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# THE TOXIC EFFECTS OF VARIOUS CONCENTRATION OF NICKEL ON SEED GERMINATION AND GROWTH OF COW PEA (*VIGNA UNGUICULATA* L. WALP

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## ABSTRACT

Nickel is available in the environment in various chemical forms due to geological, industrial and anthropogenic activities and accumulated in plants. Nickel is a trace element for normal plant growth. The high level of nickel effects germination and growth of plant. In this research, the treatment of various concentration of nickel (0.6, 1.2, 1.8 and 2.4mM) affected seed germination, seedling growth, root / shoot ratio and biomass of *Vigna unguiculata* L. Walp. Nickel treatment at all concentration produced less toxic effects on seed germination and fresh dry weight of cowpea. The seedling height, root growth and root / shoot ratio of cowpea at high concentration of nickel at 1.2 mM were significantly ($p<0.05$) decreased. Nickel treatment at 0.6 mM produced significant ($p<0.05$) reduction in dry weight of cowpea. The seedlings of *V. unguiculata* L. were also recorded for seedling vigor index and tolerance to nickel toxicity. The results showed that *V. unguiculata* seedlings has high seedling vigor index (S.V.I) and tolerance indices to nickel at 0.6 mM and lowest at 2.4 mM treatment.

**KEYWORDS:** Cowpea, nickel toxicity, seed germination, seedling growth.

## INTRODUCTION

The micronutrients are required at low concentrations for plant growth and at high concentrations are toxic (Krzesłowska, 2011; Shafiq and Iqbal, 2012). Nickel is a solid silver white hard transition element (PUBCHEM, 2018) and common uses are making stainless steel, coin and Jewellery. Nickel is an essential heavy metal element for normal plant growth (Chen *et al.*, 2009; da Silva *et al.*, 2012; Mazzafera *et al.*, 2013) but in excess quantity leads to toxicity to inhibition in seed germination, photosynthesis activities and plant growth (Eskew *et al.*, 1983; Parlak, 2016). Nickel is a naturally occurring element that found in water, soil, sediments and in the air (Kieling-Rubio *et al.*, 2012). Nickel is involved in metabolic processes, including ureolysis, hydrogen metabolism, methane biogenesis and acitogenesis (Mulrooney and Hausinger, 2003). The toxic effects of nickel treatment with different concentrations 0.025, 0.050.1 mM Ni as $Ni_2SO_4$, $6H_2O$) on root-meristem cell division in *Plantago lanceolata* (Plantaginaceae) seedlings was recorded (Pavlova, 2017).

Nickel in adequate quantities has a vital role in a large variety of physiological processes, from seed germination to productivity (Torres *et al.*, 2016). High levels of nickel can alter various metabolic activities of the plant such as the ratio of water and mineral nutrients, enzyme inhibition, functioning of the stomata, photosynthetic transport of electrons and degradation of chlorophyll molecules, consequently reducing the photosynthetic rate, growth and chlorophyll content and biological yield of plants (Bybordi and Gheibi, 2009; Yusuf *et al.*, 2011). The excess exposure is toxic for germination, root growth, seedling growth, development and metabolism in crops and higher plants (Pandolfini *et al.*, 1992; Baccouch, *et al.*, 2001; Pandey and Sharma, 2002; Gajewska *et al.*, 2006; Seregin and Kozhevnikova, 2006; Yılmaz, 2007; Gajewska and Sklodowska, 2010). Influence of nickel on nitrooxidative stress in seedlings of Basil (*Ocimum basilicum* L.) recorded (Georgiadou *et al.*, 2018).

The influence of abiotic stress due to high level of toxic pollutants on growth of plants available in scientific literature. The presence of pollutants may affect the germination and seedling growth directly or indirectly due to interaction of heavy metals (Szollosi, 2014). However, plant under stress condition is most likely to be adversely affected by high concentrations of these toxic elements. Plants have a unique role for the existence of all heterotrophic organisms (Kralova and Masarovieova, 2006).

