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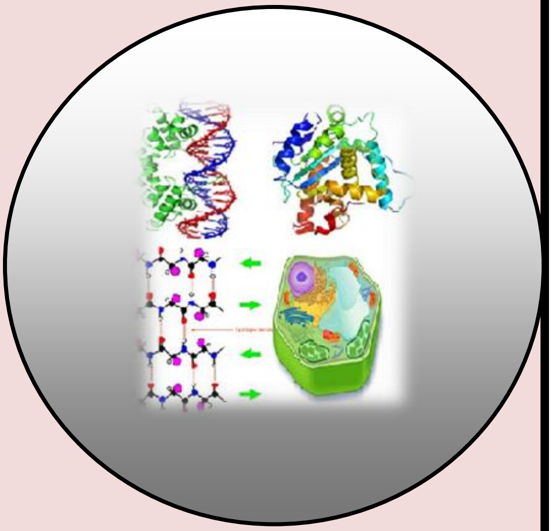

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RESEARCH PAPER

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Study of Seedling Behavior of "*Pisum sativum* L." Imposed by Lead (Pb) and Cobalt (Co) Induced Heavy Metal Stress

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ABSTRACT

The accumulation of heavy metals in soil due to uncontrolled human activities like mines, fertilizers and pesticides etc. is great ecological concern. These effects on plant were assessed in the present investigation on various physiological and biochemical parameter, by exposing pea (Pisum sativum* L.*) seedlings to cobalt and lead metal ions of various concentrations (control, 50, 100 and 150 µM). Finding of the study revealed that the heavy metals shows toxic effect on Pisum sativum* L. *in excess seviour inhibition in the physiological parameters such as seed germination, root length, shoot length, leaf area and biomass production as well as biochemical parameters (chlorophyll) also reduced but the proline content increased due to the imposition of cobalt and lead. The effect of cobalt and lead was more at higher concentrations (100 and 150 µM) than lower concentration (50 µM). Comparatively lead seems to be more toxic than cobalt in all the treatments imposed on pea. The present study will initiate optimize the toxic level of concentration of these two heavy metals to the farmer's of this region.

Key words: Cobalt, Lead, Heavy Metals and Pea.

INTRODUCTION

Nowadays, heavy metal toxicity isdangerous for food agriculture crop (Gaafar *et al.* 2012) because of Plant growth, ground cover having affected and have a negative impact on soil micro flora (Roy *et al.* 2005) and also known for many cases of diseases, disorders, malfunction and malformation of organs have been reported in human (Itumoh *et al.* 2013).However, some metals(such as Zn, Cu, Mn, Ni, and Co) are as micronutrients necessary for the plant growth and others (such as Cd, Pb and Hg) have unknown biological function (Gaur and Adholeya, 2004).Heavy metals enter in the environment by natural means and through human activities such as soil erosion, mining, industrial discharges, urban runoff, sewage discharge, pesticides and many others sources are responsible for the accumulation (Morais *et al.*2012).When heavy metals are absorbed in excess by plants either directly or indirectly they inhibit physiological processes such as respiration, photosynthesis, plant-water relationship, loss of cellular turgor, inhibiting the activity of cell and its enlargement, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass (Sanita di Topi and Gabbrielli, 1999). They disturb growth and metabolism by triggering secondary responses such as oxidative damage (Choudhary *et al.* 2004). Heavy metals (HMs) are among the major environmental contaminants and pose a severe threat to human and animal health by their long-term persistence in the environment (Jabeen *et al.* 2009), if they are excess in the food, water and in the air they may cause lot of problems (Itumoh *et al.* 2013). It is matter of special attention throughout the world because they are given toxic effects even at very low concentrations (Salama and Radwan, 2005) or at high concentrations (Stevovic *et al.* 2010).

Cobalt (Co) is essential for Rhizobium which it associates symbiotically with legume roots for N_2 fixation, however; Environmental risks of Co are managed through the establishment of environmental quality criteria and standards. Plants can accumulate small amount of cobalt from the soil. The uptake and distribution of Cobalt in plants is species-dependent and controlled by different mechanisms (Bakkaus *et al.,* 2005).many studies reported that Cobalt at toxic levels inhibit pollen germination, pollen tube growth and inhibit seed germination, causing ultra – structural changes and may cause inhibition in growth of plumule and radicals.

Lead (Pb) causes retardation of plant growth and inhibition of seed germination (Iqbal and Shazia, 2004). Pb decreases germination percentage of plant, root/shoot length, tolerance index and dry mass of roots and shoots (Mishra and Choudhari, 1998). Pb has been reported to negatively affect photosynthesis, transpiration and other physiological parameters in plants (Sharma and Dubey, 2005). Even at low concentrations, lead treatment was found to cause huge instability in ion uptake. Higher concentration of lead causes cell injury and the barrier functions of plasmalemma (Seregin *et al.,* 2004).

MATERIAL AND METHODS

The present study was carried out to determine the effect of heavy metals like Co and Pb on seed germination and seedling growth in pea (*Pisum sativum* L.). Petriplate experiments and pot experiments were carried out during summer season under laboratory and net house conditions at Botany Department, School of Life Sciences, Dr. B.R. Aambedkar University, Agra at an average maximum temperature 35-40ºC and minimum of 20-25ºC and humidity ranging between 78-98% to provide controlled conditions and. The seeds surfaces were sterilized by 2% sodium hypo- chlorite (NaOCl) solution to prevent any fungal contamination and then washed with three times with distilled water. The seeds were sown in earthen pots containing equal quantities (2kg) of washed and acid treated sand. The sand was treated with Evans and Nason nutrient solution. Metal treatments of Co and Pb were prepared using cobalt chloride ($CoCl_2$) and lead chloride ($PbCl_2$) solutions with concentrations of50, 100 and 150 µM with three replicates in green house. The samples were taken from two week old seedlings for physiological and biochemical analysis.

