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Analysis of Physiological and Biochemical Parameters of Wheat, Maize and Sweet Pea Under Copper Stress

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Abstract

A Studies were made to assess the impact of copper (Cu) on seed germination, seedling growth (shoot and root length), fresh weight, total chlorophyll content and Seedling Vigor Index (SVI) of wheat, maize and Sweet Pea at various concentration (5, 25, 50, 75 and 100 ppm) for 21days in laboratory conditions. It was found that lower concentration of copper (5 and 25 ppm) could help in growth and survival of these plant species while higher concentration (50, 75 and 100 ppm) adversely affected these edgermination seedling growth and total chlorophyll content following 21day Cuex posure.The order of SVI was recorded to beas sweet pea>maize>wheat. The exposure of Cu on these plants, total chlorophyll, total amylase,total protein and total sugar of seedlings decreased under laboratory conditions. However, the catalase and peroxidase activities were increased at all the concentration. Physiological and biochemical per turbations of these plants seedling are alter eddue to Cutoxicity.

Keywords: *Chlorophyll, Fresh weight, Copper, Toxicity, Biochemical, Physiological*

1. Introduction

Copper is considered as an essential element for all living organisms including plants (Bouazizi *et al.*, 2007; Upadhyay and Pandey, 2008a). Copper is an essential metal for plants. It plays a key roles in photosynthetic and respiratory electron transport chains, in ethylene sensing, cell wall metabolism, oxidative stress protection and biogenesis of molybdenum cofactor. Thus, deficiency in the copper supply can alter essential functions in plant metabolism. On the other hand, copper during decades has been used in agriculture as an antifungal agent and it is also extensively released into the environment by human activities that often cause environmental pollution.

Copper found in the environment as hydratedionic species, forming complex compound swith in organic and organic legends. Subsequent nutritional studies have demonstrated that copper and other metals are essential for optimal growth of plants and animals (Woolhouse, 1983). Biological organisms require certain metals for theirgrowth and metabolism and so, they evolved an appropriate uptake mechanism for metals. Some plant species havecapacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an

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ecophysiological adaptation in meta liferous soil. Indeed, heavy metals are not naturally degraded, but they progressively accumulated in soil and plants and exert biochemical alterations in the flora and fauna. This apparent toxicity to plants varies with plant species, specific metal concentration, chemical form, soil composition and pH (Pandey *et.al.*, 2001; Upadhyay and Pandey, 2008a).

Heavy metals play an essential role as components of metalloproteins, as cofactors in enzymatic catalysis, and in a wide array of other cellular processes. At higher concentration however, they become phytotoxic, inhibit leaf chlorosis and reduce growth. Heavy metals may be bound or accumulated by particular plants, which may increase or decrease the mobility and prevent the leaching of heavy metals into groundwater. The heavy metal absorb by plants is translocated to the shoots, causing physiological , biochemicaland structural damage and even cell death depending on the concentration in the cell sap (Upadhyay and Pandey, 2008).

A researches revels that increase of free amino acids (Mazen, 2004) and inhibition of nitrate reductase activity (Luna *et al.*, 1997; Fernandes and Henriques, 1991) decrease in chlorophyll content and inhibition of growth (Ralph and Burnchett, 1998; Fargasova, 2001). In fact, metabolic changes in plants can serve as a suitable indicator of copper toxicity (Chen *et aI.*, 2002; Li and Xiong, 2004). The aim of the present study was to investigate themorphological, physiological / biochemical impact of Cu on the plant species wheat *(Triticumaestivum*L.cv. PB343), maize (*Zea mays* Sunder, 4125) and Sweet pea *(Pisumsativum)* under laboratory conditions.

