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Effect of Cu and Cd on some biochemical components of *Fissidens biformis* Mitt

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Abstract: **Objective of present study was to examine the changes in different biochemical components in *Fissidens biformis* when it was exposed to Cu or Cd stress at various concentrations (100, 200, 300, 400ppm for Cu and 5, 10, 15, 20ppm for Cd) for prolonged number of days (10, 20 and 30). *F. biformis* when exposed to Cu the amino acids, proteins and phenols have shown significant acceleration in their contents on 10th, 20th and 30th day till 200ppm and on 20th day till 400ppm only in amino acids and proteins. On 30th day decrease in content was observed in 400ppm in amino acids, in 300 and 400ppm in proteins and phenols. Whereas in case of protease, acid phosphatase and peroxidase there was increase in activity during all the days up to 300ppm and the decrease in activity was observed from 20th day at 400ppm till 30th day compared to control. The presence of content observed in control remained almost same in all the components. Cd being non-essential and much toxic even in low concentrations, has shown comparatively less increase in amino acids, protein and phenolic content as well as protease, acid phosphatase and peroxidase activity till 10th day in almost all the concentrations, and thereafter a significant decrease in all the components was observed at all the concentrations on 20th and 30th day.**

After examining the changes in *F. biformis* during prolonged exposure to both the metals, the net amount of Cu (92.85ppm) and Cd (0.32ppm) absorbed by *F. biformis* has also been examined using AAS (Atomic Absorption Spectrophotometer).

Keywords: **Moss, Heavy metals, Copper, Cadmium, Biochemical parameters, AAS (Atomic absorption spectrometer).**

1. INTRODUCTION

Among the particulate pollutants the heavy metals are considered most important as heavy metal is a collective name for metallic element of high atomic weight (>50) and relative density more than 7. Heavy metals are of great importance for research motive due to their toxicological importance to human health, plants, and animals (Jarup, 2003; Azevedo and Lea, 2005; Almeida *et al*., 2007). Due to increasing industrialization, urbanization and rigorous agriculture increasing contamination of heavy metals in soil has become a matter of concern.

Though Cu is an essential micronutrient for all the organisms. It becomes toxic at its elevated concentrations, whereas Cd is toxic even at lower concentrations. Anthropogenic activities are the main source of copper in the environment and its elevated concentrations create a metal pollution affecting the flora and fauna.

Bryophytes play very important role in various ecosystems due to their capacity to accumulate various metal ions, and the possibility of their use as bio-monitoring agents as well as bio-indicating agents. In bryophytes especially mosses possess distinctive features (small size, ability to survive long periods of toxicity, poikilohydric habitat, simple structure and high regeneration potential, low nutritional requirement and short life cycle) which coupled with their ability to tolerate high concentrations of heavy metals which make them unique among the higher plants (Pathania, 2005). Mosses accumulate heavy metal ions as well as chemical substances mainly from surrounding atmosphere (Fernandez *et al*., 2004) due to their surface volume ratio and limited or no cuticle development (Brown, 1984). In addition to this, bryophytes have high counter gradient mechanisms for the accumulation of metal ions in their tissues (Carginale *et al*., 2004). Excessive

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concentration of heavy metals in the soil environment affect the growth of the plants, leading to changes in 'biomolecules' levels and interfere with many enzymatic activities related to various metabolic and development processes (Ahsan *et al*., 2007; Kuriakosa and Prasad, 2008; Kachout *et al*., 2009; Zhang *et al*., 2009; Rahoui *et al* ., 2010).

As Cu and Cd bind on the cell surface as well as enter within the cells, the ions of these metals start interacting with the functional groups of proteins, nucleic acids, polysaccharides, etc. and substitute for other metal ions already bound with these functional groups. Due to this interaction many metabolic disorders arise, and it is generally difficult to confirm whether the disorders are of primary or are of secondary origin (Seregin and Ivanov, 2001). Excess Cu and Cd induce complex changes in plants at genetical, biochemical and physiological levels, ultimately leading to phytotoxicity. The most common symptoms are (1) decreased plant growth which may lead to plant death by interfering uptake of nutrients (Sandalio *et al*., 2001; Drazkiewicz *et al*., 2003); (2) destruction of photosynthesis via deterioration of chlorophyll (Sandalio *et al*., 2001); (3) and inactivation of many enzymes (Greger and Ogren, 1991; De Filippis and Zeigler, 1993) as well as aggregation of protein complexes (Horvath *et al*., 1996); (4) changes in the amount of reactive oxygen species (ROS) and the activity of antioxidant enzymes (Sandalio *et al*., 2001; Schützendübel and Polle, 2002). The ROS have the major role in damaging biomolecules of cell wall and plasma membrane. The damage caused by ROS is called as oxidative stress. In response to ROS plants have developed active defense mechanism to cope with oxidative stress (Okamoto *et al*., 2001).

