparticle and AMF on corn (CV. *Maxima*) Zn availability in a soil contaminated with petroleum hydrocarbons and Pb.

Materials and Methods

To investigate the effects of Zn oxide nanoparticles and AMF on the corn Zn concentration of the plants cultivated in the soil contaminated with petroleum hydrocarbons and Pb, soil samples with low calcium carbonate and organic carbon levels were obtained from the field research of Islamic Azad University, Arak Branch in Arak, Iran. Selected physicochemical properties of the studied soil samples are presented in Table 1.

Table 1. Selected physicochemical properties of studied soil samples

Properties	Unit	Amount
Soil texture	---	Sandy Loam
pH	---	7.3
EC	dS/m	1.4
Calcium carbonate	%	5
Pb availability	mg/kg	ND*
Cd availability	mg/kg	ND
Zn availability	mg/kg	0.2

*ND: not detectable by atomic absorption spectroscopy (AAS)

Treatments involved the application of 0% (Zn_0) and 1% (w/w) (Zn_1) Zn oxide nanoparticles,[18] and soil pollution was with crude oil at the rates of 0% (P_0), 1.5% ($P_{1.5}$), and 3% (w/w) (P_3). The soil samples were polluted with zero (Pb_0), 400 (Pb_{400}), and 600 (Pb_{600}) mg Pb/kg soil[19] in the presence (AMF^+) and absence of AMF (AMF^-). Table 2 shows the summary of the treatments used in the experiments.

Table 2. Treatments used in experiments

Treatments	Unit	Level
ZnO nanoparticle	%(W/W)	0 and 1
Pb	mg/kg soil	0, 400 and 600
AMF*	-------	+AMF and -AMF
Crude oil	% (W/W)	0, 1.5 and 3

**Arbuscular mycorrhizal* fungi

The Zn oxide nanoparticles were purchased form the research institute of the petroleum industry in Iran. Table 3 shows the selected properties of Zn oxide nanoparticles.

Table 3. Selected physical properties of Zn oxide nanoparticles in study

Properties	Unit	Amount
Purity	%	99
Particle size	nm	<50
Specific area	m^2/g	>82
Molar Mass	g/mol	81.4

The present study was conducted as a factorial experiment in the layout of a completely randomized block design. The least significant difference (LSD) test was applied to determine the differences between the mean values using the SAS software.

The soil samples were contaminated with the mentioned levels of Pb and crude oil and incubated for one month. After one month to the equilibrium, the soil samples were treated with 0% and 1% (w/w) Zn oxide nanoparticles and incubated for one month. Following that, the samples were sterilized by autoclaving at the temperature of 120 °C for one hour.

To preparation the AMF inoculum, the rhizosphere soil around the root corn in the preliminary experiment was collected as a soil containing mycorrhiza. After that, all of the soils were re-inoculated with indigenous soil microbial suspension (50 ml/kg), which was prepared from an aqueous suspension of the soils contains inoculum (2.5% w/v), filtered twice through Whatman No 1 filter paper. In this stage, soils are free of AMF spores. To have AMF treated soils, half of the pots inoculated with 20 g of AMF inoculum, which was placed in a layer at a depth of 3 cm from the soil surface.[20] Following that, the corn seeds were planted, and after 60 days of the experiment, the plants were harvested, and the soil Pb, Cd and Zn concentration was measured using atomic absorption spectroscopy (AAS).

The petroleum hydrocarbon contents in the soil samples were extracted using a soxhlet extractor with 1:1 (v/v) dichloromethane and n-hexane mixture (150 ml) within 24 hours.[21] The concentration of polycyclic aromatic hydrocarbons (TPHS) in the soil samples was determined in the extracts via GC using a Delsi DI 200 gas chromatograph equipped with a direct injection port and an FID detector at the temperature of 340 °C.