2. Materials and Methods

The certified seeds Wheat *(Triticumaestivum*, L. cv. PBW343-A), Maize *(Zeamays* Sunder, 4125-B) and Sweet pea *(Pisumsativum-C)* were procured from A.N.D Agriculture andTechnology University, Kumarganj, Faizabad. They were stored in glass-stoppered bottles. The seeds of uniform size, weight and color were selected for experiment and were surface sterilized (0.1 % $HgCI_2$ solution) for two min. and then thoroughly washed with distilled water.To alcohol-sterilized petridishes (10 cm) kept lined with filter paper, 5 mL of nutrient solution (Hewitt, 1966) followed by 10 mL of different concentrations (5, 25, 50, 75 and 100 ppm) ofcopper sulphate ($CuSO_4.5H_2O$) were added separately for each category of seed. A control set of each experiment was also run simultaneously with distilled water (without copper sulphate). Each treatment was replicated at least three times. The plumule radical emergence was taken as the criteria for germination. The percentage of germination, average shoots and root length, fresh weight of seedlings and total chlorophyll content were recorded. The total chlorophyll content of leaves was estimated by the method of Arnon (1949) and the values have been expressed as mg/g of fresh weight (fw). The physiological impact of Cu on some biochemical parameter in seedlings is recorded after 21 days exposure of Cu under laboratory conditions.

Germination (%) = No. of germinated seeds x 100/total No. of seeds

Moistur e(%) = Fresh weight – dry weight x 100 / Fresh weight

The Seedling Vigor Index (SVI) of these plants was calculated by using the following formula-

SVI = Germination % x hypocotyls length (mm)/100

In order to analyze amylase activity in seedlings, 2.5 % extract of seedling was prepared in 10 ml distilled water with help of mortar and pestle using a pinch of acid wash sand in dark and low temperature condition. This solution was used for the estimation of amylase activity (total amylase, a -amylase and β-amylase) in terms of mg starch hydrolyzed/g fresh weight of tissue by the method of Katsuni and Fekuhara (1969) with slight modifications. For the estimation of catalase activity, 2.5 % extract of seedling was used under the standard method of Euler and Josephson (1927). The 2.5 % extract was used for the estimation of peroxidase activity by the method of Luck (1963) in the terms of ΔO.D./g fresh weight of tissues. The 2.5% extract of plant tissue was used for Protein estimation in terms of μg protein/g fresh weight of tissue by the method of Lowry *et al.* (1951). Total sugar content in plant tissue was determined using phenol reagent by the method of Dubais *et al*. (1956).

3. Results and Discussion

Table 1 has summarized the impact of copper on morphological (Percent germination, shoot length, root length, seedling fresh weight) and total chlorophyll content in Wheat, Maize and Sweet pea. Seed germination was adversely affected by the treatment of copper. The percent seed germination and seedling growth (shoot and root length) were significantly increased at lower concentration (5 and 25 ppm) and simultaneously a gradual decrease in at higher concentration was recorded. All the concentration of copper except 5 and 25 ppm caused a gradual decline in seed germination. The maximum decline was found in Wheat, Maize and Sweet pea at the concentration of 100 ppm. At maximum concentration (100 ppm), the germination of seed decreased to 32%, 44% and 41% in Wheat, Maize and Sweet pea, respectively (Figure 1).