Present study was undertaken (1) to assess the Cu or Cd tolerance capacity of *Fissidens biformis*; (2) determine the effect of both metals on the content of amino acids, proteins, phenols and activities of protease, acid phosphatase and peroxidase; (3) assess the uptake of Cu and Cd by *F. biformis* using AAS (Atomic Absorption Spectrophotometer).

2. MATERIALS AND METHODS

Collection: The moss to be studied along with substrata (soil) was collected from Mcleodganj, Dharamshala, H.P., alt. 2082m.

Experiment: In the in-vitro conditions the plant material after separating from the substratum (soil) was washed several times with tap water and then spotted under binoculars to remove other unwanted materials. The separated plant material was then washed with the distilled water to avoid any type of clinging soil particles. Then the plant was kept in clean petri-dishes.

Chemical treatments: The concentrations made for copper were 100, 200, 300 and 400 ppm and for cadmium were 5, 10, 15 and 20 ppm. The concentrations were standardized on the basis of observed concentrations in both soil and control. Each metal concentration (2 ml) was sprayed on the selected moss plant after transferring the plants into the sterilised petriplates. The process of spray was repeated after every fifth day till 30th day and the observations were taken after every 10th day. To make the extract of the metal treated moss after selected time period, the moss was washed with distilled water and then air dried at room temperature then the analyses of all the parameters were done. The contents of Cu^{++} and Cd^{+++} were evaluated in the moss by using Atomic Absorption Spectrophotometer (AAS).

3. RESULTS

Fissidens biformis was exposed separately to different concentrations of Cu (100, 200, 300 and 400 ppm) and Cd (5, 10, 15 and 20 ppm) for 10, 20, and 30 days respectively. The different parameters showed different results due to Cu or Cd created stress. The changes in control (without treatment) were negligible during all the days (Fig. 1. a-f).

Amino acid content: In case of Copper, an increase in amino acid content was observed till 30th day in 100 and 200ppm. In 300ppm the amino acid content increased till 20th day and thereafter it showed decrease. With 400ppm treatment the content increased till 10th day after that there was a decline.

Whereas with Cd treatment amino acid content increased up to 15ppm till 10th day, up to 10ppm till 20th day and up to 5ppm till 30th day, thereafter content started to decrease

Protein content: The protein content increased till 30th day up to 200ppm. In 300ppm it accelerated till 20th day and after that it started to reduce. Whereas in 400ppm the increase in content was observed till 10th day and further exposure to Cu till 20th and 30th day led to decrease in content.

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With Cd the increase in protein content was noticed in all the concentrations till 10th day. On 20th day the increase in content was noticed up to 10ppm and after that content declined. On 30 day a rapid decline in protein content was noticed in 10, 15 and 20ppm.

Phenolic content: The acceleration in phenolic content was noticed during all the days with 100 and 200 and 300ppm of Cu till 20th day and with 400ppm the content increased till 10th day after that a decrease in the content was noticed. Minor changes in control were observed during all the days.

With Cd phenolic content increased up to 10ppm only till 30th day. There was no significant increase noticed in higher concentrations (15, 20ppm).

Protease activity: Enhanced protease activity was observed during all the days (10, 20 and 30) in 100, 200 and 300ppm. In higher concentration (400ppm) increase was noticed only on 10th day and after that the activity showed decline on 20th and 30th days in 400ppm.

with Cd, elevation in protease activity was noticed up to 20ppm on 10th day and up to 10ppm till 20th day after that a fall in the activity was noticed. A rapid decline in activity was observed on 30th day in all the concentrations.

Acid Phosphatase and peroxidase activity: With Cu both acid phosphatase and peroxidase showed an increase in activity upto 400ppm till 10th day and in 100, 200, 300ppm till 20th and 30th day.

With cadmium acid phosphatase showed an increase in activity during 10th day upto 20ppm, upto 15ppm till 20th day and upto 10ppm till 30th day. The activity started to decline on 20th day in 15ppm and on 30th day in 15, 20ppm.

