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EFFECT OF HEAVY METALS ON PISUM SATIVUM LINN.

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ABSTRACT
A pot experiment was carried out to study the responses of *Pisum sativum* L. growing in Copper, Zinc and Lead treated soil. Three different soil concentrations of each heavy metal were prepared from salts of these metals (100, 200 and 400 mg/Kg for Cu, Zn and Pb) bearing twenty replicates for each heavy metal and for control . Percentage of germination was found lowest (60%) in Zn 400 mg/kg and found highest (87.5%) in Cu 100 mg/kg and 100% in the control. A decrease in plant height ($p<0.05$) and the total number of leaflets ($p>0.05$) was observed in all the treatment plants compared to the control (except Pb 200 mg/kg). Severe chlorosis, necrosis and senescence were observed in most of the treatment plants. Drastic fluctuations were observed in Chlorophyll content, regarding the concentration of chlorophyll a and b in the treatment plants, compared to control. In the first estimation (after 30 days of germination of seeds), high Proline content was observed in all the treatment plants compared to the control (except Cu 100 mg/kg and Zn 200 mg/kg), and in second estimation (after 60 days of germination of seeds), the amounts were decreased except in a few of the treatments compared to the data obtained in first estimation. Experiment showed that higher the concentration of heavy metals the more toxic effect to *Pisum sativum*. Zinc and Lead showed more toxicity to the plant compared to Copper.

KEY WORDS: Toxicity, Germination, Chlorophyll, Proline.

INTRODUCTION
Heavy metal contamination affects the biosphere in many places worldwide. Being primary producers, green plants accumulate contaminants from the soil and atmosphere (Harrison and Chirgawi 1989), plants can contribute elements in harmful concentrations through the food chain (Welch *et al*. 1993). Metal concentrations in soil range from less than 1 mg/kg (ppm) to as high as 100,000 mg/kg, whether due to the geological origin of the soil or as a result of human activity (Blaylock and Huang, 2000). Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plant species (Claire *et al*. 1991; Fernandes and Henriques, 1991). Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on live plants (Thompson *et al*. 1997; Paivoke and Simola, 2001). Most of these studies have been conducted using seedlings or adult plants (Lee *et al*.1999; Gratton *et al*. 2000). In a few of the studies, the seeds have been exposed to the contaminants (Claire *et al*. 1991; Xiong, 1998).
Unlike other heavy metals, such as cadmium, lead and mercury, copper is not readily bioaccumulated and thus its toxicity to man and other mammals is relatively low. On the contrary, plants in general are very sensitive to Cu toxicity, displaying metabolic disturbances and growth inhibition (Fernandes and Henriques, 1991). Copper absorbed by plants, apparently accumulates in roots, even in cases where roots have been damaged by toxicity (Adriano, 2001). Excess Cu inhibits a large number of enzymes and interferes with several aspects of plant biochemistry, including photosynthesis, pigment synthesis, and membrane integrity. At the whole plant level Cu is an effective inhibitor of vegetative growth and induces general symptoms of senescence. The high toxicity of Cu to plants has led to the evolution of several strategies of defense. Among the most important ones is the production of Cu-complexing compounds (Fernandes and Henriques, 1991).
The global average Pb concentration of soils ranges from 2 to 100 mg/kg (Siegel, 1998). Pb is among those elements, which at elevated concentrations constitute a potential threat to the environment and human health (OECD, 1996). Globally, children living in poorer quarters often suffer from acute Prenatal and childhood exposure to Pb which causes neuropsychological and social problems and anaemia (Lanphear, 1998; Needleman, 1999). Reduced fertility in women exposed to Pb has been attributed to interaction between Pb and oestrogen metabolism and to the mutagenicity of Pb (Tchernitchin *et al*., 1998). Zn despite its role as one of the essential micronutrients, at enhanced concentration in the environment, also becomes a hazard to living beings (WHO, 2001). The growth-improving ability of Zn on *Aspergillus* was recognized in 1869-1870 (Brown *et al*., 1993; Kiekens, 1995). Soil Fertility and health of soil microflora show sensitivity to soil Zn (Chaudri *et al*., 1993; Bruins *et al*., 2000). The potential for high toxicity of Zn lies in its function as a micronutrient, ready uptake by plants, and high soil and *in-planta* mobility (Longnecker and Robson, 1993).

MATERIALS AND METHODS
Study site
The study was carried out in Tezpur University campus (lat 26º41´50.1´´N, long 92º49´56.3´´E, elevation 56.2

msl) in Assam, India during January 2009 to March 2009. The average minimum temperature of the study area (Tezpur, Assam) ranges from 12.44ºC to 20.36ºC and maximum temperature from 24.55ºC to 29ºC, relative humidity ranges from 60- 82.2% and rainfall 4.81mm-34mm.

