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Physiological and biochemical changes in *Vigna unguiculata* (L.) Walp. due to nickel stress

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Abstract

Environmental pollution due to industrial, mining and agricultural activity as well as transportation, leads to production of high amounts of contaminants like heavy metals into surface water and soils which ultimately leaches to ground water and also affects the biosphere. In the present investigation, germination study was conducted in cowpea [*Vigna unguiculata* (L.) Walp.] In order to find out the changes in germination, different developmental stages in terms of its growth, physiological and biochemical alteration due to nickel stress. The seeds of cow pea were germinated in variable nickel concentrations ranging from 0-100 mg/l of nickel. In another experiment conducted in pot culture revealed that, parameters the growth parameters and different physiological and biochemical parameters were decreased whereas only free proline content were increased with increase in concentration of nickel. It was also observed that seeds of cow pea showed better result in terms of growth and different physiological and biochemical parameters in 20 ppm of nickel at different interval of days like 10th, 20th and 30th day of growth of seedling thereby indicating that nickel within 20mg/kg could facilitate the plants growth and it is subsequently affect the seedling when it exceeds from 20 mg/kg of nickel in soil.

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Introduction

Dispersal of industrial and urban waste generated by human activities is a major environmental concern which causes the contamination of soil. The surface deformation, caused by mining and surface construction damage, is becoming a problem. This is drawing more and more attention to underground mining causing surface deformation (Wang, 2005; Zhang, 2013). Contaminations of agricultural soils due to trace metal in agricultural soil are major environmental problems today (Chand *et al.*, 2012). Uptake of metals by plants can have strong adverse impact on both plants as well as animals through the food chain (Sadon *et al.*, 2012). There are thirty five metals responsible for occupational and residential exposure among which twenty three are heavy metals. Heavy metals can include elements lighter than carbon and can exclude some of the heaviest metals (Duffus, 2002).

Nickel is just one of a variety of ubiquitous trace metal emitted to the environment from both natural and anthropogenic sources. Sukinda is the only indigenous resources of Nickel ore of India which produces the lateritic nickel ore (Sahoo, 1998). Nickel is considered as a micronutrient for plants as it is required at very low concentration by plants (Brown *et al.*, 1987).

According to International Agency for Research on Cancer, Nickel is reported as one of the toxic heavy metals and it is also considered as human carcinogens (IARC, 1990). As Nickel is one of the heavy metal pollutants, it is of interest to study the effect of nickel on alteration of growth and development along with different physiological and biochemical parameters of cow pea (*Vigna unguiculata*).

Materials and methods

Vigna unguiculata (L.) Walp. Is commonly called cow pea and a popular vegetable plant particularly in tribal rich pockets of the states of Odisha. Seeds of cow pea were obtained from Orissa University of Agriculture and Technology, Bhubaneswar to be used as the experimental material. Seeds of cow pea were surface sterilized with 0.1% mercuric chloride and washed thoroughly with tap water and then with distilled water. 100 uniform seeds of cow pea were germinated in petridish with different nickel concentration and it was incubated for five days and then number of germinated seeds were counted and percentage of germination were calculated. Seedling vigour indices (Baki and Anderson, 1973) were also calculated before the fresh plants samples were kept in an oven at 60 ^{0}C for 48 hours to measure the dry weight.

Besides, experiment was carried out in pot culture in which seeds were sown in pre-treated soil treated with different concentration of nickel ranging from 20 mg/kg to 100 mg/kg with a nickel less soil and different growth and biochemical parameters like total chlorophyll content (Arnon, 1949), total protein content (Lowry *et al.*, 1951) and free proline content (Bates *et al.*, 1973) were estimated in plants at 10, 20 and 30 days after treatment. All the experiments were done in triplicates and the data was statistically analyzed and standard error of mean (SEM) was calculated.

Results and discussion

The data presented in Table1 revealed that the germination percentage was decreased at higher concentration of nickel and this reduction at higher concentrations may be attributed to the interference of nickel ions during the process.

