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## Research Paper

# Physiological and biochemical responses induced by Nickel to plants growing at nickel polluted soil

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## Abstract

An experiment was conducted in three different plants like a cereal crop plant [*Oryza sativa* L.], an oil yielding plant [*Arachis hypogea* L.] and a pulse crop plant [*Macrotyloma uniflorum* (Lam.) Verdc.] to assess the effect of nickel on seed germination, growth and biochemical parameters. The seeds of all the test plants were germinated in six different concentrations of Nickel chloride solution ranging from 0.0 to 100 mg/l of nickel. It was observed that seedling vigour index, metal tolerance indices were decreased and the percentage of phytotoxicity was increased with increasing level of nickel. The pot culture experiment revealed that, the growth parameters and the percentage of moisture content in plant were reduced with increase in concentration of nickel and in contradiction, seedlings exhibited better result in terms of growth in 40 ppm of nickel at 30<sup>th</sup> day (developmental stage) and 100<sup>th</sup> day (matured stage) of treatments thereby indicating that Nickel at or below 40 mg/kg had stimulating effect on the seedling growth. Similarly the total chlorophyll content, total soluble protein content were decreased although free proline content was increased with increase in concentration of nickel at 30<sup>th</sup> day (developmental stage) and 100<sup>th</sup> day (matured stage) of treatment. Hence, 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, Plant, Germination, Growth parameter, Biochemical parameter

## Introduction

Nickel (Ni) is a micronutrient required at extremely low concentration by plants<sup>1</sup>. It is considered as one of the toxic heavy metals, and is known for its harmful effects on the environment where it bio-accumulates and poses a severe threat to human and environmental health. All kinds of nickel compounds, except for the metallic nickel, have been classified as human carcinogens by International Agency for Research on Cancer<sup>2</sup>. The present study examines the extent of impact of Ni on both developmental stage and matured stage in growing plants [*Oryza sativa* L., *Arachis hypogea* L. and *Macrotyloma uniflorum* (Lam.) Verdc]. The phytotoxic effects of varying concentrations of nickel on the growth, development and biochemical changes in plants supplemented to increasing levels of nickel was assessed.

## Material and Methods

### Collection of seeds

Seeds of all the plants were obtained from the seed testing laboratory, Orissa University of Agriculture and Technology (O.U.A.T), Bhubaneswar, Odisha, India.

### Physiological and biochemical analysis

The present study was undertaken with Nickel (Nickel Chloride) at 20, 40, 60, 80 and 100 mg/l levels along with control (untreated). Seeds of all the test plants were surface sterilized with 0.1% mercuric chloride and washed carefully with tap water and then with distilled water. Hundred identical sized seeds of each plant were placed in petri-dishes of 10 cm diameter with different level of nickel chloride solution (20, 40, 60, 80 and 100 mg Ni/l) and one with control at a constant temperature of 26 °C. The seeds were submerged in 10 ml of test solutions and distilled water two times in a day. Each treatment was replicated five times. The number of seeds germinated in each treatment was counted on 5<sup>th</sup> day of sowing and the total percentage of germination was calculated. Metal tolerance index<sup>3</sup>, seedling vigour index<sup>4</sup> and percentage of phytotoxicity were calculated. In pot culture experiment seeds of test plants were sown in pre-treated soil (soil treated with 20, 40, 60, 80 and 100 mg Ni/kg of soil with a nickel less soil) and different growth and biochemical parameters like chlorophyll content<sup>5</sup>, protein content<sup>6</sup> and free proline content<sup>7</sup> were estimated in plants at 30th day and 100th day of treatment. All the experiments were repeated five times and the data was statistically analyzed and standard error of mean (SEM) was calculated.

### Results and Discussion

Effect of Nickel treatments on plant growth inhibition has been reported by several authors<sup>8</sup>. Uptake of Ni by plants shows retarded growth, damage to cell wall, cell membrane as well as 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 phyto-toxic impacts with special reference to biochemical parameters in both developing stage (30 days after treatment) and in matured stage (100 days after treatment) of all test plants.

#### Impacts of nickel on seed germination of three different plants

In this experiment, it was found that the germination percentage (Figure1) and radical length (Figure2) at 5<sup>th</sup> day of treatment were decreased with rising concentration of nickel by which seedling vigour index (Figure3), metal tolerance indices (Figure4) were reduced and the percentage of phytotoxicity (Figure5) were increased with increasing concentration of nickel. Since, germination is the most crucial phase of plant development, the seed germination can be frequently used as an indicator of early response of the plants in the unfavourable environment<sup>9</sup>. Ni inhibits all energy requiring cellular processes during germination<sup>10</sup> thus, slow down emergence of radicle and plumules.

