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Accumulation of Cu at different concentration in tea plant (*Camellia sinensis*(L.)O.Kuntze).

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Abstract: *Tea is the most popular beverage in the world. Tea is considered a beneficial drink as it contains many essential nutrients. But the intake of heavy metals even in a small amount causes adverse action in human health along with the plants. The heavy metals are present in the nutrient media itself which are required for plant growth, other important sources are pesticides and fertilizers used in the field for better production. In the present study the tea cultivars (S_3A_3) were collected from the Rosekandy tea estate, Silchar, Assam and were analyzed for the accumulation of Cu at different level on the different organs of the plants. The accumulation of Cu had a strong positive correlation with the Cu concentration. The order of deposition of Cu are in following manner roots>leaves>stems with the exposure of treatment days. From the present study we have concluded that even low amount of deposition of Cu at higher concentration causes adverse affect by retarding the growth of a plant with many other physiological characters. And this may lead to the adverse action of biological characters.*
Key words: *Accumulation, adverse effects, Cu stress, oxidative stress, toxicity.*

I. Introduction

Plants require many heavy metals as their essential source of nutrition which is provided as growth media (e.g. Cd, Cu, Fe, Mn, Ni, and Zn)which play a vital role in metabolic processes. But excess uptake of these metals can cause adverse effect by inhibiting the plant metabolism [1]. With the development of modern industries and agriculture, the accumulation of heavy metal has become one of the most harmful pollutants. Tea is one of the most popular beverages in the world.A few publications are available on the presence of heavy metals in tea and analytical difficulties associated with the separation from tea leaves [2].Copper is an essential elements for growth of plants, lack of Cu can cause defects in growth [3][4].Trace level of copper is essential for human health also. But copper can occur in very high concentration which is used as pesticides in agriculture and field to protect the crops from the attack of pests[5]. Cu can translocate to the tissues through the roots and can damage the tissues by crossing the toxic level. Where it can inhibit the stomatal closure, chlorophyll pigmentation as a whole can affect the physiological character [6]. There is also much evidence showing soil microbial community changes due to metal contamination of soils [7]. Metal hyperaccumulation acts as a remedy for metabolic defects in plant. Plants exposed to metal toxicity suffer from oxidative stress. Not only Fenton-type metals, such as Cu, induce oxidative damage , but also metals like Cd,Al, or Zn that do not change valence within plant cells [8][9][10].

II. Materials And Methods:

2.1: Sample collection:
Three months tea cultivars (S_3A_3) were collected from the Rosekandy Tea Estate, Silchar, Assam.The collected cultivars were transferred to the Hoagland solution for allowingthem to get stable for 7 days [11].Then the plants were placed in a plastic platform in 500 ml jar containing 400 ml of Hoagland solution; so that the roots were immersed in the solution and the shoot were above the platform.Then the plants were treated with $CuSO_4$ at different concentration of 50µm, 100 µm 200µm, 300µm, 400µm, 500µm, 600µm in the nutrient solution. The control plants were left as untreated, comprising only the nutrient solution. The top four leaves were collected for measuring the accumulation of Cu concentration after 2nd, 4th,and 7thday of the treatment.

2.2: Plant harvest and Cu analysis:

During harvest the plants were rinsed with distilled water. The roots, shoots and the leaves were separated. Then they were oven dried at 70°C for 48 hrs to a constant weight, after which dry weight of roots, shoots and leaves were determined by electronic balance. The plant tissues were ashed at 500°C for several hours and then cooled down. The 0.5g of the coarse powder transferred into a beaker. Add 5-10ml of the mixture of nitric acid (HNO_3) and perchloric acid ($HCLO_4$) (4:1). The samples were then heated till the solution become colourless and then allowed for cooling and then transferred it into conical flask. Then we washed the container with nitric acid solution. To dilute the same solvent we added the washing solution in the flask by adjusting it to 20ml and then shake vigorously. The blanks were treated in the same process to minimize the analytical errors. The samples were analyzed for the accumulation of Cu by flame atomic absorption spectroscopy. Data were rounded off suitably according to the value of standard deviation from measurements in triplicates.

Figures and Tables:

The results which were obtained for analysis of tea cultivars by AAS showed the accumulation of copper at different concentration in the different parts of the plant with respect to the treated days.