In case of peroxidase increase in activity was noticed upto 10ppm till 30th day and upto 15ppm till 10th day. Decrease in activity was also noticed in 20ppm on 10th day, in 15ppm on 20th day and in 10, 15 and 20ppm on 30th day.

The initial increase in content as well as activity was might be due to capacity of moss to survive under stress and antioxidative (phenols and peroxidases) defense mechanism which helps the plant to survive under stress conditions by deactivating ROS mechanism.

Metal uptake of Cu and Cd by *F. biformis* when exposed for prolonged periods (10, 20 and 30 days) under different concentrations: In control the copper content in soil substratum observed was 9.625ppm and in plant body of *F. biformis* it was 1.08ppm. After the prolonged exposure 10, 20 and 30 days respectively of *F. biformis* to Cu stress (100, 200, 300 and 400ppm) the revealed content of copper in *F. biformis* was: 7.52ppm on 10th day, 23.27ppm on 20th day and 37.06ppm on 30th day in 100ppm of Cu exposure.

In 200ppm, the absorbed content was 14.44ppm on 10th day, 39.02ppm on 20th day and 61.11ppm on 30th day. In case of 300ppm, the absorbed concentration was 42.95ppm on 10th day, 72.64ppm on 20th day and 92.5ppm on 30th day. In case of 400ppm, the absorbed concentration was 72.12ppm on 10th day, 81.06ppm on 20th day and 92.85ppm on 30th day respectively (Fig. 2).

In soil substratum of *F. biformis*, the Cd content was 16.8ppm and in plant body it was 0.146ppm.

Under 5ppm of Cd exposure the absorbed content was 0.161ppm on 10th day, 0.186ppm on 20th day and 0.211ppm on 30th day. In case of 10ppm it was 0.183ppm on 10th day, 0.234ppm on 20th day and 0.275ppm on 30th day. In case of 15ppm the absorbed content by *F. biformis* was 0.210ppm on 10th day, 0.254ppm on 20th day and 0.316ppm on 30th day. And in 20ppm the absorbed concentration was 0.240ppm on 10th day, 0.295ppm on 20th day and 0.320ppm on 30th day (Fig. 3), Cd exposure getting detrimental with the increasing concentrations.

It is revealed that *F. biformis* could absorb more amount of copper as compared to cadmium and survived better in copper toxicity compared to cadmium toxicity.

4. STATISTICAL ANALYSIS

t-test and paired t-test, One way ANOVA and Post hoc (Dunnett test) test for multiple comparison have been applied to compare the control with each concentration at every time interval 10th, 20th and 30th day (significance value < .05=significant).

