



## EFFECTS OF EDTA ON LEAD ABSORPTION BY MANGO (*Mangifera indica*) SEEDLINGS REPLANTED IN HYDROPONIC SOLUTIONS

<sup>1</sup>Dagari, M. S. and <sup>2</sup>Ehumadu, C. V.

<sup>1</sup> Department of Chemistry, Federal University, Gashua, Nigeria

<sup>2</sup> Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria

Correspondence author: 08065497722, 08023714912; [dagaribuk@gmail.com](mailto:dagaribuk@gmail.com)

### ABSTRACT

*This research investigates the effects of EDTA on absorption of lead by mango (*Mangifera indica*) seedlings grown in hydroponic solutions. Eight week old seedlings were carefully collected from the garden of the Department of Forestry and Wild Life, Kano University of Science and Technology Wudil, replanted in hydroponics with varying concentrations of  $Pb^{2+}$  and EDTA. All plants grown in treated hydroponics died before control plants. The harvesting time varied highly significantly ( $Pr < 0.0001$ ) with concentrations of  $Pb^{2+}$  or EDTA. The pH values of treated hydroponics were significant with increase in concentration of  $Pb^{2+}$  before replanting ( $Pr = 0.0239 < 0.05$ ) and highly significantly ( $Pr < 0.0001$ ) after harvest. The weights of plants decreased insignificantly with increase in concentrations of  $Pb^{2+}$  ( $Pr = 0.0784 > 0.05$ ) and EDTA ( $Pr = 0.2548 > 0.05$ ). Both shoot and root lead accumulated highly significantly ( $Pr < 0.0001$ ) in all treated hydroponics and control. The lead translocation factor decreased highly significantly ( $Pr < 0.0001$ ) in treated plants compared to control. The values were less than 1.00 which signified increased retention of lead in mango roots with very little translocation to the shoots.*

**Key Words:** EDTA, Hydroponic, Seedling, Mango, Leaf.

### INTRODUCTION

Lead (Pb) is one of the widely distributed and most abundant toxic elements in the soil. It exerts adverse effects on morphology, growth and photosynthetic processes of plants (Huang *et al.* 1974; Miller *et al.*, 1975; Khan and Khan, 1983). The degree to which root elongation is inhibited depends upon the concentration of lead and ionic composition and pH of the medium (Goldbold and Hutterman, 1986). Lead is also known to affect photosynthesis by inhibiting activity of carboxylating enzymes. High Pb concentration also induces oxidative stress by increasing the production of reactive oxygen species ROS in plants (Reddy *et al.*, 2005). Significant increases in lead content of cultivated soils have been observed near industrial areas. Lead tends to accumulate in the surface ground layer and its concentration decreases with soil depth (De Abreu *et al.*, 1998). It is easily taken up by plants from the soil and is accumulated in different organs. Lead is considered a general protoplasmic poison, which is cumulative, slow acting and barely noticeable. Soil contaminated with lead cause sharp decrease in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton, 1980). Studies of lead uptake in plants have shown that roots have the ability to take up significant quantities of lead at the same time restricting its translocation to the above ground parts

(Lane and Martin, 1997). This idea was capsized by the demonstration of *Zea mays* L. plants translocating and accumulating significant quantities of lead in the leaves in a concentration dependent manner (Miller and Koeppe, 1971). Lead accumulates in the surface layers of soils and therefore it is difficult to reliably measure the proportion of soil lead directly available to plants. Its availability depends on soil conditions. Lead binds to organic material in soil. Soil particle size and cation exchange capacity as well as plant factors such as roots surface area; root fluid discharge, mycorrhization and rate of transpiration affect the availability and uptake of lead (Davies, 1995). The absorption of lead in soil follows the Langmuir relation and increases with increasing pH ranging from 3.0 to 8.5. However it was reported that in soil with a pH between 5.5 and 7.5, lead solubility is controlled by phosphate or carbonate precipitates and insignificant lead is available to plants even if they have the genetic capacity to accumulate it (Blaylock *et al.*, 1997).