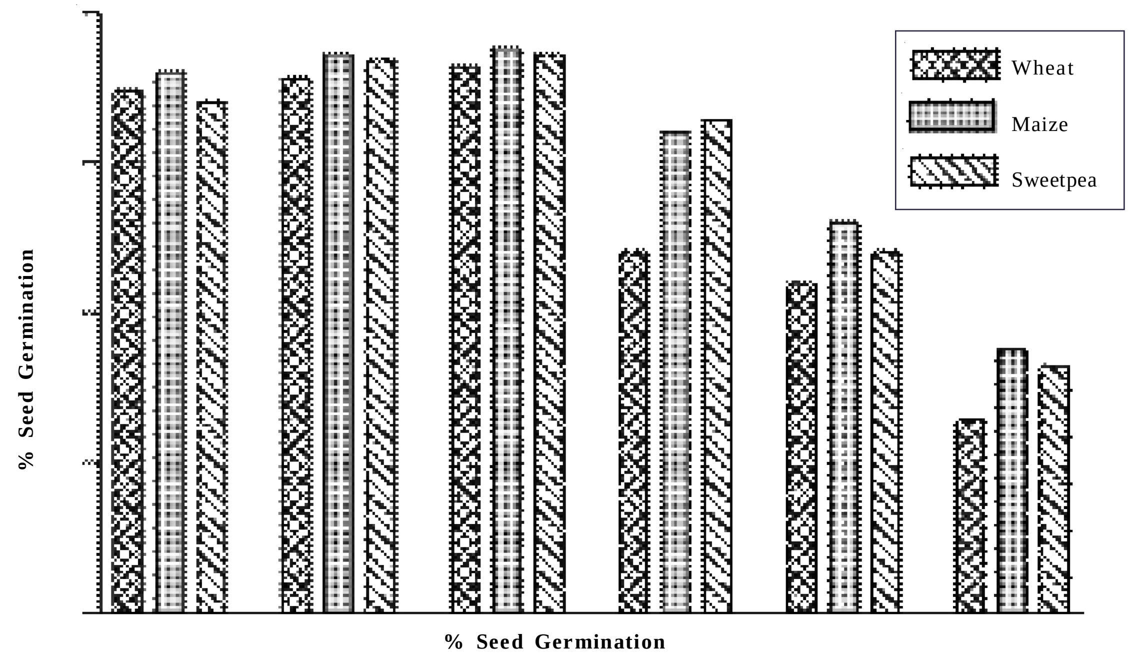

Figure 1: Percent Seed Germination in Plant Wheat Maize and Sweet Pea

Shoot growth was highly affected by the treatment of copper and significant reduction was recorded at 100 ppm. At maximum concentration (100 ppm) the shoot length decreased to 2.4, 2.1 and 1.7 cm in Wheat, Maize and Sweet pea, respectively. The root growth was moderately affected by the treatment of copper. Shoot length is maximum at 25 ppm concentration of copper that is 5.8,4.2 and 4.9 cm in wheat, maize and sweet pea, respectively. Thus it is clear that maize show saless phytotoxicity than sweet pea and wheat. Similar observation was reported by Prasad (1990) and Singh *et al.* (2006).

Table 1: Effect of Copper on Seed Germination and Seedling Growth of Wheat, Maize and Sweet Pea

S.No.	Treatment (PPM)	Wheat					Maize					Sweet Pea				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
1	Control	87	5.3	2.8	1.20	0.310	90	3.7	3.5	1.25	0.260	85	4.3	4.1	1.0	0.286
2	5	89	5.5	2.9	1.31	0.318	93	4.2	3.7	1.30	0.272	92	4.8	4.7	1.2	0.310
3	25	91	5.8	3.2	1.42	0.332	94	4.2	3.9	1.38	0.276	93	4.9	4.2	1.1	0.289
4	50	60	5.1	2.4	0.90	0.201	80	4.0	2.9	1.01	0.252	82	3.7	3.3	0.95	0.301
5	75	55	3.1	3.1	0.81	0.168	65	2.4	2.2	0.96	0.232	60	2.1	2.2	0.79	0.231
6	100	32	2.4	2.1	0.30	0.131	44	2.1	1.4	0.51	0.125	41	1.7	1.5	0.46	0.151

Note: a-Percent germination, b-Shoot length (cm), c-Root length (cm), d-Seedling fresh weight (g), e-Total chlorophyll (mg/g.fw); Table showing % change of seed germination, length of shoot, root and total chlorophyll contents for 21 days after exposure of different concentration of copper sulphate solution.

Copper toxicity was damaged plant roots, with symptoms ranging from disruption of theshoot and root layer and reduced root hair proliferation, to severe deformation of root structure. The maximum reduction was found at 100 ppm concentration, i.e., 2.1, 1.4 and 1.5 in Wheat, Maize and Sweet pea. The result obtained at 5 and 25 ppm invariable better as compared to thecontrol. The root length is maximum at 25 ppm in sweet pea (4.2) followed by maize (3.9) and wheat (3.2). At toxic level plant root were injured and plant were severely stunted or killed. Chen *et al.*, (2002) reported that copper sulfate induced inhibition in root growth of rice seedlings islikely due to cell wall stiffening related to H_2O_2 dependent per oxidase catalyzed formation ofcrosslinkingamongcell wall polymers.

