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EFFECT OF ZINC AND CADMIUM ON CHLOROPHYLL CONTENT OF MOSSES *BRACHYTHECIUM RUTABULUM* AND *MNIUM CUSPIDATUM*

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Abstract: **Bryophytes, presently survived in three lineages, i.e., mosses, liverworts and hornworts, are an ancient group of early land plants. Its unique features like lack of roots system, unistratose leaves, ion-exchange capacity and mode of nutrients uptake make it a distinctive plant group. Since plants of this group, particularly mosses, possess remarkable ability to absorb heavy metals from the atmosphere, they play a significant role as a sensitive indicator of environmental pollution as evidenced by changes in death rate, apparent injury, chlorophyll reduction and cell size reduction in leaf. The present investigation was carried out to study effect of various phytotoxic concentrations of metals - Zinc and Cadmium (for varying length of time) on the photosynthetic activity of mosses *Brachythecium rutabulum* and *Mnium cuspidatum*. It has been observed that exogenously supplied Zinc and Cadmium significantly stressed the oxidative enzymes as well as the photosynthetic pigments of *Brachythecium rutabulum* and *Mnium cuspidatum*. The chlorophyll content was studied in two above named species of mosses collected from different localities of Shimla (Himachal Pradesh). It has been found that the total chlorophyll content is more in *Brachythecium rutabulum* than in *Mnium cuspidatum* when treated with zinc in comparison with cadmium. Chlorophyll-a and Chlorophyll-b were maximum in *Brachythecium rutabulum* and minimum in *Mnium cuspidatum* when treated with Zinc and Cadmium separately.**

***Keywords: Brachythecium rutabulum*, *Mnium cuspidatum*, Zinc, Cadmium and Heavy metals.**

1. INTRODUCTION

The green pigment chlorophyll, present in higher plants, green algae and even in bryophytes play a vital role in photosynthesis. Chlorophyll molecules exhibited in and around photosystem immersed in the thylakoid membrane of the chloroplast help plants in the absorption of energy from light to drive photosynthesis.

When bryophytes were irrigated with heavy metals, significant changes have been seen in chlorophyll-a, chlorophyll-b and even in total chlorophyll content. Bryophytes are green land plants which are simple both morphologically and anatomically and of paraphyletic origin. They appear to be more sensitive to water pollution than air pollution and exhibit visible injury manifestations even in the presence of negligible quantities of pollutants. They are among the only land plants which can accumulate heavy metals and even rare earth metals to a high level. Experimentally, it was observed that older tissues of the plants could concentrate more metallic ions as compared to younger tissues. Their capabilities to grow on substrates or area which are inhospitable to higher plants exclude them from intense competition which confers an added advantage to serve as good bio-indicators. Such bryophytic species act as good bio-indicators and also serve as a "warning signals" concerning the effect of soluble heavy metals in environment.

There are three types of bioindicators, namely;

a. **True indicators:** pollutants damage the plants proportionally to the concentration of the pollutants.

b. **Accumulators:** accumulate potentially toxic pollutants.

c. **Scales of indicators species:** pollutants leads to disappearance or appearance of certain species.

Bryophytes, especially mosses are an important lineage of bryophytes with unique life history, poikilohydrous habit, antimicrobial properties, potential for soil management and as an indicator for environmental pollution, are an essential component for our ecosystem. Mosses are ubiquitous as they are provisioned with some morphological and also structural adaptive strategies, enabling them to grow successfully where they occur. Interestingly these bryophytes, especially mosses lock up heavy metals which are locked in their skeletal system (unlike any other plant group). The propensity of mosses to concentrate these heavy metals depends mainly upon the total leaf surface and thin-walled parenchymatous tissues.

Expansion of industrialization and the absence of environmental regulation have led to a rise in various toxic pollutants in the environment. This has brought to focus the urgent need for continuous monitor of air quality check either through instruments or by employing bioindicators/ biomonitors. Mosses and lichens are considered as most suitable plant material for atmospheric and experimental deposition of heavy metals because of a short life span, easy availability and lack of true root system and can be grown easily even in the laboratory for the experimental purpose (Uniyal 2010).

2. MATERIALS AND METHODS

Brachythecium rutabulum is a dioicous plant. The plant is glossy yellowish to golden green in loose, straggling patches.

Upper leaves of *Mnium cuspidatum* are more crowded while lower leaves are smaller and placed wide apart.

These plant species do not easily desiccate in adverse conditions and has ethnic medicinal value that is why these mosses are selected for experimental purpose.

Fresh moss species were collected in plastic polythene bags from different areas of Shimla (himachal pardesh). The collected plants material was cleaned by removing their soil and other materials attached to it. It was stored in Petri-plates under specific room temperature. Various phytotoxic concentrations of metals (10ppm, 20ppm, 40ppm, 60ppm, 80ppm, 100ppm, 150ppm, 200ppm, 350ppm, and 500ppm) Zn and Cd were provided to collected plant materials till 75 days.

The chlorophyll estimation was done by using the method of Arnon(1949). The fresh plant samples from petri plates were washed with distilled water and dried with blotting paper. 250mg fresh plant materials were homogenized in 10 ml of 80% acetone in cold pestle and mortar in the dark. A pinch of Magnesium carbonate was added to neutralize the acids released during extraction. The extract was filtered through Whatman No.1 filter paper using Buchner's funnel under suction. The final volume of filtrate was made to 25ml with 80% acetone. Approximate absorption of chlorophyll-a is at 663nm while chlorophyll-b has approximate absorbance at 645nm. Absorbance was read at 663nm and 645nm wavelengths on a double-beamed spectrophotometer using 80% acetone as a blank.

Statistical analysis:

Statistical analysis for chlorophyll parameters was performed with program Graph pad Prism version 5 using one way ANNOVA ($p<0.05$) for multiple comparisons with overall significance level of 0.05. Based on ANNOVA results, tukey's test for main comparison at 95% confidential intervals was applied. All experimental were carried in three independent trials and in triplicates. Values are represented as mean ± standard deviation.

3. RESULTS

Photosynthetic activity of mosses *Brachythecium rutabulum* and *Mnium cuspidatum* had been evaluated. When these moss species were sprayed regularly with different concentrations of heavy metals Cd and Zn, apparent changes were seen in the color of the mosses from green to light green, to yellow and ultimately turning brown. It was observed that heavy metals profoundly affect the chloroplast of these two species. Transformation of pigment was observed in both the species after 15 days, but prominent changes were seen after 30 days. Distinctive changes were observed in regimes sprayed with 200, 350, 500ppm (part per million).