Recent evidence suggests that the addition of chelating agents to the soil (i.e. ethylenediamine-tetracetic acid (EDTA) and structural analogues) increases the phytoavailability of Pb and other heavy metals by forming water-soluble chelate-heavy metal complexes (Huang *et al.*, 1997).

The main drawback of chelate induced phytoextraction is that EDTA forms chemically and microbiologically stable complexes that pose a threat of groundwater contamination (Grčman *et al.*, 2001).

## MATERIALS AND METHODS

### Growth Conditions of the Mango Seedlings

Eight week old Mango (*Mangifera indica*) seedlings were carefully collected from the garden of the Department of Forestry and Wild life, Kano University of Science and Technology, Wudil, on Monday, 25th November, 2014 at 12:30pm. They were identified at the Department of Plant Science, Bayero University Kano.



The seedlings were uprooted from their planting bags, washed thoroughly with tap water to remove excess soil and rinsed three times with deionised water. A control hydroponic was prepared by carefully transferring 1.28cm<sup>3</sup> of 0.10mol dm<sup>-3</sup> KNO<sub>3</sub>, 5.15cm<sup>3</sup> of 0.10mol dm<sup>-3</sup> FeCl<sub>3</sub>.6H<sub>2</sub>O, 11.35cm<sup>3</sup> of 0.10mol dm<sup>-3</sup> H<sub>3</sub>BO<sub>3</sub>, 5.00cm<sup>3</sup> of 0.05mol dm<sup>-3</sup> MgSO<sub>4</sub>.H<sub>2</sub>O, 3.57cm<sup>3</sup> of 0.05mol dm<sup>-3</sup> Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O, 5.00cm<sup>3</sup> of 0.05mol dm<sup>-3</sup> Na<sub>2</sub>H<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, 0.17cm<sup>3</sup> of 0.0075mol dm<sup>-3</sup> KI and 23.10cm<sup>3</sup> of 0.05mol dm<sup>-3</sup> MnSO<sub>4</sub>.H<sub>2</sub>O into a 500cm<sup>3</sup> volumetric flask. The volume was made to mark with deionized water (Peralta *et al.*, 2000).

Lead(II) ion in the concentrations 0.0000, 0.0025 and 0.025 mol dm<sup>-3</sup> as Pb(NO<sub>3</sub>)<sub>2</sub> and 0.000, 0.005, 0.025 and 0.1000 mol dm<sup>-3</sup> EDTA were added to the control mixture to prepare 500cm<sup>3</sup> each of different hydroponic treatments in triplicates. The solutions were carefully transferred into clean labeled 750cm<sup>3</sup> plastic containers. The seedlings were replanted on Tuesday 20th January, 2014 by 2.00pm (IITA, 1979). They were monitored in the garden at the Department of Pure and Industrial Chemistry, Bayero University, Kano. They were harvested separately, on Saturday, 31<sup>st</sup> January, 2015 by 3:00pm. However, seedlings

in the controls and hydroponics containing 0.0025mol dm<sup>-3</sup> and 0.025mol dm<sup>-3</sup> Pb(NO<sub>3</sub>)<sub>2</sub> were only harvested on Tuesday, 3<sup>rd</sup> February, 2015 by 3pm.

The roots and shoots of the harvested seedlings were ground to fine powder. Porcelain crucibles were washed, dried and ignited on a hot electric plate for 5minutes. Based on availability, 0.25g (root) and 1.00g (shoot) were separately weighed into the crucibles and gently heated on the hot electric plate until the smoking ceased. They were then ashed at 450°C in a muffle furnace to constant weight. The ash was cooled in a desiccator, dissolved in 0.10M HNO<sub>3</sub>, filtered into a 50cm<sup>3</sup> volumetric flask and made to the mark. The Pb<sup>+2</sup> content in the roots and shoots was analyzed using Atomic Absorption Spectrophotometer AA-6800 Shimadzu at 217.0nm. The concentration of Pb<sup>+2</sup> was reported as mg/kg dry weight (IITA, 1979).

