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# EFFECTS OF ANTHRANILIC ACID ON NICKEL ABSORPTION BY OLIVE (*Olea europaea*) SEEDLINGS REPLANTED IN HYDROPONIC SOLUTIONS

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*ABSTRACT*
***The effects of anthranilic acid on absorption of $Ni^{2+}$ by olive (Olea europaea) seedlings treated with hydroponic solutions were investigated. Ten week old seedlings were collected from the garden of the Department of Forestry and Wild Life, Kano University of Science and Technology Wudil. Concentrations of $Ni^{2+}$ in the hydroponics were varied from 0.000 to 0.025moldm$^{-3}$ and anthranilic acid from 0.000 to 0.100moldm$^{-3}$. The seedlings were replanted and kept in the garden of the Department of Pure and Industrial Chemistry Bayero University Kano. The weights of plants decreased significantly ($Pr = 0.0359 < 0.05$) with increase in concentration of $Ni^{2+}$ and insignificantly ($Pr = 0.8366 > 0.05$) with increase in concentration of anthranilic acid The harvesting time varied highly significantly ($Pr < 0.0001$) with concentrations of $Ni^{2+}$ or anthranilic acid. The pH values of treated hydroponics before planting and after harvest were highly significantly ($Pr < 0.0001$) lower than control pH. Concentrations of nickel accumulated ($Pr < 0.0001$) in all treated hydroponics and control. There was a significant decrease ($Pr = 0.0002 < 0.05$) in nickel translocation factor in all plants grown in treated plants compared to control. The values were less than 1.00 which indicated increased retention of nickel in olive root with very less movement to the shoot.***
***Key Words: Anthranilic acid, Hydroponic, Olive, Nickel, Concentration.***

## INTRODUCTION

Nickel (Ni) is considered to be an essential ultra micronutrient for plant metabolism. The element is the active center of urease and cofactor of a superoxide dismutase isoform (Kupper and Kroneck 2007). Due to its very low natural occurrence in plants (0.05-10 µg g-1 dry weight), Ni deficiency is rare relative to the excess caused by metal mining and smelting (Nieminen et al. 2007). Excess Ni in plants becomes toxic in some species and in such cases alters various physiological processes. The most obvious symptoms of Ni toxicity in plants are growth inhibition, chlorosis, necrosis and wilting (Pandey and Sharma, 2002; Kazemi et al. 2010). Although Ni is not generally considered to be a redox-active metal, high concentrations of Ni stimulate the production of reactive oxygen species (ROS) such as $O_2{\cdot}^-$ and $H_2O_2$ (Gajewska and Sklodowska, 2007) and induce oxidative stress evident from peroxidation of membrane lipids (Baccouch *et al,* 2001; Kazemi *et al,* 2010; Wang *et al,* 2010). Lipid peroxidation can also be initiated by enzyme activity such as that of lipoxygenase (LOX) (Mishra and Choudhuri 1999) and is believed to be a factor affecting growth inhibition in plants exposed to heavy metals, including Ni (Baccouch *et al,* 2001).

Nickel(II) ion in the concentrations 0.0000, 0.0025 and 0.025 moldm$^{-3}$ as $Ni(NO_3)_2.6H_2O$ and 0.000 ,0.005, 0.025 and 0.1000 moldm$^{-}$

This research was aimed at investigating the effects of anthranilic acid on nickel absorption by olive *(olea europaea*) seedlings replanted in hydroponic solutions.

## MATERIALS AND METHODS

### Sampling

Ten week old olive *(Olea europaea)* seedlings were collected from a garden at the Department of Forestry and Wild life, Kano University of Science and Technology, Wudil, Kano ,Nigeria. They were identified at the Department of Plant Science, Bayero University Kano

### Replanting of Seedlings

The olive 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$^3$ of 0.10moldm$^{-3}$ $KNO_3$, 5.15cm$^3$ of 0.10moldm$^{-3}$ $FeCl_3.6H_2O$, 11.35cm$^3$ of 0.10moldm$^{-3}$ $H_3BO_3$, 5.00cm$^3$ of 0.05moldm$^{-3}$ $MgSO_4.H_2O$, 3.57cm$^3$ of 0.05moldm$^{-3}$ $Ca(NO_3)_2.4H_2O$, 5.00cm$^3$ of 0.05moldm$^{-3}$ $Na_2H_2P_2O_7$, 0.17cm$^3$ of 0.0075moldm$^{-3}$ KI and 23.10cm$^3$ of 0.05moldm$^{-3}$ $MnSO_4.H_2O$ into a 500cm$^3$ volumetric flask The volume was made to mark with deionized water ( Peralta *et al, 2000*).

