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PHYSIOLOGICAL RESPONSES AND TOLERANCE EFFICIENCY OF *Spinacea oleracea* L. UNDER HYDROPONIC Ni^{2+} STRESS CONDITION.

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ABSTRACT
An indoor EDTA and HNO_3 enriched environment was created by an injection system with timing and varying concentration control. Spinacea oleracea was selected hydroponic plant exposed to various doses of Ni^{2+} (0, 1000, 2000 and 4000 mg/L) as Na_2EDTA at (0, 500 and 3000 mg/L) and (0,500 and 3000 mg/L) HNO_3 in different combinations for 6 days with 10-hour-treatment each day. This study used modified Hoagland nutrient culture in a screen house to provide an ideal environment for comparing the efficiency of chelate-assisted and unchelated phytoextraction of Ni^{2+} by S. oleracea. Changes in morphological characteristics including leaf damage rate to evaluate morphological resistance to Ni^{2+} uptake and proline contents was observed. Changes in fresh biomass were 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 proline accumulation in shoots increased significantly ($P < 0.05$) with increasing Ni^{2+} concentrations.

Keywords: proline content, fresh biomass, hydroponic, chelate, *Spinacea oleracea*.

INTRODUCTION

The micronutrients are indispensable for biogenesis, proper functioning of nucleic acid, chlorophyll, and hydro carbonates for stress resistance. Some heavy metals play an important role in plants cells as micronutrients (Rengel, 2004), while others have stimulating and harmful effects on plants even in trace concentrations (Nyitrai *et al*., 2007; Kovacs *et al.*, 2009). Nickel is essential for plant in low concentration but high concentration may be detrimental. Nickel is naturally occurring in soil, dust and surface water with concentration lower than 100 and 0.005 ppm, respectively (Chen *et al.*, 2009). Nickel is released to the environment from various anthropogenic activities, like metal mining, smelting, vehicle emission, fossil fuel burning, and disposal of household, industrial and municipal wastes, fertilizer and organic manures. Ni plays a significant role in transportation of nitrogen to seeds (Hussain *et al*., 2013). To estimate the stress induced (proline) to the seedlings by the uptake, accumulation of Ni^{2+} at different concentrations of Ni^{2+} and different concentration of EDTA and HNO_3 and observe changes in the fresh biomass of the *Spinacea oleracea.* The uptake of Ni in plants is carried out mainly by root systems via passive diffusion and active transport (Seregin *et al*., 2006). The ratio of uptake between active and passive transport varies with species, (Dan *et al*., 2002; Vogel-Mikus *et al*., 2005) Secondary active transport of chelated Ni^{2+} is possible, and corresponding proteins that specifically bind $Ni^{2+,}$ such as HoxN (high-affinity nickel transport protein, a permease) (Eitinger *et al*., 2000). The uptake of Ni by plants depends on Ni^{2+} concentrations (Chen *et al*., 2009). Soil pH values below 5.6 seem to favour the absorption of Ni and is largely due to the fact that exchangeable Ni content of soil increases with the increasing soil acidity (Sengar *et al*., 2008).

Spinacia oleracea commonly known as spinach, or "roundleaf spinage", is a staple of the early American vegetable gardens. It is a relatively quick-growing vegetable and easy to maintain. Spinach is in the classification system Family Amaranthaceae. *Spinacia oleraceae* being its official scientific classification name is an annual flowering plant that grows up to about 30 cm long (Kiple, 2002).

METHODOLOGY

Study Area

The *Spinacia oleracea* seeds were planted and raised for Eight weeks. The coordinates of the farm are latitude 8^0 22', to 9^0 25'N and longitude 11^0 57 to 12^0 00'E (figure 1).

Hydroponic procedure

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.

