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## RESEARCH ARTICLE

## Effect of Nickel and Zinc Metal on Biomass and Growth Rate of Some Pulses.

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**Abstract**

Metals are essential micro molecules required by living cell for biochemical reactions. Several metals are essential and useful to cells while few show adverse effects on living forms. Nickel and zinc are commonly counted as important metals to plant cells but sometimes their higher concentration is harmful to plant cells. In the present study was focused on study of effects of nickel and zinc on biomass and growth rate of three widely cultivated pulses i.e. *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*. It was found that higher concentration of zinc showed no reduction in biomass and growth rate of all three investigated plants. However, nickel poses adverse effect on biomass and growth rate of experimental plants at high concentrations.

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**Introduction:-**

Life on this planet has evolved in the presence of metals. Metals have been mined and used since ancient times. Cells learned to make use of the more abundant metals in the Archean oceans at an integral component in their structure and function. Today, we inherit these as the essential metals. The industrial era has seen a sharp increase in both the amounts and variety of metals that have application in industry (Clarkson, 1995).

All things in nature ultimately succumb to decay. Much of this is a natural consequence of the laws of thermodynamics. Many molecules degrade by the action of oxygen, halogens and radicals naturally found in the environment (Shmaefsky and Tucker, 2001).

Modern industry is to a large degree, responsible for contamination of the environment. Lakes, rivers and oceans are being overwhelmed with bacteria, and wastewater. Among toxic substances reaching hazardous levels are heavy metals (Vieira and Volesky, 2000).

Many uses of heavy metals in several applications lead to their wide distribution in soil, slit, waste and wastewater. Such a pollution of the environment by toxic metals and radio nucleotides arises as a result of many human activities, largely industrial, although sources such as agriculture and sewage disposal also contribute (Diels *et al.*, 2002).

Now men is facing most dangerous ecological problem of pollution of environment especially with heavy metals. Today the problem of pollution is from all the nooks and corners of the world and became a threat to the existence of man on the earth. The effect of heavy metals on the components of ecosystem is well known. They affect flora, fauna and other abiotic components. Certain heavy metals may cause severe injury and health hazards; because metals are omnipresent in environment occurring in varying concentrations in parent rock, soil, water, air and all biological matter (Soni and Bhuvra, 2015).

Heavy metals are among the conservative pollutants that are not subject to bacterial attack or other breakdown or degradation process and are permanent additions to the environment (El - Nady and Atta, 1996; Igwe and Abia, 2006).

These metal contaminants pose adverse health effects to those who live near these polluted sites. Breathing, eating, drinking, and skin contact are all possible exposure routes for metal contaminants. Metals such as mercury, lead, and arsenic, potentially can be toxic to the kidneys, decrease mental capabilities, and cause weakness, headaches, abdominal cramps, diarrhea and anemia (USEPA, 2004). Chronic exposure to these pollutants can cause permanent kidney and brain damage (USEPA, 2004; Adeniji, 2004).

To solve the water pollution problem by toxic heavy metal contamination resulting from human's technological activities has for long presented a challenge (Vieira and Volesky, 2000).

A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, and complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals.

A complete understanding about noxious effects caused by the release of toxic metals into the environment and emergence of more severe environment protection laws, have encouraged studies about removal/recovery of heavy metals from aqueous solutions using bio-sorption. Adsorption, ion exchange, precipitation and complexation with organic matter are mechanisms that limit the amount of metal leaching through surface water or groundwater (Cossich *et al.*, 2002).

At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species (Adeniji, 2004).

There are about 50 metals that are studied with respect to the toxicological importance to plants, animals and man. Such metals accumulate in soil to reach the plant through roots during water absorption and cause serious adverse effect on plants viz., inhibition of seed germination, growth of seedlings and reduction of yield. From these studies it is revealed that at international level there is awareness about the detrimental effect of various heavy metals on the plants (Soni and Bhuva, 2015; Soni *et al.*, 2016).

Though some investigations have been carried out in India throwing light on various aspects of the accumulation and effect of heavy metals in plants, yet such study is not sufficient especially in certain agricultural plants of Gujarat state.

Although a number of techniques have been developed to remove metals from contaminated soils, many sites remain contaminated because economic and environmental costs to clean up those sites with the available technologies are too high (Nascimento *et al.*, 2006).

