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EDTA and Nitric Acid Responses on Nickel Uptake, Translocation Factor and Pigments on *Spinacia oleracea* L. Replanted Seedlings in Hydroponic Solution

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ABSTRACT: The effects of Na_2EDTA and HNO_3 on Ni^{2+} uptake by *Spinacia oleracea* seedlings replanted in hydroponic culture in a greenhouse was investigated. Eight week old seedlings, were exposed to various doses of Ni^{2+} (0, 1000, 2000, and 4000 mg/L) as $NiSO_4$, at (0, 500 and 3000 mg/L) Na_2EDTA and (0, 500 and 3000 mg /L) HNO_3 in different combinations. There was a substantial increase in nickel uptake in chelated treatments ($p < 0.05$) compared to unchelated treatments of same concentrations of Ni^{2+}. So, chelation enhanced Ni^{2+} uptake in *S. oleracea*. During the exposure, antioxidant defense system helped the plant to protect itself from the damage. Due to increasing nickel uptake by the plant, the photosynthetic pigments (i.e chlorophyll a, chlorophyll b and Caretenoids) gradually declined. In this study, *Spinacia oleracea* Seedlings and contents of the photosynthetic pigments (chlorophyll a, chlorophyll b and Caretenoids) of both chelated and unchelated hydroponic treatments were investigated. Changes in photosynthetic pigments was significant ($p < 0.05$) with respect to addition of EDTA and HNO_3 at different concentration to different concentrations of Ni^{2+} compared to unchelated treatments of same concentrations of Ni^{2+}. The Ni^{2+} induced translocation factor was also determined which increased significantly ($P < 0.05$) with increasing Ni^{2+} concentrations.

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Keywords: Nickel, Translocation Factor, Hydroponic, EDTA, Nitric acid, *Spinacia oleracea*, Pigment.

Wastewater from many sources, including industries, kitchen sinks and mining sites has been found to contain traces of nickel (Carocci *et al*., 2016). Tobacco smoking is linked to nickel sources as research reveals that each cigarette contains a nickel amount of 1.1 - 3.1 µg; present as nickel carbonyl (Genchi *et al*., 2020). The most stable states of nickel in the environment is the +2 oxidation state (Ni^{+2}), other valences include -1, 0, +3 and +4. Natural sources of nickel include dust, mass creeping of rocks, volcanic eruptions and soil. Nickel exposure can be through dietry intake of some vegetables such as spinach, carrot, green beans and tomato (Carocci *et al*., 2016). Human exposure to highly nickel-concentrated environments may cause a variety of health effects. Accumulation of nickel and nickel compounds in the body through chronic exposure may be responsible for some of these deadly diseases to human beings, such as kidney failure, lung fibrosis, cardiovascular diseases and cancer of the respiratory tract. Nickel is essential for plant in low concentration but high concentration is toxic. Nickel is naturally occurring in soil and surface water with concentration lower than 100 and 0.005 ppm, respectively (Chen *et al.,* 2009). Nickel is an essential micronutrient for some higher plants, it acts as enzyme co- factor and is beneficial for plants in trace quantities, but 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 in plants (Younis *et al*., 2015). This research is a demonstration of the extent of damage caused and the stress level undergo by *Spinacia oleracea* Seedlings under nickel toxicity for a particular treatment and the effects of various doses of Na_2EDTA on nickel at it stable form. Ethelene diamine tetra acetic acid (EDTA) is a multi-dentate ligand which when donate its lone pair of electrons to a central metal ion such as Ni^{+2} to form a chelate. Chelating agents can react with metal ions and influence metal phytotoxicity and phytoextraction (Hassan *et al*., 2020). EDTA is often used to enhance metal uptake by plants in field and some hydroponic experiments (Zhou *et al*., 2020). Also, chelating agents helps in translocating metal from roots to shoot system of plants (Chen *et al*., 2020). Chlorides within its permissible limit by WHO is used in water purification in Nigeria, with this; different chloride concentrations are often present in aquatic environments. Chloride can increase metal solubility, which may enhance metal bioaccumulation in shoots and roots of plants; however, only a few studies have been conducted on the effects of chloride on nickel (He *et al*., 2020). Studies on the effects of chloride on Ni phytoremediation are also limited.

