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Effect of Nitric Acid on Uptake of EDTA-Ni^{2+} Complex by Spinach (*Spinacea oleracea l.*) Seedlings Replanted in Hydroponic Solution

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Abstract: *The aim of the research was to determine the effect of nitric acid (HNO_3) on uptake of EDTA-Ni^{2+} complex by spinach (spinacea oleracea l.)seedlings replanted in Ni^{2+} spiked hydroponic solutions in a greenhouse, Eight weeks old seedlings were exposed to various doses of Ni^{2+} (0, 1000, 2000, and 4000 mg/L) as $NiSO_4$, Na_2EDTA (0,500,and3000mg/L) and HNO_3 (0,500 and 3000mg /L) in different combinations. After 4 day exposure the plants were harvested and dried. The Ni^{2+} uptake was determined by flame atomic absorption spectrometry.The results showed that increasing the concentration of Ni^{2+} in the nutrient solutions significantly increased ($p<0.05$) the uptake and accumulation of Ni^{2+} in plant shoots and roots, giving rise to a significant change ($p<0.05$) in the weights of spinach seedlings. The effect of nitric acid was dosage dependent, increasing the biomass at low concentration and decreasing at higher dosage. Nitric acid increased EDTA-Ni^{2+} uptake and acumulation in spinach shoots and roots at all concentrations. The Ni^{2+} ion accumulated more in roots than shoots.Therefore, HNO_3 enhanced the formation of the EDTA-Ni^{2+} complex and therefore increased the toxic effect of Ni^{2+} on spinach (spinacea oleracea).*

Keywords: *Greenhouse; Hydroponic solutions; Disodium-EDTA, Nitric acid; Spinach (spinacea oleracea l.*

1. INTRODUCTION

Nickel (Ni) is an essential micronutrient for some higher plants (Younis *et al*., 2015). It acts as a co-factor of enzymes. It is beneficial for animals in trace quantities, but its higher concentrations pose toxic effects to plant growth. High nickel levels in plants reduce the rate of metabolic activities and decrease water and nutrient uptake (Gajewaska *et al*., 2006). Nickel at low levels is important for plant growth, plant senescence, nitrogen metabolism, seed germination and plant disease resistance (Hussain *et al* 2013). However, Ni can be phytotoxic when soluble forms of Ni are present in soil in excess.

Plants generate reactive oxygen species (ROS) such as $.O_2^-$, O_2, .OH, H_2O_2 under heavy metal stress (Pflugmacher, 2004) and over production of these ROS in plants causes oxidative damage to proteins, DNA and lipids (Sigh and Panday 2011). ROS also affect the antioxidative defense

system in plant cells. Therefore, to scavenge ROS and to avoid oxidative damage, plants possess enzymatic and non-enzymatic antioxidants (Sigh and Panday 2011).

Nickel has both natural and manmade sources of emission. The natural source include weathering of rocks. Many compounds of Ni like oxides, hydroxides and acetates are released into the environment as a result of devastating human activities (Cempel and Nikel, 2006). In most plants it is found at the level of 0.1-6 ppm (on dry weight basis), with a wide threshold range of 40 -246 ppm toxicity symptoms, dependent upon the plant species (Sigh and Panday 2011). The toxic effects of Ni in plants include decreased shoot and root growth and reduction in leaf area (Shaw *et al*., 2004). The growth of *Zea mays* seedling was decreased with elevated concentration of Ni^{2+} (Bhardwaj *et al*., 2007). The germination of pigeon pea was decreased by 20% in a 1.5 mM solution of Ni^{2+} and the germination percentage was decreased in proportion to the concentration of Ni^{2+} (Rao and Sresty, 2000). The mass of wheat shoot was decreased by 20 and 26% with Ni application of 100 and 200 µM respectively (Gajewska and Sklodowska, 2008).
Nitric acid (HNO_3) affects soil because of its nitrogen atoms. In small concentrations, it can be beneficial to most plants as a fertilizer, combining with other elements to form nitrates (Considine 2002). The acidity of soil influences the physical properties of the soil, the availability of certain minerals to plants, and the biological activity of the soil. Consequently, it strongly influences plant growth. Acidic pH increases the absorption of nickel in soil (Branka *et al.,*2009).

Spinach is selected for this study due to its high human consumption as a nutrient and capacity to uptake and store heavy metals (Duke and Ayensu ,1985; Palada and Crossman, 1999).

