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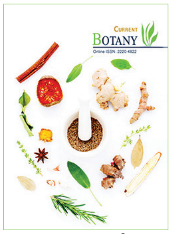

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Effects of copper bioaccumulation on growth and biochemical constituents of the seedlings of *Casuarina equisetifolia* L.

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ABSTRACT

Accumulation of heavy metals in the soil causes a significant impact on the biological processes in the plants. In the present study, the impact of bioaccumulation of copper (Cu) on the growth and biochemical constituents of the seedlings of *Casuarina equisetifolia* was assessed. The results showed that *C. equisetifolia* is a hyper-accumulator of Cu. The roots of the *C. Equisetifolia* significantly accumulated ($p < 0.05$) greater levels of Cu than stem and leaves. Similarly, the seedlings exposed to different concentrations of Cu showed differential height and collar diameter. The higher concentrations of Cu significantly ($p < 0.05$) hindered the fresh and dry weights of seedlings. It was noticed that accumulated Cu caused a significant decrease in levels of total carbohydrates, proteins and chlorophyll contents in *C. equisetifolia* seedlings.

KEYWORDS: *Casuarina*, copper, accumulation, biochemical constituents, biological processes

INTRODUCTION

Heavy metals have become the major contaminants of the environment nowadays, which raised concerns across the globe (Suman *et al.*, 2018). Generally, heavy metals reach the soil through natural and manmade activities, causing serious environmental pollution. Copper is one of the heavy metals found in contaminated soil, primarily released by industries and is considered to be deleterious to human health due to its long-term persistence in the environment (Shrivastava, 2009). However, copper is an essential element for some physiological and biochemical processes of living organisms including plants. But, its accumulation in excessive levels in soil can be toxic and cause a biological imbalance in plants (Lone *et al.*, 2008; Lange *et al.*, 2017). It is obvious that, contamination of Cu in agricultural fields creates concerns about crop productivity and food safety (Saleem *et al.*, 2020). Recently, many plant species have been employed as hyper accumulators for a wide range of heavy metals in the soil (Saleem *et al.*, 2019). The plant species used under heavy metal contaminated soils are assumed to produce higher biomass and possess heavy metal accumulation traits. Since, many fast-growing forest tree species are found to be efficient phytoremediation agents (Rathna Kumari & Raveesha, 2021), an attempt was made to evaluate the efficacy of *C. equisetifolia*, a fast-growing tree species with prolific seedling ability in bioaccumulation of Cu and its influence on its growth and biochemical constituents of the plants.

MATERIALS AND METHODS

Experiments on bioaccumulation of soils treated with Cu and its influence on growth and biochemical changes were conducted in *Casuarina equisetifolia* seedlings in potting conditions. The seeds of *Casuarina equisetifolia* obtained from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, germinated and used in the experiments. Further, the seedlings of 3 to 5 cm height were transplanted into a potting medium filled with a sufficient quantity of air-dried soil, supplied with necessary nutrients. The commercially available Copper Sulphate (analytical grade) was used in the study. The potting medium was supplied with each concentration of copper sulphate (50 to 400 ppm). Later, the seedlings of one-month-old were transplanted into the earthen pots of 2000cc capacity. Suitable management practices, including sufficient irrigation and weed control were performed during the experiments.

After six months, the shoot (stem and leaves) and roots of each test seedlings were harvested. The height of the seedlings was measured using calibrated scale and the collar diameter (mm) was measured with electronic vernier caliper (Mitutoyo, CD-6"). The data on fresh and dry weights of the seedlings were recorded using a digital scale. The quantitative estimation of total sugar, protein and chlorophyll in the leaf was carried out by spectrophotometric methods. The total sugar levels were estimated by the Anthrone reagent method (Hedge & Hofreiter, 1962). Similarly, the total protein contents of the leaf samples of seedlings were carried out using Lowry *et al*., (1951) and the total chlorophyll content was estimated using Arnon's method (1949).

At first, the roots were separated from soil and washed under running water. The oven-dried plant tissues were ground carefully using an electric grinder and passed through a 1.0 mm mesh strainer. The ground plant tissue samples (0.5 g) were digested in a heated di-acid mixture of concentrated HNO3 and HCl (3:1, v/v). The concentrations of Cu as in the samples were measured with a flame atomic absorption spectrometer (GBC, Germany). The experimental data were analyzed using SPPS 20 and the mean values of each treatment in four replications were expressed in mean± standard error. The differences between the treatments were tested statistically at 0.05% and probability by one way ANOVA.