### STATISTICAL ANALYSIS

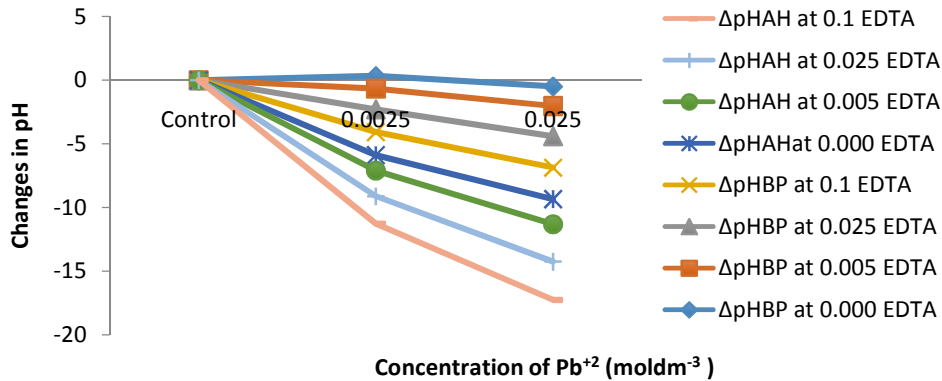
The data were analyzed through one-way analysis of variance (ANOVA) to determine the effects of treatments, and least significant difference (LSD) tests were performed to determine the statistical significance of the differences between means of treatments.

**RESULTS AND DISCUSSION**

**Determination of Field Data**

Changes in pH of hydroponic solutions before planting and after harvest, time of harvest and dry weights of plants were determined (IITA, 1979).

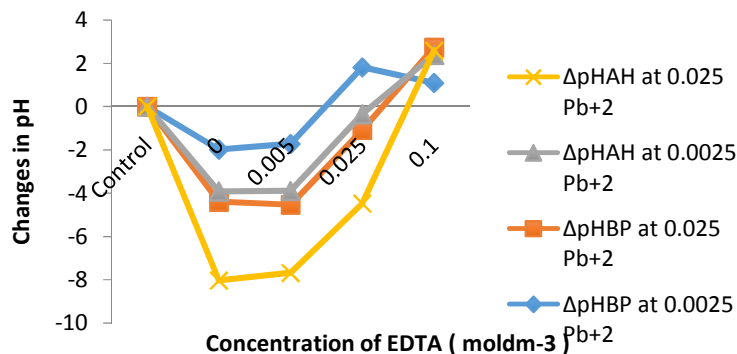
The changes in pH before planting ( $\Delta pH_{BP}$ ) and pH after harvest ( $\Delta pH_{AH}$ ) were determined by subtracting the respective pH values of the control solutions from the pH values of the given treatments.



**Fig. 1: Changes in pH of Hydroponics Before Planting and After Harvest...**

Fig. 1 shows the changes in pH of hydroponic mixtures before planting ( $\Delta pH_{BP}$ ) and after harvest ( $\Delta pH_{AH}$ ) with increase in concentration of  $Pb^{2+}$ . A negative change in pH indicates that

the pH of a treatment is less than the pH of control. There was a significant correlation ( $Pr = 0.0239 < 0.05$ ) between  $\Delta pH_{BP}$  and  $Pb^{2+}$ , a highly significant difference ( $Pr < 0.0001$ ) between  $\Delta pH_{AH}$  and  $Pb^{2+}$ .

