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Original article

Assessment bioavailability of selected heavy metal concentration and biological control parameter changes in two soils (case study: polluted and non-polluted sites in Iran)

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A B S T R A C T

Heavy metal pollution of soil is a serious concern, because such metals have toxicological importance in ecosystems, animal and human health. Metal toxicity depends on the chemical forms in which they exist. The aim of this research is investigation of biological factors and chemical forms of zinc and cadmium in two soils. We used two types of soil (polluted and non-polluted) in pot experiments that were conducted under greenhouse condition (similar to actual condition) as randomized complete factorial design. In according to this aim, the effect of this different on the soil biological factors, chemical properties, cadmium and zinc speciation was investigated in 21 and 49 days after treatment ancubation. Speciation was assayed with four individual extraction methods (distilled water, Ca(NO3)20.2M, DTPA+TEA 0.005M and HNO3 4N) in the bulk soil. The results showed that elements extractable concentration is following that Zn and Cd (HNO3)> Zn and Cd (DTPA+TEA)> Zn and Cd (Ca (NO3)2)> Zn and Cd (distilled water) in two soils. By approximately 91% and 61% of zinc and cadmium concentration is extractable with HNO3, respectively. These results showed one of the factors that caused to dangerous decrease may be related to be in unavailable and residual forms.In this study, zinc is more percent than cadmium in unavailable forms (i.e extractable with HNO3).Non-polluted soil is more microbial activity than polluted soil. Polluted soil was less microbial respiration rate (44%) and dehydrogenase activity (50%) than non-polluted soil.

1. Introduction

Soil is dynamic, lively and natural having essential role in various eco systems. The concentration of heavy metals in the soil solution plays a sensitive role in controlling of ions availability for plants. Thus, two types of investigation include overall toxicity and content of one element which has a greater role to contamination can help further understand the effects of certain heavy metal toxicity (Chaperon and Sauve, 2007).

Although zinc (Zn) is an essential micronutrient for plants, it is also phytotoxic if present in excess amounts in growth medium. The cadmium bioavailability is also strongly related to the specific characteristics of the various soil conditions under cultivation (Kabata-PendiasandPendias, 2001). There is evidence indicating high Cd impurity in some of Zn sources which have been commonly marketed as commercial Zn fertilizers in Iran (Afyuni et al., 2007). Cadmium and zinc are elements that have similar geochemical and environmental properties including similar ionic structure and electro negativities. Their chemical similarity can lead to interaction between Cd and Zn during plant uptake, transport from roots to the aerial parts, or accumulation in edible parts but they play quite different roles in the plant's metabolism. Zinc is a micronutrient, whereas Cd is toxic and ordinarily is found at very low concentrations in the plant (Moustakas et al., 2011). Cadmium exceeding the World Health Organization's recommendations is less than 0.2 mg kg-1 (Schnoor et al., 2004). Kabata-Pendias and Pendias (2001) expressed that concentration of zinc is equivalent to 100-500 mg kg-1.Therefore, it is urgent to develop cost-effective solutions to remediate soils that are contaminated with heavy metals. Bioavailability of heavy metals in the environment for organisms depends on soil properties, including pH, phosphorus and organic matter (Merry, 2001; Alloway, 2008). There are many movable and available elements for absorbable in the soils having low pH, compared to alkaline and neutral soils. Rapid decomposition of organic matter in the soil (such as added fertilizers) caused to composition of zinc-organic matter complex. This complex is available for roots and activity in the soil. Also, sustainable of this complex depends on type organic matter in soil (Alloway, 2008). Understanding and controlling the chemical composition of soil is critical to optimum utilization of nutrients in agricultural ecosystems and also for heavy metals control which is done through determination of chemical forms indicating variation in environmental mobility and bioavailability, Savonina et al., (2005). Understanding the behavior of heavy metals in soil-plant systems, especially focusing on the number of chemical forms and some of them bioavailability to the plant is an ongoing research objective. Bioavailability of metals in soil is normally related to its different chemical forms in that plants and other organisms are able to absorb over time, Chaignon et al., (2009). The 'bioavailable' element can be best described as that portion of soil element available for intake by a given organism. Consequently, the available fraction to plants is the amount that can be taken up by the roots of a given species or cultivar. It is well known that element bioavailability in soil depends on soil properties (pH, amount and nature of organic matter, soil minerals) (Zhang et al., 2001).