The seedling vigour index were increased at lower concentration and decreased at higher concentration, along with the significant decrease in radicle length of cow pea seedling indicated that low concentration of nickel was beneficial for the growth of cow pea seedling whereas at higher concentrations nickel stress suppressed the seedling growth.

The growth parameters and percentage of moisture content were found to be better in 20 ppm concentration which further showed decrease trend with increasing concentration of nickel (Table 2, 3 and 4).

Table 1. Effect of different concentrations of Nickel on seed germination, radicle length and Seedling Vigour Index of *Vigna unguiculata* (L.) Walp. After 5 days of treatment.

Treatments	Germination Percentage	Radicle Length (cm)	Seedling Vigour Index
Control	99±0.4653	2.34±0.1043	231.66
20ppm	85±1.9786	1.4±0.9978	119
40ppm	60±2.5648	0.96±0.6542	57.6
60ppm	55±3.2565	0.92±0.0976	50.6
80ppm	15±5.6040	0.32±0.0458	4.8
100ppm	5±5.9896	0.14±0.0754	0.7

Table 2. Effect of different concentrations of Nickel on root length, shoot length and percentage of moisture content of *Vigna unguiculata* (L.) Walp. Seedling after 10 days of treatment.

Treatments	Root Length (cm)	Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Moisture content of Shoot (%)	Root Fresh Weight (g)	Root Dry Weight (g)	Moisture content of Root (%)
Control	12.4 ± 0.864	11.6 ± 0.786	1.987 ± 0.096	0.350 ± 0.074	82.373	0.635 ± 0.061	0.122 ± 0.006	80.645
20ppm	12.9 ± 0.974	12.2 ± 0.976	2.145 ± 0.076	0.367 ± 0.043	82.876	0.669 ± 0.043	0.126 ± 0.005	81.098
40ppm	11.8 ± 0.768	10.1 ± 0.769	1.832 ± 0.093	0.316 ± 0.042	82.725	0.564 ± 0.051	0.121 ± 0.002	78.569
60ppm	9.8 ± 0.765	9.3 ± 0.895	1.326 ± 0.265	0.299 ± 0.063	77.463	0.405 ± 0.011	0.104 ± 0.004	74.348
80ppm	5.3 ± 0.897	6.2 ± 0.512	0.604 ± 0.652	0.156 ± 0.009	74.114	0.245 ± 0.008	0.073 ± 0.003	69.675
100ppm	4.1 ± 0.765	5.7 ± 0.056	0.264 ± 0.564	0.070 ± 0.005	73.485	0.184 ± 0.006	0.059 ± 0.002	67.695

Similarly different physiological and biochemical parameters studied in the investigation were also found to be beneficial in 20 mg/kg of soil and decreased with the gradual increase in concentration of nickel (Fig. 1 & Fig. 2). Besides, the free proline content in the cow pea seedlings was found to be increased with increasing concentration of nickel (Fig. 3).

Table 3. Effect of different concentrations of Nickel on root length, shoot length and percentage of moisture content of *Vigna unguiculata* (L.) Walp. Seedling after 20 days of treatment.

Treatments	Root Length (cm)	Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Moisture Content of Shoot (%)	Root Fresh Weight (g)	Root Dry Weight (g)	Moisture Content of Root (%)
Control	17.432 ± 0.986	16.743 ± 0.869	2.968 ± 0.085	0.629 ± 0.012	78.796	0.854 ± 0.096	0.200 ± 0.008	76.569
20ppm	18.257 ± 0.998	17.357 ± 0.840	3.157 ± 0.078	0.647 ± 0.013	79.513	0.898 ± 0.084	0.202 ± 0.009	77.462
40ppm	13.835 ± 0.865	14.457 ± 0.564	2.868 ± 0.043	0.630 ± 0.009	78.042	0.501 ± 0.036	0.125 ± 0.008	74.987
60ppm	10.721 ± 0.658	12.527 ± 0.536	1.965 ± 0.023	0.454 ± 0.008	76.897	0.386 ± 0.039	0.108 ± 0.007	71.976
80ppm	7.276 ± 0.668	10.472 ± 0.487	0.986 ± 0.011	0.256 ± 0.008	73.985	0.306 ± 0.041	0.096 ± 0.007	68.549
100ppm	5.163 ± 0.402	9.364 ± 0.398	0.475 ± 0.014	0.135 ± 0.006	71.532	0.197 ± 0.008	0.067 ± 0.003	65.986