Removal of heavy metals from waste material by use of biological way is the new era of solving heavy metal pollution. Biosorption, bioremediation, bioaccumulation, phyto-remediation, phyto-accumulation etc. are the few ways of heavy metal removal.

As a rule in nature anything that is present on this earth should be either degraded out or recycled. Heavy metals cannot be degraded out but they can be recycled by changing their ionic stage. Any kind of biomass can be easily degraded out in nature (Soni and Thanki, 2014; Soni and Bhuva, 2015; Soni *et al.*, 2016).

Several modes of biotechniques are named as, biosorption, phyto-sorption, bioaccumulation, phyto-accumulation, bio-extraction, phyto-extraction, rhizofiltration and rhizodegradation, microorganism stimulation and mobilization, phyto-stabilization and phyto-volatilization etc.

Phytosorption is the technique where plants or plant materials are used to absorb heavy metals. In phyto-accumulation technique plants are used to absorb heavy metals and they are stored in plant parts.

Contamination of heavy metals in biosphere increased drastically since 1900 and expressed severe health and the environmental problems throughout the world (Nriagu, 1979; Ensley, 2000). Blaylock and Hwang (2000) suggested that the plants that are used for phytoextraction have tolerance towards the metal(s) targeted and efficient to translocate them from below ground parts to areal parts.

The present work was focused towards the toxic effects of copper and ferrous metal on biomass and growth rate on widely cultivated pulses *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia*.

### Material and Methods:-

The study was carried out in Rajkot city area (22° 17' Lat. And 70° 49' Lon.). Experiments on seedling emergence and seedling growth were performed on a coarse loam soil found in the natural habitats where the selected plants cultivated by seed germination. Soil was collected from natural habitats, air dried and passed through a 2 mm sieve. For the study of the effect of nickel and zinc on plant growth rate and biomass development, the soil was mixed with heavy metal salts and prepared for the cultivation of experimental plants. The nickel and zinc metals were used in the form of nickel sulphate salt ( $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ) and zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) respectively. The metal salts were mixed in eight different lots of soil (each lot of 10kg) at 2.4, 4.7, 7.1, 9.4, 11.8, 14.2, 16.5 and 18.9 grams for nickel and 2.5, 5.0, 7.6, 10.0, 12.6, 15.1, 17.6 and 20.2 gram for zinc to get 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mm concentrations of metal salts.

The soil mixed with metal salt was placed in polyethylene bags and cultivation of experimental plants was carried out in these bags. The soil without metal salt was control. The initial metal concentration of control soil was negligible and considered as zero. Tap water was added to the soil in polyethylene bags to field capacity and then allowed to dry for 6 days.

The seeds of *Glycine max*; *Vigna unguiculata* and *Vigna aconitifolia* were collected from Sanjiv Agro Center, Rajkot.

Metal salt mixed soils were then raked with fingers and seeds were sown after surface sterilization with  $\text{H}_2\text{O}_2$ . Ten seeds were sown in each bag at the depth of about 8 -10 mm in evening. Immediately after sowing soils were watered and then after watering was carried out at alternate days.

All the seedlings in each bag for each metal concentration were allowed for germination. The study was carried out twice. The results are average of the study of these two sets of germination.

After specific time duration plants were harvested in such a way that the tap root and root hairs were not damaged or damage was minimum. Soil particles were removed from the root by gentle washing.

The plants collected for the study brought in the laboratory, washed with water and carefully blotted on the blotting sheets after washing to remove moisture on their surface. The length of entire plant was measured. The mean of 20 measurements was calculated as final reading. The growth rate of control and treated plant was studied on the basis of length of entire plant five weeks after the germination.

The method of Hunt (1978) was used to study the biomass of experimental plants. The fresh weight of root, stem and leaves was determined separately after blotting in the laboratory. They were cut into small pieces after weighing and placed in brown paper bags separately and kept in oven at 80° C for a period of 8 days for uniform drying. The dry weight of these organs was recorded.

### Result and Discussion:-

#### Effect of nickel on fresh weight:-

The fresh weight in root of *Glycine max* was 11.25 gm which was reduced due to the treatment of nickel. The lowest fresh weight was found at 1.2 mm nickel concentration (Table 1). In stem and leaf the amount of fresh weight in control was 14.92 and 3.78 gm respectively. This was decreased by different concentrations of nickel (Table 2 and 3). Germination was not observed at 1.4 and 1.6 mm nickel concentrations (Table 1 - 3).

