Effect of Pb, Ni and Co in growth parameters and metabolism of *Phaseolus aureus* Roxb.

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Abstract:

The concentrations 2.5,5, and 10 mM of Pb, Ni and Co have been experimented with mung bean *Phaseolus aureus* Roxb. seeds. There is a significance changes in all studied parameters of growth except in percentage velocity coefficient of germination. Pb & Ni at 10mM caused decreased in seed germination significantly. There is no growth by Ni at 10mM. Lengths of stem, root, & leaf area inhibited by all concentrations except by Co at 2,5 & 5mM. Dry weight of stem & leaf declined by Pb at 10mM, while dry weight of root declined by all concentrations. Chlorophyll content enhanced by all concentrations except by Pb (5mM) which declining chlorophyll content significantly. Protein content decreased & proline content increased significantly by Pb & Ni at 2.5 mM also by Co at 10mM.

الخلاصة :

جربت التراكيز 2.5، 5 و10 مليمولر لكل من Pb و Ni و Co في بذور الماش *Phaseolus aureus* Roxb. وكانت كل مؤشرات النمو المدروسة ذات فروق معنوية، باستثناء النسبة المئوية لمعامل سرعة الإنبات إذ لم تحصل فيها فروقات معنوية. خفض Pb و Ni بالتركيز 10 مليمولر النسبة المئوية للإنبات معنويا، ولم يحدث نمو لبذور الماش بوجود النيكل بالتركيز 10 مليمولر. ثبط طول الساق والجذر ومساحة الورقة معنويا بتأثير جميع التراكيز باستثناء تركيزا Co (2.5 و5 مليمولر) إذ لم يؤثرا معنويا. قلل Pb بالتركيز 10 مليمولر الوزن الجاف للساق والورقة في حين انخفض الوزن الجاف للجذر بتأثير جميع التراكيز. زاد محتوى الكلوروفيل بتأثير جميع التراكيز عدا Pb بالتركيز 5 مليمولر إذ قلل محتوى الكلوروفيل معنويا. انخفض محتوى البروتين مع زيادة محتوى البرولين معنويا بتأثير Pb و Ni بالتركيز 2.5 مليمولر وبتأثير Co بالتركيز 10 مليمولر.

Introduction

Heavy metals contamination attracted great interest of many scientists (Lasat, 2002) because they are one of the most important environments pollutants. In recent years, many low cost sorbets such as algae, fungi, bacteria & plants have investigated for their biosorption capacity towards heavy metals (Chehregani & Malayeri, 2007).

Lead (Pb) is a major environmental pollutant of worldwide concern. The toxic effects of Pb rest mainly in its ability to react with functional groups such as sulfhydryl, carboxyl & amine, leading to decrease or loss of activity of many enzymes that are important for cell functions (Salt *et al*, 1995).

Nickel (Ni) is an essential trace element in plants because of its important in the metalloenzyme urease (Gerendas *et al*, 1998), however, exposure to excessive Ni can cause toxic effects on plants as in toxic effect on pollen germination (Levent Tuna *et al*, 2002) or in the inhibition of growth & many parameters of plants (Al-Jubouri, 2008).

Cobalt (Co) is essential for *Rhizobium* which it associates symbiotically with legume roots for N_2 fixation, free living nitrogen – fixing bacteria, however, there is no evidence that Co has a direct role in the metabolism of higher plants (Marschner, 1995). Many studies report that Co & many heavy metals at toxic levels inhibit pollen germination, pollen tube growth (Levent Tuna *et al*, 2002) & inhibit seed germination, causing ultra – structural changes & may cause inhibition in growth of plumula & radicals (Ayaz & Kadioglu, 1997).

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The current study represent data on the effect of three metals (Pb, Ni, & Co) in seed germination, seedlings growth & the contents of chlorophyll, protein & proline of mung bean leaves.

Materials & Methods

Seeds of *Phaseolus aureus* Roxb., were obtained from local market (Babil). The seeds were surface sterilized with 1% of sodium hypo chloride to prevent any fungal contamination and germinated in growth chamber, with 25± 2°C, 60-70 % humidity & continuous light with light intensity 1500-1800 lux. Twenty five seed were grown in plastic pots(5 cm height &11 cm diameter), which contain saw dust. The sawdust was thoroughly washed with boiling water twice & dried.

Metals solutions of Pb, Ni & Co were prepared using lead, nickel & cobalt chlorides with concentrations 10, 5, & 2.5 mM, in addition to distil water (d.w.) as control. 100 ml of each solution was added to each replication.

The percentage of germination & velocity coefficient of germination measured after 3 days. Other parameters measured at 10 days old, which were length of stems & roots, dry weight of stems, roots, & leaves. Leaf area measured by planimeter, chlorophyll content was estimated according to Arnon 's (1949) technique using Optima Sp-300 spectrophotometer. The protein was estimated by buiret method, while proline was estimated according to Bates *et al.*, (1973) method. All data were statistically analyzed by one –way analysis of variance (ANOVA) followed by a least significant differences (L.S.D.) at probability level 0.05.