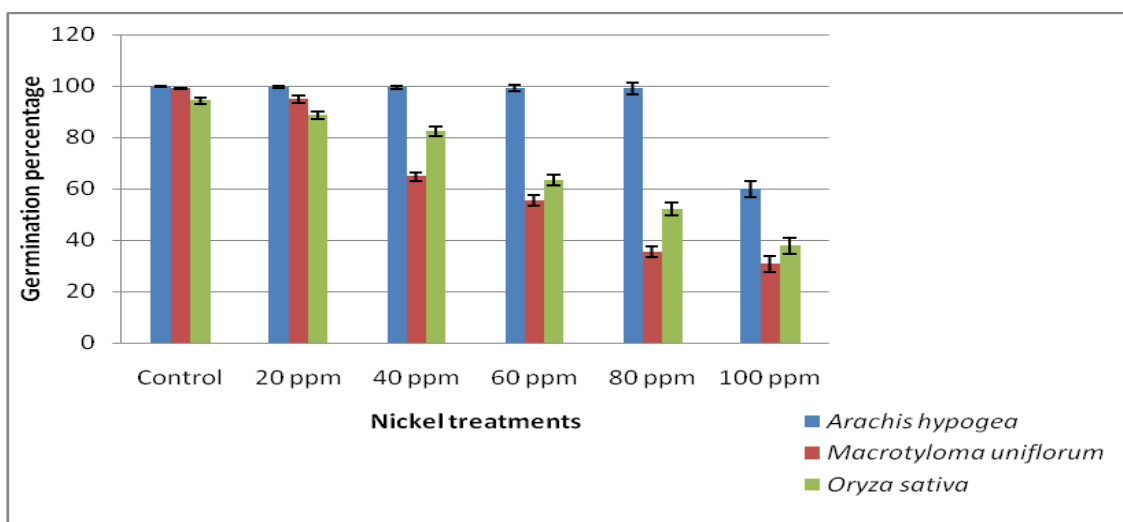


Figure 1: Impacts of Nickel on seed germination of three different plants on 5<sup>th</sup> day of treatment

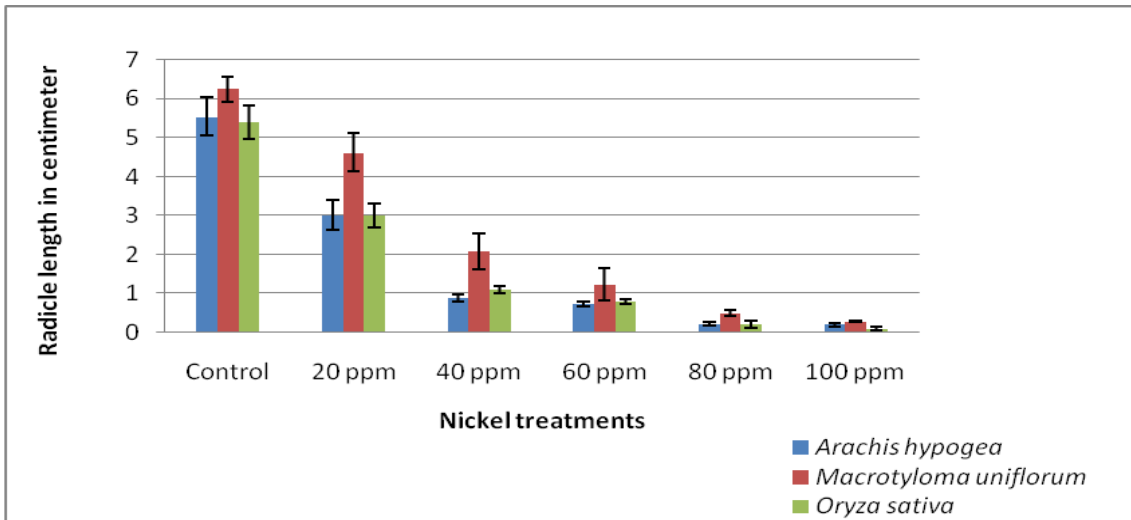


Figure 2 : Impacts of Nickel on radicle length of three different plants on 5<sup>th</sup> day of treatment