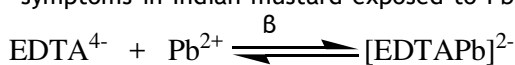


**Fig. 2: Changes in pH against Concentration of EDTA**

Fig.2 shows a significant effect ( $Pr = 0.0004 < 0.05$ ) of EDTA on  $\Delta pH_{AB}$  at different concentrations of  $Pb^{2+}$ . However, an insignificant correlation ( $Pr = 0.1293 > 0.05$ ) was observed between EDTA and  $\Delta pH_{AH}$ . The different hydroponic mixtures gave variable pH changes which agreed with the reports of Blaylock *et al* (1997), Wong and Lau (1985). Vassil *et al* (1998) reported that toxicity symptoms in Indian mustard exposed to Pb and

EDTA were strongly correlated with the presence of free protonated EDTA in solution. Laurie *et al* (1991) reported a significant correlation between EDTA and  $Pb^{2+}$  which was attributed to the formation a Pb-EDTA complex at pH 5.2 to pH 7.7. Increasing the concentration of EDTA favoured the formation of the complex.

The equilibrium reaction is represented below,



$$\text{Where } \beta = \frac{[EDTAPb]^{2-}}{[EDTA^{4-}][Pb^{2+}]} = 9.12 \cdot 10^{-19} \text{ dm}^3 \text{ mol}^{-1}$$

The change in harvesting time ( $\Delta HT$ ) for a given treatment was determined by subtracting the

harvesting time of the treatment from the harvesting time for control plant.

A negative value of  $\Delta$ HT showed that the plant was harvested earlier than the control plant. Figs.3 and 4 show that addition of  $Pb^{2+}$  and EDTA changed the harvesting time highly significantly ( $Pr < 0.0001$ ). Increasing the concentration of  $Pb^{2+}$  from 0 to  $0.0025 \text{ mol dm}^{-3}$  resulted in harvesting the plant 1hour earlier than control. When the concentration was further changed to 0.025, the plant was

harvested 5 hours earlier than control. The effect of EDTA on harvesting time was also investigated. The plant was harvested 121hours earlier than control at  $0.100 \text{ mol dm}^{-3}$  EDTA and  $0.0025 \text{ mol dm}^{-3} Pb^{2+}$ . When the concentration of  $Pb^{2+}$  was further changed to  $0.025 \text{ mol dm}^{-3}$ , the plant was harvested 125.5hrs earlier than control.