RESULTS

Accumulation of Cu in *Casuarina* Seedlings

The levels of accumulation of Cu were recorded in the root and shoot of *C. equisetifolia* seedlings (Table.1). It was found that, the bioaccumulation of Cu was significantly ($p < 0.05$) increased with an increase in the concentration of Cu in the soil. Comparatively, roots accumulated greater levels of Cu than stem and leaves. The maximum and minimum levels of Cu accumulated in roots were 4.443 and 0.098 ppm, respectively. Similarly, the Cu was also moderately accumulated in shoot parts *viz* stem and leaves, on exposure to different concentrations of Cu. The maximum and minimum levels of Cu accumulated in stem and leaves were 1.653 and 0.043ppm and 2.993 and 0.060ppm, respectively.

Influence of Cu on Growth and Biomass of the Seedlings

The Influence of different concentrations of Cu on the height and collar diameter of the *C. equisetifolia* seedlings was recorded (Table 2). The observations showed that, though the height, collar diameter and fresh and dry weight of the seedlings were increased initially with an increase in Cu concentrations, they gradually decreased with an increase in concentrations of Cu. The maximum and minimum height and collar diameter of seedlings recorded were 68 and 22 cm and 4.76 and 2.77 mm, respectively. Similarly, the maximum and minimum fresh and dry weights of seedlings recorded were 25.00 and 9.43g, 10.84 and 4.50g, respectively.

Table 1: Accumulation levels of Copper in stem root and leaves of *Casuarina equisetifolia* seedlings

Treatments (ppm)	Copper (ppm)		
	Root	Stem	Leaves
50	0.098^{e}*	0.043^{fg}	0.060^{d}
100	0.240^{e}	0.166e^{f}	0.180^{d}
150	0.446^{de}	0.283^{de}	0.346^{d}
200	0.866^{de}	0.416^{cd}	0.620^{cd}
250	1.627^{cd}	0.550^{c}	0.843^{cd}
300	2.690^{bc}	0.760^{b}	1.773^{bc}
350	3.110^{b}	0.860^{b}	2.610^{ab}
400	4.443^{a}	1.653^{a}	2.993^{a}
SE±	0.810	0.082	0.761
$CD_{(0.05)}$	1.402	1.812	1.332
p-value	1.4E-06	4.15E-15	0.0001

*Different letters in the column indicate significant differences at 5% probability level

Effect of Cu on Biochemical Constituents of the Seedlings

Effect of Cu on biochemical constituents *viz* levels of total sugars, proteins and chlorophyll content were recorded in the seedlings of *C. equisetifolia* treated with different concentrations of Cu (Table 2). The results showed different levels of biochemical constituents in different concentrations of Cu. The maximum and minimum levels of total sugars and proteins recorded in the seedlings were 2.50 and 2.15 mg/g and 1.07 and 0.55 mg/g in control and 400 ppm respectively. Similarly, the maximum and minimum levels of chlorophyll content recorded were 0.37 and 0.11 mg/g in control and 400 ppm concentrations respectively.

DISCUSSION

From the above experiments, it is apparent that heavy metals are taken up by the roots of the plants from the soil and subsequently transported to shoots. The greater levels of Cu accumulation in *C. equisetifolia* species may be attributed to the well-developed detoxification mechanisms (Cui *et al*., 2007). The findings of the study showed that, Cu ions taken up by *C. equisetifolia* from the contaminated soil are retained primarily in the roots and only a small proportion was translocated to stem and leaves. The present results are confirmatory with the findings of Pahalawattaarachchi *et al*., (2009) who found better accumulation of Cu in roots than shoot. Greater accumulation of metals in roots is a strategy to protect the stem and leaves of the plant from metal toxicity by acting as a barrier for metal translocation. Similarly, Fernandes and Henriques (1991) reported that, Cu tolerant plants prevent Cu from reaching stem and leaves by keeping it in their roots. The differential accumulation of heavy metals in plants is also determined by the mobility and solubility of metals (Guilizzoni, 1991).