In the present study, chlorophyll contents of Wheat, Maize and Sweet pea plant seedlingswas significantly reduced in the order of wheat>sweet pea>maize because of presence of >25 ppm Cu concentration (Figure 2). In particular seedlings indicating less synthesis and destruction ofchlorophyll (Prasad, 1990 ; Pandey and Neraliya, 2002). Upadhyay and Pandey (2008a) have reported that higher concentration of Cu can cause decreased chlorophyll content. Higher concentration of Cu seriously interferes with the percent germination, shoot length, root length, fresh weight of seedling and total chlorophyll contents, thus having phytotoxicity. This high tolerance may be due to its greater life period than that of other two plants. It appears that the lower concentration can act as a nutrient for better survival.

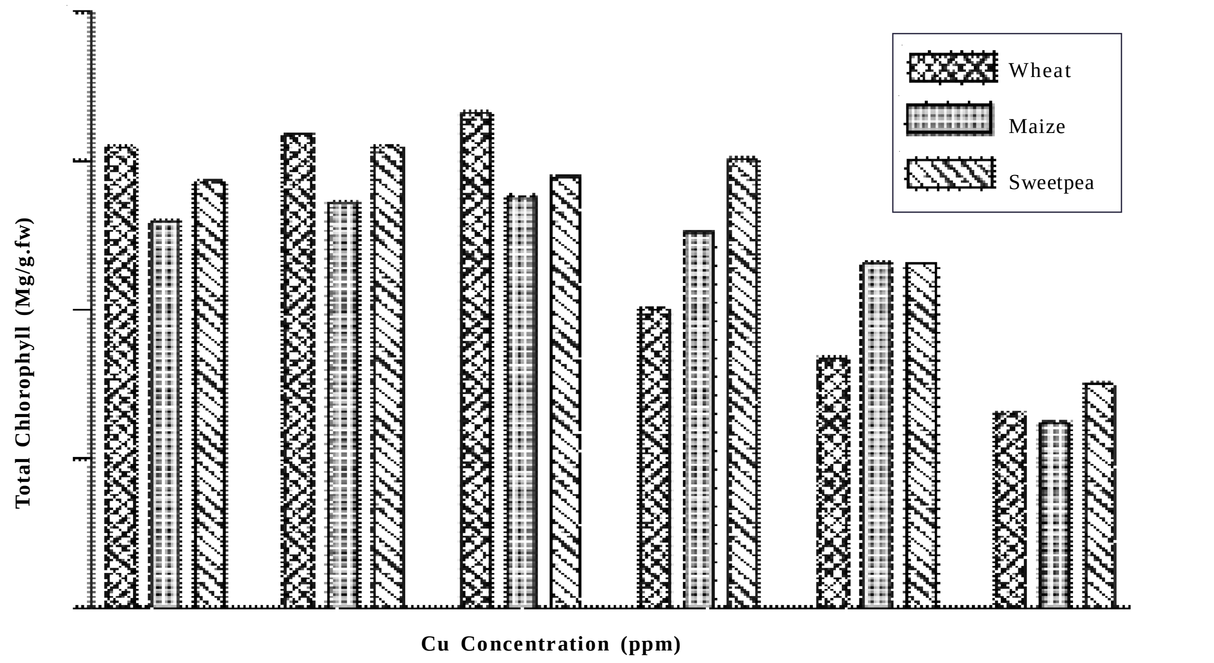

Figure 2: Total Chlorophyll Content in Three Plant Species (Wheat, Maize and Sweetpea)

The order of Seedling Vigor Index (SVI) was found to be as Sweet pea>Maize>Wheat. TheSVI was increase at 5 and 25 ppm Cu concentration while decrease at higher concentration (50, 75 and 100 ppm) in all the three plant (wheat, maize and sweet pea) (Figure 3).