Plant sample

The plant sample taken for experiment is *Pisum sativum* Linn. (Family- Leguminoceae), an annual climbing herb, commonly known as green pea. The plant is chosen to carry out the experiment for its short life cycle (one year). It is a cool season crop grown in many parts of the world.

The Experimental Design

A pot experiment was conducted using heavy metals. The experiment was designed to identify the differences in the germination and growth of *Pisum sativum* under three heavy metal stress. The measurements determined were (a) germination, (b) plant height and number of leaves, (c) chlorophyll content in leaves and (d) proline content in leaves.

Three heavy metal salts $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and Pb $(CH_3COO)_2$. $3H_2O$ were taken for treatment in soil. Three different concentrations were used and mixed thoroughly with soil of definite amount (1kg) that were passed through a 2 mm sieve (oven-dried base). The three concentrations prepared were- 100mg/kg, 200mg/kg and 400mg/kg of soil for Cu, Zn and Pb. One control was also prepared without applying any heavy metal to compare the affects. For all the concentrations of Cu, Zn, Pb, and for control, 20 replicates each were taken making a total of 200 pots. The soils treated with different concentrations of heavy metals were filled at a definite amount in plastic pots.

Seeds of pea (*Pisum sativum* L.) were soaked in cold water for about 6 hours, and then two viable seeds were sown in each pot containing different concentrations of the three heavy metal treated soils. Each pot was supplied with 150 ml of tap water every alternate day to moisten the soil. The experiment was conducted in a green house wherein on average the temperature was a maximum 26.5ºC and minimum 21ºC, relative humidity was 64% and light intensity was 26,400 lux. The physico-chemical properties of the soil used to conduct the experiment was given in table I.

TABLE I. Physico-chemical properties of the soil

SOIL PARAMETERS	VALUES	SOIL PARAMETERS	VALUES
Soil Texture	Sandy clay loam	Soil Potassium (mg/kg)	126.8
Sand (%)	62.4	Total Organic carbon (%)	0.093
Silt (%)	18	Soil Sulphate (mg/kg)	17.75
Clay (%)	19.6	Soil Nitrate (mg/kg)	47.55
Bulk density (g/cm^3)	1.42	Available Nitrogen (%)	0.0173
Water Holding capacity (%)	39.6	Soil Phosphorous (mg/kg)	0.8
Soil moisture content (%)	6.82	Copper (ppm)	0.861
Soil pH	6.51	Zinc (ppm)	0.42
Soil conductivity (µS/cm)	81.83	Lead (ppm)	0.986

Observation regarding the germination of the seed was done from the first day of sowing of seeds.

Growth and Development of the Plants

Growth and development was recorded in terms of plant height and number of leaflets. After one week of germination, the readings/observations were taken in weekly intervals. Any symptom of toxicity in the form of chlorosis, necrosis, drying and senescence were noted for different treatment plants during the growth stages.

Estimation of Chlorophyll and Proline Content in the Plant Leaves

Chlorophyll content in the fresh plant leaves was estimated (mg Chlorophyll/gm of fresh leaf weight) as per Witham *et al*., 1971 and Bansal *et al*., 2002.

Free proline in plants is said to play a role under induced, cold, drought, salt and physiological stress condition. Amount of free proline is estimated by Ninhydrin method (Bates *et al*., 1973) and expressed as µM proline per gram tissue. Two estimations were carried out for chlorophyll and proline, one after 30 days and one after 60 days of germination of seeds.

Statistical analysis: Data were analyzed with one way ANOVA for height and number of leaves of treatment plants and control.

RESULTS AND DISCUSSION

Germination of seeds

Germination of seeds started on the 3rd day after sowing of seeds. In control 38 seeds were germinated on 3rd day. Germination percentage was found lowest (60%) in concentration of Zn having 400 mg/kg. Number of germinated seeds increased in the 7th day of observation. Both the seeds sown were failed to germinate in two sample pot of Pb 400mg/kg and one sample pot of Zn 400 mg/kg. Germination percentages of seeds till the 14th day of observation were given in table II. There was no more germination after the 14th day of sowing of seed.

TABLE II. Number of seeds germinated (Total number of seed sown per treatment = 40)

Heavy metals	Treatments	Germination %
Nil(Control)	Control	100
	100 mg/kg	87.5
Copper (Cu)	200 mg/kg	62.5
	400 mg/kg	65
	100 mg/kg	82.5
Zinc (Zn)	200 mg/kg	80
	400 mg/kg	60
	100 mg/kg	82.5
Lead (Pb)	200 mg/kg	80
	400 mg/kg	72.5

Increase in plant height

The plant heights in control were gradually increased from the first observation (2/1/09) to the last observation (13/3/09) and the rate of increase in height was almost uniform in the successive observations till two months (Fig 1,2, &3). The height of the plants that were grown in different concentrations of the four heavy metals was much less as compared to the control. It has observed that, the height of the plants gradually decreased ($F= 5.206$; $P<0.05$) as the concentration of Cu increased from 100-400mg/kg (Fig 1).