Measurements

(i) Physiological parameters

The physiological parameters were measured from plant sample such as:-

- Germination percentage
- Root and shoot length (Early seedling growth)
- Leaf area
- Root development
- Biomass production

(II) Biochemical Parameters

- **Chlorophyll estimation**

For estimation of chlorophyll in leaves, Brougham (1960) method was adopted.Following Arnon's (1949) technique, the amount of chlorophyll a and b was determined by measuring the optical density (OD) on a Beckman Du-2 spectrophotometer at 663 nm and 645 nm. The check reading was taken out at 652 nm.The chlorophyll content was estimated by using the following standard formulae. The results are expressed as average values.

$$\text{Total chlorophyll mg per gm tissue} = \frac{20.2\,(\text{OD}\,645) + 8.02\,(\text{OD}\,663) \times \text{v}}{1000 \times \text{w}}$$

$$\text{Chlorophyll a mg per gm tissue} = \frac{12.7\,(\text{OD}\,663) - 2.69\,(\text{OD}\,645) \times \text{v}}{1000 \times \text{w}}$$

$$\text{Chlorophyll b mg per gm tissue} = \frac{22.9(\text{OD}\,645) - 4.68(\text{OD}\,663) \times \text{v}}{1000 \times \text{w}}$$

Where,

OD = optical density of chlorophyll extract on specific induced wavelength

V= final volume of extract in a mixture of acetone and ethyl alcohol.

W= fresh weight of tissue (gm)

- **Proline estimation**

For the estimation of proline in leaves and shoots, Bates *et al.* (1973) method was followed.

RESULTS

The effect of Cobalt and Lead were elucidated on *Pisum sativum* L.to observe its consequences on plant growth, physiological, biochemical and biomass characteristics of *Pisum sativum* L.. The pea plants were grown as per protocol described in material and methods.As per the results obtained in petridish experiments and pot experiments,which were conducted in green house of Department of Botany, Agra.

1. Effect of Cobalt and Lead on Seed Germination of Pea

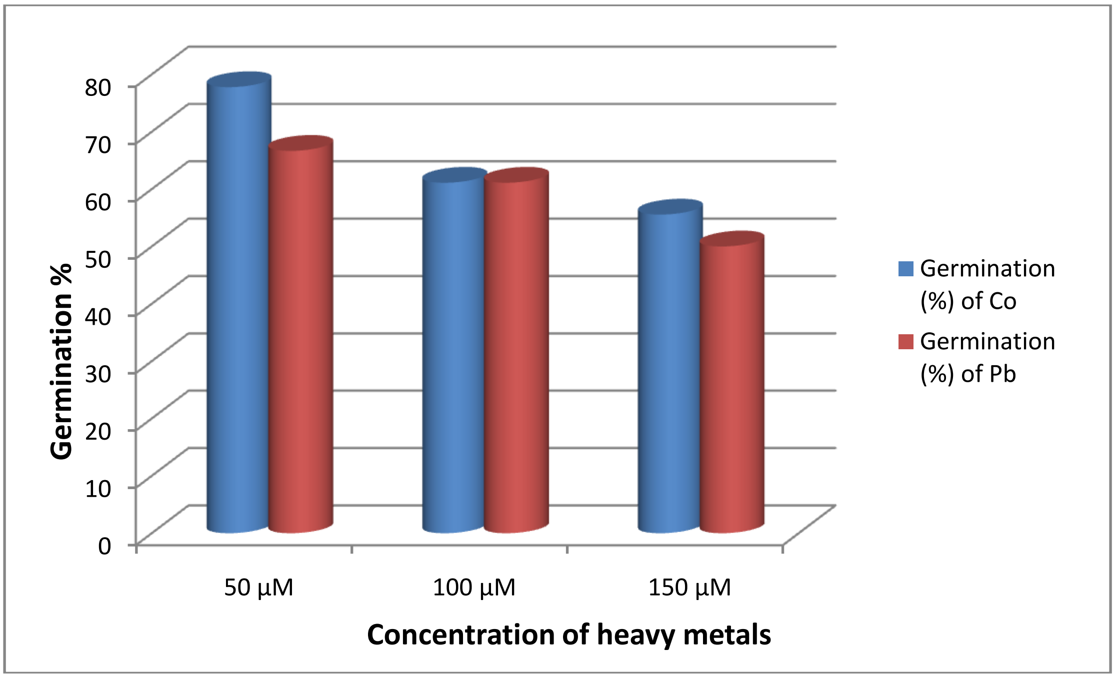
The low seed germination of plant can be attributed to the accelerated breakdown of stored nutrients in seeds and alterations of selection permeability properties of cell membrane, due to negative effect of heavy metals (Shafiq *et al*., 2008).

The pea represented gradual loss in germination upon treating with different Cobalt solutions. The differential germination percentage was recorded in pea in case of control and also upon treating with various cobalt solutions. (Jayakumar *et al*., 2008; Khan and Khan, 2010).

The variation in germination percentage was found to be 89% under normal condition. However, 88.89% germination reduced to the level of 77.78, at 50 µM, 61.11, at 100 µM and 55.56% at higher concentration of 150 µM of Cobalt (Graph 1).

On the other hand upon treated with Lead, pea seedlings shows more drastic decrease in seed germination. The differential germination percentage was recorded in control and also upon treating with various Lead solutions. Pb toxicity has been reported to retard the radical emergence via enhanced protein and carbohydrate contents, affecting the activity of peroxidases and polyphenol oxidases, oxidizing ability of roots and overall lowering of carbohydrate-metabolizing enzymes α-amylases, β-amylases, acid invertases and acid phosphatases (Singh et al., 2011). The germination percentage was found in the range of 89% under normal condition free from heavy metal treatment. However, 89% germination reduced to the level of 66.67%, 61.11%and 50% in case differentially treated with Lead levels i.e., 50, 100 and 150 µM respectively (Graph 1).

Graph1. Effect of Co and Pb on germination % of Pea (*Pisum sativum* L.).

