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Physiological and biochemical responses induced by Nickel to *Arachis hypogea* L.

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Abstract

An experiment was conducted in groundnut [Arachis hypogea L.] to find out the effect of nickel on germination, growth and biochemical parameters. The seeds of groundnut were germinated in six different concentrations of Nickel chloride solution having 0-100 mg/l of nickel. It was observed that seedling vigour index, metal tolerance indices were reduced and the percentage of phytotoxicity was increased. The pot culture experiment revealed that, the growth parameters and the percentage of moisture content of in plant were decreased with increase in concentration of nickel and in contradiction, seedlings showed better result in terms of growth in 40ppm of nickel at 10th, 20th and 30th days thereby indicating that Nickel within 40mg/kg had stimulating effect on the seedling growth. Similarly the total chlorophyll content, total soluble protein content were decreased but free proline content was increased with increase in concentration of nickel at 10th , 20th ,30th and 100th days after treatment. So, it could be concluded that Nickel at lower concentration had an inducing effect on plant growth and inhibited the same at higher concentrations.

Keywords: Nickel, groundnut, germination, growth parameters, biochemical parameters.

Introduction

Trace metals contamination of agricultural soils has become one of the most significant environmental problems today[1]. Metal contamination of agricultural soils by atmospheric deposition or by disposal of sewage sludge constitutes a risk of either leaching of metals into the ground water or excessive accumulation in top soil. Metal uptake by crop plants can have strong adverse impact on human health through the food chain[2]. There are 35 metals that concern us because of occupational and residential exposure; 23 of these are the heavy elements or "heavy metals". Heavy metal can include elements lighter than carbon and can exclude some of the heaviest metals[3]. Nickel is just one of a variety of ubiquitous trace metal emitted into the environment from both natural and anthropogenic sources. The compounds such as nickel acetate, nickel carbonate, nickel hydroxide and nickel oxide are used in a variety of industrial process. These compounds ultimately accumulate in the soil and environment, and can be easily taken up by plants. Thus they can enter into the food chain and cause deleterious effects to animals and human.

Nickel (Ni) is a micronutrient required at very low concentration by plants[4]. It is one of the toxic heavy metals, and is recognized for its negative effects on the environment where it bio-accumulates and poses a serious threat to human and environmental health. All nickel compounds, except for the metallic nickel, have been classified as human carcinogens by International Agency for Research on Cancer[5]. As Nickel is an important heavy metal pollutant, it is of interest to study its effect on the germination and seedling growth of groundnut (*Arachis hypogea* L.), an important major oil seed crop of Odisha.

The present investigation assesses the extent of Ni bio-accumulation in groundnut plant (*Arachis hypogea* L. var. Smruti). Groundnut is the major oilseeds crop accounting for 45% of oilseed area and 55% of oilseeds production of the country.The selected variety of the groundnut has a lifespan of about 100days. The experiment was carried out in pot culture experiments under nickel stress at variable concentrations. The objective of the study was mainly to assess the phytotoxic impacts of different concentration of nickel in growing groundnut plant (*Arachis hypogea* L. var. Smruti) with periodical investigation at its developing stages (10, 20, 30days) and at matured stage (100days) of Ni treated plants. The phytotoxic effects of varying concentrations of nickel on the growth, development and biomolecular changes in groundnut plants supplemented to increasing concentrations of nickel was assessed.

Material and Methods

Seeds of groundnut were obtained from Orissa University of Agriculture and Technology (O.U.A.T), Bhubaneswar. The present study was undertaken with Nickel (Nickel Chloride) at 20, 40, 60, 80, and 100 mg Ni/L along with control (untreated). Seeds of groundnut were surface sterilized with 0.1% of mercuric chloride and washed thoroughly with tap water and then with distilled water. Uniform sized seeds were placed in petridishes of 10 cm with different concentrations of Nickel Chloride solution (20, 40, 60, 80 and 100 mg Ni/L) and one with control at a constant temperature of 26ºC. The seeds were submerged in 10 ml of test solutions and distilled water twice a day. Each treatment was replicated five times. The number of seeds germinated in each treatment was counted on 5 days after sowing (DAS) and the total germination percentage was calculated. Tolerance index[6] and vigour index of seedlings were calculated[7]. The root and shoot length of seedlings in various nickel levels were measured on 10th days after showing (DAS). The plant samples were kept in an oven at 60ºC for 24 hours and the dry weights were taken by using electronic balance.

