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The effect of two humic substances on the growth and lead uptake of corn in calcareous soil

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ABSTRACT- In the past few decades, accumulation of heavy metals, such as lead (Pb), in soils has increased as a result of human activities.The environmental hazard associated with soil enrichment in heavy metals is related to their mobility and plant availability. Application of various materials such as humic may influence the amount of Pb taken up by plant. Four levels of each of liquid and solid commercial humic substances were applied in this study which were performed as two completely randomized factorial design experiments in a calcareous soil polluted with three levels of Pb. Results of a greenhouse study with corn (*Zea mays* L., cv Hido) showed that the two commercial humic substances with different chemical composition, affected dry mater yield differently. Influences of humic substances on dry mater yield were most likely dependent on their chemical composition. Maximum dry mater yield was observed at manufacturer's recommended levels of humic substances. But, the solid humic treatments had no significant effect on dry mater yield. Application of both liquid and solid commercial substances significantly increased uptake of Pb in corn shoots. Addition of humic substances significantly increased the enrichment factor in both experiments but affected the micronutrient uptake only slightly.

INTRODUCTION

The problem of environmental pollution with heavy metals is becoming increasingly urgent. Heavy metals are those with the specific density over 5 Mg m^{-3} and the relative atomic mass above 40 (Seregin and Ivanove, 2001). They are introduced into soil and natural waters and involved in a number of chemical, physical, and biological processes that determine their concentration. Heavy metals are considered serious pollutants because of toxicity, persistent, and non-degradable conditions in the environment (Mohiuddin et al., 2010). Lead (Pb) is one of the most widespread pollutants in soil and is a non-essential element that occurs naturally in the environment. However, the highest concentrations found in nature are the result of human activities (Adriano, 2001) and metals from anthropogenic sources tend to be more mobile than those from pedogenic or lithogenic sources (Shakeri and Modabberi, 2014). Despite the regulatory measures adopted in many countries to the input of Pb into environment, it continues to be one of the most serious global environmental and human hazards. Since many of the Pb pollutants are indispensable to modern life, soil contamination with Pb is not likely to decrease in the near future (Sharma and Dubey, 2005). Metal toxicants which affect the immune system may contribute to an increased incidence of autoimmune diseases, infectious diseases, and cancer (Mishra, 2009). One strategy to minimize adverse effects of Pb toxicity on crop production is to develop plant species with higher genetic ability to tolerate the Pb toxicity. All plants have the potential to extract metals from soil, but some plants have shown the ability to extract, accumulate, and tolerate high levels of heavy metals, which would be toxic to other organisms (Maywald and Weigel, 1997). Also according to Nabulo et al. (2008), uptake of trace metals from soil differs from plant to plant and from site to site. The bioavailability of a metal depends on its chemical form, which is strongly affected by the presence of natural and anthropogenic ligands. The complexation of metals with ligands can drastically change the physico–chemical and biological properties of the metal species (Gao et al., 1999). Humic acid may increase the bioavailability of soil heavy metals to plants (Marschner and Kalbitz, 2003). It may contain organic macromolecules with an important role in the bioavailability and transport of heavy metals (Lagier et al., 2000) because it competes for free metal ions to form soluble complexes and reduce metal adsorption onto soil surfaces (Antoniadis and Alloway, 2000). The organometallic complexes of humic acid and heavy metals could be taken up by the plant roots (Evangelou et al., 2004). The interactions of humic acid are different in their extents and ways of affecting the mobility, bioavailability, degradation, and phyto-toxicity of heavy metals (Lagier et al., 2000; Clapp et al., 2001). The humic substances in the soil have multiple effects that can greatly benefit plant growth (Sangeetha et al., 2006). It may have both direct and indirect effects on the plant growth (Chen and Aviad, 1990). Indirect effects involve improvements of the soil properties such as aggregation, aeration, permeability, water holding capacity, and micronutrient transport and availability (Tan, 2003). Direct effects are those which require uptake of humic substances into the plant tissue resulting in various biochemical effects (Escobar et al., 1996). However, their activity in promoting plant growth is not completely known (Ulukan, 2008).