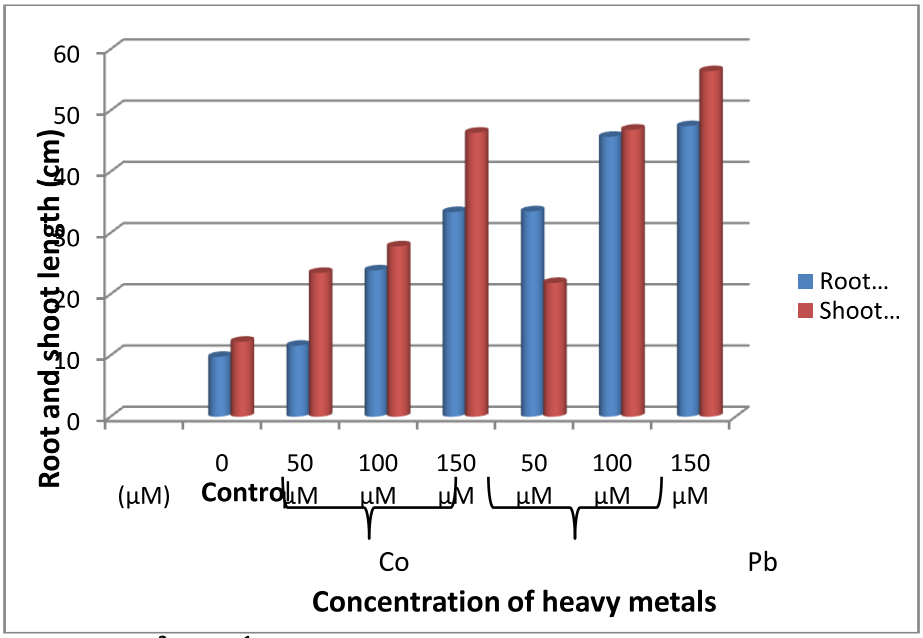


2. SEEDLING GROWTH

The data indicate that seedlings unaffected (control) by Cobalt and Lead acquired radical length maximum throughout till 21 days after germination. Data indicate down-regulated shoot length at the lower level of treatment caused about 23.47% loss with 50µM concentration of Cobalt while the 100 and 150µMCobalt applications further down-regulate the shoot length to 27.81 and 46.37% compared to control 12.23**.** Similarly, the down-regulation in root length was found to 11.58, 23.88 and 33.41% on treatment of metal solutions of 50,100 and150µM concentrations of Cobalt respectively as compared to Control 9.76 (Graph 2).In case of cobalt the results are in accordance with the findings of several other workers, where a decrease in early root and shoot length has been observed in various other crops with increasing of cobalt stress. Phytotoxicity study of Co in barley (*Hordeumvulgare* L.), oilseed rape (*Brassica napus* L.) and tomato (*Lycopersicon esculentum* L.) has recently shown the adverse effect on shoot growth. Various researchers also reported phytotoxity of Cobalt in various crops, for example (Imtiyaz *et al*., 2014) in *Glycine max*., (Jayakumar *et al.,* 2009 and Hemantaranjan *et al.,* 2000), in Tomato (Gad, 2005), in *Nitzchiaperminuta* (El-Sheekh *et al.,* 2003), in *Calothrixfuscs* (El-Naggar *et al*., 1999), in *Vignamungo* (Jayakumar and Vijayarengan; 2006), *Vigna radiate* (Jaleel *et al*; 2009), in Legume (Khan and Khan; 2010), in tomato (Gopal*et al.,* 2003).
Both shoot and root have been found to be down-regulated in relation to levels of Lead concentrations. The lower level of Pb application (50 µM) cause effectively to down-regulate root and shoot growth to 21.83 and 33.53respectively as compared to control root and shoot lengths(9.76 and12.23 respectively).

Nearly, 46.86% loss in shoot length could occur in case seedlings were treated with 100µMof lead till the termination of the observation (21days). Higher concentration causes more decline of about 56.42% as compared to control (12.23) (Graph 2).
Similarly, the down-regulation in root length recorded was about 45.70 and 47.44% on test treatments of 100 and 150µM respectively compared to control (9.76).
The reduction in the plant height might be mainly due to the reduced root growth and consequent lesser nutrient and water transport to the above parts of the plant. This is due to the fact that heavy metals accumulated on root due to binding of metals on the cell wall of root and retard cell division and cell elongation (Woolhouse, 1983).Root growth inhibition has also been associated with reduced size of the root meristem upon root emergence due to metal suppression of cytokinesis and cell elongation mechanisms (Obroucheva *et al.,* 1998). Interactions between Pb and nucleic acids have also been linked with the inhibition of cell division and though the majority of affects attributed to these associations are reversible with time, they have the largest effect within 1 day of exposure to Pb (Breckle, 1991). Lead was also reported to retard cell division and differentiation and also reduce their elongation thus affect the plant growth and development (Kastori*et al*., 1993).

Graph 2. Effect of Cobalt and Lead on root and shoot length (cm) of pea (*Pisumsativum* L.).