2. MATERIALS AND METHODS

2.1 Growth Conditions and Treatments

Seeds of spinach (*Spinacea oleracea l.*) were obtained from the International Institute of Tropical Agriculture (IITA), Tarauni station, Kano state, Nigeria. They were grown in experimental farm of Bayero University, Kano for eight (8) weeks. The seedlings were carefully harvested, washed with tap water to remove excess soil, and rinsed three times with deionized water before replanting in hydroponic solution and kept in a green house. The conditions of the greenhouse were 65% constant relative humidity, 16/8 h day/night regime under 600 µ mol m−2 s−1 of light intensity, and day/night temperatures of 38/20°C. Plants were supplied with 10% Hoagland nutrient solution (pH 1.57-6.82).
The hydroponic solution was prepared based on (Hoagland and Arnon, 1950) and had the following composition:1000cm^3 Hoagland solution was prepared by pipetting 2.5cm^3 of 2$moldm^{-3}$ KNO_3 ,2.5cm^3 of 1$moldm^{-3}$ $Ca(NO_3)_2.4H_2O$, 1.5cm^3 of 0.5 $moldm^{-3}$ $FeCl_3.6H_2O$, 1cm^3 of 2 $moldm^{-3}$ $MgSO_4.7H_2O$, 1cm^3 of 1$moldm^{-3}$ NH_4NO_3, 1cm^3 of H_3BO_4, 1cm^3 of $MnCl_2.4H_2O$, 1cm^3 of $ZnCl_2.4H_2O$, 1cm^3 of $CuSO_4.5H_2O$, 1cm^3 of $Na_2MoO_4.2H_2O$, 0.5cm^3 of 1$moldm^{-3}$ KH_2PO_4 (pH6) into a 1000cm^3 volumetric flask. The solution was made to mark with deionized water.

The control was prepared by transferring 500cm^3 of 10% Hoagland solution into 500cm^3 volumetric flask. Other treatments were prepared by addition of nickel in four levels (0, 1000, 2000, and 4000 mg/L) as $NiSO_4$, Na_2EDTA (0,500,and 3000mg/L) and HNO_3 (0,500 and 3000mg /L) in different combinations to the nutrient solution. The experiment was conducted in twelve (12) treatments in triplicates. The plants were harvested after 4 days and washed with tap water followed by deionized water then wiped with filter paper. The plant biomass before replanting in the hydroponic solution and after harvest were measured.

Figures 1 -4 show the maps of sampling and planting sites.

