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EFFECT OF IRON AND CHROMIUM ON PHOTOSYNTHETIC PIGMENTS OF MOSSES *MNIUM CUSPIDATUM* HEDW. AND *THUIDIUM CYMBIFOLIUM* DOZY & MOLK.

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ABSTRACT

Mosses are exceedingly appealing as model organisms for plant science studies because of their minute size, simple morphology, great regeneration capacity, little nutritional requirement and rapidness in completing their life cycle. Since they are one of the most primitive land plants, they play a crucial role in the study of plant evolution, especially when it comes to illuminating the processes through which their aquatic predecessors evolved into becoming terrestrial. In order to efficiently absorb metals and other nutrients from their surroundings, mosses have developed a highly effective uptake mechanism. The purpose of the present research was to determine how the different phytotoxic metal concentrations of each of the selected two heavy metals (Iron and Chromium) supplied over variable periods of time, affected the photosynthetic activity of two mosses - *Mnium cuspidatum* and *Thuidium cymbifolium*. Regeneration experiments were carried out to assess the toleration limit of Iron (1000 ppm in *Mnium* and 600 ppm in *Thuidium*) and Chromium (70 ppm in *Mnium* and 60 ppm in *Thuidium*). Chromium proved to be more phytotoxic than Iron even at low concentrations as it is non-essential in plant growth and metabolism. Iron, on the other hand, being quite essential in plant metabolic pathways, but it becomes phytotoxic at higher concentrations. The chlorophyll and carotenoid contents were assessed in both the moss species under the control as well as under the phytotoxic concentrations of Iron and Chromium within the toleration range. Total chlorophyll and carotenoid contents were more in *Thuidium* as compared to *Mnium*.

***Keywords*:** Mosses, *Mnium cuspidatum*, *Thuidium cymbifolium,* pollution biomonitoring, heavy metals, Chromium, Iron.

Introduction

Bryophytes especially the mosses have been widely used as biomonitoring agents indicating that the mosses have the ability to survive under abnormally high quantities of heavy metals as compared to vascular plants. Mosses have been deemed quite useful in biomonitoring as well as bioindicating studies (Ruhling and Tyler, 1970; Pakarinen, 1978; Aceto *et al*., 2003). Various heavy metals like Iron, Copper and Zinc play an important role in plants as well as in humans (Wintz *et al*., 2002; Yruela, 2009; Stanton *et al*., 2022).

Majority of metals in the mosses are accumulated extracellularly rather than being absorbed.

High concentrations of heavy metals usually affect the plants in two ways. On one hand, heavy metals disrupt the uptake of essential minerals with which they compete for absorption. Secondly, after the uptake, heavy metals get accumulated in plant tissues and cells, thereby, affecting the plant metabolism (Woolhouse *et al*., 1983; Turner, 1997). Hence, different bryophyte species have been observed to respond differently to heavy metal-induced stress (Stanković *et al*., 2018).

Heavy metal stress affects numerous physiological and metabolic pathways in plants. Chlorophyll content is frequently evaluated in plants to determine the impact of environmental stress, as changes in pigment content are associated with visible indications of plant injury and photosynthetic output (Parekh, 1992). Carotenoids play a significant photoprotective role in photosynthetic organisms which aids in the survival of organisms.

The present study deals with the effect of heavy metals Iron and Chromium on chlorophyll and carotenoid content of two selected mosses, an acrocarpic. *Mnium cuspidatum* and a pleurocarpic *Thuidium cymbifolium.* Acrocarpic moss *is the* type of moss *in which the archegonia, and hence the capsules, are borne at the tips of stems or branches. Acrocarpous mosses usually show little or no branching and typically grow in erect tufts. Pleurocarpic moss, on the other hand,* is the form of moss wherein archegonia, and thus capsules, are borne on short, lateral branches, rather than at the tips of stems or branches. The larger surface area of pleurocarpic mosses enables them to absorb and accumulate more pollutants than acrocarpic mosses (Gerdol *et al*., 2002).

Iron plays an important role in plant metabolism since it is an integral part of several enzymes and electron carriers (Couturier *et al*., 2013; Rout *et al*., 2015; Kobayashi *et al*., 2018). Chromium, on the other hand, is not usually observed as an essential element in plant growth and metabolism, it is a toxic heavy metal which can negatively affect the plant growth and metabolism (Satyakala *et al*., 1992; Tiwari *et al*., 2009; Rodriguez *et al*., 2012) since it has been observed that plants often acquire components from environment that are not considered essential.

Chromium and Iron belong to borderline group of heavy metals and are capable of binding to either oxygen or nitrogen - sulphur centres. These metals become toxic when found in excess.

Acrocarpic moss *is the* type of moss *in which the archegonia, and hence the capsules, are borne at the tips of stems or branches.*

Materials and Methods

The selected mosses, *Mnium cuspidatum* and *Thuidium cymbifolium* were collected in polythene bags from unpolluted sites of Shimla in Himachal Pradesh. Fresh plant materials were brought to laboratory, washed thoroughly with distilled water to remove soil and any other adherents. 15 g of each moss sample was put in petri dishes. The moss samples were sprayed with different concentrations of Iron and Chromium in ppm (parts per million) and the observations were done for 60 days after an interval of 15 days (15, 30, 45, 60 days). Three replicates were taken of each plant sample. In order to prevent ultrastructural changes or cell damage resulting from dehydration, the plant samples collected had been kept moist by spraying them with distilled water on a regular basis (Ascaso and Galvan, 1976) along with Half Knop's solution in order to maintain them at laboratory conditions. Estimation of Chlorophyll content was done using the method of Arnon (1949). Extraction was done in 80% chilled acetone. 250 mg of fresh plant material was homogenized in dark in cold mortar with pestle. The extract was filtered through Whatman No.1 filter paper under suction using Buchner's funnel under suction. Final volume of the filtrate was made to 25 ml with 80% acetone. The filtrate was then transferred to a conical flask wrapped with black paper to prevent photo-oxidation of the pigments. Absorbance was noted at 663 nm and 645 nm on a double beam spectrophotometer using 80% acetone as a blank.

Carotenoid content was estimated following the method given by Allen and Kirk (1965). Extract was prepared in similar manner as in the case of chlorophyll content. The absorbance was noted at 480 nm using double beam spectrophotometer.

Results were analysed statistically and data were expressed as Mean ± SD. Statistical significance was calculated using Two-way ANOVA followed by Tukey's test for multiple comparisons. $P \leq 0.05$ was considered as significant. GraphPad Prism (ver. 8.0.1) software was used for all analyses.

Results

The photosynthetic activity of mosses *Mnium cuspidatum* and *Thuidium cymbifolium* under normal (control) conditions and under the impact of each of the two selected heavy metals (Iron and Chromium) was evaluated. Under normal conditions, *Thuidium cymbifolium* had higher chlorophyll and carotenoid content than *Mnium cuspidatum*. The regeneration studies were performed to assess the maximum toleration of each heavy metal in both the selected moss species. No regeneration was observed in *Mnium cuspidatum* beyond 1000 ppm concentration of Iron (Fig.1) and 70 ppm concentration of Chromium (Fig.2), respectively. In case of *Thuidium cymbifolium*, regeneration was not observed after 600 ppm of Iron (Fig.3) and 60 ppm of Chromium (Fig.4). Hence, the experiments to assess photosynthetic pigments under the effect of Iron and Chromium were performed within the toleration range of both the mosses.