The results obtained from present findings are depicted in figures 1 to 12, show different time intervals effect on chlorophyll-a, chlorophyll-b as well as on total chlorophyll content of *Mnium cuspidatum* and *Bracythecium rutabulum* when treated with different concentration of Zn and Cd. It is observed that samples irrigated with lower ppm concentrations i.e. 10, 20, 40 and 60 contained more chlorophyll content than when treated with 350, 500 ppm. It was evident from the study that in the first fifteen days there was degradation of chl-a, chl-b as well as total chlorophyll content. However, from 15-30 days a rapid increase was observed. After 30 days there was a progressive decrease in studied photosynthetic pigment till the end of the experiment. Similar pattern was observed in the control samples which were irrigated with distilled water.

Figures 1 to 3 show the value of chlorophyll concentrations of *Mnium cuspidatum* when treated with different concentrations of Zn in ppm after different time intervals. Control sample contains more chlorophyll contents then treated samples. Total chlorophyll content, chl-a and chl-b show significant changes under Zn stress as compared to control. Contradictorily, chl-a concentration was significantly higher than chl-b and total chlorophyll content.

Figures 4 to 6 show the value of concentration of chlorophyll contents of *Brachythecium rutabulum* when exposed to Zn stress. It was found that lower concentrations of Zn in ppm (10, 20, 40 and 60) show higher chl-a then control upto 30 days. Rapid degradation was seen after 30 days, upto the end of the experiment. When treated with Zn upto 200ppm, the total chlorophyll content and chl-b was higher than in the control till 15 days.

Figures 7 to 12 show the results of both *Mnium cuspidatum* and *Bracythecium rutabulum* when subjected to Cd stress. Higher accumulation of Cd was seen in the mosses for upto 75 days, but significant decrease in chlorophyll contents was reported in samples. At the same time control samples contained more chlorophyll content in both the species in comparison to the treated samples.

Exposure to lower concentration of Zn slightly increased the chlorophyll content and also the rate of photosynthesis. No marked effect has been analyzed when subjected with medium concentrations of Zn, while higher concentrations decreased the green pigment and also the chlorophyll content. Moreover, samples treated with Zn show the higher value of chl-a, chl-b and even total chlorophyll content than sample analyzed with Cd, because Cd has no biological function and even when present at lower concentration are extremely toxic and are easily accumulated by mosses (Milone *et al*. 2003).

Morphologically bryophytes are separated into two groups; Acrocarpous and Pleurocarpous mosses. Pleurocarpous mosses grow parallel to substratum while acrocarpous mosses usually unbranched and typically grow in erect tufts. It was analyzed during experiment that *Brachythecium rutabulum* which is pleurocarpous moss have more ability to absorb heavy metals than acrocarpous moss i.e, *Mnium cuspidatum*. Interspecies comparisons between *Brachythecium rutabulum* and *Mnium cuspidatum* are displayed in figures A, B and C.

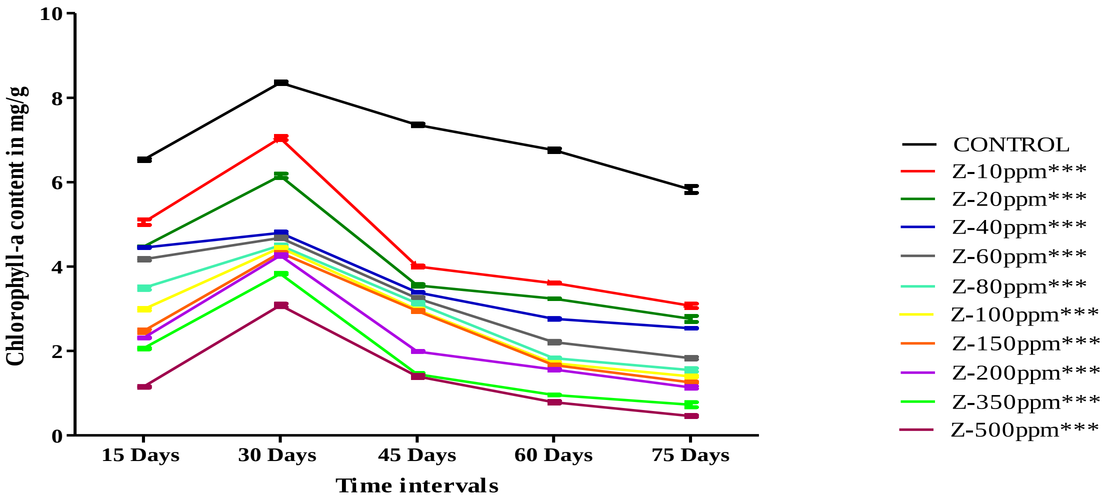

Figure 1 – Effect of different concentrations of Zinc (Z) on chlorophyll-a content (mg/g) in *Mnium cuspidatum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing control with different concentrations of Zinc (ppm), where $R^2=0.957$ and highly significant correlation ($P < 0.0001$) being indicated by (***) .

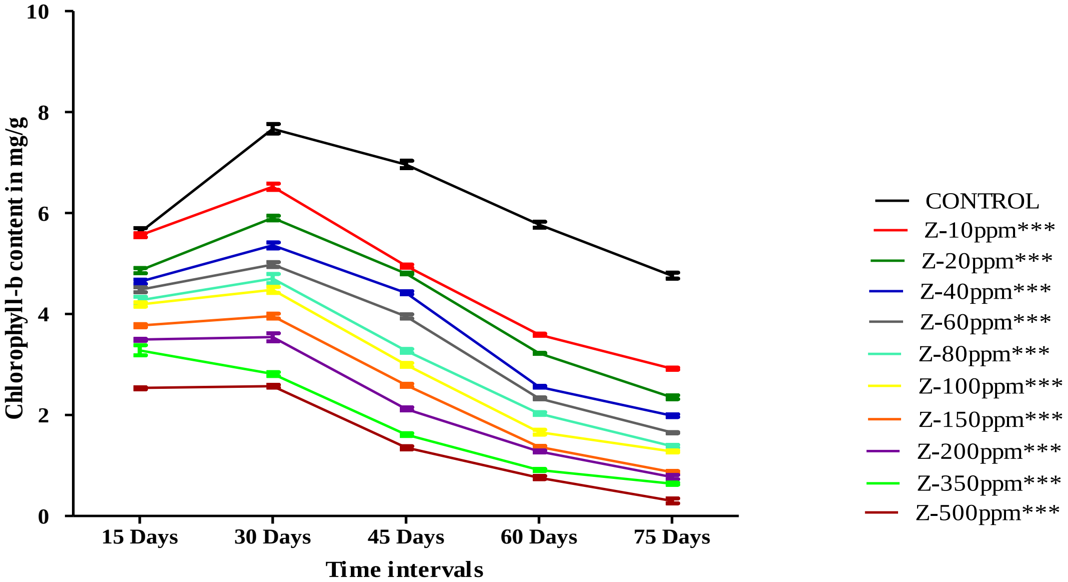

Figure 2 – Effect of different concentrations of Zinc (Z) on chlorophyll-b content (mg/g) in *Mnium cuspidatum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing control with different concentrations of Zinc (ppm), where R^2=0.939 and highly significant $P< 0.0001$ being indicated by (***).