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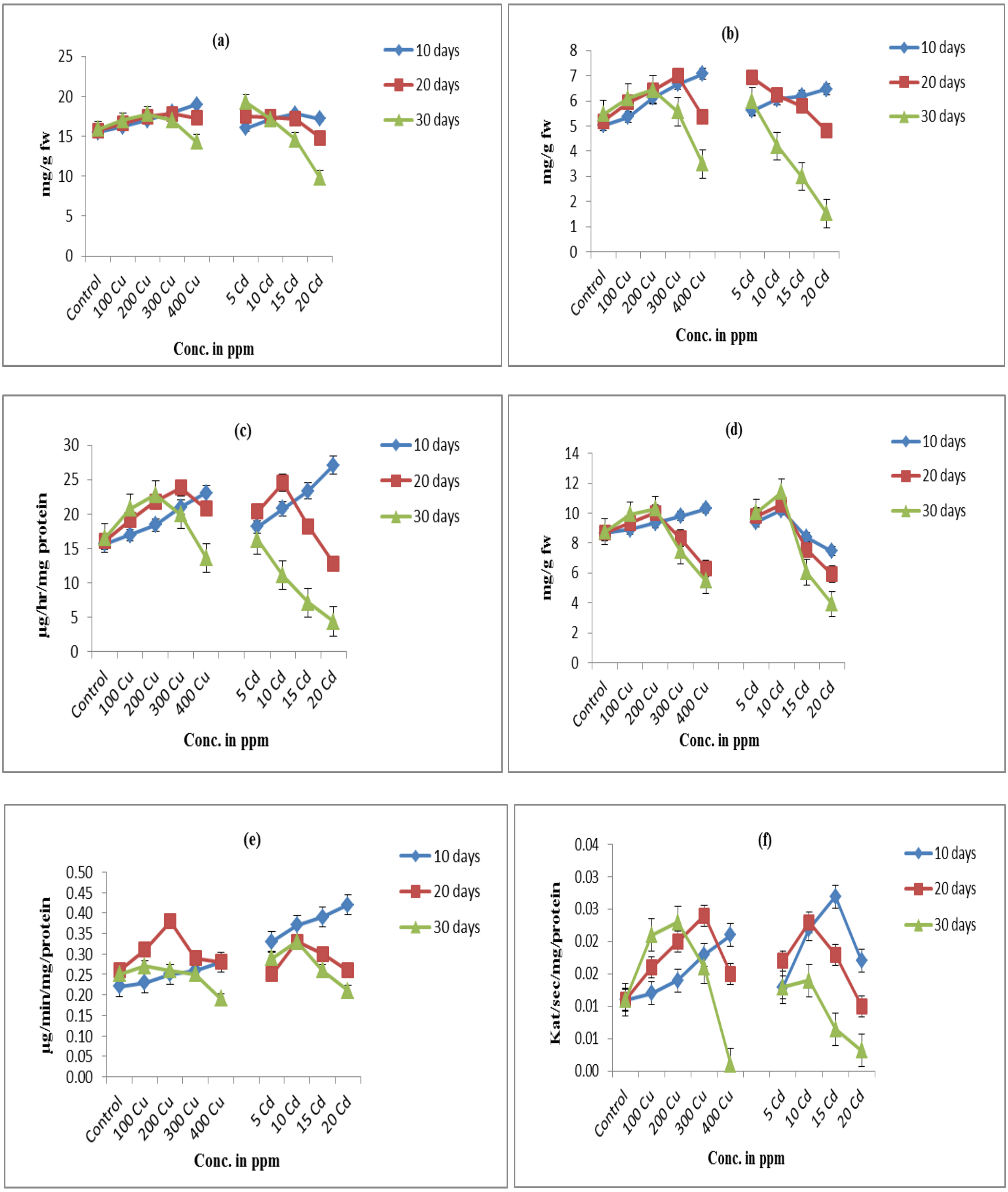

Fig. 1. a-f: (a) Amino acid content, (b) Protein content, (c) Protease activity, (d) Phenolic content, (e) Acid phosphatase activity, (f) Peroxidase activity in *F. biformis* as affected by exposure to different concentrations of Cu or Cd.

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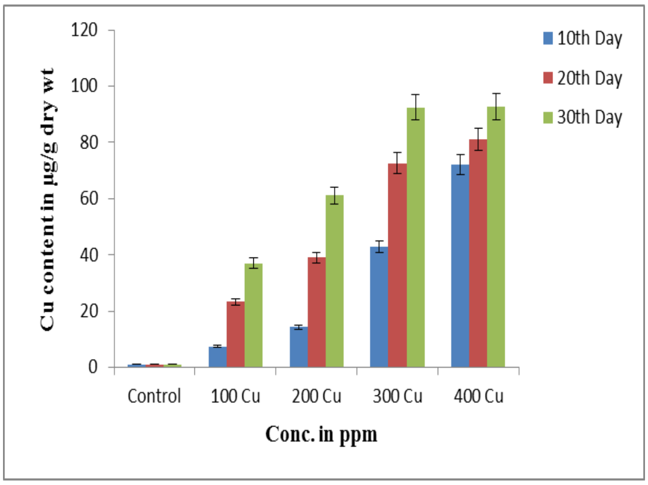

Fig. 2. Uptake of Cu by *f. biformis* exposed to different concentrations (100, 200, 300 and 400 ppm) of copper.

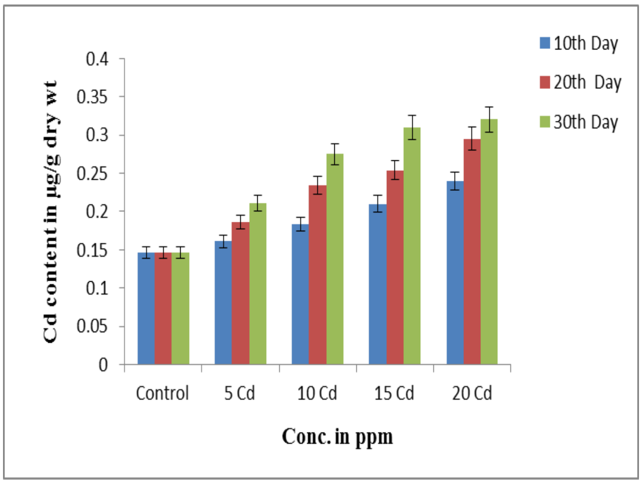

Fig. 3. Uptake of Cd by *f. biformis* exposed to different concentrations (5, 10, 15 and 20 ppm) of cadmium.