Metal availability to plants is also affected by soil characteristics such as pH, clay, calcium carbonate content, dissolved organic carbon (DOC), and humic acids. Humic substance is the second most dominant fraction, accounting for 3–28% of dissolved organic carbon (Imai et al., 2002), present in both municipal effluents and reclaimed water (Lingbo et al., 2005). Soils of Fars province of Iran are mostly calcareous having high pH and clay and calcium carbonate contents. Gharaie (2009) and Gharaie et al. (2002) determined the Pb retention capacities of 20 selected calcareous soils of the province to be very high ranging from 31,000 to 46,000 mg Pb kg^{-1}.

The objectives of this study were to investigate the effects of humic substances on Pb availability, plant growth, and Pb uptake in calcareous soils, which could provide some insights into our understanding of the potential risk of Pb pollution on such soils.

MATERIALS AND METHODS

Soil Analysis

The mainsample was taken from the surface layer (0-30 cm) of a soil (fine, mixed (calcareous), mesic, Typic Calcixerepts) from Fars province located in southern Iran, air-dried, sieved, and subsamples were taken to determine its selected physico-chemical properties. The texture was determined with the hydrometer method (Bouyoucos, 1962); pH and electrical conductivity (EC) were measured in saturation extract; calcium carbonate equivalent (Loppert and Suarez, 1996), cation exchange capacity (Summer and Miller, 1996), and organic matter content (Nelson and Sommers, 1996) were also determined. Total nitrogen was determined according to the Kjeldahl method (Bremmer, 1996). Available potassium (K) was extracted by ammonium acetate and determined with flame photometer (Helmek and Sparks, 1996). Sodium bicarbonate-extractable P was determined by spectrophotometer according to the Olsen et al. (1954). Available micronutrients and initial Pb were extracted with DTPA (Lindsay and Norvell, 1978) and determined by atomic absorption spectrometry. Electrical conductivity and pH of humic substances were measured in 1:5 water extract. Similarly, other properties of the substances were measured. Some chemical and physical properties of the soil and humic substances used are given in Tables 1 and 2, respectively.

Experimental Design

In order to investigate the effect of two humic substances on plant Pb uptake, this study was performed as two completely randomized design factorial experiments with three replicates under greenhouse conditions in a calcareous soil polluted with three levels of Pb (100, 200, and 300 mg Pb kg^{-1} as $Pb(NO_3)_2$) at the time of planting. The no Pb treatment (i.e., 0 mg Pb kg^{-1}) was intentionally not included because the retention capacity of the soils of Fars province for Pb is very high (Gharaie, 2009; Gharaie et al., 2002) and, thus, including such rate of Pb would produce plants with practically zero mg Pb kg^{-1} and its use, therefore, would seem to be a waste of time and energy. The treatments included four rates of a liquid commercial humic substance 12% (Humaster Starter®*), hereafter referred to as HS, (equivalent to 0, 4, 8, and 16 kg ha^{-1}) in the first experiment that were applied at two equal installments, one at planting time and the next at 4 weeks after emergence. In the second experiment, four levels of a solid commercial humic substance 80% (Pars Humic®*), hereafter referred to as HP (equivalent to 0, 100, 200, and 400 kg ha^{-1}) were applied at the time of planting. The recommended manufacture's rates were 4 and 100 kg ha^{-1} for HS and HP, respectively and we, therefore, used both the lower and higher than the manufacture's recommended rates in our study.

Table 1. Selected properties of the soil used

Properties	Quantities
Texture	Silty clay
Sand, %	4
Silt, %	54
Clay, %	42
Cation Exchange Capacity (CEC), cmol$_+$ kg^{-1}	13
pH	7.82
EC, dS m^{-1}	0.74
Calcium Carbonate Equivalent, %	38
Organic matter, %	0.98
Total nitrogen (N), %	0.12
Sodium bicarbonate extractable P, mg kg^{-1}	15
DTPA-extractable Mn, mg kg^{-1}	6.4
DTPA-extractable Cu, mg kg^{-1}	1.4
DTPA-extractable Fe, mg kg^{-1}	4.5
DTPA-extractable Zn, mg kg^{-1}	0.68
DTPA-extractable Cd, mg kg^{-1}	0.06
DTPA-extractable Pb, mg kg^{-1}	0.5

*Manufactured by Golsang Kavir Yazd Co., Yazd, Iran (www.golsangkavir.com). Using any trademarked product in this study does not imply its approval by Shiraz University.

