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EFFECT OF COPPER AND FERROUS METAL ON BIOMASS AND GROWTH RATE OF SOME PULSES

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Abstract: Metals are one of the micro molecules required by living cell to do their biochemical functions. Out of a list of metals few are very important and useful to cells while few are required in very minute amount and their higher concentration harm or cause adverse effects on living forms. Copper and ferrous are commonly counted as essential metals to plant cells but sometimes their higher concentration is harmful to plant cells. In the present study effects of copper and ferrous were studied 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 copper and ferrous showed reduction in biomass and growth rate of all three investigated plants.

Keywords: Copper, Ferrous, Effects, Growth Rate, Biomass

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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.

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.

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.

Several modes of biotechniques are named as, biosorption, phyto-sorption, bioaccumulation, phyto-accumulation, bio-extraction, phyto-extraction, rhizofilteration 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*.

MATERIALS AND METHODS

The study was carried out in Rajkot city area (22^{o} 17' Lat. and 70^{o} 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 copper and ferrous on plant growth rate and biomass development, the soil was mixed with heavy metal salts and prepared for the cultivation of experimental plants. The copper and ferrous metals were added in form of copper sulphate salt ($CuSO_4$) and ferrous sulphate ($FeSO_4$ $7H_2O$) and mixed in eight different lots of soil (each lot of 10kg). To get 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mM concentrations of metal salt 1.95, 3.0, 6.0, 7.8, 9.74, 11.7, 13.6 and 15.6 grams of copper sulphate and 2.8, 5.5, 8.3, 11.1, 13.84, 16.6, 19.4 and 22.1 grams ferrous sulphate was used respectively.

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 H_2O_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^{o} C for a period of 8 days for uniform drying. The dry weight of these organs was recorded.

RESULTS

Presence of heavy metal affects growth performance of plants. They showed marked differences in fresh weight and dry weight of root, stem and leaf. The data regarding the fresh weight and dry weight are presented in Table 1 to 12.

Effect of copper on fresh weight

In *Glycine max* fresh weight of root was 11.25 gm, of stem was 14.92 gm and of leaf was 3.39 gm in control condition. It was gradually decreased by increasing copper concentration in the treatment (Table 1 - 3). The lowest fresh weight was found at 1.6 mM copper concentration in all three organs.

In *Vigna unguiculata* the root fresh weight was 13.32 gm, stem fresh weight was 18.85 gm and leaf fresh weight was 5.78 gm in control condition. This was gradually decreased due to the increase in copper concentration in treatment except in root at 0.2 mM copper concentration (Table 1 - 3).

In *Vigna aconitifolia* the root fresh weight was 12.29 gm, stem fresh weight was 16.89 gm and leaf fresh weight was 4.59 gm in control. In root of *Vigna aconitifolia* the proportion of fresh weight was higher than control at lower concentration of copper (0.2 mM copper). It was gradually decreased when copper concentration was increased from 0.4 mM to 1.6 mM copper

(Table 1). In stem the fresh weight was reduced by increasing copper concentrations. Similar results were obtained for leaves also (Table 2 and 3).

Effect of copper on dry weight

In *Glycine max* dry weight of root was 9.68 gm, of stem was 12.83 gm and of leaf was 2.92 gm in control condition. The root dry weight was higher than control at 0.2 mM copper concentration which was gradually decreased when concentration was raised from 0.4 - 1.6 mM copper (Table 7). Stem and leaf dry weight was gradually decreased by increasing the copper concentration in the treatment (Table 8 and 9). The lowest dry weight of all three organs was found at 1.6 mM copper concentration (Table 7 - 9).

In *Vigna unguiculata* the root dry weight was 11.46 gm, stem dry weight was 16.21 gm and leaf dry weight was 4.97 gm in control condition. This was decreased due to the treatment of different concentrations of copper in all three organs except root where at lower copper concentration (0.2 mM) the dry weight was higher than control (Table 7 - 9). The maximum decrease was at 1.6 mM copper concentration (Table 7 - 9).

In *Vigna aconitifolia* the root dry weight was 10.57 gm, stem dry weight was 14.52 gm and leaf dry weight was 3.94 gm in control. In root of *Vigna aconitifolia* the proportion of dry weight was higher than control in treatment of lower copper concentration (0.2 mM). In all other copper treatments the root dry weight was reduced (Table 7). In stem and leaf the dry weight was reduced due to increase in copper concentration in the treatment (Table 8 and 9).

