A STUDY OF HEAVY METAL BIOACCUMULATION EFFECTS ON CHLOROPHYLL CONTENT OF THE TWO AQUATIC BRYOPHYTES *Taxiphyllum barbieri* (JAVA MOSS) and *Vesicularia montagnei* (CHRISTMAS MOSS)

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CERTIFICATE

This is to certify that the investigatory project entitled **"A STUDY OF HEAVY METAL BIOACCUMULATION EFFECTS ON CHLOROPHYLL CONTENT OF THE TWO AQUATIC BRYOPHYTES *Taxiphyllum* barbieri (JAVA MOSS) and *Vesicularia montagnei* (CHRISTMAS MOSS)** "submitted in partial fulfillment of the requirements for the award of Degree of Master of Philosophy in Botany, is an authentic record of the research work carried out by **ANU JOSE (SMP20BOT001)** under the supervision and guidance of **Dr.Elsam Joseph,** Associate Professor, Department of Botany, St.Teresa's College (Autonomous), Ernakulam. I further certify that no part of the work embodied in the project has been submitted for the award of any other degree or diploma.

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TABLE OF CONTENTS

Sl.No.	Title	Page . No.
1.	CHAPTER 1	10
1.1	INTRODUCTION	10-15
1.2	OBJECTIVES	16
1.3	CHAPTER 2	17
2.	REVIEW OF LITERATURE	17-25
2.2	CHAPTER 3	26
3.	MATERIALS AND METHODS	26-38
3.1	CHAPTER 4	39
4	OBSERVATION AND RESULTS	39-57
4.1	DISCUSSION	58-66
5	SUMMARY	67
6	CONCLUSION	68
7	REFERENCES	69-75

LIST OF TABLES

SL.NO.	TITLE	PAGE NO
1.	Heavy metals present in water sample, *T.barbieri* and *V.montagnei*	45
2.	Comparative amount of chlorophyll content in *T.barbieri* and *V.montagnei*	46
3.	Correlation between Cadmium accumulation and chlorophyll content in *T.barbieri*	50
4.	Correlation between Cadmium accumulation and chlorophyll content in *V.montagnei*	51
5.	Correlation between lead accumulation and chlorophyll content in *T.barbieri*	52
6.	Correlation between lead accumulation and chlorophyll content in *V.montagnei*	53
7.	Correlation between Zn accumulation and chlorophyll content in *T.barbieri*	54
8.	Correlation between Zn accumulation and chlorophyll content in *V.montagnei*	55
9.	Correlation between Cu accumulation and chlorophyll content in *T.barbieri*	56
10.	Correlation between Cu accumulation and chlorophyll content in *V.montagnei*	57

LIST OF FIGURES

SL.NO.	TITLE	PAGE NO.
1.	Images of *Vesicularia montagnei*	40
2.	Images of *Taxiphyllum barbieri*	40
3.	Images of leaf apex of *V. montagnei*	40
4.	Microscopic images of *T. barbieri*	40
5.	Images of internal leaf cells- *V. montagnei*	41
6.	Images of internal leaf cells- T. *barbieri*	41
7.	Images of thallus of *V. montagnei*	41
8.	Images of thallus of *T. barbieri*	41
9.	*V. montagnei* growing in uncontaminated water	42
10.	*T. barbieri* growing in uncontaminated water	42
11.	*V. montagnei* exposed to known concentration of heavy metals	42
12.	*T. barbieri* exposed to knownconcentratio of heavy metals	42
13.	Indication of chlorosis before and after heavy metal treatment in both *V. montagnei* (Christmas Moss) and *T. barbieri* (Java Moss)	43
14.	Images of estimation of chlorophyll content before heavy metal treatment	44
15.	Images of estimation of chlorophyll after heavy metal treatment	44

16.	Polluted water collection from Company padi, Eloor region	44
17.	Graphical representation of comparative amount of chlorophyll content in both *T.barbieri* and *V.montagnei*	46
18.	Graphical representation of chlorophyll content variation in response to Cadmium chloride exposure	48
19.	Graphical representation of chlorophyll content variation when treated with Lead chloride exposure	48
20.	Graphical representation of chlorophyll content analysis after exposure to Zinc Chloride	49
21.	Scattered diagram showing negative correlation between Cadmium accumulation and chlorophyll content in *T.barbieri*	50
22.	Scattered diagram showing negative correlation between Cadmium accumulation and chlorophyll content in *V.montagnei*	51
23.	Scattered diagram showing negative correlation between Lead accumulation and chlorophyll content in *T.barbieri*	52
24.	Scattered diagram showing negative correlation between Lead accumulation and chlorophyll content in *V.montagnei*	53
25.	Scattered diagram showing negative correlation between Zinc accumulation and chlorophyll content in *T.barbieri*	54
26.	Scattered diagram showing negative correlation between Zinc accumulation and chlorophyll content in *V.montagnei*	55
27.	Scattered diagram showing negative correlation between Copper accumulation and chlorophyll content in *T.barbieri*	56
28.	Scattered diagram showing negative correlation between Copper accumulation and chlorophyll content in *V.montagnei*	57

Abstract

Heavy metal pollution in aquatic ecosystems is a serious issue of concern. It can harm both aquatic organisms and biodiversity. Several studies revealed that bryophytes, especially mosses, can effectively accumulate heavy metals like Cadmium (Cd), Cu, Pb and Zn etc. These heavy metal accumulation in the bryophytes turn out to have an effect on chlorophyll content in the cells. The study envisages the bioaccumulation of heavy metals – Cd, Cu, Pb & Zn in two aquatic mosses *Taxiphyllum barbieri* / Java moss (T.*barbieri*) and *Vesicularia montagnei* / Christmas moss (V. *montagnei*). The effect of the heavy metal accumulation in the cells and its effects on the chlorophyll content was also studied in detail. Plant materials were grown in polluted water collected from the polluted site (Eloor industrial area). Then the heavy metals were analyzed and quantified by standard sampling tests. It is observed that mosses which were grown in polluted water get accumulated with Cd, Cu, Pb and Zn at different rates and amount of chlorophyll content also varied with respect to the heavy metal deposition. The study revealed that the heavy metal accumulation and chlorophyll content in both T.*barbieri* and V. *montagnei* are negatively correlated. This study paved the way for application of T.*barbieri* and V. *montagnei* for effective removal of heavy metals from the polluted water.