Soil Preparation and Planting

Experimental units were pots containing 2.5 kg soil which received a uniform application of 20 mg P kg^{-1} soil as Ca $(H_2PO_4)_2.H_2O$, 50 mg nitrogen (N) kg^{-1} soil as urea (one half of N was added at planting and the other half at 4 weeks after emergence), 10 mg Fe kg^{-1} soil as Fe-EDDHA, and 10 mg Zn and Mn kg^{-1} soil as their sulfate forms. Corn (*Zea mayz* L., cv Hido) was planted in pots and kept under 80% field capacity soil moisture condition by daily adding water to a constant weight. Six corn seeds were planted and were thinned to three uniform stands one week after emergence.

Table 2. Selected properties of the humic substances used

Properties	HS	HP
pH	9.3	4.1
EC, dS m^{-1}	4.8	2.3
Total nitrogen (N), %	12.8	5.2
Total organic carbon (TOC), %	24.67	30.09
Sodium bicarbonate-extractable P, mg kg^{-1}	8.7	27.2
Available K (Ammonium acetate-extractable), mg kg^{-1}	14,500	250
Mn* , mg kg^{-1}	22.7	8.7
Cu* , mg kg^{-1}	7.8	14.7
Fe* , mg kg^{-1}	1591	377.2
Zn* ,mg kg^{-1}	5.2	9.1
Cd* , mg kg^{-1}	3.25	0.12
Pb* , mg kg^{-1}	20.45	ND

*1:20 HS: water extractable for HS; DTPA- extractable for HP, ND = Not detectable

Plant Analysis

Eight weeks after emergence, shoots were harvested, washed with a jet of water, rinsed with distilled water, dried at 65°C, weighed, and dry-ashed at 550°C. The ash was dissolved in 2M HCl and the concentrations of Pb, Fe, Cu, Mn, and Zn were determined using atomic absorption spectrophotometer. Shoot dry matter yield, Pb concentration, and uptake (concentration × dry matter yield) were used as plant responses. Enrichment factor (EF) was calculated as the ratio of plant shoot concentration to soil concentration ([Metal]$_{shoot}$ / Metal]$_{soil}$) (Branquinho et al., 2007).

Statistical Analysis

Data were analyzed using SPSS 20 and SAS 9.13 software and the mean value of plant responses was compared statistically using Duncan's Multiple Range Test at probability level of 0.05.

RESULTS AND DISCUSSION

Dry weight of shoots is shown in Fig. 1. Application of Pb first increased dry matter yield (DMY) (although not significant at the $P \leq 0.05$) and then decreased it significantly, in both HS and HP treatments. John et al. (2008) reported that 1 mg L^{-1} of Cd and 1 mg L^{-1}of Pb increased growth in *Lemna polyrrhiza* at the end of 30-day growth period by 13% and 28% as compared to that of control, respectively, but 30 and 40 mg L^{-1} of Cd and Pb proved to be toxic, affecting the plant growth severely. Sharma and Dubey (2005) stated that the toxic level of Pb in soil exerts adverse effect on morphology, growth, and photosynthetic processes of plants. High level of Pb in plant also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. Jarrah et al. (2014) investigated the efficiency of arbuscular mycorrhizal fungus and EDTA on Pb phytoremediation by sunflower in a calcareous soil and stated that increasing Pb levels decreased shoot dry weight in nonmycorrhizal plants and in both the absence and/or presence of EDTA.

In both experiments, at low level of Pb, addition of humic substances (especially at the recommended level) significantly increased corn DMY (Fig. 2).

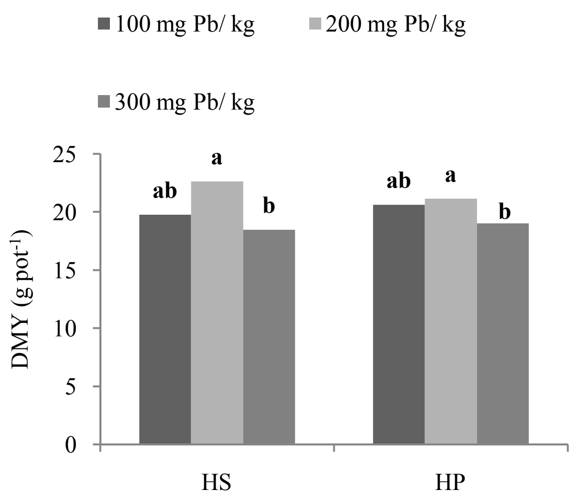
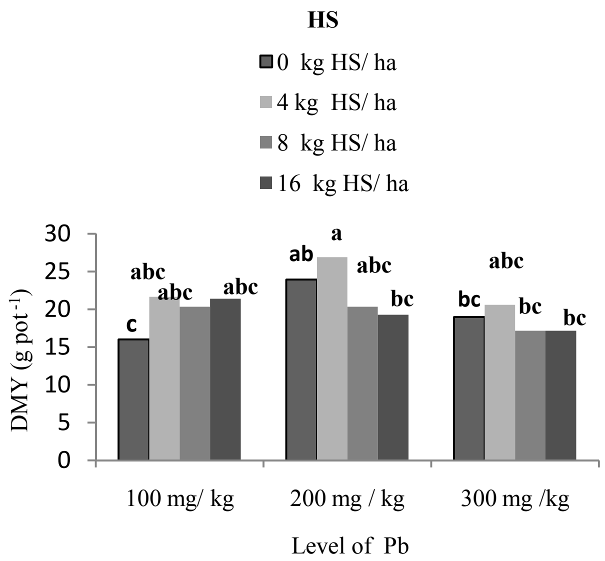


