MUTHTHARASI – Art Literary Culture Tamil Journal,Madurai
முத்தரசி – கலை இலக்கியப் பண்பாட்டுத் தமிழ் ஆய்விதழ், மதுரை
SPL.EDITION : VI VOLUME : II
E.ISSN : 2584 - 1238 அக்டோபர் - 2025

Alleviation of heavy metal stress by bioadsorbents

Gowsalya Devi, A.P

* Assistant Professor of Botany, The Standard Fireworks Rajaratnam College for Women, Sivakasi.

devikanth73@gmail.com Mobile - 9443119605

Abstract

Environmental pollution is not a new phenomenon, yet it remains the world's greatest problem facing humanity, and the leading environmental causes of morbidity and mortality. Man's activities through urbanization, industrialization, mining and exploration are at the forefront of global environmental pollution. Huge amount of waste water, the effluent are released from all industries. Most of the effluents and wastes contain heavy metals in an amount sufficient enough to cause toxicity to crop plants (Khan and Siddhu, 2006). Wastewater may contain various heavy metals including Zn, Cu, Pb, Mn, Ni, Cr and Cd, depending upon the type of activities, it is associated with. The impact of copper sulphate (2mM, 4mM, 6mM, 8mM and and 10mM) on the morphometric, biochemical and enzymatic characteristics of Trigonella foenum-graecum L. was analyzed after 21 days. The reduction in all the growth characteristics and biomass accumulation was parallel with reduction in carbohydrate and pigment content. The protein content and nitrate reductase also decreased with increasing concentration of copper sulphate. An increased leaf nitrate, free amino acids, L-proline, peroxidase and catalase indicated the stress nature of the plant. Applications of low cost environment friendly bioadsorbent (Azolla) alleviate the stress caused due to heavy metal toxicity. A significant increase in germination percentage, growth, biochemical and enzymatic characteristics was noted in the bioadsorbent treated experimental plant than when they were treated with various concentration of heavy metal alone.

Key words: Heavy metal, phytotoxicity, catalase, peroxidas, Bioadsorbent, Azolla.

http://Creativecommons.org//license/by/4.0/.

INTRODUCTION

In the current anthropocene, environmental pollution is a global problem that is inextricably linked with rapid industrialization and urbanization. Industrialization is an important tool for the development of any nation. Sources of heavy metal pollution are mainly by point sources such as emission, discharge from industries, mining and non-point sources such as excessive use of insecticides / pesticides / fertilizers. Some common heavy metal contaminants are Cd, Cr, Cu, As, Hg, Pb and Ni, and they occur either naturally in soil or due to the excessive use of agricultural chemicals, urban waste and contaminated water (Yadav et al., 2010). Mining, metallurgy, agrochemicals, agricultural and urban wastes and industrial applications are the main source of copper to the environment. Copper (Cu) is one of the essential micronutrient for the growth of the plants (Kabir et al., 2009) and helps to maintain cellular homeostasis. Elevated amount of copper damage the internal structure and ultimately inhibits the growth of the plant.

Fenugreek (Trigonella foenum-graecum L.) locally known as, methi belonging to the family- Leguminosae and Sub family-Papilionacea It is cultivated worldwide as a semiarid crop. Fenugreek is generally cultivated as leafy vegetable, condiment and medicinal purpose. Its fresh and tender leaves contain iron, calcium, protein, vitamins and essential amino acids.

Azolla is a free floating water fern, which occurs in the symbiotic association with N_2 fixing blue green alga Anabaena azollae. It has high rate of N_2 fixation making its biomass rich in nitrogen and protein. It is mainly used as green manure in agriculture and also used for bioremediation of waste water and reclamation of saline soils. Both living as well as the non-viable biomass of Azolla can be used for reclamation of heavy metal. It can uptake and accumulate nutrients several times more than its requirement then slowly releases these nutrients as it decomposes. Azolla exhibits a remarkable ability to concentrate metals Cu, Cd, Cr, Ni, Pb and nutrients directly from pollutants.

http://Creativecommons.org//license/by/4.0/.