The assessment of bioavailability is difficult, as the concentration of the element in the soil solution represents only a portion of exchangeable and slightly complexed metal forms and does not give a measure of the total labile pool, Cattani et al., (2006). Exchangeable forms, which can be extracted from soil matrix using salt, is believed to be the most important, if not the only, bio available fraction for plant root accumulation, Sparks(1983). The metals associated with minerals such as carbonates or oxides can be released under acidic or reducing conditions and changes in pH and Eh can thereby affect the bioavailability of trace metals, Marschner and Romheld (1996). Determination of different forms of the metals with extraction of them and quantifying their values in a certain phase is done using different extractants. It has been shown that water could extract soluble components, while calcium nitrate could extract water soluble and exchangeable, and DTPA could extract water soluble components, exchangeable and those adsorbed on organic matter, and nitric acid could extract all the above fractions plus components on clay minerals, oxides and secondary minerals, Alloway (1990). Use of these extractants in association with the basic concepts of speciation. Chemical speciation plays a vital role in the solubility and potential bioavailability of metals in soils (Tandy et al., 2004).Dahiya et al (1991) showed that more than 80 percent of the total zinc is in residual forms, while 0.6% distributed in soluble and exchangeable in soil. Yang et al (2010) observed a significant portion of the zinc and cadmium is unavailable in the soil. In addition,

Microbial biomass is one of the indicators in soil that are sensitive to increasing of heavy metals concentrations. It was used for investigting of the quality of soil fertility. In addition to, microbial pobulation changes influenced on Short-term (Rajapaksha et al., 2004) and long-term pollution (Smolders, 2003). Also, effects of metals toxic on microbial population is different in according to pollution background and variations of soil (Sandaa et al., 1999). So,assessment of these elements had significant role in the affecton the biological activity detection in soil. Dehydrogenase is one of the most important oxidoreductases enzyme that being as indiator of biological activity. It does oxidoreduction processes inside of the living cells (Salazar et al., 2011).

In this research, single chemical extractions were performed to speciation cadmium and zinc and selected biochemical factors in two soils samples with varying site. They were near and distant of industerial factory with same land use and physico-chemical properties that under cultivation wheat.

2. Materials methods

2.1. Soil sampling

The experiments were conducted in a greenhouse in two soils, which was collected from surface horizon of a sandy clay loam soil (0-30cm) of a wheat field located at near the city of Zanjan (near of industrial factory), northwest of Iran and the other of Taleghan (distant of industrial factory), north of Iran. Geographic position include Soil1: Longitude 36˚ 5.3′ 21″ N and Latitude50˚28.9′ 12″ E, Soil2: Longitude 36˚ 40′ 18.8″ N and Latitude 48˚21′ 3″ E. Quality of crops growth in polluted soil (Sample 1 and sample 2) is shown in Figure1.

Fig. 1. Aspect of sampling locations
(Sample 1: distant of industrial factory and Sample 2: near of industrial factory).

2.2. Initial soil materials

The soil was air-dried and passed through a 2-mm sieve. Particle-size distribution determined by hydrometer method (Bouyoucos, 1962). Soil pH_e and EC_e were measured in saturated extract (Thomas., 1996; Rhoades., 1996). Organic matter (OM) was determined by Walkley-Black method (Nelson and Sommers, 1996). Available Fe, Zn, Cu, Mn and Pb were determined by HNO3 4N extraction (Changet al., 1984).

2.3. Treatment design

In according initial zinc concentration (HNO_3 extractable) and with using $ZnSO_4.H_2O$, zinc concentration in soils received three levels (250, 375 and 500 mg/kg). After adding salt (zinc sulfate), boxes were maintained for three months in 25˚C temperature and moisture (70% MWHC) at 60% air humidity in incubator, drying–wetting periods was carried out. After This period, boxes were transported to growth chamber of soil science engineering of Tehran university (Figure 2-a). Soil sampling was conducted in two times that include 21 and 49 days after incubation period.