In root, stem and leaf of *Vigna unguiculata* the fresh weight was found lower than control in all treatments of nickel (Table 1 - 3).

In *Vigna aconitifolia* the root stem and leaf fresh weight was gradually decreased by increasing the concentration of nickel in the treatment. It was always lower than control (Table 1 - 3).

**Effect of nickel on dry weight:-**

In root of *Glycine max* dry weight was lower than control due to the treatment of different concentrations of nickel except at 0.6 mm nickel where the dry weight was slightly higher than control (Table 4). In the stem of *Glycine max* the dry weight was lower than control at 0.2 and 0.4 mm nickel concentration. It was 11.85 mg at 0.6 mm nickel concentration and gradually decreased when concentration was increased from 0.8 to 1.2 mm nickel concentration (Table 5). In leaf dry weight was decreased by the treatment of different concentrations of nickel. Germination was not observed at 1.4 and 1.6 mm nickel concentrations (Table 5 - 6).

In root, stem and leaf of *Vigna unguiculata* and *Vigna aconitifolia* the dry weight was reduced and was lower than control in all treatments of nickel (Table 4- 6).

**Effect of zinc on fresh weight:-**

*Glycine max* root fresh weight was lower than control in all zinc concentrations in treatment except at 0.2 mm zinc where slightly higher fresh weight was observed than control (Table 7). In stem also fresh weight was lower than control except at 0.2 and 0.4 mm zinc concentration where fresh weight was slightly higher than control (Table 8). Fresh weight of leaf was observed lower than control (Table 9).

In root, stem and leaf of *Vigna unguiculata* the fresh weight was gradually decreased by increasing the concentration of zinc in the treatment and was lower than control (Table 7 - 9).

Similar results were obtained for root, stem and leaves of *Vigna aconitifolia*. The lowest fresh weight was observed at 1.6 mm zinc concentration (Table 7 - 9).

**Effect of zinc on dry weight:-**

The root dry weight of *Glycine max* was lower than control in all zinc treatments except at 0.2 and 0.4 mm zinc (Table 10). The stem dry weight was 12.83 gm in control condition which was decreased by increasing the zinc concentration (Table 11). The leaf dry weight was decreased by increasing zinc concentration in the treatment (Table 12).

In *Vigna unguiculata* root, stem and leaf dry weight was decreased by increasing the concentration of zinc in the treatment except at 0.2 mm zinc treatment to the root. At this lower concentration root dry weight was higher than control (Table 10 - 12). Similar results were observed about root; stem and leaf dry weight of *Vigna aconitifolia* (Table 10 - 12).

**Effect of nickel on growth rate:-**

In all three investigated plants the growth rate was reduced by increasing the concentration of nickel in the treatment (Table 13 - 15). Germination was not observed at 1.4 and 1.6 mm nickel concentrations in *Glycine max* (Table 13).

**Effect of zinc on growth rate:-**

The growth rate in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was higher than the control plants at lower concentrations of zinc. The growth rate was affected and decreased at the higher concentrations of zinc (Table 13 - 15).

**Table 1:** Effect of nickel on root fresh weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	11.25	16.49	13.53
0.2	10.93 + 0.10	16.23 + 0.06	13.12 + 0.02
0.4	10.51 + 0.10	15.46 + 0.06	12.30 + 0.02
0.6	11.52 + 0.10	14.90 + 0.06	11.69 + 0.02
0.8	9.89 + 0.10	13.58 + 0.06	10.88 + 0.02
1.0	8.76 + 0.10	13.25 + 0.06	10.26 + 0.02
1.2	7.43 + 0.10	13.48 + 0.06	9.54 + 0.02
1.4	N. G.	13.29 + 0.06	9.02 + 0.02
1.6	N. G.	13.50 + 0.06	8.60 + 0.02

**Table 2:** Effect of nickel on stem fresh weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	14.92	21.13	18.41
0.2	12.70 + 0.10	20.22 + 0.06	17.71 + 0.01
0.4	11.75 + 0.10	19.95 + 0.06	17.10 + 0.01
0.6	13.78 + 0.10	19.12 + 0.06	16.58 + 0.01
0.8	11.69 + 0.10	17.12 + 0.06	15.99 + 0.01
1.0	10.56 + 0.10	17.20 + 0.06	15.28 + 0.01
1.2	9.28 + 0.10	17.02 + 0.06	14.68 + 0.01
1.4	N. G.	17.57 + 0.06	14.07 + 0.01
1.6	N. G.	17.04 + 0.06	13.50 + 0.01