Keywords: Bioaccumulation, Heavy metals , *Taxiphyllum barbieri* , *Vesicularia montagnei* .

Chapter 1

INTRODUCTION

Bryophtes are referred as the amphibians of kingdom plantae. They are the sole simplest and primitive group between algae and Pteridophytes. Bryophytes are autotrophic cryptogames comprising nearly 25,000 species. Taxonomically, they are classified into four classes, the hornworts (Anthocerotopsida), the liverworts which includes to classes (Marchantiopsida, Jungermanniopsida) and the mosses (Bryopsida).

The life cycle of bryophytes shows an alternation between sporophytic and gametophytic generations which are distinct in form and function. The real plant is represented by the gametophytic generation, which is the most advanced haploid generation in the entire plant kingdom (Gilbert, 1968). About 25,000 species of bryophytes thrive in mostly moist habitats, although some board in deserts.

They form the main flora of adverse environments like the tundra, where their small size and endurance to desiccation offer distinct advantages. They usually lack lignin and not posses actual tracheids (xylem cells specialized for water conduction). Rather, water and nutrients move around inside specialized conducting cells. Although the term non-tracheophyte is more specific, bryophytes are popularly called non-vascular plants (Mary Ann Clark *et al.,* 2018).

Bryophytes are green land plants which have no specialized vascular system. They are very simple in both morphologically and anatomically. Like vascular plants, the growth potential of bryophytes are not thoroughly polarized. They acquire nutrients directly from

substances dissolved in the moist environment. Nutrients are absorbed directly from the substratum by means of diffusion through the cells of gametophyte (Le Blanc & Rao, 1975).

Unlike other vascular plants, bryophytes have the potential to absorb and retain heavy metal pollutants in higher quantities compared to those absorbed by other plant groups growing in the same habitat. These plants can trap and avert recycling of such pollutants in the ecosystem for a long periods of time. Analysis and studies of such plants gives an design about the degree of metal pollution and how they are sensitive to pollution and reveal visible symptoms of impairments even in the presence of minute quantities of pollutants. This set out as an good indicators of the degree of pollution and also of the nature of pollutant (Govindapyari *et al* ., 2010).

Bryum capillare and *Ceratodon purpureus* are heavy metal hyperaccumulating species, especially they can accumulate iron, lead, copper, manganese, and zinc. The order of average values of heavy metal accumulation was in the order:- For mosses *Bryum capillare*: Iron> Zinc> Lead> Copper> Manganese> Nickel> Cadmium & *Ceratodon purpureus*: Iron> Zinc> Lead> Copper> Maganese> Nickel> Cadmium, while in the ash substrate it was: Iron> Lead> Cadmum> Zinc> Manganese> Nickel (Vukojevic, 2005) .

Heavy metals are elements having atomic weights between 63.5 g/mol and 200.6 g/mol but the word also includes other toxic metals and metalloids (Shanab *et al*., 2012). Trace amounts of some of these elements, such as cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc are needed by living organisms but excess is typically detrimental. Cadmium, chromium, mercury, lead, arsenic, and antimony are non-essential heavy metals.

These can significantly modify the biochemical processes in living organisms. Heavy metal pollutants are arised from several industrial activities, transported by runoff water which pollutes the water sources & downstreams. Heavymetal pollution is specifically derived from from metal processing, wastewater from mining, tanneries, pharmaceuticals, pesticides, organic chemicals, plastics, lumber and wood products (Neetha *et al.,* 2016).

Freshwater ecosystems pollution badly affect aquatic biota. As a response to contaminations, bryophytes have response and they interact with metabolic and physiological

level since they lack roots and other similar systems (Tyler, 1990 & Yurukova *et al.*, 1996). Negative effects of heavy metal pollution can be obvious and detected before the alteration of growth and development , which are the commonly used parameters for the assessment of the heavy metal toxicity in plants (Wolterbeek, 2002). These effects consist not only alterations in the ultrastructure but also the changes in the plant physiological processes and other attributes (Sun *et al*., 2009, Canivet *et al.,* 2015).

Moreover, as a result of heavy metal stress, chloroplast of bryophytes undergo modifications including ultrastructural changes, alternations of the chloroplast shape and thylakoid organization (Choudhury and Panda, 2005) and the presence of the stromal plastoglobules in them (Basile *et al*., 2009).

Diaz *et al*., noted that there are a number of aquatic bryophytes that can accumulate relative high concentrations of contaminants in their bodies and, because of this ability, they have been recommended as bio-indicators in water (Diaz *et al*., 2012). These are non-vascular plants that differ from vascular plants because of their direct uptake pathway through the vegetative thallus. Probably because of this characteristic, aquatic mosses have been shown to accumulate more heavy metals (HM) than vascular plants at contaminated sites (Esposito *et al*., 2012 & Esposito *et al*., 2018).