Results and Discussion

Evaluation of the effects of Zn oxide nanoparticle (Fig. 1-a) and the presence or absence of AMF (Fig. 1-b) indicated a significant increase in the plant Zn concentration (P=0.05). However, contamination with Pb (Fig. 1-c) or crude oil (Fig. 1-d) adversely affected the shoot Zn concentration. It should be noted that the role of soil properties on heavy metal availability cannot be ignored.[22]

According to the obtained results, using the Zn oxide nanoparticles had no significant effect on the soil pH, which has been reported to be a major advantage in environmental studies (data not shown). Heavy metal solubility is affected by soil pH as the reduction of pH has been reported to increase Pb availability in soil.[20]

The interaction effects of applying ZnO nanoparticles, the presence of AMF and soil Pb pollution on shoot Zn concentration was significant (P=0.05), as, applying 1% (W/W) ZnO nanoparticles in Pb polluted soil that contains 1.5%(W/W) crude oil significantly increased the Plant Zn concentration by 13% in the presence of AMF (Table 4) that may be related to the interaction effects of Pb and Zn. In a study in this regard, Sadeghi *et al.* evaluated the interactive effects of Zn and heavy metals on the growth and chemical composition of canola (*Brassica napus* L. cv. Hyola) in loamy sand soil, concluding that the increased application of a Zn fertilizer caused a significant reduction in the heavy metal concentrations of the plant, which could be attributed to the interactive effects of Zn with heavy metals.[23] In this regard, the findings of Abid *et al.* are consistent with the results of the present study.[24]