Pulses are the most important source of vegetable protein in Pakistan. The popular legume grain is cultivated on 5% of the total cropped area. Among pulses, *Vigna unguiculata* is a major pulse crops grown in the country (P.A.R.C., 2018). *V.ungu*. L.Walp, its common name is cowpea. The Cowpea (*Vigna unguiculata*) (L.) Walp.) is an annual herbaceous legume crop, cultivated for its edible seeds or for fodder and originated from Central Africa (Feedipedia, 2018). High variation in plant height, branches per plant, pods per plant, grain yield, biomass and harvest index of cowpea during tracking agronomic diversity collected from 31 districts of three provinces across Pakistan was recorded (Iqbal *et al.*, 2017).

Plants are an important part of living organisms. The ecologist are researching on the impact of heavy metals on plant growth since last few years. A significant increase of nickel in the environment is resulting nickel pollution problem. The objective of the study was to assess the toxicity and tolerance of nickel on seed germination percentage and early seedling growth performance of an important annual and warm season bean crop cowpea V.unguiculata (Fig. 1) successfully cultivating in Pakistan.

## MATERIALS AND METHODS

The healthy and certified seeds of bean crop cowpea (*Vigna unguiculata* L. Walp.) were obtained from the local market. In order to analyze the nickel toxicity for seed germination and early seedling growth, a Petri plate experiment was conducted in laboratory with four replications including one control. The petriplates were arranged in complete randomized block design. The petri plates having 9 cm diameter size thoroughly washed and sterilized in autoclave. Ni solution medium was prepared by dissolving $NiSO_4.6H_2O$ into distilled water. The treatment with 0 mM nickel was consider as control. The seeds were surface sterilized with 0.25 of sodium hypchlorite (NaOCl) for one minute to prevent any fungal contamination and placed on Whatman No. 42 filter paper. The cowpea seeds imbibed for 30 minutes in sterilized distilled water to overcome any type of dormancy. The seeds were exposed to different ($NiSO_4.6H_2O$) concentrations ranging 0.0 mM, 0.6 mM, 1.2 mM, 1.8mM and 2.4 mM salt into distilled water.

There were four different concentrations (0.6, 1.2, 1.8 and 2.4 mM) of nickel used. Ten seeds of cowpea were placed on the filter paper in petriplates and moistened. Initially with 5 ml of $Ni^{+2}$ respective treatments. The control was also moistened with equal amount of distilled water. During the experiment, the average range of minimum and maximum temperature and relative humidity were in between 26.5 to 39 ºC and 55 to 74%, respectively. After emergence of seedlings, all the petri plates were kept in light. The solution of petriplates was replaced on alternate days with 3 ml of $Ni^{+2}$ solution. To assess the effect of nickel on seedling growth the samples of seedling were collected after seven days. The seed germination, root, shoot, seedling length, seedling fresh and dry weight, root / shoot ratio, seedling vigor and metal tolerance index were recorded. The seedlings were dried at 80 ºC for 48 hours in oven and then the dried weight of root and shoot were taken of the corresponding samples.

Seedling vigor index (S.V.I.) was determined as per the formula given by Bewly and Black (1982).

$$\text{Metal tolerance index} = \frac{\text{Mean root length in } Ni^{+2} \text{ treatment}}{\text{Mean root length in control treatment}} \times 100$$ (Iqbal and Rehmati, 1992)

## STATISTICAL ANALYSIS

The data were statistically analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test using personal computer software packages SPSS version 14.0.

## RESULTS AND DISCUSSION

Nickel is a heavy metal and getting global concern due to its nature of toxicity impact on plant growth at higher concentration. In this study seed germination, root, shoot and seedling length, fresh weight, dry weight, root / shoot ratio and seedling vigor index performance of (*Vigna unguiculata* L. Walp.) were carried in different concentrations (0, 0.6, 1.2, 1.8 and 2.4 mM) of Nickel (Fig.1 - 4).

