

Effect of metallic pollutants (cobalt, nickel, lead) on growth performance and biomass accumulation of MPT'S *Acacia mangium*

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ABSTRACT

Experiment was conducted to study the effect of metallic pollutants viz., Cobalt, Nickel and Lead on growth performance and biomass accumulation of *Acacia mangium* a multipurpose tree species. Growth performance studies revealed that the concentrations of Cobalt Chloride significantly affect the shoot and root length, leaf number, Collar diameter and nodulation of growing seedling and found to be decreased with increasing the concentrations of Cobalt chloride. The shoot and root length, leaf number, Collar diameter and nodulation of rhizobium were significantly affected by different concentrations of Nickel Chloride. Different concentrations of Lead Chloride significantly influenced the growth of shoot, root, number of leaves and nodulation and the highest growth performance for all the parameters was seen in control treatment but there was gradual decreased with increasing the concentration. In case of biomass accumulation studies, different levels of Cobalt chloride, Nickel Chloride and Lead chloride in soil showed statistically significant variation in leaves dry matter of above ground parts and below ground parts, total plant along with shoot / root ratio and found control was superior for maximum biomass accumulation.

Key words: Cobalt, Nickel, Lead, growth, biomass, *Acacia mangium*.

Since the down of the industrial revolution, mankind has been introducing numerous hazardous compounds in to the biosphere. These hazardous pollutants consist of a variety of organic compounds and heavy metals, which pose serious threat not only to human health but also to other flora and fauna of the earth. The metals commonly found in the environment beyond the critical level as a result of human activities includes Cu, Zn, Ni, Pb, Cd, Co, Hg, Cr and As. There is an urgent need to reduce excess metals present in soil, sediments and water bodies so as to prevent environmental contamination. Recently, scientists and engineers have started to generate cost effective technologies that includes the use of micro-organisms and live plant in the cleaning process of polluted areas. Several studies have been conducted in order to evaluate the effect of different heavy metal concentration on live plants. Most of these studies have been conducted using seedlings or adult plants. *Acacia mangium* is medium to tall, spreading tree. It is widely planted in the Pacific and elsewhere as a forestry tree and for other purposes. It is

fast growing nitrogen fixing tree species of humid tropical. It tolerates degraded areas moist to wet sites. Now it is widely cultivated in South East Asian countries. Therefore, present study is aimed to determine effect of metallic pollutants on growth performance and biomass accumulation of multipurpose tree species *Acacia mangium* for the development of eco-friendly environment.

MATERIALS AND METHODS

Experiment was conducted in the experimental field of Department of Forestry, IGKKV, Raipur in completely randomized block design with seven concentrations replicated thrice. Fast growing and nitrogen fixing multipurpose tree species *Acacia mangium* was selected for the study. Uniform sized seeds of tree species was selected and treated with hot water (85°C) for breaking the hard seed coat dormancy. Seed was kept in this water and left for overnight (Agrawal, 2003) after which uniform swelled seeds were selected and sown three

seeds in each container. After emergence and establishment of seedlings, thinning was done to maintain one seedling in each container. Seven concentrations consisting of 0, 100, 200 500, 700, 1000 and 2000 ppm of metallic pollutants like Cobalt chloride, Nickel chloride and Lead chloride were used on the basis of per bag of soil on dry weight basis. The application of different treatments of each pollutant was given and mixed separately in the soil of each bag, so that it could be homogeneous. Thus, multiplying of 100 ml stock solution of each concentration of each pollutant was prepared in the lab for 60 bags and one treatment without any pollutant served as control.

Soil was prepared thoroughly by crushing into uniform fine grade. The sand was cleaned and washed thoroughly to get uniform size particles. Mixture of vertisols and sand were prepared in 1:1 ratio to fill in the polybag of 9.5 x 24cm size having capacity of 1.30 kg. Care was taken to keep soil free from weeds and other soil borne pathogens. The soil, sand and bore well water are filled in the bags and analyzed for their physical and chemical characteristics and as well as concentration of Cobalt, Nickel and Lead chloride in Department of Forestry I.G.K.V. and National Mineral Development Corporation Raipur. Height of seedlings was measured in centimeters from the base of the plant to the tip of the shoot with the help of standard meter scale. Collar diameter of the seedling was recorded near the base of stem in millimeter with help of digital Vernier caliper. The number of leaves obtained in each treatment was counted. The leaf area was calculated by using graphic methods along with dry weight. Root lengths of the seedlings were measured in centimeter by standard meter scale from lowest tip of the root of the seedling to the start of base of the shoot. Number of nodules per plants was recorded by simple count method. Leaves, shoots, and roots were reported from each sample plant and weighed to record the fresh weight in grams. The sample of leaves, shoot and root were dried in hot air oven for 24 hrs. at 80⁰C and again weighed for their dry weight for the observations of biomass accumulation.