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MATERIALS AND METHODS

Hydroponic Culture: Eight week old *S. oleracea* seedlings were carefully collected from Department of Agronomy farm, Bayero University, Kano. The seeds of *S. oleracea* were sterilized in a 10% H_2O_2 solution for 15 min and then washed with distilled water before they were soaked in distilled water for 8 hours at 50^0C and were sown on artificially. The seeds germinated at room temperature in a greenhouse at 65% relative humidity, 13 hour light/11 hour dark photoperiod (photosynthetically active radiation 600 μ mol m^{-2} s^{-1} with day/night temperatures 39/23°C). Each plants were supplied with 300 ml modified Hoagland nutrient solution with pH (1.57 - 6.82). After five days of exposure, the roots were extracted carefully after germination. Roots and shoots of similar size were selected, dried at room temperature for two weeks and then placed in a dark polythene bag for further analysis. Translocation Factor (TF) was calculated as reported by Yoon *et al*. (2006).

$$TF = \frac{\text{Ni2+ content of shoot}}{\text{Ni2+ content of root}} \times 100$$

AAS determination of Ni was carried out after the roots and shoots were prepared using mixed acid (HNO_3/HCl) as carried out by Adolfo *et al*. (2020).

Determination of Pigment Content (Chlorophyll A, Chlorophyll B and Caratenoid): The estimation of pigments content in both control and treated plants were carried out according to the method of Gitelson (2020). 2.0 g of dried leaf tissue of each sample were homogenized using 80% acetone. The homogenates was centrifuged for 10 minutes and the supernatant was collected. The residue was again extracted and the supernatant was pooled together. The extraction process was repeated until the residue became colourless. The volume of the combined supernatant was recorded. The absorbance of solution was then measured at 645 nm, 663 nm and 470 nm for both Chlorophyll A, Chlorophyll B and Caratenoid respectively using a UV –visible spectrophotometer.

The amount of Chlorophyll A, B and Caretenoid were calculated using the relation:

$$\text{Chlorophyll A (mg } g^{-1}) = [(11.75 \times A663) - (2.350 \times A645)] \times \text{Vol. of acetone}$$

$$\text{Chlorophyll B (mg } g^{-1}) = [(18.61 \times A645) - (3.960 \times A663)] \times \text{Vol. of acetone}$$

$$Carat = \frac{1000\ A470 - 2.270\ \text{Chloro A} - 81.4\ \text{Chloro B}}{227}$$

Where Carat = Caratenoid; Chlor A = Chlorophyll A and Chloro B = Chlorophyl B

Statistical Analysis: All data presented in this study are the mean values of three replicates. Statistical analysis was performed using Excel 2010 software and significance test were performed using One-way ANOVA at 95% confidence level.

RESULTS AND DISCUSSION

Nickel content in Spinacia oleracea Seedlings: Nickel concentration in roots and shoots of spinach plants were significantly increased ($p<0.05$) when plants were exposed to nickel at varied concentration (0, 1000, 2000 and 4000 mg/L) relative to control plants. Nickel concentration in roots and shoots gradually increased with increasing nickel concentration in the hydroponic treatments. The root accumulated the largest in all treatment. Application of EDTA and HNO_3 at constant concentration (500 mg/L) in Fig 3.1A significantly increased ($p< 0.05$) the nickel concentration in roots and shoots as compared to treatment without EDTA and HNO_3.. The highest uptake of Ni^{2+} was observed in Fig 3.1C when concentration of EDTA and HNO_3 was raised to 3000mg/L. The effect of Na_2EDTA and HNO_3 was visible between 24 – 48 hours. Some of the prominent symptoms were bleaching of leaf margin, chlorosis in leaves, browning of root tips and broken off roots indicating phytotoxicity.