Fig. 1. Effect of different levels of Pb on corn dry matter yield

Humic substances may promote growth by increasing nutrients uptake in calcareous soils (Tan, 2003). Using the data presented in Fig. 2 shows that each kg ha^{-1} of HS may increase DMY by 0.845 g pot^{-1} whereas the comparable figure for HP is only 0.019 g pot^{-1}. This means that HS (humic in solution form) was much more efficient in enhancing the plant growth. Khan et al. (2006) also stated that the use of humic acid in watering solution under certain circumstances improved the growth of wheat plants. At high level of Pb, addition of humic substances to contaminated soil decreased DMY (Fig. 2).



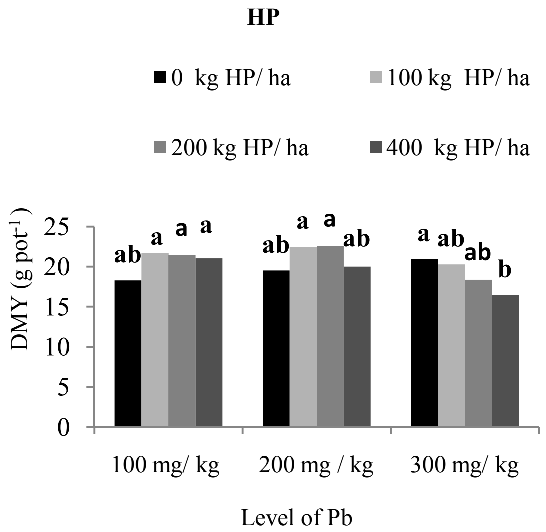


Fig. 2. Effects of liquid (HS) and solid (HP) humic substances on dry matter yield at different levels of Pb

This result is perhaps due to formation of Pb-chelates by humic materials resulting in higher Pb concentration in plant tissues (Table 3). Khan et al. (2006) stated that the influences of humic substances on plant growth were most likely dependent on soil metal contents. Under a condition when soil was in short supply of micronutrients, application of humic substances may mobilize soil nutrients and improve plant mineral nutrition and, as a result, this may improve plant growth. Whereas for soils contaminated with potentially toxic levels of metals, humic substances may enhance the phytotoxicty of metals. In general, maximum DMY was observed at medium levels of humic substances. However, HP treatments had no significant effect on DMY except at 300 mg Pb kg^{-1} where 400 kg HP ha^{-1}significantly decreased DMY (Fig.2).

The effect of treatments on plant Pb concentration is shown in Table 3. Increasing Pb levels significantly increased Pb concentration of plants. The relatively low plant Pb concentration even at the 300 mg Pb kg-1 treatment is in agreement with the findings of Gharaie (2009) and Gharaie et al. (2002) who reported the Pb retention capacity of calcareous soils of Fars province to be very high.

In both experiments, addition of humic substances significantly increased concentration of Pb (Table 3). Khan et al. (2006) also reported that Cd and Pb concentrations in plant tissues were significantly higher at higher rates of humic acid. Zhang et al. (2013) stated that the effects of humic acids on metal availability to plants in soil were pH-dependent, with inhibitory and stimulatory effects on acid and alkaline soils, respectively. Lorestani et al. (2011) reported that concentrations of Pb in the plant samples collected from the site (the industrial town of Vian, Hamedan province in Iran) were variable, ranging from 260 mg kg^{-1} to 3420 mg kg^{-1} in roots and 15 mg kg^{-1} to 2880 mg kg-1 in shoots, with the maximum level found in the roots of *C. arvense* and shoots of *S. soda*.