	2nd day of treatment		
Concentration	Roots (µg/g)	Leaves (µg/g)	Stems (µg/g)
control	36.48±0.06	33.26±0.01	30.18+0.16
50 µm	32.44±0.09	31.55±0.12	28.03±0.02
100 µm	29.70±0.02	25.71±0.08	22.98±0.23
200 µm	27.16±0.17	21.15±0.16	19.29±0.03
300 µm	24.78±0.08	18.75±0.04	15.98±0.13
400 µm	21.40±0.15	17.78±0.12	11.03±0.02
500 µm	19.80±0.01	16.11±0.15	10.98±0.13
600 µm	15.65±0.51	11.24±0.25	9.87±0.23

Table 1: Accumulation of Cu on roots, leaves and stems on 2nd day at different concentration.

	4th day of treatment		
Concentration	Roots (µg/g)	Leaves (µg/g)	Stems(µg/g)
control	32.32±0.17	28.93±0.31	25.03±0.17
50 µm	28.81±0.01	22.15±0.12	21.09±0.23
100 µm	27.15±0.07	16.31±0.33	15.98±0.21
200 µm	26.41±0.04	16.97±0.28	14.32±0.11
300 µm	21.23±0.11	15.78±0.04	13.32±0.14
400 µm	18.87±0.33	13.34±0.14	11.32±0.02
500 µm	15.93±0.42	13.21±0.32	10.03±0.04
600 µm	13.79±0.02	10.04±0.11	9.03±0.02

Table 2: Accumulation of Cu on roots, leaves and stems on 4th day at different concentration.

	7th day of treatment		
Concentration	Roots (µg/g)	Leaves (µg/g)	Stems (µg/g)
control	26.93±0.03	25.18±0.06	22.09±0.04
50 µm	21.39±0.01	19.60±0.12	16.21±0.02
100 µm	20.25±0.42	13.98±0.04	11.03±0.01
200 µm	18.90±+0.02	15.91±0.03	11.63±0.12
300 µm	14.13±.21	11.42±0.26	9.21±0.11
400 µm	13.38±0.54	14.55±0.16	9.03±0.25
500 µm	11.76±0.15	9.63±0.01	8.32±0.13
600 µm	9.17±0.02	6.02±0.20	3.02±0.04

Table 1: Accumulation of Cu on roots leaves and stems on 7th day at different concentration.

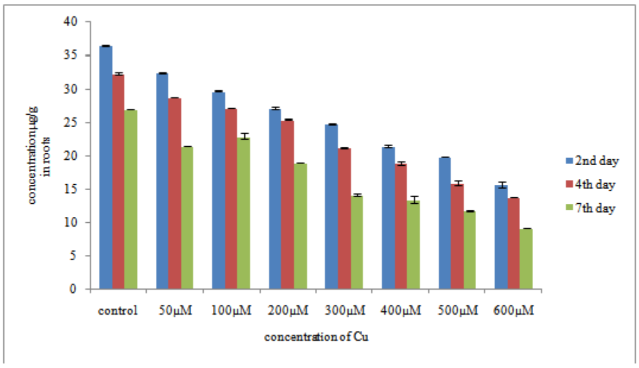


Fig1: concentration of Cu in roots of $S_3A_3$with respect of exposure days.

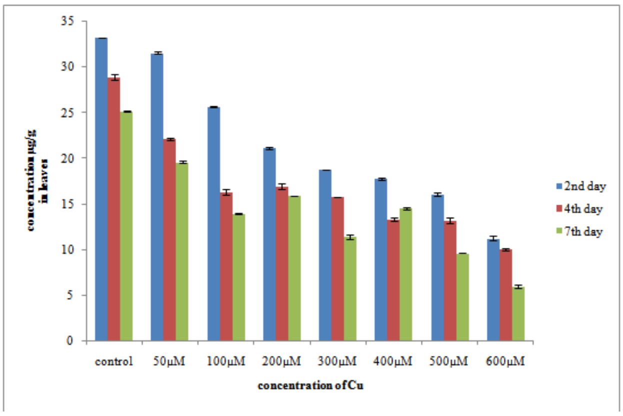


Fig2: concentration of Cu in leaves of S_3A_3 with respect of exposure days.