Experiment was framed as per CRD design and the data generated from the experiment was compounded and tabulated for its statistical analysis as per the standard statistical / package. Lotus-123 Spreadsheet software (Lotus smart sheet-123/ MS

office Excel) was used for all the mathematical and statistical calculation.

RESULTS AND DISCUSSION

Growth Performance of *Acacia mangium*

Growth performance in plants of *Acacia* species under different pollutants was recorded for their shoot and root length, collar diameter, number of leaves and nodules, dry weight accumulation in leaves, above ground and below ground parts. *Acacia mangium* an exotic species and behaved more or less similarly. Similar results were observed by Susilawati and Setiadi (2003) in their preliminary research on natural hybrids of *Acacia mangium*, they found that mother trees and their seedlings showed intermediate and similar growth behavior as these species has relationship. Similar results were observed by (Chidumayo, 2005) in their study of exotic v/s indigenous tree species. Chaukiyal *et.al.* (1999) also found similar results in *Acacia's* for nodulation.

Effect of Cobalt Chloride: *Acacia mangium* is an exotic species and prefers humid tropical environment. Concentrations of Cobalt Chloride significantly affect the shoot and root length, leaf number, Collar diameter and nodulation of growing seedling (Table 1 & Fig 1) and found to be decreased with increasing the concentrations of CoCl₂. The numerically highest shoot length (19.48 cm), root length (54.87 cm), number of leaves (15.98 plant⁻¹), collar diameter (3.14 mm) and number of nodules (9.84 plant⁻¹) was measured in control (T₁) followed by 100 ppm of CoCl₂ with 2 to 8 % reduction in growth. Number of nodules per plant was statistically on par between 200 and 500 ppm. The higher concentration *i.e.* 2000 ppm of CoCl₂ gave least shoot length (10.07 cm) and root length (36.49 cm), leaf number (8.81plant⁻¹), collar diameter (1.90 mm) and number of nodules (6.22 plant⁻¹). The reduction in all the growth characteristics was ranged up to 66.4 to 51.7 percent at 2000 ppm concentration of CoCl₂ with maximum in shoot length and minimum in root length. Similar impression of Cobalt Chloride was observed by Peralta *et.al.*, (2000) in case of *Medicago sativa* crop. In legumes Cobalt is required for symbiotic fixation of nitrogen in very-very small quantity (Ahmed and Evans, 1959) otherwise caused toxicity to plants if it exceed certain low levels. The retardation in different growth parameter of leguminous tree species under various concentrations of Cobalt Chloride may either be low mitotic activities in the meristematic some or be

inhibition of cell enlargement resulted growth inhibition in both the conditions (Arey and Jagetiya, 1998). Chatterjee and Chatterjee (2000) studied the phytotoxicity impact of Cobalt, Chromium and Copper on cauliflower that excess these metals inhibited the concentration of most of the macro and micro nutrients, particularly P, S, Mn, Zn translocation were affected most significantly leading to decrease water potential, transpiration rates and increased diffusive resistance and relative water contents in leaves and finally reduced the growth and productivity of plants.

Effect of Nickel Chloride: Data on shoot and root length, leaf number, Collar diameter and number of nodules per plant of *Acacia mangium* is presented in Table 2 & Fig 2. Perusal of showed that the shoot and root length, leaf number, collar diameter and nodulation of rhizobium were significantly affected by different concentrations of Nickel Chloride. The maximum shoot length (19.48 cm), root length (54.87 cm), number of leaves (15.98 plant⁻¹), collar diameter (3.14 mm) and number of nodules (9.84 plant⁻¹) were measured for control followed by 100 ppm Nickel Chloride *i.e.* (shoot length (18.40 cm), root length (53.29 cm), number of leaves (15.39 plant⁻¹), collar diameter (2.94 mm) and number of nodules (9.23 plant⁻¹), respectively. Gabriella and Anton (2002) narrated that capacity of tolerance the particular heavy metals by any plant species are known. Significantly indicators plants are used as higher accumulator plants (Baker *et.al.*, 1994) for low concentration but on increasing the level of concentrations the inhibitory effect imposed significant decrease in growth. The presence of Nickel marked suppression in total nitrogen and phosphate content mobilization. Singh (1985) reported more or less similar result in case of *Vigna radiata*. Also studied the level of toxicity of various heavy metals with similar results where increasing concentration of Chromium, Uranium and Silver for *Triticum aestivum* reflected in reduction of chlorophyll content.

