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Effects of Nickel Toxicity on the Indices of Germination and Ca^{2+} ATPase Activity in Cowpea Plant (*Vigna unguiculata*)

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ABSTRACT: Despite the essential role in plants, toxicologists have considered Nickel (Ni) toxicity as an environmental threat to biological systems upon over-exposure. The study examined the phytotoxicity of Ni at 0, 50 and 100 ppm concentrations on fresh weight, length *and* growth rate of plant as well as leaf Ca^{2+} ATPase activity using Cowpea seedlings grown in contaminated soil for seven (7) days of exposure. The study revealed no significant alterations ($p > 0.05$) in the fresh weight (1.97±0.16 g and 1.42±0.22 g) of cowpea seedlings exposed to 50 and 100 ppm Ni-contaminated soil relative to the control vehicle (2.05±0.12 g). In each case, the mean length (9.12±0.88 cm), growth rate (53.75±0.45 %) and leaf Ca^{2+} ATPase activity (55.90±1.49 units/mg protein) of cowpea seedlings grown in soil samples treated with 100 ppm Ni showed marked significant decrease ($p < 0.05$) in relation to their controls, but these parameters measured in cowpea seedlings of soil samples treated with 50 ppm Ni showed no significant difference ($p > 0.05$) as compared with the control vehicles. Therefore, the phytotoxicity of Ni on the measured parameters was observed to occur significantly at 100 ppm with adverse effects on plant length, plant growth rate and leaf Ca^{2+} ATPase activity.

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Soil pollution by agricultural, industrial and other anthropogenic activities has placed human subjects at health risk in view of their positions along the food chain. Heavy metals, particularly nickel (Ni) compounds employed in the present study, are known soil contaminants with increasing toxicological concerns on the health status of exposed biological systems throughout the world today with the plants called "Producers" being the prime targets. A number of reports stated the hyperaccumulation of Ni in soil with increasing solubility and availability in the mobile form (Ni^{2+}) to plants under the influence of acidic pH, (Seregin and Kozhevnikova, 2006; Pandey and Gopal, 2010). Besides, Ni uptake by plants was reported to be positively influenced by its ionic strength, amount of metal compound in solution and phosphate content in soil. Here, a considerable increase in the levels of the mentioned factors causes a proportionate increase in the absorption of Ni into the plant with highest accumulation observed to occur in the root (Morrison *et al.*, 1980; Sengar *et al.*, 2008). However, soil organic matter and fertilizers considerably constrain the uptake of Ni and other heavy metals' contaminants by plants (Sengar *et al.*, 2008). Kabata-Pendias and Pendias (2001) highlighted that 0.1-0.5 ppm of Ni^{2+} ions available in soil to plants may bring about phytotoxic effects. Studies have shown that Ni interacts with Fe metabolism and other mineral nutrients with similar chemical disposition in plants to induce tissue necrosis, chlorosis, wilting and growth inhibition brought about by the diminution of these mineral nutrients (Pandey and Sharma, 2002; Kopittke *et al.*, 2007). On the other hand, cell division in the aerial parts of plants, especially the roots were known to be subjected to inhibition by elevated levels of Ni (Robertson and Meakin, 1980; Seragin *et al.*, 2001; Bhalerao *et al.*, 2015). Although, the mechanism of growth inhibitory response to Ni toxicity were not adequately supported, but number of researchers established that Ni toxicity at high levels significantly reduced the growth and development of plants: this was linked to a decreased state of cell wall plasticity (Bhalerao *et al.*, 2015). This empirical observation was further consolidated by the inhibitory function of the metal on the photosynthetic rate of plants underlined with reduced biosynthesis of chlorophyll pigment, interruption of chloroplast structure, retardation of enzymatic activities in Calvin pathway and overall crop yield respectively (Krupa and Baszynski, 1995; Molas, 1997). The toxicological assessment of Ni on plant respiration was considered at low and high levels, where it was underscored in earlier studies that low Ni levels stimulated respiration rate in the tissues of plants and elevated concentration