MATERIALS AND METHODS

Seed Collection and treatment

Healthy and viable seeds of Trigonella foenum-graecum L. were procured from a seed vendor, certified by Tamilnadu Seed Certification Department, Rajapalayam, Tamilnadu. Seeds were surface sterilized with 0.1% mercuric chloride for 2 minutes and rinsed twice with distilled water. To evaluate the germination efficiency, spread the seeds on the sterilized petridishes lined with filter paper and irrigated with 5ml of different concentration of copper sulphate (2mM, 4mM, 6mM, 8mM and 10mM) and also amended with bioadsorbent respectively.Triplicates were maintained for each concentration along with control.

Seedling treatment

In the first set of experiment, Surface sterilized seeds were sown and allowed to grown in different earthen pots containing uniformly mixed garden soil (sand, red soil and black soil (1:1:1 ratio)). After 07 days, seedlings of Trigonella foenum-graecum L. were treated with various concentration of copper sulphate (2mM, 4mM, 6mM, 8mM and 10mM). After 15 days of heavy metal treatment, various morphometric, biochemical and enzymatic characteristics were analyzed. Azolla, an aquatic fern was grown in plastic troughs and were then harvested and allowed to dry, powdered and used as bioadsorbent. In the second set of experiment, 10 days old seedlings of Trigonella foenum-graecum L. were treated with bioadsorbent- Azolla biomass (5gm) integrated with various concentration of copper sulphate. Triplicates were maintained for both control and experimental plants.

Growth and Biochemical parameters.

Various morphometric characters such as root length shoot length, fresh and dry biomass [Arts H. H and Marks P.L. (1971)] after 21 days of sowing were measured. 21 days old plants of Trigonella foenum-graecum L. were used for analyzing the biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid [Wellburn and

http://Creativecommons.org//license/by/4.0/.

Lichtenthaler (1984)] Total soluble sugar [Jayaraman,(1981)] Protein [Lowry et al., (1951)] free amino acid content [Jayaraman, (1981)] Proline [Bates et al., (1973)] leaf nitrate [Cataldo et al., (1978)] invivo nitrate reductase activity [Jaworski (1971)] Catalase activity [Kar and Mishra, (1976)] Peroxidase [Addy and Goodman, (1978)]

Statistical analysis

Growth parameters were determined with ten independent replicates. Biochemical characters and enzymatic assay were carried with five replicates. The data were reported as mean and in parentheses represent the percent activity.

Table I- Effect of various concentration of copper sulphate on the growth of Trigonella foenum-graecum L.

S.No	Parameters	Control	Concentration of Copper sulphate				
		(Water)	2mM	4mM	6mM	8mM	10mM
1.	Shoot length (cm)	15.4 (100)	14.62 (95)	12.28 (80)	11.7 (76)	10.04 (65)	9 (58)
2.	Root length (cm)	12.84 (100)	11.9 (93)	10.34 (81)	9.6 (75)	7.5 (58)	6.9 (53)
3.	Fresh weight (gm)	0.512 (100)	0.498 (97)	0.47 (92)	0.352 (67)	0.324 (63)	0.278 (54)
4.	Dry weight (gm)	0.328 (100)	0.208 (63)	0.19 (58)	0.136 (41)	0.088 (27)	0.074 (23)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table I-A Effect of various concentration of copper sulphate with bioadsorbent on the growth of Trigonella foenum-graecum L.

http://Creativecommons.org//license/by/4.0/.

S.No	Parameters	Control (Water+ Bioadsorbent)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Shoot length (cm)	16.26 (100)	15.04 (92)	14.1 (87)	12.24 (75)	11 (68)	10.5 (65)
2.	Root length (cm)	13.38 (100)	12.04 (90)	11.6 (87)	10.34 (77)	8.68 (65)	7.82 (58)
3.	Fresh weight(gm)	0.604 (100)	0.508 (84)	0.49 (81)	0.376 (62)	0.342 (57)	0.296 (49)
4.	Dry weight(gm)	0.354 (100)	0.248 (70)	0.228 (64)	0.166 (47)	0.122 (34)	0.112 (31)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table II Effect of various concentration of copper sulphate on the pigment content of Trigonella foenum-graecum L.

S.No	Parameters	Control (Water)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Chlorophyll – a (mg/g LFW)	0.222 (100)	0.211 (95)	0.200 (90)	0.190 (86)	0.176 (79)	0.170 (77)
2.	Chlorophyll – b (mg/g LFW)	0.326 (100)	0.295 (90)	0.276 (85)	0.258 (79)	0.240 (74)	0.221 (68)
3.	Total chl (mg/g LFW)	0.173 (100)	0.162 (94)	0.156 (90)	0.145 (84)	0.136 (79)	0.125 (72)
4.	Carotenoids	7.067	6.649	6.276	5.296	4.848	4.819

http://Creativecommons.org//license/by/4.0/.