Fig. 1 : *Mnium cuspidatum* showing regeneration under Iron stress at 1000 ppm.

Fig. 2 : *Mnium cuspidatum* showing regeneration under Chromium stress at 70 ppm.

Fig. 3 : *Thuidium cymbifolium* showing regeneration under Iron stress at 600 ppm.

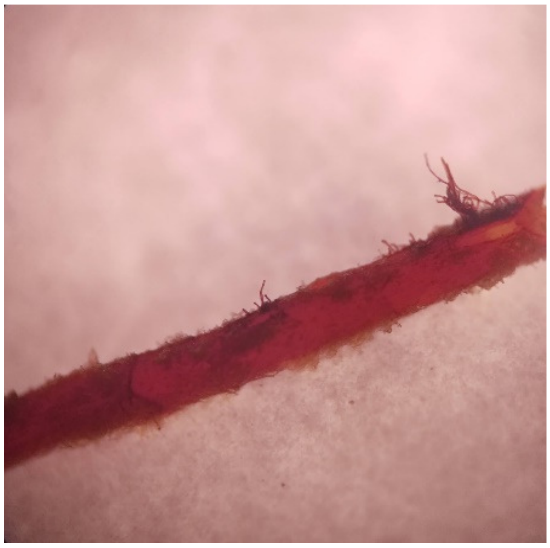

Fig. 4 : *Thuidium cymbifolium* showing regeneration under Chromium stress at 60 ppm.

The plants of *Mnium cuspidatum* were treated with varying concentrations of Iron (100-1000 ppm) over a period of 60 days to observe the changes in the concentrations of chlorophyll-a (Fig.5), chlorophyll-b (Fig.6), total chlorophyll content (Fig.7) and carotenoid content (Fig.8).

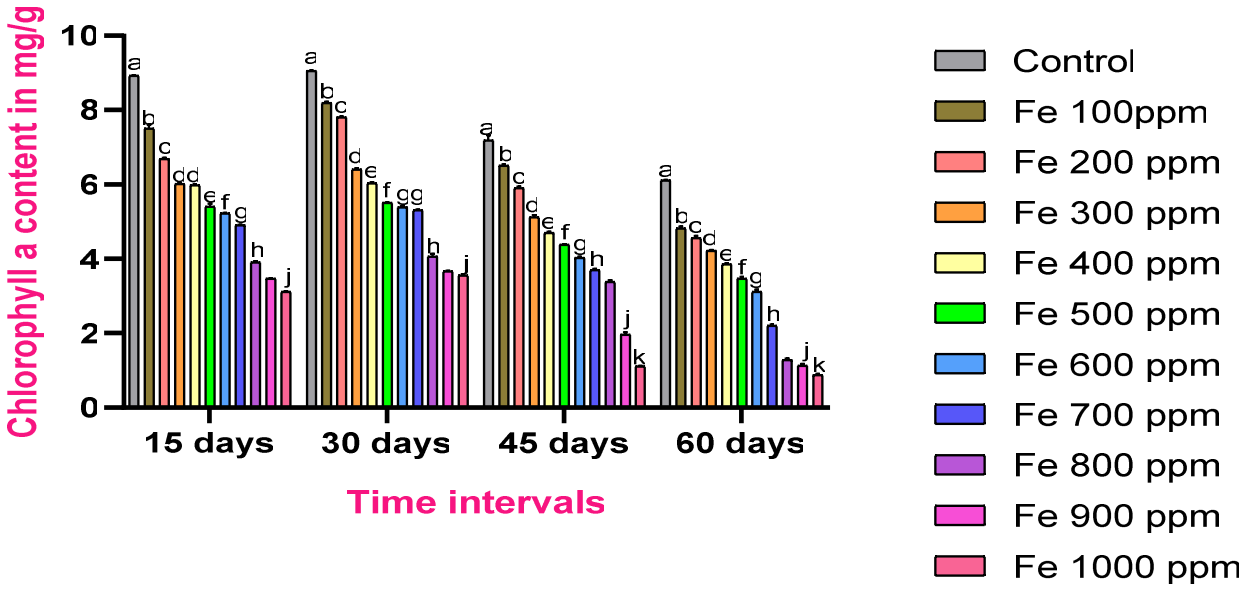

Fig. 5 : Effect of different concentrations of Iron (Fe) on chlorophyll -a content (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by two-way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

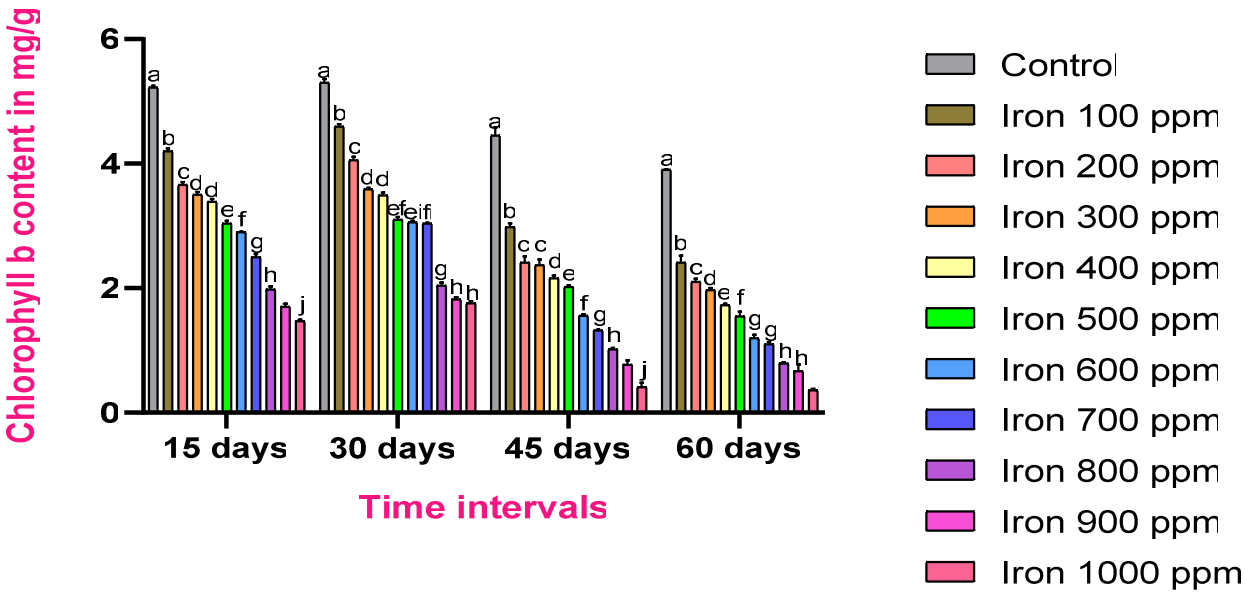

Fig. 6 : Effect of different concentrations of Iron (Fe) on chlorophyll -b content (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by two-way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