In pot culture experiment seeds were shown in pretreated soil (soil treated with 20,40,60,80 and100 mg Ni/kg of soil with one control i.e. nickel less soil) and different growth parameters and biochemical parameters like total chlorophyll content[8], total soluble protein content[9] and free prolin content[10] were observed in plants at 10, 20, and 30 and 100 days after treatment (DAT).

All the experiments were done in triplicates and the data were statistically analyzed and standard errors of mean (SEM) was calculated.

Results and Discussion

Germination studies: There was concentration dependant decrease in number of seed germinated for nickel treatment in *Arachis hypogea* L. Least number of seed germination was noticed in treatments of 100ppm. The effect of nickel on germination, seedling vigour index and metal tolerance index are presented in table-1.

Effect of Nickel on growth parameters: The comparative changes in the growth parameters were observed in *Arachis hypogea* L. plants of 10, 20, 30 days old (developing stage) grown in pot culture treated with different concentrations of nickel (0ppm, 20ppm, 40ppm, 60ppm, 80ppm and 100ppm). Some of the salient features of the present investigation are described as follows.

The root and shoot length also increased remarkably with increase in Ni^{2+}concentrations up to 40ppm but deteriorated at higher concentrations. The root and shoot length of the seedlings treated with 40ppm was the highest as compared to all other Ni^{2+}treatments whereas it was minimum in 100ppm-Ni^{2+} treatment. The percentage of moisture content gradually decreased with increase in the concentrations. However, the seedlings treated with 40 ppm of nickel had maximum moisture content in 10, 20 and 30 days old and in 100 ppm plants resulted in least moisture content.

Effect of Nickel on Biochemical parameters of *Arachis hypogea* L.: The comparative changes in total chlorophyll content, and biochemical alterations (soluble protein content and free proline content) were observed in *Arachis hypogea* L. plants of 10, 20, 30 days old (developing stage) grown in pot culture treated with different concentrations of nickel (0ppm, 20ppm, 40ppm, 60ppm, 80ppm and 100ppm) described in figure-1, 2 and 3.

Total chlorophyll contents were decreased with increase in concentration after 10, 20, 30 days of treatment on the plants. At 10 days after treatment it was observed that the total chlorophyll content were decreased as 3.301 to 2.269 mg/g fresh wt. in control and 100ppm respectively. Similarly at 20 days after treatment it was observed that the total chlorophyll content were decrease as 3.350 to 2.486 mg/g fresh wt. in control and 100ppm respectively and also at 30 days after treatment it was observed that the total chlorophyll content were decrease as 4.348 to 2.797 mg/g fresh wt. from control to 100ppm respectively. Similarly in samples of100 days after treatment, it was observed that the total chlorophyll content was lowered to2.405from 4.148 mg/g fresh wt. in control to 100ppm respectively.

Table-1
Effect of Nickel on seedling vigour index, metal tolerance index and percentage of phytotoxicity on 5 days after treatment of the *Arachis hypogea* L

Treatments	Germination Percentage	Radicle length (in cm)	Seedling Vigour Index	Metal Tolerance Index	PercentageofPhytotoxicity
Control	99±0.8944	5.54±0.1299	548.46	100	0
20 ppm	99±1.4142	3.02±0.1506	298.98	54.512	45.487
40 ppm	99±1.6733	0.89±0.1368	88.11	16.064	83.935
60 ppm	99±2.0000	0.74±0.1437	73.26	13.357	86.642
80 ppm	99±2.0000	0.23±0.0334	22.77	4.151	95.848
100 ppm	60±3.1622	0.20±0.0066	19.8	3.610	96.389