	(mg/g LFW)	(100)	(94)	(89)	(75)	(69)	(68)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table II-A Effect of various concentration of copper sulphate with bioadsorbent on pigment content of Trigonella foenum-graecum L.

S.No	Parameters	Control (Water+ Bioadsorbent)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Chlorophyll – a (mg/g LFW)	0.212 (100)	0.202 (95)	0.194 (91)	0.180 (85)	0.168 (79)	0.157 (74)
2.	Chlorophyll – b (mg/g LFW)	0.342 (100)	0.317 (93)	0.296 (87)	0.282 (82)	0.261 (76)	0.241 (70)
3.	Total chlorophyll (mg/g LFW)	0.180 (100)	0.171 (95)	0.161 (89)	0.153 (85)	0.142 (79)	0.136 (76)
4.	Carotenoids (mg/g LFW)	7.162 (100)	6.867 (96)	6.295 (88)	5.870 (82)	5.259 (73)	4.826 (67)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table III Effect of various concentration of copper sulphate on the biochemical characters of Trigonella foenum-graecum L.

S.No	Parameters	Control (Water)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Total soluble sugar	20.322	17.25	12.41	5.16	3.06	1.77

http://Creativecommons.org//license/by/4.0/.

	(mg/g LFW)	(100)	(85)	(61)	(25)	(15)	(9)
2.	Protein (mg/g LFW)	14.872 (100)	13.15 (88)	9.58 (64)	6.76 (45)	3.81 (26)	1.84 (12)
3.	Free Amino acid (mg/g LFW)	7.348 (100)	9.20 (125)	10.62 (145)	11.85 (161)	14.77 (201)	16.63 (226)
4.	Proline (mg/g LFW)	7.511 (100)	10.26 (137)	11.64 (155)	16.26 (217)	19.73 (263)	27.6 (367)
5.	Leaf Nitrate (µg/hrs)	7.42 (100)	11.5 (155)	15.31 (206)	18.8 (253)	22.73 (306)	26.46 (357)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table III-A Effect of various concentration of copper sulphate with bioadsorbent on biochemical characters of Trigonella foenum-graecum L.

S.No	Parameters	Control (Water+ Bioa)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Total soluble sugar (mg/gLFW)	24.193 (100)	20.32 (84)	17.25 (71)	8.752 (36)	5.967 (25)	3.387 (14)
2.	Protein (mg/g LFW)	52.851 (100)	39.20 (74)	33.55 (63)	26.54 (50)	23.35 (44)	20.280 (38)
3.	Free Amino acid (mg/g LFW)	1.777 (100)	2.725 (153)	4.266 (240)	5.807 (327)	6.992 (393)	8.098 (456)
4.	Proline (mg/g LFW)	1.28 (100)	1.311 (102)	1.72 (134)	2.017 (158)	2.075 (162)	2.564 (200)
5.	Leaf Nitrate	5.333	7.333	11	14.22	18.11	21.333

http://Creativecommons.org//license/by/4.0/.

	(µg/hrs)	(100)	(138)	(206)	(267)	(340)	(400)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table IV Effect of various concentration of copper sulphate on the enzymatic activities of Trigonella foenum-graecum L.

S.No	Parameters	Control (Water)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Nitrate reductase (µ mole/30 min)	0.627 (100)	0.530 (85)	0.433 (69)	0.352 (56)	0.258 (41)	0.183 (29)
2.	Catalase (N mole ml)	0.005 (100)	0.006 (120)	0.007 (140)	0.008 (160)	0.009 (180)	0.010 (200)
3.	Peroxidase (µ mole/g LFW)	86.369 (100)	130.904 (152)	226.720 (263)	288.798 (334)	351.430 (407)	398.110 (461)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Table IV-A Effect of various concentration of copper sulphate with bioadsorbent on the enzymatic activities of Trigonella foenum-graecum L.