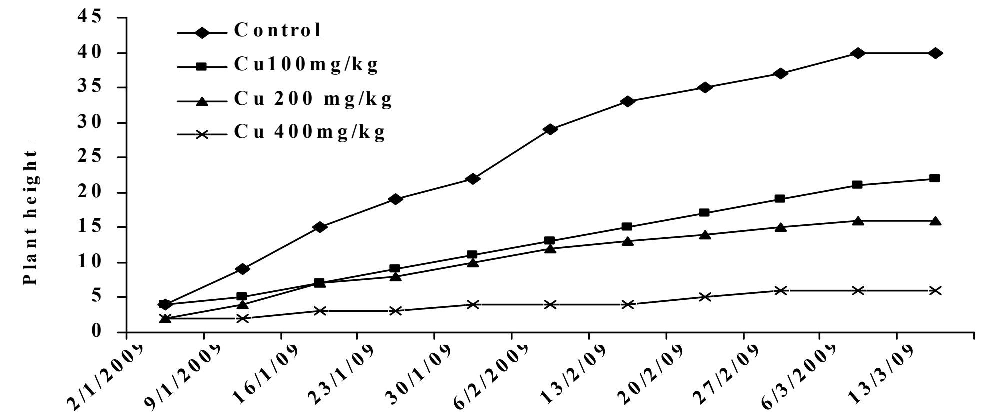

Fig. 1: Growth in terms of height (cm) of *Pisum sativum* L. growing in control and Cu treated soil.

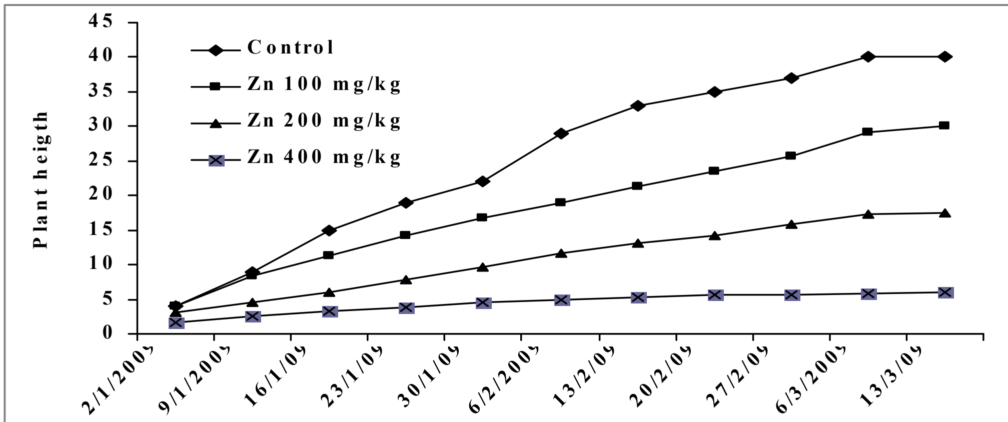

Fig. 2: Growth in terms of heigth (cm) of *Pisum sativum* L. growing in control and Zn treated soil.

This may be due to the accumulation of Cu in the plant tissue which has various metabolic effects on plant. Plants in general are very sensitive to Cu toxicity, and displays Cu toxicity by growth inhibition even at a very smaller concentration due to metabolic disturbances (Fernandes and Henriques, 1991). Towards the 10th observation, the growth in terms of height increment declined. The flowering and podding were also delayed as the concentration of Cu increased in the treatments.

Similarly, in Zn treated soils the heights of the plants were gradually declined when the concentration increased from lower to higher. The heights of the plants in the three treatments were significantly different (F=16.03; P<0.05). In first observation, Zn showed an increase in growth rate This may be due to the fact that, Zn is an essential micronutrient and is mobile in plants. The potential for high toxicity of Zn lies in its function as a micronutrient, ready uptake by plants, and high soil and *in-planta* mobility (Longnecker and Robson, 1993). It was observed that, at 400mg/kg Zn concentration, after the 4th observation the height increment was negligible. This reveals that at higher concentrations than the optimum requirement, Zn become toxic to plants.

In the treatments of Pb, growth inhibition was much higher as compared to Cu and Zn. The height of the plants as compared to the other treatments i.e. Cu and Pb (Fig 2).

in the three treatment plants also differ significantly (F=11.573; P<0.05). But interestingly, it was observed that at the concentration of Pb 200 mg/kg the growth rate was higher as compared to 100 and 400 mg/kg (Fig 3).