Table 3. Effects of different levels of applied humic substances and Pb on corn shoot Pb concentration (μg g^{-1}dry mater)

Level of Pb (mg kg^{-1})	Level of HS (kg ha^{-1})				Mean
	0	4		16	
100	1.6d	0.6e	1.7d	2.4d	1.6B
200	2.3d	5.6ab	5.4ab	5.5bc	4.7A
300	4.1c	4.8bc	5.3ab	6.0b	5.0A
Mean	2.7C	3.6BC	4.1AB	4.6A	
	Level of HP (kg ha^{-1})				
	0	100	200	400	
100	1.3e	1.2e	3.0de	4.8cd	2.6B
200	2.3e	2.7e	2.8de	2.8de	2.7B
300	5.4bc	7.3ab	6.5abc	6.5abc	6.9A
Mean	3.0B	3.7AB	4.7A	4.7A	

Means in each row or column followed by the same lowercase or capital letters are not significantly different (P <0.05) by Duncans Multiple Range Test

The total uptake of Pb by corn plants also increased by increasing application of Pb (Fig. 3); however, the highest total Pb uptake in the first experiment was observed in the treatment with 200 mg Pb kg^{-1} which could be due to the increase in DMY observed at 200 mg Pb (Fig. 1).

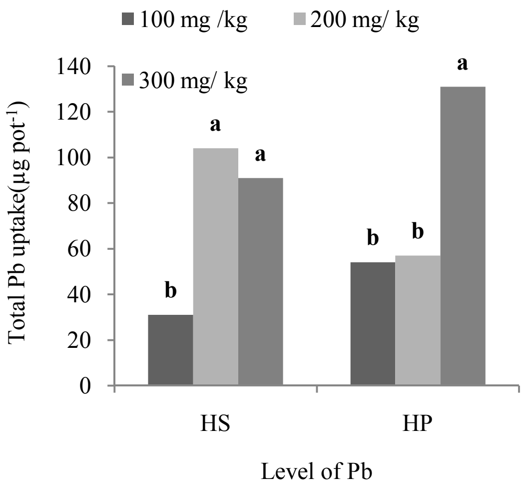


Fig. 3. Effect of levels of Pb on corn shoot total Pb uptake

The main effect of humic substances application was to significantly increase the uptake of Pb in corn shoots, in both experiments (Fig. 4). Lead uptake is a function of Pb concentration and DMY, thus, for example, in the second experiment, humic substances had no significant effect on DMY but increased Pb concentration. Uptake also increased by increasing the use of humic substances. At the recommended level of HS (4 kg ha^{-1}), Pb uptake was 60% higher as compared to that of control and Pb uptake at recommended level of HP (100 kg ha^{-1}) was 26% higher than that of control. Strawn and Sparks (2000) stated that the correlation between organic matter in the soils, and the percentage of Pb desorbed from the soils suggests that it plays an important role in slow desorption reactions of Pb from soil materials.

In both experiments, application of humic substances increased enrichment factor (EF) significantly, although EF values were very low (EF<1) in all treatments (Fig. 5). The ability of metal uptake by plant from contaminated soils was evaluated by the EF in a study done by Yadav et al. (2009). This study assumed that plants with EF values > 1 are accumulators, while plants with EF values < 1 are excluders. Since the EF values observed in our study were all less than 1 (Fig. 5), it may be concluded that corn plants acted as an excluder plant under the conditions of our experiment. Metal excluders accumulate heavy metals from substrate into their roots but restrict their transport and entry into the aerial parts (Malik and Biswas, 2012).

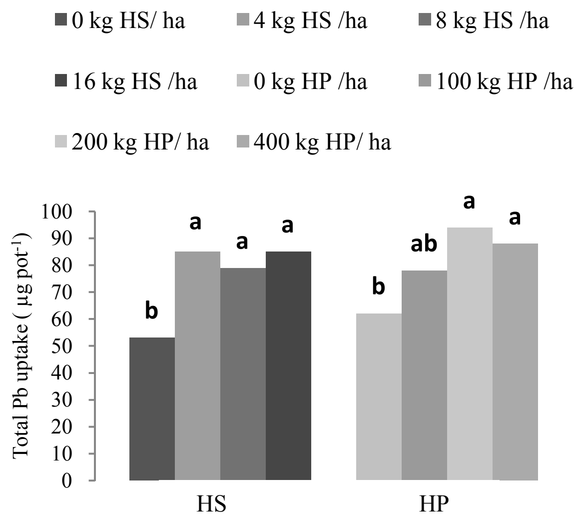


Fig. 4. Effect of different levels of humic substances on corn shoot total Pb uptake