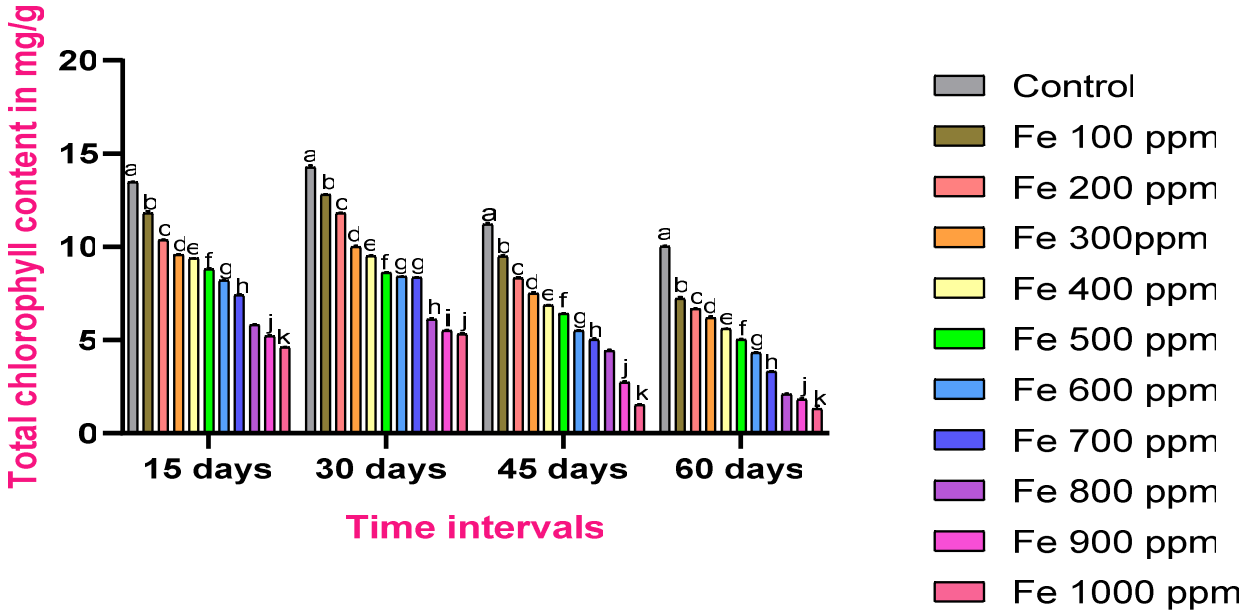

Fig. 7 : Effect of different concentrations of Iron (Fe) on total chlorophyll content (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$)

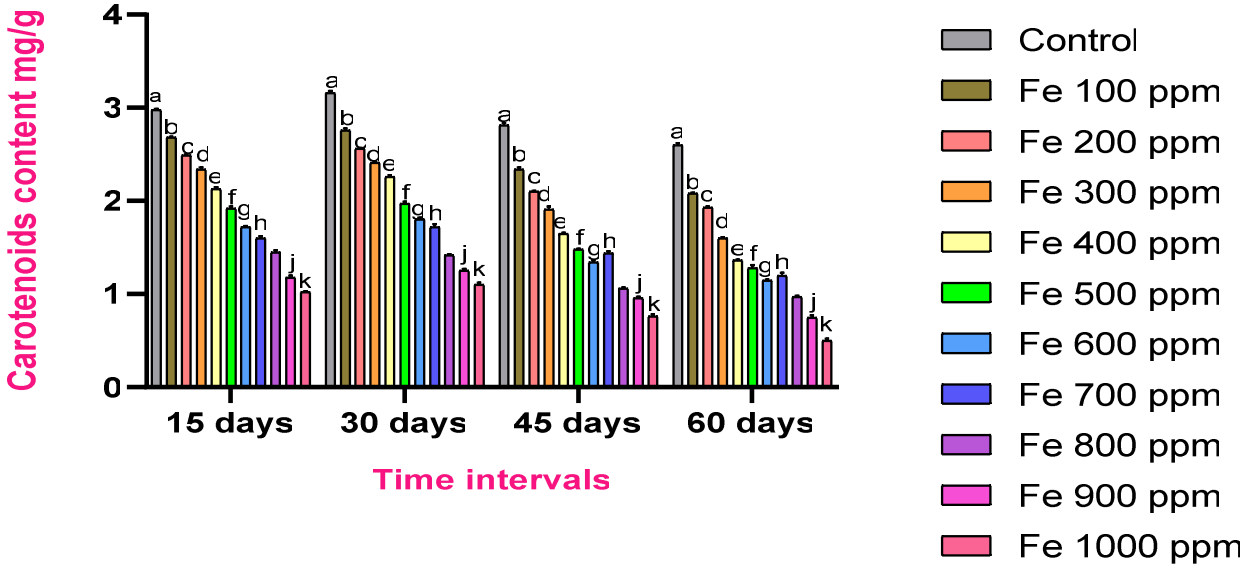

Fig. 8 : Effect of different concentrations of Iron (Fe) on carotenoid content (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by two-way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

Gradual increase in all the pigments was observed up to 30th day in the control as well as the treated samples, after which consistent decline was noticed. The highest chlorophyll-a (8.20±.02mg/g),

chlorophyll-b (4.60±.03mg/g), total chlorophyll content (12.80±.02mg/g) and carotenoid content (2.76±.02mg/g) were observed at the concentration of 100 ppm of Iron on the 30th day.

Variations in the concentrations of chlorophyll-a (Fig.9), chlorophyll-b (Fig.10), total chlorophyll content (Fig.11) and carotenoid content (Fig.12) of *Mnium cuspidatum* were noticed when treated with varying concentrations of Chromium (20-70 ppm) over a period of 60 days.

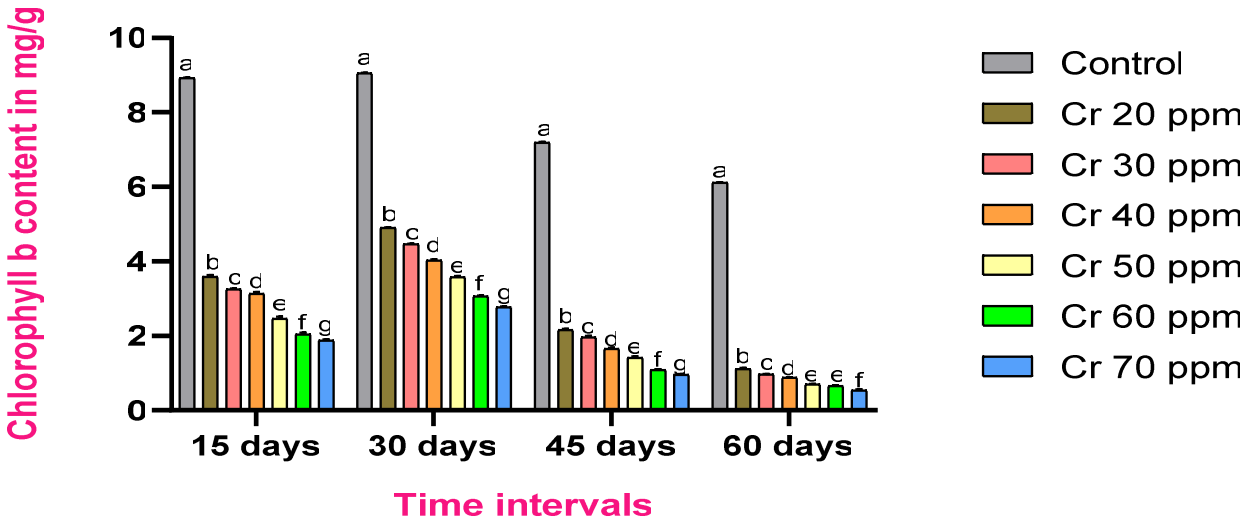

Fig. 9 : Effect of different concentrations of Chromium (Cr) on chlorophyll-a (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

