



زانكۆی سه‌لاحه‌دین - هه‌ولێر  
Salahaddin University-Erbil

# **Effect of Lead and Zinc on Soil Biochemical Properties**

**A Research Project**

***By:***

**ASAAD ABDULLAH SULIMAN**

**AHMED TAHA OTHMAN**

***Supervisor by:***

***Assist. Prof. Dr.* NASHMEEL SAEED KHUDHUR**

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## ABSTRACT

Effect of soil pollution with Pb and Zn on soil enzymatic activities was studied under a laboratory experiment. For this purpose, the enzymes dehydrogenase, urease and nitrate reductase were assessed. Different concentrations of Pb and Zn were tested including: 50, 100, 150, 200, 250 and 300 ppm. The incubation period of the study was 2 months in 25 °C. Main results showed that lead has reduced both dehydrogenase and urease activities as the concentration increased while it has increased nitrate reductase activities in response to the increased doses of Pb. Whereas, Zn increased dehydrogenase, whereas decreased both urease and nitrate reductase activities in response to the increased doses. Thus it was clarified that heavy metals in the soils may either show antagonistic or synergistic effects on soil biochemical properties.

**Keywords:** Soil; Pollution; Heavy metals; Enzymatic activities.

## 1. INTRODUCTION

Pollution of the environment with toxic heavy metals is spreading throughout the world with industrial progress (Zwolak et al., 2019) that cause deleterious effects on soils, water and air (Khudhur et al., 2018). Environmental pollution and human exposure associated with heavy metals are attributed to different anthropogenic activities that include mining, industrial production, and the use of metal-containing compounds in domestic and agricultural settings (Semu et al., 2019).

Soil pollution by heavy metals represents a threat to the environment and food security due to the fast growth of industry and agriculture, and the disruption of natural ecosystems by anthropogenic pressure linked to the growth of human populations (Sarwar et al., 2017). Contamination of soil with toxic heavy metals is spreading throughout the world with industrial progress. Various industrial, agricultural and military operations have released huge amounts of toxic heavy metals into the environment with deleterious effects on soils, water and air (Khudhur et al., 2018, Khudhur and Khudhur, 2015).

Soil contamination with Pb and Zn can disrupt the activity of enzymes responsible for biomass decomposition and nutrient cycling (e.g. P, N, S, C). Biochemical activities are

robust indicator of soil fertility and quality. Lead and Zinc in soil generally disrupt soil enzymatic activity. Heavy metal ions can act as either cofactors or inhibitors in enzymatic pathways. It has been estimated that about half of all enzymes require a metal cofactor to be active and functional (Nagajyoti et al., 2010).

Metal ions are essential for the catalytic action of some enzymes. Metal ions contribute to the catalytic process through their ability to attract or donate electrons. Some metals bind the substrate by coordination links. Others contribute to maintain the tertiary and quaternary structures of the enzyme molecule (Prejanò et al., 2020).

Enzyme activity is a measure of microbial metabolism (Kaczyńska et al., 2015). The biochemical activity of soil is affected by several factors, including the presence of heavy metals, which penetrate the soil from various sources and modify its properties (Adugna and Abegaz, 2016). Enzyme activities have been used as indicators of soil quality and changes in biogeochemical function. Since enzymes catalyze all biochemical transformations, measurements of soil enzyme activities are useful indicators of biological activity as well as to understand how human activity is changing biogeochemical cycles in ecosystems (Bossio et al., 2005). Soil itself has no any enzyme activity for solubilization as well as mobilization of minerals. But the huge number of microorganisms present in soil makes it possible to recycle the nutrients from both organic and inorganic substances (Nath and Samanta, 2012).

Excessive uptake of metals by plants may produce toxicity in human nutrition, and cause acute and chronic diseases. For instance, Cd and Zn can lead to acute gastrointestinal and respiratory damages and acute heart, brain and kidney damages. High concentrations of heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Chibuike and Obiora, 2014).