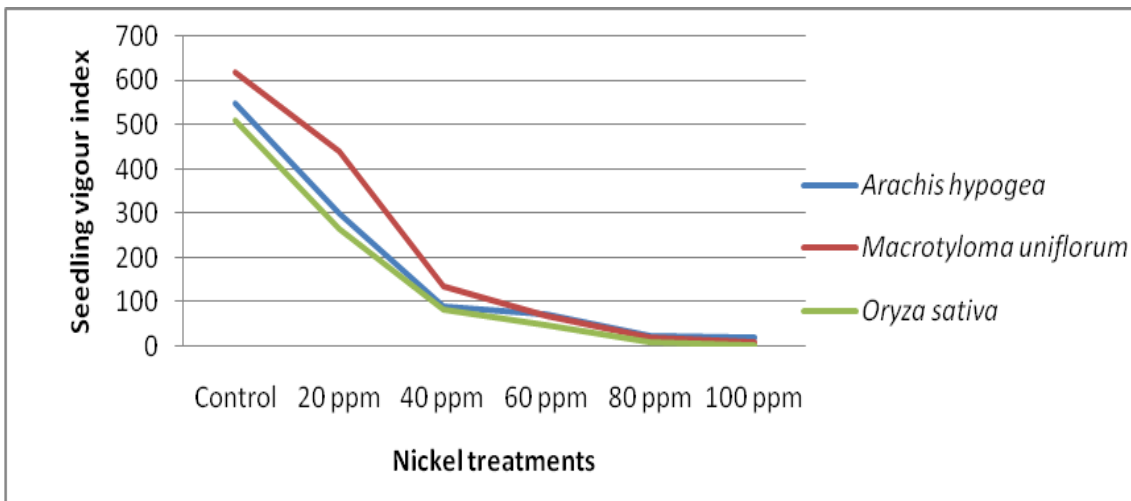


Figure 3: Impacts of Nickel on seedling vigour indices of three different plants on 5<sup>th</sup> day of treatment

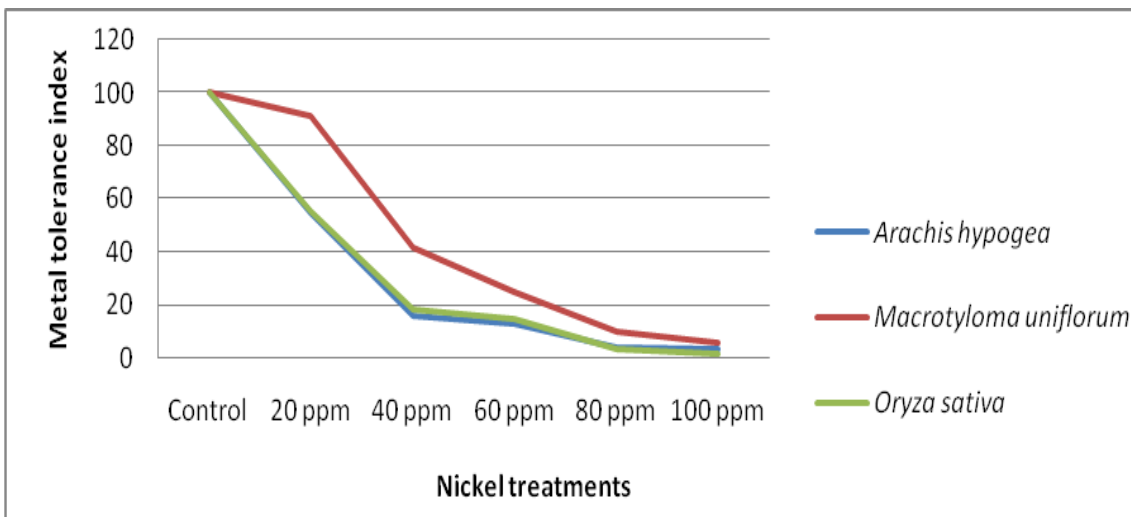
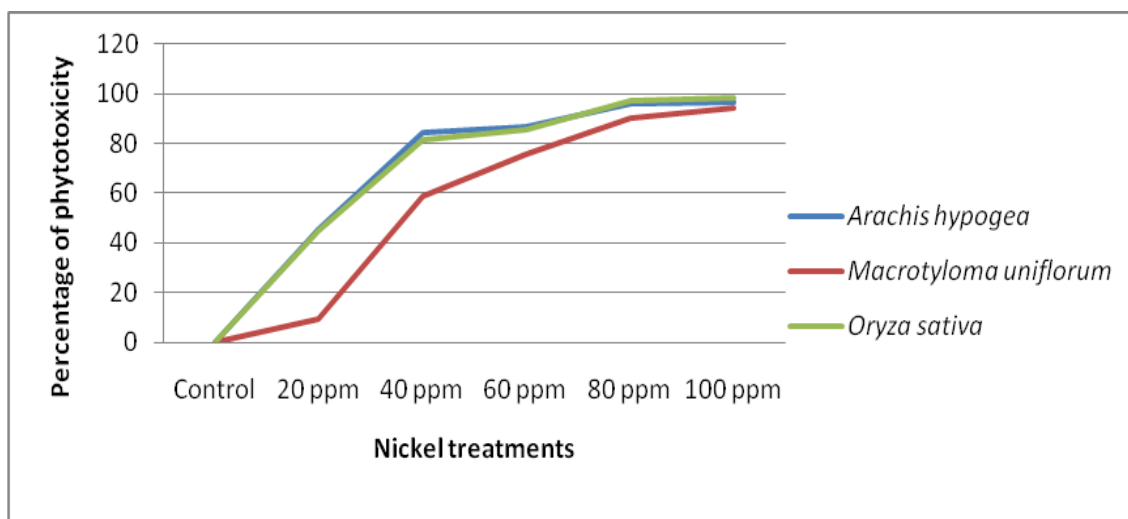