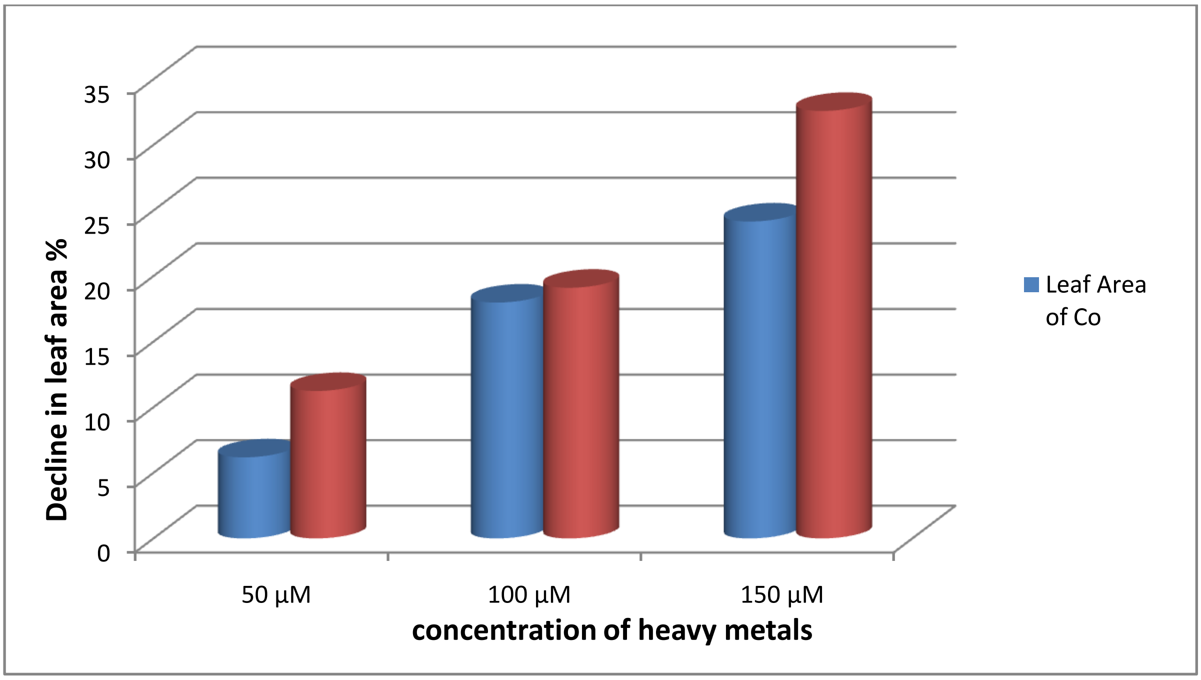
3. TOTAL LEAF AREA (cm^2 $plant^{-1}$)

Leaf area is an important variable for most eco physiological studies in terrestrial ecosystems concerning light interception, evapotranspiration, photosynthetic efficiency, fertilizers and plant growth. Leaf area mentions to the surface lamella in terms of length and width. More the leaf area, higher will be the photosynthesis which in turn increases the productivity. Thus leaf area is directly related with productivity. Data presented in graph 3showed the toxic effect of both of these metals on leaf area of *Pisum stavium* L. which was measured by the assistance of graph paper.
The highest leaf area of $1.78cm^2$ was observed in control seedlings. Cobalt and Lead exhibited more inhibitory effect at 150 µM and reduced he leaf area to 24.16% in comparison to control i.e., $1.78cm^2$.At 100µM concentrations of Cobalt, the loss in leaf area was recorded up to 17.98%, followed by reduction of about 6.18% in accordance with the control plants $1.78cm^2$.
On the other hand, lead caused more inhibition on leaf area in comparison to control ($1.78cm^2$) leaf area down regulated upto11.24% in plants subjected to Co treatment at 50µM concentration.

At higher concentrations of 100µM and 150µM, the loss recorded was upto19.11 and 32.59% respectively in comparison of control seedlings. It is evident that both these metals show negative effect on leaf area in all concentration. However lead displayed more toxic effect to leaf area at all concentrations as compared to the cobalt (Graph 3).

The reduction of leaf area was observed due to imposition of metal stress found by various workers (Panda and Khan, 2003; Wahid and Ghani, 2007) while working on several crop plants. Vijayrangavan *et al*., (2011) reported that the total leaf area of cowpea were maximum at control plants and get decreased with increase in Cd level in the soil. Similar observations were made by Schutzendubel *et al*., (2002) in *Populuscanescesus*, Faizan *et al*., (2011) on *Cicerarietinum* and by Zhou and Qui, (2005) in *Sedum alfredii*. Decrease in the leaf area of plant of higher concentration of heavy metals may be due to decrease activities of many enzymes involved in the fixation of Co_2, changes in the thylakoid organization, reduction of chlorophyll contents and inhibition of photosynthetic activities and disturbing the interaction of chlorophyll molecules into the stable complex (De-Fillippis and Ziegler, 1993; Fodor *et al*., 1996).

Graph 3: Effect of Cobalt and Lead and on leaf area (cm^2) of pea (*Pisumsativum* L.).



4. ROOT DEVELOPMENT

Heavy metals are known to reduce and disturb root system. Result presented in table indicated the effect of cobalt and lead on root development. It has been observed during the course of experiment that the number of primary roots was not affected much by various concentrations of cobalt and lead, while secondary and tertiary roots were affected. Lead showed the more pronounced deleterious effects than cobalt on root development of pea plants.

Data presented in the graph 4 shows inhibitory effect of cobalt on secondary root system of *Pisum stavium* L. (pea). At application of 50 µM concentration of cobalt solution, test plants show down regulation in number of secondary roots to about 11.91% decrease over control (19.67) while at 100 µM, data displays a decrease of 20.34% as compared to control. At Higher concentrations (150 µM) secondary roots was reduced and showed a decrease of about 28.83% in comparison to control seedlings under Co imposition (Graph 4).