Metals at high concentrations are toxic for living organisms (Oukarroum, 2016). Several reports indicates that plants exposed to metal at high concentration induced toxicity and showed inhibition of germination and growth. In the present study, nickel toxicity showed an important factor governing seed germination of V. unguiculata (Fig. 1). Seed germination was highly decreased as the nickel concentration increased upto 2.4 mM in the medium. Similarly, $Ni^{2+}$ treatment affected all indexes of germination and growth stage of *Brassica napus* L. reported by Yang and Zhao (2013). Nickel treatments caused a considerable significant reduction ($p<0.05$) in all seedling growth parameters as compared to control. This reduction was found less at 0.6 mM Ni treatment, as compared to 1.2 mM, 1.8 mM, and 2.4 mM nickel concentrations, respectively. There was a significant ($p<0.05$) reduction in the seedling growth including root and shoot of cowpea was recorded with increasing concentrations of $Ni^{+2}$ at 1.2 mM in the medium. Omoregie (2010) suggested that the seedling growth retardation could be related due to decrease in cell enlargement and division. Furthermore, the toxic effects of Ni on germination and plant growth might be due to its interference with other essential metal ions and induction of oxidative stress (Chen *et al.*, 2009). The nickel sulfate solutions treatments at 200 ppm highly affected the seed germination percentage, seedling growth, dry matter yield and changes in biochemical contents of total sugar, protein and pigments of *Abrus precatorius* L. (Vyasm, 2017). Root growth is an important growth variable and found highly affected by different concentrations of Ni treatment. The results showed that Ni treatment at 0.6 mM produced the significant effect on root length of *V. unguiculata* as compared to control. An increase in concentration of Ni treatment at 1.2 mM was found responsible for significant reductions in shoot and seedling length of *V. unguiculata*. The reduction in the root length of *V. unguiculata* with the increase in concentration of nickel in substrate provides further evidence that the nickel in excess may be inhibitory to plant growth and development. Toxic metal ions enter cells by means of the same uptake processes as essential micronutrient metal ions. Patra *et al.*, (2004) concluded that excessive concentrations of metals result in phytotoxicity through changes in the permeability of the cell membrane and replacement of essential ions. The roots has the ability to supply water and nutrients to the plants, regulate the growth and performance of root, and shoot (Blackman and Davies, 1985). The phytotoxicity of nickel treatment at 2.4 mM showed damaging effects on shoot length as compared to control. The results of this investigation have shown that nickel treatment found more toxic for shoot than root development of cowpea.