Figure 4: Impacts of Nickel on metal tolerance indices of three different plants on 5<sup>th</sup> day of treatment



**Figure 5: Impacts of Nickel on percentage of phytotoxicity of three different plants on 5<sup>th</sup> day of treatment**

#### Impacts of nickel on seedling growth of three different plants

The pot culture experiment indicated that, the growth parameters and the moisture content in plant were decreased with increase in concentration of nickel and in contradiction, seedlings showed better result in terms of growth in 40 ppm of nickel at 30<sup>th</sup> day (developmental stage) and 100<sup>th</sup> day (matured stage), thereby indicating that Nickel at or below 40 mg/kg had stimulating effect on the seedling growth (Table.1, 2 and 3). Similar results were reported on the effect of cadmium in *Triticum aestivum*<sup>11</sup>, chromium in *Salvia sclarea* and cobalt and zinc in *Pennisetum americanum* L. and *Parkinsonia aculeata* L.<sup>12</sup>.

**Table 1: Effect of Nickel on root length, shoot length and percentage of moisture content of *Arachis hypogea* L. plants at 30<sup>th</sup> day of treatment**

Ni Concentration	Root length (in cm)	Shoot length (in cm)	Shoot fresh weight (in g)	Shoot dry weight (in g)	Moisture content (%)	Root fresh weight (in g)	Root dry weight (in g)	Moisture content (%)
Control (00 ppm)	22.46	17.26	6.872	1.239		2.351	0.470	
	±	±	±	±		±	±	80.00
	0.98	1.04	0.089	0.008	81.970	0.006	0.008	
20 ppm	25.46	18.16	8.119	1.450		3.266	0.625	
	±	±	±	±		±	±	80.863
	0.94	0.85	0.088	0.008	82.140	0.008	0.007	
40 ppm	25.92	18.68	7.385	1.266		2.529	0.486	
	±	±	±	±		±	±	80.782
	0.85	0.65	0.076	0.007	82.857	0.004	0.009	
60 ppm	20.34	15.08	4.334	0.934		1.573	0.385	
	±	±	±	±		±	±	75.524
	0.76	0.59	0.053	0.009	78.449	0.007	0.005	
80 ppm	18.98	13.66	3.167	0.716		1.365	0.338	
	±	±	±	±		±	±	75.238
	0.83	0.43	0.009	0.006	77.391	0.006	0.006	
100 ppm	14.58	14.58	2.742	0.674		0.608	0.204	
	±	±	±	±		±	±	66.440
	0.26	0.45	0.006	0.003	75.419	0.004	0.004	

Values of 5 replicate ±SEM

**Table 2: Effect of Nickel on root length, shoot length and percentage of moisture content of *Macrotyloma uniflorum* (Lam.) Verdc. at 30<sup>th</sup> day of treatment**

