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The Effect of Chelation on Translocation of Lead (Pb^{2+}) to the shoots of Tomato Seedlings (*Lycopersicon esculentum mill*) Replanted in Hydroponic Solution

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Abstract: *The effects of Chelation on Pb^{2+} uptake by tomatoes (Lycopersicon esculentum mill) seedlings replanted in hydroponic solutions in a greenhouse was investigated. Six weeks old seedlings, were exposed to various concentrations of Pb^{2+} (0.0025, 0.005, 0.0075, and 0.025mg/L) and control (0.000). EDTA (0.025mM). From 0.0025 to 0.025mg/L Pb^{2+}, there was a substantial increase in translocation of Pb^{2+} in chelated treatments ($p<0.05$) compared to unchelated treatments of same concentrations of Pb^{2+}. So, chelation enhanced Pb^{2+} uptake by tomatoes (Lycopersicon esculentum mill). The Pb^{2+}-induced proline accumulation in shoots was also determined. The proline content increased significantly ($p<0.05$) with increasing Pb^{2+} concentrations. Free proline is known to accumulate in plants under heavy metals exposure and is considered to be involved in stress resistance.*

Keywords: *Proline, Hydroponic, Chelation, Greenhouse, Ethylene Diamine Tetra Acetic Acid [EDTA].*

1.0 INTRODUCTION

Lead is a chemical element with the symbol 'Pb', atomic number of eighty two (82) and relative atomic mass of 207.2. It is the heaviest member of carbon family. Even though a member of the carbon family, lead (Pb) looks and behaves very differently from carbon. The carbon family consists of the five elements in group (IVA) of the periodic table. Lead has been around for thousands of years. It is not possible to state when humans first found the element. No one is quite sure how lead got its name. The word has been traced to manuscripts that date to before 12th century. Romans called the metal *plumbum.* The chemical symbol; 'Pb' comes from this element. Compounds of lead are occasionally called by this old name, such as plumbous chloride. Lead infrequently exists as a pure element in the earth. The most common ore of lead is galena, or lead sulfide (PbS). Anglesite, or lead sulfate ($PbSO_4$); cerussite, or lead carbonate ($PbCO_3$); and

mimetite ($PbCL_2$ o $Pb_3(AsO_4)_2$) are the other ores of lead. It is very resistant to corrosion but tarnishes upon exposure to air. It dissolves slowly in water. It reacts rapidly with hot acids but slowly in cold acids. Due to protective oxide layer, lead does not react with sulphuric acid and hydrochloric acid. However, it reacts with nitric acid to form nitrogen oxide and lead nitrate

$3Pb_{(s)} + 8\ HNO_3 \longrightarrow 2NO_{(g)} + 3Pb(NO_3)_{2(s)} + 4H_2O_{(l)}$

It reacts sparingly with oxygen in the air to form lead (II) monoxide

$2Pb_{(s)} + O_{2(g)} \longrightarrow 2PbO_{(s)}$

At room temperature, lead reacts actively with halogens

$Pb_{(s)} + Cl_{2(s)} \longrightarrow PbCl_{2(s)}$

2.0 Materials and Method

2.1 Source Of Tomato (Lycopersicon esculentum mill) Seeds

The tomato (*Lycopersicon esculentum Mill*) seeds were obtained from the International Institute of Tropical Agriculture (IITA) station, Tarauni, Kano state, Nigeria. The seeds were planted and monitored for eight weeks, from the 21st of August to 28th of October, 2017 at the Department of Agronomy farm, Bayero University, Kano. The coordinates of the farm are latitude 8° 22', to 9° 25'N and longitude 11° 57 to 12° 00'E. They were washed with tap water to remove excess soil, and rinsed three times with deionise water before replanting in hydroponic solution and kept in a greenhouse at 65% relative humidity, 13hrs/day 11hrs/night under 600 µ mol m^{-2} s^{-1} of light intensity, and day/night temperatures 39/23°C. Plants were supplied with the Hoagland nutrient solution (pH 6.0-6.3) which contained the following nutrients: 1mM KH_2PO_4, 2mM $MgSO_4 \cdot 4H_2O$, 5mM KNO_3, and 5mM $Ca(NO_3)_2 \cdot 4H_2O$ and 9µM $MnCl_2 \cdot 4H_2O$, 4.6 µM H_3BO_3, 0.8 µM $ZnSO_4 \cdot 7H_2O$, 0.3 µM $CuSO_4 \cdot 5H_2O$, and 0.1 µM $H_2MoO_4 \cdot H_2O$. Iron was supplied as Fe-EDTA (1.8 mM). Lead (Pb) was also supplied as $Pb(NO_3)_2$, at the following concentrations 0.0025moldm^{-3} to 0.025moldm^{-3} and 0.25mol/dm^3 of EDTA (Dagara M.S. and Umar G.W. 2016).