Values of 5 replicates ± SEM

Table-2
Effect of Nickel on root length, shoot length and percentage of moisture content on10 days after treatment on *Arachis hypogea* L

Treatments	Root Length (in cm)	Shoot Length (in cm)	Shoot Fresh Weight (in gram)	Shoot Dry Weight (in gram)	Moisture Content (in %)	Root Fresh Weight (in gram)	Root Dry Weight (in gram)	Moisture Content (In %)
Control	17.1± 0.87	13.4± 0.56	7.414±0.032	1.039 ± 0.01	85.985	3.820 ± 0.02	0.802 ± 0.002	79.005
20 ppm	18.2 ± 0.54	13.5 ±0.61	7.805 ± 0.02	1.074 ± 0.01	86.231	3.916 ± 0.07	0.786 ± 0.006	79.928
40 ppm	18.6 ± 0.57	13.9 ±0.09	7.585 ± 0.04	0.996 ± 0.09	86.868	3.825 ± 0.04	0.768 ± 0.004	79.921
60 ppm	13.2± 0.67	10.8 ±0.08	6.154 ± 0.01	0.935 ± 0.01	84.806	2.575 ± 0.02	0.672 ± 0.004	73.902
80 ppm	9.6 ± 0.86	8.4 ± 0.05	3.927 ± 0.02	0.806 ± 0.02	79.432	1.862 ± 0.01	0.548 ± 0.003	70.569
100 ppm	5.1 ± 0.07	5.6 ± 0.04	3.115 ±0.07	0.684 ± 0.01	78.037	0.926 ± 0.01	0.302 ± 0.005	67.386

Values of 5 replicates ± SEM

Table-3
Effect of Nickel on Root length, Shoot Length and Percentage of Moisture content of 20days of *Arachis hypogea* L.plants

Treatments	Root Length (in cm)	Shoot Length (in cm)	Shoot Fresh Weight (in gram)	Shoot Dry Weight (in gram)	% of Moisture Content	Root Fresh Weight (in gram)	Root Dry Weight (in gram)	% of Moisture Content
Control	19.69 ±0.874	15.43 ± 0.56	3.141 ± 0.032	0.586 ± 0.006	81.343	0.163 ± 0.021	0.033 ± 0.002	79.754
20 ppm	21.32 ±0.549	16.12 ± 0.61	3.183 ± 0.021	0.593 ± 0.013	81.369	0.166 ± 0.072	0.033 ± 0.006	80.120
40 ppm	21.85 ±0.576	16.52 ± 0.09	3.157 ± 0.043	0.579 ± 0.009	81.659	0.164 ± 0.043	0.032 ± 0.004	80.487
60 ppm	15.46 ±0.675	11.12 ± 0.08	2.964 ± 0.012	0.568 ± 0.013	80.836	0.146 ± 0.021	0.030 ± 0.004	79.452
80 ppm	12.08 ±0.867	10.34 ± 0.05	2.589 ± 0.009	0.524 ± 0.020	79.760	0.135 ± 0.011	0.029 ± 0.003	78.518
100 ppm	9.24 ± 0.076	8.61 ± 0.04	2.254 ± 0.007	0.499 ± 0.004	77.861	0.062 ± 0.009	0.020 ± 0.005	67.741