**Table 3:** Effect of nickel on leaf fresh weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	3.78	6.43	5.22
0.2	3.66 + 0.05	6.16 + 0.03	5.03 + 0.01
0.4	3.63 + 0.05	5.99 + 0.03	4.79 + 0.01
0.6	3.37 + 0.05	5.73 + 0.03	4.54 + 0.01
0.8	2.92 + 0.05	5.22 + 0.03	4.33 + 0.01
1.0	2.91 + 0.05	5.13 + 0.03	4.08 + 0.01
1.2	3.01 + 0.05	5.12 + 0.03	3.84 + 0.01
1.4	N. G.	5.12 + 0.03	3.61 + 0.01
1.6	N. G.	4.94 + 0.03	3.30 + 0.01

N.G. = no germination

**Table 4:** Effect of nickel on root dry weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	9.68	14.18	11.63
0.2	9.40 + 0.09	14.06 + 0.03	11.53 + 0.01
0.4	9.04 + 0.09	13.29 + 0.03	10.57 + 0.01
0.6	9.91 + 0.09	12.81 + 0.03	10.05 + 0.01
0.8	8.31 + 0.09	11.41 + 0.03	9.03 + 0.01
1.0	7.27 + 0.09	11.00 + 0.03	8.52 + 0.01
1.2	6.09 + 0.09	11.05 + 0.03	7.83 + 0.01
1.4	N.G.	10.63 + 0.03	7.21 + 0.01
1.6	N.G.	10.53 + 0.03	6.71 + 0.01

**Table 5:** Effect of nickel on stem dry weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	12.83	18.17	15.83
0.2	10.92 + 0.09	17.39 + 0.03	15.23 + 0.01
0.4	10.11 + 0.09	17.16 + 0.03	14.71 + 0.01
0.6	11.85 + 0.09	16.44 + 0.03	14.26 + 0.01
0.8	9.82 + 0.09	14.38 + 0.03	13.43 + 0.01
1.0	8.76 + 0.09	14.28 + 0.03	12.68 + 0.01
1.2	7.61 + 0.09	13.96 + 0.03	12.04 + 0.01
1.4	N.G.	14.05 + 0.03	11.26 + 0.01
1.6	N.G.	13.29 + 0.03	10.53 + 0.01

**Table 6:** Effect of nickel on leaf dry weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	3.25	5.53	4.49
0.2	3.15 + 0.05	5.30 + 0.01	4.32 + 0.01
0.4	3.12 + 0.05	5.15 + 0.01	4.12 + 0.01
0.6	2.90 + 0.05	4.93 + 0.01	3.90 + 0.01
0.8	2.45 + 0.05	4.39 + 0.01	3.63 + 0.01
1.0	2.41 + 0.05	4.26 + 0.01	3.39 + 0.01
1.2	2.47 + 0.05	4.20 + 0.01	3.15 + 0.01
1.4	N.G.	4.10 + 0.01	2.88 + 0.01
1.6	N.G.	3.85 + 0.01	2.57 + 0.01

N.G. = no germination

**Table 7:** Effect of zinc on root fresh weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	11.25	14.59	12.78
0.2	12.58 + 0.10	14.55 + 0.02	12.34 + 0.01
0.4	11.20 + 0.10	13.74 + 0.02	11.93 + 0.01
0.6	10.84 + 0.10	13.08 + 0.02	11.43 + 0.01
0.8	10.69 + 0.10	11.81 + 0.02	10.68 + 0.01
1.0	10.50 + 0.10	11.39 + 0.02	10.08 + 0.01
1.2	10.37 + 0.10	11.14 + 0.02	9.31 + 0.01
1.4	10.17 + 0.10	10.74 + 0.02	8.82 + 0.01
1.6	10.03 + 0.10	10.70 + 0.02	8.56 + 0.01