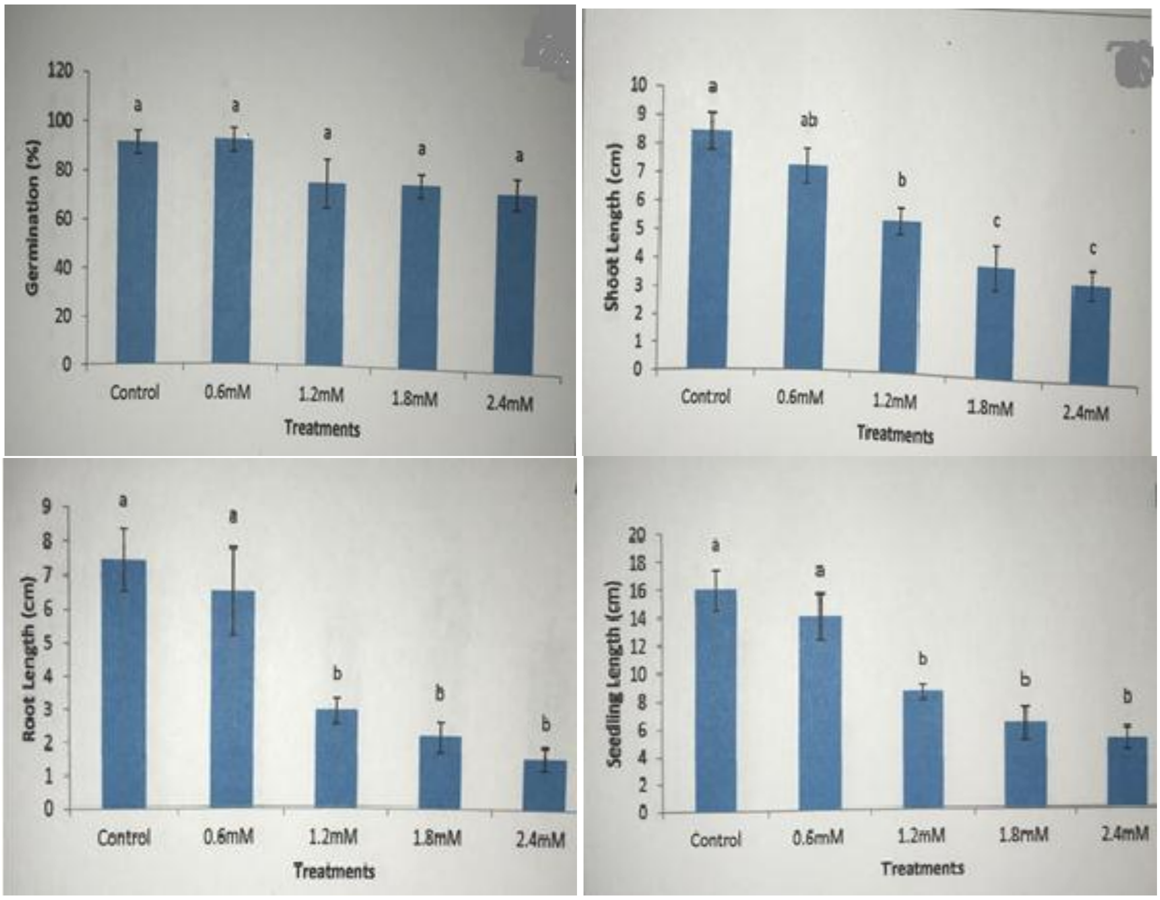
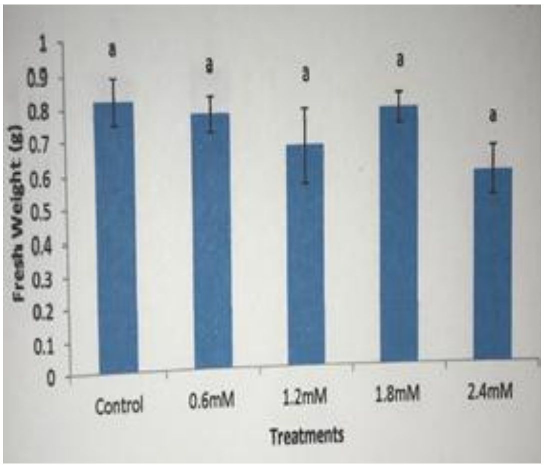



**Fig. 1: Effects of different concentrations of nickel (Control 0.0 Mm, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on seed germination (%), shoot, root and seedling length (cm) of *V. unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test.**

The results of nickel toxicity on seedling fresh and dry weight of cowpea shown in (Fig. 2). The changes in seedling dry weight of *V. unguiculata* seedlings using varying concentrations of nickel were observed. The effects of increasing level of nickel on fresh and dry weight of cowpea was more. Ni treatment at all concentration decreased fresh and seedling dry weight of *V. unguiculata*. Nickel treatment at 0.60 mM concentration was less toxic for biomass production of cowpea. Ni treatment at 1.2 mM concentration further decreased the seedling dry weight of *V. unguiculata* as compared to control. The significant decrease in seedling fresh and dry weight of *V. unguiculata* due to metal toxicity of nickel was also observed. Malan and Farrant, (1998) examined the toxic effects of nickel chloride in nutrient solution markedly reduced plant and biomass and seed production of soybean (*Glycine max*).

The treatment of nickel in *V. unguiculata* provided evidence that the trace element in nutrient medium if present in excess may be inhibitory to plant growth and development especially at more than 2.4 mM. Bauddh

(2015) also recorded the effect of increasing level of nickel in the range of 25 to 150 mg Ni $kg^{-1}$ on biomass of cow pea .Toxicants accumulate in the plant when soluble forms are present in high quantities. In another investigation, The presence of increasing Ni concentrations (0.0004–0.08 mM) significantly elevated wheat root biomass. At the same time, the lower Ni doses (0.0004 and 0.04 mM) did not change markedly, but the highest dose of this element used (0.08 mM) decreased shoot biomass (Matraszek *et al.*, 2016).