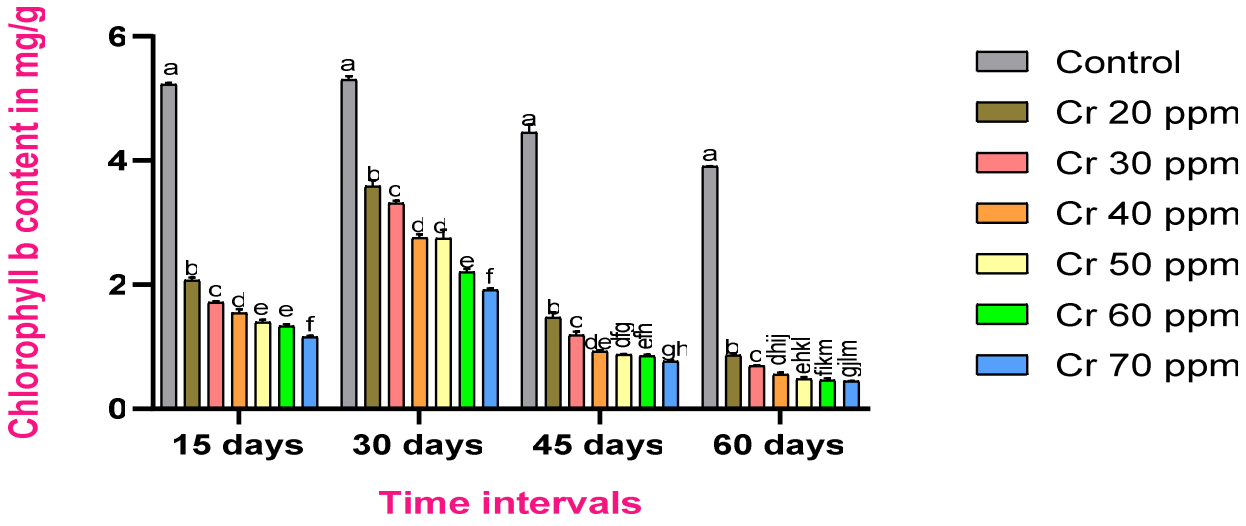

Fig. 10 : Effect of different concentrations of Chromium (Cr) on chlorophyll-b (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

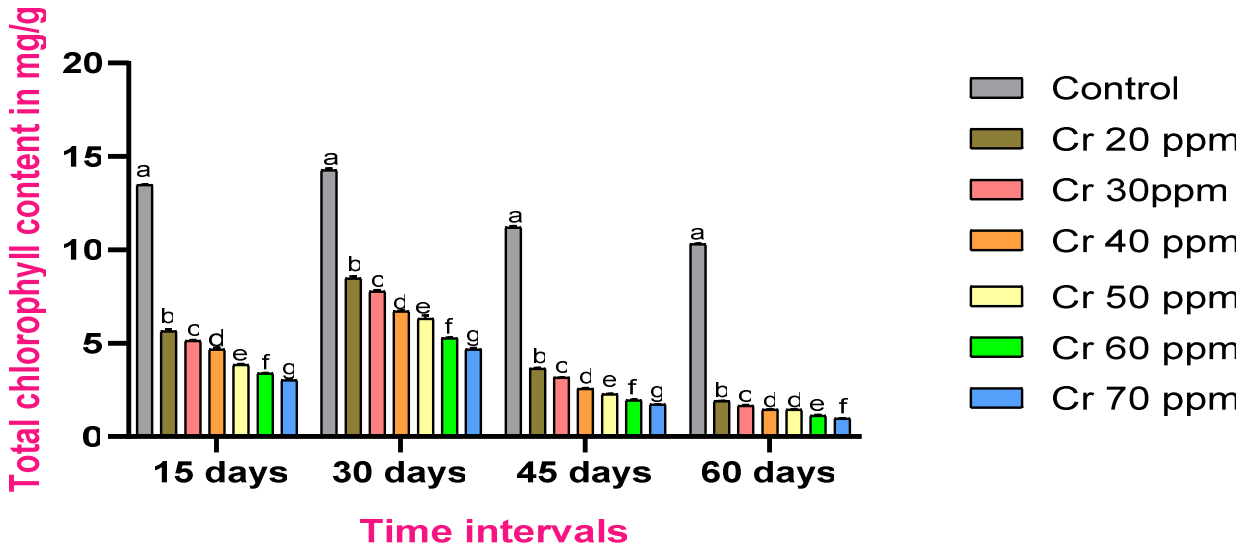

Fig. 11 : Effect of different concentrations of Chromium (Cr) on total chlorophyll (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

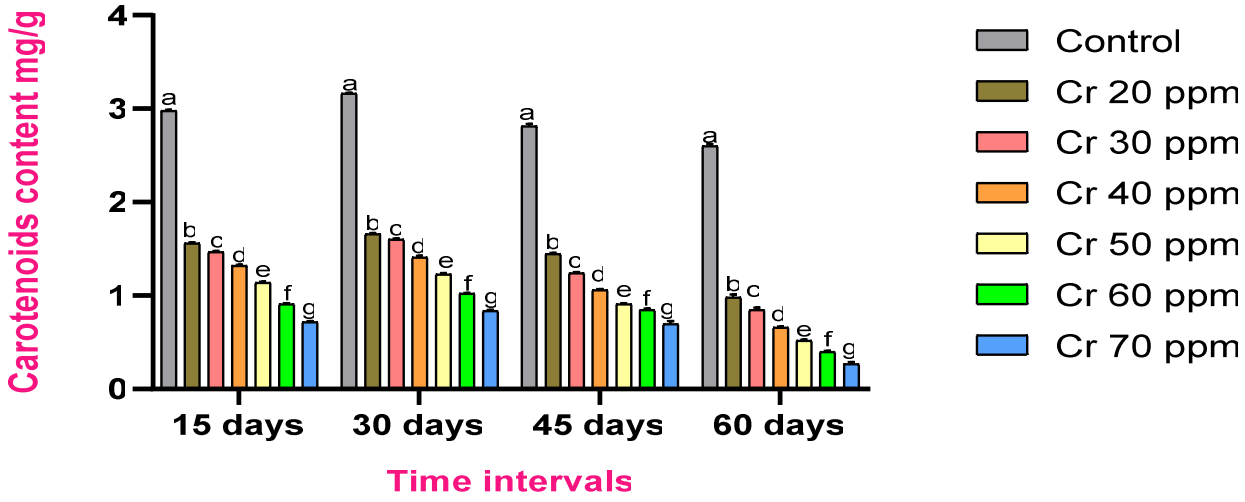

Fig. 12 : Effect of different concentrations of Chromium (Cr) on carotenoid content (mg/g) in *Mnium cuspidatum* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

Gradual increase in all the pigments was observed up to 30th day in all the treated samples, after which gradual decrease was observed. Highest chlorophyll-a (4.90±.01mg/g), chlorophyll-b (3.58±.10mg/g), total chlorophyll content (8.48±.09mg/g) and carotenoid content (1.66±.01mg/g) were observed at the concentration of 20 ppm on the 30th day.

The plants of *Thuidium cymbifolium* were treated with varying concentrations of Iron (100-600 ppm) over a period of 60 days in order to observe how the concentrations of chlorophyll-a (Fig.13), chlorophyll-b (Fig.14), total chlorophyll content (Fig.15) and carotenoid content (Fig.16) changed.