Several studies evidenced the ability of *Leptodictyum riparium* isolated from polluted freshwater to serve as bioindicator, accumulating several heavy metals such as chromium, lead, and zinc (*Drepanocladus* spp) (Koch *et al*., 2000) and *Warnstorfia fluitans* appeared particularly efficient in the uptake of arsenic (Sandhi *et al.,* 2000). *Funaria hygrometrica* accumulates great quantities of lead (Itouga *et al.,*2017) but less than 10 species are known for the accumulation of various heavy metals.

Water Relations of Bryophytes

Bryophytes have two mechanisms of water movements. In endohydric bryophytes, the internal movement of water is through the central cylinder whereas in ectohydric bryophytes, the external movement of water is along the surface of the leafy thallus. Due to the absorption of water from the external capillary spaces, the endohydric mosses maintain a specific water level.

Mixohydric bryophytes possess both internal and external movement of water. Parenchyma, hydroids, leptoids and stereids are the primary cells which take part in movement of water and sugars. While hydroids conduct water, leptoids direct sugars organised as in tracheophytes, with the water conducting cells encircled by the sugar conducting cells. Movement of water takes place by a mechanism of diffusion pressure deficit. Bryophytes have remarkable abilities to absorb, retain and recover the loss of water (Glime,2017).

Economic and ecological importance of bryophytes

Bryophytes have medicinal values. In the ancient times, various mosses such as *Bryum, Mnium*, and *Philonotis* were crushed and applied as a poultice. A paste from *Riccia* sps is applied externally to cure ringworm. Diplophyllin from Liverwort *Diplophyllum* is significantly effectful against human epidermoid carcinoma.

Mosses are definite sources of antibiotics. Peat humic acid posses antiviral activity against herpes simplex virus types 1 and 2. Extracts of *Porella, Pallavicinia* and *Reboulia* have antimicrobial activity. Some bryophytes show anticancerous properties. Extract of *Polytrichum juniperinum* has anticancerous activity against Sarcoma. Anti-leukemic activity of Marchantin – A from *Marchantia polymorpha* and Ricardin from *Ricardia multifida* has also been reported.

Bryophytes such as *Sphagnum* has been widely used in horticulture as soil additives, ground cover, dwarf plants and as seedling beds. In European countries, Methane is produced on a largescale from *Sphagnum*. Therefore, mosses have wide potential uses as fuels (Sharma, 2014). Bryophytes as indicator of heavy metal pollution. *Peat mosses* and *Atrichum undulatum* are bioindicators which are sensitive to air pollution. Copper mosses such as *Mielichhoferia sps, Mercega ligulata and Dryptodon stratus* are indicators of copper content in the substrate.

Bryophytes can prevent soil erosion by forming a cover on soil and retain the moisture content of the soil. I t als contain essential oil and sesquiterpenoids etc. Bryophytes are good indicators of environmental conditions. *Funaria hygrometrica* in an area indicate good base

saturation. *Ceratodon purpureus* indicates good drainage and high amount of nitrogen (Sharma,2014)

Various studies revealed that bryophytes especially mosses can effectively bioaccumulate heavy metals. *Taxiphyllum barbieri* and *Vesicularia montagnei* are such aquatic mosses which have the the capabilities to absorb heavy metals properly. The present study aimed to analyse the effect on chlorophyll content with respect to heavy metal acccumulation.

About Study material- mosses

Taxiphyllum barbieri, known as Java moss or Bogor moss, which belonging to the family Hypnaceae. It is endemic to southeast Asia, it is commonly used in freshwater aquariums. It adhere to rocks, roots, and driftwood. In the wild, it grows in humid riparian areas (Goffinet *et al*., 2008). Java moss is a bright green leafy thallus that forms dense, carpet. The plant's stems are short, reaching nearly a height of 4 inches, and are covered by small 0.7-inch overlapping leaves. Java mosses don't have true roots. Instead, the plants have tiny sticky rhizoids which can anchor them in place.

The plants grow slowly, at a pace between 1 and 1.5 inches per month. When grown underwater, Java moss which grown in water have smaller greener leaves than grown on land. Young, newly planted moss is more brighter than old moss.Java Moss can be readily identifiable by the presence of many long branches with distantly spaced, lateral branchlets. The small, flattened leaves are organised on two sides of the stem and branches.

When tied to driftwood or rocks, the long branches of the plant become more copiously produced. Observed under the microscope, the shape of leaf varies from oblong to oblong-lanceolate, with a short and wide leaf tip. Leaf cells are long and narrow, with thin- to thick walls. The leaf margins are toothed in nature. At the base of leaf blade, two short "veins" (or costae) can be clearly seen. The species is dioecious and perennial. It forms loose mats of unevenly arranged branches. Leaves are two ranked and flat.

Since this plant is largely used as a decorative plant in aquariums, it is known that the thallus can grow in very variable light (from 150 µmol/m^2 s to shaded discontinuous light) and varying temperature (15–35 °C) conditions. The mechanical characteristics of the gametophyte mats attracted our attention.

They can be removed from water and exposed to air for many hours or compressed and handled with no notable damage to the organism integrity and vitality. The moss can be propagated ex situ and packed to adapt filtering containers of the desired volume and shape where it may accumulate particular quantities of heavy metals within a few hours, representing a new possibility for water eco-sustainable remediation. He confirmed in his study about the known capacity of mosses to accumulate heavy metals and then we approached the question of whether moss biomasses can be grown to enhance removal of specific pollutants and be used as filtering material to rapidly reduce water contamination (Papadia *et al*., 2020).