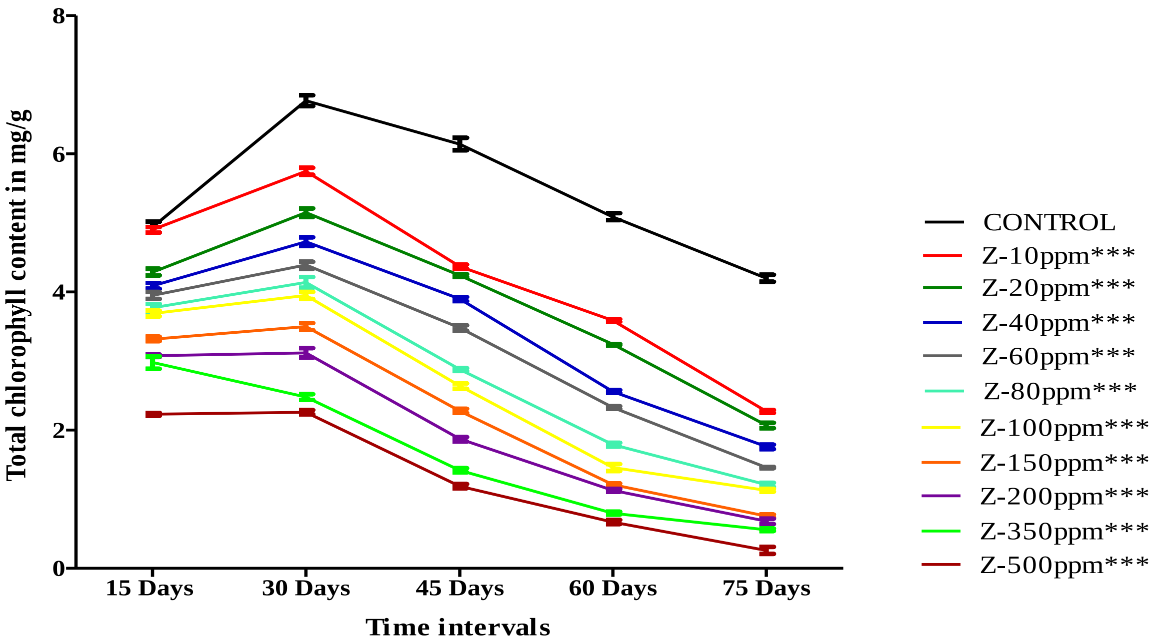

Figure 3 – Effect of different concentrations of Zinc (Z) on total chlorophyll content (mg/g) in *Mnium cuspidatum* at different time intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing control with different concentrations of Zinc (ppm), where R^2=0.937 and highly significant $P< 0.0001$ being indicated by (***) .

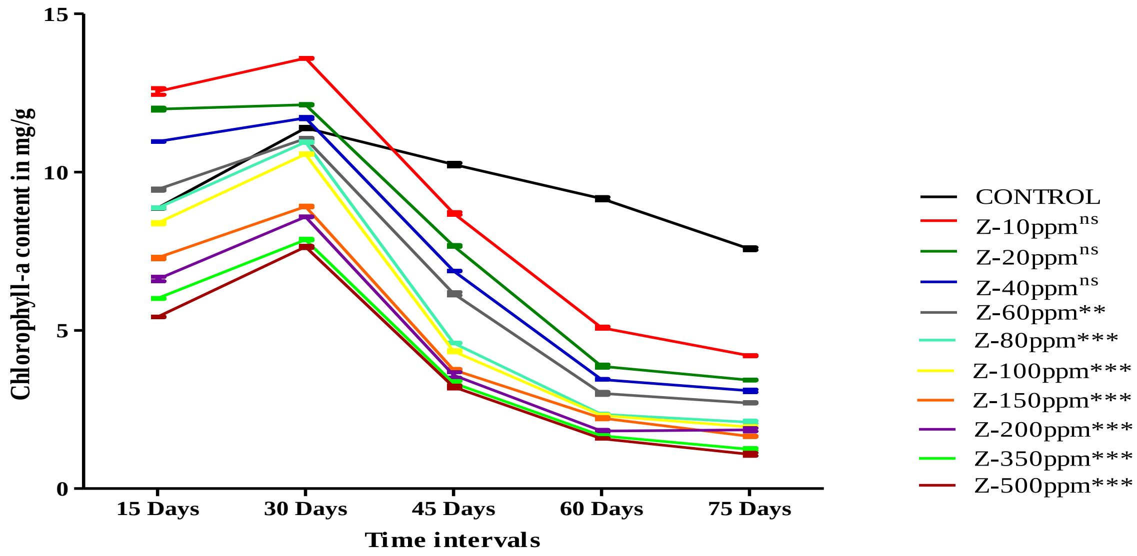

Figure 4– Effect of different concentrations of Zinc (Z) on chlorophyll -a content (mg/g) in *Brachythecium rutabulum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing control with different concentrations of Zinc (ppm), where R^2=0.801 and highly significant correlation P< 0.0001 being indicated by (***) , P< 0.001 being indicated by (**) while ns for not significant.

