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LEVELS OF INDUCED FREE PROLINE IN CHELATED AND UNCHELATED HYDROPONICALLY GROWN LETTUCE (*Lactuca sativa* L.)

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ABSTRACT

It is well described that under stress conditions many plant species accumulates proline as adaptive response to adverse conditions. In this study, eight weeks old Lactuca sativa L. seedlings were transplanted in nutrient solution containing various doses (0.000, 0.0025, 0.005, 0.0075, and 0.025mg/L) of Pb^{2+} and Cd^{2+} and constant concentration of EDDS (0.0025M). The effects of these treatments of Pb^{2+}, Cd^{2+}, combination of Pb^{2+} + Cd^{2+} and with or without EDDS (Ethylene diamine disuccinate) were compared based on the level of stress induced to the seedlings. Free proline contents induced due to stress by uptake of Pb^{2+} and Cd^{2+} were investigated in both unchelated and chelated treatment. The results obtained showed that the effect of increasing concentration of Pb^{2+} in hydroponic solution leads to decrease in the level of stress induced to the seedlings by EDDS while the lower the Cd^{2+} concentration in the hydroponic solution the more the level of stress induced by EDDS to the plant. The highest level of stress caused to the seedlings by unchelated and chelated Pb^{2+} solution are 68.294±5.642; 72.681±14.740 $\mu molg^{-1}$ respectively while that of Cd^{2+} are 28.962±8.703; 48.263±2.455 $\mu molg^{-1}$ respectively. From this, it is evident that Pb^{2+} induced more stress to the plants than Cd^{2+} in the presence and absence of EDDS ($p<0.05$).

Keywords: Proline, Ethylenediaminedisuccinic Acid (EDDS), Lead, Cadmium, Lettuce.

INTRODUCTION

Among the lot of plant species, macrophytes are known to take up a great amount of pollutants by surface adsorption and or absorption and to incorporate them directly into their tissues (Espinoza-Quinones *et al*., 2009). The exudation of organic compounds by roots and their influence on the solubility of essential and toxic ions through their effects on microbial activities, root growth dynamics, acidification and chelation is a well-known phenomenon (Nigam *et al.,* 2000). Due to this plant strategy, various organic compounds are used for induced phytoremediation methods to increase the amount of water soluble and exchangeable risk element forms. Cadmium and lead are among the most habitually discussed environmental pollutants. However, they are absorbed and accumulated in different plant parts, especially in the roots (Malkowski *et al.,* 2002).

Proline is a proteogenic amino acid and accumulates both under stress and non-stress conditions as a beneficial solute in plants. Recent discoveries point out that proline plays an important role in plant growth and differentiation across life cycle (Kishor *et al.,* 2015). The amino acid proline has a unique biological role in stress adaptation. Proline metabolism is manipulated under stress by multiple and complex regulatory pathways and can profoundly influence cell death and survival in microorganisms, plants and animals (Zhang and Becker, 2015). It is well described that under stress conditions many plant species accumulates proline as adaptive response to adverse conditions (Verbruggen and Hermans, 2008). Although a clear-cut relationship between proline accumulation and stress adaptation has been questioned by some authors, (Hare and Cress, 1997). It is generally believed that the increase in proline content following stress injury is beneficial for the plant cell. Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important roles. Heavy metal stress leads to proline accumulation, (Alia and Saradhi, 1991; Zengin and Muzuroglu, 2005).

Proline accumulation in plant tissues has been suggested to result from a decrease in proline degradation, an increase in proline biosynthesis, a decrease in protein synthesis or proline utilization, and hydrolysis of proteins (Charest and Phan, 1990; Zengin and Muzuroglu, 2005).

This research was carried out to determine the level of stress caused by cadmium and lead to lettuce plant and to ascertain which of the heavy metals causes more stress to the plant, also to establish the concentration at which both metals indicate the highest intensity of the stress.