The results of the present study indicated that different concentrations of nickel stress could affect seed germination in *Vigna unguiculata* plants. Being the most crucial stage of plant development, the germination of seeds can be used as an indicator of early response of the plants in adverse environmental condition (Singh *et al*., 2006). During germination Ni inhibits all cellular processes (Hall, 2002) thus, slow down growth of plumules and radicles. Similar inhibition of germination at higher concentrations was observed by Mahalakshmi and Vijayarengan (2003) with cobalt treatment in *Vigna mungo* (L.) Hepper. But the results obtained from the germination studies of *Vigna unguiculata* showed higher seedling growth and dry weight at 40 mg/kg nickel level in the soil.

Table 4. Effect of different concentrations of Nickel on root length, shoot length and percentage of moisture content of *Vigna unguiculata* (L.) Walp. Seedling after 30 days of treatment.

Treatments	Root Length (cm)	Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Moisture Content of Shoot (%)	Root Fresh Weight (g)	Root Dry Weight (g)	Moisture Content of Root (%)
Control	21.765 ± 1.254	20.564 ± 1.013	3.31 ± 0.841	0.768 ± 0.084	76.768	0.931 ± 0.082	0.245 ± 0.008	73.657
20ppm	22.953 ± 1.302	22.247 ± 1.154	3.965 ± 0.437	0.850 ± 0.064	78.546	0.978 ± 0.063	0.248 ± 0.009	74.548
40ppm	16.478 ± 0.897	18.357 ± 0.954	3.013 ± 0.654	0.722 ± 0.059	76.021	0.754 ± 0.027	0.210 ± 0.008	72.104
60ppm	12.932 ± 0.876	16.368 ± 0.967	2.932 ± 0.138	0.656 ± 0.008	73.983	0.401 ± 0.008	0.121 ± 0.006	69.853
80ppm	8.731 ± 0.432	14.362 ± 0.758	1.460 ± 0.095	0.423 ± 0.008	70.967	0.322 ± 0.009	0.107 ± 0.007	66.674
100ppm	5.912 ± 0.451	11.591 ± 0.657	0.564 ± 0.041	0.176 ± 0.006	68.752	0.205 ± 0.002	0.075 ± 0.001	63.028

The values of growth parameters indicated that nickel had a significant stimulating and nutritional effect at 20 mg/kg concentration which is beneficiary for plant growth and beyond 40 ppm concentration adverse impact on seedling growth was observed.