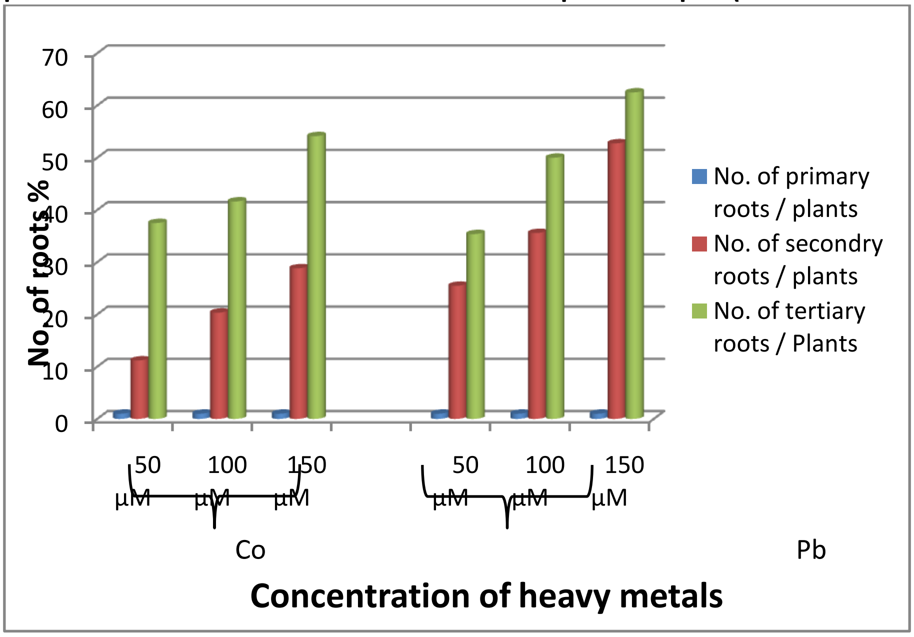
However lead imposition on seedlings showed more drastic inhibitory effect on root development than cobalt. The lowest percentage of secondary roots was recorded at 150µM concentration of lead and showed a decline of about 52.75%, followed by 35.59 % decline at 100 µM concentration of lead. At 50 the down regulation recorded was 25.48% as compared to control seedlings (19.67) (Graph 4).

On the other hand tertiary roots are also reduced by all the metal concentrations of Co and Pb on test plants. At 50 µM concentration of cobalt, tertiary roots were reduced up to 37.50% as compared to control (8), while at 100 µM and 150µMconcentration of Co, tertiary roots were reduced up to 41.63% and 54.13% respectively. Lead also shows the same pattern of reduction in tertiary root system. Lowest percentage of tertiary roots was recorded in 150µM concentration of lead and showed a reduction of about 62.50%, followed by decline of 50.00% at 100µM concentration of lead as compared to control. At 50µMconcentration the reduction over control was about 33.38%. Higher concentration shows higher reduction than all the other concentrations. (Graph 4)

The decrease in root development at higher concentration of Co could be attributed to an inhibition of vegetative organ growth in some plant species. Shafiq and Iqbal (2006) determined decrease in root development of *Cassia siamea*. Goldbold and Kettner (1991) observed a significant decrease in primary, secondary and tertiary root growth of *Piceaabies*. Similarly, these results are in accordance with the finding of other workers Gad (2005 b) on tomato plants Jayakumar *et al*; (2006) on *Vignamungo*, Jaleel *et al.* (2009) in *Vigna radiate* studied the inhibition of primary, secondary and tertiary root development by heavy metal, Imtiyaz *et al*., 2014 studied the inhibition of roots by heavy metals in *Glycine max.*

Marcnano *et al*., (2002) have suggested that the morphological and structural effects caused by metal toxicity in plants was due to decrease in root tip formation, suppression of elongation growth rate of cells affecting the ultra structure of meristematic cells and inhibition of the size of plant cells of inter cellular spaces.

Graph 4. Effect of Cobalt and Lead on root development of pea (*Pisum sativum* L.)



5. Biomass and Moisture Content vs. Cobalt Levels

Heavy metals are known to reduce the biomass production. Imposition of heavy metals viz. cobalt and lead in *Pisum sativum* L. plants produced a significant decrease in dry weight of both root and shoot. It was observed that in comparison to cobalt, lead caused more deleterious effects on biomass production.

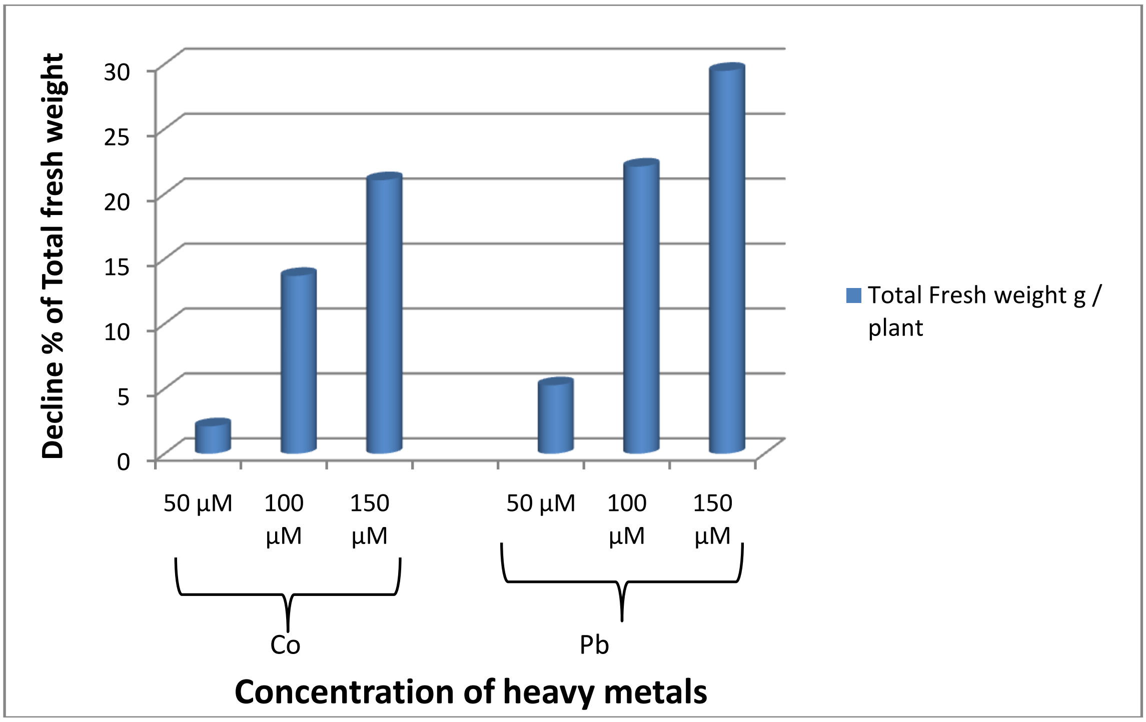
The highest fresh weight 0.95 was observed at control seedlings devoid of any metal concentration of cobalt and lead. At 50 µM Co, the reduction was about to 2.11 %, followed by 13.69 at 100 µM concentration as compared to control (0.95). At the highest concentration of cobalt 150 µM more toxic effect was observed on the fresh weight and showed a decline of about 21.06% as compared to control (0.95) (Graph 5).