Christmas Moss (*Vesicularia montagnei* (Bel.) Broth.). The common name of this plant is due to its mature fronds hang down and overlap each other like the branches of a Christmas tree. Being aquatic, the moss can form long triangular fronds. Its more or less regularly pinnate to subpinnate branches are attribute.

The leaves are round to broadly oval in shape with an short and sharp apex, and the leaf cells are broad and short in outline, and with thin walls. The two leaf costae are also a bit apparent. In its natural dwelling, Christmas Moss grows on shaded wet bank of stream and creeks, and also on wet shaded ground in forest.

It is a broad species in tropical Asia (Tan *et al*., 2005). Christmas moss is endemic to the tropical areas of Asia and it can be found all throughout Japan, Thailand, India, and also the Philippines. This bryophyte is typically found immersed underwater on shaded riverbanks, creeks, and streams. Christmas moss will be seen hooked up to to rocks, branches, drowned tree boles, or simply floating around.

Just like other mosses, it possess a rhizoid root-like system, which implies it consumes nutrients through its leaves and stems. *Vesicularia montagnei* prefers cooler flowing water that ranges anywhere from 65 to 77 degrees Fahrenheit. *Vesicularia montagnei* is a creeping moss extensively spread in the Asian tropics, where it is found mainly, on moist shaded riverbanks and on damp forest soil.

Its popular name, Christmas moss, because of its shoots that have a little ressemblance like the branches of a true fir tree. They have a rather regular growth habit and have dense lateral shoots (under low light, this moss does not furcate well, though). Fronds of submersed

plants are triangular in outline and an overhanging habit, bu not arched down as *Vesicularia ferriei* (weeping moss).

The leaves are around 1 to 1.5 mm long and they are almost exactly right angle to the stem. The immersed form has nearly round to wide-oval leaves and an acute, sharp-pointed and rather short tip. Under a microscope, the leaf cells can observed. They are relatively broad (only 2 to 3 times longer than than wide) and thin-walled. In contrast to this, *Vesicularia dubyana* have ressemblance to *V. montagnei*, shows an overall more irregular and more loosely branching habit under the same conditions, the leaves tend to be more shorter and are narrower, and the leaf cells are more elongated (Flow grow, 2006).

Present study aimed to evaluate the effect on chlorophyll content with respect to bioaccumulation of heavy metals by the two aquatic mosses *Taxiphyllum barbieri* and *Vesicularia montagnei*. It can be used as a criteria to check the phytoremediation capability of the selected mosses towards heavy metal pollution.

OBJECTIVES OF THE STUDY

The main objectives of the study were,

- ✓ To study the effects of heavy metal accumulation in the aquatic bryophytes *Taxiphyllum barbieri* and *Vesicularia montagnei*.
- ✓ Estimation of chlorophyll content with respect to heavy metal accumulation
- ✓ To check the heavy metal bioaccumulation capacity of the two selected mosses in vitro

Chapter 2

REVIEW OF LITERATURE

Charophytes and bryophytes are early-diverging streptophytes widely used for biomonitoring purposes, as they are able to endure high concentrations of toxic metals /metalloids without showing any clear heavy damage. Special focus will be given to strategies including Cadmium vacuolar sequestration, cell wall immobilization and mechanisms that help to achieve detoxification. After analysing the effects of metal/ metalloid pollution and accumulation on the morpho-physiological characters of charophytes and in fact bryophytes can be fundamental for optimizing their use as phytomonitors and/or phytoremediators (Erika Bellini *et al*., 2021).

Seeds of *Avena sativa* were allowed to grown in the dark in a wet vermiculite at 22/sup 0/C. The first formed leaf was cut into pieces when the seedlings were 16 days old. These leaf pieces were taken in a 25-ml Erlenmeyer flasks, containing 10 ml glycylglycine with and without PbCl/sub 2/, and preincubated in the dark for 1 h. The flasks were kept for 72 h at 21/sup 0/C in a water bath and lluminated at 7000 lux, and shaken 40 times a minute. Chlorophyll was extracted and the concentration of chlorophyll a and chlorophyll b were determined. Synthesis of both types of chlorophyll a and chlorophyll b was reduced in proportion to the increasing amounts of lead ions. Minute concentrations as low as 50 mu.M PbCl/sub 2/ had a statistically remarkable effect. Comparing to chl a and chl b, at lower

concentrations (0-500 mu.M) synthesis of chlorophyll b was inhibited profoundly than that of chlorophyll a; at higher concentration the chlorophyll a and chlorophyll b got varied (Hampp and Lendzian , 1974).

Absorption of phytotoxic amounts of metal by higher plants or algae will cause the inhibition of several enzymes, and additionally cause increase in activity or induction of others. There are two mechanisms of enzyme inhibition : (1) binding of the metal to sulphydryl groups and involved within the catalytic action or disturb structural integrity of enzymes, and (2) deficit of an necessary metal in metalloproteins or metal-protein complexes, finally that combined with replacement of the noxious metal for the deficient element. Accumulation of metals within the cellular compartment of the enzyme might be requirement for enzyme inhibition in vivo. The induction of some enzymes are thought to play a significant role within the stress metabolism evoked by metal phytotoxicity.

Peroxidase induction is an example for the inhibition of enzyms by metals.It is likely to be related to oxidative reactions at the biomembrane; several enzymes of the intermediary metabolism might be stimulated to compensate for metalsensitive photosynthetic reactions (Van Assche & Clijsters,1990).