Results and Discussion

Table 1 shows a significant decrease in percentage of germination caused by Pb & Ni at 10mM.This result agreed with Ashraf & Ali (2007) & confirm that germination of seeds was gradually delayed in the presence of increasing concentrations of Pb. At the same time, there are no significance changes in percentage of germination by Pb & Ni at 2.5mM & by all concentrations of Co. These results were agreed with (Al-Jubouri, 2008), who indicate that germination of seeds was favored with all concentrations of Pb & Ni until starting the growth of stem cells. Khan & Khan (2010) showed that higher concentrations of Co (200 & 400ppm) caused declining in seed germination of chickpea. There are no significant changes in velocity coefficient percentage of germination at all treatments, while the lengths of stem & root were decreased significantly by all concentrations except at 2.5 & 5mM of Co, which is not change leaf area significantly too. Co in this stage of growth may be has enhancing effect especially at low concentrations. This enhancing effect of Co extended to chlorophyll content (table 3). Present results were agreed with Tayakumar *et al.*, 2009. They indicated that Co at low concentrations has sobbed beneficial values on soybean. Ni at 10 m M (table 1) causes seeds emergency through the seed coat, but failed to elongate, this inhibition of seedlings growth at this concentration due to the heavy metal toxicity has been previously reported by Al-Yemeni & Al-Helal (2002).

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Table 1: Effect of Pb, Ni & Co in percentage of germination,% velocity coefficient of germination, stem length, root length, & leaf area of mung bean.

Metal	Concentrations (mM)	% germination	% velocity of germination	Stem length (cm)	Root length (cm)	Leaf area (cm2)
Pb	control	97.3	58.5	6.3	6.5	3.2
	2.5	92.0	58.2	3.4	1.4	2.1
	5	94.7	55.6	2.6	1.5	1.4
	10	86.7	60.7	0.8	0.2	1.3
Ni	2.5	94.7	54.0	1.9	1.0	1.8
	5	96.0	59.1	2.3	1.1	2.9
	10	89.3	53.0	0.0	0.0	0.0
Co	2.5	100	59.1	4.8	5.4	3.7
	5	97.3	57.0	5.7	6.1	4.0
	10	97.3	65.5	3.3	2.0	2.8
L.S.D(0.05)		6.58	Non significance	1.69	1.53	0.83

Dry weight of stem & leaf decreased by Pb at 10mM, whereas root dry weight decreased by all concentrations of heavy metals significantly (table2) . These results reflect the toxicity effect of Pb at 10mM on the shoot, which is noticed by (Miranda *et al*., 2000&Nosalewics *et al*., 2008), as well as, reflect the differentiation in metals accumulation & partitioning which is may be varied in parts of plant (Pahalawattaarachchi *et al*., 2009) depending on type of metals.

Table 2: Effect of Pb, Ni, & Co in dry weight of stem, root & leaf of mung bean.

Metal	Conc. (mM)	Dry weight (mg)		
		stem	Root	Leaf
Pb	control	7.0	21.8	9.7
	2.5	8.2	10.0	8.4
	5	4.9	7.2	9.7
	10	3.5	2.0	3.0
Ni	2.5	6.5	4.9	7.8
	5	6.4	4.4	6.9
	10	0.0	0.0	0.0
Co	2.5	5.7	11.4	5.0
	5	6.0	10.8	8.2
	10	5.3	7.4	9.5
L.S.D(0.05)		2.38	5.08	3.14

Table 3 shows that all concentrations caused increasing in chlorophyll content except at 5mM of Pb which decreased chlorophyll content significantly. This result illustrated by Zengin &Munzuroglu (2005) too, they showed inhibition of chlorophyll content with increasing concentrations of heavy metals. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness & photosynthetic productivity (Parakh *et al*., 1990).

Pb & Ni at 2.5mM increased in proline & decreased in protein content significantly. These results agreed with Panda (2003) who referred to increase the proline content in *Taxithellium* sp. when treated it with low concentrations of some elements as ZnCl(0.001μM). An increase in proline accumulation may help in osmoprotection. Pandey & Sharma (2002) indicated that when cabbage exposure to each Co^{2+}, Ni^{2+}, & Cd^{2+}, water potential & transpiration rate decreased which associated with increasing in diffusive resistance showing development of

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water stress. This was further substantiated by enhanced accumulation of proline in leaves. High concentration (10mM) caused reversed results.

Co at 2.5 & 5mM causing decrease in protein content & increase in proline content, while Co at 10mM enhanced proline content & decreased protein content significantly. Some authors indicated that protein content has not been changed significantly with increasing of proline content (Ghezelbash *et al*., 2008), whereas ShafiTantrey & Agnihotri (2010) referred to occur inhibition of proline content by Cd & Hg treatments (10 & 25 μmol/l) in *Cicer arietinum* L.. In this study, although there was a differences in contents of proline & protein after treated the mung bean seeds with different concentrations of Pb, Ni, & Co, protein content increase in contrast with decrease in proline content & vis versa. These results showed that proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, & (d) hydrolysis of proteins (Chrest & Phan, 1990).

Table 3: Effect of Pb, Ni, & Co in chlorophyll, protein and proline contents of mung bean.

Metal	Conc. (mM)	Chlorophyll mg/g f.w.	Protein mg/g f.w.	Proline μmole/g
Pb	control	0.88	343.5	70.2
	2.5	1.66	30.2	115.7
	5	0.27	309.8	50.6
	10	0.59	391.8	10.0
Ni	2.5	1.66	116.6	125.7
	5	1.32	424.7	7.9
	10	0.0	0.0	0.0
Co	2.5	1.43	306.3	7.3
	5	1.38	215.1	21.9
	10	0.86	152.1	96.6
L.S.D (0.05)		0.41	117.4	41.8

Conclusions

Based on the results, Pb had more effect than Ni which is more effect than Co in all physiological parameters in this research. These reflect the toxicity of Pb>Ni>Co. Co may be important in enhancing the seeds germination, leaf area, & chlorophyll content of mung bean. Generally, after treated with deferent concentrations of Pb, Ni, & Co, protein content still in high level in contrast with low level of proline content or vice versa

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