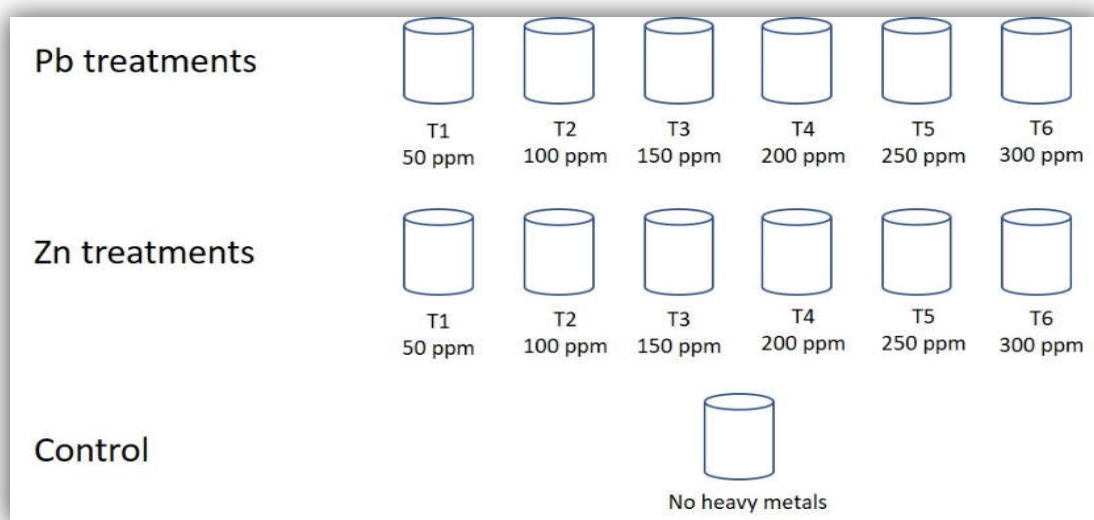
## The main aims of the study

Present study aimed to investigate the effects of lead and zinc on the following:

1. Soil dehydrogenase activity.
2. Soil urease activity.
3. Soil nitrate reductase activity.

## 2. MATEREAL AND METHODS

Surface soil sample (0-15 cm depth) from unpolluted area was collected. Then, the soil was air-dried, crushed and sieved through 2-mm stainless sieve to remove debris (Pansu, 2006). Then a laboratory experiment was set up according to the following: Thirteen beakers were filled with 300 grams of soil. Then each beaker was treated separately with the prepared heavy metals according to (Figure 1).



**Figure 1:** Diagram of the experiment set up.

After two weeks of incubation in the laboratory conditions (25 °C), samples were analyzed for enzymatic activities.

### **2.1. Estimation of dehydrogenase**

The dehydrogenase activity was determined by the modified procedure of Casida 1977 given by (Khudhur, 2018). For 5 g of soil in a test tube, 2.5 ml of sterile distilled water and 1ml of 3% aqueous solution of triphenyl tetrazolium chloride (TTC) was added and incubated at 30 °C for 24 hours. The triphenyl tetrazolium formazone end product was measured at 485 nm. The results expressed as  $\mu\text{g TPF}\cdot\text{g}^{-1}$  dry soil.24h<sup>-1</sup>.

### **2.2. Estimation of urease**

Urease activity was determined by the modified method of Hoffmann and Teicher 1961 described by (Khudhur, 2018). For 1 g of soil, toluene, citrate buffer (pH 6.7) and 10% urea substrate solution were added and incubated for 3 hours at 37 °C. The formed ammonium was determined spectrophotometrically at 636 nm (Bashour and Sayegh, 2007). Results expressed as  $\mu\text{g NNH}_4^+\cdot\text{g}^{-1}$  dry soil.3h<sup>-1</sup>.