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of Ni competitively inhibited the associated enzymes of the electron transport chain in the mitochondria of plant tissues (Singh *et al.*, 2001; Sengar *et al.*, 2008). A toxicological report showed that Ni accumulation above the threshold limit inhibited both enzymatic and non-enzymatic antioxidant defense system thereby promoting oxidative stress in plants (Smeets *et al.*, 2005). This evidently suggested significant induction of reactive oxygen species (ROS) in plants with stimulated oxidation of biomolecules and breakdown of chlorophyll pigments (Rasmusson *et al.*, 2007; Bhalerao *et al.*, 2015). During Ni stress, studies have shown a significant suppression of superoxide dismutase (SOD) activity, but a significant enhancement of ascorbate peroxidase (APX) activity was observed in the leaves of wheat plant with a corresponding mop-up of hydrogen peroxide (H_2O_2) (Rao and Stresty, 2000; Gajewska *et al.*, 2009; Bhalerao *et al.*, 2015). Furthermore, the evaluation of Ni toxicity was known to indirectly decline the activities of some enzymes in plants through interference with nutrient uptake from soil samples. This observation was reported by El-Shintinawy and El-Ansary (2000) that the cultivation of *Beta vulgaris* plants in Ni-contaminated soil caused a reduced absorption of nitrate from the soil, which in turn suppressed the activity of nitrate reductase. A similar report showed that high levels of Ni in soil significantly diminished the activities of glutamate synthetase and alanine aminotransferase since their expressions were nitrate-dependent (Seregin and Ivanov, 20001). However, low levels of Ni in the soil may show no disruption of nutrient uptake into plant tissues, and this may in turn stimulate the activities of the aforesaid nutrient-dependent enzymes.

In studies relating to Ni toxicity, a variety of plants were employed for investigative effects of the metal on physiological, morphological, biochemical and molecular indicators (Sengar *et al.*, 2008; Pandey and Gopal, 2010; Bhalerao *et al.*, 2015). However, cowpea plant was carefully selected for the present study based on its rapid growth rate, frequent consumption by locals and potential sensitivity to Ni toxicity. Hence, the present study was aimed at assessing the effects of Ni toxicity on the indices of germination (weight, length and, growth rate of plant) and Ca^{2+} ATPase activity respectively.

MATERIALS AND METHODS

Chemical Reagents: All chemicals (trichloroacetic acid, sodium carbonate, ammonium molybdate, nickel sulphate, disodium adenosine triphosphate, ascorbic acid, sodium hydroxide, folin-ciocalteu reagent, hydrated copper (ii) sulphate, sodium potassium tartrate, common salt, hydrochloric acid and tricarboxylic acid) used in the present study were of analytical grade.

The experimental Plant: Cowpea seeds (*Vigna unguiculata L.*), popularly known as "Beans", were procured in a single batch from a local market around the environment of Delta State University, Abraka, Nigeria. These uninfected seeds used in the present study were sown in soil supplemented with nickel sulphate ($NiSO_4$) at graded concentrations for seven (7) days.

Preparation of Soil Samples, Cultivation of Seeds and Sampling: Humus soil was obtained and sieved to remove debris and other unwanted materials. Exactly 1.40 kg of soil samples were weighed each into sixteen black polythene bags. By randomized design, 200 ml of deionized water was applied to four of the polythene bags only as the control vehicle while 200 ml of 50 and 100 ppm, in each case, were used to contaminate four polythene bags per experimental set. Three cowpea seeds were cultivated per bag and water was added daily to keep the soil moist for 7 days. After the experimental period, the cowpea seedlings were harvested with their fresh weights, growth lengths and growth rates measured and recorded. Daily records of percentage germinations were taken and seeds, which failed to sprout were considered zero percent germinated Each seedling growth rate was measured after 7 days and the plant length from the soil level to the terminal bud. Also, 7 days old cowpea seedlings were uprooted, washed with clean water, dried and weighed immediately using precision electronic balance (Setra BL-4105). The leaves were further homogenized in pre-chilled mortar and pestle using normal saline, centrifuged at 15,000 g for 10 minutes and supernatants were collected for biochemical analysis.

