Effect of Toxic Metal Copper on Biophysical and Biochemical Parameters of *Vigna mungo* and its Adsorption by Egg Shell Powder

Shabana Askari, Syeda Sadaf Altaf*
Jinnah University for Women, Nazimabad, Karachi, Pakistan
*Email: sadaf_syeda@hotmail.com

ABSTRACT

INTRODUCTION

Now a day, there has been a rising ecological and global public health concern associated with environmental contamination by the metals (Paul *et al*., 2012). According to the literature reviewed the heavy metals sources include geogenic, industrial, agricultural, pharmaceutical and domestic discharges (Akpor 2014). Insufficient copper results in stunting of plants. In copper elevated soil crops might receive the toxic substances from the soil and might cause serious health issues in people consuming them (Sarmishta, 2014). Increased concentration of Cu may reduce soil fertility having adverse effects on crop yield and quality (Dumestre *et. al*., 1999). When copper sulfate is applied excessively, soil copper levels become toxic to plants (Singh *et al*., 2007; Houshmandfar & Moraghebi, 2011). *Vigna mungo* (Black gram) is one of the frequently grown pulse crops throughout the world. In Pakistan, it is grown as a minor crop and used as food. It contains iron, calcium, magnesium and vitamins, they are highly nutritive food.

Egg shells as a wastage from hatcheries, homes and fast food industries are readily collected in plenty. Eggshells contain calcium and trace amounts of other micro elements, and can efficiently increase plant growth and yield (Chavan 2015). Ca+ plays a very important role in plant growth and nutrition, as well as in cell wall deposition. Ca+ neutralizes cell acids as well. Ca+ insufficiency in root tips, young leaves, and shoot tips shows distorted growth due to improper cell wall formation.

OBJECTIVES

Cost is actually an important parameter for comparing the adsorbent materials. The question behind the current research is that, how much effectively egg shell can absorb toxic metal “copper” from plant *Vigna mungo*?