Effect of Lead Chloride: Growth performance of seedlings grown under different concentration of PbCl₂ is presented in Table 3 & Fig 3. It is evident from the data that different concentrations of PbCl₂ significantly ($P \leq 0.05$) influenced the growth of shoot, root, number of leaves and nodulation in *Acacia mangium*. The highest growth performance for all the parameters were observed in control treatment but there was gradual

decrease with increasing the concentration and the growth was reduced up to 45 to 63.3 per cent at higher concentration of PbCl₂ 2000 ppm. At 100 ppm concentration of PbCl₂ shoot length (18.20 cm), root length (52.00 cm), leaves number (15.30 plant⁻¹), collar diameter (2.90 mm) and number of nodules (8.87 plant⁻¹) were reduced by 3 to 10 per cent. However, the least shoot length (9.90 cm), root length (34.41 cm), leaves number (8.70plant⁻¹), Collar diameter (1.83 mm) and number of nodules (4.44 plant⁻¹) were recorded at 2000 ppm. Lead is a biological non-essential element and it interferes with the general metabolism of plant particularly in synthesis of chlorophyll and photosynthesis rate (Singh 1988) and ultimately growth performance of plant is affected. Similar results were also observed in exhaustive maize crop by Kalimuthu and Sivasubramanian (1990) when crops were grown after seed soaking in different concentrations of Lead. Al-Yemini (2001) analyzed the process of reduction in root and shoot length with Lead in *Vigna radiata*, in presence of higher Lead content in cell, retarded the cell division and differentiation and reduce their elongation and effect the plant growth and development (Lane *et.al.*, 1978 and Kasturi *et.al.*, 1991). The differential response in root and shoot might be due to more rapid accumulation in root than shoot (Shaukat *et.al.*, 1999) in case of *Parkinsonia acculeata*.

BIOMASS ACCUMULATION IN *Acacia mangium*

Accumulation of dry matter in the form of biomass in growing plant is the final outcome of performance which survived in a given eco system either having a positive or negative relationships to available resources or living neighbors. Here in the study, the application of metallic pollutants *viz.*, Chlorides of Cobalt, Nickel and Lead known for creating toxicity but there be a differences in the uptake of metal for species (Nandakumar, *et.al.*, 1995).

Effect of Cobalt Chloride: Perusal of data presented in Table 1 revealed that the maximum dry matter accumulation in seedling of *Acacia mangium* was recorded in control at 90 DAS for leaves (0.85 g), above (1.59 g), and (0.78 g) below ground parts, total plant (2.37 g) with their 2.03 shoot / root ratio followed by 100 ppm treatment, 0.78 g, 1.43 g, 0.69 g, 2.12 g with 2.07 shoot / root ratio respectively. Treatments 500 ppm and 700 ppm were statistically on par, for leaves and above ground dry matter. Shoot



Fig 1. Effect of Cobalt Chloride on growth performance of *Acacia mangium*



Fig 2. Effect of Nickel Chloride on growth performance of *Acacia mangium*



Fig 3. Effect of Lead Chloride on growth performance of *Acacia mangium*

Table 1: Growth performance of *Acacia mangium* with different concentration of Cobalt Chloride in soil at 90 DAS

