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EFFECTS OF ETHYLENE DIAMINE DISUCCINIC ACID (EDDS) ON LEAD (Pb²⁺) AND CADMIUM (Cd²⁺) UPTAKE BY LETTUCE (Lactuca sativa L.) SEEDLINGS PLANTED IN HYDROPONIC SOLUTIONS

¹Dagari, M. S., ²Musa, M. S.^{*} and ²Maude, F. M.

¹Department of Chemistry, Faculty of Science, Federal University Gashua, Yobe State, Nigeria. ²Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, College of Natural and Pharmaceutical Sciences, Bayero University Kano, P.M.B. 3011, Nigeria.

*Correspondence author: <u>m_smusa@yahoo.com</u>; <u>msmusa.chm@buk.edu.ng</u>; +2348060899099

ABSTRACT

This research work was carried out to investigate the effects of ethylene diamine disuccinate (EDDS) on the absorption of cadmium and lead by hydroponically grown lettuce (Lactuca sativa L.). Samples were prepared through digestion (dry ashing) method and the analytes quantitatively analyzed using Atomic Absorption Spectrophotometric (AAS) technique. The effects of EDDS on Pb²⁺ and Cd²⁺ uptake by lettuce (Lactuca sativa L.) seedlings replanted in hydroponic solutions for 72 hr in a greenhouse were investigated. Two months old seedlings were exposed to various doses of Pb²⁺ and Cd²⁺ at constant concentration of EDDS. For unchelated treatments, increase in concentrations of Pb²⁺ and Cd²⁺ in the solution increases the uptake by the plants relative to control. Pb²⁺ contents in the roots showed more accumulation than in shoots (p<0.05) while Cd²⁺ content in the shoots was higher than in roots. Addition of 0.0025M EDDS to the solutions of Pb^{2+} and Cd^{2+} inhibits the uptake of the metal ions at the following concentrations (0.0025, 0.005 and 0.0075 mg/L) when compared to unchelated treatments of same concentrations. At 0.025 mg/L of Pb²⁺ and Cd²⁺, chelation substantially enhances their uptakes in both shoot and root of the plant (p<0.05). Compared to the unchelated treatments, chelation suppresses the translocation of Pb²⁺ and Cd^{2+} to the shoots of the plant (p<0.05), but there was a better translocation of Pb^{2+} (1.257) and Cd²⁺ (0.953) at 0.025 mg/L, thus indicating that, at very high concentration of the metals the plant was hyper-accumulator. For hydroponic solutions containing the combination of both metals; $Pb^{2+} + Cd^{2+}$, Pb^{2+} uptake was more in the roots than in the shoots while Cd²⁺ content in the shoots showed more absorption than the roots for both chelated and unchelated treatments. Also, it was observed that chelation suppresses the uptake of Pb^{2+} and Cd^{2+} in the presence of one another by both root and shoot. Furthermore, increasing the concentrations of $Pb^{2+} + Cd^{2+}$ in chelated treatments of hydroponic solutions results to a better translocation of Pb²⁺ while the translocation of Cd²⁺ to the shoot was enhanced. Generally, addition of EDDS to the hydroponic mixtures affected to a large extent the availability of Pb^{2+} and Cd^{2+} to the growing seedlings. Keywords: Ethylene diamine disuccinate, greenhouse, hydroponic solution, lettuce, metals.

INTRODUCTION

Any metallic element that has relatively high density and is toxic or poisonous even at low concentration refers to the term "Heavy Metal". In plants cells, some heavy metals play a crucial role as micronutrients (Rengel, 2004), while others even in trace concentrations have an invigorating and evoking effects on plants (Nyitrai *et al.*, 2007; Kovacs *et al.*, 2009). An alternative classification of these metals is based on their coordination chemistry. In this regard, the metals are categorized into two classes, namely: 'essential (class A) and non-essential

(class B)' heavy metals. Non-essential (class B) heavy metals such as mercury (Hg), silver (Ag), lead (Pb), nickel (Ni), cadmium (Cd), and arsenic (As) are highly toxic. However, high concentrations of both essential and nonessential heavy metals in the soil can result to growth inhibition and symptoms of toxicity in most plants (Hall, 2002) as cited by Zengin and Munzuroglu, (2005). In hydroponic systems containing the heavy metal ions, the whole roots of plant are exposed to these ions. Heavy metal ions are also absorbed directly to the other parts of the plants (Nieboer and Richardson, 1980).