$^3$Anthranilic acid(AA) were added to the control mixture to prepare 500cm$^3$each of different hydroponic treatments in triplicates.

The solutions were carefully transferred into clean labeled 750cm$^3$ table water plastic bottles. The seedlings were replanted on Wednesday 21st January, 2015 by 2.00pm (IITA, 1979 ) .

The seedlings were monitored in the garden at the Department of Pure and Industrial Chemistry, Bayero University, Kano. They were harvested separately, washed with tap water, rinsed thoroughly with deionized water and then dried in sealed plastic bags.

**Plant Tissue Analysis**

The various parts 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$^{O}$C in a muffle furnace to constant weight. The ash was cooled in a desiccator, dissolved in 0.10M $HNO_3$, filtered into a 50cm$^3$ volumetric flask and made to mark. $Ni^{+2}$ content in the roots and shoots was analyzed using Atomic Absorption Spectrophotometer at 232.0nm. The concentration of $Ni^{+2}$ was reported as mg/kg dry weight (IITA,1979).

**Data Analysis**

The data were analyzed through one-way analysis of variance (ANOVA) using SAS (Statistics for applied sciences) software to determine the effects of various treatments. Least significant difference (LSD) tests were performed to determine the statistical significance of the differences between means of treatments at 95% probability level.

## RESULTS AND DISCUSSION

### Determination of Field Parameters

### Change in Harvesting Time (ΔHT)

The change in harvesting time for a given treatment is compared with the corresponding control value. A negative value shows that the plant was harvested earlier than the control plant. .Fig.1 shows the change in harvesting time against concentration of added $Ni^{2+}$.

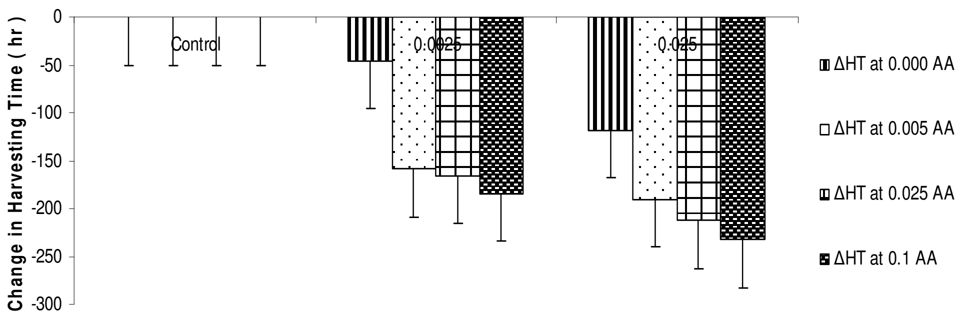


**Fig.1 : Change in Harvesting Time against Concentration of $Ni^{+2}$**

The change in harvesting time for control plant was zero. All other treatments gave negative values for ΔHT. Those plants died and were harvested much earlier than control.. The change in harvesting time caused by addition of different concentrations of $Ni^{2+}$ was highly significant ($Pr < 0.0001$). The phytotoxic effects of $Ni^{2+}$ ion might be responsible for the earlier death of the seedlings, a result consistent with the findings of Brune and Dietz (1995).

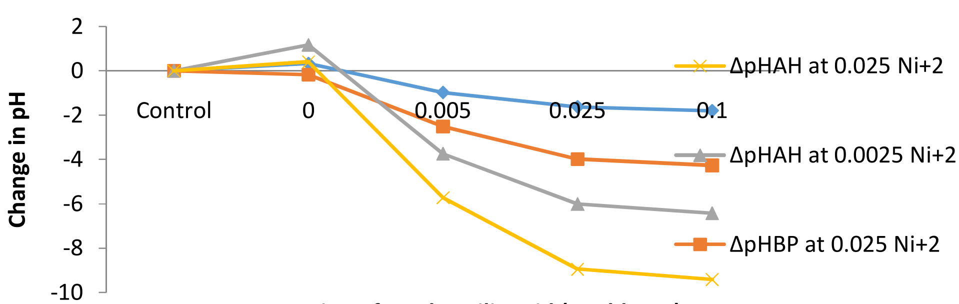


**Fig.2: Change in pH against Concentration of Anthranilic acid**

**Changes in pH before Planting ($\Delta pH_{BP}$) and after Harvest ($\Delta pH_{AH}$)**

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

This is supported by the report of Pandey and Sharma (2002) who found that nickel toxicity on growth and metabolism of plants depended on the pH of the nutrient solution.