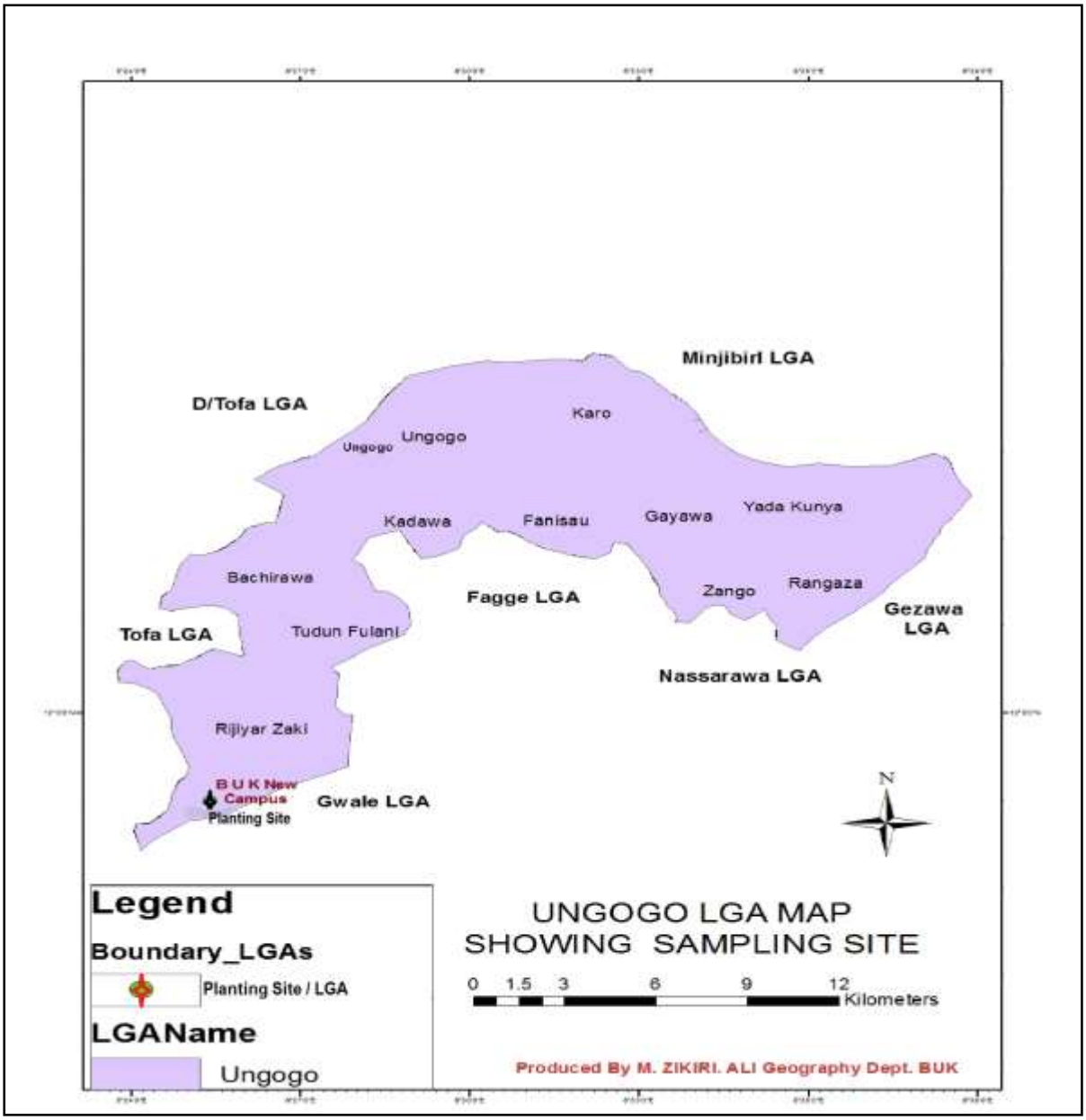

Figure 1: The map of Ungogo LGA showing planting site (New Campus Bayero University, Kano).

Translocation Factor (TF) was calculated as reported by Yoon *et al*. (2006).

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

Working Standard and Estimation of Proline

Proline standards of 0, 20, 40, 60 and 80 µg/ml were prepared by serial dilution of 0, 2, 4, 6, and 8 cm^3 of 100 µg/ml proline stock solution in 100 cm^3 volumetric flasks respectively. 0.5 g of fresh leaf was homogenized in 10.0 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman paper. 2.0 mL of the filtrate was then mixed with 2.0 mL of acid-ninhydrin and 2.0 mL of glacial acetic acid and heated at 100°C for 60 min. The reaction was terminated in an ice bath and 4mL of toluene was added to the mixture and contents of tubes were stirred for 20s (Gajewska *et al*., 2006). Absorbance of the pink red upper phase was recorded at 520 nm against toluene blank using