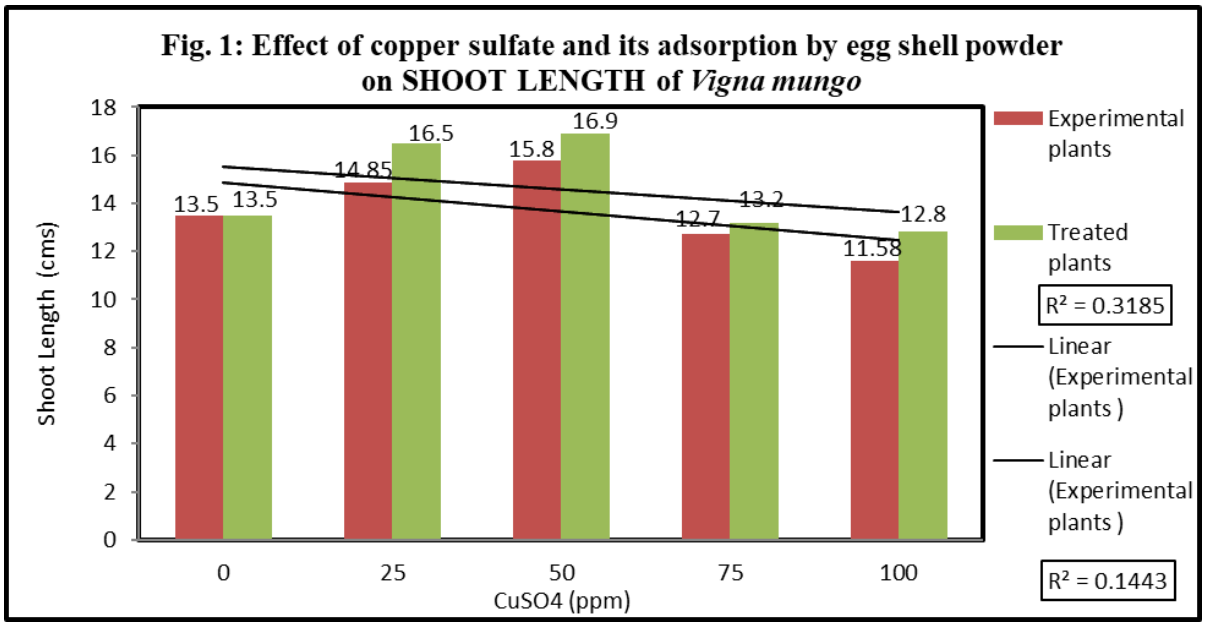
METHODOLOGY

The present research was planned to evaluate the adsorption potential of egg shell powder for toxic metal “Copper” in *Vigna mungo*. Cost is a major factor deals with the use of adsorbent in a large scale. The seeds were sown in randomized block design supplied with different concentrations of Copper as $CuSO_4$/Kg soil (0, 25, 50, 75 and 100ppm). Adsorption of copper was carried out by the application of 10gm egg shells powder/ kg soil to all applied levels of copper. The plants were harvested after 25 days. Various biophysical and biochemical parameters like shoot length, root length, Carbohydrate content and Total protein were pragmatic to evaluate the efficiency of egg shell powder for adsorption of CuSO4 in *Vigna mungo*. Aim of current research was mainly focused on the improvement of crop productivity of agricultural land by adsorbing toxic metal copper from the soil by a free of cost adsorbent (Eggshells) which may be collected in bulk amount from hotels, restaurants and bakeries and off course from the residential areas.

RESULTS

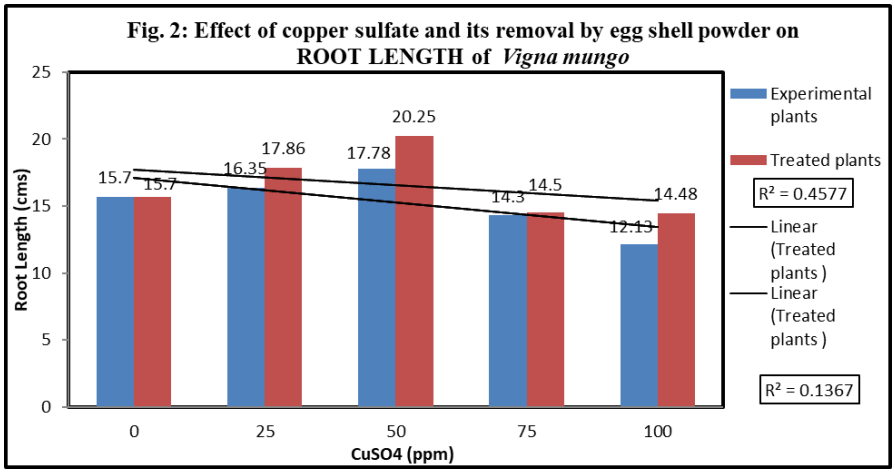
Shoot Length

Present study showed that the shoot length of *Vigna mungo* increased at lower concentrations of $CuSO_4$, whereas it was significantly inhibited at higher concentrations as compared to control (Sarmishta, 2014). According to the data presented in Figure **1**, shoot length of *Vigna mungo* growing in various concentrations of copper sulfate was 14.85±0.66, 15.8±1.39, 12.7±0.39 and 11.58±0.87 and it was 13.5±0.49 at 0 ppm $CuSO_4$. Copper is involved in lignin synthesis and in some other vital functions too. But extensive Cu supply to soil and leaves showed significant decrease in root and shoot length (Sarmishta, 2014; Sonmez *et al*., 2006; Sheldon & Menzies, 2005). Reduction in shoot length was appreciably improved by the application of egg shell powder to *Vigna mungo* growing in all applied levels of copper up to 16.5±0.43, 16.9±0.91, 13.2±0.86 and 12.8±0.52 as compare to experimental plants. This improvement may attribute to the presence of macro and micronutrients and most importantly calcium ions in egg shell powder which activates various enzymes and cell wall formation (Li, 2017; Peter, 2005).