It may be due to the cause that, in soil with pH from 5.5 to 7.5, lead solubility is controlled by phosphate or carbonate precipitates that constrain plant lead uptake, even if plants have the capacity to accumulate it (Blaylock *et al*., 1997). In all the concentrations of Pb, compared to the number of leaves, the heights of the plants were very less and the lengths of internodes were very short.

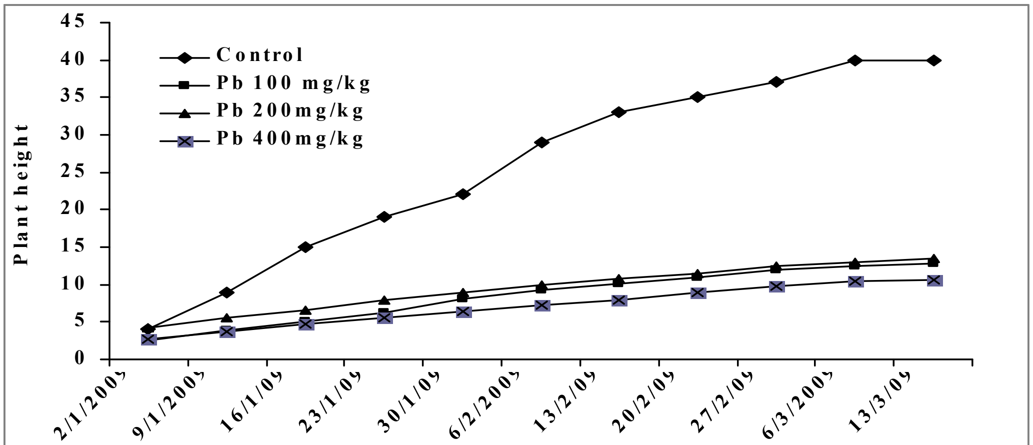

Fig 3. Growth in terms of height (cm) of *Pisum sativum* L. growing in control and Pb treated soil.

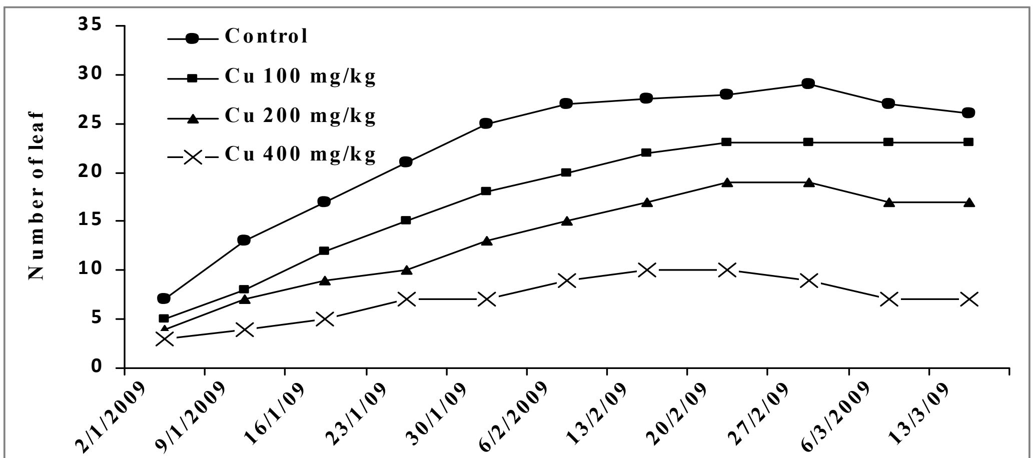

Fig. 4: Number of leaves (leaflets) of *Pisum sativum* L. growing in control and Cu treated soil.

Increase in number of leaflets

The numbers of leaflets were less in all the treatments than the control. Up to the 7th week, the number of leaflets increased at a faster rate, but decreased thereafter. A similar pattern of gradual decrease in the number of leaflets from lower to higher concentration was observed in the heavy metals except Pb (Fig 4,5,6), though the differences in leaf number were insignificant (F=1.454, P>0.05; F= 2.53, P>0.05 and F=1.74, P>0.05) in the three concentration. Towards the 9th observation, the number of leaflets become very less due to chlorosis. Chlorosis was observed in 400mg/kg concentration of all the heavy metals.

In case of Zn, the decrease in leaflet number is slightly less as compared to the Cu treated plants. Zn 200mg/kg showed a gradual increase in the number of leaflets up to 9th observation (Fig 5). This may be due to some stimulatory effect of Zn on plant. But at Zn 400mg/kg concentration, the number of leaflets suddenly decreased after 9th observation. This happened may be due to the toxic affect of Zn at higher concentration.

The number of leaflets in 200 mg/kg of Pb was more compared to Pb 100 and Pb 400mg/kg (Fig 6). Leaf damage has been seen remarkably from 8th observation onwards.