Barley (*Hordeum vulgare* L., cv. Hemus) plants were full-grown in nutrient solution with 54 µM Cd^{2+} for 12 days. An exposure with Cd^{2+} inhibited the expansion of young barley plants. The important factor for limiting plant growth was net assimilation rate, shriveled photosynthetic rate and accelerated dark respiration rate. The primary reasons for the reduced photosynthetic rate was the lower chlorophyll and carotenoid content. Cd^{2+} conjointly reduced the water potential and transpiration rate of barley plants but relative water content in leaves of the treated plants were not considerably modified (Vassilev *et al*., 1998).

Chlorosis taken place when the plants irrigated with heavy metal solutions. Due to heavy metal exposure their bright green colours changed to light green, yellow or brown while the control remained green throughout the period of study. Now it is clear that heavy metals affected the chloroplast negatively. The change in colouration started at the

4th week of experiment in after irrigated with 5mg/l Cd, Fe and Pb and 6th week irrigated with Va and 8th week in samples irrigated with Cu. The trend of this result implies that Cd, Fe and Pb are more deleterious to the chloroplast than Va and Cu. The cells and nuclei of the control remained uninjured throughout the period of study but the cell started collapsing and becoming distorted during the 4th week for Va treatment, 6th week for Cu, Cd, Fe and Pb treatments (Fatoba and Udoh, Emem ,2008).

To study the effect of Cd, Cu, Ni, Pb and Zn on the moss *Rhytidiadelphus squarrosus* metals were supplied as single salt solutions between 10 μM and 0·1 M. on the moss *Rhytidiadelphus squarrosus.* Results showed insignificant alteration to respiratory rates but photosynthetic rates get reduced drastically, and some membrane damage also taken place and are assessed by K leakage. Two distinct morphological forms of the moss posses different responses to heavy metal. Storage of materials decreased after 30 mins exposure to heavy metals, resulted in a further decrease in the photosynthetic rate. When the the total heavy metal recovered from the moss and photosynthetic reduction were studied, it also expressed a similar pattern. Movement of metal from extracellular exchange sites into the protoplast was also studied with storage after exposure. There was a linear relationship between photosynthetic decline and intracellular heavy-metal concentration, regardless of the duration of exposure or morphological nature of the material used. Photosynthetic reduction on storage can be considered as a response to additional metal stress rather than a progressive deterioration of the physiological process (Brown and Wells, 1990).

Heavy metals have deleterious effects on plant growth and metabolism. Heavy metal toxicity affects photosynthesis. In vivo and in *vitro* photosynthetic CO_2 fixation are also affected by heavy metals. Most of the heavy metals are inhibitory to photosystem (PS) II than PS I, being less sensitive in isolated chloroplasts. The target site of heavy metals seems to be at the oxidizing side of PS II. Other possible inhibition sites include photophosphorylation and/or enzyme activity. Inhibition in vivo is due to the multiple effects of these metals.

The direct effect of heavy metal is visible on the stomatal closure followed by chloroplastic changes. Long-term exposure resulted in reduced leaf growth, decreased

photosynthetic pigments, changed chloroplast structure, and reduction in enzyme activities for CO_2 assimilation (Sheoran and Singh, 1993).

The important functions of plant roots include the absorption of water and inorganic nutrients, supporting the plant body and fixing it to the ground, storage of food and nutrients, and vegetative reproduction. Roots are also the primary contact site for heavy metal ions and higher amount of metals get accumulate in them than in aboveground plant parts. An excessive absorption of metal ions in tissues may induce change in the water absorption from the soil, and cause sequential decrease in water content of roots. It has been experimentally proved that water deficit in the roots are also an indication of exposure to Cd, Ni and Zn (Barceló and Poschenrieder ,1990 & Nagajyoti *et al*., 2010).

To study carbon assimilation and chlorophyll (Chl) A content, blue green algae *Spirulina platensis* and *Anacystis nidulans* were grown in artificial solution treated with Cu and Cd in concentrations of 0.01, 0.1, 1.0 and 10 ppm . The species were treated with concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 ppm to check the absorption of metals with exposure time. Carbon assimilation and Chl A content exhibitted responses proportional to the concentration, the general form $y = K + n \ln C$, where C implies the concentration of metal in ppm, while just in case of uptake the relation was $y = KC''$; (where C is the molar concentration x 10^{-6} of the metal).

The n values in uptake was found to be < 1 indicating a non-Langmuir kind of absorption. The concentration of metals decreased with metal concentration in the medium (Azeez and Banerjee, 2008).

Metal accumulation in the plant bodies increased with excess metal concentrations. Cu accumulation have a significant inhibitory effect on chlorophyll-a, chlorophyll-b, and total chlorophyll in the mosses and the leafy liverwort. A little decrease in chlorophyll content in both *Thuidium* species, but a significant decrease in leafy liverwort, was reported after Zn and Pb accumulation. A reduction in the chlorophyll a is reported *T. sparsifolium*; and chlorophyll-b and total chlorophyll in *T. delicatulum.* After the accumulation of Cu+Zn+Pb ions together from mixed metal solution, all chlorophyll contents decreased insignificantly in *P. striatus.* When higher concentrations Cu+Zn+Pb ions treated together, the ratio of chlorophyll-a to -b decreased more rapidly in both *Thuidium* species than Cu, Zn, or Pb ions were alone. This

suggested a destructive effect of Cu metals on the chlorophyll contents of both *Thuidium* species. High concentrations of Cu can activate oxidative damage and change cell-membrane properties by lipid peroxidation, thereby demonstrating the inhibitory effect on the enzymes involved in chlorophyll production (Shakya *et al*., 2008).