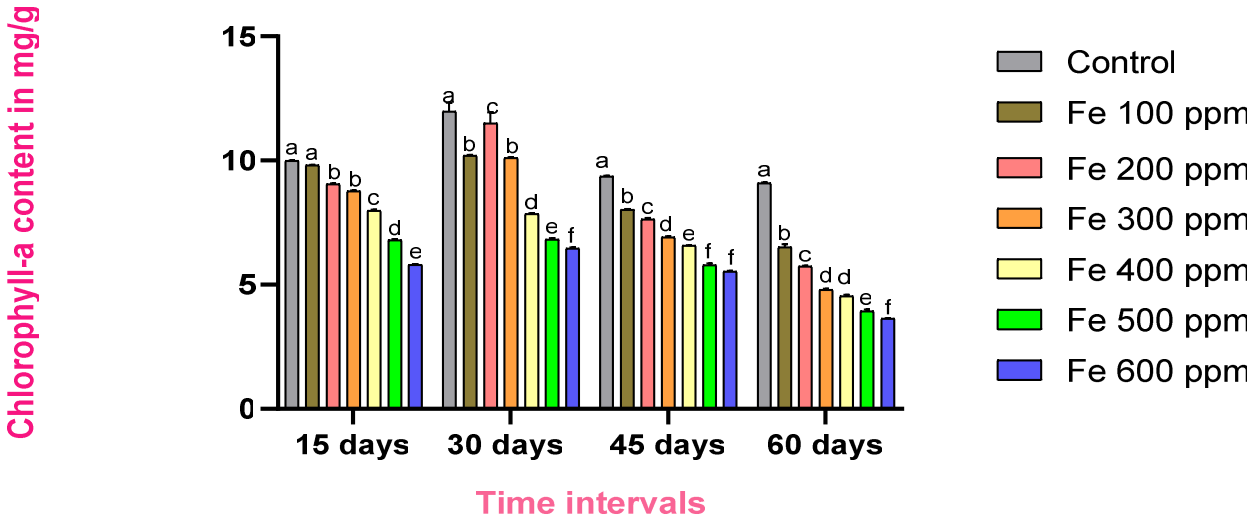

Fig. 13 : Effect of different concentrations of Iron (Fe) on chlorophyll-a (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

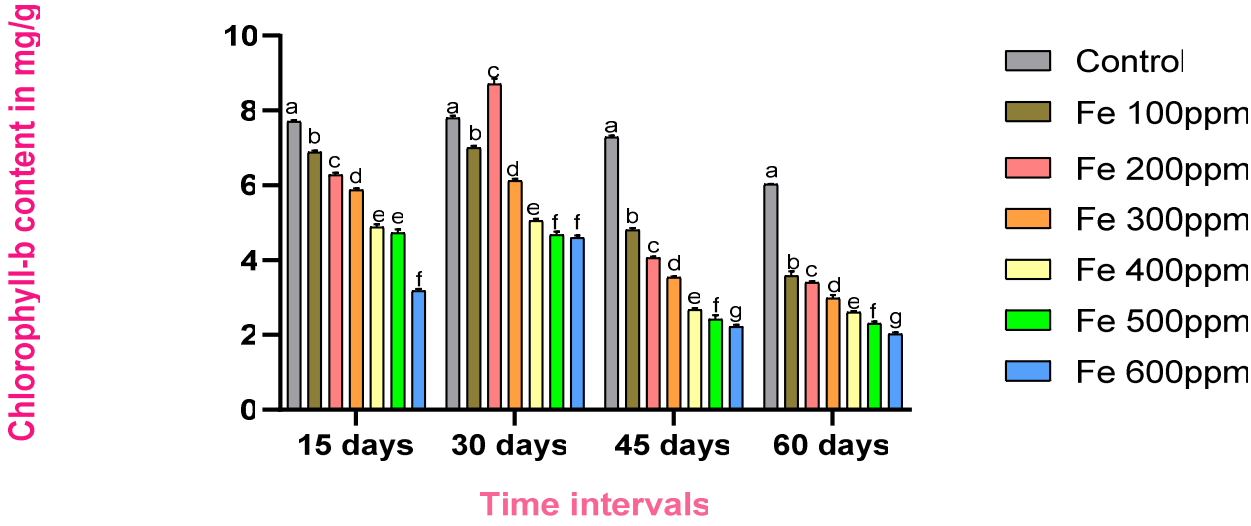

Fig. 14 : Effect of different concentrations of Iron (Fe) on chlorophyll-b (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

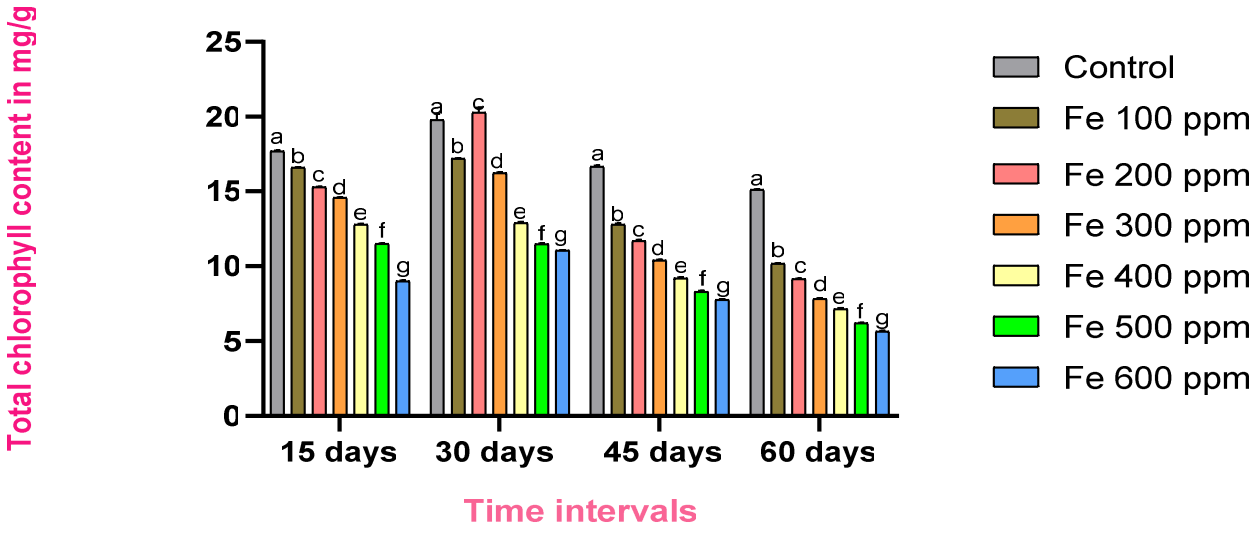

Fig. 15 : Effect of different concentrations of Iron (Fe) on total chlorophyll (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