Fig. 2 shows the pH changes before planting and after harvest against the concentrations of anthranilic acid.

The changes in pH of the various hydroponic treatments which were highly significant (Pr<0.0001) facilitated the absorption of sufficient quantities of $Ni^{2+}$ to kill the growing seedlings.

**Change in Plant Weight ($\Delta WP$)**

The change in plant weight for all treatments including the control is the difference between the weight of plant before replanting and the weight of plant after harvest. A negative value of $\Delta WP$ indicates a decrease in plant weight. Fig. 3 shows the changes in plant weight with concentration of $Ni^{2+}$ .

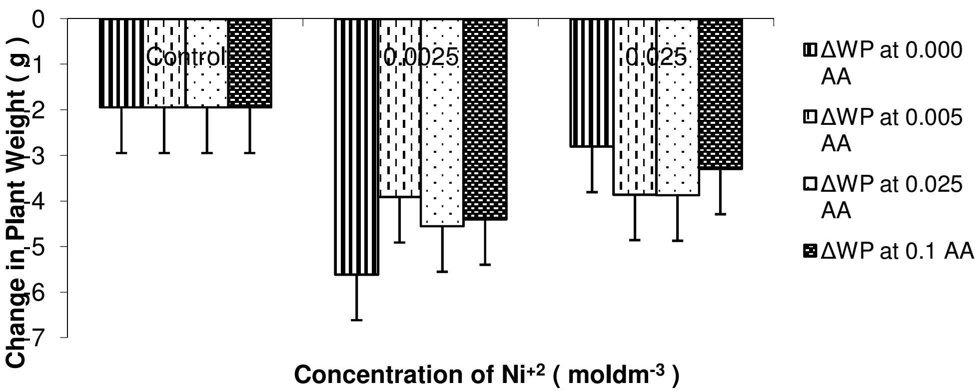


**Fig.3: Change in Plant Weight against Concentration of $Ni^{+2}$**

The dependence of the change in biomass of plant species on concentrations of toxic metals in nutrient solution was reported by Pandey and Sharma (2002). In this work, a significant change in plant weight (Pr=0.0359 < 0.05) was observed in all treatments including the control.

**Plant Parameters**

**Changes in Concentrations of Root and Shoot $Ni^{2+}$**

Changes in concentrations of root and shoot $Ni^{2+}$ accumulated in Olive seedlings growing in different hydroponic solutions are shown in Fig. 4.These changes which were highly significant (Pr < 0.0001) were determined by subtracting the control values of $Ni^{2+}$ in shoot and root from the values in various treatments.