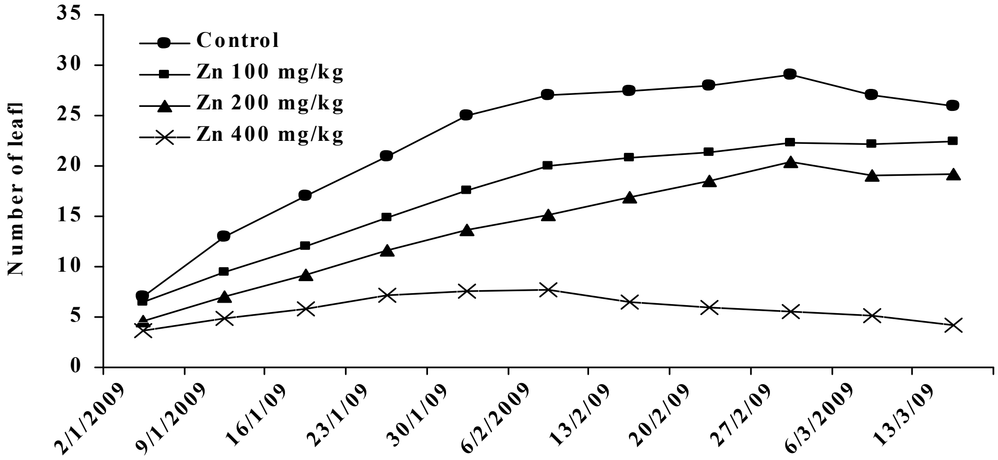

Fig. 5: Number of leaves (leaflets) of Pisum sativum L. growing in control and Zn treated soil

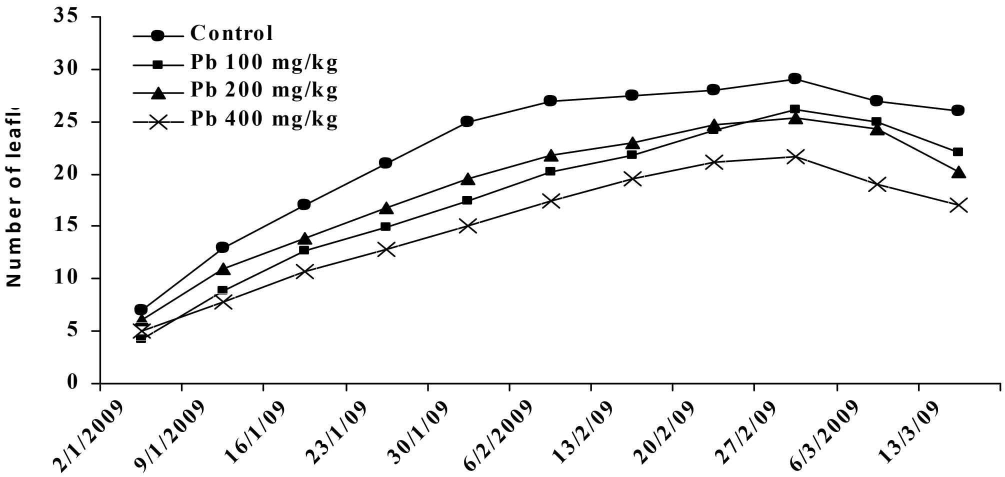

Fig. 6: Number of leaves (leaflets) of Pisum sativum L. growing in control and Pb treated soil.

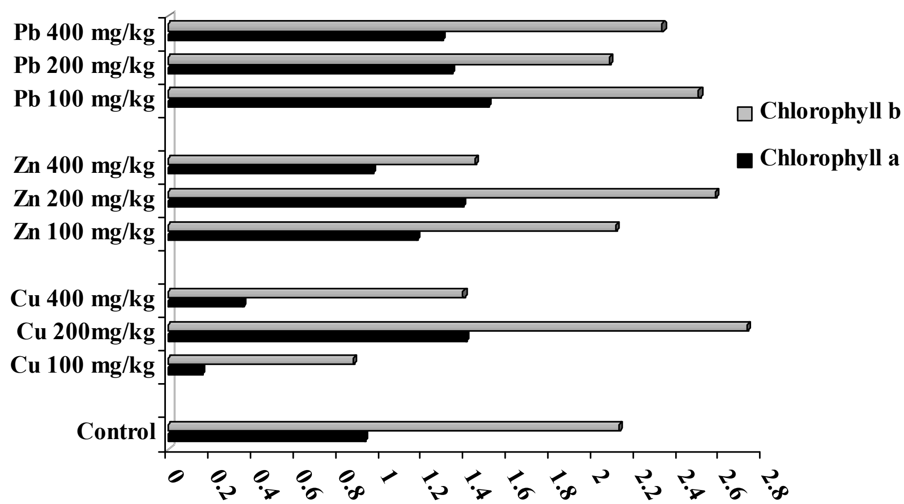

Fig. 7: Concentration (mg chl/gm of fresh leaf weight) of chlorophyll a and b in control and the treatments (after 30 days of germination of seeds).