2.4. Sample analysis

The soil samples were air-dried at room temperature and purified by passing through a 2 mm nylon sieve. To measure the amount of Zn and Cd available, 10 gram soils with 20 gram DTPA + TEA was shaken two hours and the solution was filtered with what man 42 filter paper, Lindsay and Norvel (1978). For calcium nitrate extractable Zn and Cd, 10 gram soil with 50 mL 0.2 Mcalcium nitrate was shacked for two hours and the solution was filtered, Menchet al.,(1994). Water soluble Zn and Cdofasolution was collected in soil: extractant of1:1.5ratiousing20gramsoilwith30mldistilled water shacked for two hours and the solution was filtered, Abreuet al.,(2006). Tome asure the amount of Zn and Cd extractable with nitricacid, 2gram soils with 15mL4N HNO_3,wasdigestedfor12hoursinwaterbathandthen filtered and collectedin 50mL volumetricflask, Changet al., (1984). Zn and Cd contents extracted were determined using atomic absorption spectrometery (Schimadzo model AA-670)and ICP (Perkin Elemer Optima 2100) were reported as mg/kgdry soil. Because biological samples had not different in two times, it was sampled only at the end of experiment. CO_2 content from respiration rate was determined by back titration with residual NaOH and reported in terms of (mgCO_2/ gdry soil week) (Alef and Nannipieri., 1995) (Figure 2-b). Dehydrogenase activity (DHA) assay was based on using of 2,3,5-triphenyltetrazolium chloride (TTC) which was reduced to triphenyl for mazan (TPF), a red colored compound. It was reported (µg TPF/g dry soil24h) (Öhlinger., 1996) (Figure 2). Soil $pH_{1:5}$ (soil: distilled water 1:5) was measured in saturated extract (Thomas., 1996; Rhoades., 1996). Organic matter (%OM) were determined by Walkley-Black method (Nelson and Sommers, 1996).

Fig. 2. Aspect of factors regulating in the growth chamber (a) and assesment biological parameters (b).

2.5. Statistical analysis

The data were subjected to statistical analysis using SAS9.2software computer. LSD's multiple-ranged test was also performed to identify the homogenous sets of data. All statements reported in this study are at the p<0.05 levels. Draw graphs was taken placed with Excel 2010 program.

3. Results and discussion

Although the most of physical and chemical properties of soil that used in the experiment before treatment for both soils were similar, but the concentrations of heavy metals except iron and copper of them (due to soil samples location) were different (Table 1).

3.1. Assessment of bio-chemical factors

Factors affecting on toxicity of heavy metal in soil-crop system include soil type which includes soil pH, organic matter content and other soil chemical and biochemical properties (Islam et al., 2007). In soil environments, sorption/desorption reactions as well as chemical complexation with inorganic and organic ligands, both biotic and abiotic, are of great importance in controlling their bioavailability, leaching and toxicity. These reactions are affected by many factors such as pH, nature of the sorbents, presence and concentration of organic and inorganic ligands (Violante et al., 2010).

Table1

Some physicochemical propertiesof soils.

Soil	pHe	ECe[a]	Texture	OM[b]	Fe	Zn	Cu	Mn	Pb
		(d/sm)		(%)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Sample 1	8.4	1.1	**Sandy clay loam**	0.85	242.5	38.26	18.8	25.8	15.5
Sample 2	8.2	1.3	**Sandy clay loam**	1.47	220.7	223.7	21.9	327.5	227

[a]Electrical Conductivity of soil saturation extracted /[b]Organic matter.