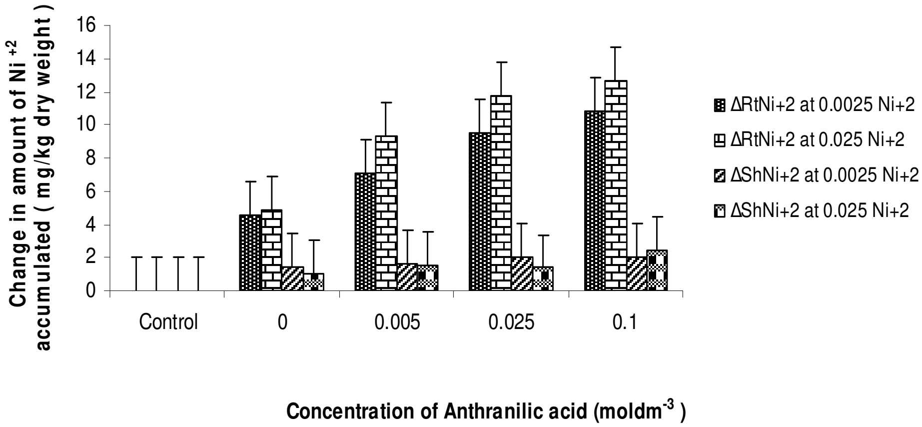


**Fig.4: Change in amount of $Ni^{+2}$ against Concentration of Anthranilic acid**

The roots are important for element uptake in plants (Sharma and Gaur, 1995). Higher amount of $Ni^{2+}$ can be rapidly taken up and accumulated by the plant root system (Ali *et al.,* 2008). In this work, the highest concentration of $Ni^{2+}$ taken up by the root of *Olea europaea* seedlings was 12.697mg/kg above the control. Madhava and Sresty (2000) reported that the uptake of $Ni^{2+}$ by plant was concentration dependent.

**Change in Translocation Factor (ΔTF )**

The translocation factor or mobilization ratio was calculated by dividing the concentration of nickel in the shoot with the corresponding root concentration. TF < 1 and TF > 1 respectively, at low and high capacity to translocate nickel from the roots to the shoots (Zhang *et al,* 2000). Fig.5 shows the changes in $Ni^{2+}$ translocation factor.

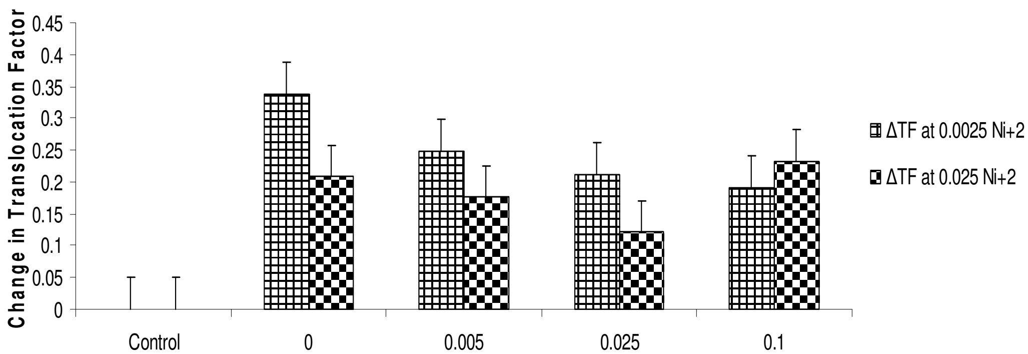


**Fig. 5: Change in Translocation Factor against Concentration of Anthranilic acid**

There was a significant change in $Ni^{2+}$ translocation factor (Pr = 0.0002 < 0.05). This was determined by subtracting the $Ni^{2+}$ translocation factor in a particular treatment from the control value. Fig.5 indicates that the $Ni^{2+}$ accumulated was largely retained in the roots (TF ratio < 1). This result agrees with the findings of (Hozhina *et al.,* 2001)who reported that protective barriers exist to prevent the penetration of $Ni^{2+}$ from the roots to the shoots. The high $Ni^{2+}$ concentrations would cause weak plant growth, leading to depression, metabolic disorders and chlorosis (Kabala *et al,* 2008).

**CONCLUSION**

The effects of anthranilic acid on $Ni^{2+}$ absorption by olive seedlings replanted in hydroponic solutions was studied. It was found that addition of 0.000 to 0.025moldm$^{-3}$ $Ni^{2+}$ and 0.000 to 0.100moldm$^{-3}$ anthranilic acid to various hydroponic solutions affected the growth of replanted olive (*Olea europaea*) seedlings. Seedlings grown in treated solutions died earlier than those grown in control. The weights of harvested plants decreased due to the phytotoxic effects of nickel. The rate and extent of translocation of nickel within the plants depended on the concentrations of $Ni^{2+}$ and anthranilic acid in the hydroponic mixtures. The highest concentration of nickel taken up by the root was 12.697mg/kg above the control. This was given by the seedling grown in a solution treated with 0.025moldm$^{-3}$ of $Ni^{2+}$ and 0.100moldm$^{-3}$ anthranilic acid. The TF ratio for all seedlings was less than 1.00 which indicated that the $Ni^{2+}$ accumulated was largely retained in the roots. This could be used in phytoremediation technique to remove $Ni^{2+}$ pollutants from the environment or to decrease their toxicity . The technique has many advantages which include low economic costs and the possibility of being applied to soils, causing a minimum environmental impact.

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