Lead in excess brings about several toxicity symptoms in plants e.g. chlorosis, blackening of the root system and stunted growth. Lead hinders photosynthesis, interferes with mineral nutrition and water balance, alters hormonal status and exerts influence on membrane structure and permeability (Sharma and Dubey, 2005). Generally, to a great extent, lead inhibits germination of seed, elongation of root, growth of seedling, plant growth, transpiration, chlorophyll production, water and protein content (Pourrut et al., 2011). Lead absorption studies in plants have indicated that roots are capable of taking up significant quantities of lead while at the same time seriously hindering its movement to above ground parts (Lane and Martin, 1977).

Cadmium is among the most hazardous metals in view of the fact that it has a high proficiency to move and the little concentration in which its consequences on plants turns out to be visible (Barceló and Poschenrieder, 1990). Irrespective of the different ability of metal ions to translocate in plants, the concentration of metal is basically higher in roots than in the shoots (Ramos et al., 2002). Usually, very little quantity of cadmium ion is transported to the shoots and most of it is retained in the roots (Cataldo et al., 1983). The common symptoms of Cd toxicity in plants are mainly chlorosis, necrotic lesions, stunted growth, wilting and disruptions in mineral nutrition and carbohydrate metabolism (Sharma, 2005).

Lettuce (*Lactuca sativa* L.) is an annual plant of the daisy family, *Asteraceae*, it is a rich source of vitamin K and vitamin A and a moderate source of folate and iron. Contaminated lettuce is often a source of bacterial, viral, and parasitic outbreaks in humans. The four main types of lettuce are; Romaine lettuce, Butterhead Lettuce (Boston and Bibbs), Red and Green Lettuce and Crisphead Iceberg lettuce (Hall, 2002).

Ethylenediaminedisuccinate; EDDS $(C_{10}H_{16}N_2O_8)$ is a chelating agent. It has been shown to be easily biodegradable (Meers *et al.,* 2008), to form strong complexes with transition metals and radionuclides (Jones and Williams, 2001). Metal complexes are easily taken up by plants compared to free metal ions which suffer diffusional constraints. (Degryse *et al,* 2006).

Hydroponic is the growing of plants in nutrient solution without an inert medium (as soil) to provide mechanical support. Hydroponic systems are categorized as *open* (i.e. once the nutrient solution is delivered to the plant roots, it is not reused) or *closed* (i.e. surplus solution is recovered, replenished, and recycled), (Saulawa, 2015). Hydroponic growing (as opposed to soil growing) allows you to control the nutrient levels for your plants directly.

This research work is precisely aimed at carrying out investigations on the effects of ethylenediaminedisuccinate (EDDS) on the of cadmium and absorption lead by hydroponically grown lettuce (Lactuca sativa L.).

MATERIALS AND METHODS Treatment of seedlings

Eight weeks old lettuce (Lactuca sativa L.) 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: 0.0075 moldm⁻³KI, 0.10 moldm⁻³ H₃BO₃ 0.10 moldm⁻³ FeCl₃.6H₂O, 0.05 moldm⁻³ MnSO₄.H₂O, 0.05 moldm⁻³ Ca(NO₃)₂ .4H₂O, 0.05 moldm⁻³ Na₂H₂P₂O₇ 0.10 moldm⁻³ KNO₃, 0.05 moldm⁻³ MgSO₄.H₂O. Lead and Cadmium in (0, 0.0025, 0.005, 0.0075 and 0.025 mg/L) each as $Pb(NO_3)_2$ and $Cd(NO_3)_2$ respectively were added to 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.