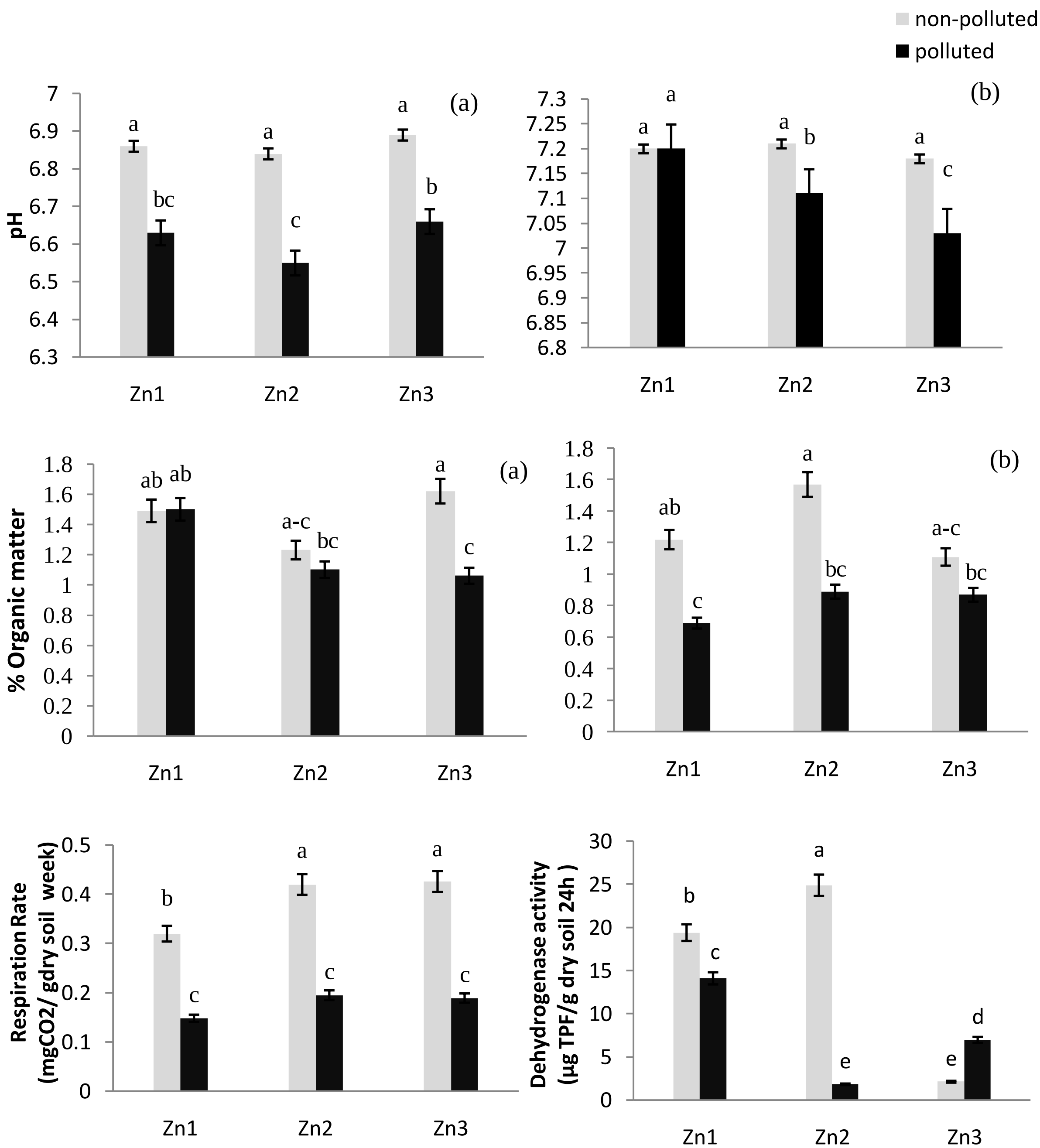
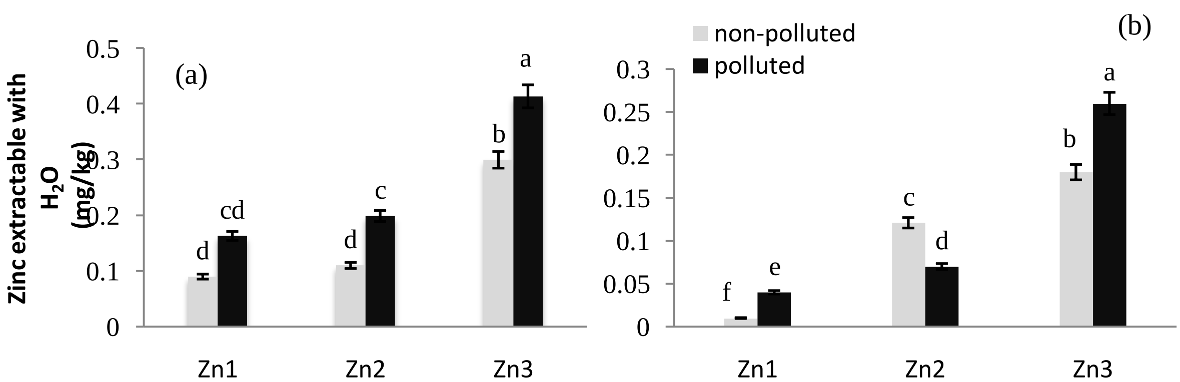


Fig. 3. Changes of biological control (Respiration Rate and Dehydrogenase activity) in 49 days and chemical parameters (pH and %Organic matter) after ancubation period 21(a) and 49 (b).

In according to figure 3, it was observed that beunstable condition in pH of polluted soil in two times (21 and 49 days)($p<0.05$). Increase of zinc sulphate levels caused to decreasepH of polluted soil in 49 days. Over time, the amount of sulfate soil increased as a result of sulfur fertilizer oxidation into the soil and pH decreased (Jiang et al., 2003; Najafi et al., 2013).It was related to sulfate anion in in pH decrease. Differencein changes of soil pH was due to differences in soil buffering capacity. Non-polluted soil had more organic matter due to greater microbial activity (1.37% vs. 1.01%), higher pH (7.13 vs. 6.86), thus it has a higher buffering capacity. Soil 1more percent organic matter than in soil 2 in all levels (except of the first level). This is agreement with the results of dehydrogenase and microbial respiration. Increase of microbial activity causes to further degradation of plant components into organic materials. Kim et al (2010) reported that significant correlation between dissolved organic carbon and microbial population in the soil. In the end of experiment, degradation amount in polluted soil had severer decrease than non-polluted soil. Actually, organic matter changed from 0.85% to 1.37% in the non-polluted soil, while from 1.47% to 1.01% in the polluted soil. Microbial activities including microbial respiration rate and dehydrogenase in non-polluted soil was more than polluted soil. Only in non-polluted soil that affected by higher concentration ofzinc,dehydrogenase wasdecreased severely by (70%) in 500 mg zinc kg^{-1}soil.Thiscould be related to the buffering capacityandsoil effectin maintaining of zinc bioavailability and concentration for microorganism. In this zinc level, increase of zinc bioavailability was observed in polluted soil (zinc exteractable with distilled water, $Ca(NO_3)_2$ and DTPA). These results were agreement with Aliasgharzad et al (2010). In some soils, addition of Zn increased bio-secretions that include severel enzyms (Xu et al., 2007).