Effect of ferrous on fresh weight

The fresh weight of root was found lower than control in *Glycine max* at all ferrous treatments. In stem of *Glycine max* also the fresh weight was decreased by the treatment of ferrous and was lower than control. In leaves also the fresh weight was reduced due to all concentrations of ferrous in the treatment (Table 4 - 6).

In root, stem and leaf of *Vigna unguiculata* and *Vigna aconitifolia* also the fresh weight was reduced and found lower than control by ferrous treatments (Table 7 - 9).

Effect of ferrous on dry weight

The root, stem and leaf dry weight of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was affected by the treatment of various ferrous concentrations and was lower than control (Table 10 - 12).

Effect of copper on growth rate

The length of *Glycine max* was decreased as the concentration of copper increased in the treatment. The lowest growth rate was found at 1.6 mM copper concentration (Table 13). In *Vigna unguiculata* and *Vigna aconitifolia* also the length of entire plant was reduced due to

increase in copper concentration in the treatment. The length of *Glycine max* and *Vigna aconitifolia* was slightly higher than control at 0.2 mM copper concentration (Table 13 - 15).

Effect of ferrous on growth rate

Ferrous has inhibitory effect on growth rate. Different concentrations of ferrous reduced the growth rate in *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* (Table 13 - 15).

Table 1: Effect of copper on root fresh weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	11.25	13.32	12.29
0.2	11.44 ± 0.02	13.78 ± 0.01	12.61 ± 0.01
0.4	10.79 ± 0.02	12.59 ± 0.01	11.69 ± 0.01
0.6	10.64 ± 0.02	11.87 ± 0.01	11.26 ± 0.01
0.8	9.81 ± 0.02	10.63 ± 0.01	10.22 ± 0.01
1.0	9.78 ± 0.02	10.14 ± 0.01	9.96 ± 0.01
1.2	8.72 ± 0.02	9.58 ± 0.01	9.15 ± 0.01
1.4	8.34 ± 0.02	9.04 ± 0.01	8.69 ± 0.01
1.6	8.22 ± 0.02	8.84 ± 0.01	8.53 ± 0.01

Table 2: Effect of copper on stem fresh weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	14.92	18.85	16.89
0.2	14.37 ± 0.02	17.64 ± 0.01	16.01 ± 0.01
0.4	13.49 ± 0.02	17.53 ± 0.01	15.51 ± 0.01
0.6	12.79 ± 0.02	17.22 ± 0.01	15.01 ± 0.01
0.8	11.84 ± 0.02	16.96 ± 0.01	14.40 ± 0.01
1.0	10.91 ± 0.02	16.53 ± 0.01	13.72 ± 0.01
1.2	10.89 ± 0.02	15.32 ± 0.01	13.11 ± 0.01
1.4	10.61 ± 0.02	15.18 ± 0.01	12.90 ± 0.01
1.6	10.43 ± 0.02	14.98 ± 0.01	12.71 ± 0.01

Table 3: Effect of copper on leaf fresh weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	3.39	5.78	4.59
0.2	3.27 ± 0.01	5.41 ± 0.01	4.34 ± 0.01
0.4	3.22 ± 0.01	5.26 ± 0.01	4.24 ± 0.01
0.6	2.81 ± 0.01	5.02 ± 0.01	3.92 ± 0.01
0.8	2.63 ± 0.01	4.88 ± 0.01	3.76 ± 0.01
1.0	2.36 ± 0.01	4.59 ± 0.01	3.48 ± 0.01
1.2	2.14 ± 0.01	4.24 ± 0.01	3.19 ± 0.01
1.4	1.92 ± 0.01	3.98 ± 0.01	2.95 ± 0.01
1.6	1.47 ± 0.01	3.56 ± 0.01	2.52 ± 0.01

Table 4: Effect of ferrous on root fresh weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	11.25	18.39	14.27
0.2	10.97 ± 0.33	17.92 ± 0.14	13.89 ± 0.01
0.4	10.85 ± 0.33	17.18 ± 0.14	12.66 ± 0.01
0.6	10.74 ± 0.33	16.72 ± 0.14	11.95 ± 0.01
0.8	9.82 ± 0.33	15.36 ± 0.14	11.07 ± 0.01
1.0	9.90 ± 0.33	15.12 ± 0.14	10.44 ± 0.01
1.2	10.93 ± 0.33	15.82 ± 0.14	9.78 ± 0.01
1.4	11.14 ± 0.33	15.85 ± 0.14	9.21 ± 0.01
1.6	11.17 ± 0.33	16.29 ± 0.14	8.64 ± 0.01