S.No	Parameters	Control (Water+ bioabsor)	Concentration of copper sulphate				
			2mM	4mM	6mM	8mM	10mM
1.	Nitrate reductase (µ mole/30 mn)	0.675 (100)	0.636 (94)	0.530 (79)	0.433 (64)	0.352 (52)	0.258 (38)
2.	Catalase	0.003	0.004	0.005	0.006	0.007	0.008

http://Creativecommons.org//license/by/4.0/.

	(N mole ml)	(100)	(133)	(167)	(200)	(233)	(266)
3.	Peroxidase (µ mole/g LFW)	8.502 (100)	13.765 (162)	18.623 (219)	23.143 (272)	27.53 (324)	28.744 (338)

Values are average of three observations. Values in parentheses are percentage activity with respect to control.

Result and Discussion

Effect of five different concentrations (2mM, 4mM, 6mM, 8mM and 10mM) of copper sulphate on the growth, biochemical and enzymatic activities are represented in Table I, II, III & IV.

Germination percentage and growth parameters decline gradually with increasing concentration of copper sulphate. The germination was found to be 95%, 84%, 73% and 63% in 2mM, 4mM, 6mM and 8mM respectively. The minimum percentage of germination was observed at 10mM as 52 %. The result coincides with the findings of Ramasubramanian et al., 2015 in cowpea. The decrease in seed germination may be due to the breakdown of stored food material in the seed or the toxic effect of copper. Similar results were reported in Sorghum (Metwali et al., 2013) and in Triticale (Brezoczki and Fillip 2017) subjected to copper stress. Minimum shoot length of 62% and root length of 18%was observed at 10mM. The reduction in shoot length persists due to retardation of cell division, differentiation and elongation Metwali et al., 2013 in Sorghum. It was revealed that reduction in root growth was primarily attributed to the binding of heavy metals to sulfhydrl groups which regulates cell division in plants (Verma et al., 2011). Copper affected root more than shoot attributed to the fact that roots receive copper ions in soils via apoplastic transport, resulting in higher accumulation of copper in the roots (Drazkiewicz et al., 2005).A maximum reduction of fresh weight and dry weight was observed in 10mM concentration. According to Manivasaga perumal et al., 2011 a reduction in biomass in higher concentration of heavy metal might be due to inhibiting rate of photosynthesis, low

http://Creativecommons.org//license/by/4.0/.

level of protein formation as well as hampered carbohydrate translocation. In Polygonum convolvulus reduction in biomass, seed production and plant mortality was observed due to Cu toxicity (Mohnish pichhode 2015).

Similarly chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content diminished with increasing concentration of the heavy metal.Copper dust imparts adverse effect on various photosynthetic processes in the leaves of many tree species (Mohnish Pichhode 2015). Excess amount of Cu is cytotoxic and induces stress which leads to retardation in plant growth and chlorosis (katare et al., 2015). High concentrations of heavy metals degrade photosynthetic enzymes activities and photosynthetic electron transport chain was blocked which resulted in reduction of chlorophyll content (Thapar et al., 2008).

Sugar content was decreased from 87% to 50% from 2mm to 10mM respectively when compared to control is due to the photosynthetic inhibition or stimulation of respiration rate. Reduction may be due to copper induced alternation of carbohydrate metabolism which was homologous to the findings of Ramasubramanian et al., (2015). Gradual decline in protein content was found to be observed with the increasing concentration of copper which might be due to the interference of copper in the enzyme activity, involved nitrogen metabolism. (Tandon and Srivastava 2004). Accumulation of copper in the experimental plants may induce lipid peroxidation in them and fragmentation of protein occurs due to toxic effects of reactive oxygen species which resulted in reduced protein content.

Free amino acid ranges from 16.632mg/g/LFW in 10mM concentration treatment to 9.204mg/g/LFW in 2mM concentration. A reduction in soluble protein level eventually leads to an increase in free amino acid content. Proline content ranges from 27.6mg/g/LFW in 10mM concentration treatment to 10.266mg/g/LFW in 2mM concentration which was homologous to the findings of Ramasubramanian et al., 2017 and Murugalakshmi Kumari 2017 with heavy metal treated seedlings. Increase in leaf nitrate values in 2mM, 4m,M, 6mM, 8mM and 10mM

http://Creativecommons.org//license/by/4.0/.

treatments were 155%, 206%, 253%, 306% and 357% respectively homology to the findings of Duraipandian et al., 2016 in Eleusine coracana. It may be attributed to low photosynthetic rate which limits energy and declining power potential for physiological activities.