Treatments	Shoot length (cm)	Collar diameter (mm)	Leaf number plant ⁻¹	Root length (cm)	Nodules No./plant	Dry weight gm plant ⁻¹				
						Leaves	Above ground	Below ground	Total	Shoot /root
T₁ (0)	19.48	3.14	15.98	54.87	9.84	0.85	1.59	0.78	2.37	2.03
T₂ (100)	18.43	2.92	15.70	52.14	9.20	0.78	1.43	0.69	2.12	2.07
T₃ (200)	16.49	2.74	15.03	50.39	8.78	0.68	1.25	0.61	1.86	2.04
T₄ (500)	14.63	2.55	14.10	47.04	8.50	0.60	1.09	0.55	1.64	1.98
T₅ (700)	13.41	2.45	12.85	43.50	7.22	0.57	0.99	0.46	1.45	2.15
T₆ (1000)	11.60	2.35	10.89	40.71	6.85	0.46	0.73	0.38	1.21	1.92
T₇ (2000)	10.07	1.90	8.81	36.49	6.22	0.31	0.55	0.27	0.81	2.03
SEm ±	0.15	0.03	0.05	0.27	0.10	0.01	0.05	0.01	0.03	
SEd ±	0.21	0.04	0.08	0.39	0.14	0.02	0.07	0.02	0.05	
CD at 5%	0.45	0.08	0.17	0.83	0.30	0.04	0.14	0.04	0.10	

* Values in parameters are concentration of CoCl₂ in ppm

Table 2: Growth performances of *Acacia mangium* with different concentration of Nickel Chloride in soil at 90 DAS

Treatments	Shoot length (cm)	Collar diameter (mm)	Leaf number plant ⁻¹	Root length (cm)	Nodules No./plant	Dry weight gm plant ⁻¹				
						Leaves	Above ground	Below ground	Total	Shoot /root
T₁ (0)	19.48	3.14	15.98	54.87	9.84	0.85	1.59	0.78	2.37	2.03
T₂ (100)	18.40	2.94	15.39	53.29	9.23	0.74	1.37	0.72	2.07	1.90
T₃ (200)	17.80	2.80	14.70	50.87	8.70	0.66	1.23	0.65	1.88	1.89
T₄ (500)	16.11	2.67	13.50	48.44	8.31	0.57	1.08	0.58	1.66	1.86
T₅ (700)	13.33	2.56	11.83	47.88	7.88	0.49	0.92	0.54	1.46	1.70
T₆ (1000)	11.50	2.35	10.35	47.26	7.44	0.38	0.65	0.46	1.11	1.41
T₇ (2000)	9.95	1.94	8.95	45.37	6.61	0.25	0.38	0.36	0.74	1.05
SEm ±	0.13	0.02	0.06	0.28	0.10	0.01	0.02	0.01	0.03	
SEd ±	0.18	0.03	0.08	0.40	0.15	0.02	0.02	0.02	0.04	
CD at 5%	0.38	0.07	0.17	0.85	0.32	0.03	0.06	0.04	0.08	

* Values in parameters are concentration of NiCl₂ in ppm

Table 3: Growth performances of *Acacia mangium* with different concentration of Lead Chloride in soil at 90 DAS

Treatments	Shoot length (cm)	Collar diameter (mm)	Leaf number plant ⁻¹	Root length (cm)	Nodules No./plant	Dry weight gm plant ⁻¹				
						Leaves	Above ground	Below ground	Total	Shoot /root
T₁ (0)	19.48	3.17	15.98	54.20	9.84	0.85	1.59	0.78	2.37	2.03
T₂ (100)	18.20	2.90	15.30	52.00	8.87	0.70	1.33	0.67	2.00	1.98
T₃ (200)	16.61	2.70	14.65	48.63	8.29	0.63	1.18	0.59	1.77	2.00
T₄ (500)	14.96	2.46	13.43	46.38	7.46	0.55	1.03	0.50	1.53	2.06
T₅ (700)	13.20	2.37	11.30	43.11	6.87	0.46	0.87	0.43	1.30	2.02
T₆ (1000)	11.40	2.29	10.14	39.49	5.40	0.29	0.58	0.24	0.82	2.41
T₇ (2000)	9.90	1.83	8.70	34.41	4.44	0.14	0.27	0.13	0.40	2.07
SEm ±	0.16	0.02	0.03	0.40	0.19	0.02	0.02	0.01	0.03	
SEd ±	0.22	0.03	0.04	0.57	0.27	0.02	0.03	0.02	0.04	
CD at 5%	0.48	0.06	0.10	1.21	0.19	0.05	0.06	0.04	0.09	

* Values in parameters are concentration of PbCl₂ in ppm

/root ratio was least (1.92) at 1000 ppm, however, least dry matter was accumulated for leaves (0.31 g), above ground (0.55 g), below ground (0.27 g) and total (0.81 g) at 2000 ppm. Rehab and Wallace (1978) that, addition of Cobalt caused the reduction in the dry weight of cotton plants significantly. Cobalt concentrations for cultivation of moong found toxic effect even at very lower concentration and ultimately lead to reduction in dry matter production. Agrawal *et.al.* (1977) reported the restriction in biomass of barley and cauliflower due to abnormal metabolism due to excess supply of Cobalt, Chromium and Copper in the soil (Chatterjee and Chatterjee, 2000).