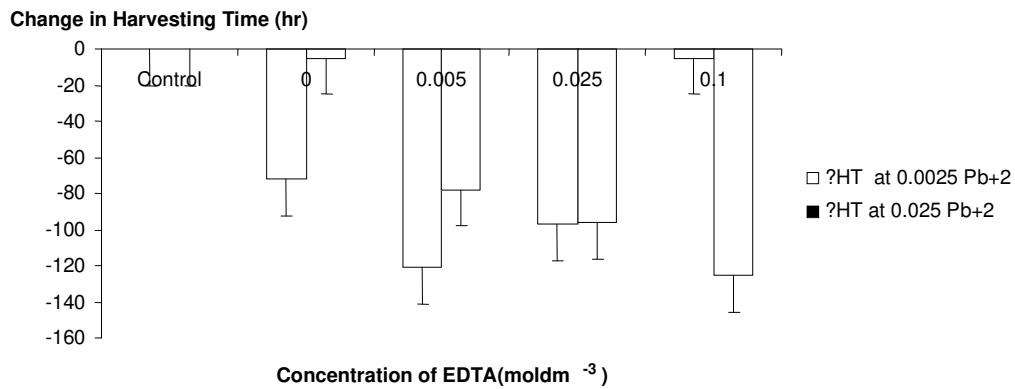


Fig.3: Change in Harvesting Time against Concentration of EDTA

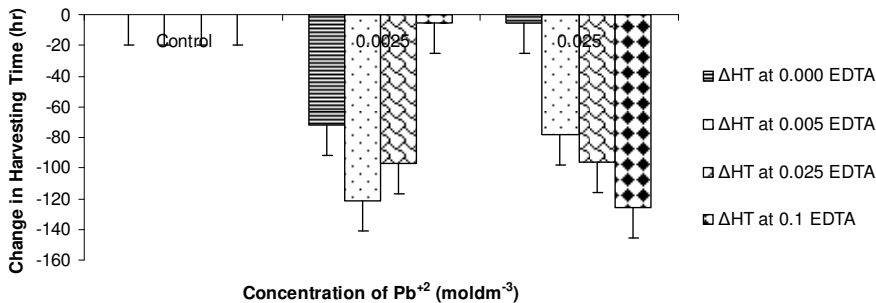


Fig.4: Changes in Harvesting Time against Concentration of  $Pb^{2+}$

The death of plants in treated hydroponics earlier than control seedlings could be due to the poisonous effect of lead. According to Johnson and Eaton (1980), lead is considered a general protoplasmic poison, which is cumulative, slow acting and subtle.

The changes in plant weights for all treatments including the control were determined by subtracting the weight of the plant before replanting from its weight of plant after harvest.

Figs.4 and 5 show that addition of  $Pb^{2+}$  ( $Pr = 0.0784 > 0.05$ ) and EDTA ( $Pr = 0.2546 > 0.05$ ) caused insignificant changes in plant weights.

The change in plant weight ( $\Delta$ WP) for the control was  $-0.321 \pm 0.045 \text{ g}$ . Values of  $\Delta$ WP for  $0.0025$  and  $0.025 \text{ mol dm}^{-3}$  were  $-11.227 \pm 2.581$  and

$-7.435 \pm 4.579 \text{ g}$ . The change in plant weight was also investigated for a given concentration of  $Pb^{2+}$  at different concentrations of EDTA. For  $0.0025 \text{ mol dm}^{-3} Pb^{2+}$ , the values of  $\Delta$ WP varied from  $-11.227 \pm 2.581$  to  $-2.632 \pm 1.214 \text{ g}$  as the concentration of EDTA was changed from 0.000 to  $0.100 \text{ mol dm}^{-3}$ . The corresponding values of  $\Delta$ WP for  $0.025 \text{ mol dm}^{-3} Pb^{2+}$  were  $-7.435 \pm 4.579 \text{ g}$  at  $0.000 \text{ mol dm}^{-3}$  and  $-3.268 \pm 1.301 \text{ g}$  at  $0.100 \text{ mol dm}^{-3}$  respectively.