Similarly the maximum dry weight 0.264 was noted in control seedlings. At 50 µM dry weight was shows a decline of about 9.85 %, followed by 24.63% and 31.82 % at 100µM and 150 µM concentration of cobalt as compared to control plants (Graph 6).

On the other hand lead shows more toxic effect than cobalt. At 50 µM concentration the down regulation in fresh weight recorded was 5.27 % as compared to control. The down regulation of fresh weight was more at higher concentrations of lead. At 100 the down regulation of fresh weights was about 22.11% followed by more decrees at 150µMisconcentration of lead with decline of about 29.48 % as compared to control (0.95) (Graph 5).

Similar results of decline in dry weight biomass were observed with control seedlings showing highest accumulation biomass of about0.264 gm/plant. The decline occurs in same manner as occurred in fresh weight. Higher concentration of 150 µM is of lead treated test plants showed lowest % of biomass with decline of about 41.29% followed by 24.25 % at 100µMconcentration, of lead treated test plants in comparison to control (0.264). At 50 µM dry mass was also declined to 11.75% after the due course of time (Graph 6).

Graph 5: Effect of Cobalt and Lead on the Biomass production (Fresh weight) of pea (*Pisum sativum* L.)



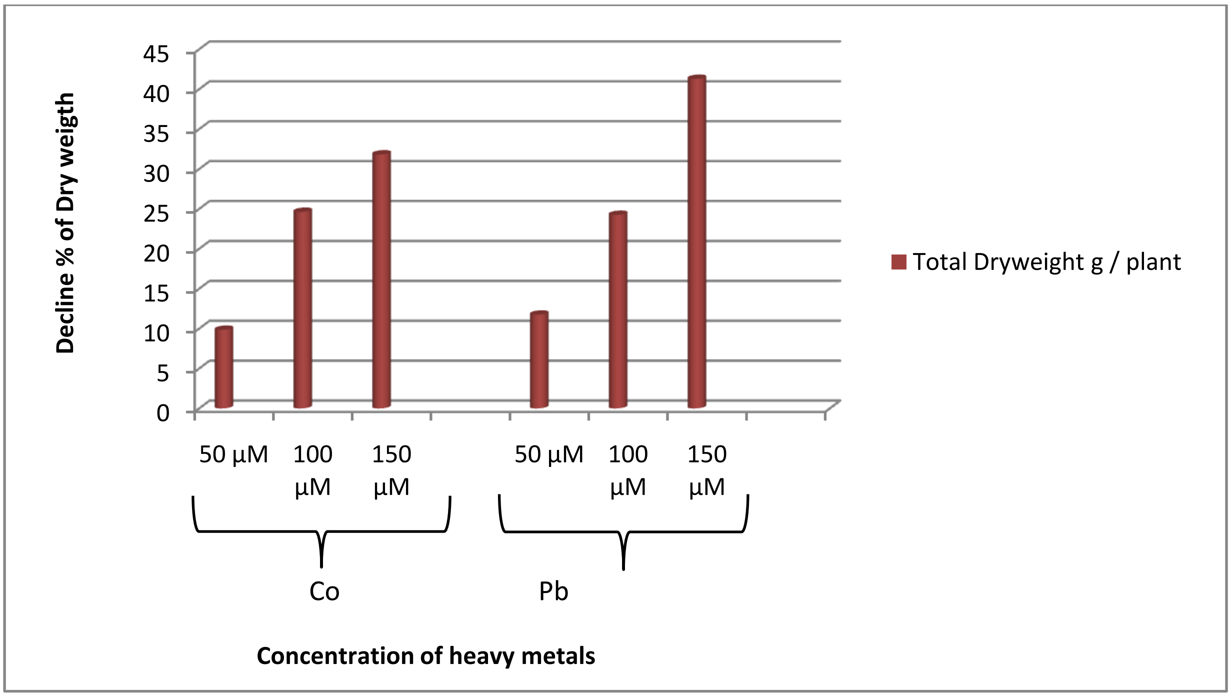
Reduction in biomass accumulation is often reliable indication on the plants sensitivity to various stress, represents the cumulative effects of damaged or inhibited physiological functions. The exposure of heavy metal stress caused significant reduction in biomass accumulation (Usha and Nathawat, 1993).

Our findings are similar to the results observed by several other workers on various plants (Imtiyaz *et al.,* 2014; Hosseini *et al*., 2007; Kamel, 2008). They have shown that reduction in biomass production under the influence of heavy metals (Cd and Cr) may be due to the impairment of uptake and translocation of nutrients and water in aerial plant parts.

Decrease in fresh weight of plants might be due to heavy loss of moisture. Decrease in dry weight might be due to accumulation of certain nutrients, reduction in photosynthesis and chlorophyll 'a' synthesis as suggested for cowpea (Joshi *et al*., 1999).

Biomass of *Allium cepa* also showed a decrease up to 50% on 70 DAS. Reduction in biomass of a plant is a clear indication of contamination and the effect of heavy metal stress. Reduction in biomass accumulation could be due to a possible retention of Cd in plant system, resulting in physiological damage to the plant. Similar results were given by Weigel and Jager (1980).

Graph 6: Effect of Cobalt and Lead on the Biomass production (dry weight) of pea (*Pisum sativum* L.)