Effect of Nickel Chloride: Different level of Nickel Chloride in soil showed statistically significant variation in leaves dry matter of above ground parts and below ground parts, totals plant along with shoot / root ratio of *Acacia mangium* (Table 2). Irrespective to treatments of Nickel Chloride, control was superior for maximum biomass accumulation followed by 100ppm NiCl₂ treatment. Higher concentrations of Nickel Chloride, *i.e.* 2000 ppm gave least dry matter accumulation for leaves (0.25 g), above ground parts (0.38 g), below ground parts (0.36 g), with total of 0.74 g/plant). The Shoot/Root ratio was found to be

maximum in control (2.03) followed by 100 ppm (1.90) and it decreased with increasing concentration up to 1.05 at 2000 ppm. Singh (1983, 1985) studied the rate of Nickel on *Vigna radiata* and *Luffaa egyptica* where, the dry weight of seedlings decreases with increasing the concentration of Nickel in the soil. Such inhibitory effect of Nickel on seedling growth and its biomass accumulation was due to binding of metal with sulphhydryl group of proteins, which lead to check the mobilization of nitrogen and phosphors through enzymes (Jerome and Ferguson 1972). Sharma (1982) also studied similar results in case of using Mercury. Peralta *et.al.* (2000) also reported similar results in preliminary study of alfalfa with several doses of Cd, Cr, Cu, Ni and Zn where higher concentrations reduced the growth and dry matter.

Effect of Lead Chloride: Results presented in Table 3 showed that biomass accumulation was reduced with application of concentrations of Lead Chloride. The mean values for dry matter accumulation for leaves above ground part, below ground part and the total plant showed significant variation in plant of *Acacia mangium* at 90 DAS where these values were ranged from 0.14 -0.85 g, .027-1.59 g, 0.13-0.78 g, 0.40-2.37 g respectively. The maximum dry matter was

accumulated in control (0 ppm) and minimum in 2000 ppm PbCl₂. All the results were in decreasing order with increasing the concentration of PbCl₂. The Shoot/Root ratio was maximum in 1000 ppm (2.41) and followed by 2000 ppm (2.07) while the minimum was in 100 ppm (1.98) respectively. Lead has been identified as an important metal which caused severe lethal effect on mankind directly or indirectly through edible plant material. It causes anti-vital role in growth and development of plants mostly. Al-Yemeni *et.al.* (2001) reported the similar trend of biomass accumulation in case of *Vigna ambaconsis* and worked the metabolic activities during the process of germination and growth of seedlings of *Acacia farnesiana* (Al-Yemeni and Al-Helol, 2000).

CONCLUSION

The present study leads to the conclusion that the significant inhibitory effects of metallic pollutants *viz.*, Cobalt Chloride, Nickel Chloride and Lead Chloride on growth performance, biomass accumulation of *Acacia mangium*. The application of Cobalt, Nickel and Lead Chlorides inhibited the performance of *Acacia mangium*. Increasing the concentrations from 100, 200, 500, 700, 1000 to 2000 ppm there was decrease in the growth performance (shoot and root length, leaves number, Collar diameter number of nodules), biomass accumulation (dry weight of leaves, shoot and root).

REFERENCES

Arey, N.C. and B.L. Jagetiya. 1998. Toxic effect of Cobalt on Barley growth in soil culture. *Environment and Development*, 193-198.

Agrawal, R.L. 2003. Seed technology. *Oxford & IBH Publishing Co. Pvt. Ltd.* pp. 550-551.

Agrawal, S.C., S.S. Bisth and C.P. Sharma,. 1977. Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barely. *Canadian J. Botany*, 55: 1299-1307.

Ahmed, S. and H.J.Evans. 1959. Biochemistry, Biophysics Research Communication. pp. 272-275.

AL-Yemeni, M.N. 2001. Effect of Cadmium, Mercury and Lead on seed germination and early

seedling growth of *Vigna ambacensis* L. *Indian J. Plant Physiol.*, 6(2): 147-151.