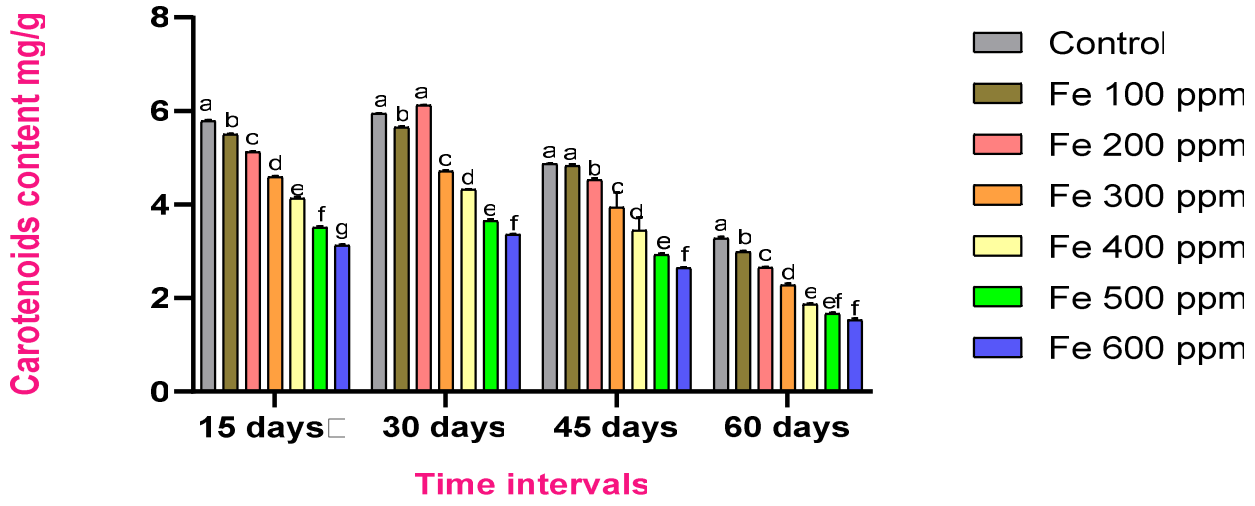

Fig. 16 : Effect of different concentrations of Iron (Fe) on carotenoid content (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Iron (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

The highest chlorophyll-a content (11.50± .45mg/g), chlorophyll-b (8.70±.15mg/g), total chlorophyll content (20.29±.30mg/g) and carotenoid content (6.12±.01mg/g) was observed in the treated sample at the concentration of 200 ppm on the 30th day. After that, gradual decrease in all the mentioned pigments was observed in the control as well as the treated samples throughout the experiment.

Thuidium cymbifolium when treated with varying concentrations of Chromium (20-60 ppm) over a period of 60 days was observed to show notable variations in the concentrations of chlorophyll-a (Fig.17), chlorophyll-b (Fig.18), total chlorophyll content (Fig.19) and carotenoid content (Fig.20).

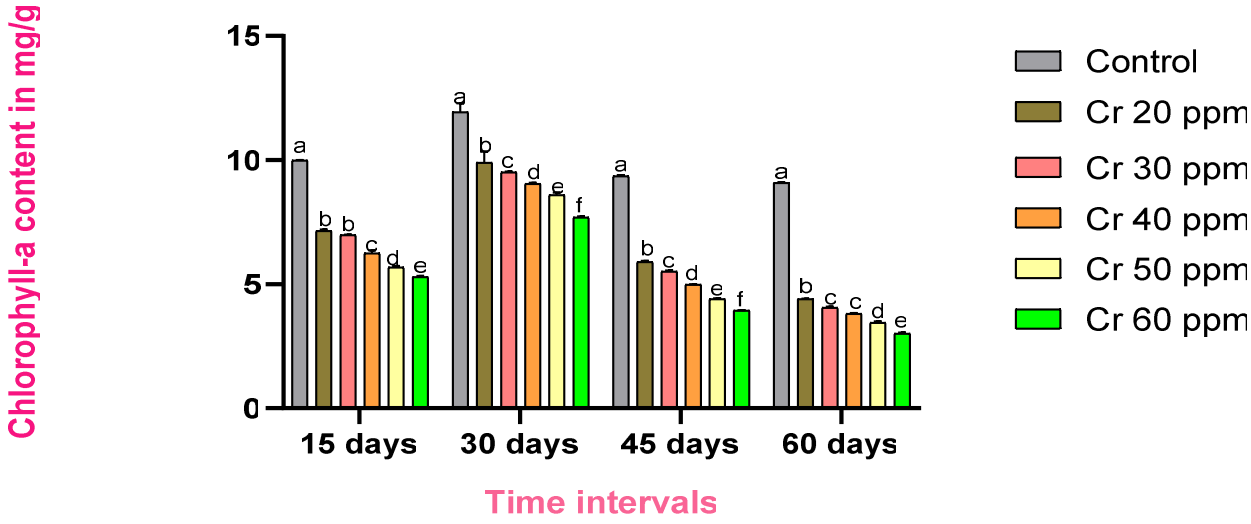

Fig. 17 : Effect of different concentrations of Chromium (Cr) on chlorophyll-a (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

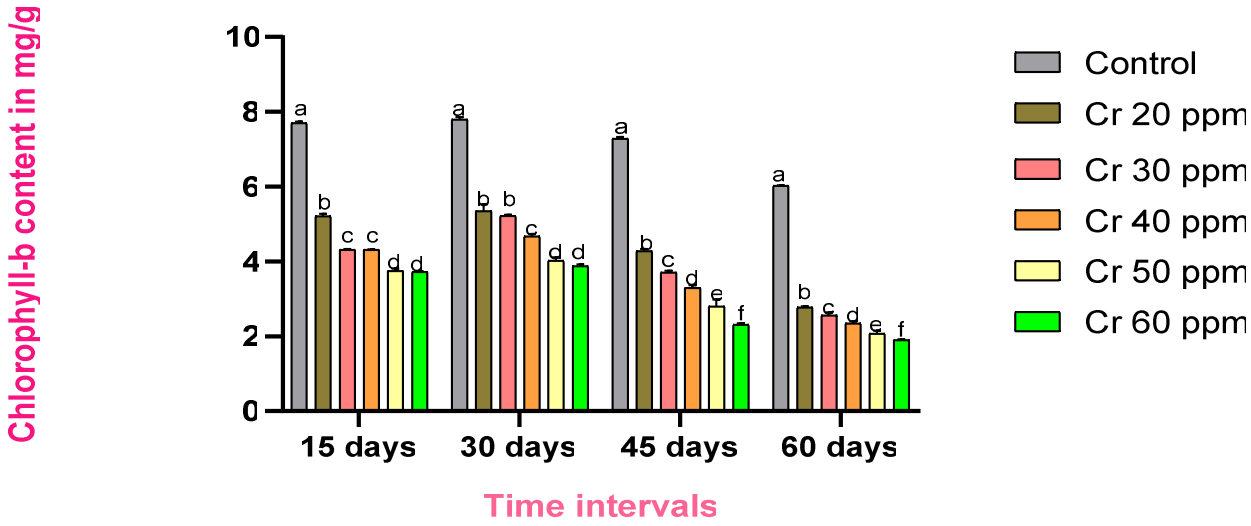

Fig. 18 : Effect of different concentrations of Chromium (Cr) on chlorophyll-b (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