MATERIALS AND METHODS

Treatment of seedlings

Eight weeks old lettuce (*Lactuca sativa*) seedlings were carefully harvested from Department of Agronomy farm, Bayero University, Kano in October, 2017. They were washed with tap water to remove excess soil, and rinsed three times with deionized water before replanting in different concentrations of hydroponic solution as reported by Dagari and Umar (2016) which contains: 0.0075moldm^{-3} KI, 0.10moldm^{-3} H_3BO_3, 0.10moldm^{-3} $FeCl_3.6H_2O$, 0.05moldm^{-3} $MnSO_4.H_2O$, 0.05moldm^{-3} $Ca(NO_3)_2$ $.4H_2O$, 0.05moldm^{-3} $Na_2H_2P_2O_7$, 0.10moldm^{-3} KNO_3, 0.05moldm^{-3} $MgSO_4.H_2O$. Lead and Cadmium in five levels (0, 0.0025, 0.005, 0.0075 and 0.025 mg/L) each as $Pb(NO_3)_2$ and $Cd(NO_3)_2$ were introduced into the nutrient solution. The concentration of EDDS used was 0.0025M. Each treatment in triplicates was allowed to stand for five days, after which the plants were harvested and subjected to physiological and biochemical analysis.

Plant Analysis

The lettuce *(Lactuca sativa)* seedlings were harvested after five days exposure to Hydroponic solutions kept in a greenhouse at 65% relative humidity, 13hrs/day 11hrs/night under 600µmol m^{-2} s^{-1} of light intensity, and day/night temperatures 39/23°C, and washed first with tap water, followed by 1% HNO_3 acid and finally rinsed with deionised water (Dagari and Umar, 2016).

Proline Content Determination

The roots and shoots were separated and dried under laboratory temperature for two (2) weeks. The shoots were ground with wooden mortar and pestle to a fine powder and the proline content in the shoots was determined by the method recommended by Bates *et al.,* (1973).

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 $p<0.05$.

RESULTS AND DISCUSSION

To estimate the extent of stress undergone by lettuce (*Lactuca sativa* L.) seedlings when subjected to various concentrations of Pb^{2+} in the range from 0.000-0.025 mg/L for chelated (0.0025M EDDS) and unchelated treatments of hydroponic solutions, Figure 1 shows Pb^{2+} induced proline (µmolg^{-1}) accumulation in the shoots. The change in the proline production due to the addition of Pb^{2+} was linear from 0.000 to 0.005mg/L (24.575±9.511, 53.971±19.861 and 68.294±5.642 µmolg^{-1}) respectively, i.e. proline accumulation by the plant is proportional to added concentration of the Pb^{2+}. This change in proline production is significant (*$P < 0.05$*). The contents of proline declined from 0.0075 mg/L (53.860±22.567 µmolg^{-1}) to 0.025 mg/L (20.668±2.163 µmolg^{-1}) which has the lowest production. This may be due to high concentration of Pb^{2+} in the solution.

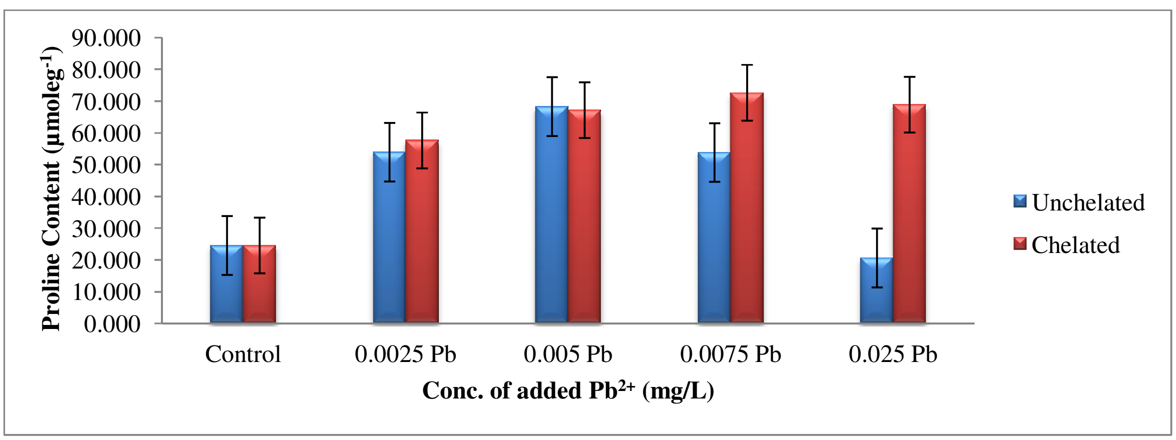

Figure 1: The extent of stress induced on Lettuce (*Lactuca sativa*) Seedlings in chelated (EDDS 0.0025M) and unchelated treatments of hydroponic solutions containing different concentrations of Pb^{2+}