Ni concentration	Root length (in cm)	Shoot length (in cm)	Shoot fresh weight (in g)	Shoot dry weight (in g)	Moisture content (%)	Root fresh weight (in g)	Root dry weight (in g)	Moisture content (%)
Control (00 ppm)	19.8	20.1	2.091	0.495		1.047	0.267	
	±	±	±	±		±	±	74.475
	0.985	1.045	0.089	0.008	76.312	0.006	0.008	
20 ppm	20.4	21.5	2.145	0.471		1.198	0.287	
	±	±	±	±		±	±	75.976
	0.945	0.858	0.088	0.008	78.041	0.008	0.007	
40 ppm	18.3	18.9	1.609	0.384		0.812	0.212	
	±	±	±	±		±	±	73.875
	0.856	0.657	0.076	0.007	76.134	0.004	0.009	
60 ppm	16.5	17.1	1.268	0.342		0.675	0.192	
	±	±	±	±		±	±	71.549
	0.768	0.592	0.053	0.009	73.028	0.007	0.005	
80 ppm	15.6	16.4	1.086	0.310		0.469	0.141	
	±	±	±	±		±	±	69.932
	0.835	0.438	0.009	0.006	71.454	0.006	0.006	
100 ppm	14.2	15.9	0.932	0.289		0.389	0.124	
	±	±	±	±		±	±	67.876
	0.265	0.459	0.006	0.003	68.991	0.004	0.004	

Values of 5 replicate ±SEM

**Table 3: Effect of Nickel on root length, shoot length and percentage of moisture content of *Oryza sativa* L. at 30<sup>th</sup> day of treatment**

Ni concentration	Root length (in cm)	Shoot length (in cm)	Shoot fresh weight (in g)	Shoot dry weight (in g)	Moisture content (%)	Root fresh weight (in g)	Root dry weight (in g)	Moisture content (%)
Control (00 ppm)	13.8	12.4	1.715	0.368		0.712	0.160	
	±	±	±	±		±	±	77.465
	0.874	0.564	0.032	0.006	78.534	0.021	0.002	
20 ppm	14.2	12.9	1.914	0.370		0.769	0.154	
	±	±	±	±		±	±	79.985
	0.549	0.612	0.021	0.013	80.673	0.072	0.006	
40 ppm	13.3	12.1	1.362	0.324		0.604	0.154	
	±	±	±	±		±	±	74.543
	0.576	0.099	0.043	0.009	76.183	0.043	0.004	
60 ppm	11.8	11.2	1.032	0.268		0.502	0.131	
	±	±	±	±		±	±	73.876
	0.675	0.084	0.012	0.013	74.031	0.021	0.004	
80 ppm	8.8	9.8	0.869	0.232		0.212	0.063	
	±	±	±	±		±	±	70.876
	0.867	0.051	0.009	0.020	73.302	0.011	0.003	
100 ppm	6.4	8.1	0.663	0.199		0.102	0.032	
	±	±	±	±		±	±	68.297
	0.076	0.043	0.007	0.004	70.037	0.009	0.005	

Values of 5 replicates ±SEM

**Impacts of nickel on physiological and biochemical parameters of three different plants**

Chlorophyll content and soluble protein content were decreased while free proline content was increased with increase in concentration of nickel at 30<sup>th</sup> day (developmental stage; Figure 6, 7 and 8) and 100<sup>th</sup> day (matured stage; Figure 9, 10 and 11) of treatment. 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 earlier reported<sup>13</sup>. Total soluble protein concentrations were found to decline in the leaves of the plant with the increase in nickel concentration. It might be due to the degradation of proteins in plants which could result in inhibition of nitrate reductase activity<sup>14</sup> and it could be correlated with decreased photosynthetic activity, nitrogen metabolism and nucleic acid damage. Proline seems to be the only amino-acid that accumulates to a great extent in the leaves of many plants under stress. Higher proline content was recorded in leaves of seedlings treated with increasing concentrations of nickel. Hence, proline accumulation under such condition might also be operative as usual in osmotic adjustment whereas accumulation of proline in tissue can be taken as dependant marker for genotypes tolerant to stress.