**Table 8:** Effect of zinc on stem fresh weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	14.92	19.76	17.49
0.2	15.82 + 0.12	18.67 + 0.02	16.69 + 0.01
0.4	15.85 + 0.12	18.50 + 0.02	16.15 + 0.01
0.6	13.78 + 0.12	17.98 + 0.02	15.64 + 0.01
0.8	13.70 + 0.12	17.02 + 0.02	15.04 + 0.01
1.0	13.73 + 0.12	16.80 + 0.02	14.34 + 0.01
1.2	13.64 + 0.12	16.00 + 0.02	13.73 + 0.01
1.4	13.50 + 0.12	16.13 + 0.02	13.37 + 0.01
1.6	13.44 + 0.12	15.80 + 0.02	13.02 + 0.01

**Table 9:** Effect of zinc on leaf fresh weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	3.55	6.04	4.84
0.2	3.43 + 0.01	5.71 + 0.01	4.61 + 0.01
0.4	3.38 + 0.01	5.55 + 0.01	4.46 + 0.01
0.6	3.03 + 0.01	5.31 + 0.01	4.16 + 0.01
0.8	2.75 + 0.01	5.02 + 0.01	3.98 + 0.01
1.0	2.58 + 0.01	4.81 + 0.01	3.72 + 0.01
1.2	2.49 + 0.01	4.59 + 0.01	3.45 + 0.01
1.4	2.39 + 0.01	4.44 + 0.01	3.21 + 0.01
1.6	2.07 + 0.01	4.11 + 0.01	2.83 + 0.01

**Table 10:** Effect of zinc on root dry weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	9.68	12.54	10.99
0.2	10.82 + 0.06	12.72 + 0.01	11.12 + 0.01
0.4	10.08 + 0.06	11.81 + 0.01	10.26 + 0.01
0.6	9.32 + 0.06	11.25 + 0.01	9.83 + 0.01
0.8	9.25 + 0.06	9.92 + 0.01	8.76 + 0.01
1.0	9.16 + 0.06	9.45 + 0.01	8.37 + 0.01
1.2	8.91 + 0.06	9.13 + 0.01	7.63 + 0.01
1.4	8.74 + 0.06	8.59 + 0.01	7.06 + 0.01
1.6	8.28 + 0.06	8.35 + 0.01	6.67 + 0.01

**Table 11:** Effect of zinc on stem dry weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	12.83	16.99	15.04
0.2	12.61 + 0.06	16.06 + 0.01	14.35 + 0.01
0.4	12.33 + 0.06	15.91 + 0.01	13.89 + 0.01
0.6	11.85 + 0.06	15.46 + 0.01	13.45 + 0.01
0.8	11.51 + 0.06	14.30 + 0.01	12.63 + 0.01
1.0	11.40 + 0.06	13.94 + 0.01	11.90 + 0.01
1.2	11.18 + 0.06	13.12 + 0.01	11.26 + 0.01
1.4	10.80 + 0.06	12.91 + 0.01	10.69 + 0.01
1.6	10.48 + 0.06	12.33 + 0.01	10.16 + 0.01

**Table 12:** Effect of zinc on leaf dry weight

Concentration(mm)	<i>Glycine max</i> (gm)	<i>Vigna unguiculata</i> (gm)	<i>Vigna aconitifolia</i> (gm)
Control	3.05	5.19	4.16
0.2	2.95 + 0.01	4.91 + 0.01	3.97 + 0.01
0.4	2.91 + 0.01	4.78 + 0.01	3.84 + 0.01
0.6	2.61 + 0.01	4.56 + 0.01	3.58 + 0.01
0.8	2.31 + 0.01	4.21 + 0.01	3.35 + 0.01
1.0	2.14 + 0.01	3.99 + 0.01	3.08 + 0.01
1.2	2.04 + 0.01	3.76 + 0.01	2.83 + 0.01
1.4	1.91 + 0.01	3.55 + 0.01	2.57 + 0.01
1.6	1.62 + 0.01	3.21 + 0.01	2.21 + 0.01

**Table 13:** Effect of heavy metals on *Glycine max* growth rate

Concentration (mm)	Length of plant (cm)		
	Control	Nickel	Zinc
Water	52.00	-	-
0.2	-	50.60 + 0.01	53.15 + 0.28
0.4	-	48.50 + 0.01	52.80 + 0.28
0.6	-	53.30 + 0.01	50.10 + 0.28
0.8	-	45.70 + 0.01	49.20 + 0.28
1.0	-	40.50 + 0.01	48.80 + 0.28
1.2	-	34.40 + 0.01	47.50 + 0.28
1.4	-	N.G.	46.40 + 0.28
1.6	-	N.G.	46.20 + 0.28