UV- visible spectrophotometer. The concentration of proline was determined from a standard curve and calculated on a dry weight basis as follows:

$$\textbf{Proline } [\mu g /g \textbf{ dry weight } (DW)] = \frac{(\mu g/mL \text{ proline} \times \text{vol.of toluene} \times \text{vol.of SS acid})}{(g/DW \times 115.5)}$$roline [µg /g dry weight (DW)](µg/mL proline × vol. of toluene × vol. of SS acid)/(g/DW × 115.5)

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

Change in Weight of Spinach Seedlings

The morphological parameters monitored in the different treatments include changes in the fresh biomass of all treatment. Figure 2 shows changes in weight of spinach seedlings before and after harvest.

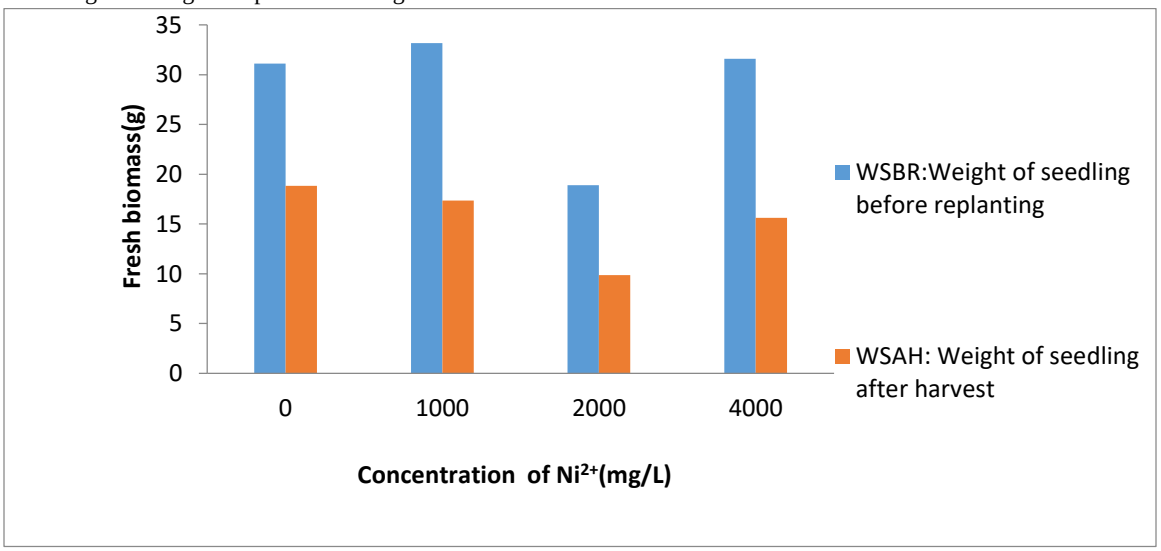

Figure 2: Effect of addition of 0.0 mg/L of HNO_3/Na_2EDTA on fresh biomass of *S. oleracea* Seedlings replanted in hydroponic mixture.

Application of different doses of Ni^{2+} significantly changed ($p<0.05$) the weights of *S. oleracea* seedlings. The highest value was obtained at 1000 mg/L Ni^{2+} which decreased when Ni^{2+} was increased to 2000 mg/L and then increased at 4000 mg/L. Increasing the concentration of Ni^{2+} from 0 to 1000 mg/L at constant concentration (500 mg/L) EDTA and HNO_3 (Figures 3 and 4) decreased the fresh biomass which gradually increased as Ni was increased to 2000 mg/L and decreases as Ni was increased to 4000 mg/L. Similar trend was observed when Na_2EDTA and HNO_3 concentrations was raised to 3000 mg/L