Harvesting and Pretreatment of the Lettuce Plant

The lettuce *(Lactuca sativa* L.) seedlings were harvested after five days exposure to Hydroponic solutions kept in a green house at 65% relative humidity, 13 hr/day 11 hr/night under specified light intensity, and day/night temperatures 39/23°C, and washed first with tap water followed by 1% HNO₃ acid and finally rinsed with deionised water (Dagari and Umar, 2016).

Plant Samples Analysis

The roots and shoots were separated and dried under laboratory temperature for two (2) weeks. They were ground with wooden mortar and pestle to a fine powder. A washed dried porcelain crucible was placed on a hot electric plate for 5 min. Based on availability, 0.25 g (root sample) and 1.00 g (shoot 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°C for 3 hr.

The ash was cooled in a desiccator, dissolved in 0.10M HNO₃ acid, filtered into 50 cm³ volumetric flasks and made to mark, (Dagari and Umar, 2016). The Cd²⁺ and Pb²⁺content in the roots and shoots were analyzed using Atomic Absorption Spectrophotometer (Bulk Scientific AASAccusys 211, USA) at 228.8 nm and 283.2 nm respectively. The concentration of Cd²⁺ and Pb²⁺was reported as mgkg⁻¹ dry weight.

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 standard deviation, SD. Statistical significance was assumed at p<0.05.

RESULTS AND DISCUSSION

The results of the present work are presented in Figures 1 - 12. Figures 1-3 show the results of lead (Pb²⁺) absorption by the root and shoot of lettuce (Lactuca sativa L.) seedlings replanted in unchelated and chelated treatments of hydroponic solutions in the range of 0.0025 mg/L to 0.025 mg/L also the translocation of Pb^{2+} from the root to shoot of the seedlings. In figure I above, Pb²⁺ content was measured in the shoots and roots of the Lactuca sativa. From the results obtained the effects of increasing concentrations of Pb²⁺ in the range of From Figure 1, lead concentration in the solution increases the uptake by the plants relative to control. Pb²⁺ contents in the roots showed more accumulation than in shoots (P<0.05). From 0.0025 - 0.0075 mg/L of Pb²⁺, the uptakes were 3.380±1.440, 0.203±0.177; 11.037±2.166, 7.953±2.596; and 15.230±3.442, 9.233±8.182 mg/kg for the roots and shoots respectively. These higher concentrations of Pb^{2+} in the roots was attributed to bio concentration factor in which the metal ion cluster around the root for uptake by the plant. This finding is related to that of Rashid *et al.* (2014) where metals content was measured in the roots and shoots of lettuce in which the root and shoot Cd^{2+} and Pb^{2+} contents increase linearly in response to increasing concentrations of cadmium (Cd^{2+}) and lead (Pb^{2+}) in the solution.

Sudden change occurred at 0.025 mg/L where the uptake of the root was lower than that of the shoot 21.277 \pm 15.486, 27.957 \pm 4.328 mg/kg. This exceptional absorption may be due to high concentration of Pb²⁺ in the solution. This finding is supported by free-ion activity hypothesis which states that "the uptake of trace metals by plants is commonly assumed to depend on the free metal-ion activity, rather than the total concentration of dissolved metal" (Degryse *et al.*, 2006).

Figure 2 shows the effects of addition of 0.0025M of EDDS (chelate) to the Pb solution relative to the control. From 0.0025-0.0075 mg/L of Pb²⁺, chelation inhibits the uptake of the Pb²⁺ compared to unchelated treatments of same concentration (p < 0.05). At 0.025 mg/L of Pb²⁺ chelation enhances the uptake of Pb²⁺ in both shoot (32.063±6.191 mg/kg) and root (25.503±0.250 mg/kg) of the plant (p < 0.05). From the result above, chelation suppresses the uptake of Pb²⁺. This finding is similar to that of Zhao *et al.* (2010) in which EDDS was found to suppress the uptake of Pb²⁺ by *Zea mays* L.



Figure 1: Roots and shoots Pb²⁺ uptake a **Figure 2:** Roots and shoots Pb²⁺ uptake at different concentrations in unchelated hydroponic different concentrations in chelated (EDDS nixtures. 0.0025M) hydroponic mixtures.