Table 5: Effect of ferrous on stem fresh weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	12.83	22.49	19.32
0.2	12.41 ± 0.25	21.77 ± 0.12	18.73 ± 0.01
0.4	12.38 ± 0.25	21.41 ± 0.12	18.06 ± 0.01
0.6	11.50 ± 0.25	20.26 ± 0.12	17.53 ± 0.01
0.8	8.74 ± 0.25	17.21 ± 0.12	16.94 ± 0.01
1.0	9.50 ± 0.25	17.60 ± 0.12	16.21 ± 0.01
1.2	10.23 ± 0.25	18.04 ± 0.12	15.62 ± 0.01
1.4	11.61 ± 0.25	19.00 ± 0.12	14.78 ± 0.01
1.6	11.29 ± 0.25	18.27 ± 0.12	13.97 ± 0.01

Table 6: Effect of ferrous on leaf fresh weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	4.01	6.81	5.60
0.2	3.90 ± 0.31	6.61 ± 0.12	5.44 ± 0.01
0.4	3.87 ± 0.31	6.43 ± 0.12	5.12 ± 0.01
0.6	3.71 ± 0.31	6.16 ± 0.12	4.91 ± 0.01
0.8	3.09 ± 0.31	5.43 ± 0.12	4.67 ± 0.01
1.0	3.23 ± 0.31	5.45 ± 0.12	4.44 ± 0.01
1.2	3.53 ± 0.31	5.64 ± 0.12	4.23 ± 0.01
1.4	3.81 ± 0.31	5.81 ± 0.12	4.00 ± 0.01
1.6	3.88 ± 0.31	5.76 ± 0.12	3.77 ± 0.01

Table 7: Effect of copper on root dry weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	9.68	11.46	10.57
0.2	9.84 + 0.01	11.85 + 0.01	10.84 + 0.01
0.4	9.28 + 0.01	10.83 + 0.01	10.05 + 0.01
0.6	9.15 + 0.01	10.21 + 0.01	9.68 + 0.01
0.8	8.24 + 0.01	8.93 + 0.01	8.58 + 0.01
1.0	8.12 + 0.01	8.42 + 0.01	8.27 + 0.01
1.2	7.15 + 0.01	7.86 + 0.01	7.50 + 0.01
1.4	6.67 + 0.01	7.23 + 0.01	6.95 + 0.01
1.6	6.41 + 0.01	6.90 + 0.01	6.65 + 0.01

Table 8: Effect of copper on stem dry weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	12.83	16.21	14.52
0.2	12.36 ± 0.01	15.17 ± 0.01	13.76 ± 0.01
0.4	11.60 ± 0.01	15.08 ± 0.01	13.34 ± 0.01
0.6	11.00 ± 0.01	14.81 ± 0.01	12.90 ± 0.01
0.8	9.95 ± 0.01	14.25 ± 0.01	12.10 ± 0.01
1.0	9.06 ± 0.01	13.72 ± 0.01	11.39 ± 0.01
1.2	8.93 ± 0.01	12.56 ± 0.01	10.75 ± 0.01
1.4	8.49 ± 0.01	12.14 ± 0.01	10.32 ± 0.01
1.6	8.14 ± 0.01	11.68 ± 0.01	9.91 ± 0.01

Table 9: Effect of copper on leaf dry weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	2.92	4.97	3.94
0.2	2.81 ± 0.01	4.65 ± 0.01	3.73 ± 0.01
0.4	2.77 ± 0.01	4.52 ± 0.01	3.65 ± 0.01
0.6	2.42 ± 0.01	4.32 ± 0.01	3.37 ± 0.01
0.8	2.21 ± 0.01	4.10 ± 0.01	3.15 ± 0.01
1.0	1.96 ± 0.01	3.81 ± 0.01	2.88 ± 0.01
1.2	1.75 ± 0.01	3.48 ± 0.01	2.62 ± 0.01
1.4	1.54 ± 0.01	3.18 ± 0.01	2.36 ± 0.01
1.6	1.15 ± 0.01	2.78 ± 0.01	1.96 ± 0.01