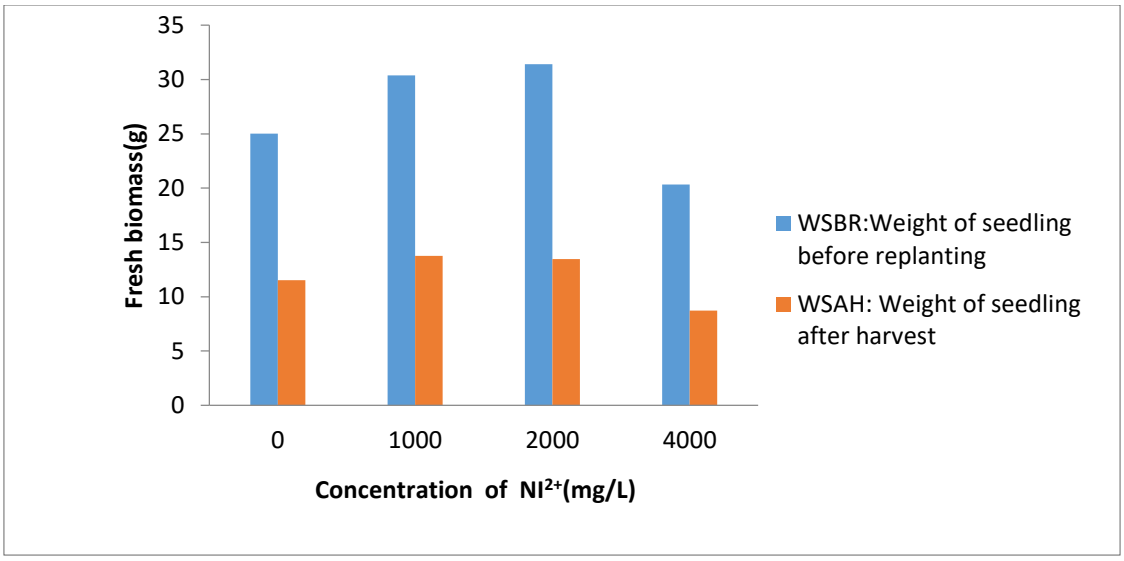

Figure 3: Effect of addition of 500 mg/L of HNO_3/Na_2EDTA on fresh biomass of *S. oleracea* Seedlings replanted in hydroponic mixture.

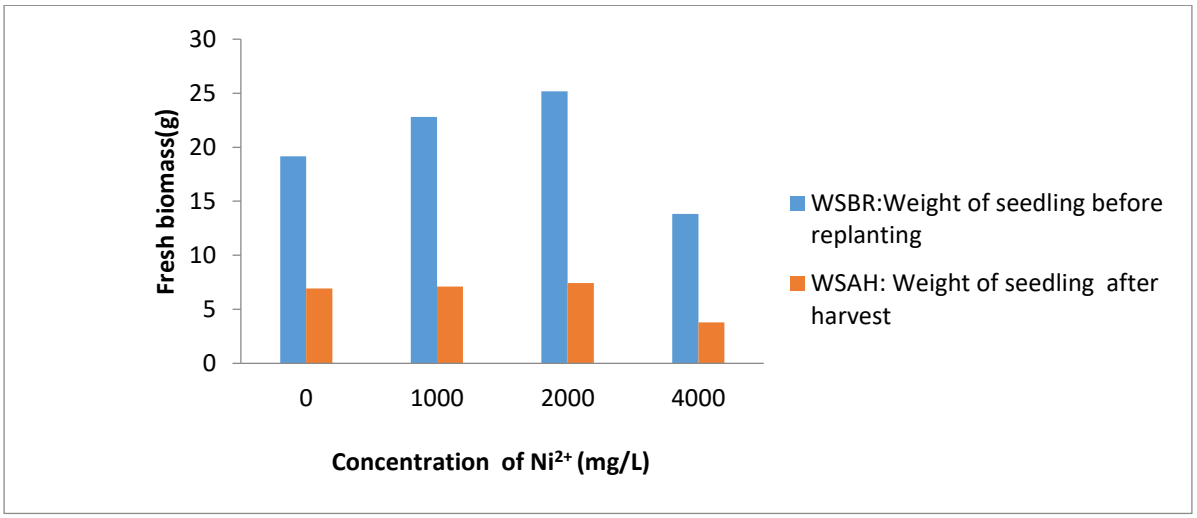

Figure 4: Effect of addition of 3000 mg/L of HNO_3/Na_2EDTA on fresh biomass of *S. oleracea* Seedlings replanted in hydroponic mixture.