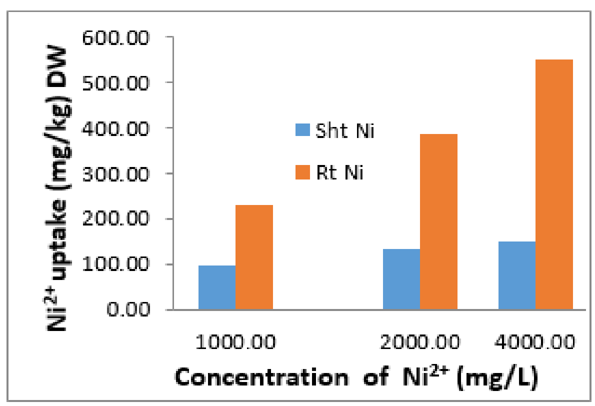

Fig 3.1: Effect of addition of 0 mg/L HNO_3 on Absorption of Ni^{2+} by *S. oleracea* Seedlings replanted in Hydroponic mixture at 0 mg/L Na_2EDTA.

Translocation factor of Ni^{2+} in S. oleracea Seedlings: Translocation factor (TF) is the capacity of a plant to transfer metal from its roots to shoots. The TF of *S. oleracea* plant significantly decreased ($p<0.05$) when the plant was exposed to nickel at varied concentration (0, 1000, 2000 and 4000mg/l). At 0 mg/L Na_2EDTA and HNO_3, the TF gradually increases as Ni concentration increased from 0 to 1000mg/l and slightly decreases as Ni concentration increases to 2000 and 4000mg/l respectively. This is in conformity with the work of Yoon *et al*. (2006). Fig 3.2 shows the translocation factor of Ni by the *S. oleracea* seedlings. Similar trend was observed when Na_2EDTA and HNO_3 concentrations was raised to 500 mg/L and 3000 mg/L respectively.

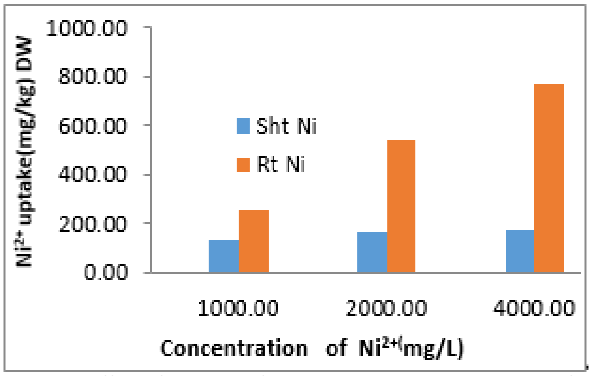

Fig 3.1B: Effect of addition of 500 mg/L HNO_3 on Absorption of Ni^{2+} by *S. oleracea* replanted in Hydroponic mixture at 500 mg/L Na_2EDTA

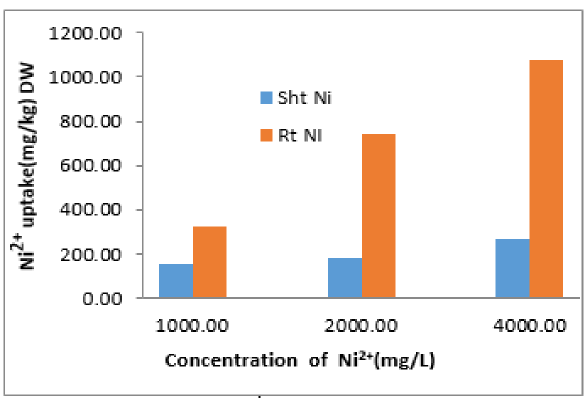

Fig 3.1C: Effect of addition of 3000 mg/L HNO_3 on Absorption of Ni^{2+} by *S. oleracea* Seedlings replanted in Hydroponic mixture at 3000 mg/L Na_2 EDTA.

