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Toxic Effects of Copper on Growth of *Enydra fluctuans* DC.

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Abstract—*The effects of copper (Cu) on the growth of an aquatic macrophyte, Enydra fluctuans were studied. The plants were exposed to different concentrations of Cu (0.11,0.51,1.09,2.54 and 3.03 mg L^{-1}) for 15 days. Various growth parameters such as fresh weight, dry weight, shoot height, number of new leaves, leaf fall, and number of new nodes were studied. At higher concentrations i.e., 2.54 and 3.03 mg L^{-1} of Cu, the growth of the plant was significantly inhibited from 5^{th} day of exposure compared to that in control. With the increase in concentrations of Cu, there was significantly less increase in the number of new leaves, nodes, and shoot height while number of leaf fall was increased. On the contrary, growth as well as fresh and dry weights were enhanced at lower Cu concentrations (0.11 and 0.51 mg L^{-1}). One way ANOVA revealed significant ($P<0.05$) differences among control and Cu-exposed plants for all the growth parameters except dry weight. Effects on the plants were concentration and time dependent. Since the plant could survive at all Cu concentrations at the total exposure period of 15 days, it reflected its tolerance level upto 3.03 mg L^{-1} of Cu concentration. Therefore, the results suggest that E. fluctuans can be effectively used for biomonitoring of Cu polluted aquatic bodies using all these growth parameters.*

Keywords: *Enydra fluctuans;Copper; Toxicity; Growth.*

1. Introduction

Discharge of sewage into different water bodies such as ponds, lakes, rivers, etc. from various industrial sector resulting in water pollution is becoming a matter of great importance for developing countries like India [27]. In aquatic macrophytes, heavy metals are considered to accelerate senescence [13,14] and because of its bio-accumulative and persistent nature, heavy metals arewater contaminants of great concern [24,29,7]. Copper is an essential plant micronutrient, which participates in metabolic pathways of plants, acting as a precious component of various enzymes and co-enzymes [20]. However, copper can become very toxic at higher concentrations affecting development of the plant because of its interference with several physiological processes directly or indirectly [28,18]. Plant species get affected differently by excess amount of copper depending on the stages of plant growth at which copper was used, the duration of action as well as the various copper concentrations[20].

Aquatic macrophytes are found in different ecosystems ranging from terrestrial to marine water[4] and they are highly productive in terms of biomass [5].For the purpose of proper monitoring and assessment of toxic heavy metals in water bodies, aquatic macrophytes can act as a suitable and appropriate models [22]. *Enydra fluctuans* belonging to the family Asteraceae, is a semi aquatic edible plant species found in tropical and sub tropical regions.It normally grows in water channels, rice fields, fish pond margins, along drains and similar places, and blocks water courses due to its proliferation[1]. It is known that many plant species have the capacity for the remediation of heavy metals from contaminated water bodies[26,25]. Work has been done on *Brassica* species to determine the plant's tolerance of heavy metals (Zn and Cu) and its toxic effects on different growth parameters.The results revealed that dry weight of the plant tissues was significantly decreased and growth of the plant was also reduced when exposed to heavy metals[8]. Changes in morphological parameters in relation to heavy metal toxicity were also studied in many different plants such as *Typha domingensis*[12], *Ceratophyllumdemersum*[17], *Lemna Polyrrhiza* [15].

In view of the above facts, our objective in the present study was to examine the morphological parameters of leaves, shoots and nodesin *E. fluctuans* exposed to different concentrations of copper.

2. Materials and methods

Enydra fluctuans was collected from a water body in Cachar district, Assam, India, and the plants were washed thoroughly with tap water. Stock cultures were grown according to [11]. The plants were grown in hydroponic tubs till new branches developed. New branches were planted in pots carrying soil flooded with 50% Hoagland nutrient media. Fully grown and healthy shoots of similar weight and height of the same mother plant were cut, cleaned with tap water and acclimatized in 50% nutrient media (Hoagland) at 25 - 27˚C for one week, 12 h light with an intensity of 100-120 µmol^{-2}s^{-1} and 12 h dark period.

With the chronic toxicity test, experiment was conducted with an exposure period of 15 days. The acclimatized plants were exposed to 0.11, 0.51, 1.09, 2.54 and 3.03 mg L^{-1} of Cu in a set of experiments. Plants grown in 50% Hoagland nutrient media were maintained as control without added metals. In these experiments, toxicological endpoints such as changes in fresh weight(FW), dry weight (DW), shoot height (SH), number of new leaves(NL), number of new nodes (NN), and leaf fall (LF) with respect to those noted at the starting point of the experiment (0 days) were observed on 5th, 10th and 15th day of the experiment. At the end of 15 days experiment, all the Cu treated and control plants were removed, washed with distilled water and remaining water was blotted before recording fresh weight of plants. After this, the plants were dried in hot air oven till constant weight at 60˚C.The results obtained for copper-exposed plants were compared with that from the control plants. The changes in the length of shoot height (SH) were measured using thread and a meter scale (well marked) and also the number of new nodes, leaves, leaf fall were measured at an interval of 5 days. While measuring proper care was taken to make sure that the plants did not experience any stress. The test solution was changed every week during toxicity tests. Statistical significance of differences was tested by One-way ANOVA, with multiple comparisons made by Tukey tests. SPSS 20 software for Windows was used for performing all statistical analysis.