### **2.3. Estimation of nitrate reductase**

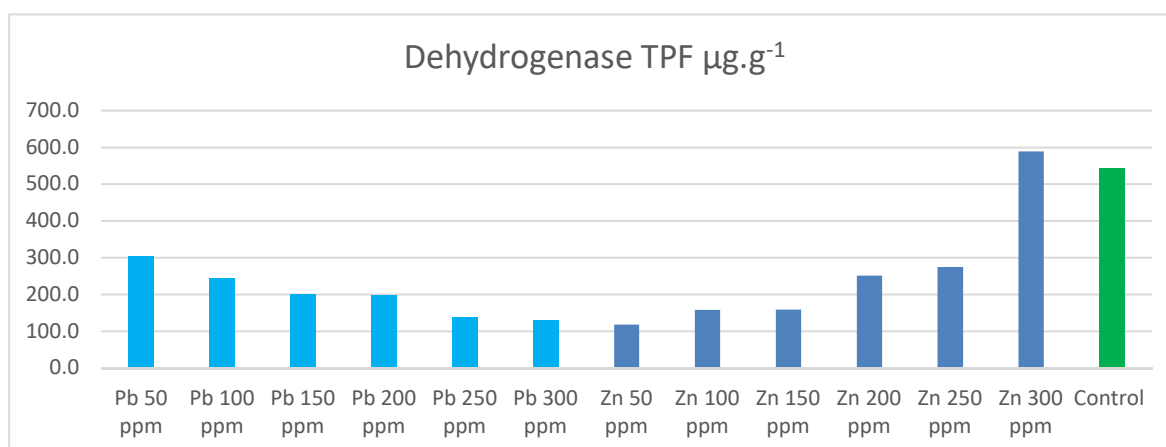
Nitrate reductase was estimated according to (Nath and Samanta, 2012). Into 150 ml conical flasks, 50 ml of peptone water media containing 1% KNO<sub>3</sub> were poured and then inoculated with 5 grams of different soil samples. The flasks were all incubated at 30 °C for 3 hours and then 10 ml of each soil suspensions were centrifuged at 5000 rpm for 10 minutes and 1 ml of the supernatants were treated with 1 ml of sulphanilamide. After 20 minutes, 1 ml of N (naphthyl) Ethelene Diamine Dihydrochloride (NEDD) was added to each sample and left for development of a pink color. Intensity of the pink color was measured at 540 nm and un-inoculated media (with sulphanilamide and NEDD) used as blank. Results expressed in  $\mu\text{g N-NO}_2\cdot\text{g}^{-1}$  dry soil.3h<sup>-1</sup>.

### **2.4. Statistical analysis**

Results were analyzed using Microsoft excel 2019. Data is reported as mean values in histogram presentations.

### 3. RESULTS AND DISCUSSION

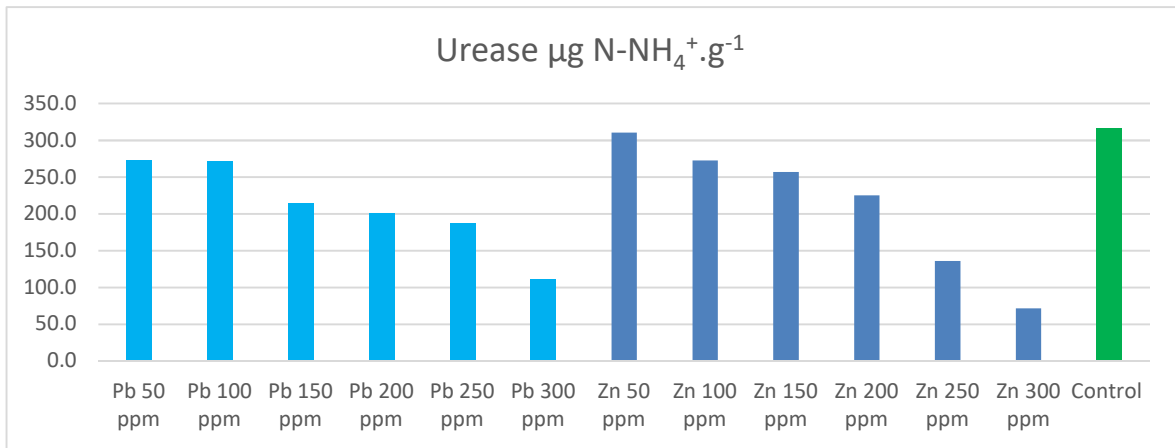
Present results are expressed in Figures 2-4. Dehydrogenase activity was decreased as Pb concentrations increased indicating the toxicity of lead to dehydrogenase so the highest dehydrogenase activity was 303.7 TPF  $\mu\text{g}\cdot\text{g}^{-1}$  during 50 ppm of Pb treatment. Whereas increasing Zn concentrations enhanced dehydrogenase activity and the highest activity was 589.2 TPF  $\mu\text{g}\cdot\text{g}^{-1}$  during 300 ppm of Zn treatment (Figure 2). Since, dehydrogenase is an enzyme that is particularly sensitive to the action of toxic compounds and it can indicate type and significance of pollution in soils (Nwaogu et al., 2016). Therefore, in our studies, the low activity of dehydrogenases in the soils is an indicator of decreased microbiological activity in the environment due to lead effects (Khudhur, 2018).



**Figure 2:** Dehydrogenase activity (TPF  $\mu\text{g}\cdot\text{g}^{-1}$ ) under the effects of both Pb and Zn during the study.