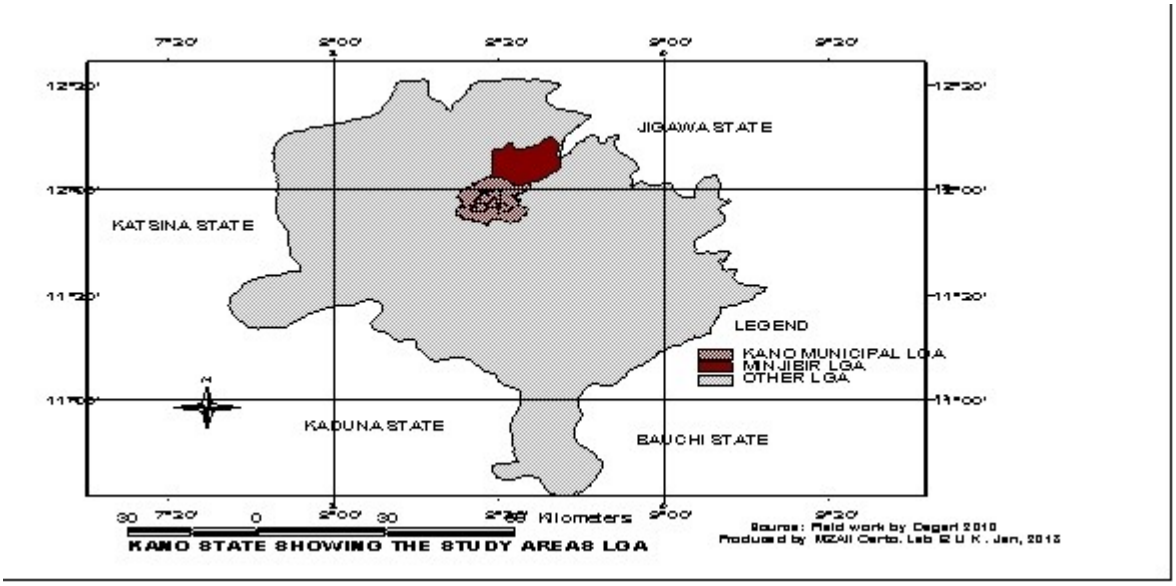


Fig 1: Map of Kano State Showing the Study Local Government Area

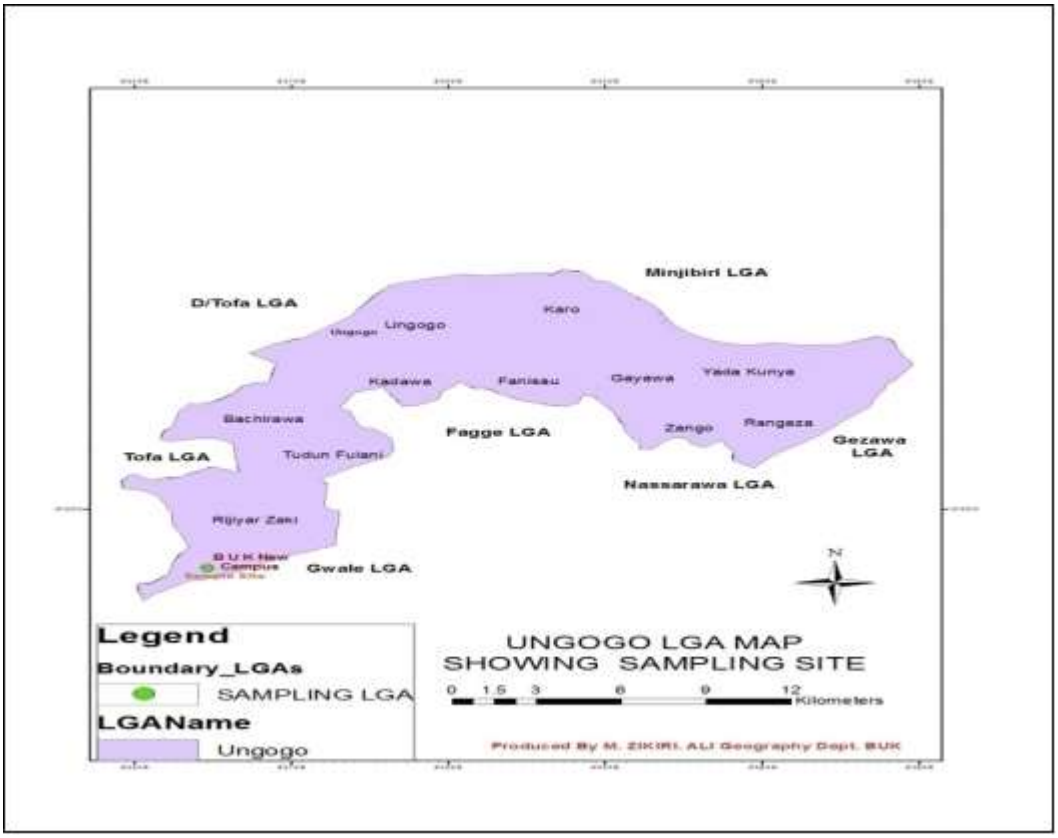


Fig 2: Map of Kano state

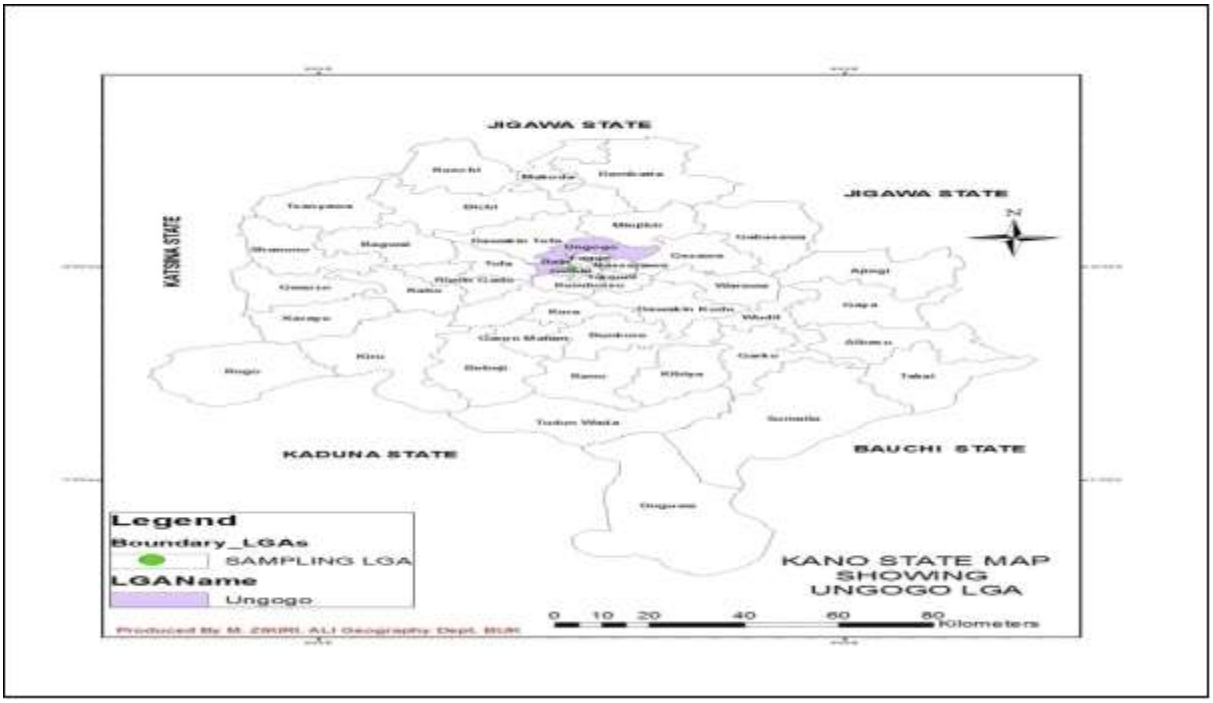