3.2. Bioavail ability of Cd and Zn in the polluted and non-polluted after ancubation period

One step extraction procedure was used to determine the chemical forms of cadmium and zinc in the soils after period ancubation and zinc sulphate application. Assessment of the bioavailability of heavy metals in polluted soils using extraction is based on the assumption that metal bioavailability decreases with each successive extraction step in the procedure (Adamma et al., 2014).



With adding levels of zinc, amount of zinc exteractable with four extractants increasesd in two times. Polluted soil was more zinc bio availability than non-polluted soil (Figure 4). In both soils, zinc extractable with DTPA was decreased from 10 to 8% (from 21 to 49 days) and was transformed to residual forms. Generally, a significant part of zinc was extracted with HNO3 (90-92% of all) (Figure 5). Iyengar et al (1981) observed that the most of total zinc was in residual forms. While zinc extractable with distilled water and exchanged forms was less 1% of all zinc in soil. Liang et al (1990) exported that the most of zinc concentration was residual forms (60-99%). It was possible due to zinc transformation from activited and unstable to stable forms during time. Zn were distributed almost evenly across the residual fractions and also recorded high mobility factors (Adamma et al., 2014).

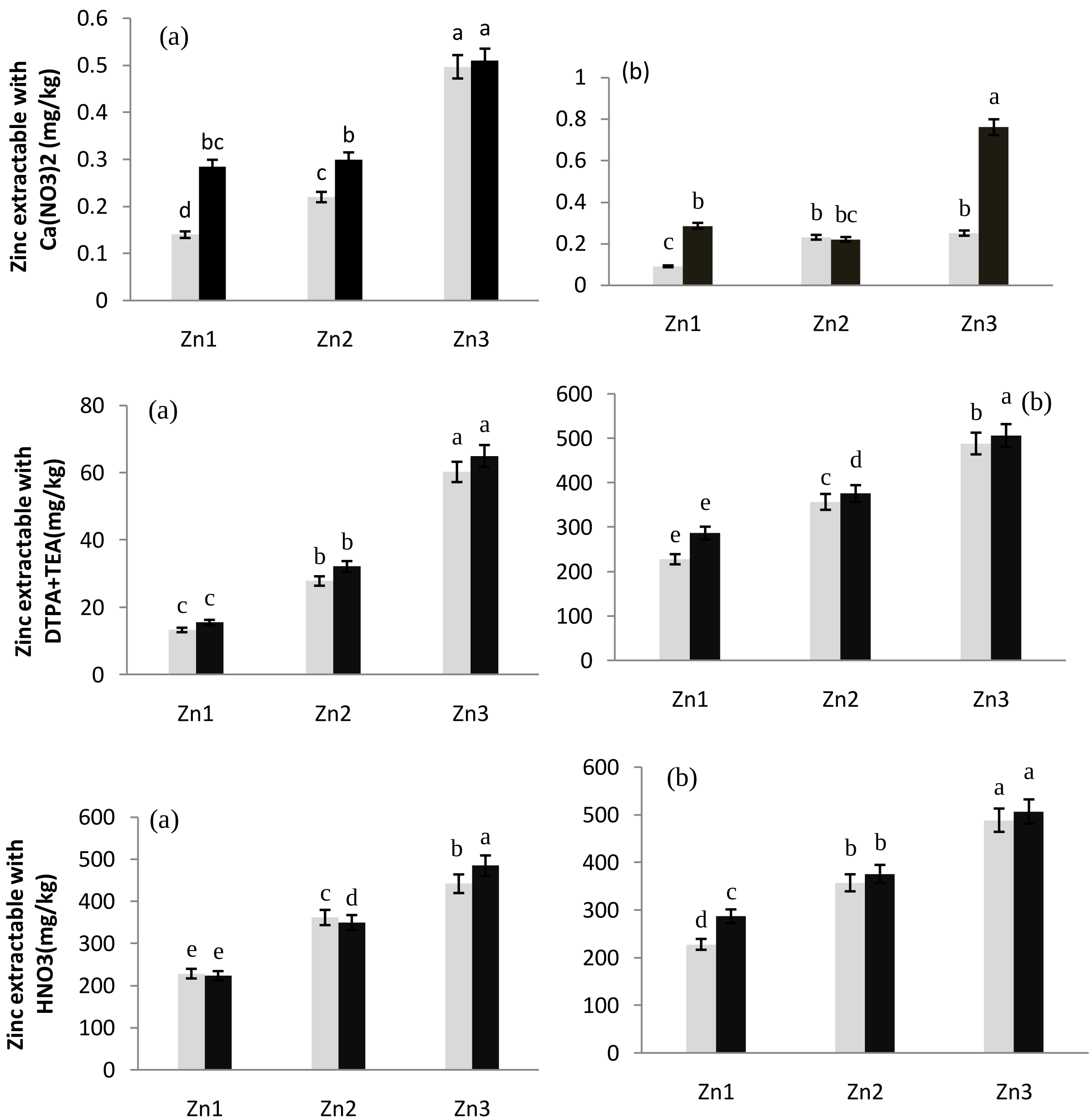
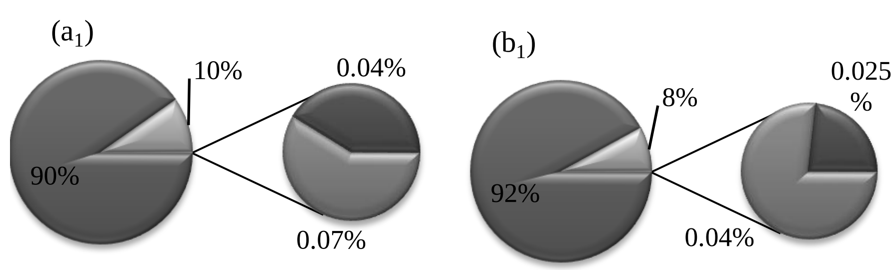