2.2 Translocation Factor

Translocation factor (TF): is defined as the ratio of the concentration of a metal in the aerial part of a plant to its concentration in the root, Chen *et al.* (2012).This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant. It is represented by the ratio:

$$TF = \frac{Concentration\ of\ metal\ in\ the\ shoot}{Concentration\ of\ metal\ in\ the\ root}$$

2.3 Proline Content Determination

The proline content in the shoots was determined by the method recommended by Bates *et al.* (1973).

2.4 Atomic Absorption Spectrophotometric Determination of Pb^{2+} in Roots and Shoots of Harvested Tomatoes (*Lycopersicon esculentum Mill*) Seedlings.

After five days exposure, the tomato (*Lycopersicon esculentum Mill*) seedlings were harvested and washed first with tap water, followed by 1% HNO_3 and finally rinsed with deionised

water. The roots and shoots were separated and oven dried at 60°C for 48 hours. They were ground with wooden mortar and pestle to a fine powder. A washed dried porcelain crucible was ignited on a hot electric plate for 5minutes. 2g of each sample was accurately weighed into the crucible and gently heated on hot electric plate until the smoking ceased. It was then transferred and ashed to constant weight in a muffle furnace at 550^0C for 4hours. The ash was cooled in a dessicator, dissolved in 0.10M HNO_3, filtered into $50cm^3$ volumetric flask and made to mark. The Cu^{2+} content in the roots and shoots was analyzed using Atomic Absorption Spectrophotometer (Buck Scientific, Model 210VGP) at 324.7nm. The concentration of Cu^{2+} was reported as mg g^{-1} dry weight (Dagara M.S. and Umar G.W. 2016).

2.5 Statistical Analysis

Analysis of variance (ANOVA) using the SPSS software was performed to check the accuracy and validity of the results. Data were expressed as mean followed by SD. Statistical significance was assumed at $p<0.05$.

3.0 Results and Discussion

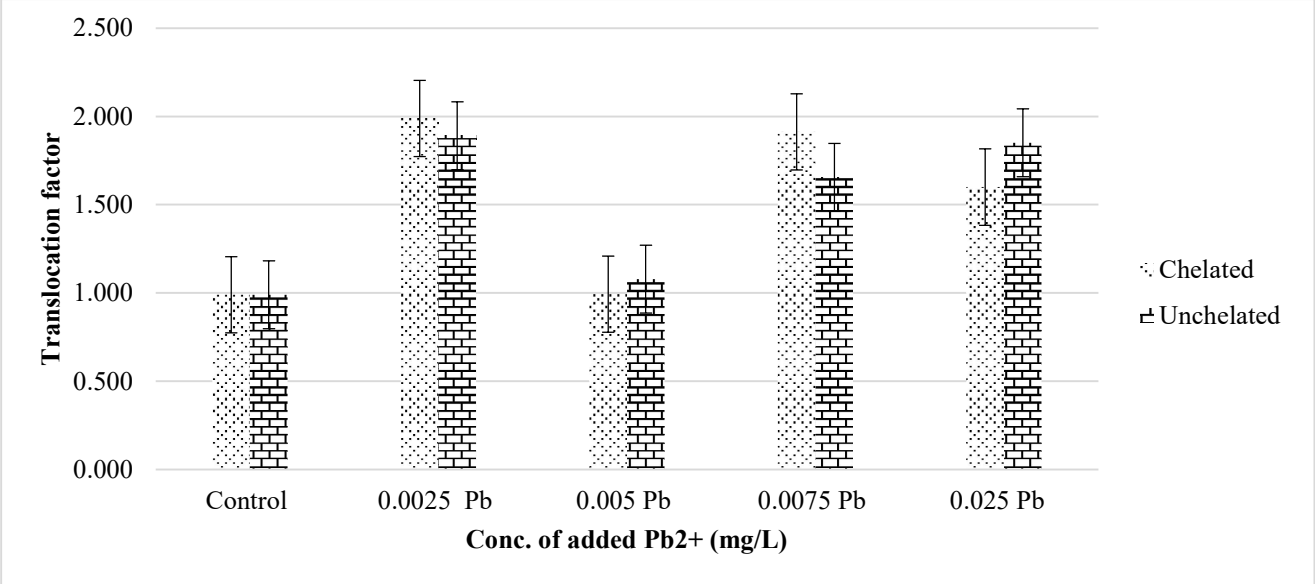

Figure 3.1: Translocation Factor (TF) of Pb in Chelated and Unchelated Treatments by Seedlings Replanted in Hydroponic Solution.