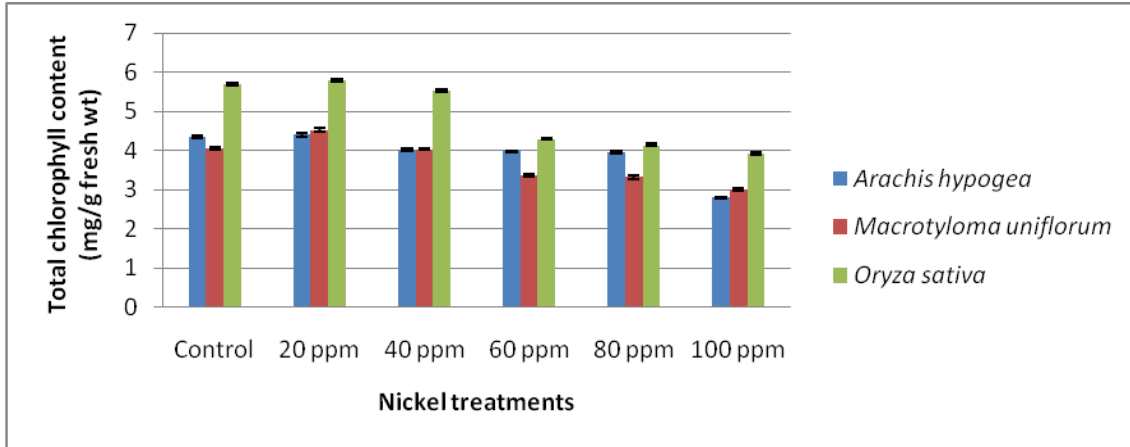


Figure 6: Impacts of Nickel on total chlorophyll content of three different plants after 30<sup>th</sup> day of treatment

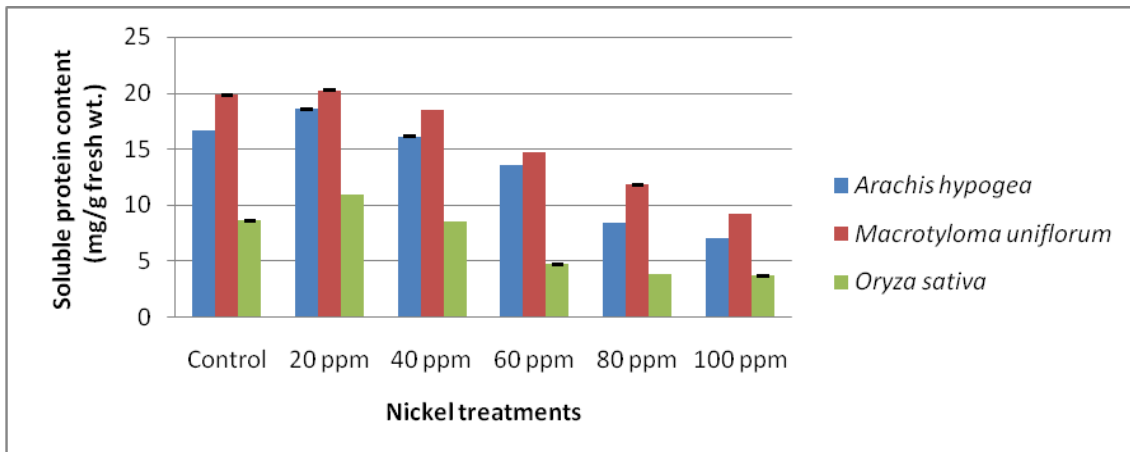
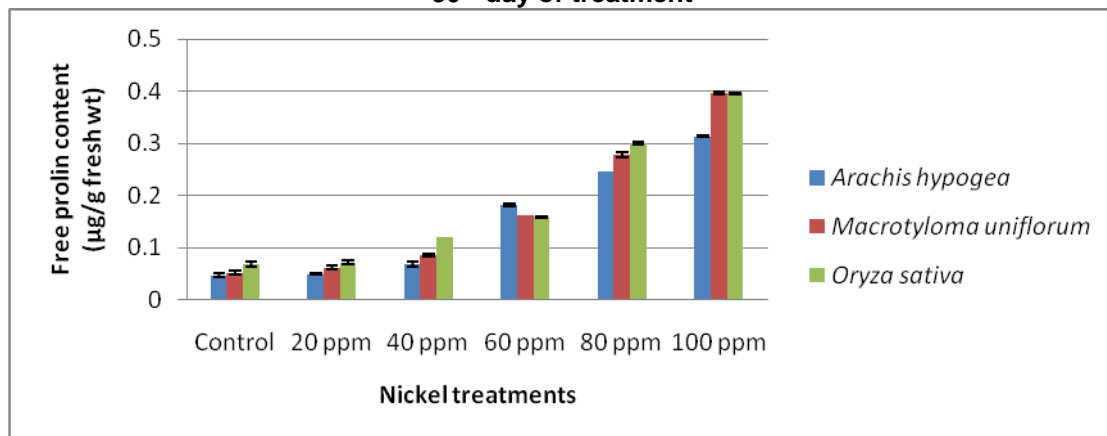
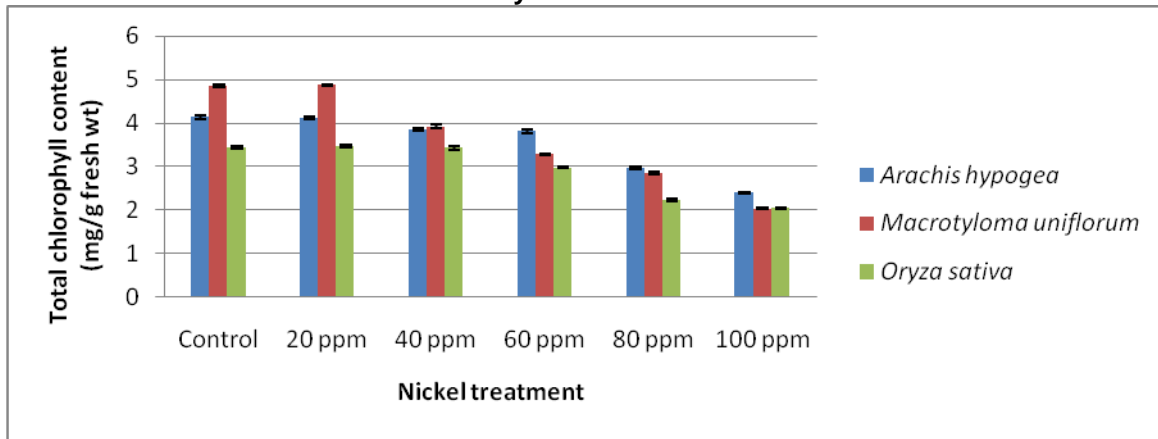