Changes in the chlorophyll content

The chlorophyll concentration was found to be increased in most of the treatments compared to control during the first observation i.e. 30 days after sowing of seeds (Fig 7). In the second observation (60 days after sowing of seeds) the chlorophyll contents estimated were very less in all the treatments because of severe chlorosis and necrosis but this amount is still higher than the control in most of the treatments (Figure 8). In the first observation, the *chlorophyll a, chlorophyll b* and total chlorophyll concentrations were highest in Cu 200 mg/kg (4.123mg chl/gm of fresh leaf weight) treatment. But in the second observation, Chlorophyll a concentration was found highest in treatment Pb 200mg/kg (0.61mg chl/gm of fresh leaf weight) and chlorophyll b and total chlorophyll concentration found highest in Pb 100 mg/kg treatment. This change in the chlorophyll a/b ratio observed in pea plants grown in different heavy metal treated soils may be due to alterations in photosystem II (Young *et al*., 1996).

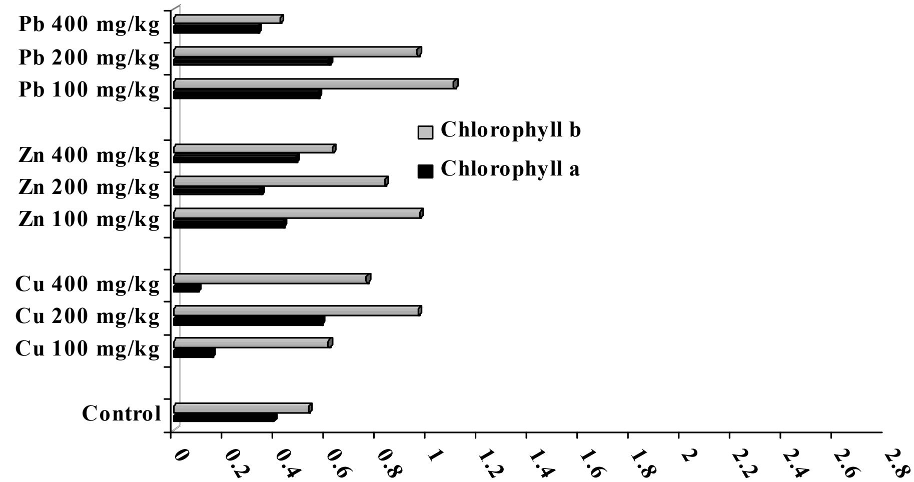

Fig. 8: Concentration(mg chl/gm of fresh leaf weight) of chlorophyll a and b in control and the treatments (after 60 days of germination of seeds).

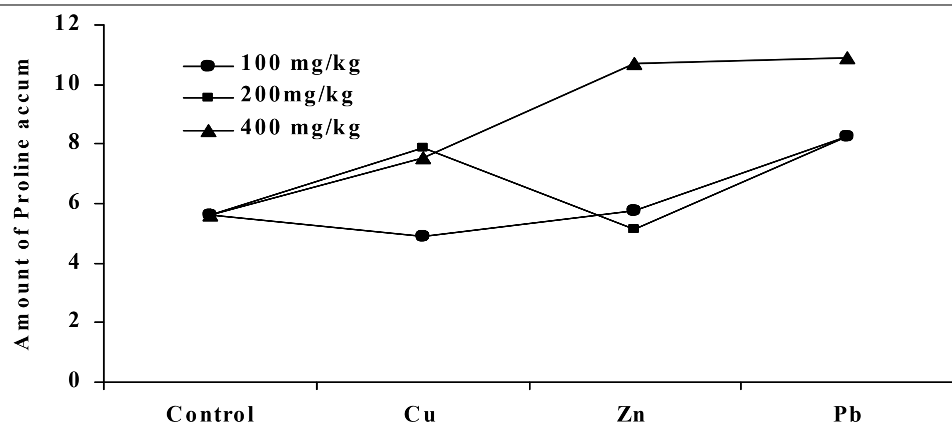

Fig. 9 : Proline concentration in the plant leaves in control and in all the treatments (After 30 days of germination of seeds).

Changes in the proline content

The proline estimation showed an increase in the first observation (30 days after sowing of seeds) in most of the treatments compared to the control (Figure 9). Proline content reached highest in the treatment of Pb 400mg/kg (10.88µmole proline/g tissue) in the first observation. In Cu 100mg/kg, the concentration of proline is found to be least (4.91µmole proline/g tissue). Proline concentration decreased in a small amount in Cu 100 mg/kg and Zn 200 mg/kg compared to the control.