Values of 5 replicates ± SEM

There was a gradual decrease in protein content with rise in different levels of concentrations due to Nickel stress. Here protein content was produced in maximum amount when plants are subjected at treatment of 20ppm and least amount when plants are subjected at treatment of 100ppm. at 10, 20, and 100 days after treatment. The total protein content (mg/g fresh wt) was decreased with increased concentration if nickel such as: 40 ppm – 4.972, 60 ppm – 4.743, 80 ppm – 3.648 and 100 ppm – 3.110 whereas the control was 5.448 but there was slightly increased in 20 ppm – 7.059 and after 20 days of treatment, it was also decreased with increased concentration of nickel such as: 40 ppm – 5.339, 60 ppm – 4.632, 80 ppm – 3.562 and 100 ppm – 3.358 whereas the control was 6.079 but a slight increase was resulted in 20 ppm – 8.468. In 30 days after treatment, it was also decreased with increased concentration of nickel such as: 40 ppm – 8.486, 60 ppm – 4.708, 80 ppm -3.835 and 100 ppm – 3.660 whereas the control was 8.587 but a slight increase in 20 ppm – 10.932. Similarly after 100 days of treatment, it was also decreased with increased concentration of nickel such as: 40 ppm – 15.738, 60 ppm – 11.268, 80 ppm – 7.034 and 100 ppm – 6.678 whereas the control was 16.134 but a slight increase was resulted in 20 ppm – 18.698.

Table-4
Effect of Nickel on Root length, Shoot Length and Percentage of Moisture content of 30days of *Arachis hypogea* L. plants

Treatments	**Root Length (in cm)**	**Shoot Length (in cm)**	**Shoot Fresh Weight (in gram)**	**Shoot Dry Weight (in gram)**	**% of Moisture Content**	**Root Fresh Weight (in gram)**	**Root Dry Weight (ingram)**	**% of Moisture Content**
Control	22.46± 0.98	17.26± 1.04	6.872± 0.089	1.239± 0.008	81.970	2.351± 0.006	0.470± 0.008	80.00
20 ppm	25.46± 0.94	18.16± 0.85	8.119± 0.088	1.450± 0.008	82.140	3.266± 0.008	0.625± 0.007	80.863
40 ppm	25.92± 0.85	18.68± 0.65	7.385± 0.076	1.266± 0.007	82.857	2.529± 0.004	0.486± 0.009	80.782
60 ppm	20.34± 0.76	15.08± 0.59	4.334± 0.053	0.934± 0.009	78.449	1.573± 0.007	0.385± 0.005	75.524
80 ppm	18.98± 0.83	13.66± 0.43	3.167± 0.009	0.716± 0.006	77.391	1.365± 0.006	0.338± 0.006	75.238
100 ppm	14.58± 0.26	14.58± 0.45	2.742± 0.006	0.674± 0.003	75.419	0.608±0.004	0.204± 0.004	66.440

Values of 5 replicates±SEM

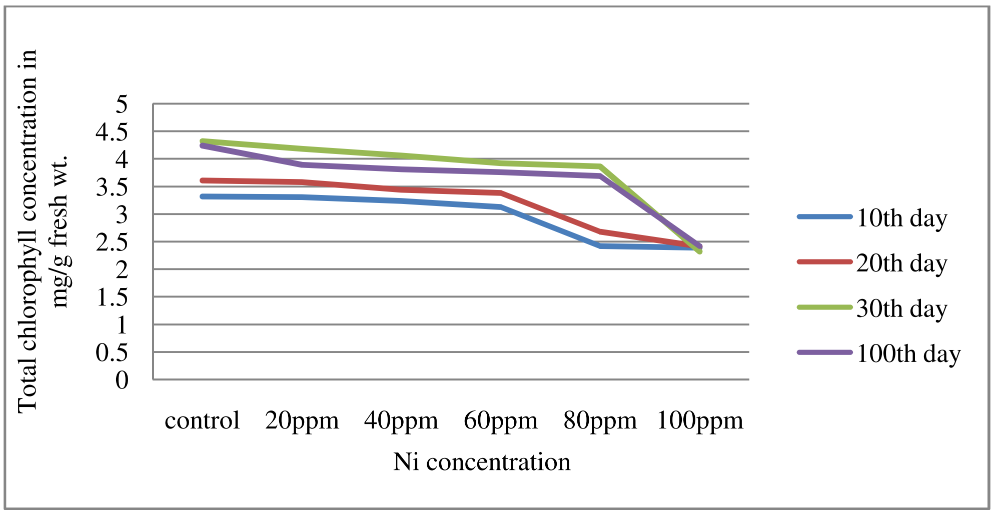

Figure-1
Effect of Nickel ions on chlorophyll content of *Arachis hypogea* L. plants grown in pot culture experiments