Table 10: Effect of ferrous on root dry weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	9.68	15.81	12.27
0.2	9.43 ± 0.28	15.41 ± 0.05	11.95 ± 0.01
0.4	9.33 ± 0.28	14.77 ± 0.05	10.89 ± 0.01
0.6	9.24 ± 0.28	14.37 ± 0.05	10.28 ± 0.01
0.8	8.25 ± 0.28	12.90 ± 0.05	9.30 ± 0.01
1.0	8.22 ± 0.28	12.55 ± 0.05	8.67 ± 0.01
1.2	8.96 ± 0.28	12.97 ± 0.05	8.02 ± 0.01
1.4	8.99 ± 0.28	12.68 ± 0.05	7.37 ± 0.01
1.6	9.34 ± 0.28	12.71 ± 0.05	6.74 ± 0.01

Table 11: Effect of ferrous on stem dry weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	11.03	19.34	16.62
0.2	10.67 ± 0.18	18.73 ± 0.07	16.11 ± 0.01
0.4	10.64 ± 0.18	18.41 ± 0.07	15.53 ± 0.01
0.6	9.89 ± 0.18	17.43 ± 0.07	15.08 ± 0.01
0.8	7.35 ± 0.18	14.46 ± 0.07	14.23 ± 0.01
1.0	7.88 ± 0.18	14.61 ± 0.07	13.45 ± 0.01
1.2	8.39 ± 0.18	14.80 ± 0.07	12.81 ± 0.01
1.4	9.29 ± 0.18	15.20 ± 0.07	11.82 ± 0.01
1.6	8.80 ± 0.18	14.25 ± 0.07	10.90 ± 0.01

Table 12: Effect of ferrous on leaf dry weight

Concentration (mM)	*Glycine max* (gm)	*Vigna unguiculata* (gm)	*Vigna aconitifolia* (gm)
Control	3.45	5.86	4.81
0.2	3.35 ± 0.21	5.69 ± 0.06	4.68 ± 0.01
0.4	3.33 ± 0.21	5.53 ± 0.06	4.40 ± 0.01
0.6	3.19 ± 0.21	5.30 ± 0.06	4.23 ± 0.01
0.8	2.60 ± 0.21	4.56 ± 0.06	3.92 ± 0.01
1.0	2.68 ± 0.21	4.53 ± 0.06	3.69 ± 0.01
1.2	2.89 ± 0.21	4.63 ± 0.06	3.47 ± 0.01
1.4	3.05 ± 0.21	4.65 ± 0.06	3.20 ± 0.01
1.6	3.02 ± 0.21	4.49 ± 0.06	2.94 ± 0.01

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

Concentration (mM)	Length of plant (cm)		
	Control	Copper	Ferrous
Water	52.00	-	-
0.2	-	52.90 ± 0.01	50.70 ± 0.32
0.4	-	49.80 ± 0.01	50.15 ± 0.32
0.6	-	49.10 ± 0.01	49.65 ± 0.32
0.8	-	45.40 ± 0.01	48.30 ± 0.32
1.0	-	45.20 ± 0.01	47.80 ± 0.32
1.2	-	40.30 ± 0.01	47.70 ± 0.32
1.4	-	38.50 ± 0.01	46.90 ± 0.32
1.6	-	37.60 ± 0.01	46.55 ± 0.32

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

Concentration (mM)	Length of plant (cm)		
	Control	Copper	Ferrous
Water	70.00	-	-
0.2	-	67.40 ± 0.02	67.70 ± 0.03
0.4	-	63.30 ± 0.02	65.30 ± 0.03
0.6	-	60.00 ± 0.02	58.50 ± 0.03
0.8	-	55.50 ± 0.02	40.80 ± 0.03
1.0	-	51.20 ± 0.02	31.30 ± 0.03
1.2	-	51.10 ± 0.02	26.20 ± 0.03
1.4	-	49.80 ± 0.02	25.50 ± 0.03
1.6	-	48.90 ± 0.02	24.70 ± 0.03