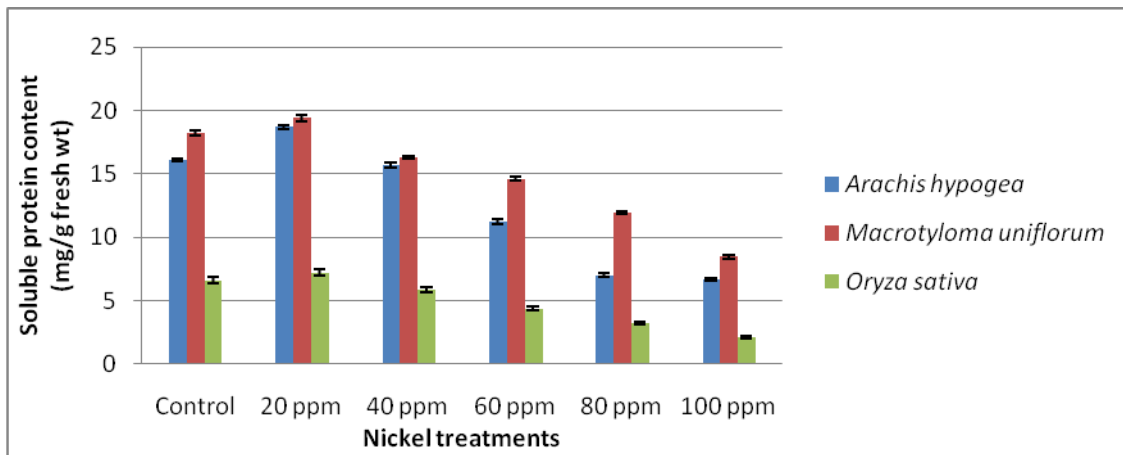
Figure 7: Impacts of Nickel on soluble protein content of leaves of three different plants after 30<sup>th</sup> day of treatment