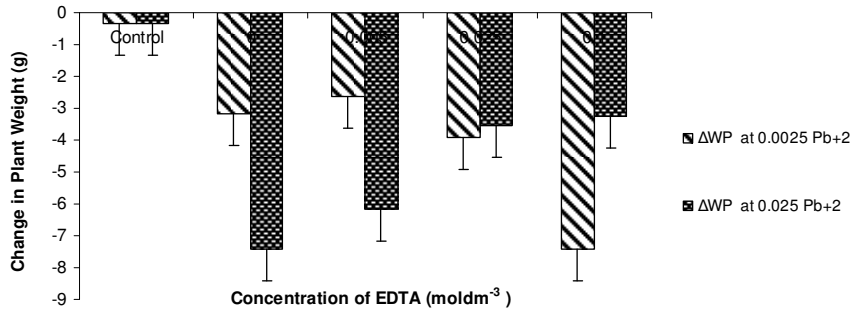


Fig.5: Change in Plant Weight against Concentration of EDTA

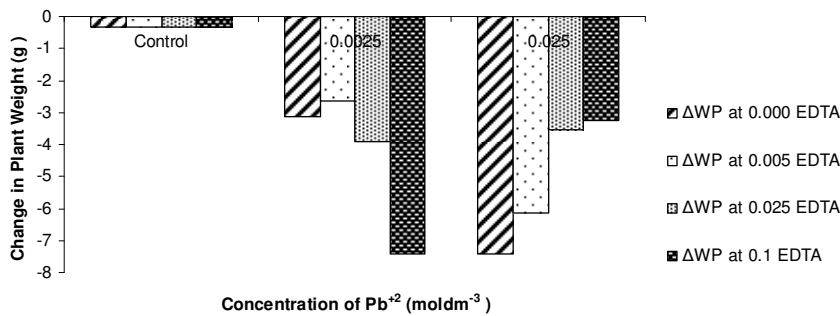


Fig.6: Change in Plant Weight against Concentration of Pb<sup>2+</sup>

The decrease in weights of plants harvested in all treatments agreed with the works of several authors. Breckie(1991), reported that absorbed lead resulted in reduction in growth rate of roots and change in branching pattern. A considerable decrease in dry weights of plant parts is observed under Pb treatment (Krobrukhv *et al.*, 2004). Lead toxicity inhibits germination of seeds, retards growth of seedlings, root/shoot length, tolerance index and dry mass of roots and shoots. Very high Pb concentrations may eventually lead to cell death (Mishra and Choudhari, 1998).

**Determination of Plant Parameters**

The effects of EDTA at a given concentration of Pb<sup>2+</sup> on changes in root Pb and shoot Pb are shown on Fig.7. Addition of 0, 0.005, 0.025 and

0.100moldm<sup>-3</sup>EDTA at 0.00250moldm<sup>-3</sup> Pb<sup>2+</sup> gave the values of ΔRtPb as 6.383±0.638, 15.106±0.737, 19.362±0.737 and 20.071±1.720 respectively. The corresponding values of ΔShPb were 3.138±0.184, 5.355±0.111, 8.954±0.154 and 6.099±0.215 respectively. When the concentration of Pb<sup>2+</sup> was maintained at 0.025moldm<sup>-3</sup> and EDTA varied from 0, 0.005, 0.025 and 0.100moldm<sup>-3</sup>, the values of ΔRtPb were -12.128±2.128, 16.809±2.790, 21.277±0.638 and 21.418±0.860 respectively. The corresponding values of ΔShPb were 3.723±0.372, 2.660±0.160, 4.149±0.415 and 4.450±0.494 respectively. The correlations of ΔRtPb and ΔShPb with EDTA were highly significant (Pr< 0.0001).

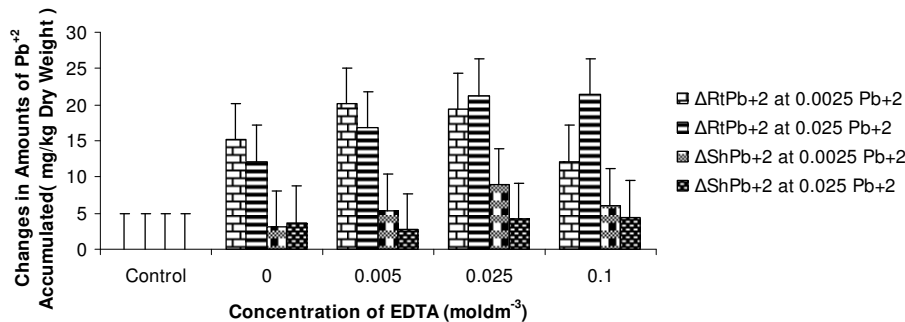


Fig. 7: Changes in Amounts of of Pb<sup>2+</sup> Accumulated against Concentration of EDTA

Fig.8 shows the changes in root ( $\Delta RtPb$ ) and shoot ( $\Delta ShPb$ ) lead in mango seedlings replanted in various hydroponic mixtures. These changes were determined by subtracting the corresponding control values from the values of individual treatments. For  $0.0025\text{mol dm}^{-3}$   $Pb^{2+}$ ,  $\Delta RtPb$  and  $\Delta ShPb$  were  $6.383 \pm 0.638$  and  $3.138 \pm 0.184$  mg/kg

respectively. When the concentration of  $Pb^{2+}$  was increased to  $0.025\text{mol dm}^{-3}$ , the root and shoot Pb increased by  $12.128 \pm 2.128$  and  $3.723 \pm 0.372$  mg/kg respectively. These increases in root Pb and shoot Pb were highly significant ( $Pr < 0.0001$ ) with respect to  $Pb^{2+}$ .