Tomato plants (*Lycopersicum esculentum* Mill. cv. Moneymaker) grown on nutrient solution containing cadmium showed reduction in the net photosynthesis, chlorophyll concentration and other accessory pigments. In the chloroplasts isolated from cadmium treated plants, photoreduction - photosystem II and I activity ($H_2O \rightarrow$ methyl viologen) were inhibited to about 60%. The photosystem II activity, was measured by 2,6-dichlorophenolindophenol. When 1,5-diphenylcarbazide was used as artificial electron donor, no cadmium effect was observed. Photosystem I activity had no detrimental effects by cadmium treatment. The ultrastructure of chloroplasts in cadmium-treated plants was vitiated similar to senescence response. The main symptom of cadmium action was the appearance of large plastoglobules and a disorganization of the lamellar structure, especially grana stacks. Transfer of cadmium-treated plants into a medium with increased manganese level leads to grana stacking and restoration of photosystem II activity (Baszynki *et al*.,1980).

The concentrations of Cd, Cu, Pb, and Zn in sediments, water, and plant parts of six aquatic vascular plant species:- *Ceratophyllum demersum, Echinochloa pyramidalis, Eichhornia crassipes, Myriophyllum spicatum ,Phragmites australis and Typha domingensis* growing naturally within the Nile system (Sohag Governorate), were evaluated.

The concentrations of heavy metals in water, sediments, and plants follow the same trend: Zn > Cu > Pb > Cd which reflects the biomonitoring capabilities of the investigated plant species. Two-way ANOVA was used to measure the variation of heavy element concentrations in water and sediments in relation to site and season where $p = < 0.05$. However, minimal variations were observed in the concentrations of Pb and Cd in sediments in relation to season and of Cu and Zn in relation to site.

Results also showed that the selectivity of the heavy elements for the plant material varied significantly ($p < 0.05$) with species variation. The accumulation capability of the

studied species were arranged in a order : *C. demersum* > *E. crassipes* > *M. spicatum* > *E. pyramidalis* > *T. domingensis* > *P. australis*. On the basis of the concentrations of metals, roots of all the studied species contain higher concentrations of Cu and Zn than shoots and leaves were acquired the highest concentrations of Pb. Cd concentrations among different plant organs were closer except in M. *spicatum,* where the highest Cd concentrations were recorded in the leaves (Manal Ahmed Fawzy *et al*., 2012).

Phytoremediation is a convenient and less harmful tool to remove heavy metals from polluted water, however it is are restricted by the selection of plants ready to adapt filtration of polluted water in terms of area and physiological needs. Biomasses are usually suggested for bioremediation. Due to gametophyte characteristics, aquatic mosses can act as live filtering materials. The potential for phytoremediation of Hypnales aquatic mosses has been poorly studied compared to aquatic macrophytes. More than pollutant removal, their potential is usually indicated as a tool for bioindication and environmental monitoring. When phytoremediation has been considered, adaptability of mossess to different needs were neglected without proper attention. The *Taxiphyllum barbieri* grown in two different light conditions, was tested with high concentrations Pb, Cd, Zn, Cu, As, and Cr. This moss produced dense mats with few culture needs. The entire experimental setup confirmed the capacity of the moss to accumulate heavy metals accordingly to their physiology and then demonstrated that a significant proportion of heavymetals was accumulated within a few hours (Paride Papadia *et al*., 2020).

The leaves of dark-grown seedlings of barley (*Hordeum vulture*) were used to check the effect of cadmium on the biosynthesis of chlorophyll. Cadmium inhibited the synthesis of chlorophyll by affecting the synthesis of 5-aminolacvulinic acid and the protochlorophyllide reductase ternary complex with its substrates. Cd^{2+} had no action on the constituent enzymes that catalyse the synthesis of free protochlorophyllide from 5aminolaevulinic acid. The results obtained were proved that Cd^{2+} could inhibit the formation of chlorophyll by reacting with essential thiol groups in both the protochlorophyllide reductase protein and the enzyme(s) which were occurred in the light dependent synthesis of 5-aminolaevulinic acid (Stobart *et al*.,1985).

Moss is considered as pollution indicator due to its sensitive to heavy metals. However, it is unaware about the physiological parameters which indicate metal contaminations more

rapidly and non-invasively. This work examined the effects of heavy metals on physiological parameters and photosynthetic activities of two moss species grown in moist soil surface. They suggested that characters such as anthocyanin accumulation pattern and chlorosis pattern and two chlorophyll fluorescence parameters reflect metal species groups, concentrations and differences between the two moss species. In other words, metal contaminations could be roughly evaluated visually by the naked eye. *Eurhynchium eustegium* showed higher enzymatic and non-enzymatic anti-oxidative abilities and photosynthetic protein contents than those of *Taxiphyllum taxirameum*, indicating their differential metal tolerance. Anti-oxidative abilities and photosynthetic proteins were rarely used as ideal indicators (Yang-Er Chen *et al*., 2015).

Aquatic bryophytes are ecologically important especially in mountain streams, adapted to the adverse environmental conditions. Bryphytes could successfully inhabit other hostile aquatic habitats, such as deep lakes with light starvation. In this work ecophysiology of pigments in aquatic bryophytes such as the functioning of the xanthophyll cycle and the use of chlorophyll fluorescence as a vitality index, are investigated. The photosynthetic pigments chl-a and beta-carotene were present in the core complex of PSII. Between the spectrophotometrically-determined concentrations of chl-b and phaeophytin-a there existed a close correlation in aquatic bryophytes probably derives from the characteristic presence of both pigments in PSII. The genetic influence on pigment composition can occur in the colour of the different species, which is sometimes used as a taxonomical aid. Pigment variations can be used as a tool for nutrient deficiencies, toxicities or antagonisms (Javier Martinez-Abaigar *et al*., 1998).