Special Conference Edition, November, 2019 According to Chen *et al.* (2012) Translocation factor (TF) is defined as the ratio of the concentration of metal in the shoot to its concentration in the root.

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

Concentration of metal in the root Figure 3 shows that the translocation factor (TF) directly proportional to the is added concentration of Pb²⁺ from (0.0025-0.025 mg/L) in unchelated treatment, thus, the TF gradually increases with increase in concentrations of Pb²⁺ (0.060±0.809, 0.721±2.381, 0.606±5.812, 1.314±9.907) relative to control whereas, in chelated treatment, it was observed that the TF was almost constant (0.301±0.259, 0.394±0.142 & 0.362±0.161) between 0.0025-0.0075mg/L of Pb²⁺, hence, chelation (EDDS) suppresses translocation of Pb²⁺ to the shoots of the plant, though a sudden increase was observed at 0.025 mg/L where the TF was (1.257 ± 3.221).

Figure 4 shows the Cd²⁺ content measured in the shoots and roots of the plant. From the results obtained the effects of increasing concentrations of Cd²⁺ in the range of 0.000-0.025 mg/L in the solution increases the uptake by the plants (P < 0.05) relative to control. At these concentrations (0.0025-0.0075 mg/L) of Cd²⁺, the Cd²⁺ uptake in the shoots was higher than in roots. The uptakes at 0.0025, 0.005 & 0.0075 mg/L were 7.107±1.537, 1.463±0.325; 6.333±2.145, 2.667±0.327; and 11.750±1.200, 3.813±1.070 mg/kg respectively. Suddenly a change occurred at 0.025 mg/L where the uptake of the shoot was lower than that of the root 9.750±0.779, 15.007±7.854 mg/kg, this exceptional absorption of Cd²⁺ may be due bio concentration factor. The changes in the Cd²⁺ uptake by both shoots and roots of the plant were significant (*P*<0.05) when compared to the control.

Figure 5 shows the effects of addition of 0.0025M of EDDS (chelate) to the Cd solution relative to the control. The uptake by both shoots and roots were almost constant from 0.0025 to 0.0075 mg/L Cd²⁺ (4.073±1.146, 2.483±0.268; 4.547±0.804, 2.207±0.395; and 5.283±0.653, 2.813±0.681) mg/kg which shows that chelation suppresses the uptake of the Cd²⁺ by both shoots and roots compared to unchelated treatments of same concentration (*P>0.005*). At 0.025 mg/L of Cd²⁺, chelation enhances the uptake of Cd^{2+} by both shoot (15.587±0.810 mg/kg) and root (16.350±7.859 mg/kg) of the plant. Compared to the control and other concentrations (0.0025-0.0075 mg/L), this change is very significant (P < 0.05).



Figure 3: The translocation factor (TF) in different concentrations of Pb²⁺ in lettuce seedlings (*Lactuca sativa* L.) transplanted in chelated (EDDS 0.0025M) and unchelated treatments.



Figure 4: The root and shoot Cd^{2+} uptake in unchelated treatments of hydroponic mixtures containing different concentrations of Cd^{2+} .

Figure 6 shows the effects of translocation factor (TF) against concentration of added Cd^{2+} . TF changes significantly with concentration of added Cd^{2+} relative to the control (*P* < 0.05). From 0.0025 to 0.0075mg/L of Cd^{2+} , a relatively good fraction of the absorbed Cd²⁺ was translocated to the shoots in unchelated treatments (4.856±0.931, 2.375±1.236, 3.081 ± 1.135) more than the chelated treatments (1.640±0.707, 2.060±0.600, 1.878±0.667). The least concentration of added Cd^{2+} (0.0025mg/L) had the highest change in translocation factor (4.856±0.931) for **Figure 5:** The root and shoot Cd^{2+} uptake in chelated treatments (EDDS 0.0025M)of hydroponic mixtures containing different concentrations of Cd^{2+} .

unchelated treatments while the highest concentration of added Cd²⁺ (0.025mg/L) had the lowest change in translocation factor for both unchelated (0.650±4.317) and chelated treatment (0.953±4.335). There were significant differences (P < 0.05) for the changes in translocation factor for unchelated and chelated treatments when compared to the control (0.284±0.477).