Addition of 0.0025M EDDS to the different concentrations of Pb^{2+} (0.000, 0.0025, 0.005, 0.0075 mg/L) results into substantial increase in the proline production by the plants (24.575±9.511, 57.674±3.434, 67.232±7.621, 72.681±14.740 μmolg^{-1}) which indicates that EDDS induces more stress to the plant. At 0.025 mg/L concentration of Pb^{2+} sudden change in the proline production occurred (68.941±3.702 μmolg^{-1}). Thus, the changes in the proline production are very significant ($P < 0.05$) when compared to control. In Figure 2, when plants were exposed to various concentrations (0.0025, 0.005 and 0.025 mg/L) of Cd^{2+}, the changes in the proline production were almost constant (28.962±8.703, 28.075±9.285 and 24.815±5.541 μmolg^{-1}). The difference was insignificant (p>0.05) between 0.0025 and 0.025 mg/L but there was significant difference ($P<0.05$) compared to the control. Whereas at 0.025 mg/L, the difference was insignificant ($P>0.05$) compared to the control. Though, it was observed that the lower the concentration of Cd^{2+}, the higher the amount of proline in the shoots. At 0.0075 mg/L, proline content of the plant was lower (6.529±5.585 μmolg^{-1}) compared to other Cd^{2+} treatments, the difference was significant ($p<0.05$).

The addition of 0.0025M EDDS led to a significant increase ($p<0.05$) in the proline contents at 0.0025 mg/L (48.263±2.455 μmolg^{1}) and 0.0075 mg/L (27.152±2.445 μmolg^{-1}) more than the unchelated treatments of same concentration. This indicates that EDDS induces more stress to the plant at these concentrations. At 0.005 mg/L (22.414±3.904 μmol/g) and 0.025 mg/L (6.668±1.147 μmolg^{-1}), proline production was lower in the presence of EDDS than the unchelated treatment of same concentrations; also, there was a significant decrease relative to the control ($p<0.05$). This finding is in agreement with the findings of Hassan *et al.,* (2016). Figure 3 shows the results of the stress induced to the plant by both metals in chelated and unchelated treatments. From the results, it is shown clear that there was more proline production to the seedlings transplanted in the unchelated treatment than the seedlings under EDDS toxicity. It was observed that as the concentration of Pb^{2+} + Cd^{2+} increases, the proline accumulation declines for both chelated and unchelated. The highest proline production; 44.975±3.782, 41.171±3.700 μmolg^{-1} for chelated and unchelated treatments respectively was recorded at 0.0025 mg/L Pb^{2+} + Cd^{2+} and the lowest proline production (4.608±2.591, 18.166±4.629 μmolg^{-1}) for chelated and unchelated treatments respectively was observed at 0.025 mg/L Pb^{2+} + Cd^{2+}. From the results above, it is shown that the addition of chelant (EDDS) prevents the plants from undergoing much stress relative to the seedlings replanted in treatments without chelant (EDDS).