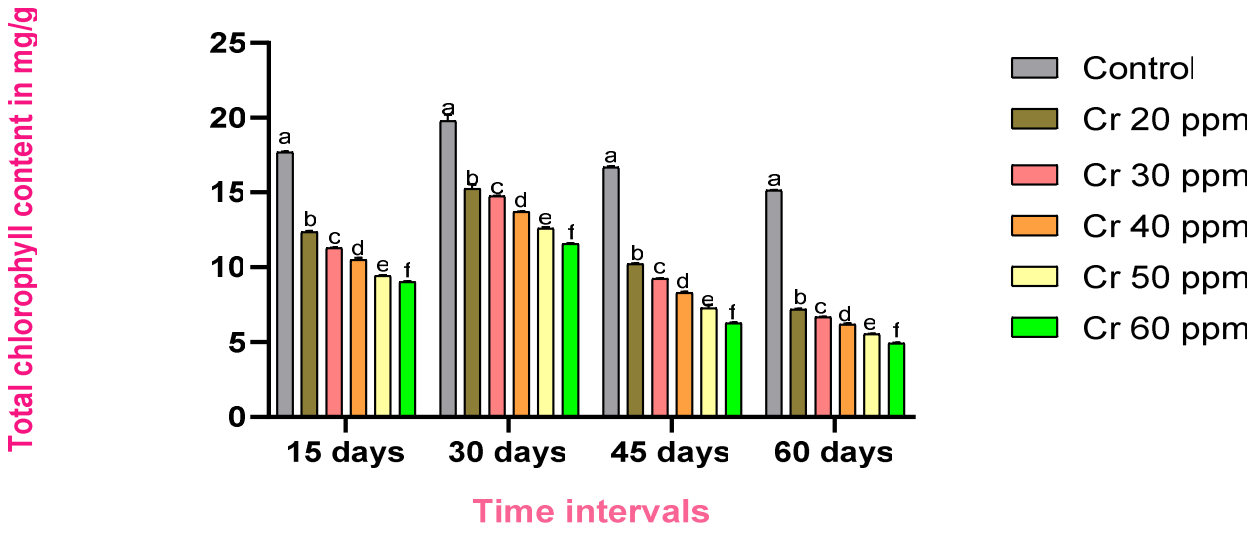

Fig. 19 : Effect of different concentrations of Chromium (Cr) on total chlorophyll (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

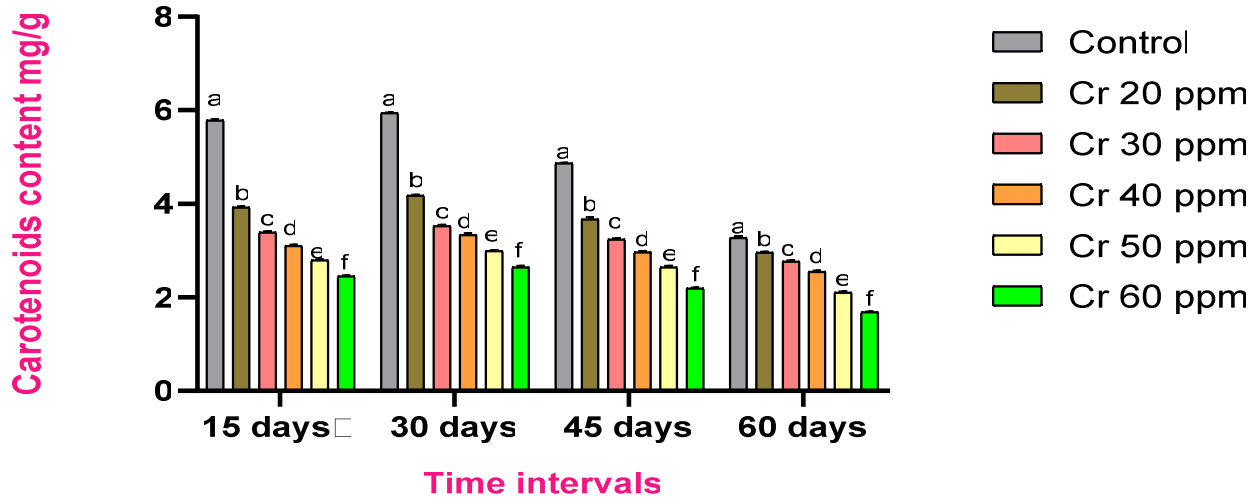

Fig. 20 : Effect of different concentrations of Chromium (Cr) on carotenoid content (mg/g) in *Thuidium cymbifolium* at 15 days intervals. Data is represented as mean ± standard deviation of three replicates. Statistical significance was analysed by one way ANOVA comparing control with different concentrations of Chromium (ppm). Values with different superscripts indicate a significant difference ($p<0.05$).

Gradual increase in all the mentioned contents was observed up to 30th day, after which a decline was noted in the control as well as the treated samples. The highest chlorophyll-a (9.90±.45mg/g), chlorophyll-b (5.35±.19mg/g), total chlorophyll content (15.25 ±.49mg/g) and carotenoid content (4.18±.01mg/g) were noted on the 30th day in the samples treated with 20 ppm concentration of chromium. Under metal treatments, total chlorophyll and carotenoid content decreased, possibly due to suppression of chlorophyll production.

The morphology of mosses was the first parameter to be influenced by the external application of heavy metals as depicted by change of coloration from dark green to light green, followed by yellow and ultimately brown. Significant alterations were observed after 30 days. After 30 days, photosynthetic pigment levels gradually decreased until the experiment ended. Interspecies comparison of *Mnium cuspidatum* and

Thuidium cymbifolium under control conditions during the exposed period, indicated that *Thuidium cymbifolium* has higher chlorophyll-a (Fig.21), chlorophyll-b (Fig. 22), total chlorophyll content (Fig. 23) and carotenoid content (Fig.24).

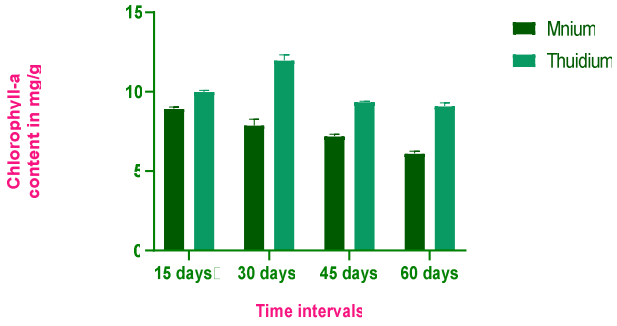

Fig. 21: Chlorophyll-a content of *Mnium cuspidatum* and *Thuidium cymbifolium* under control conditions

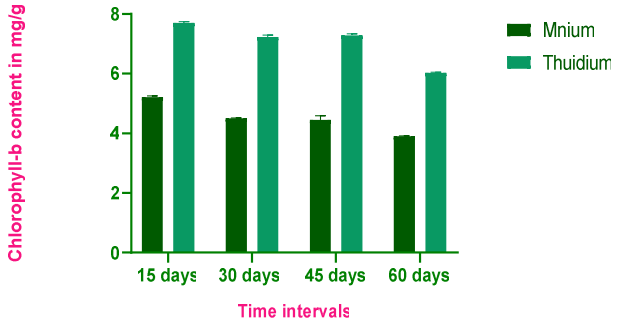

Fig. 22: Chlorophyll-b content of *Mnium cuspidatum* and *Thuidium cymbifolium* under control conditions

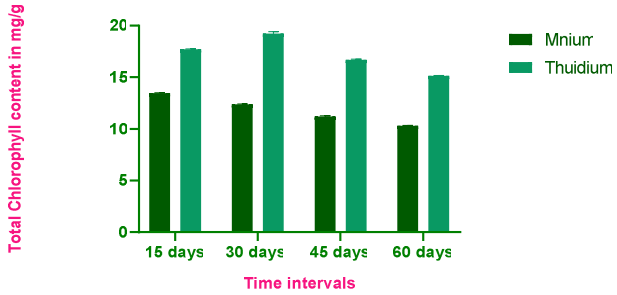

Fig. 23 : Total Chlorophyll content of *Mnium cuspidatum* and *Thuidium cymbifolium* under control conditions

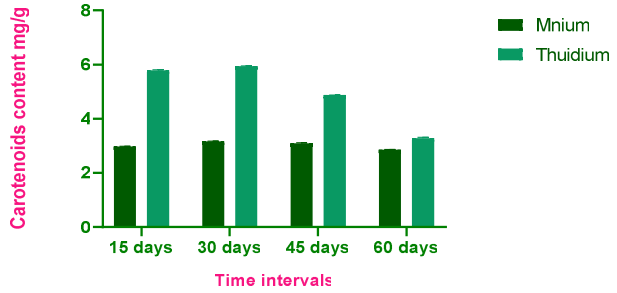

Fig. 24 : Carotenoid content of *Mnium cuspidatum* and *Thuidium cymbifolium* under control conditions

Treatment of lower concentrations of Iron slightly enhanced the chlorophyll content in the selected moss species, thereby indicating the importance of Iron in plant metabolism as a micronutrient. However, as we increase the concentration of Iron, it becomes phytotoxic, as a decrease in chlorophyll content is observed.