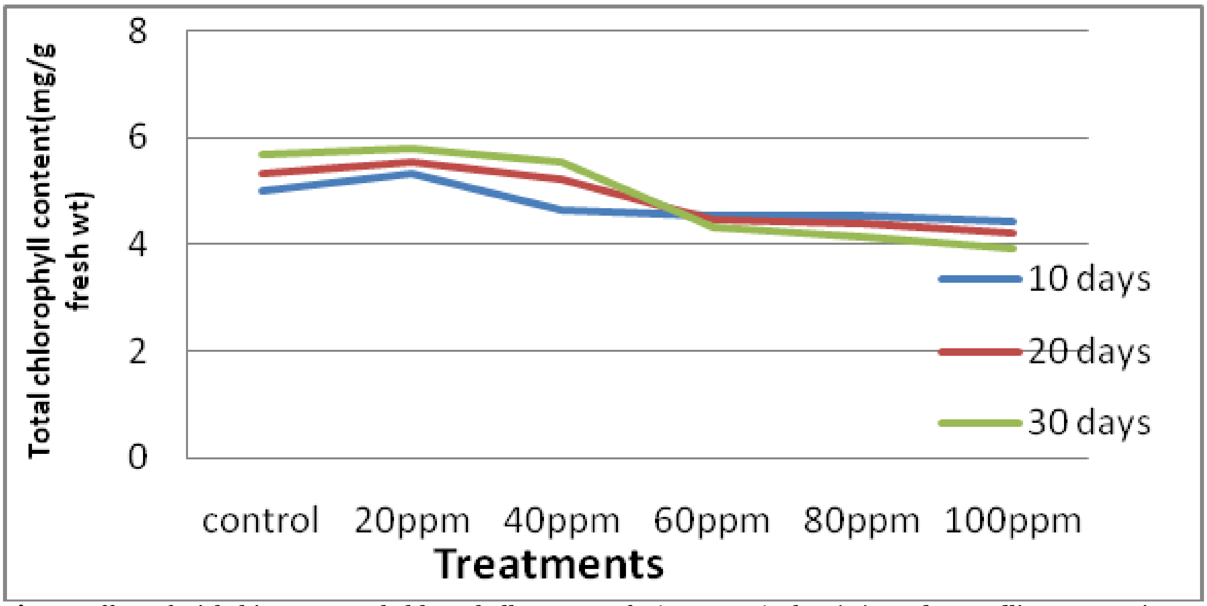

Fig. 1. Effect of Nickel ions on total chlorophyll content of *Vignaunguiculata*(L.) Verdc. seedlings grown in pot culture experiments.

The growth parameters above this concentration indicated that even a little excess of nickel over these levels had an adverse effect. From the result of this investigation, it can be concluded that nickel at lower concentration has a stimulating effect on the germination process and seedling growth of *Vigna unguiculata* and higher concentrations of nickel could inhibit the same. Similar results were reported on the effect of cadmium on *Triticum aestivum* (Kalita *et al.*, 1993), chromium on *Salvia sclarea* (Corradi, 1993) and cobalt and zinc on *Pennisetum americanum* L. and nickel stress on *Macrotyloma uniflorum* (Lam.) Verdc (Arzoo *et al.*, 2014).