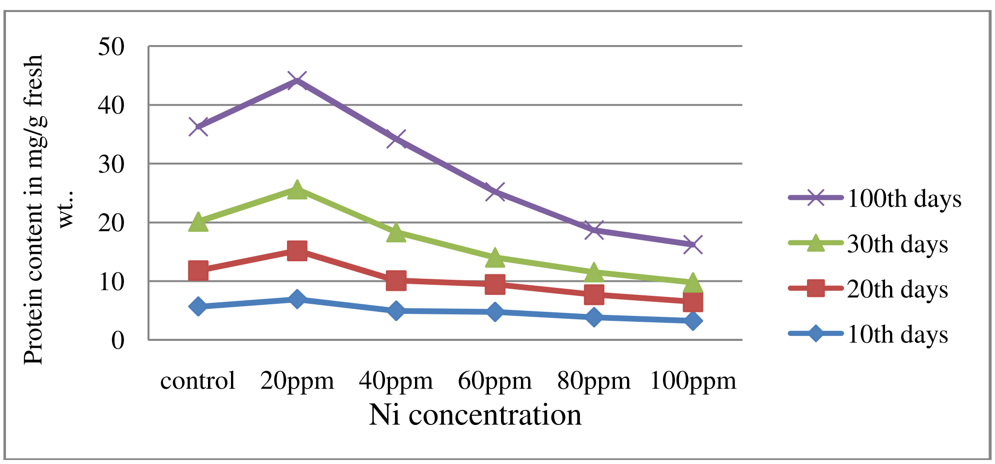

Figure-2
Effect of Nickel ions on total soluble protein content of *Arachis hypogea* L. plants grown in pot culture experiments

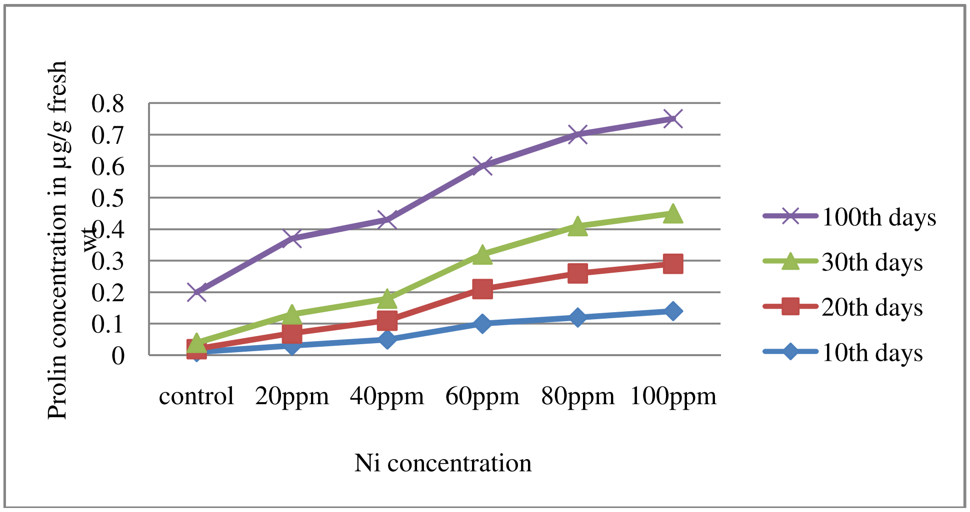

Figure-3
Effect of Nickel ions on free proline content of *Arachis hypogea* L. plants grown in pot culture experiments