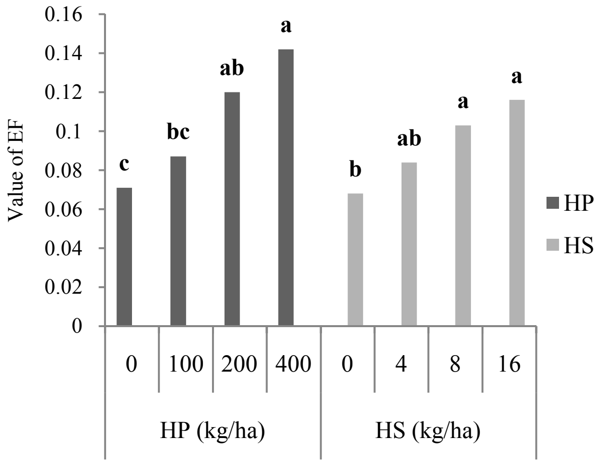


Fig. 5. Effect of different levels of humic substances on enrichment factor (EF)

Naz et al. (2013) using regression analysis showed a positive relationship between the concentrations of Pb in soil and in purslane (*Portulaca oleracea*) seedlings in all the three treatments i.e. single Pb (300, 400, and 500), Pb/Cd (300/0.5, 400/1, 500/1.5) and Pb/Zn (300/250, 400/500, 500/ 700); and stated that seedlings accumulated 35%, 31%, and 10.1% of Pb from single Pb, Pb/Cd and Pb/Zn treatments, respectively.

Zinc uptake of shoot increased by about 41% in response to the application of 16 kg HS ha^{-1} as compared to that of control; however, application of HP had no significant effect on this parameter (Fig. 6).

Application of humic substances had no significant effect on the uptake of Fe and Mn in corn shoots as compared to those of their controls (Fig. 7 and 8). Turan et al. (2011) reported that soil application of humus substance was significantly effective on maize plants uptake of Mn and Cu and also stated that the highest mean uptake of Mn was obtained with 1 g humus kg^{-1} treatment (1.797 mg pot^{-1}), and the highest amount of Cu (0.171 mg pot^{-1}) was obtained with 2 g humus kg^{-1} treatment. Sanchez et al. (2005) investigated the use of humic substances and amino acids to enhance Fe availability for tomato plants from applications of chelate FeEDDHA and stated that both organic compounds (especially the humic substances) improved Fe uptake in comparison to a control without the addition of organic materials.

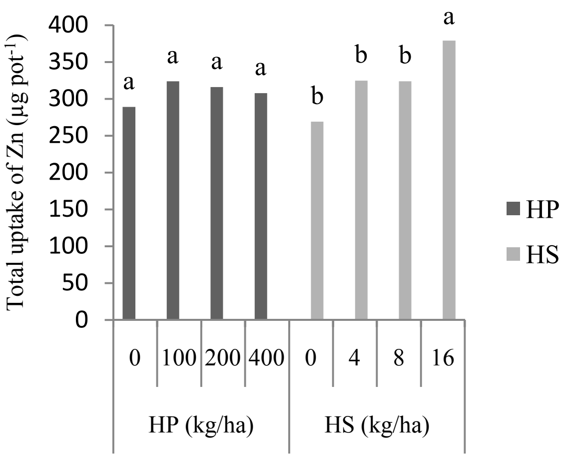


Fig. 6. Effect of different levels of humic substances on corn shoot total Zn uptake

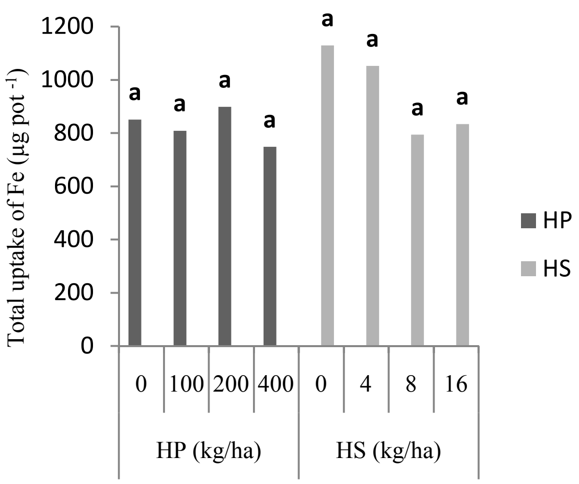


Fig. 7. Effect of different levels of humic substances on corn shoot total Fe uptake