Fig3: Map of Ungogo LGA Showing New Campus, Bayero University, Kano.

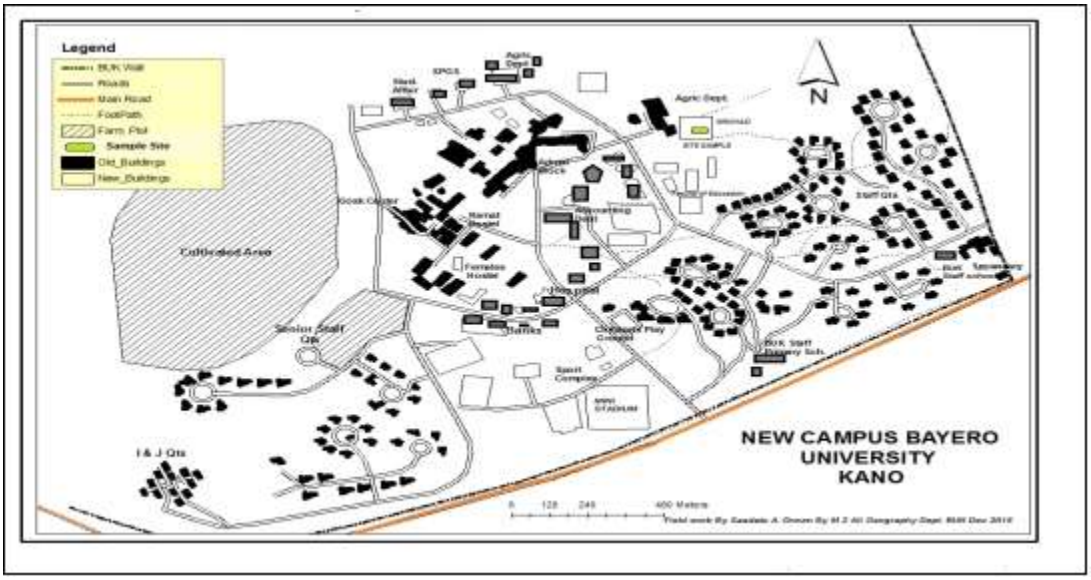


Fig 4: Map of New Campus Bayero University, Kano Showing Planting Site.

