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**RESEARCH ARTICLE**

# Studies on the physiological and biochemical parameters of Wheat, Maize and Sweet pea under copper stress

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## *Abstract*

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 and100ppm) for 21 days in laboratory conditions. It was observed that lower concentration of copper (5 and 25 ppm) could help in growth and survival of these plant species while higher concentration (50, 75 and100ppm) adversely affected the seed germination seedling growth and total chlorophyll content following 21 day Cu exposure. The order of SVI was found to be as sweet pea>maize>wheat. Following 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 perturbations of these plants seedling are altered due to Cu toxicity.



## Introduction

Copper is widely prevalent in our environment and was 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 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 occurs in the environment as hydrated ionic species, forming complex compounds with inorganic and organic legends. Subsequent nutritional studies have demonstrated that copper and other metals are essential for optimal growth of plants and animals (Upadhyay and Pandey, 2009). Living organisms require certain metals for their growth and metabolism and so, they evolved an appropriate uptake mechanism for metals. Some plant species have capacity to grow in the metal contaminated soil and accumulate elevated amount of heavy metals (hyper-accumulation) as an ecophysiological adaptation in metaliferous 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, 2008b).

Essential 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 , biochemical and structural damage and even cell death depending on the concentration in the cell sap (Upadhyay and Pandey, 2008b).

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A perusal of literature 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 ;Hacker,et al.,2003; Li and Xiong, 2004).The aim of the present study was to investigate the morphological, physiological / biochemical impact of Cu on the plant species wheat *(Triticum aestivum* L.cv. PB343), maize (*Zea mays* Sunder, 4125) and Sweet pea *(Pisum sativum)* under laboratory conditions.

## Material and Methods

The certified seeds Wheat *(Triticum aestivum*, L.cv. PBW343-A), Maize *(Zea mays* Sunder, 4125-B) and Sweet pea *(Pisum sativum-C)* were procured from A.N.D Agriculture and Technology University, Kumarganj, Faizabad. They were stored in glass-stoppered bottles. The seeds of uniform size, weight and colour 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, 5ml of nutrient solution (Hewitt,1966) followed by 10 ml of different concentrations (5, 25, 50, 75 and 100ppm) of copper 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

Moisture (%) = Fresh weight- dry weight x100 / Fresh weight

The seedling vigour 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/gm 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. / gm fresh weight of tissues. The 2.5% extract of plant tissue was used for Protein estimation in terms of µg protein/gm 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).

## Result 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 25ppm) 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 100ppm. At maximum concentration (100ppm), the germination of seed decreased to 32%, 44% and 41% in Wheat, Maize and Sweet pea, respectively (figure-1).

Shoot growth was highly affected by the treatment of copper and significant reduction was recorded at 100 ppm. At maximum concentration (100ppm) the shoot length decreased to 2.4, 2.1 and 1.7cm in Wheat, Maize and Sweet pea, respectively. The root growth was moderately affected by the treatment of copper. Shoot length is maximum at 25ppm concentration of copper that is 5.8,4.2 and 4.9cm in wheat, maize and sweet pea, respectively. Thus it is clear that maize shows a less 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. N. | 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 |

- a-Percent germination, b-shoot length (cm), c-root length (cm), d-seedling fresh weight (gm), 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.

**Table-2: Impact of Cu on biochemical constituents and physiology of three plants [ A- Wheat (*Triticum aestivum* L.cv pbw-343), B- Maize (*Zea mays* Sunder- 4125) and C- Sweet Pea (*Pisum sativum*) ] following 21 days exposure of Cu .**

| S. N. | 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 (Δ O.D./gm fw.) | 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 (µg/mg.fw) | 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) |

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 100ppm) of Cu on mentioned parameters.