Lead (Pb^{2+}) absorption by seedlings replanted in different hydroponic solutions containing equal concentration of lead (Pb^{2+}) and cadmium (Cd^{2+})



Figure 6: The translocation factor (TF) in different concentrations of Pb²⁺ in lettuce seedlings (*Lactuca sativa* L.) transplanted in chelated (EDDS 0.0025M) and unchelated treatments.

Figure 7 shows that the effect of increasing concentrations of Pb^{2+} + Cd^{2+} in the range of 0.000-0.025 mg/L in the solution is directly proportional to the uptake of Pb^{2+} by the plants. Pb²⁺ contents in the roots showed more accumulation than in shoots (P<0.05). From 0.0025-0.025 mg/L Pb²⁺ the uptakes were 2.347±0.416, 0.833±0.576; 8.387±1.331, 7.043±4.128; 16.147±4.275, 7.607±2.887; and 30.123±4.036, 15.677±7.698 mg/kg (p<0.05). These higher concentrations of $P\bar{b}^{2+}$ in the roots may be attributed to bio concentration factor in which the metal ion cluster around the root for uptake by the plant.

Figure 8 shows the effects of addition of 0.0025M of EDDS (chelate) to the Pb and Cd solution relative to the control. From 0.0025-0.0075 mg/L of Pb²⁺, chelation suppresses the uptake of the Pb²⁺ compared to unchelated treatments of same concentration (p < 0.05). This finding is similar to that of Zhao *et al.* (2010) in which EDDS was found to suppress the uptake of Pb²⁺ by Zea mays L.

At 0.025 mg/L of Pb²⁺ the uptake of Pb²⁺ was enhanced in both shoot (15.477±9.413 mg/kg) and root (10.127±2.119 mg/kg) of the plant compared to the other concentrations (p < 0.05). From the results, the uptake was more in the root than in the shoot from 0.0025 to 0.0075 mg/L which is similar to the result of the unchelated treatments of same concentrations, but at 0.025 mg/L, uptake was more in the shoot than in the root (p < 0.05). Figure 9 shows that there was a better translocation of Pb²⁺ in chelated treatments than the unchelated treatment. There was a significant difference (p < 0.05). The translocation factor for the unchelated treatment was observed to be almost constant (0.355, 0.471 and 0.520) whereas TF of Pb²⁺ (0.546, 0.927 and 1.528) for the chelated treatment is directly proportional to the added concentration of Pb^{2+} and Cd^{2+} at 0.0025, 0.0075 & 0.025 mg/L respectively. Therefore, chelation (EDDS) enhances the translocation of Pb²⁺ to the shoots of the plant and there was a significant difference relative to the control (p < 0.05).





Figure 7: Comparison of root and shoot Pb^{2+} uptake in unchelated treatments of hydroponic mixtures containing same concentrations of Pb^{2+} and Cd^{2+}

Figure 8: Comparison of root and shoot Pb^{2+} uptake in chelated treatments (EDDS 0.0025M) of different hydroponic mixtures containing same concentrations of Pb^{2+} and Cd^{2+}



20.000

Figure 9: Comparison of translocation factor (TF) of Pb^{2+} in lettuce seedlings (*Lactuca sativa* L.) transplanted in chelated (EDDS 0.0025M) and unchelated treatments containing same concentrations of Pb^{2+} and Cd^{2+}

Special Conference Edition, November, 2019 Cadmium (Cd²⁺) absorption by seedlings replanted in different hydroponic solutions containing equal concentration of lead (Pb²⁺) and cadmium (Cd²⁺)