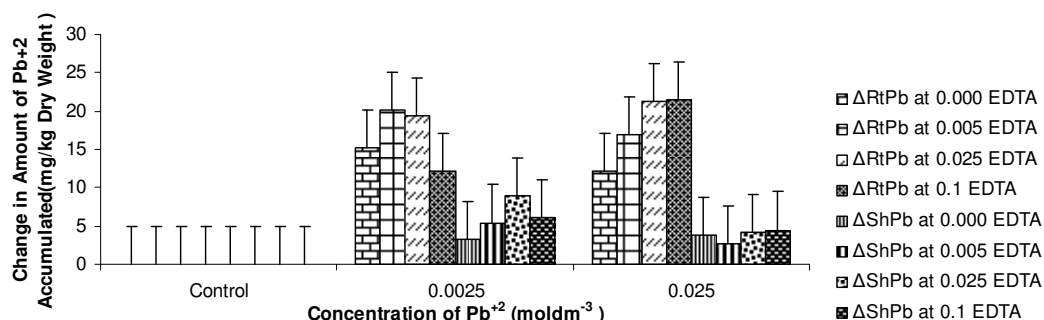


Fig. 8: Changes in Amounts of Pb<sup>2+</sup> Accumulated against Concentration of Pb<sup>2+</sup>

In all hydroponic treatments, the shoot and root lead increased considerably over the control values, which agreed with the reports of Wong and Lau (1985), MacPherson and Martin (1994), Blaylock *et al* (1997). The changes in translocation factor ( $\Delta TF$ ) were determined by subtracting the corresponding control values from the values of individual treatments. Fig. 9 shows that the change in

translocation factor caused by addition of  $Pb^{2+}$  was highly significant ( $Pr < 0.0001$ ). For  $0.0025\text{mol dm}^{-3}$   $Pb^{2+}$ , the change in translocation factor was  $0.497 \pm 0.078$ . When the concentration of  $Pb^{2+}$  was increased to  $0.025\text{mol dm}^{-3}$ , the change in translocation factor was  $0.316 \pm 0.082$ .

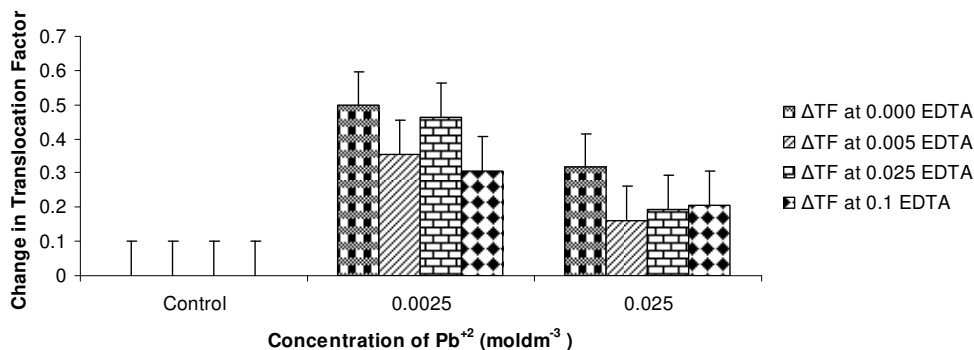


Fig.9: Change in Translocation Factor against Concentration of Pb<sup>2+</sup>

The effects of EDTA at a given concentration of  $Pb^{2+}$  on changes in translocation factor of Pb are given on Fig.10. Addition of 0, 0.005, 0.025 and  $0.100\text{mol dm}^{-3}$  EDTA at  $0.0025\text{mol dm}^{-3}$   $Pb^{2+}$  gave the values of  $\Delta TF$  as  $0.497 \pm 0.078$ ,  $0.355 \pm 0.25$ ,  $0.463 \pm 0.010$  and  $0.360 \pm 0.035$  respectively. When the concentration of  $Pb^{2+}$