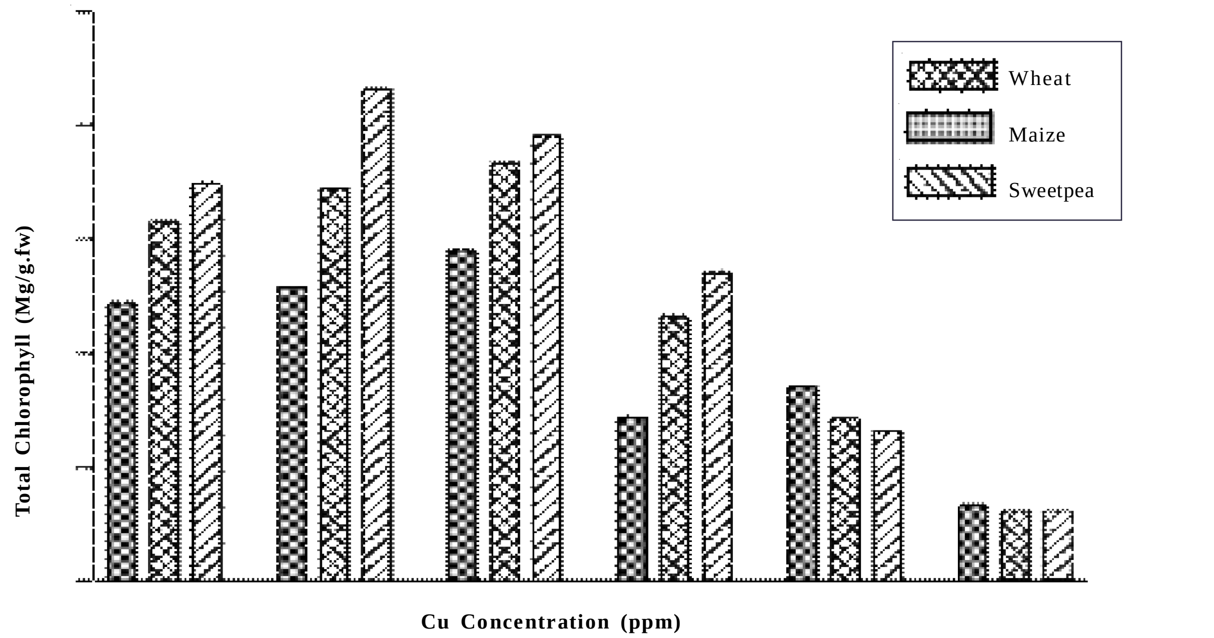

Figure 3: Seedling Vigour Index (SVI)

The decrease in the chlorophyll contents of plants after exposure of higher concentration of copper represents less synthes is and more destruction of chlorophyll under the influence of pollutants (Prasad, 1990). Singh *et al.* (2006) reported that copper concentration greater than 20 to 30 ppm have been found toxic to plants at lower concentration of copper the photo synthetic pigment were increased but at higher concentration of copper the photo synthetic pigment were decreases due to copper toxicity. The significant reduction in the total chlorophyll content athigher concentration of copper solution (i.e., 50, 75, and 100 ppm) was also recorded. Due toinhibitory effect of toxicants morphology and photosynthetic leaf area is reduced, resulting incorrespondingdecreasein photosynthesis (Pandey and Neraliya, 2002).

Since, higher concentration of copper seriously interferes with the percent germination, shoot length, root length, fresh weight of seedling and total chlorophyll content. The result obtained at 5 and 25 ppm invariably better as compared to control. It appears that these concentration of copper act as a good nutrient for plant growth.

Table 2 has summarized the impact of Cu on biochemical constituents and physiology of three plants, Wheat (*Triticumaestivum*L.cv pbw-343), Maize (*Zea mays* Sunder- 4125) and Sweet Pea (*Pisumsativum*) following 21 days exposure of Cu. The decreased amylase activity in plants under the influence of different levels of copper solution was found to be significant in comparison to control. The poor germination rate and seedling growth in treatments seems to bedue to the poor break down of starch by low amylase activity. Amylase and its important role during seed germination through hydrolysis of reserve starch and release of the energy has been reported (Nath *et al.*, 2005).

