Biological and Chemical Parameter Changes in Zinc polluted Soils (Case Study: Zanjan, Iran)

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ABSTRACT

Heavy metal pollution of soil is a serious environmental concern, because such metals have toxicological effects in ecosystems, animal and human health. Metal toxicity depends on the chemical forms in which they exist. The aim of this research was to investigate biological factors of two polluted soils (near and far from industrial factories) with three levels of zinc concentration. In both soil samples zinc contents were raised with zinc sulphate to three levels (250, 375 and 500 mg kg<sup>-1</sup> soil) based on initial HNO<sub>3</sub>-Zn content. Two types of soil (polluted and non-polluted) in a pot experiment were used under greenhouse condition (similar to actual condition) as randomized complete factorial design. The effect of pollution on soil biological factors was investigated at 49 days after treatment incubation (in according to first changes). Results indicate that the concentration of Zn, Fe, Cu, Pb and Mn was 223.7, 220.7, 21.9, 227 and 327.5 in µg g<sup>-1</sup> in the industrial land use and 38.26, 242.5, 18.8, 15.5 and 25.8 in µg g<sup>-1</sup> in the non-polluted soil, respectively. Increase of zinc sulphate levels decreased pH of polluted soil from first level (7.2) to third level zinc concentration (7.03) in 49 days (p<0.05). In non-polluted soil, organic matter percentage (% OC) decreased from 1.47% (at the beginning of experiment) to 1.22% (at day-21) and then to 0.82% (at day-49). By zinc concentration increment of Zn<sub>2</sub> and Zn<sub>3</sub>, respiration activity increased 31% compared to Zn<sub>1</sub> and non-polluted soil. Polluted soil had less microbial respiration rate (44%), dehydrogenase activity (50%) and organic matter (38%) compared to non-polluted soil.

Keywords: Dehydrogenase, heavy metal, microbial respiration rate, organic matter, pot experiment, soil respiration.

Abbreviations:
DHA: Dehydrogenase activity; OC%: Organic Matter percentage.

INTRODUCTION

The level of heavy metals and some physicochemical parameter in the soil are assayed. Soil is known to be the product of climate and living organism on rocks, and its nature is determined by the composition of parent rocks, type of climate and its age. It is essential to human because it is the top layer of the earth in which all the living things both plant and animals directly and indirectly take their food (Adefemi and Awokummi, 2009).

Pollution of the environment is one of the major side effects of human technological advancement. It results when a change in the environment harmfully affects the quality of human life and also animals, micro-organisms and plants. Therefore, soil pollution is defined as the appearance of persistent toxic compounds, chemicals, salts, or disease causing agents in soils which have adverse effects on plant growth and animal health (Pepper, 1996; Abosede, 2013).

Soil pollution with heavy metals occurs not only near mines, smelters and industrial factories; but it can also happen on agricultural land because of agricultural fertilizer and metal-based pesticides application. In Iran-zanjan province, long-term activity of industrial factories has led to the pollution of agriculture lands surface soils with heavy metals (Parizanganeh et al., 2010). Now a days, Taleghan is one of the places where agriculture is prevalent (Hosseini et al., 2012).

Many physical, chemical and biological properties of soil should be changed under the influence of contamination, but rate of biological and chemical characterisitc changes is very slow, therefore the measurement of affected index is
very important (Brady, 1996). It is urgent to develop cost-effective solutions to remediate soils contaminated with heavy metals. Bioavailability of heavy metals in the environment for organisms depends on soil properties, including pH and organic matter (Merry, 2001; Alloway, 2008). Soil is dynamic, lively and natural having essential role in various ecosystems. Although zinc (Zn) is an essential micronutrient for plants, it is also phytotoxic if present in excess amounts in growth medium. Excessive accumulation of zinc in soil will cause threat for bio-population of soil. Heavy metal pollution is significantly toxic for humans, animals, microorganisms and plants (Quartacci et al., 2005). Pendias and Pendias (2001) determined the critical concentration of zinc toxicity in soil for susceptible plant. This amount is equivalent to 100-500 mg kg⁻¹, especially 200-150 mg kg⁻¹. The concentrations caused a decrease of 25% yield percent of plants.