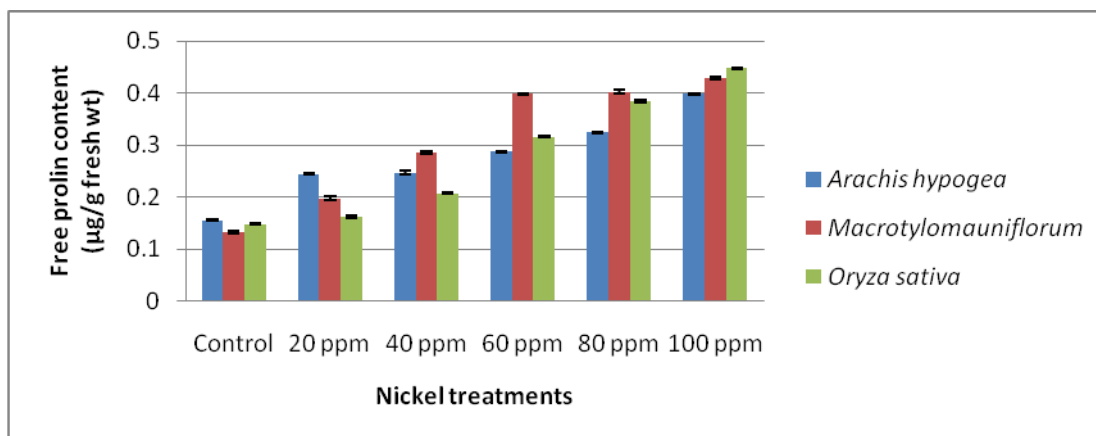
**Figure 8: Impacts of Nickel on free proline content of leaves of three different plants after 30<sup>th</sup> day of treatment**



**Figure 9: Impacts of Nickel on total chlorophyll content of leaves of three different plants after 100<sup>th</sup> day of treatment**



**Figure 10: Impacts of Nickel on soluble protein content of leaves of three different plants after 100<sup>th</sup> day of treatment**



**Figure 11: Impacts of Nickel on free protein content of leaves of three different plants after 100<sup>th</sup> day of treatment**

### Conclusion

The values of growth and biochemical parameters indicated that nickel had a significant stimulating, beneficiary and nutritional effect up to 40 mg/kg concentration for all test plants and all the parameters beyond this concentration slight excess of nickel levels had an adverse effect. From the findings 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.

### **Acknowledgment**

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### **References**

1. Brown P.H., Welch R.M. and Carry E., Nickel is a micronutrient essential for higher plants. J. Plant Physiology, 85: 801-803 **(1987)**
2. IARC (International Agency for Research on Cancer). IARC Monograph on the evaluation of carcinogenic risks to humans 49. Lyons, France IARC 318-411 **(1990)**
3. Turner R.C. and Marshal C., Accumulation of zinc by sub cellular fraction of root *Agrostis tenuis* Sibth in relation to zinc tolerance. J. New Phytologist., 71: 671-676 **(1972)**
4. Abdul Baki A.A. and Anderson J.D., Vigour determination in soybean seed by multiple criteria. J. Crop Science, 3: 630-633 **(1973)**
5. Arnon D.I., Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. J. Plant Physiologist, 24: 1-15 **(1949)**
6. Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J., Protein measurement with Folin-Phenol reagent. Journal of Biological Chemistry, 193: 265-275 **(1951)**
7. Bates L.S., Waldren R.P., Teare I.D., Rapid determination of free proline for water stress studies. J. Plant and Soil, 39: 205-208 **(1973)**
8. Chen Cuiyan, Dejun Huang, Jianquan Liu, Function and Toxicity of Nickel in Plants: Recent advance and future prospects, 37(4-5): 304-313 **(2009)**
9. Singh B.K., Walker A. and Wright D.J., Bioremedial potential of fenamiphos and chlorpyriferos degrading isolates: Influence of different environment conditions. J. Soil Biol. Biochem., 38: 2682-2693 **(2006)**
10. Hall J.L., Cellular mechanisms for heavy metal detoxification and tolerance. J. Experimental Botany, 53: 1-11 **(2002)**
11. Kalita M.C., Devi P., Bhattacharya I., Effect of cadmium on seed germination, early seedling growth and chlorophyll content of *Triticum aestivum*. J. Indian Journal of Plant Physiology, 36(3): 189-190 **(1993)**
12. Burhan S.S., Tahira A., Effect of zinc and cobalt on germination and seedling growth of *Pennisetum americanum* (L.) Schumann. and *Parkinsonia aculeata* L., J. Pakistan Journal of Biological Science, 4(5): 575-580 **(2001)**
13. Dodge J.D., Lawes G.B., Plastic ultra-structure in some parasitic and semi-parasitic plants. J. Cytobiologie., 9: 1-9 **(1974)**
14. Choudhury S. and Panda S.K., Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots, Bulg. J. Plant Physiol., 30(3-4): 95-110 **(2004)**.