Figure 5 shows the percentage decrease of fresh biomass of the *S. oleracea* Seedlings replanted in hydroponic solution. Application of different doses of Ni^{2+} ((0, 1000, 2000 and 4000mg/L) significantly decreased ($p<0.05$) the fresh biomass of *S. oleracea* Seedlings. In the absence of EDTA and HNO_3, the percentage of fresh biomass increased as nickel concentration increased from 0 to 1000 mg/L and steadily increases as Ni concentration increases to 2000 and 4000 mg/L respectively. Similar trend was observed when Na_2EDTA and HNO_3 concentrations was raised to 500 mg/L and 3000 mg/L respectively.

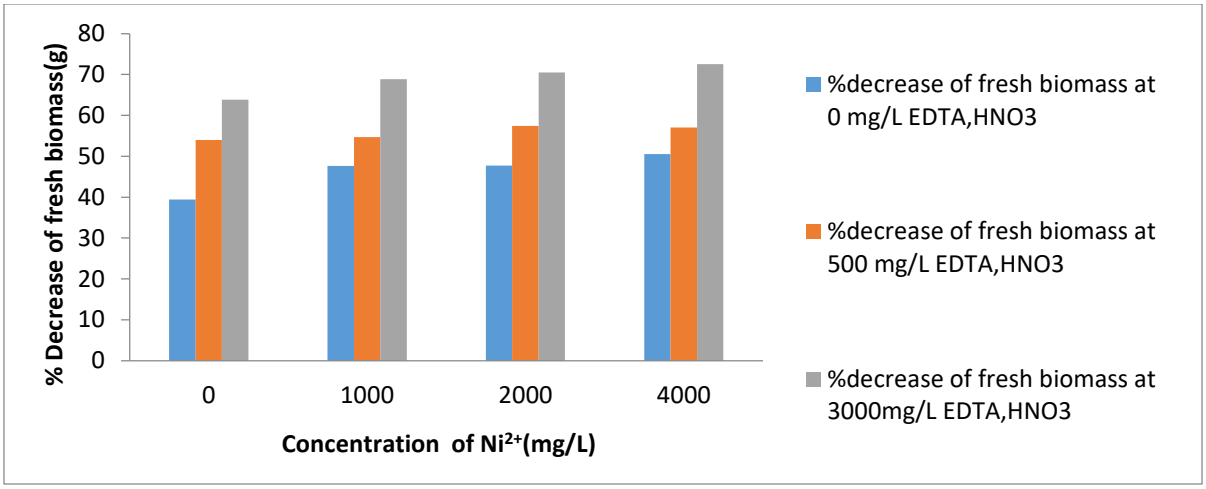

Figure 5: Percentage decrease of fresh biomass of *S. oleracea* Seedlings replanted in hydroponic mixture.

The changes in the fresh biomass were significant ($p < 0.05$) with respect to addition of EDTA and HNO_3. The change in biomass is dependent on the plant species and concentration of toxic metals in nutrient solution (Duman and Ozturk, 2009). In this work, a general decrease in plant weight was observed in all treatment including the control. Chelate-assisted phytoextraction is proposed as an effective approach for the removal of heavy metals from contaminated soil through the use of high biomass plants. The major problem hindering plant remediation efficiency is that some of the metals are immobile in soils and their availability and phytoextraction rates are limited by solubility and diffusion to the root surface (Tijana *et al*, 2010). Synthetic chelators, such as ethylenediamine tetraacetic acid (EDTA), have been used to artificially enhance heavy metal solubility in soil solution from the soil solid phase and thus to increase phyto-availability of heavy metals. The addition of chelators into the soil induces phytoextraction and translocation of heavy metals from the roots to shoots of plants (Meers *et al.*, 2008). The use of chelator is especially important for induced phytoextraction of nickel. This result is also supported with the findings of Giordan *et al* (2005) and Mamdouh *et al* (2014). The roots of plants act as a barrier against heavy metal translocation possibly as a result of potential tolerance mechanism (Singh and pandey, 2011).

Uptake in root and translocation in aerial parts may be supported with low translocation factor which showed great potential for phytostabilization of nickel in root. The heavy metal accumulation in root was more than the shoot in radish and spinach (Pandey, 2006). Vajpayee *et al* (2001) also reported high accumulation of heavy metal (Cr) in root of an aquatic plant (*Vallisneria spiralis L.*) than the shoot. Thus, these findings indicate that various levels of nickel influence uptake and translocation of nickel.