Root Length

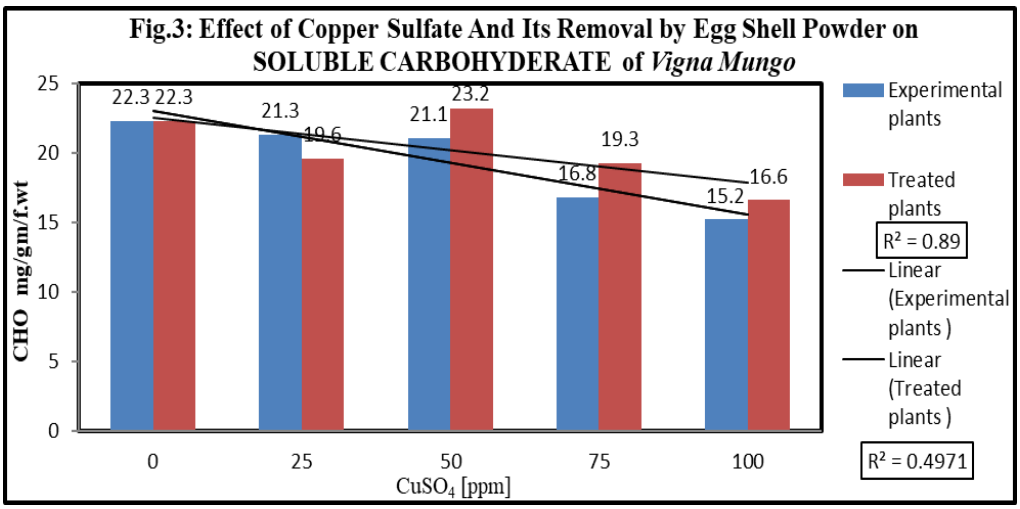
Roots grow downward and grasp the plant to their substratum, and conduct water and minerals from the soil to the aerial parts. Current data revealed that root length of *Vigna mungo* increased markedly at lower concentrations but it was significantly inhibited at higher concentrations of $CuSO_4$, upto 16.35±3.4, 17.78±2.6, 14.3±1.5 and 12.13±2.5 cms. Whereas it was found to be 15.7±0.5 in control plants. This may be due to copper to a breakdown in copper tolerance mechanism in roots (Sheldon & Menzies, 2005). High conc. of Cu is poisonous to plant roots (Askari & Khurshid, 2018: Askari *et. al*., 2017), it interferes with iron and other nutrients uptake, especially in acidic soils where pH is uncontrolled (Lamichhane *et.al*., 2018). By the application of egg shell root length of *Vigna mungo* enhanced up to 17.86±4.5, 20.25±4.1, 14.5±2.79 and 14.48±4 cms at all applied levels of $CuSO_4$ respectively (Figure **2**). Egg shell powder provides calcium ions to the plant which in the form of calcium pectate makes middle lamella which serves to bind the cells together. Calcium is also supposed to be a fundamental growth regulator of plants (Peter, 2005) as it is involved in activating certain enzymes for cellular activities (Li, 2017; White, 2003).



Soluble Carbohydrates (Yemm and Wills, 1945)

Carbohydrate is the photosynthetic product. Data presented in Figure **3** showed that Carbohydrate contents were significantly declined in *Vigna mungo* at all applied concentrations of copper sulphate as 21.3±3.7, 21.1±9.3, 16.8±1.3 and 15.2±2.06 over control where it was found to be 22.3±2.7 mg/gm/f.wt (Figure **3**). $CuSO_4$. Accumulation of copper inhibits photosynthesis in plants which ultimately causes reduction in carbohydrates (Badr *et.al*., 2004). Cu toxicity induce adverse effects of light which decreases photosynthetic activity (Inmaculada *et. al*., 1996b). Various authors suggested that the low chl. content in leaves in the presence of increased Cu level is due to a Cu-induced Fe deficiency (Pätsikkä *et al*. 2002, Inmaculada *et. al*., 1996b). Decrease in carbohydrates may attribute to the shortage of water and minerals due to root damage or decrease in root length (Figure **2**).

Reduced Carbohydrate contents due to copper accumulation were significantly improved by the application of egg shell powder upto 19.6±2.6, 23.2±9.5, 19.3±7.5 and 16.6±2.2 mg/gm/f.wt. Egg shell provides calcium to the plants which not only involved in activation of enzymes, cell wall formation but also involve in the electron transport chain of photosynthesis (Peter, 2017; Xu, 2013).



Total Protein Content (Lowry *et al*., 1951)

Data presented in Table **2** revealed that protein contents in *Vigna mungo* decline significantly at all applied levels of copper except at the concentration of 50 ppm (68.7) upto 67.25, 68.7, 56.2, 51.6 whereas it was 67.75 mg/gm F.wt. This decrease of protein contents in experimental plants may attribute to the attraction of copper for protein and enzymes, due to their mercapto ligands for the formation of chelate and hence losing their functional property or it may be due to hydrolysis of protein under copper stress. Proteins participate in numerous physiological functions in plants (Printz, 2016: Singh and Tewari, 2003) but their excess amount may be harmful or even lethal (Printz, 2016; Inmaculada *et. al*., 1996b). Application of egg shell powder to the *Vigna mungo* growing under copper stress remarkably enhanced the protein contents upto 65.93, 71.7, 56.3, 60.4 mg/gm F.wt., this improvement in protein contents may attribute to the presence of calcium and various other macro and micronutrients in egg shell powder (Figure **4**). Calcium plays a dogmatic role in maintaining and controlling membrane structures and participates in various functions like activation of enzymes and proteins they also reduce the loss of chlorophyll.