Al-Yemeni, M.N. and A.A. Al-Helol. 2000. Some metabolic changes in germinated *Acacia farnesiana* L. Seeds. *Journal of Science Engineers*, 13(7): 237-243

Baker, A.J.M., S.P. McGrath, C.M.D. Sidali and R.D. Reeves. 1994. The possibility of in-situ heavy metals decontamination of polluted soil using crop of metal accumulating plants. *Resources conservation and Recycling*, 11: 41-49.

Chatterjee, J. and C. Chatterjee, 2000. Phytotoxicity of Cobalt, Chromium and Copper in Cauliflower. *Environment and Pollution*, 109: 69-74.

Chaukiyal, S.P., K.C.H. Singh and T.C. Pokhriyal. 1999. Effect of seasonal variations on nodulations and nitrogen fixation behavior in some *Acacia* species. *Annals of Forestry*, 7 (1): 112-119.

Chidumayo, E.N. 2005. Effect of climate on the growth of exotic and indigenous trees in Central Zambia. *Journal of Biogeography*, 32 (1): 111-120.

Gabriella Mathe-Gaspar and Attila Anton, 2002. Heavy metal up take by two radish varieties. Proceeding of the 7th Hungariun congress on Plant Physiology in: *Acta Biologica Szegediensis*, 46 (3-4): 113-114.

Jagetiya, B.L. and N.C. Arey. 1998. Effect of different Nickel salts on germination in moong. *In: Environment and Development* (Ed. Grover and Thakural) Pub. Scientific Publishers, Jodhpur, 189-192.

Jerome, G. and J.F. Ferguson. 1972. The cycling of Hg through environment. *Water Research*, 6: pp-98.

Kasturi, R., N. Petrovite, A. Knezevic and D. Kabie, 1991. Effect of Lead on the morphology and anatomy of Maize plants (*Zea mays* L.). *Maticasrpska Proce Natural Science*, 87: 121-129.

Kalimuthu, K. and K. Sivasubramanian. 1990. Physiological effect of heavy metals on *Zea*

- mays (Maize) seedlings. *Indian J. Plant Physiol.*, 33(3): 242-244.
- Lane, S.D., E.S. Martin and I. E. Garrod. 1978. Lead toxicity effects on indole-3-acetic acid induce cell elongation. *Planta*, 144: 79 – 84.
- Nandakumar, P.B.A., V. Dushenkov, H. Motto and I. Raskin. 1995. Phytoextraction: The use of Plants of Remove Heavy metals from Soils. *Environmental Science Technology*, 29: 1232-1238.
- Peralata, J.R., J.L. Gardea-Torresdey, K.J. Tiemann, E. Gomez, S. Arteaga, E. Rascon, and J.G. Parsons. 2000. Study of the effect of heavy metals on seed germination and Plant growth on *Medicago sativa* Grown in solid media. *Conference on Hazardous Waste Research*, pp 135-139.
- Rehab, F.I. and A. Wallace, 1978. *Soil Science Plant Annals*, 9: pp 507-518.
- Sharma, S.S. 1982. Effect of Mercury on Germination and seedling growth of different varieties of *Phaseolusaureus*. *Indian Journal of Ecology*, 9 (1): 78-81.
- Singh, B.P.1998. Growth and Growth analysis of Bhalia (*Flemingi amacrophylla* (Wild.) O. Ktze) In relation to date of sowing and size of polythene bags in nursery. *Indian Journal of Forestry*, 21(1): 34-37.
- Singh, S.N. 1983. Effect of Nickel on germination, seedling growth in *Luffaa egyptica* L. cultivars. *Geobia*, 10: 86-88.
- Singh, S.N. 1985. Effect of Nickel on germination, seedling growth, total nitrogen and phosphate in *Vigna radiata* cultivars. *Indian Journal of Ecology*, 12(1): 162-165.
- Susilaawati, S. and D. Setiadi. 2003. Preliminary research on natural hybrid of *Acacia mangium* and *Acacia auriculiformis* in woong, central Jawa. In: (Ed-Rimbawanto, A and M. Susanta) *Proceeding of International Conference on “Advances in Genetic Improvement of Tropical Tree species”* at Indonesia, 1-3 Oct. 2002. pp 153-156.
- Shaukat, S.S., M. Mushtaq and Z.S. Siddiqui. 1999. Effect of Cadmium, Chromium and Lead on Seed germination, early seedling growth and phenolic contents of *Parkinonia acculeata* L. and *Pennisettium americanum* L. *Schumann. Pakistan Journal of Biological Science*, 2: 1307–1313.