Application of HS did not affect the uptake of Cu, except at 4 kg HS ha^{-1} rate which significantly increased this parameter by 30.5 % due to the positive effect of HS on increasing DMY and consequently Cu uptake. Application of HP first decreased Cu uptake (although not significant at the $P \leq 0.05$) and then increased it significantly (Fig. 9).

Hartz et al. (2010) conducted field trials in two years and investigated the effects of pre-transplant soil application of humic substances at 1.1 or 3.4 kg ha^{-1} rates on nutrient uptake of tomato (*Lycopersicon esculentum* Mill.) and reported that in neither year did macro- or micronutrient uptake increase with humic substance. Asik et al. (2009) stated that soil application of humus increased N, P, K, Mg, Na, and Cu uptake of wheat. Foliar application of humic acid increased Zn uptake.

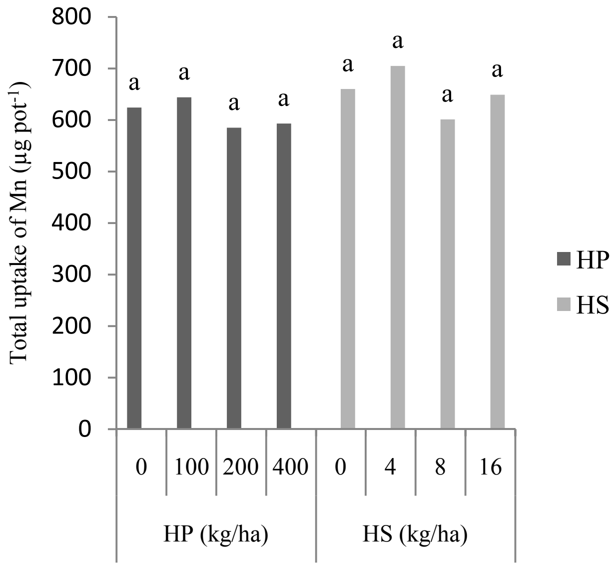


Fig. 8. Effect of different levels of humic substances on corn shoot total Mn uptake

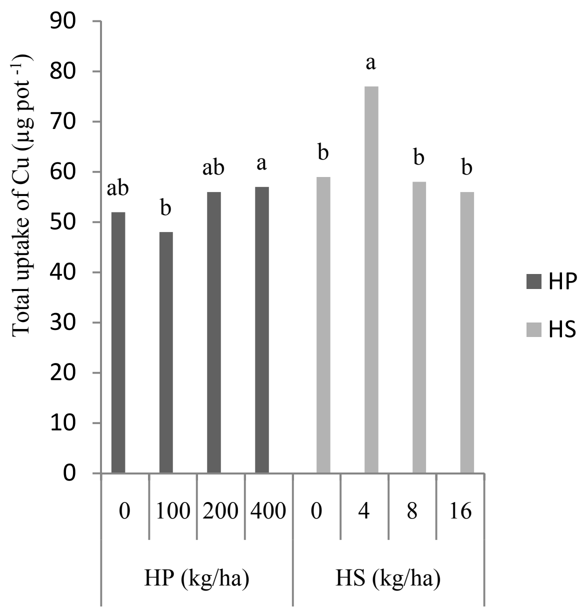


Fig. 9. Effect of different levels of humic substances on corn shoot total Cu uptake

In our study, Pb applications had no significant effect on micronutrients Fe, Cu, Mn, and Zn (Fig. 10). This might be due to the fact that Pb concentration of the shoots was very low (Table 3). Blaylock et al. (1997) reported that in soil with a pH between 5.5 and 7.5, Pb solubility is controlled by phosphate or carbonate precipitates and very little Pb is available to plants even if they have the genetic capacity to accumulate it. Sharma and Dubey (2005) stated that significant changes in nutrient contents as well as in internal ratios of nutrients occur in plants under Pb toxicity and in most cases, Pb blocks the entry of cations such as K, Ca , Mg, Mn, Zn, Cu, Fe, and anions (NO_3^-) in the root system.