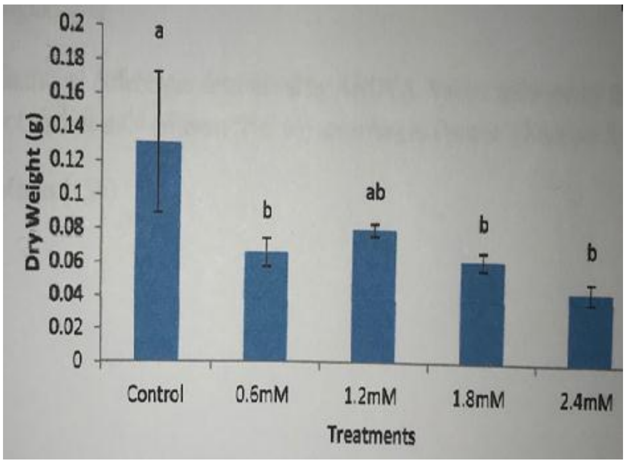



**Fig. 2: Effects of different concentrations of nickel (Control 0.0 mM, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on fresh and dry weight (g) of *V. unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.**

Ni treatment at all concentration gradually decreased root/ shoot ratio of *V. unguiculata*. Nickel treatment at 0.60 mM concentration was less toxic for root / shoot ratio. Ni treatment at 1.2 mM concentration decreased the root / shoot ratio of *V. unguiculata* as compared to control. Nickel treatment at 2.4 mM produced significant (p<0.05) impact on root / shoot ratio of *V. unguiculata*. The root / shoot ratio of *V. unguiculata* was decreased gradually with increasing the nickel concentrations upto 2.4 mM in a dose dependent manner. Ni 50 µM treatment inhibited root and coleoptile length with significant reduction in chlorophyll 'a' and chlorophyll 'b' of *Zea mays* (Negi, 2016).

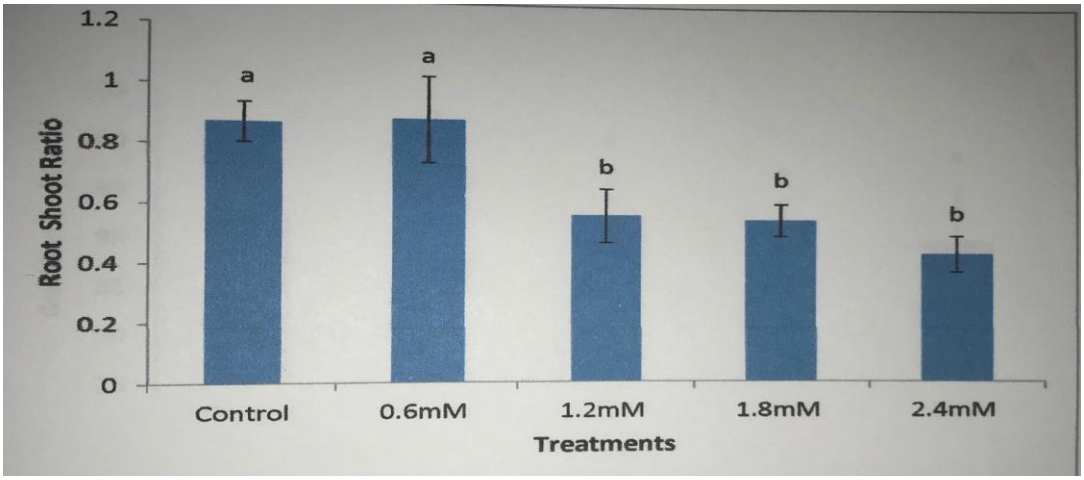



**Fig. 3: Effects of different concentrations of nickel (Control 0.0 mM, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on root / shoot ratio of *V. unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.**