2. 2 Ashing of Plant Materials

Roots and shoots were separated and washed three times with deionized water. The samples were oven dried at 80°C for 48 hours and then ground with wooden pestle and mortar. 2.0g of each sample was weighed into a porcelain crucible to which one millitre of concentrated nitric acid was added and then ashed to constant weight in a crucible kept in a muffle furnace at 480°c for 3 hours. The sample was cooled to room temperature, dissolved in 5ml of 30% hydrochloric acid and then filtered using Whatmann No. 42 filter paper. The filtrate was transferred into 50ml volumetric flask

and made to mark with de-ionized water (AOAC, 1990). Nickel in roots and shoots was analysed by flame atomic absorption spectrophotometry at 232nm. Concentration of Ni^{2+} was reported as $mgkg^{-1}$ dry weight.

2.3 Atomic Absorption Spectrophotometric Determination of Ni^{2+} in Roots and Shoots

The root and shoot extracts were analyzed for nickel at 232nm using flame atomic absorption spectrophotometry. Blank determinations were made prior to sample analysis. Concentrations of Ni^{+2} in the plant extracts were obtained in triplicates from calibration curves and expressed as mg/kg dry weight (AOAC, 1990)).

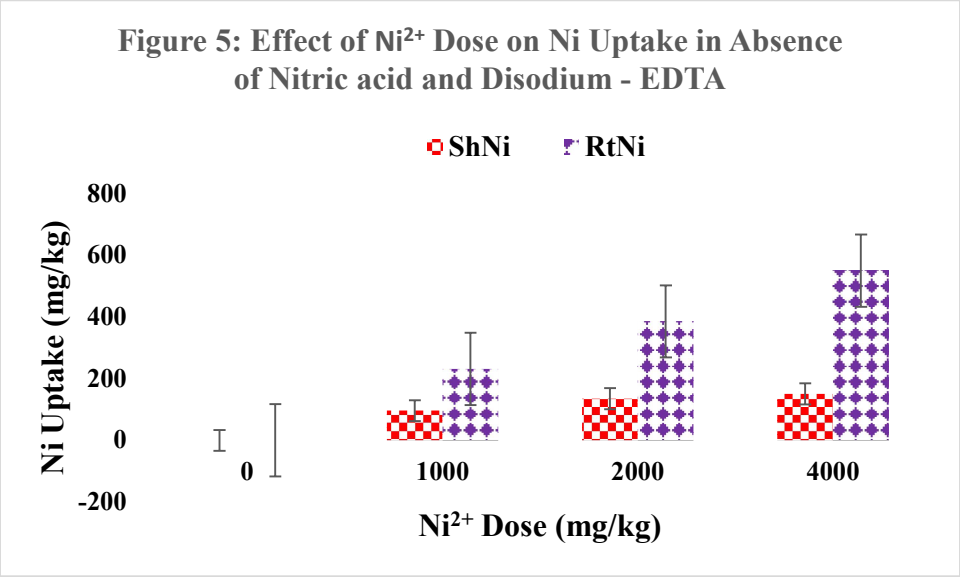
2.4 Statistical Analysis

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. Statistical significance was assumed at $p<0.05$.

3. RESULTS AND DISCUSSION

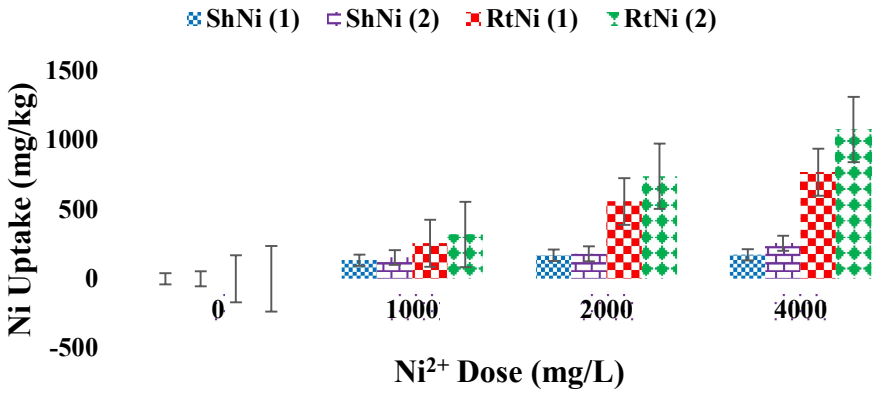
3.1 Nickel Concentration and Uptake by Spinach

Nickel concentration in roots and shoots of spinach plants were significantly increased ($p<0.05$) when plants were exposed to varied Ni^{2+} doses (0, 1000, 2000, and 4000mg/L) relative to control plants as shown in **Figure 5**. Nickel uptake in roots and shoots gradually increased with increasing Ni^{2+} concentration in hydroponic treatments. The roots accumulated the largest amount of Ni^{2+} .concentration. According to the reports by Amal and Saleh (2002) and Giordan *et al* (2005), accumulation of heavy metals was more in root than in the shoot of sun flower.