Table 2: Impact of Cu on Biochemical Constituents and Physiology of Three Plants [A-Wheat (*Triticuma estivuml*. Cv Pbw-343), B-Maize (*Zeamays* Sunder-4125) and C-Sweetpea (*Pisum sativum*)] Following 21 days Exposure of Cu

S. No.	Parameters (Value)	Control			Exposure of Different Concentrations of Copper for 21 Days															
					5 ppm			25 ppm			50 ppm*			75 ppm*			100 ppm*		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	Total Amylase (mg/g.fw)	12.5	16.6	18.3	10.2 (-18)	14.1 (-15)	16.4 (-10)	9.1 (-27)	12.8 (-23)	15.0 (-18)	8.7 (-30)	12.1 (-27)	13.5 (-26)	8.1 (-35)	11.4 (-31)	12.5 (-32)	7.3 (-42)	10.3 (-38)	10.3 (-44)
2	Catalase (ml H_2O_2 hydrolysed/gmfw)	131.3	118	137	138.2 (+5)	130.0 (+10)	142.0 (+4)	143.3 (+9)	140.2 (+19)	147.1 (+7)	149.2 (+14)	144.1 (+22)	155.2 (+13)	155.1 (+18)	150.5 (+28)	165.3 (+21)	161.5 (+23)	156.1 (+32)	170.4 (+24)
3	Peroxidase (ΔO.D./gmfw.)	40.2	23.7	16.6	47.2 (+17)	27.1 (+14)	18.5 (+11)	50.6 (+26)	29.7 (+25)	23.4 (+41)	54.1 (+35)	34.6 (+46)	24.9 (+50)	56.4 (+40)	35.8 (+51)	25.7 (+55)	61.0 (+52)	38.5 (+62)	29.1 (+75)
4	Total protein (μg/mg.fw)	78.1	53.6	75.6	65.7 (-16)	51.2 (-4)	71.3 (-6)	59.8 (-27)	50.6 (-6)	64.3 (-15)	55.2 (-29)	47.2 (-12)	60.8 (-20)	51.2 (-34)	42.3 (-21)	56.4 (-25)	47.3 (-39)	39.0 (-27)	51.5 (-32)
5	Total sugar	4.3	3.9	3.2	4.0 (-7)	3.3 (-15)	2.9 (-9)	3.6 (-16)	3.0 (-23)	2.5 (-22)	3.0 (-30)	2.6 (-33)	2.1 (-34)	2.8 (-35)	2.1 (-46)	1.8 (-44)	2.6 (-40)	1.8 (-54)	1.5 (-53)

Note: Values represent as Mean of three replicates and values in parentheses indicate % change over control (Zero%); * Negative values express inhibitory effect: Significant (p<0.01) when t-test was applied to see the inhibitory/stimulation effect of the concentrations (50, 75, and 100 ppm) of Cu on mentioned parameters.

The catalase is an enzyme involved in antioxidant defense that eliminates hydrogen peroxide. Catalase and peroxidase activity significantly increased in seedlings as well as plants as shown in Tables 2 due to exposure to 25 ppm and above producing significant higher catalase and peroxidase activity. Plants with100 ppm copper treatment showed the highest catalase and peroxidase activity levels than all other treatments. Catalase and peroxidase activity significantly decreased on exposure to copper. Copper application to agricultural land in quantities greatly inexcess of that required by crops occurs in the case of sewage sludge application (Mcbride, 1995). It is well known that catalase and ascorbate peroxidase play important role in preventing oxidative stress by catalyzing the reduction of H_2O_2 (Weckx and Clijsters, 1996).

The soluble protein contain in the plant cell is an important indicator of their physiological state. Protein is an essential component of plants whose concentration is also beaffected by the supply of the heavy metal as some enzymatic activities may be altered due to heavy metal stress which might result in alteration of protein contain showing the involvement of heavy metal in its metabolism. Total protein and sugars where significantly reduced at the higher concentration of copper exposure. Indeed, reports are available that sugar contents was decreased following exposure of heavy metals (Tripathi and Tripathi, 1999; Tandon and Gupta; 2002 and Pourakbar *et.al*., 2007).

4. Conclusion

In conclusion, as has been stated above Cu has harmful effects on the physiological and biochemical parameters of wheat, maize and sweet pea plants. In addition to these findings, the increase in Cu concentration can lead to certain irreversible impact on plants and even in higher organisms. Therefore, Cu concentration should be monitored in polluted soil before seeding for the health of plants, thereby soil productivity. The evaluation of present study is therefore very helpful in understanding the detrimental impacts of Cu on the agriculture ecosystem and this will help in preserving our lands in a high state of productivity, thus insuring a prospective future for the coming generations.

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