Figure 10 shows the Cd²⁺ content measured in the shoots and roots of the plant. From the results obtained the effects of increasing concentrations of Cd²⁺ in the range of 0.000-0.025 mg/L in the solution increases the uptake by the plants. The Cd^{2+} uptake in the shoots was higher than in roots; this result is similar to that obtained in the hydroponic treatments containing cadmium (Cd²⁺) alone. The uptakes in the shoots and roots at 0.0025, 0.005, 0.0075 and 0.025 mg/L were 5.033±1.066, 7.090±0.957, 1.297±0.040; 1.237±0.118; 5.940±1.250, 2.533±0.845; and 9.597±1.553, 6.950 ± 3.667 mg/kg respectively. It was observed that the Cd²⁺ absorption by the roots of the plants were almost constant from 0.0025-0.0075 mg/L and compared to the control, the difference was insignificant (P>0.05) whereas the changes in the Cd²⁺ uptake by the shoots of the plants were significant (P < 0.05) relative to the control.

In the chelated treatment, the Cd^{2+} uptake by plant as depicted in Figure 11 above is directly proportional to the concentrations of $Pb^{2+} + Cd^{2+}$ in the Hoagland solution (hydroponic nutrient solution developed by Hoagland) i.e. the higher the concentration of the metals in the solution, the higher the absorption by plant. The shoots of the plant showed more Cd²⁺accumulation than the roots where the uptakes were also found to be almost constant, this is in similar trend with the unchelated treatment. The changes in the uptake by the shoots of the plants were significant when compared to the control, (p < 0.05). The lowest uptakes by the shoots and roots of the plant were 2.407±0.552, 0.823±0.405 while the highest uptakes were 20.377±11.345, 4.480±0.791 respectively. In Figure 12, the translocation factor for the control was 0.284 for both chelated and unchelated treatments. At 0.0025 and 0.005 mg/L of Pb²⁺ and Cd²⁺ treatment, there was a better translocation of Cd²⁺ for unchelated treatment (3.882, 5.733) than for chelated treatment (2.923, 2.754) respectively. At 0.0075 mg/L of Pb²⁺ and Cd²⁺ treatment, the translocation of Cd²⁺ was higher for the chelated treatment (2.588) than the unchelated treatment (2.345) though the difference was very little, (p>0.05). Also at 0.025 mg/L of Pb²⁺ and Cd²⁺ treatment, there was a higher translocation of Cd^{2+} in the chelated treatment (4.548) compared to unchelated treatment (1.381), (p<0.05). There was a significant difference relative to the control, (p<0.05).



Figure 10: Comparison of root and shoot Cd^{2+} in unchelated treatments of hydroponic mixtures containing same concentrations of Pb^{2+} and Cd^{2+}

Figure 11: Comparison of root and shoot Cd^{2+} in chelated treatments (EDDS 0.0025M) of hydroponic mixtures containing same concentrations of Pb²⁺ and Cd²⁺



Figure 12: Comparison of translocation factor (TF) of Cd^{2+} in lettuce seedlings (*Lactuca sativa* L.) transplanted in chelated (EDDS 0.0025M) and unchelated treatments containing same concentration of Pb²⁺ and Cd²⁺

CONCLUSION

In this work, addition of EDDS to the hydroponic mixtures affected to a large extent the availability of Pb^{2+} and Cd^{2+} to the growing seedlings. Addition of 0.0025M EDDS to the solutions of Pb^{2+} and Cd^{2+} inhibits the uptake of the metals ions at 0.0025, 0.005 and 0.0075 mg/L when compared to unchelated treatments of same concentrations. This result is in agreement with the findings of Liu *et al.* (2008) but at 0.025 mg/L of Pb^{2+} and Cd^{2+} , chelation enhances their uptakes in both shoot and root of

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the plant, this is similar to the findings of Engelen *et al.* (2007) and Nascimento *et al.* (2006).Varying degrees of phytotoxic symptoms which include chlorosis, necrosis and reduction in dry weight of plant were observed depending on the concentration of the metals (Pb^{2+} and Cd^{2+}) and presence or absence of EDDS, though no leaf was observed to fall during the growth of seedlings. This could be due to the low concentration of the metals; Pb^{2+} and Cd^{2+} (0.000 – 0.025mg/L) and EDDS (0.0025M) used in this research.

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