Figure 3.1 above shows the effects of translocation factor (TF) against concentration of added Pb^{2+} in chelated and unchaleted treatments. The chelated treatment showed TF values which were 1.989; 0.994; 1.913; and 1.600. This shows that good fraction of Pb^{2+} Was translocated to the shoots, hence chelation enhances uptake of Pb^{2+}. These findings were similar to those of Degryse *et al*, (2006) and Mellem *et al*., (2009). Where there was a translocation of metal ion to the aerial parts of the plant. While, the treatments for unchelated Pb also showed TF values greater than 1 which were 1.892; 1.079; 1.656 and 1.852. From the results above, EDTA chelate did not significantly aid the translocation of Pb in various treatments as compared to unchelated treatments.

Fig. 3.2 below showed proline accumulation in shoots of tomato seedlings. untreated Pb^{2+} solutions showed significant increase ($P<0.05$) when compared to control. The proline content for unchelated treatments were 24.483±15.344; 56.917±29.489; 40.284±33.525; 33.016±34.764µmol proline g^{-1} respectively. While at 0.005 mg Pb L^{-1} there was peak increase in proline.

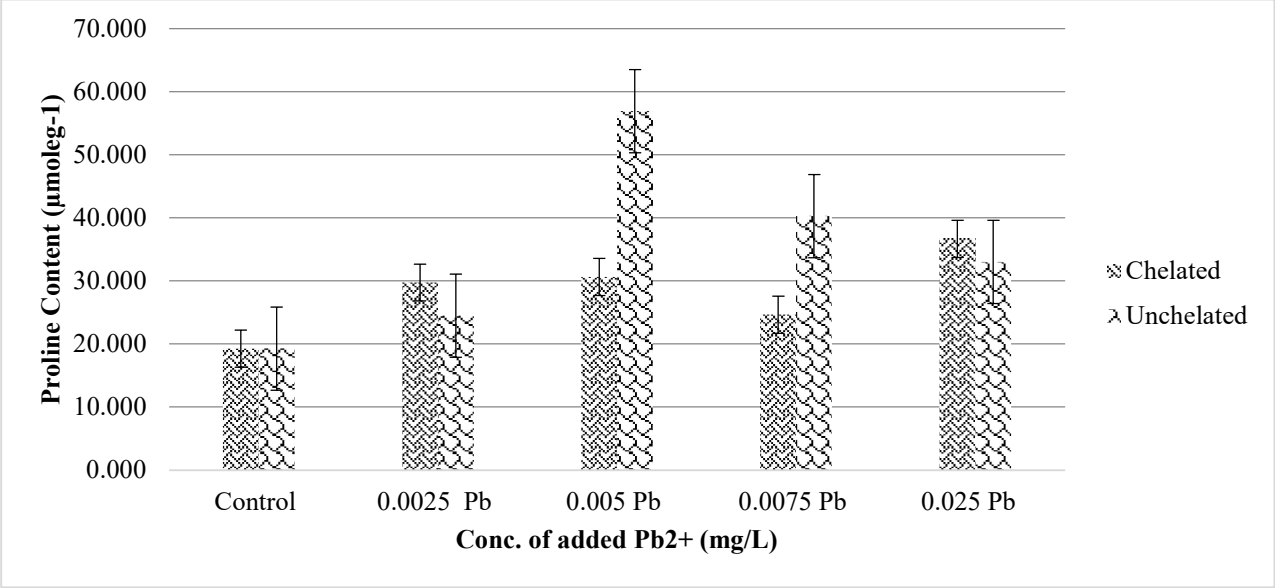

Fig 3.1: Proline Accumulation in the Shoot and Root of Tomato Seedlings (*Lycopersicon esculentum mill*) in Treated and Untreated Pb solutions Replanted in Hydroponic Solution.

3.0 Conclusion

Application of various concentrations of Pb^{2+} (0.00, 0.0025, 0.005, and 0.025mg/L) and 0.25mM EDTA to hydroponic solutions enhance the translocation of Pb^{2+} in tomato Seedlings (*Lycopersicon esculentum mill*) seedlings. Varying degrees of phytotoxic symptoms which include reduction in dry weight of plant were observed depending on the concentration of Pb^{2+} and presence or absence of EDTA these results is in agreement with the findings of Erdei *et al*, (2002), Jiang *et al.,* (2004) and Glinska *et al.,* (2013). The highest proline content was found in seedlings grown in hydroponic treated with 0.005mg/L Pb^{2+} and 0.25mM EDTA.

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