N. G. = no germination



**Table 14:** Effect of heavy metals on *Vigna unguiculata* growth rate

Concentration (mm)	Length of plant (cm)		
	Control	Nickel	Zinc
Water	52.00	-	-
0.2	-	59.60 + 0.01	71.20 + 0.06
0.4	-	46.90 + 0.01	70.35 + 0.06
0.6	-	43.30 + 0.01	69.80 + 0.06
0.8	-	34.00 + 0.01	66.90 + 0.06
1.0	-	24.00 + 0.01	61.50 + 0.06
1.2	-	14.90 + 0.01	56.30 + 0.06
1.4	-	13.60 + 0.01	50.90 + 0.06
1.6	-	12.80 + 0.01	45.90 + 0.06

**Table 15:** Effect of heavy metals on *Vigna aconitifolia* growth rate

Concentration (mm)	Length of plant (cm)		
	Control	Nickel	Zinc
Water	52.00	-	-
0.2	-	60.7 + 0.01	61.9 + 0.01
0.4	-	55.2 + 0.01	57.8 + 0.01
0.6	-	47.7 + 0.01	51.7 + 0.01
0.8	-	37.0 + 0.01	41.2 + 0.01
1.0	-	27.1 + 0.01	31.4 + 0.01
1.2	-	18.2 + 0.01	21.8 + 0.01
1.4	-	13.3 + 0.01	14.0 + 0.01
1.6	-	11.5 + 0.01	8.5 + 0.01

**Effect of nickel on growth rate and biomass:**

Although nickel is natural component of the soil, human activities such as metal processing, land application of sludge and the use of certain fertilizers can lead to an accumulation of nickel at potentially toxic levels (Kabata-Pendias and Pendias, 2001). In addition to anthropogenic pollution, nickel may also accumulate in soil naturally. For instance, soils formed from serpentine minerals often contain high nickel concentrations (Batianoff and Singh, 2001).

In plants nickel is a component of the enzyme urease and is considered as an essential micronutrient for growth (Brown *et al.*, 1987). However, excess nickel is known to be toxic and many studies have been conducted concerning nickel toxicity of various species (Mishra and Kar, 1974; Seregin and Kozhevnikova, 2006).

Few workers reported beneficial effect of nickel at lower concentration. Rao and Shantaram (2000) showed that nickel at lower level gave promotive effect on dry matter. Karagiannidis *et al.* (2002) found that nickel improved the yields and biomass in tomato plants. Brake *et al.* (2004) recorded that the tomato plant grown in soil with lower nickel concentration increased the growth and improved the fruit quality. Tripathi and Tripathi (2000) showed that nickel gave promotive effect on root and shoot growth, leaf area and biomass of *Albizia lebbek*. Rahmatullah *et al.* (2001) showed that nickel improved tomatoes, shoot and root growth. The findings of Wallace *et al.* (1997) were similar to results of Rahmatullah *et al.* (2001). Gad *et al.* (2007) explained that the lower concentration of nickel has beneficial effect on all growth parameters.

However, when present at higher concentration in the soil environment or experimental growth media, nickel becomes phytotoxic (Parida *et al.*, 2003; Gajewska and Sklodowska, 2005) and many studies have been conducted concerning nickel toxicity of various species (Mishra and Kar, 1974; Seregin and Kozhevnikova, 2006). In the present work decrease in fresh weight and dry weight of root, stem and leaves was observed in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* by increasing nickel concentration in the treatment. Kopittke *et al.* (2007) have also studied the effect of nickel concentration on the growth of *Vigna unguiculata* and found similar decrease in fresh weight and explained that the fresh weight of root and shoot was decreased at similar rate. The reduction in relative shoot and root mass by nickel treatment was also reported in *Lactuca sativa* L. (Heikal *et al.*, 1989) and



*Phaseolus vulgaris* L. (Piccini and Malavolta, 1992). In *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* the growth rate was decreased as there was reduction in length by increasing the nickel concentration in the treatment. This was in accordance with the results of Maheshwari and Dubey (2009). They reported 50 % reduction in root length and 27 % reduction in shoot length as well as 47 % decline in fresh biomass of roots and 29 % decline in shoots compared to control by the treatment of higher concentration of nickel. A significant reduction in shoot length, root length and biomass of chick pea was reported by Khan and Khan (2010) under the effect of higher nickel content in the treatment. They also noticed the suppression of the growth of the lateral roots. A comparative study of the effect of nickel on dry matter of roots and shoots of white clover, ryegrass, cabbage and maize was carried out by Yang *et al.* (1996). They found reduction in dry matter due to the treatment of higher concentration of nickel and explained that the root growth was more affected than shoot growth due to nickel treatment.