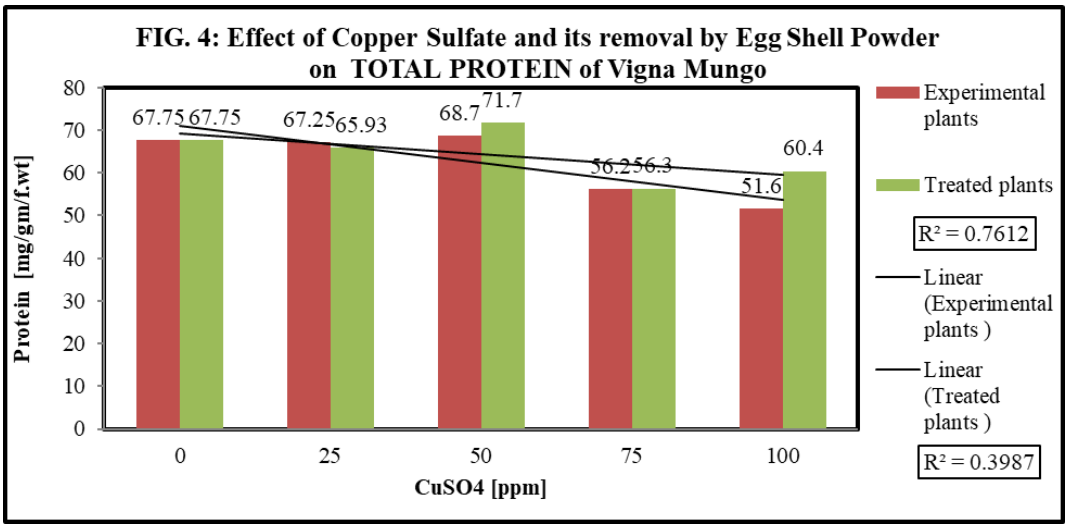


Table 1: Effect of copper on shoot length and root length of *V. mungo* & its adsorption by egg shell powder.				
Cu [ppm]	**Shoot Length (cm)**		**Root Length (cm)**	
-	**Experimental**	**Treated**	**Experimental**	**Treated**
0	13.5±0.49	13.5±0.49	15.7±0.5	15.7±0.5
25	14.85±0.66	15.5±0.43	16.35±3.4	17.86±4.5
50	15.8±1.39	16.1±0.91	17.78±2.6	20.25±4.1
75	12.7±0.39	13.2±0.86	14.3±1.5	14.5±2.79
100	11.58±0.87	12.8±0.52	12.13±2.5	14.48±4

Table 2: Effect of copper on Carbohydrates and Proteins of *V. mungo* & its adsorption by egg shell powder.				
Cu [ppm]	**Carbohydrates (mg/gm F.wt)**		**Proteins (mg/gm F.wt)**	
	Experimental	**Treated**	**Experimental**	**Treated**
0	22.3±2.7	22.3±2.7	67.75±4.3	67.75±4.3

25	21.3±3.7	18.6±2.6	67.25±7.8	65.93±57.9
50	24.1±9.3	23.2±9.5	68.7±1.2	71.7±6.7
75	16.8±1.3	19.3±7.5	51.6±15.7	56.3±9.3
100	15.2±2.06	16.6±2.2	56.2±4.9	60.4±4.9

CONCLUSION

Present study revealed the positive effect of copper on *Vigna mungo* at low concentrations (25, 50ppm) whereas it has a negative impact on plants at higher concentrations like 75 & 100 ppm. All physical and biochemical parameters were inhibited at high concentrations of Cu. With the application of egg shell powder the physical and biochemical parameters of treated plants enhanced as compare to experimental plants. It is concluded that egg shell powder is an efficient, free of cost and abundantly available adsorbent and these results strongly suggest credible reuse of calcinated eggshell in the removal of copper. Egg shell should be used as an effective fertilizer as it also contains various organic and inorganic substances.