3. Results and discussion

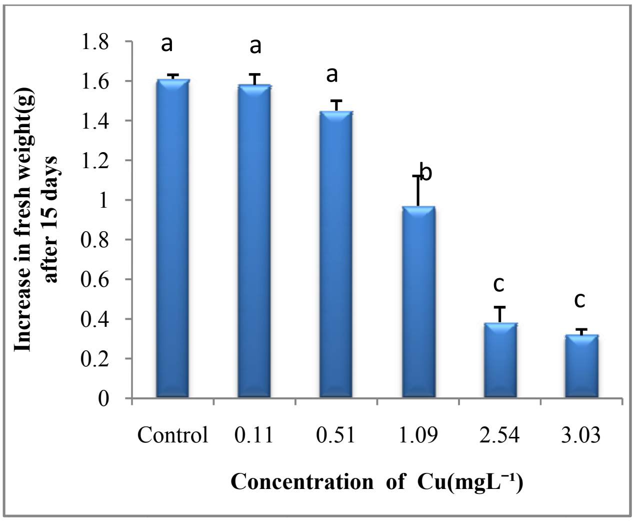

Fig. 1 Effect of Cu on increase in fresh weight (FW) of *E. fluctuans* at the end of 15 days of exposure. Values are given as mean ±SD; values with different superscript letters on bars indicate significant differences at P<0.05 among one another.

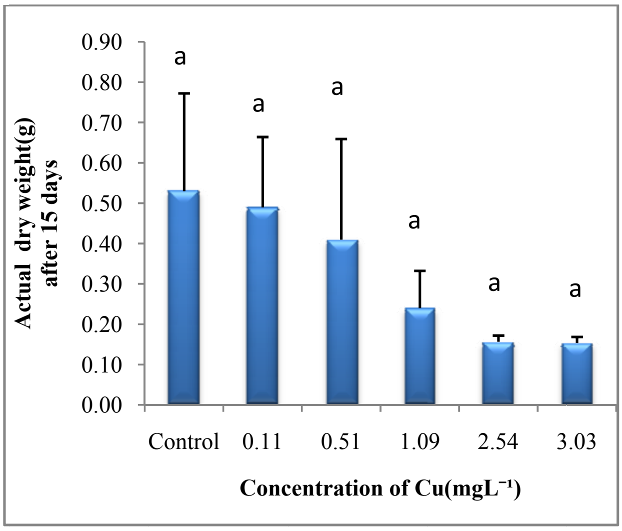

Fig 2. Effect of Cu in dry weight (DW) of *E. fluctuans* at the end of 15 days of exposure. Values are given as mean ±SD; values with different superscript letters on bars indicate significant differences at P<0.05 among one another.

Table 1. Effect of Cu on increase in Shoot Height(SH) of *E. fluctuans* at the end of 15 days of exposure. Values are given as mean ±SD; values with different superscript letters on bars indicate significant differences at P<0.05 among one another.

Cu Conc. (mg L^{-1})	Shoot Height		
	Day 5	Day 10	Day 15
Control	20.92 ± 6.71^{a}	36.08 ± 6.42^{a}	39.29 ± 5.03^{a}
0.11	23.18 ± 5.53^{a}	30.48 ± 5.39^{ab}	34.14 ± 4.48^{ab}
0.51	17.04 ± 8.95^{ab}	20.38 ± 10.45^{bc}	21.71 ± 8.57^{bc}
1.09	11.99 ± 3.89^{ac}	12.99 ± 3.78^{cd}	12.99 ± 3.78^{cd}
2.54	6.15 ± 2.76^{bc}	6.15 ± 2.76^{ce}	6.15 ± 2.76^{d}
3.03	1.94 ± 0.80^{c}	1.94 ± 0.80^{de}	1.94 ± 0.80^{d}

Table 2. Effect of Cu on number of new leaves (NL) of *E. fluctuans* at the end of 15 days of exposure. Values are given as mean ±SD; values with different superscript letters on bars indicate significant differences at P<0.05 among one another.