Free proline contents were increased with increase in nickel concentration at 10, 20, 30 and 100 days after treatment on the plants. At 10 days after treatment it was observed that the free proline content were increased from 0.002 to 0.145µg/g fresh wt. from control to 100ppm respectively. Similarly at 20 days after treatment it was observed that the free proline contents were increased from 0.003 to 0.153 µg/g fresh wt. in control and 100ppm respectively and also at 30 days after treatment, it was observed that the free proline content were increased from 0.003 to 0.169 µg/g fresh wt. from control to 100ppm respectively. Similarly 100 days after treatment it was observed that the free proline content were increased from 0.156 to 0.295 µg/g fresh wt. from control to 100ppm respectively.

Discussion: Metal toxicity has high impact and relevance to plants and consequently it affects the ecosystem, where the plants form an integral component. Plants growing in metal-polluted sites exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation. Various physiological and biochemical processes in plants are affected by metals.

Effect of Nickel treatments in plant growth inhibition has been reported by many authors[11]. Uptake of Ni by plants shows retarded growth, damage to cell wall, cell membrane and change the metabolism of plants. Keeping on the view that Ni had toxic effect on plants, the present study has been conducted with an effort to assess the phytotoxic impacts with special reference to biochemical lesions in developing stages (10, 20, 30 days) and matured stage (100 days) of groundnut (*Arachis hypogea* L.) plant. The present study provides the effect of Ni toxicity in groundnut with a potentiality of its tolerance after exposure to different concentrations of Ni. The present work signifies the potential of groundnut plant towards Ni phytotoxicity and tolerance.

Seed germination was affected by the presence of Ni and the groundnut plants showed significantly variable responses. Since, germination is the most crucial stage of plant development; the seed germination can be frequently used as an indicator of early response of the plants in the unfavourable environment[12]. Ni inhibits all energy requiring cellular processes during germination[13] thus, slow down emergence of radicle and plumules. But the results obtained from the germination studies indicated that *Arachis hypogea* L. showed higher seedling growth and dry weight at 40 mg/kg nickel level in the soil. The values of growth parameters indicated that Nickel had a significant stimulating, beneficiary and nutritional effect at 40mg/kg concentration. The growth parameters beyond this concentration indicated that a little excess of Nickel above 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 *Arachis hypogea* L. and will inhibit the same at higher concentrations. Similar results were reported on the effect of cadmium[14] (*Triticum aestivum*), chromium[16] (*Salvia sclarea*), and cobalt and zinc[16] (*Pennisetumamericanum* L. and *Parkinsonia aculeata* L.).

Total chlorophyll concentration in leaves significantly decreased with the increase in Ni concentration. The chlorophyll pigments are present in thylakoid within chloroplast, and any damage brought to these structures could lead to denaturation of these pigments. It may be suggested that observed decrease in chlorophyll content at higher concentration of nickel might be due to breakdown of thylakoid and chloroplast envelope as was previously reported[17]. Total soluble protein concentrations were found to decrease in the leaves of the plant with the increase in nickel concentration. It may be the degradation of proteins in plants which could result in inhibition of nitrate reductase activity[18] and it could be correlated with reduced photosynthetic activity, nitrogen metabolism and nucleic acid damage. Proline apparently is the only amino-acid that accumulates to a great extent in the leaves of many plants under stress. Higher proline content was observed in leaves of seedlings treated with increasing concentrations of nickel. Thus proline accumulation under such condition might also be operative as usual in osmotic adjustment while accumulation of proline in tissue can be taken as dependant marker for genotypes tolerant to stress.

Conclusion

The values of growth and biochemical parameters indicated that nickel had a significant stimulating, beneficiary and nutritional effect up to 40mg/kg concentration for *Arachis hypogea* L. and all the parameters beyond this concentration indicated that a little excess of nickel above these levels had an adverse effect. From the result of this investigation, it can be concluded that nickel at lower concentration had a stimulating effect on plant growth and will inhibit the same at higher concentrations.

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