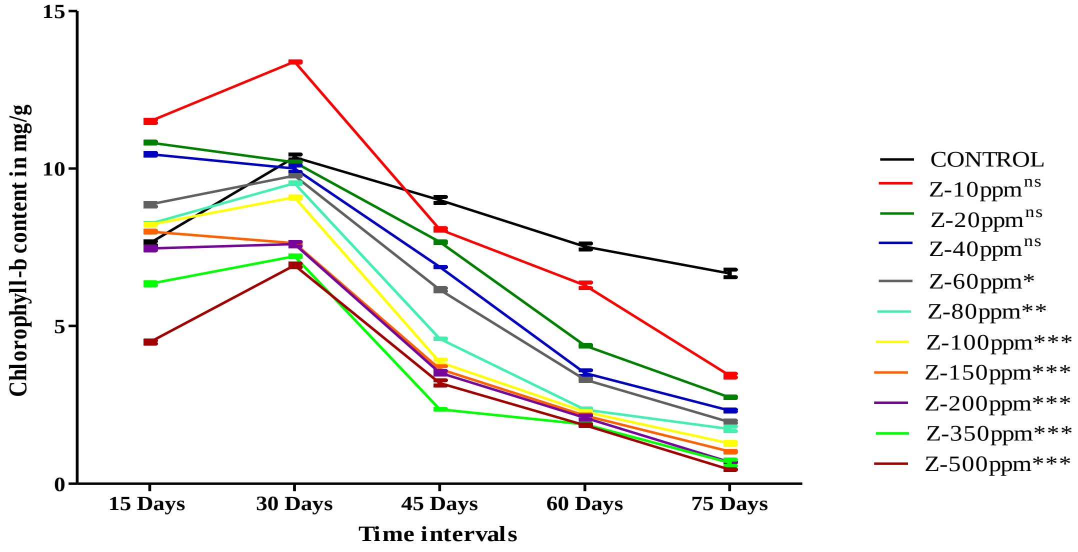

Figure 5– Effect of different concentrations of Zinc (Z) on chlorophyll -b content (mg/g) in *Brachythecium rutabulum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing control with different concentrations of Zinc (ppm), where R^2=0.794 and highly significant correlation P< 0.0001 being indicated by (***), P< 0.001 being indicated by (**), P<0.05 indicated by (*) while ns for not significant.

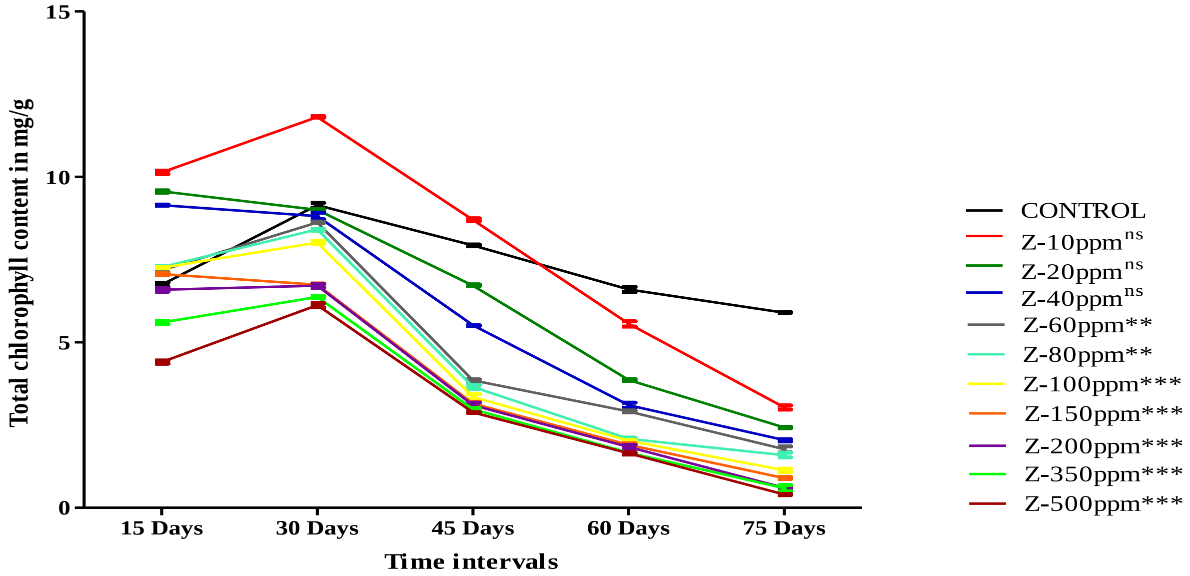

Figure 6– Effect of different concentrations of Zinc (Z) on total chlorophyll content (mg/g) in *Brachythecium rutabulum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing control with different concentratio ns of Zinc (ppm), where R^2=0.798 and highly significant correlation P< 0.0001 being indicated by (***), P< 0.001 being indicated by (**), P<0.05 indicated by (*) while ns for no t significant.

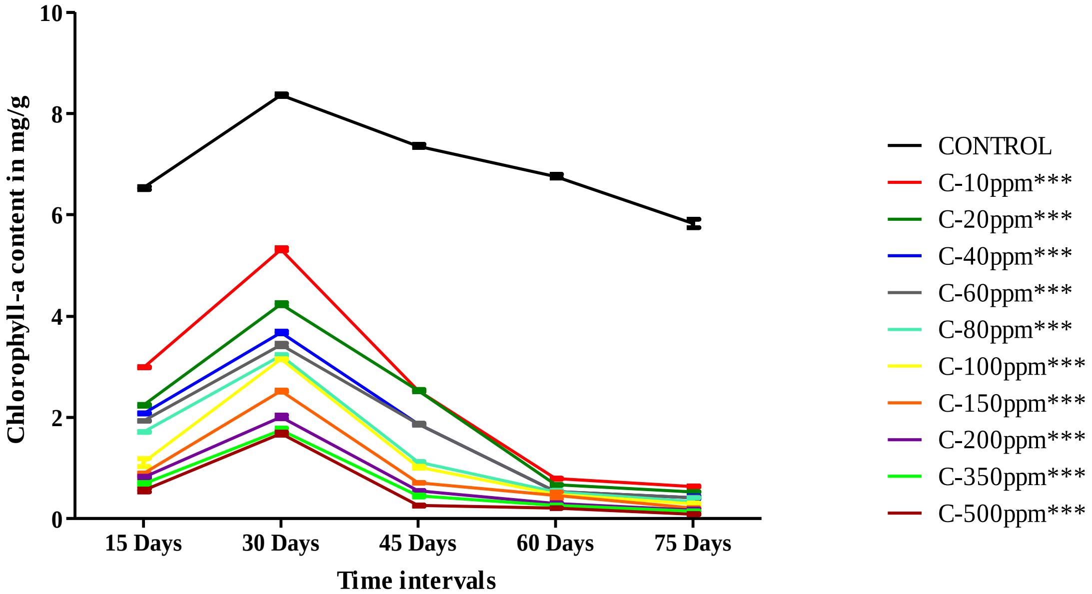

Figure 7- Effect of different concentrations of Cadmium (C) on chlorophyll -a content in *Mnium cuspidatum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing with different concentrations of Cadmium (ppm) where R^2=0.951 and highly significant p<0.0001 b eing indicated by (***).