Bryophyte were proved as promising bio-indicator of air pollution. Due to its habitat diversity, structural simplicity, totipotency, rapid rate of multiplication and high metal accumulation capacity make them an ideal organism for pollution studies.

Air polluion, specifically caused by gaseous and particulate pollutants lead to the decline and absence of bryophyte populations especially epiphytes. Bryophytes are authentic indicators and monitors of air pollution being easy to handle and show a vast range of specific sensitivity and visible symptoms to pollutants greatly exceeding that of higher plants (Govindapyari *et al*., 2010).

The two cultivars Norquay and Columbus of *Triticum aestivum* (wheat) which differ in tolerance of Mn is studied, to evaluate the toxic effects of Mn on chlorophyll content, photosynthesis and respiration. When Mn tolerant cultivar grown over a range of concentrations of Mn (0–1 000 μ*M*), they maintained a higher rates of photosynthesis and respiration, and chlorophyll *a* and chlorophyll *b* content, than the Mn-sensitive cultivar, although leaf tissues have greater accumulations of Mn. After 5 days of growth in the solution with 1000 μ*M* Mn, the photosynthetic rate get lessened to 25% of control in the sensitive cultivar and to only 75% of control in the tolerant cultivar (Macfie and Taylor, 1992).

To study the substitution of the central ion of isolated chlorophylls by heavy metal ions, in vitro and in vivo experiments with submersed water plants were carried out. It was observed that the substitution of the central atom magnesium of chlorophyll, by heavy metals (mercury, copper, cadmium, nickel, zinc, lead) in vivo is a chief damage mechanism in stressed plants. This substitution prevents photosynthetic light harvesting system in the affected chlorophyll molecules, which inturn cause breakdown of photosynthesis. This reaction depends on light intensity. In low light irradiance all the central atoms of the chlorophylls are attracted to heavy metals and heavy metal - chlorophylls being formed, some of which are more stable towards irradiance than Mg-chlorophyll. Consequently, plants remain as green even when they are dead. In high light, almost all chlorophyll decays and under such conditions most of the chlorophylls are inaccessible to heavy metal ions (Hendrik Kupper *et al*.,1995).

Rhynchostegium riparioides and (in one experiment) *Fontinalis antipyretica* were used as aquatic mosses in mesh bags to monitor heavy metal pollution after measuring concentrations in 2-cm tips of these species. Recurrent pollution events were excited by transporting moss from streams with low concentrations to ones with high concentrations of zinc, cadmium and lead and also in one case back from high to a low concentration.

The key factors that affect accumulation include position inside bag, density of packing, mesh size and differences between moss on boulders and in bags. Generally these factors influenced accumulation moderately over a wide range of treatments. The relation between time period and the moss exposed to Zinc pollution was tested : a greater proportion was lost with in first 2 h in moss exposed for 1 h than 24 h. The potential for using the moss-bag technique is

applicable : it is powerful and convenient to handle and is suggested for monitoring heavy metals in stretches of rivers where there were no natural moss populations (Kelly *et al*., 1987).

When the concentration of Cadmium in the growth medium doubled, the concentration of Cd in leaves get doubled and in chloroplasts, Cadmium concentrations were high as 3 nmol mg1 chlorophyll. This high metal concentration caused reduction in photosynthesis and chlorophylls (50%– 60%), as well as Rubisco content (35%) and activity (70%). Conspicuously, the decrease in photosynthesis assumed to follow (as with 50 m Cd) that of chlorophyll and of maximum extractable Rubisco activity. Even with this, the efficiency of PSII was still close to control values.

On the contrary, chloroplasts had visible alterations in thylakoids and a large starch accumulation. It is previously argued, that Cadmium can adhered to the cytosolic thiol-dependent enzyme F-1,6P2ase and it reduced sucrose synthesis, thus limiting phosphate recycling between cytosol and chloroplasts (Sharkey, 1990).

By means of the chlorophyll fluorescence transient OJIP, the toxic effects of cadmium on the photosynthetic apparatus of *Avicennia germinans* were studied. The chlorophyll fluorescence transients were recorded in vivo with high time resolution and analyzed per OJIP-test which quantify the performance of photosystem II. Cadmium-treated plants have decreased yield for primary photochemistry, TR0/ABS. Because of cadmium treatment, the performance index of photosystem II (PSII)- PIABS get decreased.

This performance index includes the combination of indexes of three independent parameters, (1) total number of active reaction centers per absorption (RC/ABS), (2) yield of primary photochemistry (TR0/ABS), and (3) efficiency of a trapped exciton which can move an electron into the electron transport chain (ET0/TR0). Moreover, the F0/Fv recorded highest sensitivity to the metal, thus indicating that the water-splitting apparatus of the oxidizing side of PSII is the initial site of action of cadmium. Cadmium affects several sites of photosystem II. More accurately, the main targets of cadmium keeping with the OJIP test, can be listed as a decrease in the number of active reaction centers and damage to the activity of the water-splitting complex (Daniel Gonzalez-Mendozaa *et al*., 2006).

Acceptor side of PSII have an inhibitory effect because of Cu accumulation. This happened due to induced inhibition of the Calvin cycle and down-regulation of electron transport. The plants exposed to Copper, at the end of the growth stage of primary leaves showed chlorosis and unchanged leaf area. Besides these, vital changes in acyl lipid content and well defined loss of core antenna PSII polypeptides and oxygen evolving complex subunits were observed. The low PSII activity (50% of control) is due the changes in the acceptor and donor sides of PSII and its reaction centre (Maksymiec *et al*., 1994).