(2) BIOCHEMICAL PARAMETERS

Biochemical parameters viz. pigment composition and proline content are being affected by various heavy metals. By keeping this thing in view, the effect of various concentrations (50 µM, 100 µM and150 µM) of heavy metals Co and Pb on these biochemical Parameters was estimated. The result obtained is described below:

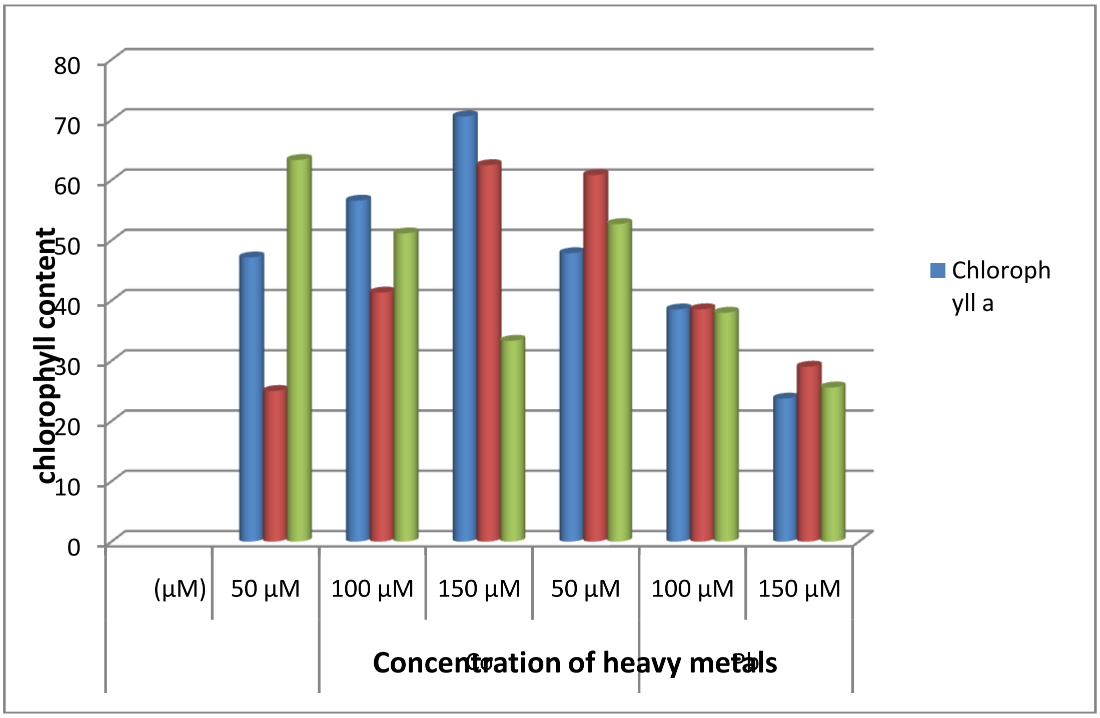
7. PIGMENT COMPOSITION

Chlorophyll is the main pigment which can help plants to photosynthesize. When plants exposure to stress, their photosynthesis will be inhibited and the concentrations of chlorophyll can directly indicate the extent of stress-induced damage in plants (Lin *et al*., 2012). Katarzyna and Smolik (2011) obtained a decreased amount of chlorophyll a, chlorophyll b and carotenoids in *Lemna minor* after the application of a Co treatment. Erdei *et al*. (2002) recorded a higher decrease in the content of chlorophyll in barley after the application of Cobalt as the shown in (Graph 7).

The chlorophyll b was found more significantly affected compared to chlorophyll a. control seedlings recorded maximum content of both chlorophyll a (0.61) and chlorophyll b (0.68). At 50µM treatment of cobalt, chlorophyll a 47.16% and 25% chlorophyll b (50 µM) were found down-regulated in pea seedlings, which could reach about56.59%and 41.33% (100 µM) after 21 days of cobalt treatment. Higher concentration of 150 shows more toxicity on pigment components and the decline was about 70.57 and 62.47% as compared to control seedlings (Graph 7). Similarly total chlorophyll was also found down-regulated in pea, which could also reach about 66.30% (150 µM) after the experimental period.

On the other hand lead shows more toxicity than cobalt on pigment systems. Highest content of pigments was observed in unaffected control seedlings. The lowest chlorophyll content and chlorophyll a(23.73%) and chlorophyll b (29.03%) content was noted at 150is concentration of lead. At 100 µM concentration, chlorophyll a and chlorophyll b was about 38.53 and 38.56% as compared to control (0.615 and 0.682). At 50 µM concentration of lead the effect was not much more and displayed the value of 44.87 in chlorophyll a and 60.85 in chlorophyll b. Lei *et al*. (2012) have also reported that Cu, Zn, Pb and Cd depressed chlorophyll and carotenoids levels and the quantum yield of PS II in *Thalassiahemprichii.*

Graph 7: Effect of Cobalt and lead on pigment composition (mg g^{-1} FW) of pea (*Pisumsativum* L.)