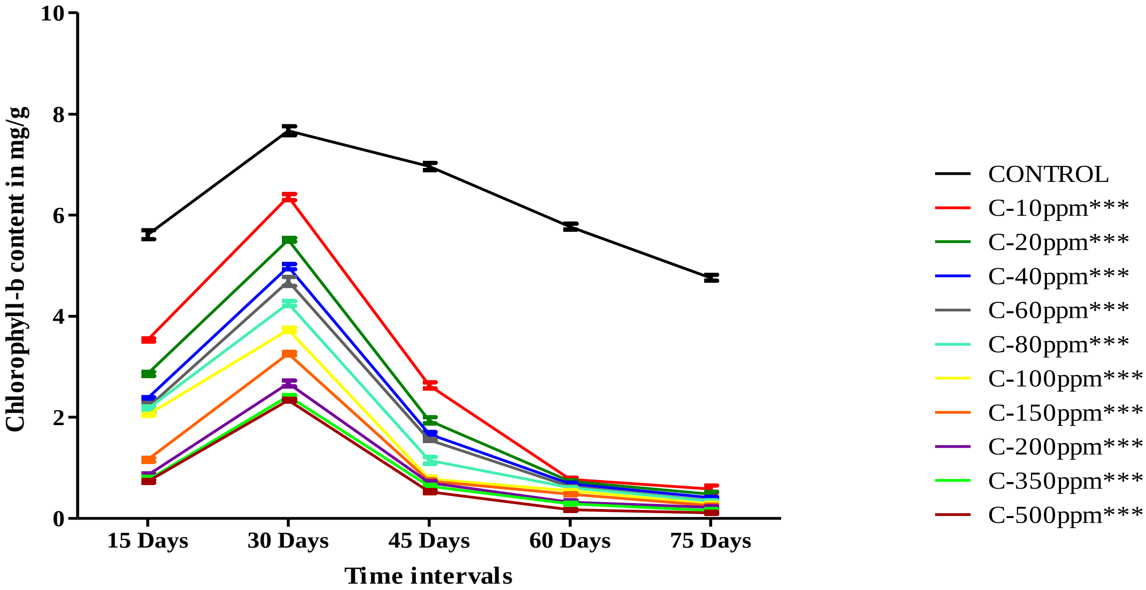

Figure 8- Effect of different concentrations of Cadmium (C) on chlorophyll-b content in *Mnium cuspidatum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing with different concentrations of Cadmium (ppm) where R^2=0.896 and highly significant $p<0.0001$ being indicated by (***) .

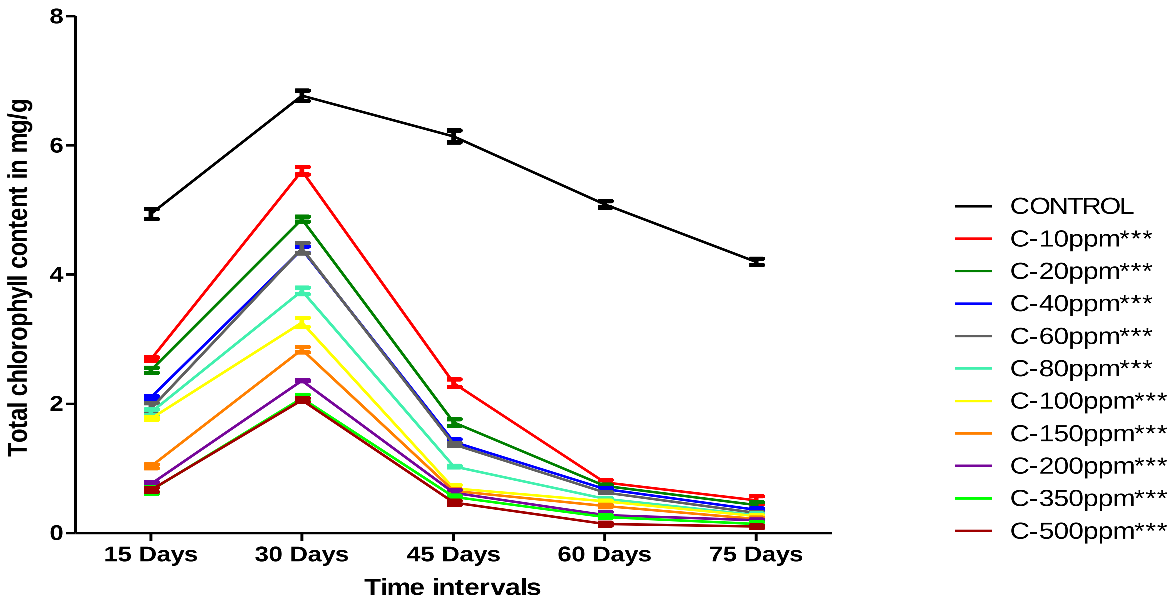

Figure 9- Effect of different concentrations of Cadmium (C) on total chlorophyll content in *Mnium cuspidatum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing with different concentrations of Cadmium (ppm) where R^2=0.899 and highly significant $p<0.0001$ being indicated by (***) .