In the second observation (60 days after sowing of seeds), increase in Proline content was observed in some of the treatments than the first observation except Cu 200 mg/kg, Cu 400 mg/kg, Zn 200 mg/kg, Zn 400 mg/mg, Pb 400 mg/kg and control (Figure 10). In all the treatments it has been seen that more accumulation of proline occurred in the highest concentration of the heavy metals. This indicates that there is more accumulation of proline when the plant suffers from severe environmental stress. Proline accumulation may reduce stress-induced cellular acidification or prime oxidative respiration to provide energy needed for recovery. High levels of proline synthesis during stress may maintain $NAD(P)^+/NAD(P)H$ ratios at values compatible with metabolism under normal conditions . This would provide precursors to support the demand for increased secondary metabolite production during stress as well as nucleotide synthesis accompanying the accelerated rate of cell division upon relief from stress, when oxidation of proline is likely to provide an important energy source for ADP phosphorylation. Thus, the extreme sensitivity of the metabolic processes of proline synthesis and degradation themselves may be of benefit by regulating metabolic processes adversely affected by stress (Hare and Cress, 1997).

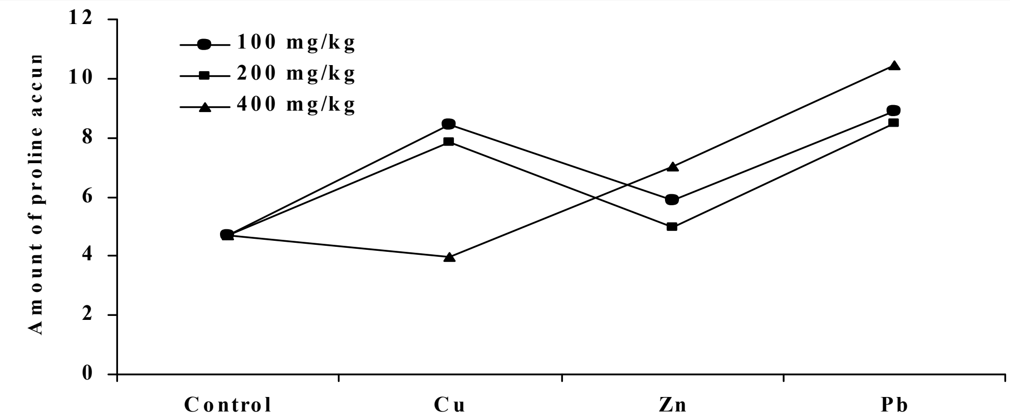

Fig. 10: Proline concentration in the plant leaves in control and in all the treatments (After 60 days of germination of seeds).

CONCLUSION

Toxic effects of heavy metals are everywhere and in almost all the plants. But the intensity of toxicity may vary from plant to plant. In the present study it has observed that, heavy metals in higher concentration are toxic to plant's growth and development in response to their physiological and morphological characteristics. The heights of *Pisum sativum* were found to be less in the treatment plants at various concentrations than the control. The numbers of leaves were also less in the plants growing in heavy metal treated soil compared to the plants grown in control condition.

From the results obtained in this study, it can be revealed that, heavy metals affect the growth and development of *P. sativum*. The heavy metals (Cu, Zn and Pb) used in the study at various concentrations have significant impact on the growth and development of *Pisum sativum* in terms of height and number of leaves.

Estimation of chlorophyll and proline content also shows a gradual change at different concentration of heavy metals thereby affects the characteristic development of *Pisum sativum L*.

Hence it can be concluded that, higher concentration of any kind of heavy metal present in the soil may affect the overall growth and development, and productivity of the plants. Indirectly, it may lead to certain health hazards due to the consumption of contaminated plants and food. Therefore, study on amount of heavy metals accumulated in different parts of the plants, growing in the contaminated soil, will provide a valuable support to understand the impact on human health.

REFERENCES

Adriano, D.C. (2001) Trace elements in the Terrestrial Environments. Springer-Verlag, Heidelberg.

Bansal, P., Sharma, P., Goyal, V. (2002) Impact of lead and cadmium on enzyme of citric acid cycle in germinating pea seeds. Plant Biology, 45, 125-127.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973) Rapid determination of free proline for water stress studies. Plant and Soil. 39, 205-207.

Blaylock ,M.J., Huang, J.W. (2000) Phytoremediation of toxic metals: using plants to clean up the environment. In: Raskin I. and B.D. Ensley (ed) Phytoextraction of metal. John Wiley and Sons, Inc, Toronto, 303.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakarova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I. (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol, 31, 860–865.