8. PROLINE CONTENT

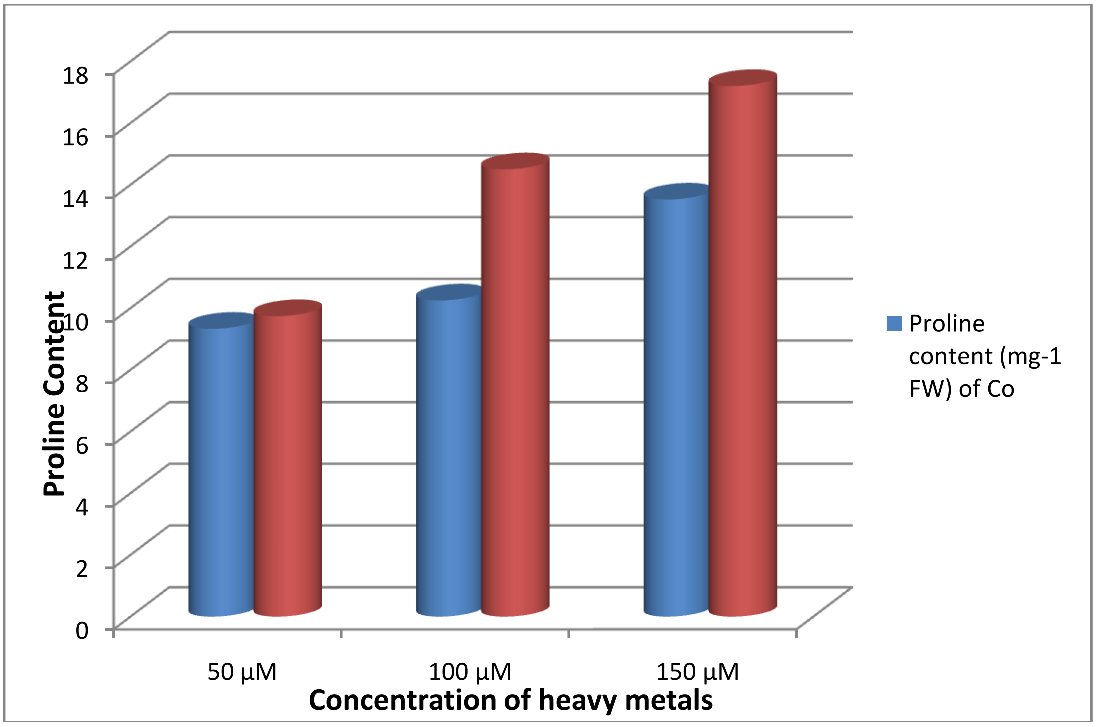
Proline, an amino-acid is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Saradhi and Alia, 1993). Proline sugar, glycine, betaine and other organic solutes are believed to improve metal tolerance by contributing to osmosis and preserving enzymes activity in presence of toxic ions (Greenway and Munns, 1980).

Plants have shown higher proline accumulation under environmental stress (Ahmad *et al*., 2008) and are considered to involve in stress resistance to give rise to a series of reaction, which generate numerous free radical by altered levels of major anions and accumulation (Alia and Saradhi, 1991). It is also an important part of structural protein and enzymes and help in repair process (Chris *et al*., 2006).

The findings of our experiment are in agreement with other workers that with the increase in stress, the proline content also increases in different plants (Singh *et al.,* 2012). Pea plants shows increase in proline content with increase in concentration of metal treatment. Test plants shows the proline content of 9.33,10.25 and 13.52mg g^{-1}proline as compared to control (8.44 mg g^{-1}proline) when treated with the 50, 100 and 150 µM concentration of Co. When treated with Pb, the proline concentration was further enhanced and it was 9.74, 14.50 and 17.2 mg g^{-1}proline when treated with 50, 100 and 150 µM of Pb as compared to control (8.44 mg g^{-1}) proline (Graph 8).

Under stress metabolism the proline is a source of nitrogen and energy for the plants. Associated with the stress, the accumulation of proline or some other organic solute may be serving as a compatible solute for maintaining the osmotic balance between the cytoplasm and vacuoles (Dar*et al.*, 2009).

Graph 8: Effect of Cobalt and lead on proline content of pea (*Pisum sativum* L.) (mg g^{-1} FW) in leaves



CONCLUSION

Germination percentage was considerably decreased by lead and cobalt in comparison to that of control. However, lead seems to be more toxic to seed germination than that of cobalt in all the three Concentrations. Lower concentration (50 µM) of both the metals (Co and Pb) show little effect on germination percentage, but higher concentrations (100 and 150 µM) delayed and decreased germination percentage to a considerable extent. The maximum effect was seen on imposition of higher concentrations of both lead and cobalt. However lead causes more toxicity and inhibits both root and shoot to greater extent. Shoot length was more inhibited than root length. However, the leaf area was less affected by the low concentrations (50 µM) of the cobalt and lead as compared to the high doses of these metals (100 and 150 µM) and lead again was more toxic as compared to cobalt. Here also lead causes more phytotoxicity on leaf area than cobalt at all the treatments. There was no effect of cobalt and lead on the growth of primary roots at all the three concentrations (50, 100 and 150 µM).

However, secondary and tertiary roots were decreased at all the concentration of heavy metals in all the three treatments used. The maximum effect of heavy metals was observer on tertiary roots as compared to secondary roots in all treatments used. Tertiary roots were found more sensitive to both these metals. Higher the treatment levels of both cobalt and lead,more was the effect in impairing shoot and root fresh biomss. However all the metal concentrations (50, 100 and 150 μM) have adversely effected the biomass production and significantly reduces the biomass content in pea. Similarly leaf area shows the same pattern of reduction in leaf area with increasing concentrationof cobalt and lead in comparision to control.The biochemical aspects such as chlorophyll ‘a’, chlorophyll ‘b’, and total chlorophyll decreases on Imposition of both heavy metals in the treated plants as compared to control. Here also lead was observed to have more deleterious effects than that of cobalt. Higher concentration of both Pb and Co caused the maximum decline in chlorophyll in test plants. Proline which could play a therapeutic role in plants seems to increase in plants under stress conditions caused by Co and Pb. The imposition of low concentration of these metals (50 μM) less amount of proline was increased and as the plants are treated with higher doses (100 and 150 μM) of Co and Pb, there seem to be an increase in the accumulation of proline in test plants of *Pisum stavium* L. Overall it can be concluded that cultivation of pea in metal polluted soils should be avoided or appropriate control measure should be adopted to maintain the heavy metal content of soil below the damage threshold level. The identification of toxic concentration of metals and tolerance indices of plant species, such as *Pisumstavium*L. would be helpful for the establishment of an environment quality standard. The findings can also contribute to better physiological fragility, the potential of pea in coordinating crop management programs in metal contaminated areas. Furthermore, research studies with different metal stresses can be helpful in the solution of various problems associated with metal pollution in agricultural regions.

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