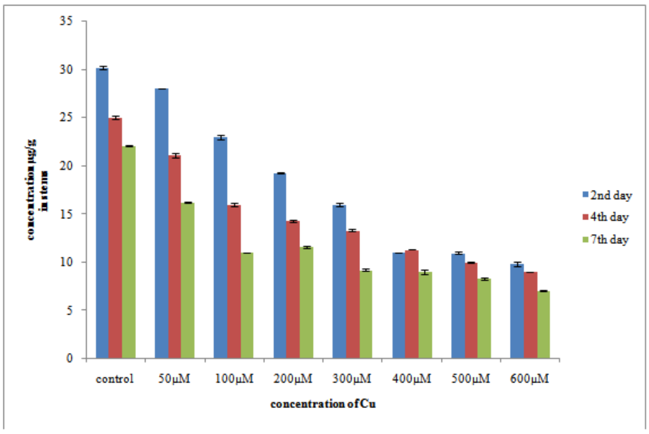
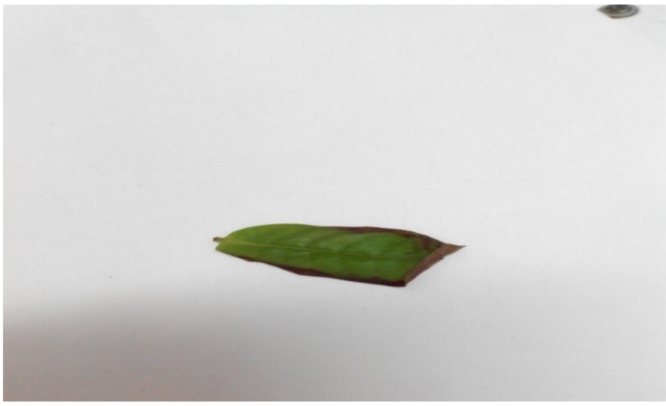


Fig3: concentration of Cu in stemsof $S_3A_3$with respect of exposure days.



(a)

(b)

(c)

Fig4: effect of leaves at higher doses. (a) At500µM on 7th day. (b) At 600 µM on 4th day (c)) At 600 µM on 7th day

III. Conclusion:

The pathogen is an obligate parasite and the crop has to be protected with frequent application of fungicides, pesticides, and fertilizers throughout the season. And these fungicides, pesticides contain a lot of heavy metals in it. Which lead to the adverse effect both in the quality and quantity of production. Many other authors have also determined the accumulation of Cu in tea leaves by Flame Atomic Absorption Spectrophotometry [12]. And many other authors have determined the accumulation of Cd, Cu, Ni, Pb in tea samples [13]. From the above experiment we have observed that with the exposure of days, at different concentration of Cu are showing different level of accumulation of Cu by the different parts of the tea cultivars. From the fig:1 we can observed that the roots are at highest levelof accumulating Cu as they are in direct contact with the stress present in the nutrient media.Form the fig:2 and fig:3 we can observed thatthe deposition of Cu in organ are in the following order roots> leaves> stems. At the higher concentration along with the span of days the deposition is at very low amount but is affecting a lot in the physiological characters. They showed different degrees of symptoms at different concentration with the exposure days. From fig: 4 the leaves first show some brown spots, yellow patches, become dry and then fallen off. From fig: 4(b)at the highest concentration less number of leaves come out, and most of the leaves became withered. The stress plants on the 2nd day is showing less amount of adverse effect whereas 4th day and 7th day plants are affected mostly. The roots suffered a lot by retarding the growth of root length. The number of new leaves slowed down with the less arrival of new stems. As a whole the growth has slowed down. The same results have been observed by many authors that the growth rate of plants slowed down with the increase in the Cr stress [14]. This adverse action of physiological characters may show adverse effect in biological characters with reduction in chlorophyll pigments.Many authors have observed the decreasing of chlorophyll content with increase in Cu concentration [15]. This also may lead to the action of ROS enzymes. With the adverse effect of CAT activity, SOD activity, POD activity so on. The decrease or unaffected in CAT activity by Cu on oat leaf was reported by [16]. The SOD activity has declined with Cr exposure [14]. There was a significant decrease in the activity of POD on exposure of Cr in tea plant [14]. But the judicious use of Cu fungicides may not cause or can cause less problems of public health and also can reduce environmental contamination. This analysis of Cu content in S_3A_3 cultivar indicated that the mean value of Cu ranged between 36.48±0.06 µg/g to 3.02±0.04 µg/g.

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