Biochemical Analysis: In each case, total protein contents in samples were evaluated using the procedure stated by Lowry *et al.* (1951). On the basis of ATP hydrolysis in which inorganic phosphate (Pi) was complexed with molybdate ion in the presence of ascorbic acid, the activity of Ca^{2+} ATPase was monitored at 700nm using Thermo-Fischer spectrophotometer (G10SUV-Vis) according to the method described by Matsukama and Takiguchi (1981).

Statistical Appraisal: The data obtained in the present study were subjected to statistical analysis, where results were expressed as Mean ± SEM (=standard error of mean) for four and eight replications. Analysis of variance (ANOVA) was employed to ascertain the degree of mean significance difference using

GraphPad Prism 8.0 software. Thus, mean values were rated significant at $p < 0.05$.

RESULTS AND DISCUSSION

The toxicity of Ni is continuously evaluated by researchers, especially toxicologists, since the toxicant is readily available in all sections of the biosphere. Its ubiquitous nature was reported to embrace air, water and soil components of the biosphere with the capacity to bioaccumulate along the food chain, where it induces a broad spectrum of toxic effects on plants and animals at the cellular and molecular levels respectively (Cempel and Nikel, 2006). It was also established that excessive accumulation of Ni compounds in farmlands renders such environment unsuitable for agricultural activities such as the cultivation of arable crops (Duarte *et al.*, 2007). These developments necessitated this study to investigate the phytotoxic effect of Ni on the germination rate as well as its effect on Ca^{2+} ATPase activity in cowpea seedlings.

Biomass of Cowpea Seedlings: Figure 1 clearly explains the effect of Ni contaminated soil on the fresh weight of cowpea seedlings. There were no significant changes ($p > 0.05$) in the fresh weights of cowpea seedlings despite the increasing concentration of Ni from 50 to 100 ppm in relation to the control vehicle. Therefore, it was inferred that exposure to this toxicant at the said concentration and experimental setting may not cause any significant alteration in the weight of plants generally.

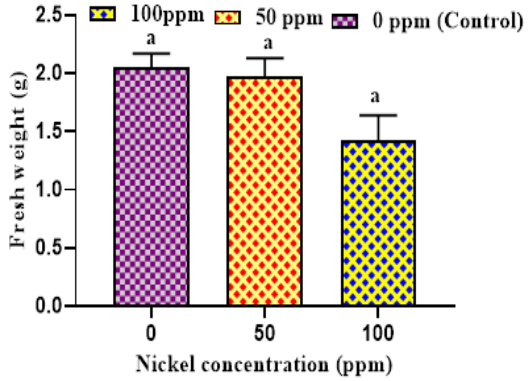

Fig 1: Effect of Nickel treated soil on fresh weight of cowpea seedlings. Each bar represents Mean ± SEM (n=4). *Bars with identical letter(s) were not significantly different ($p > 0.05$)

Germination of Cowpea Seedlings: The effect of increasing concentration of Ni contaminated soil on the growth Length of cowpea seedlings is clearly shown in Figure 2. It was observed that there was no significant impact ($p > 0.05$) on the growth length of cowpea seedlings grown in 50 ppm of Ni treated soil in relation to the control vehicle. However, exposure of cowpea seedlings to 100 ppm Ni contaminated soil provoked significant reduction ($p < 0.05$) in the growth length of the plants in relation to the control vehicle. Therefore, it may be drawn from the present study that 100 ppm of Ni compound may be toxic to the plant as it significantly reduced its length during the period of germination.