**Figure-1** Percent seed germination in plant wheat maize and sweet pea

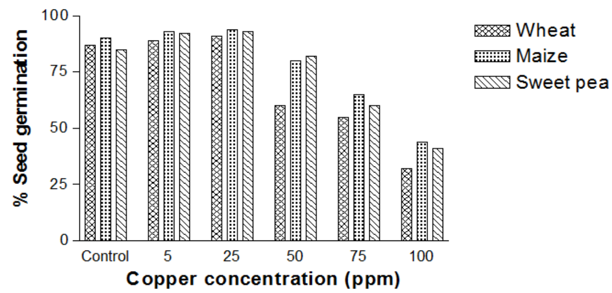



**Figure-2 Total chlorophyll content in three plant species (Wheat, maize and sweet pea)**

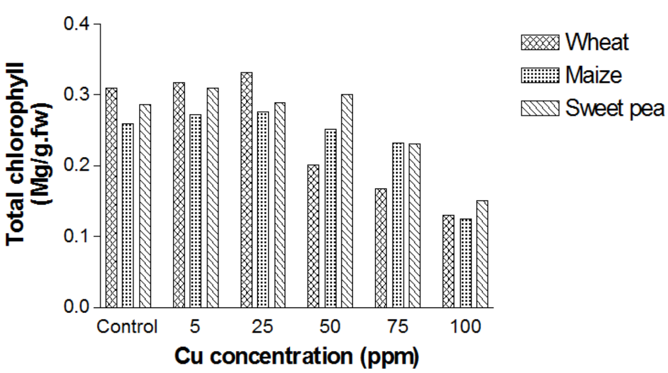
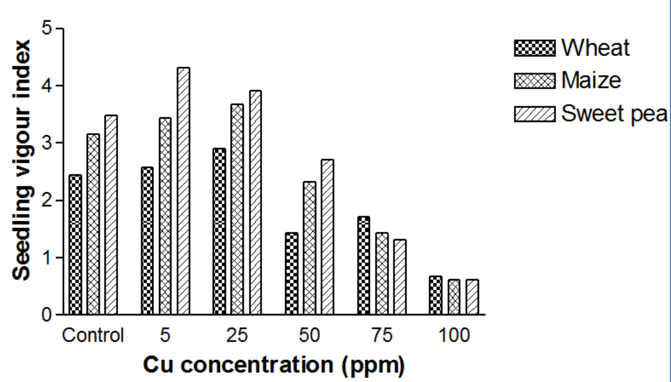



**Figure -3 Seedling vigour index (SVI)**




Copper toxicity was damaged plant roots, with symptoms ranging from disruption of the shoot and root layer and reduced root hair proliferation, to severe deformation of root structure. The maximum reduction was found at 100ppm concentration i.e. 2.1, 1.4 and 1.5 in Wheat, Maize and Sweet pea. The result obtained at 5 and 25ppm invariable better as compared to the control. The root length is maximum at 25ppm 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 is likely due to cell wall stiffening related to $H_2O_2$ dependent per oxidase catalysed formation of cross linking among cell wall polymers.

In the present study, chlorophyll contents of Wheat, Maize and Sweet pea plant seedlings was significantly reduced in the order of wheat>sweet pea>maize because of presence of >25ppm Cu concentration (figure-2). In particular seedlings indicating less synthesis and destruction of chlorophyll (Prasad, 1990 ; Pandey and Neraliya, 2002). Upadhyay and Pandey (2008) 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.

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

The decrease in the chlorophyll contents of plants after exposure of higher concentration of copper represents less synthesis and more destruction of chlorophyll under the influence of pollutants (Prasad, 1990). Singh *et al.*, (2006) reported that copper concentration greater than 20 to 30ppm 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 at higher concentration of copper solution (i.e.50, 75, and 100 ppm) was also recorded. Due to inhibitory effect of toxicants morphology and photosynthetic leaf area is reduced, resulting in corresponding decrease in 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.

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 be due 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).

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 with 100 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 in excess 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 $H_2O_2$ (Wong and bradshaw,1982;Weckx and Clijsters, 1996). 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).

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.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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