In comparison to control condition, the percent decrease in invivo nitrate reductase values in 2mM, 4mM, 6mM, 8mM and 10mM concentrations were 85%, 69%, 56%, 41% and 29% respectively. Catalase and peroxidase elevated to a greater extent with increasing concentration of the heavy metal. The observed increase in peroxidase activity can be correlated with the reduction in chlorophyll content, fresh weight and dry weight.

Effect of bioadsorbent (Azolla biomass) integrated with 5 different concentrations (2mM, 4mM, 6mM, 8mM and and 10mM) of copper sulphate on the growth, biochemical and enzymatic activities are represented in Table IA, IIA, IIIA & IVA. Addition of bioadsorbent nullify the toxicity of copper sulphate and the growth parameters such as root length, shoot length, fresh and dry weight increased in all the concentration compared to heavy metal alone treated plants. Azolla biomass has been used as a bioadsorbent for the removal of copper from industrial waste water [Bilal et al., 2013].

Percentage of seed germination and all growth parameters and a huge biomass accumulation showed increasing trend from 2mM concentration to 10mM concentration of copper sulphate treated with the bioadsorbent -Azolla biomass. This may be due to the fact that the bioadsorbent -Azolla biomass released certain adsorbed products such as nutrients and growth regulators which become available to the plants. Pigment content, soluble sugar and protein content elevated and free amino acid, proline and leaf nitrate content shows gradual reduction after the application of bioadsorbent in all the concentration indicates the active promotive nature of Azolla biomass on plant growth and metabolism. Datura biomass – bioadsorbent has the ability to relieve the toxicity of paper mill effluent and an increase in the

http://Creativecommons.org//license/by/4.0/.

pigment content was reported in Lycopersicum esculentum. [Selvarathi and Ramasubramanian (2010)]

The result of the present study clearly stated that the addition of bioadsorbent to the various concentration of copper sulphate showed improved germination percentage, root length, shoot length, biomass, biochemical parameters and enzymatic activities compared to the heavy metal alone treated plants. As Azolla biomass can efficiently remediate the toxicity of heavy metal, it can be safely used for the irrigation purpose to promote sustainable agriculture.

REFERENCES

- Addy S.K and Goodman R. K. (1978): Polyphenol oxidase activity in apple leaves inoculated with a virulent oravirulant of Erwinia amylovora. Phytopathol., 24 (3) :575-579.
- Arts H. H and Marks P.L. (1971 Arts, H. H, and Marks P.L. (1971): A summary table of biomass and net annual primary production in forest ecosystem. (editor, Young, HE) Life science and agriculture experimental station University of Marine. Ornao,Marine. USA.
- Bates L.S, Warden R.P and Teare L.D. (1973). Rapid determination of the proline in water stress studies. Plant and Soil, 39.205-208.
- Bilal,M., Jehanzeb Ali Shah, Tayyab Ashfaq, Syed Mubashar Hussain Gardazi, Adnan Ahmad Tahir, Arshid Pervez, Hajira Haroon, Qaisar Mahmood (2013): Waste biomass adsorbents for copper removal from industrial wastewater—A review Journal of Hazardous Materials 263 322– 333
- Brezoczki V M and G M Filip (2017) The heavy metal ions (Cu2+, Zn2+, Cd+) toxic compounds influence on triticale plants growth Innovative Ideas in Science 2016IOP Conf. Series: Materials Science and Engineering 200 012025

http://Creativecommons.org//license/by/4.0/.