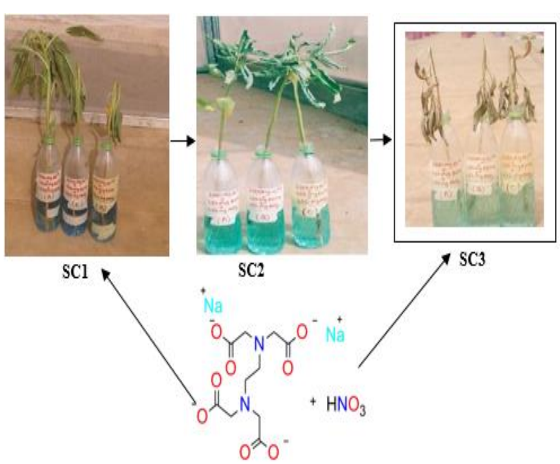

Plate 1: SC1 – SC3: Shows effect of Na_2EDTA and HNO_3 after 24 hours

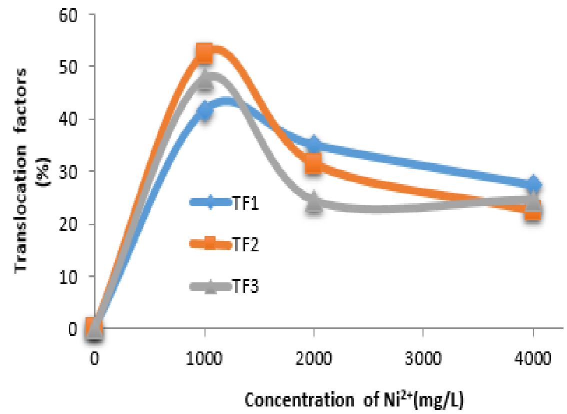

Fig 3.2: Effect of HNO_3 and Na_2EDTA, on Ni^{2+} TF in *S. oleracea* Seedlings replanted in hydroponic solution
KEY: TF1: *TF at 0 mg/L each HNO_3 and Na_2EDTA,* ***TF2:*** *TF at 500 mg/L each of HNO_3 and Na_2EDTA and* ***TF3:*** *TF at 3000 mg/L each of HNO_3 and Na_2EDTA.*

Pigment Content of S. oleracea Seedlings: The plant pigments chlorophyll A, chlorophyll B and carotenoids were determined to justify the Ni^{2+} uptake by the plants. The results revealed inverse proportion of Ni^{2+} uptake significantly *($p < 0.05$)* for chlorophyll A, chlorophyll B and carotenoids contents. Figure 3.3A shows the pigment content of Spinach seedlings in the absence of Na_2EDTA and HNO_3. The plants were exposed to nickel at varied concentration (0, 1000, 2000 and 4000 mg/L. Pigment contents in shoots gradually decreases with increasing nickel concentration in the hydroponic treatments. Application of Na_2EDTA and HNO_3 at constant concentration (500 mg/L) in Figure 3.3B significantly decreased ($p<0.05$) the pigment contents as compared to treatment without Na_2EDTA and HNO_3. Similar trend was observed in Figure 3.3C when concentration of Na_2EDTA and HNO_3 was raised to 3000 mg/L.

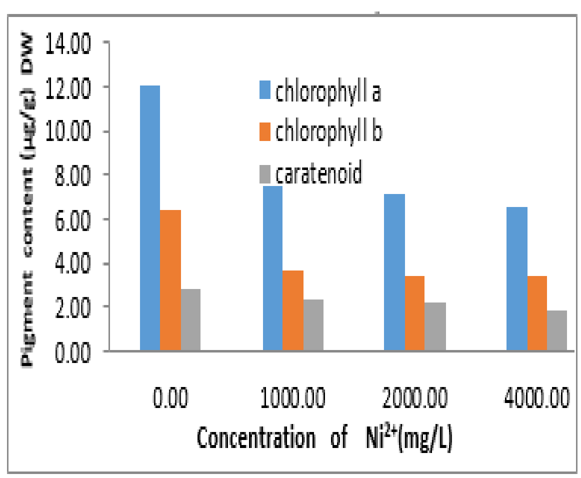

Figure 3.3A: Effect of addition of 0 mg/L HNO_3 on pigment content of *S. oleracea* seedlings replanted in hydroponic mixture at 0 mg/L Na_2EDTA