The effects of addition of HNO_3 and disodium-EDTA on Ni uptake by spinach seedlings treated with different Ni^{2+} doses are shown in **Figure 6**.

Figure 6: Effect of Ni^{2+} Dose on Ni Uptake in Presence of Nitric acid and Disodium - EDTA



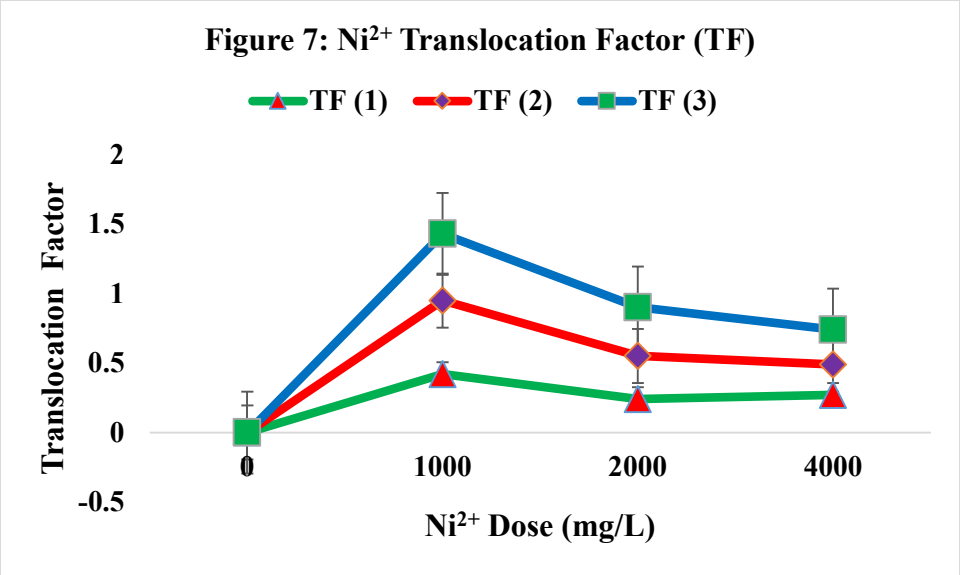
KEY:
ShNi (1): Ni in shoot in presence of 500mg/L of HNO_3 and disodium-EDTA
ShNi (2): Ni in shoot in presence of 3000mg/L of HNO_3 and disodium-EDTA
RtNi (1): Ni in root in presence of 500mg/L of HNO_3 and disodium-EDTA
RtNi (2): Ni in root in presence of 3000mg/L of HNO_3 and disodium-EDT

Increasing the concentration of Ni^{2+} (0, 1000, 2000, and 4000mg/L) in presence of 500mg/L each of disodium-EDTA and HNO_3 significantly increased ($p<0.05$) Ni uptake in roots and shoots compared with other treatments without disodium-EDTA and HNO_3 .The increase in Ni uptake might be due to chelation of Ni^{2+} with disodium-EDTA. According to Meers *et al.* (2008), chelation induces phytoextraction and translocation of heavy metals from the roots to shoots of plants.

In this study, nickel stress was found to induce visible toxicity symptoms in spinach (*Spinacia oleracea* l.) The prominent symptoms were bleaching of leaf margin, chlorosis in leaves, browning of root tips and breaking of roots. These toxicity symptoms could be attributed to high accumulation of nickel in tissues (Amal and Saleh, 2002). It has been reported that nickel ion decreases the permeability of cell membrane, inhibit root system development and cause necrosis and chlorosis (Pandey and Gautam, 2009). Symptoms of Ni toxicity were more effective with increasing Ni^{2+} concentration. In this study, the effect was more pronounced in hydroponic mixture of 4000mg/LNi^{2+} , 3000mg/L disodium-EDTA and 3000mg/L HNO_3.The toxicity increased with increasing concentrations of HNO_3 and disodium-EDTA in the nutrient solution. This result is in agreement with the reports of Jean *et al*. (2008) and Jing *et al*. (2015) who suggested that EDTA is the most effective means of increasing the uptake of Ni in plants and overcoming the diffusion limitation of metals to the root surface as well as the barrier of root to shoot translocation.