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

Concentration (mM)	Length of plant (cm)		
	Control	Copper	Ferrous
Water	61.20	-	-
0.2	-	62.8 ± 0.01	59.6 ± 0.01
0.4	-	58.2 ± 0.01	52.8 ± 0.01
0.6	-	56.1 ± 0.01	44.3 ± 0.01
0.8	-	49.7 ± 0.01	34.3 ± 0.01
1.0	-	47.9 ± 0.01	25.1 ± 0.01
1.2	-	43.5 ± 0.01	17.2 ± 0.01
1.4	-	40.3 ± 0.01	11.1 ± 0.01
1.6	-	38.5 ± 0.01	6.7 ± 0.01

DISCUSSION

Dry matter yield decrease has generally been accepted as the standard measure for comparisons of toxicity. However, occasionally other measures such as fresh weight, commencement of symptoms (Elamin and Wilcox, 1986), and metabolic responses have been used (Gherardi *et al.*, 1999).

Effect of copper on growth rate and biomass

Copper is an essential plant micro nutrient required for the protein components of overall enzymes (Marschner, 1995). However, when present in excess quantities, copper is also toxic to plant growth potentially causing damage resulting in complete inhibition of growth (Kopittke and Menzies, 2006).

According to Kopittke and Menzies (2006) and Kopittke *et al*., (2007) the higher concentration of copper inhibits the growth of root and stem. The other workers Smirnoval *et al.* (2006), Lin *et al.* (2003) and Ali *et al.* (2002) have reported decrease in root and shoot growth due to higher amount of copper in the treatment.

In the present work the decrease in fresh weight and dry weight of root, stem and leaf of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was observed when concentration of copper was increased in the treatment. Zhu and Alva (1993) and Kopittke and Menzies (2006) also found decrease in fresh weights due to higher concentration of copper and explained that

shoot growth reduction is not due to direct toxicity of copper in the shoots, but rather to nutrient deficiencies resulting from a reduced nutrient uptake by the damaged roots. Kopittke and Menzies (2006) reported reduction in growth of cow pea due to copper treatment. In the present work also reduction in growth of investigated plants was observed due to higher concentration of copper.

Effect of ferrous on growth rate and biomass

Ferrous is an essential nutrient for plants. It functions to accept and donate electrons and plays important roles in the electron-transport chains of photosynthesis and respiration (Connolly and Guerinot, 2002; Ghasemain *et al*., 2010). But ferrous is toxic when it accumulates to high levels, which can damage lipids, proteins and DNA. Plants must therefore respond to iron stress in terms of both iron deficiency and iron overload (Connolly and Guerinot, 2002).

Iron plays important role in numerous physiological functions. Soil condition leading to iron deficiency (such as calcareous soil), or increasing the uptake of ferrous (such as soil water logging) are widespread in nature (Snowden and Wheeler, 1993). Limited growth under ferrous deficiency or toxicity described by various parameters, i.e. biomass accumulation, shoot and root length, area and number of leaves, relative growth rate etc. has been reported for different plant species (Foy *et al*., 1978; Nenova and Stoyanov, 1993; Snowden and Wheeler, 1993; Dobermann and Fairhurst, 2000; De la Guardia and Alcantara, 2002; Batty and Younger, 2003).

Few workers studied the effect of various concentrations of ferrous on plants and obtained different types of results. Ghasemain *et al*. (2010) reported increase in biomass of soya bean by the treatment of 50 Kg / hector of iron. Singh (1995) and Naik (1984) also found similar results. These results have conformity with the results obtained by the study of Singh and Shaha (1990). According to Nenova (2008) ferrous deficiency in pea plant resulted decrease in biomass. Kuraev (1966) suggested that high internal iron concentrations in plant tissue reduce plant yield. In the current work the fresh weight and dry weight of root, stem and leaves of *Glycine max*, *Vigna unguiculata* and *Vigna aconitifolia* was observed lower than control due to ferrous treatment.

Nenova (2009) reported decrease in root growth of pea plant due to ferrous deficiency. Ward *et al.* (2008) observed decrease in root length under the influence of higher concentration of ferrous in *Arabidopsis*. Kuraev (1966) reported inhibition of root growth at the higher concentration of ferrous. Laan *et al.* (1991) reported that the growth of *Rumax* was decreased by increasing the concentration of ferrous ion in nutrient solution. This was accompanied by corresponding decrease in root development. In present work also decrease in growth rate of *Vigna unguiculata* and *Vigna aconitifolia* was observed by increasing the concentration of

ferrous in the treatment. The inhibition of shoot and root growth due to higher ferrous concentration was due to ferrous toxicity (Nenova, 2009).

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