REFERENCES

1. Tchounwou, Paul B., *et al*. "Heavy metal toxicity and the environment." Molecular, clinical and environmental toxicology (2012): 133-164.
2. Akpor, Oghenerobor Benjamin, Gladys Onolunose Ohiobor, and D. T. Olaolu. "Heavy metal pollutants in wastewater effluents: sources, effects and remediation." Advances in Bioscience and Bioengineering 2.4 (2014): 37-43.
3. Sarmishta Dey, P. B. Mazumder & S. B. Paul. “Effect of copper on growth and chlorophyll content in Tea plants (Camellia sinensis L.)”. International Journal of Research in Applied, Natural and Social Sciences. Vol. 2, No. 5 (2014): pp. 223-230.
4. Dumestre, A., *et al*. "Copper speciation and microbial activity in long-term contaminated soils." Archives of Environmental Contamination and Toxicology 36.2 (1999): 124-131.
5. Singh, Dharam, Kamlesh Nath, and Yogesh Kumar Sharma. "Response of wheat seed germination and seedling growth under copper stress." Journal of Environmental Biology 28.2 (2007): 409.
6. Houshm, Alireza, and Farhang Moraghebi. "Effect of mixed cadmium, copper, nickel and zinc on seed germination and seedling growth of safflower." African Journal of Agricultural Research 6.5 (2011): 1182-1187.
7. Chavan, Mayur and Sachin J. Mane. “Removal of Copper and Zinc from Aqueous Solutions by Using Low Cost Adsorbents”. International Journal of Science and Research (IJSR). 4 (7) (2015): 3076-3080.
8. Sonmez, Sahriye, *et al*. "High level of copper application to soil and leaves reduce the growth and yield of tomato plants." Scientia Agricola 63.3 (2006): 213-218.
9. Sheldon, A. R., and N. W. Menzies. "The effect of copper toxicity on the growth and root morphology of Rhodes grass (Chloris gayana Knuth.) in resin buffered solution culture." Plant and soil 278.1 (2005): 341-349.
10. Li, Z., *et al*. "The effect of CaCl 2 on calcium content, photosynthesis, and chlorophyll fluorescence of tung tree seedlings under drought conditions." Photosynthetica 55.3 (2017): 553-560.
11. White, Philip J., and Martin R. Broadley. "Calcium in plants." Annals of botany 92.4 (2003): 487-511.
12. Peter K *et al*. “A Central Regulator of Plant Growth and Development”. Plant Cell. 17(8) (2005): 2142–2155.
13. Askari, Shabana, and Hina Khurshid. "Conocarpus mediated improvement in photosynthetic pigments of Cicer arietinum growing under copper stress." RADS Journal of Biological Research & Applied Sciences 9.1 (2018): 41-49.
14. Askari, Shabana, Aisha Siddiqui, and Maria Kaleem. "Potato peel mediated improvement in organic substances of vigna mungo growing under copper stress." J Pharmacogn Phytochem 6 (2017): 1373-1378.
15. Lamichhane, Jay Ram, *et al*. "Thirteen decades of antimicrobial copper compounds applied in agriculture. A review." Agronomy for Sustainable Development 38.3 (2018): 1-18.
16. Printz, Bruno, *et al*. "Copper trafficking in plants and its implication on cell wall dynamics." Frontiers in plant science 7 (2016): 601.
17. Alaoui-Sossé, Badr, *et al*. "Effect of copper on growth in cucumber plants (Cucumis sativus) and its relationships with carbohydrate accumulation and changes in ion contents." Plant Science 166.5 (2004): 1213-1218.
18. Yruela, Inmaculada. "Copper in plants." Brazilian Journal of Plant Physiology 17.1 (2005): 145-156.

19. Pätsikkä, Eija, *et al*. "Excess copper predisposes photosystem II to photoinhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll." Plant physiology 129.3 (2002): 1359-1367.
20. Xu, Chengbin, Xuemei Li, and Lihong Zhang. "The effect of calcium chloride on growth, photosynthesis, and antioxidant responses of Zoysia japonica under drought conditions." PloS one 8.7 (2013): e68214.
21. Shaoshan, Li, Zhigang Nie, and Yan Wang. "Heavy metal induced DNA damage in plant measured by single cell gel electrophoresis." Toxicology Letters 180 (2008): S200.
22. Dumestre A, Sauve S, McBride M. *et al*. "Copper speciation and microbial activity in long-term contaminated soils". Arch Environ Contam Toxicol 36 (1993): 124-131.