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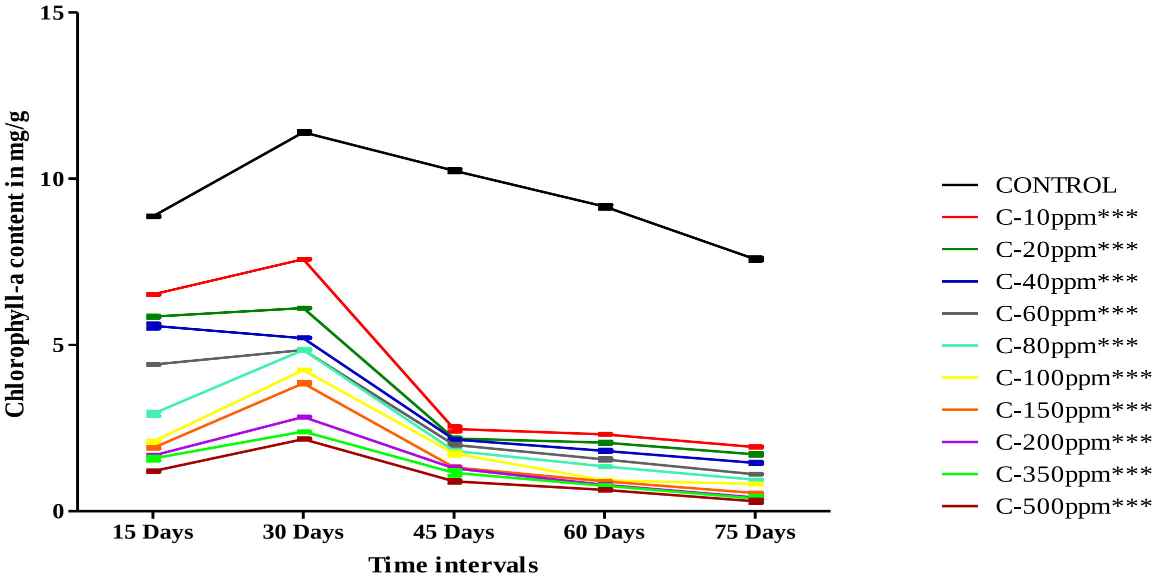


Figure 10- Effect of different concentrations of Cadmium (C) on chlorophyll-a content in *Brachythecium rutabulum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing with different concentrations of Cadmium (ppm) where R^2 =0.917 and highly significant $p<0.0001$ being indicated by (***) .

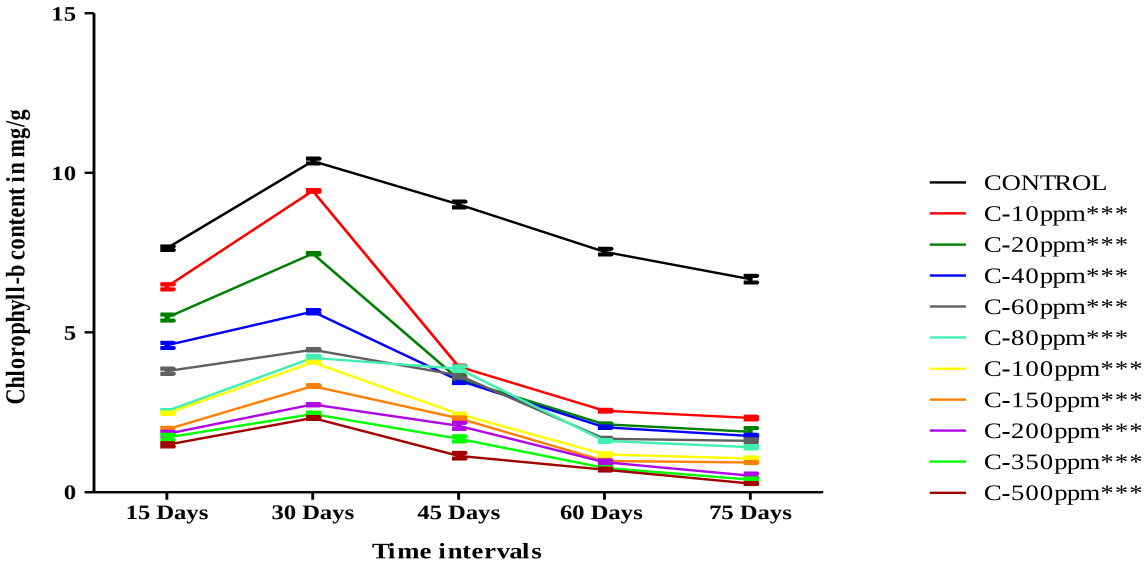


Figure 11- Effect of different concentrations of Cadmium (C) on chlorophyll-b content in *Brachythecium rutabulum* at different day intervals. Data represented as mean ± standard deviation of three replicates. Statistical significance was analyzed by one way ANOVA comparing with different concentrations of Cadmium (ppm) where R^2 =0.889 and highly significant $p<0.0001$ being indicated by (***).