3.2 Translocation Factor

The translocation factor (TF) gives the ratio of the shoot to root nickel concentration and depicts the ability of the species to translocate the metal from the roots to the shoots (Ghnaya *et al*, 2007).
TF= Ni^{2+} in shoots ($mgkg^{-1}$) / Ni^{2+} in roots ($mg\ kg^{-1}$)
Figure 7 shows Ni^{2+}translocation factor against Ni^{2+}dose at different concentrations of disodium-EDTA and nitric acid.

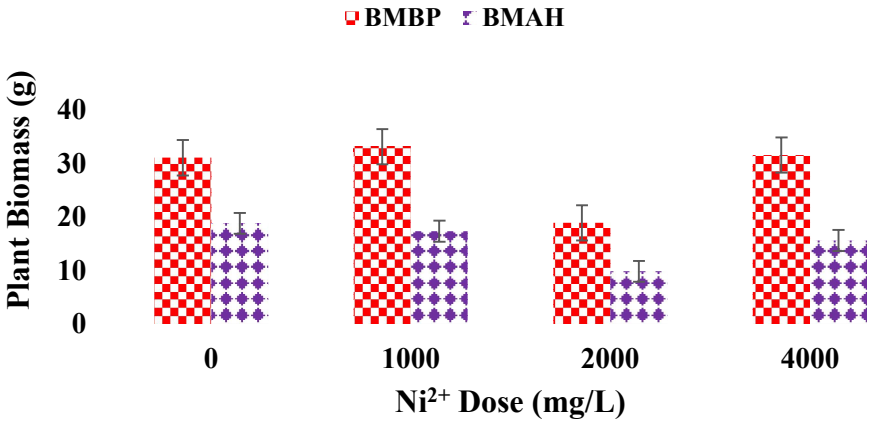


KEY:
TF (1): Ni $^{2+}$ translocation factor in absence of HNO_3 and disodium-EDTA
TF (2): Ni $^{2+}$ translocation factor in presence of 500mg/L of HNO_3 and disodium-EDTA
TF (3): Ni $^{2+}$ translocation factor in presence of 3000mg/L of HNO_3 and disodium-EDTA
TF varied significantly ($p<0.05$) with concentration of added Ni $^{2+}$.Thus a relatively good fraction of Ni $^{2+}$was translocated to the shoots. In absence of HNO_3 and disodium-EDTA, the TF at 1000 mg/L Ni $^{2+}$ was 0.42 which decreased to 0.24 at 2000 mg/L Ni $^{2+}$. In presence of 500 and 3000 mg/L each of HNO_3 and disodium-EDTA, the translocation factors at 1000, 2000 and 4000mg/L Ni $^{2+}$ were 0.53, 0.31, 0.22 and 0.48, 0.35, 0.25 respectively. From the results above EDTA aided the translocation of Ni^{2+} to the shoots. This is supported by the findings of Saima *et al,* (2010), in which EDTA was found to promote the translocation of Ni^{2+} to the shoots of sun flower (*Helianthue annuus L.).*This observation is in agreement with the report of Lombi *et al* (2001) who observed that EDTA application increased metal mobility in soil and uptake by roots, but did not substantially increase the transfer of metals (Cd, Zn, Pb, Cu) to corn shoots.
The roots of plants act as a barrier against heavy metal translocation possibly as a result of potential tolerance mechanism (Singh and Pandey, 2011).

3.3 Changes in Fresh Plant Biomass of Due to Ni^{2+} Stress

Figure 8 shows changes in fresh plant biomass against Ni $^{2+}$dose in absence of disodium-EDTA and nitric acid.