There is many mobile and available elements for absorbable in soils that have 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 the type of organic matter in the soil (Alloway, 2008). They are considered as the most sensitive ecological factors of agriculture and natural condition. Enzymes are vulnerable to high concentration of heavy metals and their measurement methods are quick, simple and cheap (Norwood et al., 2003; Cheng-Li, 2005).

Microbial biomass (bacteria and fungi) is a measurement of the living component's mass of soil organic matter. The microbial biomass decomposes plant and animal residues and soil organic matter to release carbon dioxide and plant available nutrients. Microbial biomass is one of the soil indicators that are sensitive to heavy metals concentration increment. It is used for investigating the quality of soil fertility. In addition to, microbial population changes influenced on short-term (Rajapaksha et al., 2004) and long-term pollution (Smolders, 2003). Microorganisms do not live in isolation but in complex biological communities within which exist complex interactions arising from biotic and abiotic influences (Petersen and Klug, 1994; Chefetz et al., 1996).

Besides the mentioned, 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 has a significant role in the biological activity detection in various soils. Dehydrogenase is one of the most important oxidoreductases enzyme that is an indicator of biological activity. It does oxidoreduction processes inside the living cells (Salazar et al., 2011). Chen et al. (2014) suggested that long-term heavy metal pollution decreased the microbial biomass. Heavy metals and soil physicochemical parameters were identified as environmental pollutants in some major industrial cities (Ojo et al., 2015).

The subject of the present study is assessment of biochemical factors in two soils samples of two different site (near and far from industrial factory) with same land application under wheat cultivation.

**MATERIALS AND METHODS**

**Soil Sampling:**

The experiment was conducted in a greenhouse in two soils, which were collected from surface horizon of a sandy clay loam soil (0-30 cm) of a wheat cultivated field located near the city of Zanjan (near an industrial factory - northwest of Iran) (Sample 1) and the other was located at Taleghan (far from industrial factory - north of Iran) (Sample 2). Geographic position of the soils was: Longitude of 36° 5.3' and Latitude of 50°28.9' 12" E (soil sample 1) and Longitude of 36° 40' 18.8" N and Latitude of 48°21' 3" E (soil sample 2).

**Initial Soil Materials:**

The soil was air-dried and passed through a 2-mm sieve. Particle-size distribution was determined by hydrometer method (Bouyoucos, 1962).

**Measurements:**

Soil pH and EC were measured in saturated extract (Thomas, 1996; Rhoades, 1996). Organic matter (OM) were determined by Walkley-Black method (Nelson and Sommers, 1996). Available Fe, Zn, Cu, Mn and Pb were determined by HNO₃ 4N extraction according to Chang et al. (1984).

**Soil Physicochemical Properties:**

Soil Physicochemical Properties are presented in Table 1. Although the most of physical and chemical properties of soil that was used in the experiment before treatment for both soils were similar, but the concentration of heavy metals except iron and copper (due to soil samples location) were different. Iron, zinc, copper, manganese and lead concentration were 242.5, 38.26, 18.8, 25.8, 15.5 mg kg⁻¹ in soil sample one (non-polluted) and 220.7, 223.7, 21.9, 327.5 and 227 mg kg⁻¹ in soil sample two (polluted), respectively. Soil 1 being away from the source of pollution had lower concentration of heavy metals in the soil while in soil 2 had increased clearly. Both soil texture was sandy clay loam, they were alkaline soil (pH>8). At the beginning of the experiment, soil 1 (polluted) had 0.62% organic matter higher than soil 2 (non-polluted) (Table 1).
**Table 1.** Some physicochemical properties of used soils.