Brown, P.H., Cakmak, I., Zhang, Q. (1993) Form and function of zinc plants. In: Robson A.D. (ed) Zinc in Soils and Plants. Proc. In. Symp. 'Zinc in Soils and Plants' University of W. Australia, 93-106.

Bruins, M.R., Kapil, S., Oehme, F.W. (2000) Microbial resistance to metals in the environment. Ecotoxicol Environ Safety, 45B, 198-207.

Chaudri, A.M., McGrath, S.P., Giller, K.E., Rietz, E., Sauerbeck, D.R. (1993) Enumeration of indigenous *Rhizobium leguminosarum* biovar *trifolii* in soils previously treated with metal-contaminated sew age sludge. Soil Biol Biochem 25, 301-309.

Claire, L.C., Adriano, D.C., Sajwan, K.S., Abel, S.L., Thoma, D.P., Driver, J.T.(1991) Effects of selected trace metals on germinating seeds of six plant species. Water, Air, and Soil Pollution, 59, 231-240.

Fernandes, J.C., Henriques, F.S. (1991): Biochemical, physiological, and structural effects of excess copper in plants. The Botanical Review, 57(3), 246-273.

Gratton, W.S., Nkongolo, K.K., Spiers, G.A. (2000) Heavy metal accumulation in soil and Jack pine (*Pinus banksiana*) needles in Sudbury, Ontario, Canada, Bull. Environ. Contam Toxicol, 64, 550-557.

Hare, P.D., Cress, W.A. (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regulation, 21, 79–102.

Harrison, R.M., Chirgawi, M.B. (1989) The assessment of air and soil as contributors of some trace metals to vegetable plants. Use of a filtered air growth cabinet. Science of the Total Environment, 83**,** 13-34.

Kiekens, L. (1995) Zinc. In: Alloway, B.J. (ed) Heavy Metals in Soils, Blackie Academic & Professional, London, 2, 284- 305.

Lanphear, B.P. (1998) The paradox of lead poisoning prevention. Science, 281, 1617-1618.

Lee, Y.Z., Suzuki, S., Kawada, T., Wang, J., Koyama, H., Rivai, I.F., Herawati, N. (1999) Content of cadmium in carrots compared with rice in Japan. Bull. Environ. Contam Toxicol, 63, 711-719.

Longnecker, N.E., Robson, A.D. (1993) Distribution and transport of zinc in plants. In: Robson A.D. (ed) Zinc in Soils and Plants Proc. Int. Symp. 'Zinc in Soils and Plants' University W. Australia, 27-28 Sept, 79-91.

Needleman, H.L. (1999) History of lead poisoning in the world. In: George AM (ed) Lead Poisoning Prevention & Treatment: Implementing a National Program in Developing Countries Proc. Int. Conf. Lead Poisoning Prevention & Treatment, Feb 8-10, Bangalore, 17-25.

OECD, (1996) Resolution of the Council concerning the declaration on risk reduction for lead. C (96) 42/Final, Paris.

Paivoke, A.E.A., Simola, L.K. (2001) Arsenate toxicity to *Pisum sativum:* Mineral nutrients, chlorophyll content, and phytase activity. Ecotoxicol Environ Safety (Environ Res section B), 49, 111-121.

Siegel, F.R. (1998) Geochemistry, metal toxins and development planning. In: Rose J (ed) Environmental Toxicology Current Developments, 81-107.

Tchernitchin, N.N., Villagra, A., Tchernitchin, A. (1998) Antiestrogen activity of lead. Environ Toxicol Water Qual, 13, 43-53.

Thompson, E.S., Pick, F.R., Bendell- Young, L.I. (1997) The accumulation of cadmium by the yellow pond lily, *Nuphar variegatum* in Ontario peatlands. Arch. Environ. Contam Toxicol, 32, 161-165.

Welch, R.M., Norvell, W.A., Schaefer, S.C., Shaff, J.E., Kochian, L.V. (1993) Induction of iron (III) and copper (II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status: Does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake? Planta, 190, 555-561.

WHO, Zinc. Environmental Health Criteria 221, 2001, WHO, Geneva. http://www.inchem.org/documents/ehc/ehc/ehc221.htm. Assessed 28 September 2010.

Witham, F.H., Bladydes, D.F., Delvins, R.M. (1971) Experiment in plant physiology. Van Nostrand Reinhold, New York. 245.

Xiong, Z.T. (1998) Lead uptake and effects on seed germination and plant growth in a Pb hyper accumulator *Brassica pekinensis* Rupr Bull Environ Contam Toxicol, 60, 285-291.

Young, A.J., Phillip, D., Savill, J. (1996) Carotenoids in higher plant photosynthesis. In: Pessarakli M. (ed) Handbook of Photosynthesis, Marcel Dekker, New York, 575-596.