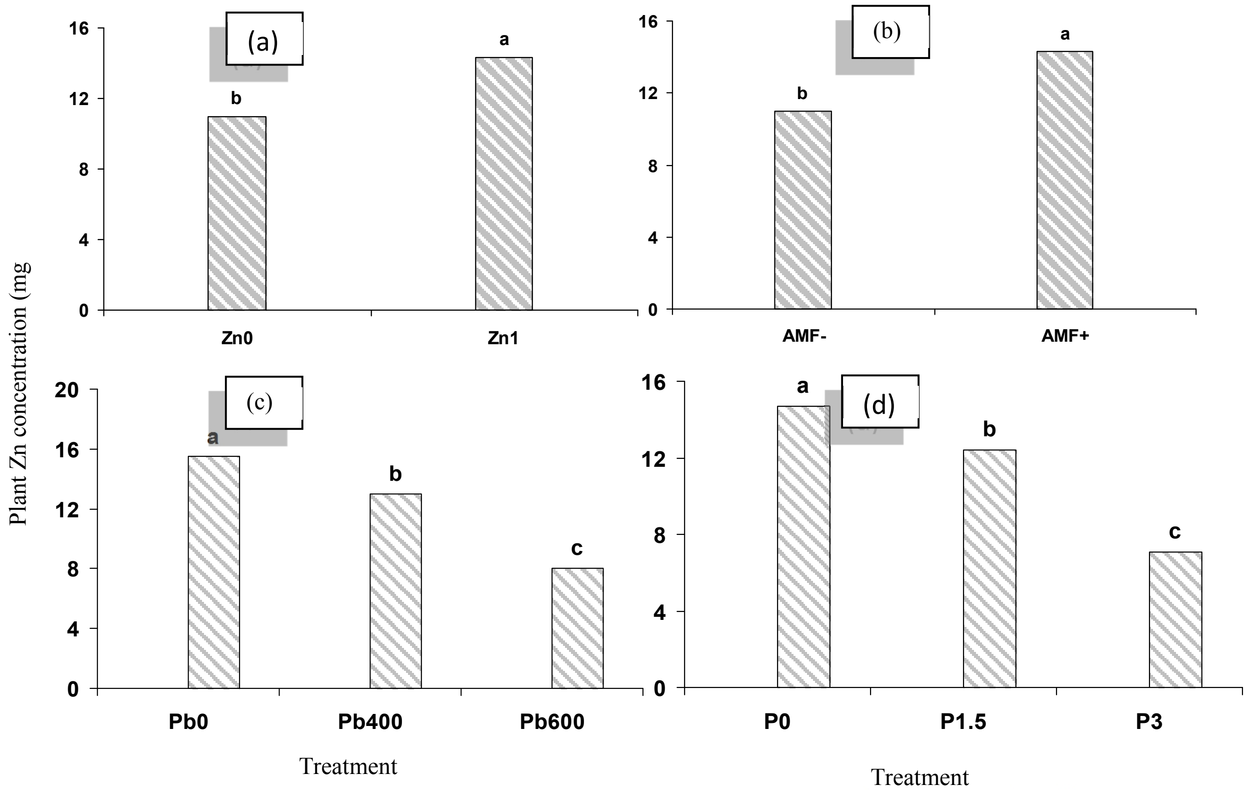

Fig. 1. The simple effect of ZnO nanoparticle (a), the presence or absence of AMF (b), Pb (c) and crude oil (d) pollution on plant Zn concentration. Pb_0, Pb_{400} and Pb_{600} are 0, 400 and 600 mg Pb /kg soil, Zn_0 and Zn_1 are 0 and 1 % (W/W) ZnO nanoparticle, $AMF^-$and AMF^+ indicate the presence and absence of *arbuscular mycorrhiza* fungus and P_0, $P_{1.5}$ and P_3 are applying 0, 1.5 and 3 % (W/W) crude oil, respectively.

Table 4. Effect of Zn oxide nanoparticles, Pb, and AMF on plant Zn concentration (mg/kg)

Treatment	AMF^-Zn_0			AMF^-Zn_1			AMF^+Zn_0			AMF^+Zn_1		
	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3
Pb_0	6.4^{r*}	7.7^{q}	5.5^{s}	12.9^{k}	13.8^{ij}	11.3^{m}	17.2^{d}	18.4^{b}	16.1^{f}	18.4^{b}	19.6^{a}	17.1^{d}
Pb_{400}	5.3^{s}	6.4^{r}	4.3^{tu}	10.7^{n}	11.6^{m}	9.3^{o}	15.6^{g}	16.4^{e}	14.1^{i}	16.9^{d}	17.7^{c}	15.0^{h}
Pb_{600}	4.1^{u}	4.6^{t}	3.1^{v}	9.1^{o}	10.5^{n}	8.3^{p}	12.4^{l}	13.7^{j}	10.5^{n}	13.5^{j}	14.9^{h}	12.3^{l}

(* Pb_0, Pb_{400}, and Pb_{600} are 0, 400 and 600 mg Pb /kg soil, Zn_0 and Zn_1 are 0 and 1 % (W/W) ZnO nanoparticle, AMF^- and AMF^+ indicate the presence and absence of *Arbuscular mycorrhiza* fungus, respectively. P_0, $P_{1.5}$ and P_3 are applying 0, 1.5 and 3 % (W/W) crude oil. Means with the similar letters are not significantly different [P=0.05], LSD test).

According to the findings of the current research, increased soil pollution with crude oil from 0% to 3% (w/w) caused a significant reduction in the plant Zn concentration as the shoot concentration of Zn significantly decreased (17.4%) in the Pb polluted soil (600 mg Pb/kg soil) in the presence of AMF when the soil pollution with crude oil increased from 1.5% to 3% (w/w). This could be due to the toxic effects of crude oil on the population of soil microorganisms. Principally, soil microorganisms could affect the physicochemical properties of soil, thereby altering Zn availability in soil. In a research in this regard, Ziółkowska *et al.* investigated the toxicity of petroleum substances in microorganisms and plants, concluding that petroleum hydrocarbon pollution adversely affected microbiological and biochemical properties.[17]

Regardless of soil pollution with heavy metals or petroleum hydrocarbons, the presence of AMF was observed to significantly increase the plant Zn concentration in the current research as the presence of AMF in the soil containing 1.5% (w/w) crude oil and 400 mg Pb/kg soil caused a significant increase in the plant Zn concentration (52%).

A highly notable finding of the present study was that at the highest level of soil pollution with crude oil, the shoot Zn concentration significantly reduced, so that the lowest shoot concentration of Zn was observed in the plants cultivated in the soil treated with 600 mg Pb/kg and 3% (w/w) crude oil without Zn oxide nanoparticles. On the other hand, the presence of AMF in the soil treated with Zn oxide nanoparticles could help increase the shoot Zn concentration, while the shoot Pb concentration significantly decreased (Table 5).

Increasing Zn concentration with decreasing plant heavy metal concentration has been reported by some researcher.[25, 26] However, no prior studies have investigated the interactive effects of petroleum hydrocarbons and heavy metals on plant Zn concentration, which was the main objective of the current research.