was maintained at  $0.025\text{mol dm}^{-3}$  and EDTA varied from 0, 0.005, 0.025 and  $0.100\text{mol dm}^{-3}$ , the values of  $\Delta TF$  were  $0.316 \pm 0.082$ ,  $0.161 \pm 0.023$ ,  $0.195 \pm 0.025$  and  $0.207 \pm 0.017$  respectively. The change in translocation factor caused by addition of EDTA at constant  $Pb^{2+}$  was insignificant ( $Pr = 0.3331 > 0.05$ ).

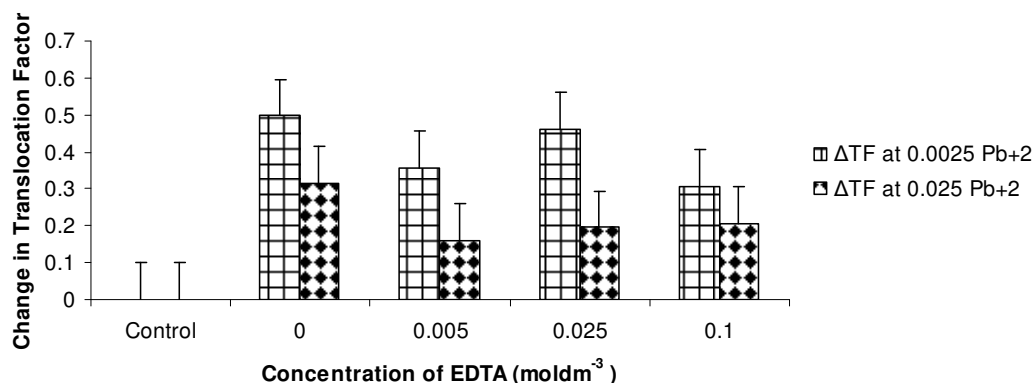


Fig. 10: Change in Translocation Factor against Concentration of EDTA

The change in translocation factor ( $\Delta TF$ ) measures quantitatively the translocation of Pb to aerial parts of the plant (Wong and Lau, 1985; MacPherson and Martin, 1994; Blaylock *et al*, 1997). The increase in shoot-root Pb ratio with increase in amount of EDTA at a given value of lead nitrate agreed with work of Blaylock *et al* (1997), who reported that Pb entered the plant and was transported to the shoot as an EDTA complex. In a similar report, Laurie *et al* (1991), proposed a possible pathway for metal uptake under the influence of complexes. It involves the absorption of the metal complex by the root cell membrane. The complex then either undergoes dissociation in the cell membrane, with free metal ion being transported to the cell while the ligand (L) goes back to the solution phase, or the metal is transported to root cells across the plasmalemma in the form of a complex. Lead retention in roots is based on its binding to ion exchangeable sites on the cell wall and extracellular precipitation, mainly in the form of lead carbonates deposited in the cell wall (Dushenkov *et al*, 1995). EDTA in combination with low pH effectively prevents cell wall

retention of Pb, thereby making it available for translocation to the shoots.

#### CONCLUSION

The growth of replanted mango (*Mangifera indica*) seedlings was affected as a result of addition of 0.000 to 0.025 mol dm<sup>-3</sup> Pb<sup>2+</sup> and 0.000 to 0.100 mol dm<sup>-3</sup> EDTA to various hydroponic solutions. There were significant changes in pH of the hydroponic solutions before planting and after harvest. The low pH values of the hydroponic mixtures favoured the formation of a

Pb-EDTA complex which entered the plant and was transported to the shoot. The death of plants with decrease in weights was due to lead poisoning, which was favoured in presence of EDTA. This research could be used in monitoring lead toxicity in plants.

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