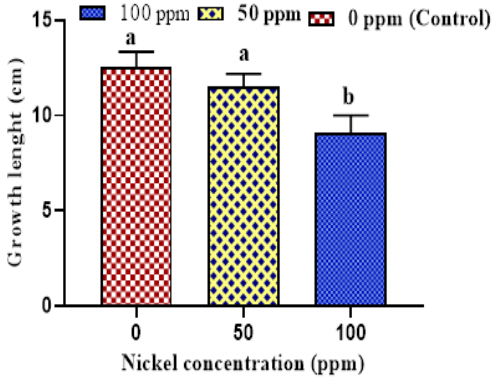

Fig 2: Effect of Ni treated soil on growth length of cowpea seedlings. Each bar represents Mean ± SEM (n=4). *Bars with different letters differ significantly ($p<0.05$)

The phytotoxicity of Ni was reported to cause a significant alteration ($p > 0.05$) in the biomass of plants (fresh and dry weights) at concentrations exceeding 150 µg/g of sample (Pandey and Gopal, 2010). The non-significant alteration ($P>0.05$) in the fresh weight of cowpea plants that was observed in the present study (Figure 1) at 50 and 100 ppm of Ni toxicity was contrary to the report presented by Khan and Khan (2010) that treatment of chickpea with 100-400 ppm of Ni caused a significant reduction ($p < 0.05$) in the biomass of the plant. However, the findings encompassing the non-significant effect of Ni toxicity on fresh weight relative to the control vehicle and regardless of dosage form correspond with the above set limit reported by Pandey and Gopal (2010). By implication, the non-significant effect of the metal on the biomass of cowpea plant may be associated with the fact that there was no significant alteration in the photosynthetic rate as well as nutrient utilization respectively. In addition, the significant reduction ($p < 0.05$) observed in the growth length of cowpea plant at 100 ppm of Ni treatment (Figure 2) suggested that Ni strongly interferes with the uptake of mineral nutrients responsible for elongation of the plant.

Growth Rate of Cowpea Seedlings: The growth rate of cowpea seedlings was investigated during exposure to varying concentrations (50 and 100 ppm) of Ni compound (Figure 3). The study showed that 50 ppm of Ni-contaminated soil indicated no significant effect ($p > 0.05$) on the growth of cowpea seedlings for four weeks, but there was marked significant decrease

($p < 0.05$) in the growth rate of the plant relative to the control vehicle at 100 ppm of Ni-contaminated soil. This also validated Ni toxicity at 100 ppm of exposure to the plant

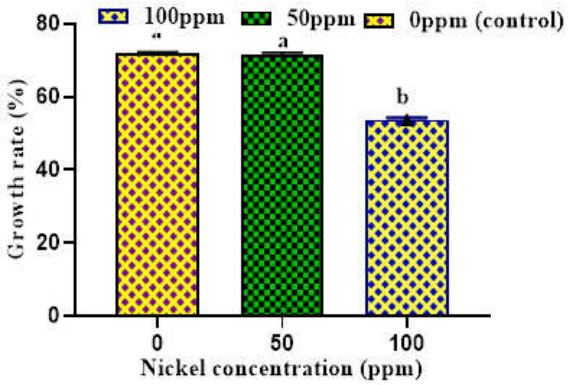

Fig 3: Effect of Nickel treated soil on the growth rate of cowpea seeds. Each bar represents Mean ± SEM (n=4). *Bars with different letters differ significantly ($p<0.05$)