Cu Conc. (mg L^{-1})	Number of new leaves		
	Day 5	Day 10	Day 15
Control	5 ± 1^{a}	6 ± 1^{a}	8 ± 1.73^{a}
0.11	4 ± 1^{a}	6 ± 1^{a}	7 ± 1^{ab}
0.51	2.67 ± 2.08^{ab}	4 ± 2^{ab}	4.33 ± 1.53^{bc}
1.09	2 ± 1^{ac}	3 ± 1^{ac}	3.67 ± 0.58^{c}
2.54	2 ± 1^{ad}	2.33 ± 1.15^{bc}	3 ± 1^{cd}
3.03	0 ± 0^{bcd}	0.33 ± 0.58^{c}	0.33 ± 0.58^{d}

Table 3. Effect of Cu on number of new nodes (NN) of *E. fluctuans* at the end of 15 days of exposure. Values are given as mean ±SD; values with different superscript letters on bars indicate significant differences at P<0.05 among one another.

Cu Conc. (mg L^{-1})	Number of new nodes		
	Day 5	Day 10	Day 15
Control	3.67 ± 0.58^{a}	5 ± 1^{a}	7 ± 1^{a}
0.11	2.67 ± 2.08^{ab}	5.33 ± 1.53^{a}	6 ± 1^{a}
0.51	2.33 ± 1.15^{ac}	3 ± 1^{ab}	3.67 ± 0.58^{b}

1.09	1.33±0.58[ad]	2±1[b]	2.33±0.58[bc]
2.54	0.67±0.58[bcd]	1.33±0.58[b]	1.33±0.58[c]
3.03	0.33±0.58[bcd]	0.33±0.58[b]	0.33±0.58[c]

Table 4. Effect of Cu on number of leaf fall (LF) of *E. fluctuans* at the end of 15 days of exposure. Values are given as mean ±SD; values with different superscript letters on bars indicate significant differences at P<0.05 among one another.

Cu Conc. ($mg\ L^{-1}$)	Number of leaf fall		
	Day 5	Day 10	Day 15
Control	0±0[a]	0±0[a]	0.33±0.58[a]
0.11	0±0[a]	0±0[a]	0±0[a]
0.51	0±0[a]	0.33±0.58[ab]	0.67±0.58[a]
1.09	0.33±0.58[a]	0.67±0.58[ac]	1.67±0.58[ab]
2.54	0.67±0.58[a]	0.67±0.58[ad]	2.67±0.58[b]
3.03	1±1[a]	1.33±0.58[bcd]	5±1[c]

Enydra fluctuans DC. showed six different types of morphological changes namely fresh weight, dry weight, shoot height, new leaf, new nodes, leaf fall at different Cu concentrations whereas the changes and extent of damage of the plant parts and other morphological symptoms were found to have increased with the increased copper concentration. Lower the concentrations of Cu, longer the occurrence of symptoms while higher the concentrations, shorter is the duration of occurrence of symptoms. It is found in the study that duration and treatment concentration play a vital role to exhibit different toxicological symptoms.

The effects of different concentration of Cu on fresh weight (FW) of*Enydra fluctuans* plants during the 15 day exposure are shown in Fig.1. The maximum increase in fresh weight was in control (1.61 g) followed by the plant exposed at 0.11 $mg\ L^{-1}$ Cu (1.58 g). FW was least at 3.03 $mg\ L^{-1}$ Cu (0.32). One way ANOVA showed significant changes in fresh weight of the plants exposed at different concentrations ($P<0.05$).The effects of different concentration of Cu on dry weight (DW) of theplants during the 15 day exposure are shown in Fig. 2. The maximum increase in dry weight was in control (0.53 g) followed by the plant exposed at 0.11 $mg\ L^{-1}$ Cu (0.49 g). DW was least at 3.03 $mg\ L^{-1}$ Cu (0.15). At all concentrations, Cu affected both the fresh and dry weight as in other study it was reported that heavy metals restrict biomass production in several plants such as *Colocasia esculenta*[3], *Lemna polyrrhiza*[15]. Again there is no significant differences in dry weight of *E. fluctuans*where other similar study also revealed that *Ocimum gratissimum* showed no significant differences between control and other heavy metal concentrations treated to the plant in dry biomass production [6]. It wasreported that in plants heavy metal exposure can affect photosynthesis as well as can alter metabolic pathways resulting in growth reduction[30].Cu toxicity also affected *Salix viminalis* L. in reduction of the plant tissue biomass [9].