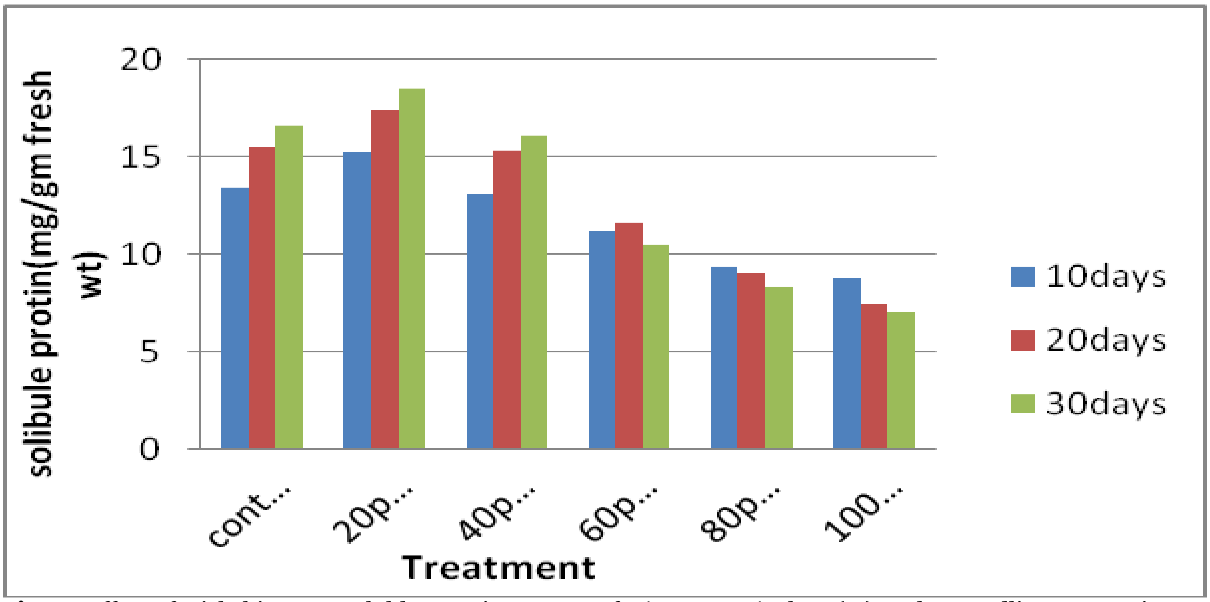

Fig. 2. Effect of Nickel ions on soluble protein content of *Vigna unguiculata* (L.) Walp. Seedlings grown in pot culture experiments.

In a study it was reported that the application of egg shell waste enhances the plant growth (Biswal *et al.*, 2019). Different physiological and biochemical changes also reported in different studies like impact of Cr+6 toxicity in plants (Pati *et al.*, 2014) and nickel toxicity in *Arachis hypogea* L. (Arzoo and Satapathy, 2015).

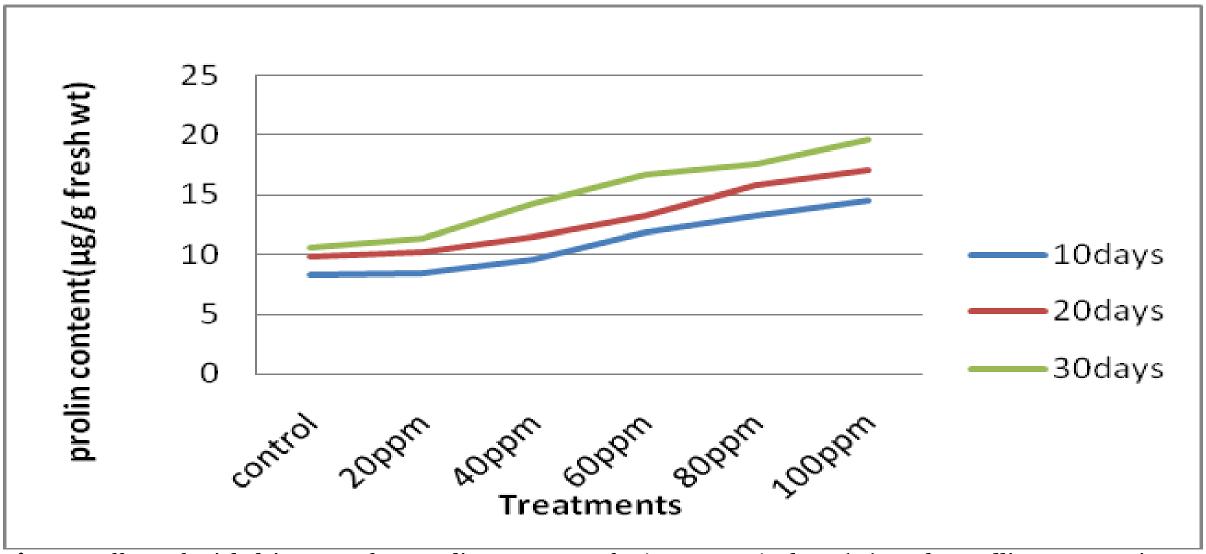

Fig. 3. Effect of Nickel ions on free proline content of *Vigna unguiculata* (L.) Walp.seedlings grown in pot culture experiments.

Conclusion

The alteration in growth and biochemical parameters of the seedling indicated that nickel had a significant stimulating nutritional effect up to 20 mg/kg concentration for *Vigna unguiculata* (L.) Walp., and all the physiological and biochemical parameters were affected adversely above this level of concentration of nickel. From the result of this investigation, it can be concluded that nickel at lower concentration had a beneficial effect on germination and growth of the test plant which was inhibited at higher concentrations. So, it can be utilized for supplement as micronutrient with controlling the limit within 20 ppm.

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