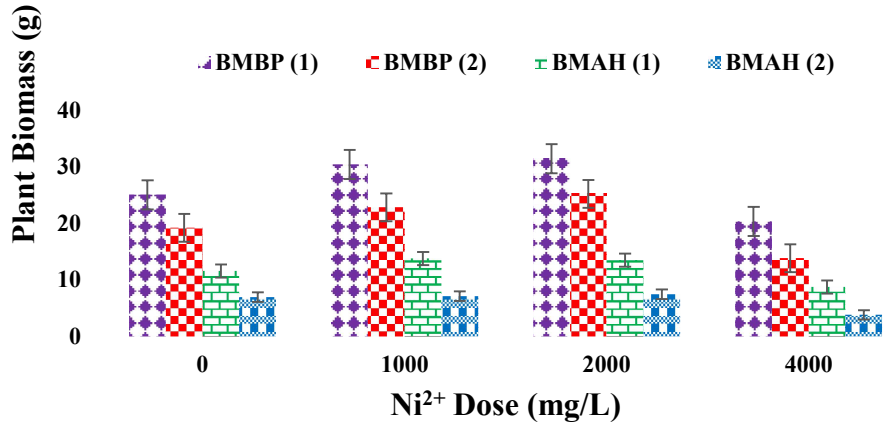
Figure 8: Effect of Ni^{2+} Dose on Plant Biomass in Absence of Nitric acid and Disodium - EDTA



KEY:
BMBP: Biomass before planting **BMAH:** Biomass after harvest

The plant biomass for control plants before planting and after harvest were 31.1 and 18.83g respectively. For 1000, 2000 and 4000 mg/L Ni $^{2+}$, the biomass before planting and after harvest were 33.17, 18.9, 31.6 and 17.37, 9.87, 15.63g respectively. Gajewaska *et al* (2006) reported that low concentration of Ni^{2+} is beneficial to plants, but higher concentrations are toxic to growing plants. **Figure 9** shows changes in fresh plant biomass against Ni $^{2+}$dose in presence of disodium-EDTA and nitric acid.

Figure 9: Effect of Ni^{2+} Dose on Plant Biomass in Presence of Nitric acid and Disodium - EDTA



KEY:
BMBP (1): Biomass before planting in presence of 500mg/L of HNO_3 and disodium-EDTA
BMBP (2): Biomass before planting in presence of 3000mg/L of HNO_3 and disodium-EDTA
BMAH (1): Biomass after harvest in presence of 500mg/L of HNO_3 and disodium-EDTA
BMAH (2): Biomass after harvest in presence of 3000mg/L of HNO_3 and disodium-EDTA

In presence of 500mg/L each of HNO_3 and disodium-EDTA, the plant biomass before planting and after harvest for control plants were 25.03 and 11.53g respectively. For 1000, 2000 and 4000mg/L Ni^{2+} dose, the plant biomass before planting and after harvest were 30.37, 31.43, 20.33 and 13.77, 13.47, 8.73g respectively. When the concentrations of each of HNO_3 and disodium-EDTA was increased to 3000mg/L, the plant biomass before planting and after harvest for 1000, 2000 and 4000mg/L Ni^{2+} dose were 22.8, 25.17, 13.83 and 7.1, 7.43, 3.8g respectively. According to (Amal and Saleh 2002), the decrease in plant biomass with increasing heavy metal concentration was due to inhibitory effect on cell division ,cell elongation and enzyme activity. The effect of HNO_3 on spinach biomass was dose dependent. The acid modified the physico-chemical properties of the hydroponic resulting in reduced Ni^{2+} stress (Starr, 2006). At higher concentration, the acid caused more harm to the semi permeable membrane and the ability of the plant to absorb nutrients (Considine, 2002).

4. CONCLUSION

The results of this study revealed that different levels of Ni^{2+} had negative effect on growth of Spinach (*Spinacea oleracea*). The effect of nitric acid was dosage dependent, increasing the biomass at lower concentration and decreasing at higher dosage. Furthermore, The addition of EDTA and HNO_3 to hydroponic solution enhanced the phytoextraction of Ni^{2+} in spinach seedlings (*Spinacea oleracea*).The potential of a plant for phytoremediation depends on its ability to produce large biomass in a short time, tolerate and accumulate higher concentration of heavy metal ($\geq$100) times–above plants that do not hyperaccumulate under the same conditions, without showing any observable symptoms in their tissues. From the result of this study, if spinach were to be used for phytoremediation of nickel, the remediation processes will need a very long time.

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Authors' Contributions
Dagari M.S.: Conceptualization, design and supervision of the research work; Editing of the write-up
Abdulsalam S.: Undertaking the research work, write-up and data analysis.

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