High percentage of decrease in seedling growth of cowpea at 1.8 mM and 2.4 mM nickel treatment provided evidence that the treatment of nickel in excess is inhibitory to plant growth and development. The permeability of metals can decreased the seedling vigor and tolerance indices of cowpea. The seedlings of *V. unguiculata* were tested for the establishment of seedling vigor index (SVI) to different level of nickel treatment (Fig. 4). The results showed that *V. unguiculata* has high tolerance to nickel at 0.60 mM (86.66%) and lowest at 0.6 mM (20.00%) of Nickel. *V. unguiculata* seedlings showed better percentage of tolerance (40.00 %) to nickel at 1.60 mM. The seedlings of *V. unguiculata* were tested for the establishment of seedling vigor index (S.V.I.) to different level of $Ni^{+2}$ treatment. The results showed that *V. unguiculata* has greater seedling vigor index was (1440) to nickel at 0.0 mM and lowest (400) at 2.40 mM of nickel. *V. unguiculata* seedlings showed moderate S.V.I. (1260) at 0.60 mM nickel treatment. *V. unguiculata* showed better SVI (480) to nickel treatment at 1.80 mM.

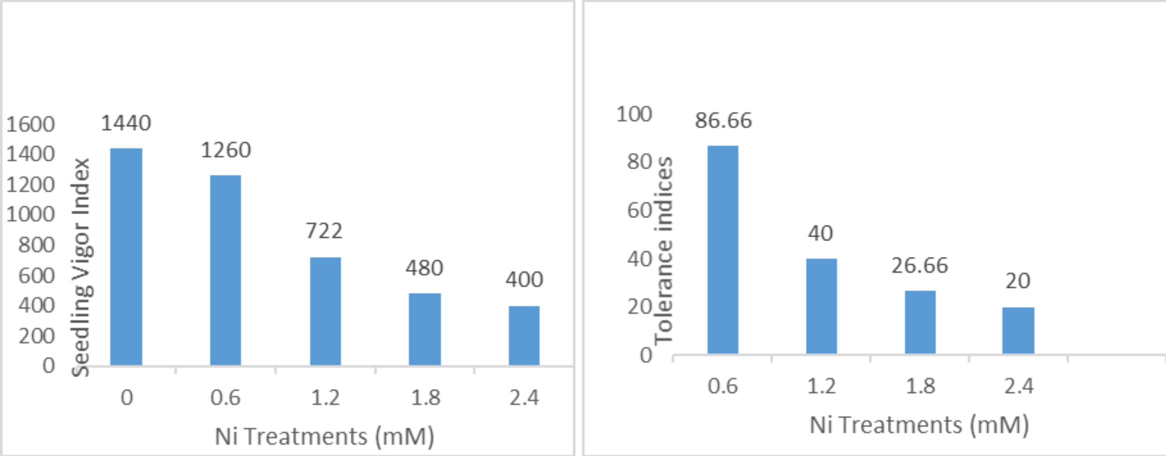



**Fig. 4: Effects of different concentrations of nickel (0,0 mM, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on seedling vigor index (S.V.I.) and metal tolerance indices of cowpea V. unguiculata (L.) Walp.**

## CONCLUSION

It was concluded that the low concentration of nickel 0.6 mM showed less toxic effects on seed germination of cowpea. However, high concentration of nickel 2.4 mM showed toxic effects on these germination and seedling growth parameters of cowpea. The ever increase in concentration of nickel in the environment suggests that, with the passage of time, Ni polluted can influence more prominently on seedling growth performance. The other seedling growth parameters of cowpea including root and shoot showed tolerance up to 0.6 mM $Ni^{+2}$ concentration. $Ni^{+2}$ treatment at 1.8 and 2.4 mM concentrations showed significant ($p<0.05$) decreasing trend in seedling dry weight of cowpea. It was also concluded that $Ni^{+2}$ treatment at 2.4 mM was detrimentally lethal effects for seedling growth of cowpea as compared to 0.6, 1.2 and 1.8mM, respectively. The seedlings of cowpea were able to survive and tolerate nickel toxicity upto 2.4mM and can be used for the Ecotoxicological studies and for monitoring in nickel-contaminated areas.

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## CONFLICTS OF INTEREST STATEMENT

There is no such conflict exists among the authors.

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