Further, the woody species produce a very high amount of biomass which facilitates the accumulation of high levels of heavy metals in their shoot system (Luo *et al*., 2015). Similarly, the tree species have a deep root system, which can effectively reduce soil erosion and prevent the dispersal of contaminated soil to the surrounding environment (Suman *et al*., 2018). The findings of the present study showed differential growth in terms of the height of the seedlings and collar diameter and biomass *viz* fresh and dry weight of the *C. equisetifolia* seedlings treated with Cu. Barbosa *et al*., (2013) found that, the decrease in growth and biomass parameters with an increase in Cu concentrations is mainly due to stress caused to seedlings due to its toxicity. The decrease in plant biomass was attributed to disturbed metabolic activities, low photosynthetic reactions and reduced uptake of essential mineral nutrients under heavy metal stress (Amin *et al*., 2019). Similarly, the toxicity of Cu reduced seedling height and

Table 2: Effect of Copper on growth and biochemical constituents of seedlings of *Casuarina equisetifolia*

Treatments (ppm)	Height (cm)	Collar diameter (mm)	Fresh Weight (g)	Dry Weight (g)	Sugars (mg/g)	Protein (mg/g)	Chlorophyll (mg/g)
Control	52.33[c*]	4.26[bc]	21.43[b]	10.69[a]	3.50[a*]	1.07[a]	0.37[a]
50	68.00[a]	4.76[a]	25.00[a]	10.84[a]	3.49[a]	1.04[a]	0.36[a]
100	65.00[b]	4.70[a]	24.00[a]	10.75[ab]	3.44[ab]	1.04[a]	0.35[a]
150	60.00[c]	4.53[ab]	22.00[b]	10.70[ab]	3.43[ab]	1.01[ab]	0.33[b]
200	54.00[d]	4.19[bcd]	16.38[bc]	9.38[abc]	3.28[ab]	0.91[abc]	0.30[b]
250	50.00[e]	4.04[cd]	16.24[bc]	8.74[bc]	3.12[ab]	0.86[bc]	0.28[b]
300	43.00[f]	3.85[d]	12.14[c]	7.97[cd]	2.85[abc]	0.77[cd]	0.20[c]
350	29.67[g]	3.15[e]	10.02[c]	6.00[de]	2.79[bc]	0.64[de]	0.16[d]
400	22.00[h]	2.77[e]	9.43[d]	4.50[e]	2.15[a]	0.55[e]	0.11[e]
$SE_{(0.05)}$	1.662	0.046	4.196	0.954	0.431	0.11	0.0143
$CD_{(0.05)}$	2.876	0.403	7.259	1.650	0.747	0.1906	0.025
p-value	2.75E-12	2.45 E- 07	4.36E-03	4.10E-06	4.29E-03	1.41E-05	1.49E-15

*Different letters in the column indicate significant differences at 5% probability level

affected the growth and development with the interference of plant metabolic activities (Turgut *et al.*, 2004).

The results obtained on the biochemical studies showed a significant effect of Cu on total sugars, proteins and chlorophyll content in the seedlings. The results indicate that, the exposure of seedlings to different concentrations of Cu showed an increase in sugar content in lower concentrations of heavy metal; however, higher concentrations decreased the levels of sugar content. It is obvious that, during stress conditions, there is a possibility of increase in sugar content in plants (Shah *et al*., 2017). Verma and Dubey (2001) found that a higher concentration of soluble sugar could possibly provide an adaptive mechanism in maintaining favourable osmotic potential under metal toxicity. The studies also showed that, there was a decrease in the total protein content in the seedlings treated with Cu and it might be due to stress caused by the Cu in the seedlings (Guo *et al.*, 2007). It was found higher chlorophyll content in control seedlings without application of Cu and was decreased with an increase in concentrations of Cu. It would be possibly due to the breakdown of chlorophyll during stress or due to inhibition of chlorophyll biosynthesis (Prasad and Prasad, 1987; Patsikka *et al.*, 2002).

Based on the results obtained in the study, it is concluded that, *C. equisetifolia* seedlings are efficient bioaccumulator of Cu. However, the accumulation of higher concentrations of Cu hinders the growth and accumulation of biomass in *C. equisetifolia* trees. In addition, Cu accumulation also causes a greater impact on biochemical *viz* total sugars, proteins and chlorophyll content thereby causing deleterious effects on the seedlings of *C. equisetifolia*.

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