The findings relating to inhibition of growth rate by exposure to 100 ppm Ni-contaminated soil (Figure 3) agreed with the report of Bhalerao *et al.* (2015) that growth inhibition was strengthened at toxic levels. The significant reduction in growth rate of cowpea seedlings observed in the present study may be attributed to the inhibition of biomolecule synthesis, enzyme activities such as protease and α-amylases activities, and utilization of food reserves (Maheshwari and Dubey, 2007; Ahmad *et al.*, 2009). Bhalerao *et al.* (2015) further highlighted that growth inhibition may be accounted for through significant reduction of cell wall plasticity brought about by enhancement of peroxidase activity for lignification process; this may be occasioned by the cofactor role of Ni during germination. Also, it was strongly stated that toxic levels of Ni may prevent the uptake of mineral nutrients such as K, Mg Zn and Fe needed for the germination of plants thereby resulting in stunted or reduced growth of plants (Barker, 2006; Ahmad *et al.*, 2007). In light of the above, the down-regulation of some enzymatic activities and other metabolic alterations were some of the reasons for the inhibitory growth response to Ni toxicity at levels, as observed in the present study.

Nickel Toxicity on Ca^{2+} ATPase Activity: The activity of Ca^{2+} ATPase in the leaves of cowpea seedlings exposed to Ni-contaminated soil at 50 and 100 ppm were monitored for four weeks (Figure 4). The data obtained clearly showed that 50 ppm Ni-contaminated soil showed no marked significant difference ($p > 0.05$) in the activity of Ca^{2+} ATPase relative to control vehicle, Contrary to the above, 100 ppm Ni-contaminated soil significantly inhibited ($p < 0.05$) the activity of Ca^{2+} ATPase in the leaves of Cowpea seedlings as compared with the control group.

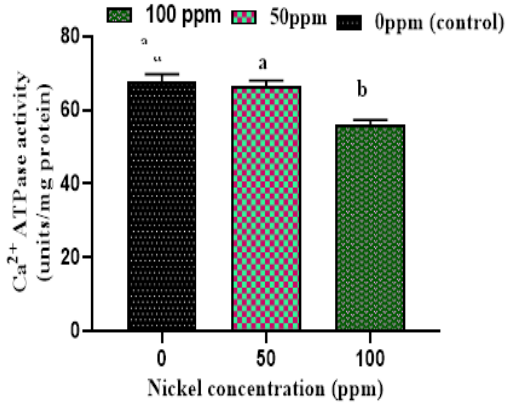

Fig 4: Effect of Nickel treated soil on Ca^{2+} ATPase activity in the leaves of cowpea seedlings. Each bar represents Mean ± SEM (n=8). *Bars with different letters differ significantly ($p<0.05$)

Ca^{2+} ATPase was reported as a classified p-type of transport protein with critical role in the transport and maintenance of homeostasis of Ca^{2+} ions in cells (Huda *et al.*, 2013). The transport process is energized by hydrolyzed ATP, which was commonly stabilized by Mg^{2+} ions. However, studies have shown that heavy metals at toxic levels competes with these essential mineral (Ca^{2+} and Mg^{2+}) ions for binding to the transport protein (Culotta *et al.*, 2005; Gatto *et al.*, 2007). Thus, in the present study, Ca^{2+} ATPase activity showed inhibitory response to high Ni concentration (Figure 4). The inhibitory response of the protein was associated with the competitive displacement of Ca^{2+} and Mg^{2+} from binding to their respective domains on the protein by the inactive redox metal (Ni), especially at 100 ppm. This further validated our findings that 100 ppm of Ni caused a significant reduction in cowpea growth rate in correlation with depleted levels of Ca^{2+} and Mg^{2+} ions in cells, and with subsequent hindrance to their functional role in the plant.

Conclusion: The present study emphasized that the effects of Ni toxicity was observed in Ca^{2+} ATPase activity, biomass and growth of cowpea plants cultivated in contaminated soil, which were explained at the morphological, physiological and biochemical levels respectively. Ni, which was reported to play an essential role in some enzymes at low level, was observed to be phytotoxic at high level to cowpea plants cultivated in contaminated soil. Therefore, sources of Ni contamination in soil must be strictly monitored to reduce its toxicity to plants.

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