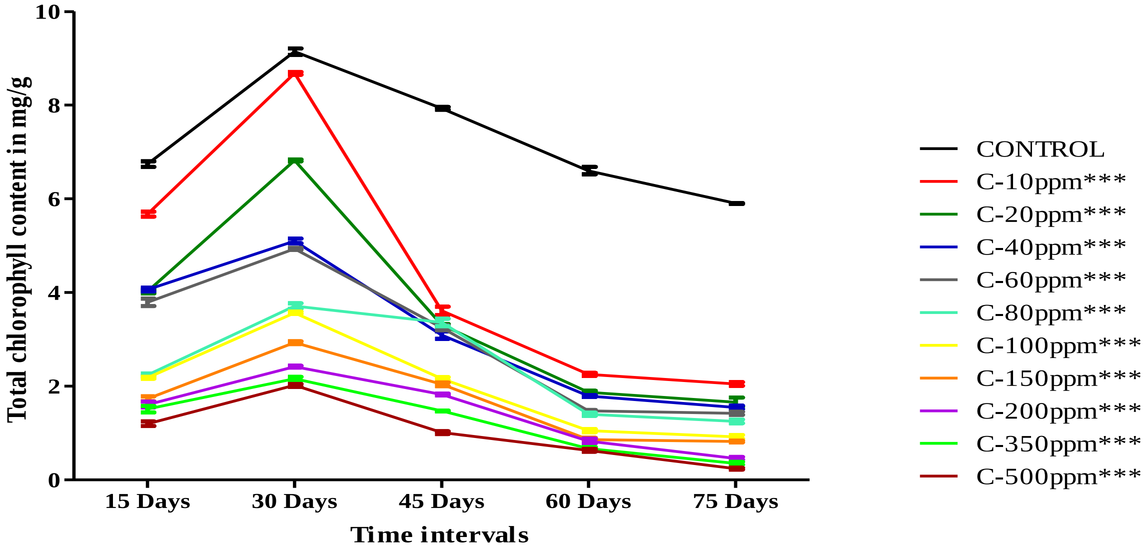
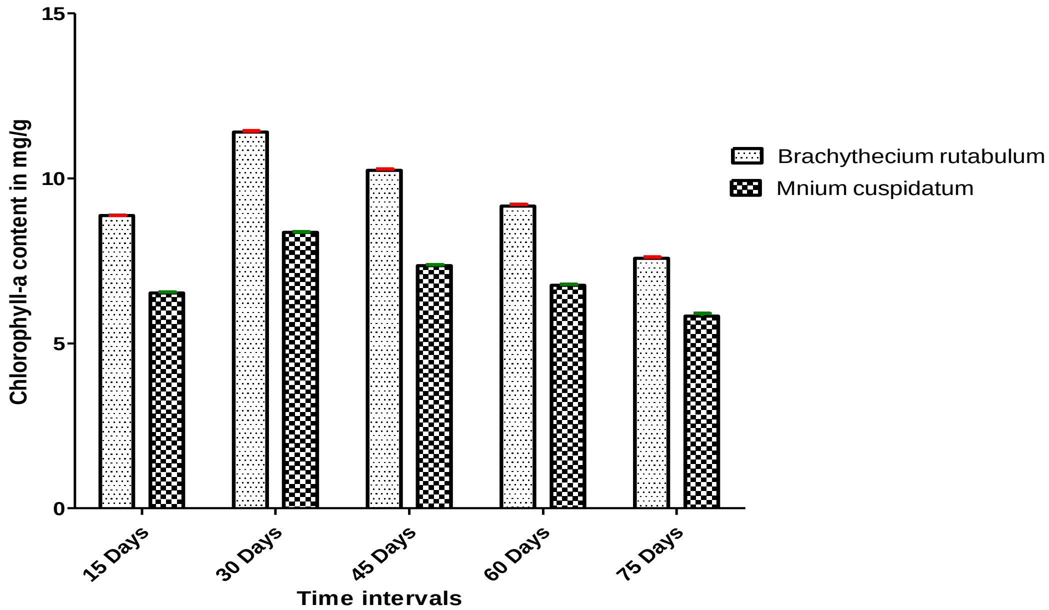
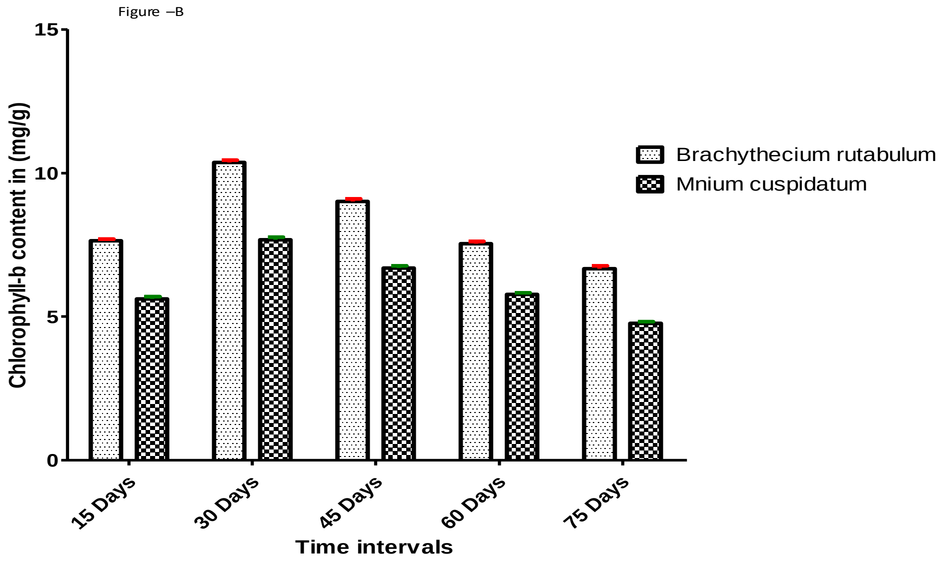


Figure 12- Effect of different concentrations of Cadmium (C) on total chlorophyll content in *Brachythecium rutabulum* at different day intervals. Data represented as mean ± standard deviation of three re plicates. Statistical significance was analyzed by one way ANOVA comparing with different concentrations of Cadmium (ppm) where R^2=0.886 and highly significant $p<0.0001$ being indicated by (***).

Figure -A





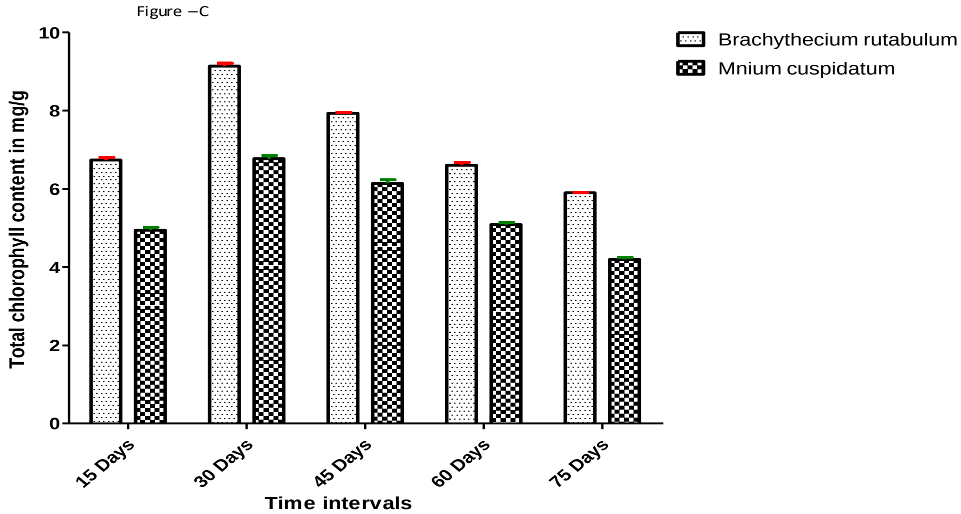


Figure A, B and C - Comparison of chlorophyll content (chlorophyll -a, chlorophyll-b and total chlorophyll) in controlled samples of *Brachythecium rutabulum* and *Mnium cuspidatum* at different day intervals. Data represented as mean ± standard deviation of three replicates .

4. DISCUSSION

Mosses *Brachythecium rutabulum* and *Mnium cuspitadum* when exposed to different concentrations in ppm of Cd and Zn for 15, 30, 45, 60 and 75 days show increase in chlorophyll contents initially, indicating that these mosses have distinguished ability to absorb heavy metals and also have greater potential to adapt to heavy metal stress. Mosses show a reduction in growth when subjected to metals stress, but on other hand it is quite excellent in countering the stress caused by heavy metals.

The deleterious effect of Zn and Cd was apparent after 30 days of treatment, whereas the evident degradation of chlorophyll contents was seen after 45 days of the experiment. The decrease in chlorophyll contents is inversely proportional to the increase in the concentration of the ppm (part per million) for both Cd and Zn.