Proline Content of *S. oleracea* Seedlings

Proline was determined to assess the level of stress and damage caused by Ni^{2+} uptake in chelated and unchelated treatments to the plants. The proline content increased substantially with increasing Ni^{2+} concentrations ($P < 0.05$). Figure 6 showed proline content of spinach seedlings in the absence of Na_2EDTA and HNO_3. The proline content increases as nickel concentration is increased from 0 to 1000, 2000, and 4000 mg/L respectively.

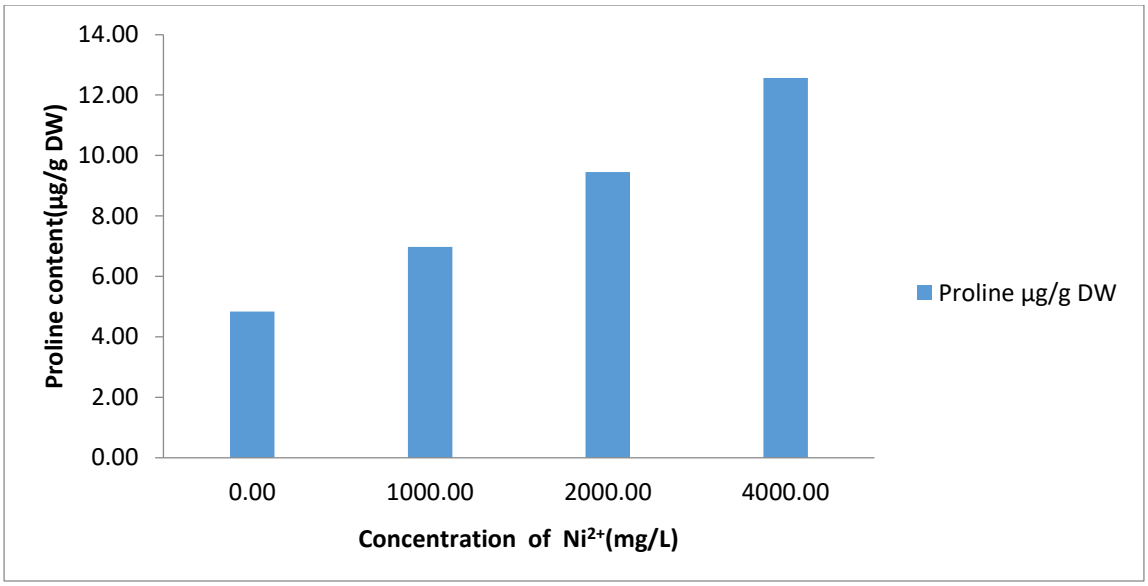

Figure 6: Effect of addition of 0.0 mg/L HNO_3/Na_2EDTA on proline content of *S. oleracea* Seedlings replanted in hydroponics mixture.

Application of Na_2EDTA and HNO_3 at constant concentration (500mg/L) in Figure 7 significantly increased ($p<0.05$) the proline content of the *S. oleracea* seedlings compared to treatment without Na_2EDTA and HNO_3. The highest proline content was observed at the application of 3000 mg/L Na_2EDTA and HNO_3 in Figure 3.2C compared to other treatment and control. The increase in the proline content was significant ($p<0.05$).