Changes in shoot height(SH) in control and Copper exposed plants on 5,10 and 15 days of exposure are shown in Table 1.On day 5, control plant showed an increase in SH by 20.92 cm during this period of exposure. However plant exposed at 0.11 $mg\ L^{-1}$ Cu show higher increase in shoot height (23.18 cm) than in control (20.92 cm). The lowest increase in shoot height was seen at 3.03 mgL^{-1}Cu (1.94 cm).On day 10, control plant showed an increase in SH by 36.08 cm during this exposure period. The lowest increase in shoot height is seen at highest concentration of 3.03 $mg\ L^{-1}$ Cu (1.94 cm).On day 15, the highest increase in SH was 39.29 cm showed by the plant in control. The changes in Shd crease gradually with increase in concentration. One way ANOVA revealed that the differences in SH was significantly different ($P<0.05$).Similar study also reported that Cu(II) when exposed to alfalfa plant at 40 ppm caused serious effect resulting in the reduction of shoot elongation (70%) in an experiment of 14 days [23].

Appearance of New Leaf (NL) in control and copper exposed plants on 5, 10 and 15 day is shown in Table 2. On day 5 of exposure, the highest NL of 5 was seen in control. At 1.09 and 2.54 $mg\ L^{-1}$ Cu NL of 2 were recorded. On day 10 of exposure, plants in control showed the highest NL (6). The lowest NL was at 3.03 $mg\ L^{-1}$ Cu (0.33). On day 15 of exposure, control plants showed the highest appearance of NL (8). The lowest was seen in plants exposed at 3.03 $mg\ L^{-1}$ Cu (0.33).One way ANOVA revealed that the differences in SH were significantly different ($P<0.05$) among control and Cu-exposed plants. From the present study it was found that the NL was seen in lower concentrations but gradually decreased with increase in Cu concentrations. Similar trend of growth was found in plants treated within 0.51-2.54 mgL^{-1} in terms of NL, where *Vigna radiata* showed increase in its overall growth at lower Cu concentrations and the reason might be as Cu has micronutrient properties as reported by [19].

Changes in the number of new nodes (NN) in control and Cu exposed plants on 5, 10 and 15 day of exposure are shown in Table 3. On the 5th day of exposure, the highest NN of 3.67 was observed in control and the lowest increment of 0.33 at 3.03 $mg\ L^{-1}$ Cu. On the 10th day of exposure, the plants exposed to 0.11 $mg\ l^{-1}$ Cu showed the highest NN of 5.33 while the control plants showed NNof 5. The lowest NN was seen in plants exposed to 3.03 $mg\ L^{-1}$ Cu with NN of 0.33. On the 15th day of exposure, the highest NN of 7 was showed by the control plants while NN is 6 in case of plant exposed at 0.11 $mg\ L^{-1}$C. The lowest was seen in plant exposed at 3.03 $mg\ l^{-1}$ Cu with NN of 0.33. One way ANOVA revealed that the differences in NN were significantly different ($P<0.05$) among control and Cu-exposed plants. Multiple comparisons using Tukey Test showed that the changes in NN at 0.11-3.03 $mg\ L^{-1}$Cu were significantly lower than those in control ($p<0.05$). Similarly it was reported that there was reduction in new nodes development due to heavy metal(Pb) stress in the stem of *Mentha piperita* L., though it was observed at 100-1500 ppm [2].

Number of Leaf Fall (LF) in control and copper exposed plants on 5, 10 and 15 day is shown in Table 4.On day 5 of exposure, the highest number of LF was seen in plants exposed to 3.03 mg L^{-1} Cu (1) while there was no leaf fall in case of control plants as well as in plants exposed to 0.11 and 0.51mg L^{-1} Cu. On day 10 of exposure, control plants and plants exposed to 0.11mg L^{-1} Cu had no leaf fallwhile the highest number of LF was observed in 3.03 mg L^{-1} Cu (1.33). LF gradually increased with increase in Cu-concentration. Plants exposed to 1.09 and 2.54 mg L^{-1} Cu had equal number of leaf fall (0.67). On day 15 of exposure, the plant in 3.03 mg L^{-1} Cu showed the highest number of LF (5), while the lowest was seen in plants exposed to0.11mg L^{-1} Cu (0). One way ANOVA revealed that the differences in LF were significantly different ($P<0.05$) among control and Cu-exposed plants. Similar study also reported that there was growth reduction, and induced leaf senescence at higher concentrations of Zincbecause of disturbance in metabolic mechanisms namely antioxidant processes, content of pigment as well as in photosynthetic activity [21,10]. It was also found that *Brassica napus* at 50 µM Pb showed reduction in number of leaves[16].

4. Conclusion

Copper at very low concentration acts as an essential micronutrient for many plants but at higher concentrations it becomes toxic. Thus, the results of this study indicated that *E.fluctuans*was tolerant to Cu toxicity and could be employed successfully in various toxicity studies in water bodies polluted with Cu using the growth parameters as tools for biomonitoring of toxic effects of copper. In view of its ability to survive at high Cu concentrations, it could also be explored for its phytoremedaition potential for Cu.

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