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>ECe* (d sm⁻¹)</th>
<th>Texture</th>
<th>OM (%)</th>
<th>Fe (mg kg⁻¹)</th>
<th>Zn (mg kg⁻¹)</th>
<th>Cu (mg kg⁻¹)</th>
<th>Mn (mg kg⁻¹)</th>
<th>Pb (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.4</td>
<td>1.1</td>
<td>Sandy clay loam</td>
<td>0.85</td>
<td>242.5</td>
<td>38.26</td>
<td>18.8</td>
<td>25.8</td>
<td>15.5</td>
</tr>
<tr>
<td>Sample 2</td>
<td>8.2</td>
<td>1.3</td>
<td>Sandy clay loam</td>
<td>1.47</td>
<td>220.7</td>
<td>223.7</td>
<td>21.9</td>
<td>327.5</td>
<td>227</td>
</tr>
</tbody>
</table>

*Electrical Conductivity of soil saturation extracted; OM: Organic matter.

**Treatment Design:**

In accordance to initial zinc concentration (HNO₃ extractable) and with using ZnSO₄·H₂O, zinc concentration in soils received three levels (250, 375 and 500 mg kg⁻¹). After adding salt (zinc sulphate), soil 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 (Fig. 1-a). Soil sampling was conducted in two times that include 21 and 49 days after incubation period.

**Sample Analysis:**

The soil samples were air-dried at room temperature and purified by passing through a 2 mm nylon sieve. Because biological samples had not different in two times, it was sampled only at the end of experiment. CO₂ content from respiration rate was determined by back titration with residual NaOH and reported in terms of (mg CO₂ per g dry soil week) (Alef and Nannipieri, 1995) (Fig. 1-b).

Dehydrogenase activity (DHA) assay was based on using of 2,3,5-triphenyltetrazolium chloride (TTC) which was reduced to triphenylformazan (TPF), a red colour compound. It was reported as μg TPF per g dry soil 24h (Ohlinger, 1996) (Fig. 1). Soil pH₁:₅ (soil: distilled water; 1:5) was measured in saturated extract (Thomas, 1996; Rhoades, 1996). Organic matter percent (OM%) were determined by Walkley-Black method (Nelson and Sommers, 1996).

**Statistical Analysis:**

The data were subjected to statistical analysis using SAS 9.2 computer software. 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. Graphs were drawn with Excel 2010 program.

**RESULTS AND DISCUSSION**

Factors affecting heavy metal toxicity in soil-crop system include soil type which itself includes soil pH, organic matter content, 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).

**Chemical Reaction (pH):**

According to Fig. 2, a significant differences was observed in three levels of zinc in polluted and non-polluted soils at two times (21 and 49 days) (p<0.05). pH average of non-polluted soil was 6.86 at 21 days after the beginning of experiment and after 49 days received 7.2. pH of polluted soil average was 6.61 at 21 days after the beginning of
experiment and after 49 days received 7.11. At 21 days after the beginning of experiment, pH was 6.86, 6.84 and 6.89 at levels of zinc including Zn1, Zn2 and Zn3 and 7.2, 7.21 and 7.18 in 49 days in non-polluted soil, respectively. At 21 days after the beginning of experiment, pH was 6.63, 6.55 and 6.66 at levels of zinc including Zn1, Zn2 and Zn3 and 7.2, 7.11 and 7.03 in 49 days in polluted soil, respectively (Fig. 2).

Increase of zinc sulphate levels caused a decrease of pH in polluted soil at 49 days. Over time, the amount of soil sulphate increased as a result of sulphur fertilizer oxidation into the soil and pH reduction (Jiang et al., 2003; Najafi, 2013). It was related to sulphate anion in pH decrease. Difference in changes of soil pH was due to differences in soil buffering capacity.

**Organic Matter Percentage:**
A significant differences in three levels of zinc in polluted and non-polluted soils at two times (21 and 49 days) was observed (p<0.05). Average organic matter percentage of polluted soil was 1.22 at 21 days after the beginning of experiment and after 49 days it was 0.82. In this soil, %OC decreased from 1.47% to 1.22% and then 0.82% at the beginning of experiment to 21 days and then 49 days. %OC of polluted soil average was 1.22% at 21 days after the beginning of experiment and after 49 days it was 0.82. At 21 days after the beginning of experiment, %OC was 1.5, 1.1 and 1.06 at levels of zinc including Zn1, Zn2 and Zn3 and %0.69, 0.89 and 0.87 in 49 days, respectively in polluted soil. At 21 days after the beginning of experiment, %OC was 1.49, 1.23 and 1.62 at levels of zinc including Zn1, Zn2 and Zn3 1.22, 1.57 and 1.11 in 49 days, respectively in non-polluted soil (Fig. 3).