CONCLUSIONS

It is concluded that
Humic substances used in this study had different effects on DMY. These differences supposedly reflect the difference between the kind and amount of different functional groups present in different humic substances, a topic which deserves further studies. Addition of humic substances at low and high level of Pb increased and decreased DMY, respectively. Application of humic substances increased Pb uptake which is mainly because of increasing bioavailability of Pb. Micronutrients uptake were not affected by Pb treatments, probably due to the fact that shoots Pb concentration was very low. The effect of humic substances on micronutrients uptake differed depending on the kind of micronutrients and humic substances. The uptake of Fe and Mn were not affected, whereas Zn and Cu uptakes increased following the application of HS.

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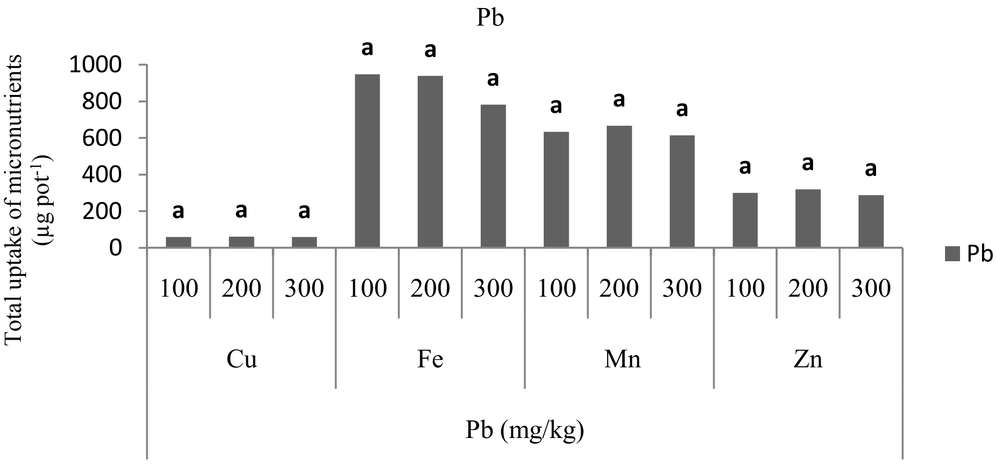

Fig. 10. Effect of different levels of Pb on corn shoot total micronutrients uptake

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تاثیر دو ماده هیومیک بر رشد و جذب سرب گیاه ذرت در یک خاک آهکی

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ذرت

چکیده- در چند دهه گذشته، در نتیجه فعالیت‌های انسانی، غلظت فلزات سنگین از جمله سرب در محیط زیست به طور آشکاری افزایش یافته است. خطر اصلی زیست محیطی ناشی از فلزات سنگین خاک مربوط به غلظت شکل‌های متحرک و قابل جذب توسط گیاه می‌باشد، کاربرد مواد مختلف از جمله مواد هیومیک ممکن است به دلیل تاثیر بر حلالیت و تحرک آن‌ها در خاک میزان جذب سرب توسط گیاه را تحت تاثیر قرار دهد. در این تحقیق به منظور بررسی اثر مواد هیومیک چهار سطح از هر کدام از مواد هیومیک تجاری مایع و جامد در دو آزمایش فاکتوریل در قالب طرح کاملا تصادفی در یک خاک آهکی آلوده شده با سه سطح سرب به کار برده شد. نتایج این تحقیق گلخانه‌ای نشان داد که تاثیر دو ماده هیومیک با ترکیبات شیمیایی متفاوت بر وزن خشک گیاه متفاوت بوده است. تاثیر مواد هیومیک بر وزن خشک گیاه به مقدار زیادی به ترکیب شیمیایی آن‌ها بستگی دارد. در هر دو آزمایش بیشترین میزان وزن خشک در سطوح توصیه شده به وسیله سازنده این مواد مشاهده شد، اما تیمارهای ماده هیومیک تجاری جامد اثر معنی‌داری بر میزان وزن خشک گیاه نداشت. کاربرد هر دو ماده هیومیک جذب سرب در گیاه را به طور معنی‌داری افزایش داد. در هر دو آزمایش، اضافه کردن مواد هیومیک فاکتور غنی‌سازی را به طور معنی‌داری افزایش داد، اما اثر کمی بر جذب عناصر میکرو داشت.