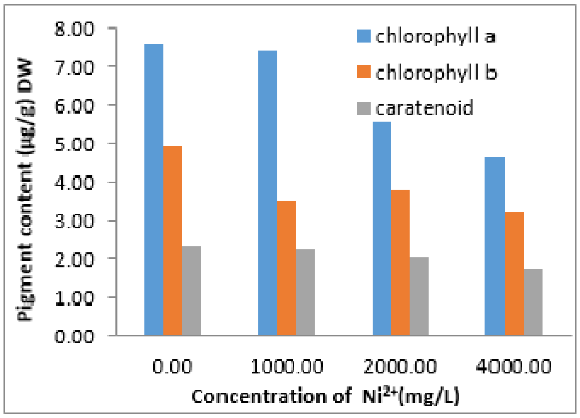

Fig 3.3B: Effect of 500 mg/L each of Na_2EDTA and HNO_3 on pigment content of *S. oleracea* seedlings replanted in hydroponic mixture

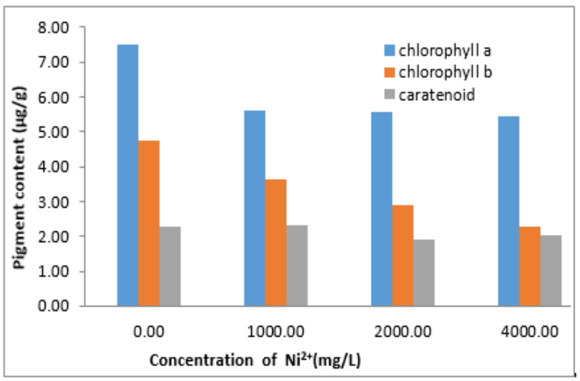

Figure 3.3C: Effect of 3000 mg/L each of Na_2EDTA and HNO_3 on pigment content of *S. oleracea* seedlings replanted in hydroponic mixture

Nickel stresses uptake: Nickel is an essential micronutrient for some higher plants (Younis *et al*., 2015). It acts as a co-factor of enzymes and it's beneficial for animals in trace quantities, but its higher concentrations pose toxic effects in plant growth. High nickel levels in plants reduce the rate of metabolic activities and decrease water and nutrient uptake in plants (Gajewaska *et al*., 2006). Nickel at low levels are 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. Furthermore, some physiological and biochemical parameters were also considered to buttress the results. In this study the spinach (*Spinacia oleracea L.*) seedlings were exposed to various doses of Ni^{2+} (0, 1000, 2000, and 4000 mg/L) supplied as $NiSO_4$, EDTA (0,500,and 3000 mg/L) supplied as Na_2EDTA and HNO_3 (0,500,and 3000mg/L) in the nutrient solution. The results of both chelate-assisted and unchelated hydroponic treatments revealed significant changes ($p<0.05$) in Ni^{2+} uptake by the plant.

The results of unchelated hydroponic treatments are supported by Saleh (2002) and Giordan *et al* (2005), they reported that the roots accumulate the largest Ni^{2+} concentration. Increase in soil solution concentration of Ni resulted in increased Ni concentration in plant tissues. The highest root uptake in unchelated hydroponic treatment was (549.87mg/kg±0.72) at 4000 mg/L.At the application of 500 and 3000 mg/Leach of EDTA and HNO_3 to various doses of nickel in the hydroponic solution significantly increased ($p<0.05$) the uptake of nickel compared to unchelated hydroponic treatments of same concentrations of Ni^{2+}.The root uptake in the chelated hydroponic treatments were 1.346 and 1.960 times higher than the unchelated hydroponic treatments at 4000 mg/L Ni^{2+} concentrations. So, chelation enhanced Ni^{2+} uptake by S. *oleracea*. The highest root uptake in chelated hydroponic treatments was 1077.46mg/kg±0.54 at 4000 mg/L + 30000 mg/L EDTA + 3000 mg/L HNO_3 ($p<0.05$). Jean *et al.* (2008) and Jing *et al.* (2015) findings both suggested that EDTA is the most effective means of increasing the uptake of Ni in plants. Chaney *et al*. (1998) proposed that EDTA and nitrilotriacetic acid (NTA) can increase bioavailability of nickel and other Elements. Many phytoextraction studies have been carried out using synthetic chelates such as EDTA and NTA in order to artificially enhance metals solubility, resulting in a marked increase in metal uptake by plants (Johnson *et al.*, 2009). According to Chen *et al* (2010), EDTA-promoted uptake of Cr in *Ipomonea aquatic*. From the result above the root accumulated the highest Ni content therefore it is probable that the formation of Ni–EDTA enhanced the mass transfer of Ni^{2+} ions to the root surface. Addition of varying amount of HNO_3 and Na_2EDTA decreased the pH significantly before and after harvest which facilitated the absorption of sufficient quantities of Ni^{2+} by the plant. Acidic pH increases the absorption of nickel in soil Zarkovic and Srdjan *(*2009). The plants were harvested at different times as they died. The control plants were the last to die. Those in other treatment died much earlier due to phytotoxicity effect of nickel. Nickel also induces visible toxicity in *S. oleracea*. The general symptoms of nickel toxicity were bleaching of leaf margin, chlorosis in leaves, browning of root tips and broken off roots. The toxicity symptoms in plants could be attributed to high accumulation of nickel in tissues (Saleh, 2002). It has been reported that nickel ion decrease the permeability of cell membrane inhibit root system development and cause necrosis and chlorosis (Pandey and Gautam, 2009).