- Cataldo D.A, Harron M, Schroader L.E and Younger V.C.(1978): Rapid colorimetirc NR determination of Nitrate in plant by nitrate salicyclic acid. Commun. Soil Sci. Plant. Analy., 6:7180.
- Drażkiewicz, M. and T. Baszyński. (2005). Growth parameters and photosynthetic pigments in leaf segments of Zea mays exposed to cadmium, as related to protection mechanisms. J. Plant Physiol. 162: 1013-1021.
- Duraipandian, M., Marisamy, K., Periyanayagi, G., Sevugaperumal, R., Ganesh, D and Ramasubramanian, V. (2016). Impact of cobalt on the growth, pigmental, some biochemical and enzymatic characteristics of Eleusine coracana (L.) Gaertn. American-Eurasian Journal of Toxicological Sciences. 8(3): 115-119.
- Jeyaraman . J. (1981): In Laboratory manual in Biochemistry, Wiley Eastern Limited, Madras, 53-55.
- Jaworski S.G. (1971): Nitrate reductase assay in intact plant tissues. Biochem. Biophy. Res. Commun., 43. 1274-1279.
- Kabir. M., Iqbal M. Z., and M. Shafiq (2009) “Effects of lead on seedling growth of Thespesia populnea L.,” Advances in Environmental Biology. 3(2):184–190.
- Katare, Jyotish & Pichhode, Mohnish & Nikhil, Kumar. (2015). Growth of Terminalia Bellirica [(Gaertn.) Roxb.] On The Malanjkhand Copper Mine Overburden Dump Spoil Material. International Journal of Research . 3. 14-24.
- Kar M and Mishra D. (1976): Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol., 57(2): 315- 319.
- Khan A.M.A., Siddhu G., (2006), “Phytotoxic effect of cadmium (Cd) on physiology of Urd bean [Vigna mungo (L.) Heeper]”, Ad Plant Sci., 19:439- 444.
- Lowry O.H, Rosenbrough N.J, Farr A.L, Randall R.J. (1951): Protein measurement with folin phenol reagent. JBio Che 193 : 265-275

http://Creativecommons.org//license/by/4.0/.

- Metwali Ehab M.R., 1Salah M.H. Gowayed, Omar A. Al-Maghrabi and Yahia Y. Mosleh (2013).Evaluation of toxic Effect of copper and cadmium on growth, physiological traits and protein profile of wheat (Triticum aestivium L.), maize (Zea mays L.) and sorghum (Sorghum bicolor L.) World Applied Sciences Journal 21 (3): 301-314,
- Murugalakshmi kumari. R (2017). Bagasse as a low cost bioadsorbent on the growth and biochemicalcharacteristics of metal treated Eleusine coracana, Gaertn International Multidisciplinary Innovative Research Journal -An International refereed e-journal - Science Issue ISSN: 2456-4613 IMIRJ, 1(2), [1-14]
- Mohnish Pichhode, Kumar Nikhil (2015). “Effect of copper dust on photosynthesis pigments concentrations in plants species,” International Journal of Engineering Research And Management 2:63-66.
- Ramasubramanian V. Duraipandian M. and R.Sevugaperumal (2015) Assessment of Copper Caused Stress in Terms of Morphometric, Pigmental, Biochemical and Enzymatic Characteristics of Vigna Unguiculata (L). J. of Advanced Botany and Zoology, V3I2. DOI: 10.15297/JABZ.V3I2.05.
- Ramasubramanian V., M. Mahalakshmi, and K. Marisamy(2017) Cadmium Toxicity Induced Changes on Morphological, Photosynthetic, Biochemical and Antioxidative Enzymes Level in Vigna mungo (L.) Hepper Environment and Ecology Research 5(6): 422-426.
- Selvarathi P and Ramasubramanian V. (2010): Phytoremedial effect of Datura metal L. on paper mill effluent and its impact on Physicochemical characteristics of Lycopersicon esculentum Mill. J. Biosci. Res., 1(2): 94-100.
- Tandon, P.K. and Srivastava, M. (2004). Effect of cadmium and nickel on metabolism during early stages of growth in gram (Cicer arietinum L.) seeds. Indian J. Agric. Biochem., 17: 31 – 34.

http://Creativecommons.org//license/by/4.0/.

- Thapar, R., Srivastava A.K., Bhargava P., Mishra Y., and L.C. Rai. (2008). Impact of different abiotic stress on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated Anabaena doliolum. J. Plant Physiol. 165: 306-316.
- Verma J. P., Vimal singh.,and Janardan yadav(2011) Effect of copper sulphate on seed germination, plant growth and peroxidise activity of Mung bean(Vigna radiata) International journal of botany 7(2): 200-204.
- Wellburn, A.R. and H. Lichtenthaler, (1984). In Advances in photosynthesis Research, (ed.Sybesma) Martinus Nijhoff, Co. The Hague., II : 9-12.
- Yadav S. K. (2010). Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S. Afr. J. Bot. 76 167–179. 10.1016/j.sajb.2009.10.007

http://Creativecommons.org//license/by/4.0/.