El-Enany *et al.* (2000) studied the effect of nickel on bean seedlings. They observed that the fresh and dry matter of roots and shoots of broad bean plants were adversely affected by higher nickel content in the nutrient solution. Similar reduction of the growth of root and shoot of maize was observed by L'Huillier *et al.* (1996) due to the higher content of nickel in the nutrient solution and reported greater effect of nickel on growth of root than shoot. They explained that this was due to the higher level of nickel in root as shown by Cataldo *et al.* (1978), Lubben and Sauerbeck (1991) and Taylor (1989) as well as due to consequence of depressed mitotic activity in the root meristem by nickel content.

Few other investigations recorded decrease in fresh weight as a result of nickel toxicity (Mocquot *et al.*, 1996; Paivoeke, 1983; Barcelo *et al.*, 1986). They observed decrease in fresh weight as a result of change in plant water status (El-Enany *et al.*, 2000). Nickel accumulation decreased water uptake or enhanced water loss, both of which may cause membrane damage. Plant cell membranes are generally considered primary sites of metal injury (Barceleo and Poschenrieder, 1990).

#### **Effect of zinc on growth rate and biomass:-**

Zinc is a necessary element for plants (Wang *et al.*, 2009) and has significant role in the seed germination (Cakmak, 2008) and production of biomass (Kaya and Higgs, 2002), plant fertilization (Pandey *et al.*, 2006) as well as it is important as a cofactor in several enzymes (Grotz and Guerinot, 2006).

Zinc can be toxic in excess for most plants with the exception of a few plant species that can hyper accumulate metals.

According to Ali *et al.* (1999) zinc is known to be toxic at higher concentration and several studies have been conducted concerning zinc toxicity of various species (Rauser, 1973). From these investigations it has been found that there was reduction in biomass and inhibition of the growth of various plant organs due to treatment of higher concentration of zinc. In several plants the growth of root and / or shoot has been decreased due to zinc effect. Wong and Bradshaw (1982) observed 63 % reduction of the root in response to 185 ppm of zinc. Ekaterina and Jeliashkova (2001) reported 30 % to 50 % reduction in root growth by 800 mg/l zinc treatment in *Cuminum cyminum* and *Satvia officinalis*. Patel *et al.* (1976) found 30% decrease in fresh and dry weight of *Chrysanthemum* seedling due to zinc treatment.

The other plants in which the growth of root and / or shoot decreased by zinc treatment are *Nigella sativum* and *Triticum aestivum* (EI - Ghamery *et al.*, 2003); *Phaseolus mungoo* (Chaoui *et al.*, 1997); *Bacopa moniera* (Ali *et al.*, 1999) and *Artemisia annua* (Khudsar *et al.*, 2004).

Balashouri, (1995) worked on *Vigna radiata* and noticed reduction in root and shoot growth due to effect of zinc. Similar results were obtained for *Vigna unguiculata* and *Vigna aconitifolia* in the present investigation. White *et al.* (1979) reported decrease in weight in soya bean due to effect of higher concentration of zinc. The decrease in fresh and dry weight of root, stem and leaves by increasing the concentration of zinc in the treatment in the investigated plants supports this result.

Patel (2008) found that zinc application retarded the growth of shoot and root in *Cajanus cajan* and *Trigonella foenum-graecum*. More retardation was observed in root growth than shoot growth in both plants. The reduction in growth is also consequence of zinc interference with nutrient uptake (Chaney, 1983 and Kaya *et al.*, 2000), specific

enzyme activities (Quariti *et al.*, 1997) and certain essential metabolic events (Tripathy and Mohanthy, 1980; Van Assche and Clijsters, 1990; Alia *et al.*, 1995).

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