On the other hand, chromium has proven phytotoxic even at very low concentrations since it is not known to have any biological role in plants.

Discussion

The investigations revealed declines in levels of photosynthetic pigments chlorophyll-a, chlorophyll-b, total chlorophyll content and carotenoids in both the selected moss species after exposure to heavy metals.

In order to efficiently absorb metals and other nutrients from their surroundings, mosses have developed a highly effective uptake mechanism. However, the majority of the metals in mosses are accumulated extracellularly rather than being absorbed and found inside the cells, making them efficient bioaccumulators. Plants irrigated with heavy metal solutions changed colour from bright green to light green and ultimately brown as also reported by Fatoba (2008).

Bhushan *et al.* (2014) found a negative connection between chlorophyll content and higher heavy metal concentrations, which is consistent with the current findings. The impact of metal accumulation on the chlorophyll content of other two mosses *Thuidium deliculatulum* and *T. sparsifolium* as well as a leafy liverwort, *Ptychanthus striatus* was studied by Shakya *et al.* (2008) where copper was found to have notable inhibitory effect on chlorophyll-a, chlorophyll-b and total chlorophyll content. A significant decrease in chlorophyll and carotenoid content of *Barbula lambarenensis* was observed by Fatoba (2008) with rise in concentrations of heavy metals (lead, copper, cadmium, iron and vanadium) over time. Similar pattern of decline of photosynthetic pigments in *Brassica juncea* was observed by John *et al.* (2009) under the influence of cadmium and lead. The photosynthetic pigments considerably declined in Hemp plant under Cadmium stress as reported by Shi *et al.* (2011).

The present investigation demonstrates that the two moss species, although phylogenetically distinct (representing separate families), showed comparable trends in chlorophyll content under exposure to the studied heavy metals. It is evident that elevated concentrations of Iron and Chromium induce a significant decline in chlorophyll content, indicating

metal-induced pigment degradation. After 30 days, a progressive reduction in the analyzed photosynthetic pigments was recorded till the end of the experiment. A comparable trend was noted in the control samples treated with half-Knop's solution. Iron treated samples demonstrated greater chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content than Chromium treated samples, attributable to Chromium's non-essential role in plant physiology.

The pleurocarpic moss, *Thuidium cymbifolium* presently efficiently accumulated heavy metals due to its large surface area and porous structure, making it reliable indicators of pollution over time. *Fabriona ciliaris* and *Leskea angustata* collected from various sites in MATV, Mexico, were evaluated for atmospheric deposition of heavy metals (Zn, Pb, Cr, Cd) and their accumulation in the moss tissues by Macedo - Miranda *et al*. (2016). *Pleurozium schreberi* has been used as a bioindicator to assess the levels of Cd, Cr, Cu, Fe, Ni, and Pb in urban regions of Russia (Yushin *et al*., 2020). Lazo *et al.* (2022) used *Hypnum cupressiforme* as a biomonitor to assess atmospheric metal deposition across Albania and identified anthropogenic factors influencing the levels of toxic metals/metalloids (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, and Zn).

The acrocarpic mosses like presently studied *Mnium cuspidatum* have also been reported as efficient biomonitors. Basile *et al*. (1995) investigated the effects of lead and colchicine on the protonemal development of *Funaria hygrometrica*, noting that the moss can absorb heavy metal cations at concentrations much higher than those in the surrounding substrate. Aceto *et al*. (2003) used the moss *Bryum argenteum* as an environmental indicator, collecting it from polluted to unpolluted mountain areas. They examined nearly 20 elements, including major (K, P, Al, Ca, Fe, Mg), minor (Mn, Na, Ti, Zn), and trace (As, Ba, Cd, Co, Cr, Cu, Li, Pb, Sr) elements. Olumayede and Ojiodu (2017) studied heavy metal concentrations (Zn, Pb, Cd, Ni, and Cu) in *Polytrichum commune* from various microenvironments at Yaba College of Technology, Nigeria, and found that some metals exceeded WHO threshold limits. They assessed the potential of using the moss as an indicator of atmospheric metal pollution and air quality.

Iron is essential for several physiological and biochemical processes in plants. It is a key component of enzymes like cytochromes in the electron transport chain and is involved in the synthesis of chlorophyll as well. But beyond toleration limit, Iron toxicity has been reported to inhibit photosynthesis along with photoinhibition in *Nicotiana plumbaginifolia* (Kampfenkel *et al*. 1995). Iron toxicity in tobacco, canola, soybean, and *Hydrilla verticilata* is associated with reduced plant photosynthesis and yield, along with an increase in oxidative stress and ascorbate proxidase activity (Sinha *et al*., 1997). Although, Iron plays a crucial role in plant cellular activities, including photosynthesis and respiration, despite this, it becomes toxic at higher concentrations (Crichton *et al*., 2002).

Chromium stress is one of the key factors affecting photosynthesis, in terms of CO_2 fixation, electron transport, photophosphorylation, and enzyme activity (Vazques *et al*., 1987). Chromium toxicity has been reported to impair the chlorophyll biosynthesis, thereby, decreasing the chlorophyll content in *Nelumbo nucifera* and *Nymphaea alba* (Vajpayee *et al*., 1999,2000). It has been reported that in peas, Chromium has a more substantial effect on photosystem1 than on photosystem 2 in isolated chloroplasts (Bishnoi *et al.,*1993a, b).

ALAD is an important enzyme in the biosynthesis of chlorophyll as it catalyzes the condensation of two molecules of 5-aminolevulinic acid (ALA) to produce porphobilinogen, a precursor of chlorophyll (Tripathi *et al*., 2011). Chromium has been reported to hamper the ALAD activity which ultimately leads to reduction of chlorophyll biosynthesis (Vajpayee *et al*., 2000).

Conclusion

The present findings suggest that pleurocarpic *Thuidium cymbifolium* is more tolerant as compared to acrocarpic *Mnium cuspidatum.* Both types of mosses have been utilized in biomonitoring, but research typically indicates that pleurocarpic mosses are more appropriate for long-term assessment of overall pollutant accumulation, whereas acrocarpic mosses are effective in detecting short-term fluctuations in pollution, especially concerning growth tips and reproduction. Also, Iron toxicity was found relatively low as compared to Chromium toxicity in both the studied mosses. The knowledge of implications of heavy metals on various physiological parameters of mosses can be of great assistance in ecophysiology.

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