Fig. 4. Zinc extractable with extractants in two soils (a = 21 and b=49 days).



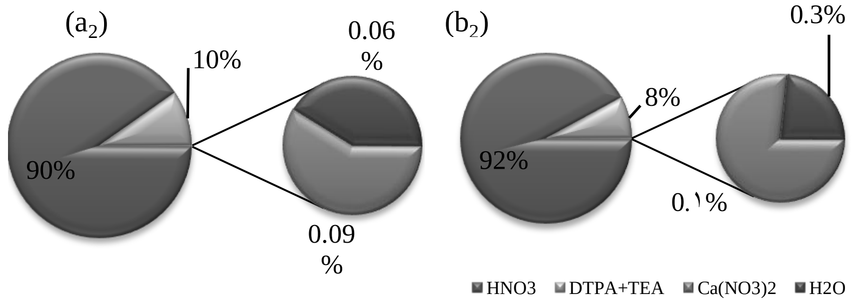
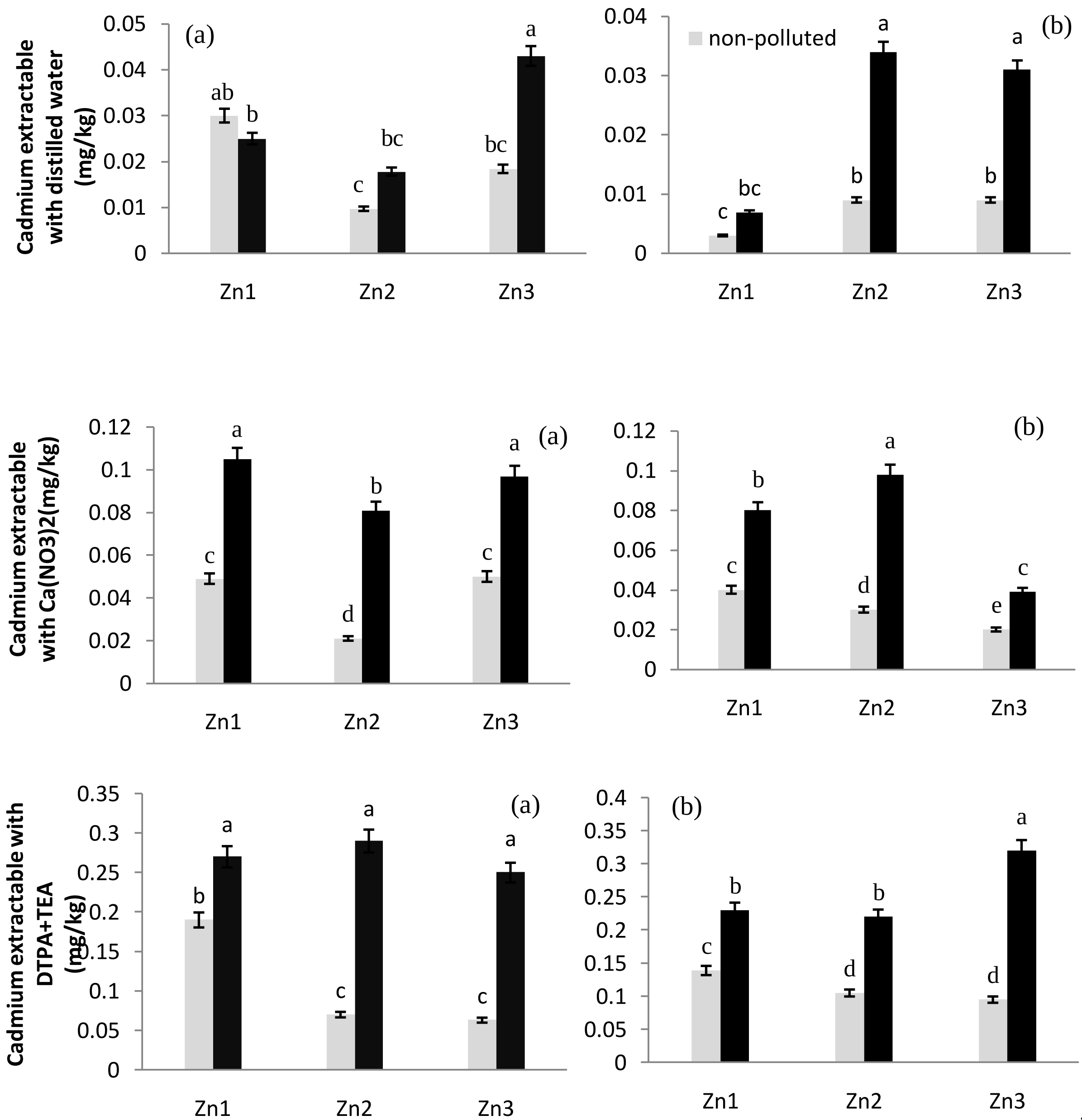


Fig. 5. Speciation of zinc in soil 1 (a1 = 21 and b1=49 days) and soil 2 (a2 = 21 and b2= 49 days).



a

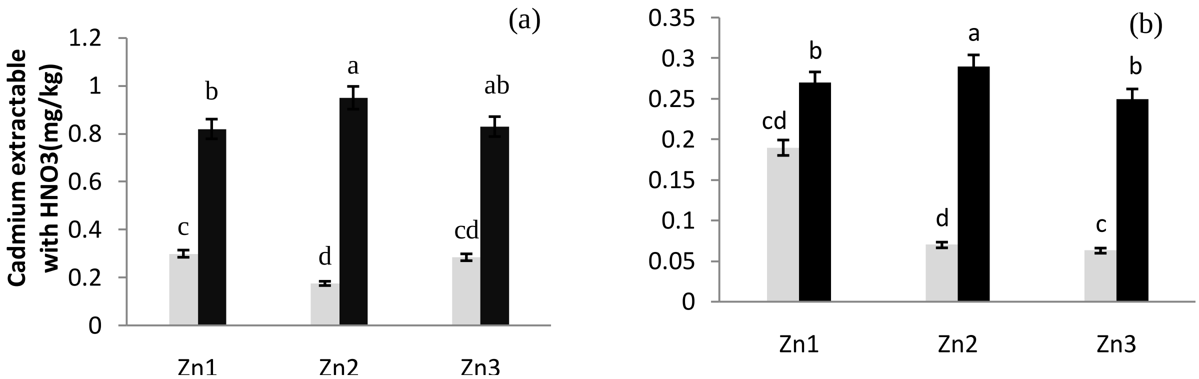


Fig. 6. Cadmium extractable with extractants in two soils (a = 21 and b=49 days).