In treated samples, *Brachythecium rutabulum* has a higher capacity to absorb these heavy metals more efficiently then *Mnium cuspidatum*; whether samples of *Brachythecium rutabulum* are irrigated with Cd or Zn, both samples had shown better absorption capacity, even the control samples of *Brachythecium rutabulum* show a higher concentration of chlorophyll contents than *Mnium cuspidatum*. The Cd and Zn stress leads to a reduction of chlorophyll content which varied with the intensity and duration of exposure. From the presentstudy, it can be safely stated that higher concentration of Cd and Zn ppm stress leads to degradation of chlorophyll contents. This finding recommended that *Brachythecium rutabulum is* more tolerant than *Mnium cuspidatum*, which further support that *Brachythecium rutabulum* is more efficient for heavy metals accumulation and can be used as bioaccumulator.

The present study suggested that although the two mosses belong to different families but the effect of both heavy metals studied show the same pattern of chlorophyll contents. The peak for chl-a was higher than chl-b and even total chlorophyll content in both the mosses when subjected to Zn and Cd stress for different days' exposure. Among all the experiments conducted, highest concentration of chl-a was observed in *Brachythecium rutabulum* when treated with Zn; while lowest concentration of total chlorophyll content was observed in *Mnium cusipdatum* when treated with Cd.

Fatoba *et al.* (2008) reported that when *Barbula lambarenensis* irrigated with 1000ppm and 2000ppm concentration of Cadmium, Copper, Iron, Lead and Vanadium, these heavy metals had negative effect on chloroplast and even change in color of the plant. Saxena and Afreen (2009) reported significant degradation of chlorophyll within 15 days when subjected to cadmium stress. Similar observation was made by Sun *et al*. (2009) where total chlorophyll content was initially under stress when treated with elevated stress conditions.

Metal accumulation in bryophytes is determined by nature of the sources of pollution, metal state and relative concentrations of metals in the environment. Plants affected by metal accumulation show mainly change in color due to alteration in the ultrastructure of the chloroplasts which leads to destruction in the biosynthesis of chlorophyll pigment.

REFERENCES

[1] **Arnon, D. 1949.** Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology. 24: 1-15.

[2] **Batagarawa, S. M.,andLawal, A. O. 2010.** Determination of Some Heavy Metals Levels in *Funaria hygrometrica* in Dutsinma Town of Katsina State, Nigeria. Journal of Applied Science and Environment Management. 14(3): 65 – 68.

[3] **Bhushan, G., Sharma, S. K., and Kumar, S., 2014.** Changes in nitrate reductase activity, chlorophyll content, proline and oxidative stress response in two moss subjected to Pb toxicity. Ethiopian International Journal of Multidisciplinary Research. **2(1):** 7-12.

[4] **Ceburnis, D., Ruhing A. and Kvietkus, K. 1997.** Extended study of atmospheric heavy metal deposition in Lithuania based on mosses analysis. Environment Monitoring and Assessment. **47(2):** 135-152.

[5] **Chopra, R. N. andKumra, P. K. 1988.**Biology of bryophytes. Wiley Eastern Limited, New Delhi.

[6] **Devmalkar V. S., Murumkar, C. V., Salunkhe, S. M and Chavan, S. J. 2014.**Studies on pigment chlorophyll isolation and estimation of different bryophytes for their biochemical properties. Journal of Natural Product and Plant Resources. **4(2):** 56-61.

[7] **Díaz S., Villares, R., María, D., Vázquez and Carballeira, A. 2013.**Physiological Effects of Exposure to Arsenic, Mercury, Antimony and Selenium in the Aquatic Moss *Fontinalis antipyretica* Hedw. Water air soil pollution. **224:** 1659-1673.

[8] **Fatoba, P. O. andUdoh, E. G. 2008.** Effect of heavy metal on chlorophyll accumulation in *Barbula lambarenensis*. Ethanobotanical Leaflet. **12:** 776-783.

[9] **Fraser, D. C. 1961b**. Organic sequestration of copper. Economic geology. **56:** 1063-1078.

[10] **Govindapyari, H., Leleeka, M., Nivedita, M. and Uniyal, P. L. 2010.** Bryophytes: indicators and monitoring agents of pollution. International Journal of Environment and Biodiversity. **1(1)**: 35-41.

[11] **Ghatge, M. M., Shaikh, S. D. and Dongare, M. 2011**. Study on the metal absorption by two bryophytes from Koyana wildlife sanctuary (India). Journal of Experimental Sciences. **2(3):** 49-50.

[12] **Kaur, S., Rao, A.and Kumar, S. S. 2010.**Studies on the effect of heavy metals on the growth of some bryophytes-I (mosses). International Journal of Pharmaceutical Sciences Review and Research. **5(3):** 102-107.

[13] **Kumar, S. S., Uniyal P. L. and Kaur, P. 1989**. Heavy metal deposition in some mosses of Garhwal Himalaya Yushania**6(3):** 9-14.

[14] **López, J. and Carballeir, A. 1993.** Metal accumulation and pigment stress of aquatic Bryophytes from the river eume, galicia (nwspain). Limnetica. **9:**1-9.

[15] **Rühing, A. and Tyler, G.1984.**Recent changes in the deposition of heavy metals in northern Europe. Water, Air and Soil Pollution. **22:** 173-180.

[16] **Rühing, A. and Tyler, G. 2004**. Changes in the atmospheric deposition of minor and are elements between 1975 and 2000 in south Sweden, as measured by moss analysis. Environmental pollution. **131(3):** 417-423.

[17] **Saxena, D. K. andAfreen. S. 2009.** Effect of Cu and Cd on Oxidative Enzymes and Chlorophyll Content of Moss *Racomitriumcrispulum.* Taiwania. 54(4): 365-374

[18] **Shotbolt, L., Buker, P. and Ashmore, M. R. 2007**. Reconstructing temporal trends in heavy metal deposition, assessing the value of herbarium moss samples. Environmental Pollution. **147:** 120-130.

[19] **Tuba, Z., Csintalan, Z. S. and Proctor, M. C. F. (1996).** Photosynthetic responses of a moss, *Tortularuralis*sp. *ruralis*and the lichens *Cladonia convolute* and *C. furcata*to water deficit and short periods of desiccation: abaseline study at present-day CO2 concentration. New Phytologist. **133:** 353-361.