%OC decreased in both soils over time (from 21 to 49 days). Plant released the carbon fixed by photosynthesis in root environment (Jones et al., 2011). This condition decreased with polluting during 21 to 49 days after the beginning of experiment in the studied soils.

**Dehydrogenase:**
A significant differences was observed for dehydrogenase in three levels of zinc in polluted and non-polluted soils (p<0.05). Dehydrogenase average was 15.46 and 7.62 μg TPF/g dry soil 24h in non-polluted and polluted soil at 49 days after the beginning experiment, respectively. At 49 days after the beginning of experiment, dehydrogenase was 19.4, 24.89 and 2.11 at levels of zinc including Zn1, Zn2 and Zn3 and 14.1, 1.81 and 6.96 μg TPF/g dry soil 24h at 49 days, in non-polluted and polluted soils, respectively (Fig. 4).

Qu et al. (2011) reported that negative interactions was found between dehydrogenase and pollution from zinc and lead in the soil. These results are in accordance with the findings of this research. Also, increased levels of zinc have shown a different trend in both soils.

**Respiration Rate:**
Among the three levels of zinc in polluted and non-polluted soils, a significant differences (p<0.05) was observed. Respiration average was 0.39 and 0.17 mgCO2 per g of dry soil week in non-polluted and polluted soil at 49 days after the beginning experiment, respectively. At 49 days after the beginning of experiment, respiration rate was 0.52, 0.42 and 0.426 at zinc levels of Zn1, Zn2 and Zn3 and 0.148, 0.195 and 0.189 mgCO2 per g dry soil week in 49 days, in non-polluted and polluted soils, respectively (Fig. 4).
Microbial activities including respiration rate and dehydrogenase in non-polluted soil was more than polluted soil. Dehydrogenase decreased severely up to 70% in 500 mg zinc kg⁻¹ in non-polluted soil. This could be related to the buffering capacity and soil effect in maintaining of zinc bioavailability for microorganism. These obtained results are in agreement with Aliasgharzad et al. (2011). In some soils, addition of Zn increased bio-secretrions that include several enzymes (Xu et al., 2007). Some similar results by other researchers are also reported. Dehydrogenase activity, respiration per unit of soil and total organic carbon increased significantly by soil pH increment. Similar results have been reported by Aoyama and Nagumo (1997) in apple orchard soils with heavy metal accumulation for the microbial biomass.

The organic carbon content in the soil samples ranged between 1.06 to 1.62% after 21 days and 0.69 to 1.57% after 49 days. Non-polluted soil had more organic matter in all levels (except of the first level) due to greater microbial activity (1.37% vs. 1.01%), higher pH (7.13 vs. 6.86), thus it has a higher buffering capacity. Nwuche and Ugoji (2008) showed that the microbial process of carbon mineralization was inhibited to varied extents by the metals. This is in agreement with the results of dehydrogenase and microbial respiration. Microbial activity increment causes further degradation of plant components into
organic materials. Kim et al. (2010) reported a significant correlation between dissolved organic carbon and microbial population in the soil. At 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, this was while in the polluted soil the change was from 1.47% to 1.01%.

Reduce of the microbial and enzymatic activity caused a decrease organic matter in soil. Reduced of organic matter increased the potential of Zn bioavailability. Zinc concentration had different effects on the two types of soil microbial activity and dehydrogenase. In each soils, the critical concentration of zinc was different for decrease of dehydrogenase activity. The reasons for reducing Zn negative effects on some biological parameters of soils is to be a significant part of zinc in non-available and solid phase and also having a nutritional role in plants. Also, by comparing the results of chemical properties, it was observed that pH and organic matter reduction are factors that happen through interaction with biological properties. Increase of other heavy metals concentration and studying their effects on biological changes could be recommended for further studies.

References


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