The TF factor: TF decreased significantly ($p<0.05$) with increasing concentration of added Ni^{2+}. Thus, a small fraction of the Ni^{2+} was translocated to the shoots at 0 mg/L each of HNO_3 and EDTA (41.71%,35.01%,27.36%) at 500 mg/L each of HNO_3 and EDTA (52.59%,31.48%,22.47%) at 3000 mg/L each of HNO_3 and EDTA(47.80%, 24.32%,24.46%). The highest TF of unchelated hydroponic treatment was 41.71±0.27% at 1000mg/l while the chelated hydroponics treatment was 52.59±0.10% at 1000mg/L + 500mg/L EDTA + 500 mg/L HNO_3. From the results above EDTA aid the translocation of Ni^{2+} from the roots to the shoots of spinach Seedlings but most of the Ni^{2+} was retained in the roots. 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. For that, they suggested that EDTA was far more efficient in overcoming the diffusion limitation of metals to the root surface than the barrier of root to shoot translocation.

Effect of Pigment: The chlorophyll A, chlorophyll B, and carotenoid content decreased substantially with increasing Ni^{2+} concentrations ($P < 0.05$). The photosynthetic contents in unchelated hydroponic treatments were relatively higher than those in chelated treatments of same Ni^{2+} concentrations. The chelated hydroponic treatments showed the highest decrease of Chlorophylls A 4.68µg/g±0.24 Chlorophylls B 3.24µg/g±0.010 and caretenoids 1.75µg/g±0.001 at 4000mg/L Ni^{2+} +500mg/L EDTA +500 mg/L HNO_3 when compared to unchelated hydroponic treatment Chlorophylls A 6.64µg/g±0.11 Chlorophylls B 3.46. µg/g±0.08 and carotenoids 1.92µg/g±0.03 at 4000mg/L Ni^{2+}. Similar findings were reported by Pandey and Sharma (2002), who reported that concentration of chlorophyll a was more reduced than that of chlorophyll b in leaves of nickel treated cabbage. Vajpayee *et al* (2001) also reported greater inhibition in chlorophyll a than chlorophyll b following exposure to heavy metal (Cr) in submerged aquatic plants. Nickel inhibits chlorophyll biosynthesis by creating nutrient imbalances, replacement of Mg^{2+} ions (Molas, 2002).The decrease in photosynthetic pigments is due to the inhibition of the activities of enzyme that play important roles in the synthesis of these pigments, such as δ-aminolevulinic acid dehydratase and proto-chlorophylli-dereductase (Younis *et al.,* 2015).

Conclusion: The effect of nickel stresses were investigated and the results clearly indicated that application of various doses of Ni^{2+} to hydroponic solution enhanced the phytoextraction of Ni^{2+} in Spinach (*Spinacia Oleracea*) Seedlings. The different levels of nickel had negative effects on growth, chlorophylls A, B, and caratenoid and physiological attributes of Spinach plants.

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