5. DISCUSSION

Present study has revealed that *F. biformis* has more potential to tolerate copper stress both concentration and duration wise (as compared to Cd) as evident from less and delayed negative impact on the selected moss. Cu is considered as an essential mineral element for plant growth but at higher concentrations it may cause toxicity in plants, whereas cadmium being non-essential, the mineral element is toxic even at low concentrations (Solanki *et al*., 2010).

In case of all the parameters the moss under study has shown different potential of toleration, as evident from changes in various biochemical parameters for Cu or Cd stress.

This capacity to tolerate Cu or Cd stress by *F. biformis* may be due to its morphology, physiology, high regeneration potential and cation exchange sites in its cell walls, surface absorption mechanism and locking of these metals in its skeletal system. Owing to the peculiar capabilities of moss, it serves as good bio-accumulator of heavy metals. (Dazy *et al*., 2009) has also reported the similar response in the aquatic moss *Fontinalis antipyretica* treated with various heavy metals (Cd, Cu, Pb and Zn) with varied concentrations 0, 0.1, 1, 10, 100 and 1000µm. and assessment of lipid peroxidation and anti-oxidative responses in the apices in the duration of 2 and 7 days has shown increased lipid peroxidation and enzymatic activity as compared to control.

Studied moss also acted as a bio-indicator due to prolonged exposure to both the heavy metals (Cu or Cd) for extended period induced complex changes in moss plant at both biochemical and physiological levels, ultimately leading to phytotoxicity. (Sandalio *et al*., 2001 and Drazkiewicz *et al*., 2003) also reported that excess of heavy metals lead to various changes at genetical, biochemical and physiological levels. Damage of cell membrane due to metal toxicity, leading to ion leakage and has created negative impact on various parameters (Brown and wells, 1990; Guschina and Harwood, 2002) and all other activities protein synthesis, chlorophyll synthesis, and enzymatic activities etc. (Cumming and Taylor, 1990).

Excessive level of heavy metals in the soil environment negatively affects the plant growth, changes the levels of biomolecules in the cells and obstruct with the activities of many key enzymes playing important roles in normal metabolic and developmental processes (Ahsan *et al*., 2007; Kuriakosa and Prasad, 2008; Kachout *et al*., 2009; Zhang *et al*., 2009).

Present study has revealed the enhanced oxidative enzymatic activities during the initial exposure to metal stress but the prolonged exposure has led to decrease in enzymatic activity that might be due to excessive generation of reactive oxygen species. It has been reported that reactive oxygen species produced during stress may undergo further reactions, catalysed by metal ions as in the fenton reaction with the increase in stress much more reactive hydroxyl radical also generated, responsible for changes in macromolecule and finally contribute to cell death (Cho and Park, 2000).

In present study, under Cu as well as Cd stress an increase in phenolic content was observed initially but prolonged stress conditions decreased the content, as it is believed that both peroxidases and phenols are interlinked and play an important role during stress conditions. As the number of peroxidases increase the phenols also increase especially flavonoids and phenylopropanoids, which are oxidized by the enzyme peroxidase, which may act as H_2O_2- scavenging or phenolics/POX system. Like peroxidases, phenols also act as antioxidants. This has been seen in the moss that both the metals caused delay in decrease of phenolic content even after the decrease of peroxidase activity. Phenols have the capacity to bind specifically copper by chelating the metal and it inhibits the production of ROS by controlling the fenton reaction (Michalak, 2006).

In addition to this due to more absorption of applied concentrations of Cu and good biochemical response *F. biformis* acts as a good bio-accumulator and as a good bio-indicator due its less uptake of applied concentrations of Cd and poor response.

Mosses are considered useful in bio-monitoring as well as bio-indicating studies of different environmental qualities due to their differential sensitivity to air born contaminants (Rühling and Tyler, 1970; Pakarinen, 1978; Aceto et al., 2003). The awareness of heavy metal impact on various physiological and biochemical mechanisms in plants can be of a great significance in Eco physiology and mosses can play a good role in maximisation of phytoremediation.

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