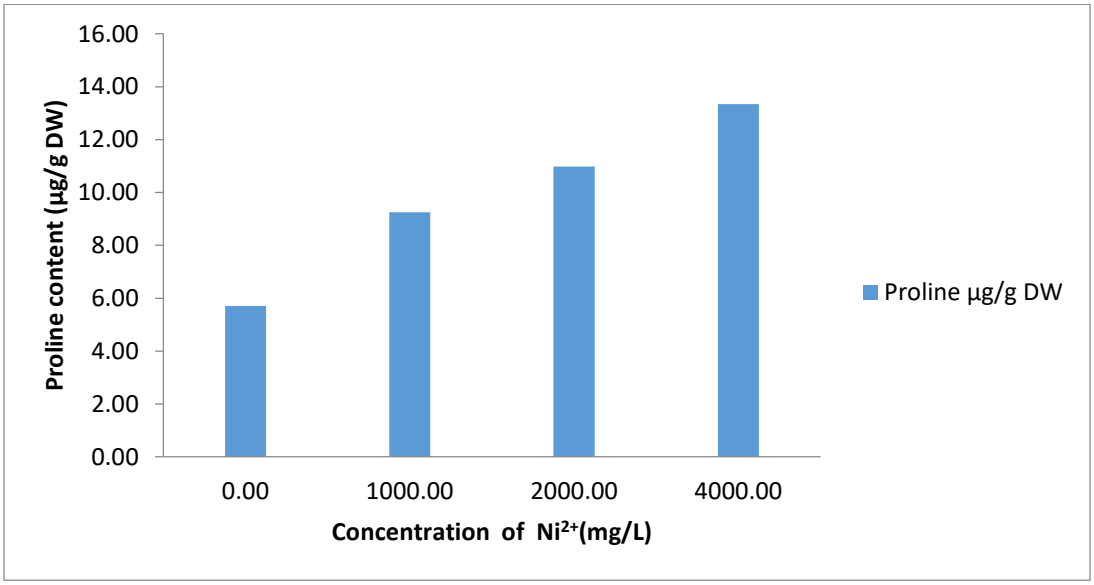

Figure 7: Effect of addition of 500 mg/L HNO_3/Na_2EDTA on proline content of *S. oleracea* Seedlings replanted in hydroponics mixture.

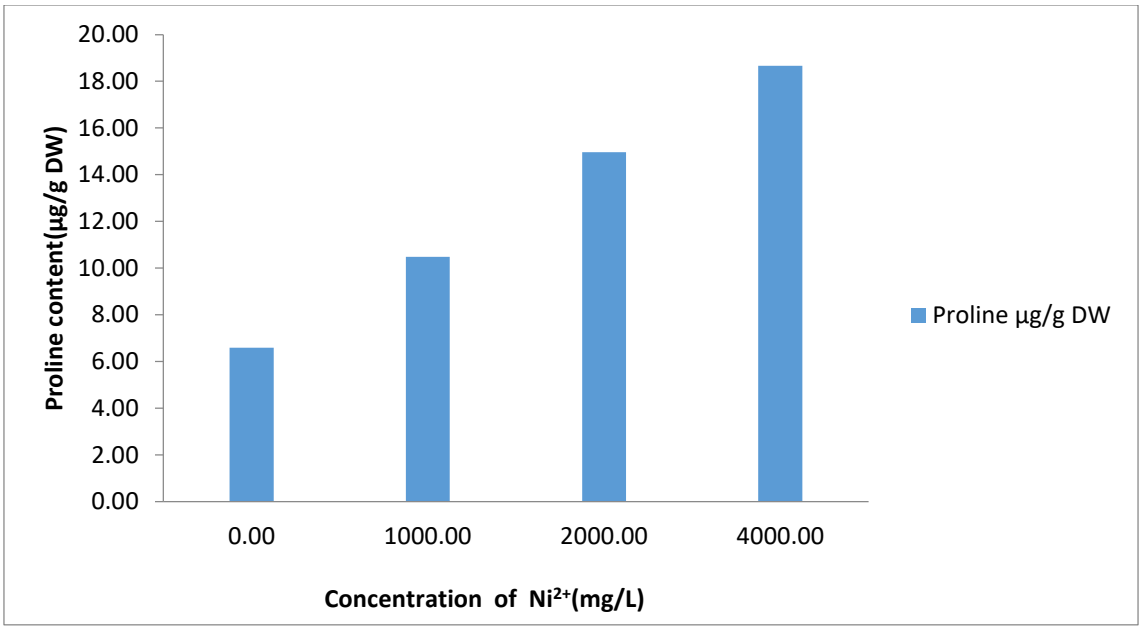

Figure 8: Effect of addition of 3000 mg/L HNO_3/Na_2EDTA on proline content of *S. oleracea* Seedlings replanted in hydroponics mixture.

CONCLUSION

Pollution of soil, water, and air due to heavy metals, is the end result of mostly man's activity as society continue to evolve. The cleaning of polluted soil can be achieved in different method. An alternative and save way is the use of plant that can uptake the accumulated heavy metals in their tissue. Hydroponic method is an easy and safe method of demonstrating the degree of phytoremediation which depends on other factors such as proline contents, fresh biomass and pigments.

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