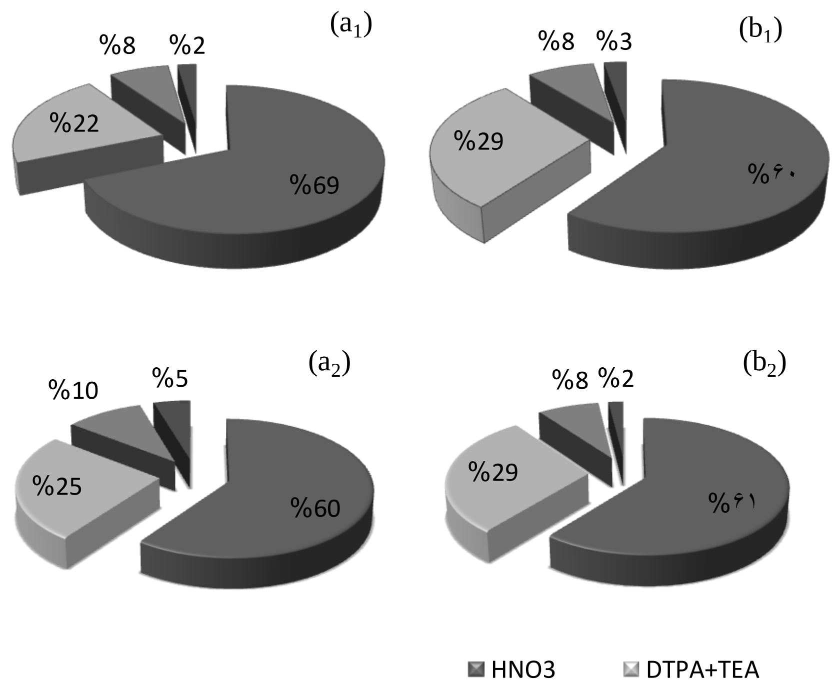


Fig. 7. Chemical forms of cadmium in two soils with four extractants.

Type soils and levels of zinc havesignificant differences on extractable cadmium by four extractants (Figure 6). These results confirmed with Chen et al., (2000). They reported that the different conditions of soils influence chemical forms of cadmium. Indeed, polluted soil had cadmium extractable concentration than non-polluted (approximately two times). It was due to nearby soil site to industerial factory. Generally, the polluted soil had more the amount of Cd extractable with extractants than non-polluted soil. It was related to site of sampling soils (polluted soil was nearby industrial factory). In the first 21 days, the amount of Cd extractable with NO_3, DTPA, Ca $(NO_3)_2$ and distilled water increased by 29%, 37%, 44% and 67% in the soil2 compared to soil1, while in 49 days, it was by 43%, 41%, 40%, 29% (Figure 6). It was peresented that more available forms was declined and added to un-available (residual) forms in the second time. Over time, Cd bioavailability was modified with affected of zinc levels. Effect of Zn levels were different in cd concentration (increase or decrease effect on its concentration). This issue justified, in according to its effects on the biochemical properties of the two times. Increase and decrease of cadmuim were due to impurity effect of cadmium in application zinc salt and interaction of Cd and Zn, respectively.

In the non-polluted soil, cadmium extractable with HNO_3 decreased from 69% to 60% and other available forms (exteractable with DTPA, Ca(NO3)2 and distilled water increased. Therefore, its bioavailability increased in non-polluted soil over time, while the polluted soil had been modified.This results showed that Cd bioavalability was more concentration in the soil that had low pH and organic matter (polluted soil; approximately3 times increase at first and second time, respectively) (Figure 7). The previous studies showed that the important role of pH in the metals bioavailability (McBride et al., 1997).Metal ions form stable complexes with organic matter and its bioavailability of these complexes may vary in solution (Fotovat et al., 1997).This result suggested that Cd forms weak complexes with organic matter and was easily removed at the initial stages of extraction, as reported by Ramos et al (1994). This disagreement is due to type of organic matter and complex power. Organic matter caused to formation of stable complex with cadmium in non- polluted soil and prevented its extraction, in addation to cadmium concentration was low concentration in distant soil from in dusterial factory.

4. Conclusion

Reduce of them icrobial and enzymatic activity caused to decrease organic matter in soil, and this issue had a special effecton the bioavailability of zinc and cadmium. Reduced of organic matter caused to increase the potential of Zn and Cd bioavailability. Increasing of zinc concentration had different effectson the two types of soil microbial activity and dehydrogenase. In each soils, the critical concentration of zinc was different in decrease of dehydrogenaseactivity.In regarded to being a significant part of zinc in non-available and solid phase and also nutritional role of zinc in plants were reasons for reduce in gits negative effectson some biological parameters of soils, while cadmium had more concentration in available forms (even at low concentrations) also not nutritional role of cadmium in plants. Also, by comparing of the results of chemical properties showed that pH and organic matter reducing were such as factors that through interaction with biological properties and chemical forms had effect on the zinc and cadmium. Increase of cadmium concentration and its effecton forms changing in order to complete this research is suggested.

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