Table 5. Effects of Zn oxide nanoparticles, Pb, and AMF on plant Pb concentration (mg/kg)

Treatment	AMF^-Zn_0			AMF^-Zn_1			AMF^+Zn_0			AMF^+Zn_1		
	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3
Pb_0	ND**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pb_{400}	65.3^{f*}	64.2^{f}	68.9^{e}	52.3^{j}	53.0^{j}	61.4^{g}	44.6^{l}	45.2^{l}	55.1^{i}	33.2^{n}	32.8^{n}	38.9^{m}
Pb_{600}	78.9^{b}	77.1^{b}	80.1^{a}	70.1^{d}	70.8^{d}	75.4^{c}	49.8^{k}	49.1^{k}	60.2^{h}	37.1^{m}	37.9^{m}	44.6^{l}

(* Pb_0, Pb_{400}, and Pb_{600} are 0, 400 and 600 mg Pb /kg soil, Zn_0 and Zn_1 are 0 and 1 % (W/W) ZnO nanoparticle, AMF^- and AMF^+ indicate the presence and absence of *Arbuscular mycorrhiza* fungus, respectively. P_0, $P_{1.5}$ and P_3 are applying 0, 1.5 and 3 % (W/W) crude oil. Means with the similar letters are not significantly different [P=0.05], LSD test), **ND: not detectable by AAS).

In the current research, the highest TPHS degradation was observed in the soil treated with 1% (w/w) Zn oxide nanoparticles containing 1.5% (w/w) crude oil in the presence of AMF (Table 6), while the lowest TPHS degradation was observed in the soil with no Zn oxide nanoparticles and crude oil pollution in the absence of AMF. Therefore, it could be concluded that the rate of TPHS degradation decreased with the increasing of soil pollution with crude oil to 3% (w/w), which could be attributed to the role of crude oil toxicity in the reduction of microbial populations.[27] On the other hand, plant Zn concentration was also decreased with increasing soil pollution to crude oil which suggest that the THPS degradation and plant Zn concentration has the same trend. Basically, microorganism needs nutritional element for their activity.[28]

In a study in this regard, Kavousi Bafti *et al.* investigated the effects of crude oil pollution and oil-degrading bacteria on some biochemical and corn growth factors, concluding that TPHS pollution could significantly decrease the activity of the microorganisms that causes TPHS degradation.[29]

Table 6. Effects of Zn oxide nanoparticles, Pb, and AMF on petroleum hydrocarbon degradation (%)

Treatment	AMF^-Zn_0			AMF^-Zn_1			AMF^+Zn_0			AMF^+Zn_1		
	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3	P_0	$P_{1.5}$	P_3
Pb_0	NC**	35.5^{m*}	30.2^{n}	NC	53.2^{ef}	50.5^{h}	NC	58.3^{c}	51.2^{g}	NC	64.5^{a}	57.4^{c}
Pb_{400}	NC	30.2^{n}	26.5^{p}	NC	49.7^{h}	43.2^{k}	NC	54.5^{e}	47.6^{i}	NC	62.1^{b}	54.3^{e}
Pb_{600}	NC	27.4^{o}	20.1^{q}	NC	45.1^{j}	40.1^{l}	NC	51.2^{g}	45.5^{j}	NC	56.1^{d}	52.3^{f}

(* Pb_0, Pb_{400}, and Pb_{600} are 0, 400 and 600 mg Pb /kg soil, Zn_0 and Zn_1 are 0 and 1 % (W/W) ZnO nanoparticle, AMF^- and AMF^+ indicate the presence and absence of *Arbuscular mycorrhiza* fungus, respectively. P_0, $P_{1.5}$ and P_3 are applying 0, 1.5 and 3 % (W/W) crude oil. Means with the similar letters are not significantly different [P=0.05], LSD test, ***NC:* not calculated).

Conclusion

According to the results, the application of Zn oxide nanoparticle significantly increased the plant Zn concentration. Furthermore, the presence of AMF could significantly increase the plant Zn concentration. On the other hand, increased soil pollution with hydrocarbons or heavy metals had significant effects on the shoot Zn concentration. Using ZnO nanoparticle has contributed to the degradation of soil crude oil components, which may be attributed to the positive role of Zn element on increasing the microbial activity and its role on soil TPHS degradation. As we did not assess the role of the other physicochemical properties of soil, type of heavy metals, and their concentrations type, it is recommended that further investigations be conducted in this regard in order to achieve accurate results.

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