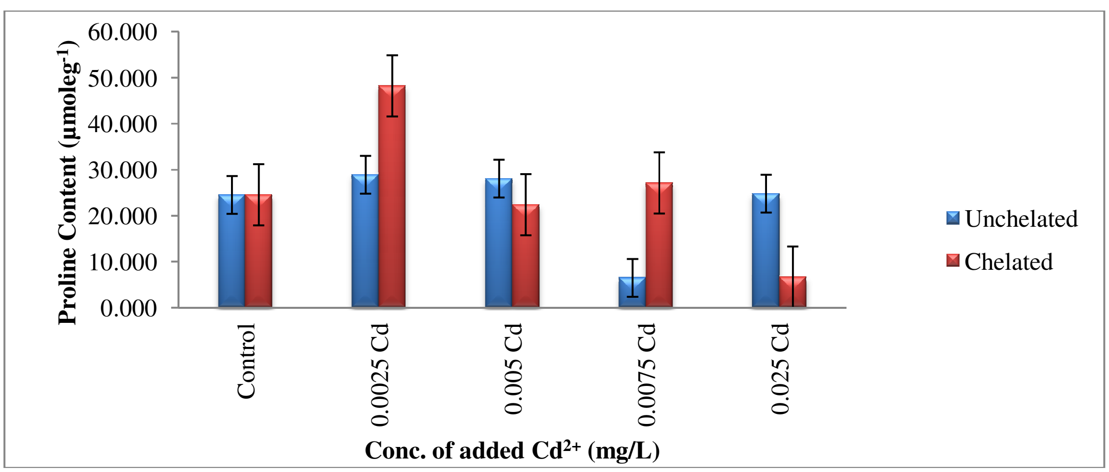

Figure 2: The extent of stress induced on Lettuce (*Lactuca sativa* L.) Seedlings in chelated (EDDS 0.0025M) and unchelated treatments of hydroponic solutions containing different concentrations of Cd^{2+}

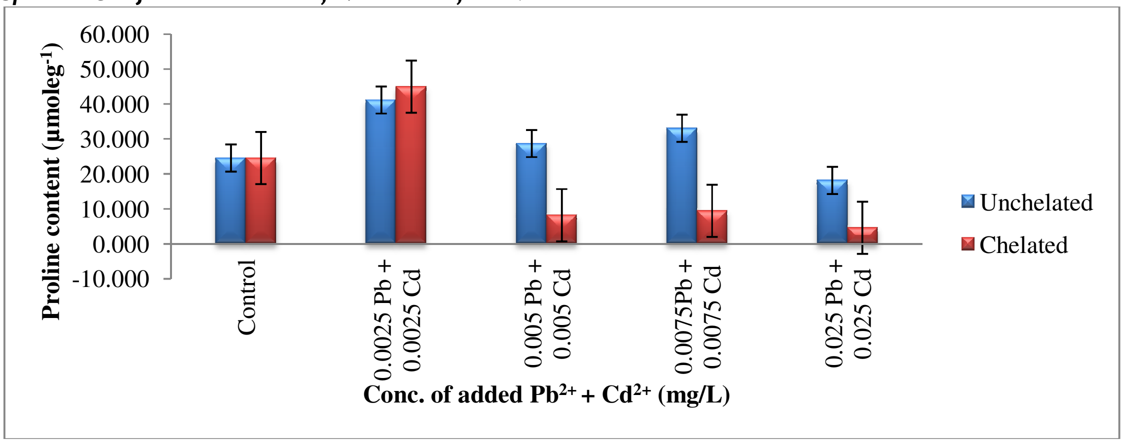

Figure 3: Comparing the extent of stress induced on Lettuce (*Lactuca sativa* L.) Seedlings in chelated (EDDS 0.0025M) and unchelated treatments of hydroponic solutions containing same concentrations of Pb^{2+} and Cd^{2+}

CONCLUSION

Proline was determined to assess the level of stress and damage caused by Pb^{2+} and Cd^{2+} uptake in chelated and unchelated treatments to the plants. Under Pb-toxicity, EDDS induced more stress to the seedlings when compared to unchelated hydroponically grown seedlings. The chelates had negative impact on Pb^{2+} uptake from nutrient solution and its accumulation. Lower chelate addition caused higher Pb^{2+} accumulation compared to higher chelate addition. Proline accumulation by seedlings under unchelated Cd-toxicity was almost constant and there was no significant difference relative to the control ($p < 0.05$). Also, the lower the concentration of Cd^{2+} the lower the proline contents of the seedlings in both chelated and unchelated treatments. Furthermore, the addition of chelant (EDDS) to hydroponic treatments containing the combination of both metals (Pb^{2+} + Cd^{2+}) prevents the plants from undergoing much stress when compared to the seedlings under unchelated (Pb^{2+} + Cd^{2+}) treatment. The highest proline accumulation was shown under Pb-toxicity for both chelated (72.681±14.740 µmolg^{-1}) and unchelated (68.941±3.702 µmolg^{-1}) treatments. The common symptoms of Pb^{2+} and Cd^{2+} toxicity in plants which are mainly; chlorosis, necrotic lesions, stunted growth and leaf wilting were observed.

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