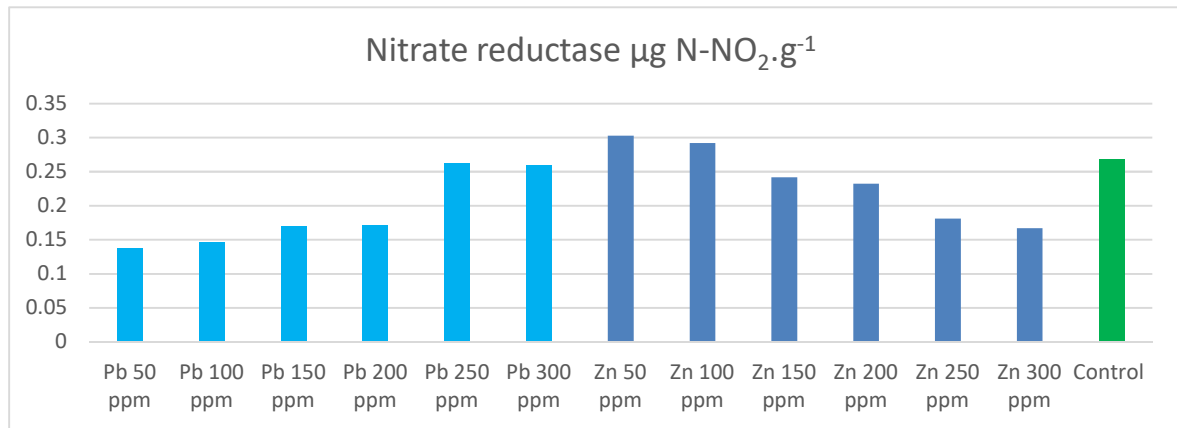
Both Pb and Zn decreased urease activity of soil as their concentrations increased as shown in (Figure 3). The highest urease values were: 272.9  $\mu\text{g N-NH}_4^+\cdot\text{g}^{-1}$  during 50 ppm of Pb treatment and 310.5  $\mu\text{g N-NH}_4^+\cdot\text{g}^{-1}$  during 50 ppm of Zn treatment. In this regard (Yan et al., 2013) found that the inhibitory effect of Pb was existed in most of the higher concentrations and with the increasing of Pb concentration in soil from 0.5 ppm to 100 ppm, the soil urease activity decreased and this confirm the present findings. Moreover,

(Yang et al., 2006) found the inhibitory effect of Zn on soil urease and catalase activities and this confirm the present findings.



**Figure 3:** Urease activity ( $\mu\text{g N-NH}_4^+.\text{g}^{-1}$ ) under the effects of both Pb and Zn during the study.

However, Pb increased nitrate reductase as concentrations increased, while decreased as Zn concentrations increased as given by (Figure 4). The highest nitrate reductase activity was  $0.259 \mu\text{g N-NO}_2.\text{g}^{-1}$  during 300 ppm of Pb treatment and the highest value was  $0.303 \mu\text{g N-NO}_2.\text{g}^{-1}$  during 50 ppm of Zn treatment. However, previous findings of (Sinha et al., 1988) observed that Pb inhibited nitrate reductase in contrast to the present finding and this may refer to the antagonistic effects of Pb with the other elements present in the soil (Hu et al., 2014), while (Singh et al., 1997) found the same observation as we observed during this study when Pb concentrations increased the nitrate reductase activities were also increased. The finding of (Trevisan et al., 2012) confirm the present results who found that Zn contamination with 350 ppm reduce nitrate reductase activity.



**Figure 4:** Nitrate reductase activity ( $\mu\text{g N-NO}_2\cdot\text{g}^{-1}$ ) under the effects of both Pb and Zn during the study.

With respect to the sensitivity of the soil samples to lead, the enzymes were arranged in the following order: dehydrogenase > urease > nitrate reductase. While the sensitivity of the soil samples toward zinc was: urease > nitrate reductase > dehydrogenase.

#### 4. CONCLUSIONS

Lead caused inhibition of dehydrogenase and urease in soil as its concentration increased, while increased nitrate reductase activity. However, zinc increased dehydrogenase activity as increased in the concentration, while decreased urease and nitrate reductase activities. The sensitivities of the soil samples to both Pb and Zn were as follow: dehydrogenase > urease > nitrate reductase and urease > nitrate reductase > dehydrogenase, respectively.

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