Chapter 3

MATERIALS AND METHODS

3.1. Plant material

Systematic position

- ***Taxiphyllum barbieri***

Kingdom: Plantae

Division: Bryophyta

Class: Bryopsida

Subclass: Bryidae

Order: Hypnales

Family: Hypnaceae

Genus: Taxiphyllum

Species: *T. barbieri*

- ***Vesicularia montagnei***

Kingdom: Plantae

Division: Bryophyta

Class: Bryopsida

Subclass: Bryidae

Order: Hypnales

Family: Hypnaceae

Genus: Vesicularia

Species: *V. montagnei*

Taxiphyllum barbieri (Java Moss) is popularly used as aquarium moss. It have root like rhizhoids and cushion or tuft of leaves on either side of stem. Leaves are lanceolate in shape with acuted ends. Numerous branches are arising from two sides of the stem. The entire plant body is thin and are green in colour.

Vesicularia montagnei or Christmas moss is also another aquarium moss. It is easily identified by its christmas tree like appearance. Christmas moss is more brightly coloured than Java moss. Leaves are arranged on either side of stem with wider base than Java moss .

Both mosses were sourced from domestically growm specimens inTrivandrum District. Mosses were collected and grown in 100% pure distilled water. Java moss and Christmas Moss were collected from aquariums and are grown in polluted water collected from Eloor region, Ernakulam. After 5 days, they were analysed for heavy metal accumulation rate and chlorophyll

content using standard protocols. An invitro condition was made with known concentration of $Cuso_4$ (1.5g/100ml), $Pbcl_2$ (1.5g/100ml), $Zncl_2$ (1.5g/100ml) and $Cdcl_2$ (1.5g/100ml). The chlorophyll content were tested for 24hrs, 48hrs, 72hrs and 96hrs using spectrophotometer. Heavy metal analysis was carried out in Polu Chem laboratories, North Kalamaserry, Ernakulam and Chlorophyll content analysis was done by using spectrophotometer in the college itself.

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Water sample and plant materials analysis for heavy metal detection

- **Detection of Copper(IS-3025(P) 42- 1992 RA2014**

The sampling and storage done as prescribed in IS 3025 (Part1) : 1986.

The sample bottles were cleaned thoroughly with dilute nitric acid (6 N) prior to the final rinsing with water. The water samples were collected and stored for 24 hrs preferably in polypropylene or chemically resistant glass containers. For preservation, the samples were acidified with concentrated nitric acid (2 ml of AR grade nitric acid to 1 litre just to bring down the pH below 2). Unacidifled samples were analysed while the acidified samples were kept in a refrigerator.

1.NEOCUPROINE METHOD

Principle

Copper (II) is reduced to copper (I) by hydroxyl amine hydrochloride and the pH of tie solution is adjusted to 5 by sodium citrate solution. Copper (I) forms a soluble yellow spectrophotometric measurement. This method is applicable in the concentration range of 0.05 to 5mg/l of copper.

Interferences

Chromium will interfere when its concentration exceeds five times that of copper. The interference from organic matter, sulphide, cyanide and chromium can be eliminated by preliminary sample treatment. The other ions present do not interfere.

Apparatus

The apparatus used for the detection was Spectrophotometer – used at 457 nm with 1 cm cell

Reagents

The reagents used were concentrated hydrochloric acid, ammonium hydroxide, chloroform, isopropyl alcohol, neocuproine solution, concentrated nitric acid, concentrated Sulphuric acid, Sodium citrate, copper solutions .

Procedure

For removing the interfering substances, 1 ml of concentrated sulphuric acid and 5ml of concentrated nitric acid ere added. Evaporated the sample to dense white sulphur trioxide fumes on a hot plate. Repeated the treatment with 5ml or concentrated nitric acid and 5 ml of hydrogen peroxide and again evaporated the solution to complete dryness. Then the residue is dissolved with 80 ml of water, boiled, cooled and filtered. The pH was adjusted with dropwise addition of ammonium hydroxide to 4 to 6. After that 0-2 ml of hydrochloric acid was added and diluted to 100 ml.

Extraction

50 ml of the acidified sample or filtrate (5.5.1) were transferred to a 125 ml separating funnel. Then 5ml of hydroxylamine-hydrochloride solution were added to 10 ml of sodium citrate solution and 10 ml of neocuproine solution. Shaken well. After the addition of 20 ml

ochloroform and the entire solution is shaken for 1 minute. Then the aqueous and chloroform layers separated after few minutes. The chloroform layer was collected in a dry flask. Again, they were repeated with separate 20 ml aliquot of chloroform. Then the extracts were combined and diluted to 50 ml with isopropyl alcohol. Prepare A reagent blank was prepared by treating 50ml of double distilled water.

The optical density of the sample solution at 457 nm against the reagent blank was measured. Treat 50 ml portions of standard solutions containing 0·05, 0-1, 0.5, 1·0, 5.0 mg/lof copper were treated in similar way as above. The absorbance versus copper concentration were plotted for the standards to get a calibration graph. The concentration of copper in the sample were read from the calibration graph.

Calculation

Copper, mg/l$= \frac{M}{V} \times 1000$

Where, M -= mass in mg of copper in the sample, and

V = volume of sample in millilitre

2.ATOMIC ABSORPTION SPECTROPHOTOMETRY METHOD (Direct)

Principle

The copper content of the sample was determined by atomic absorption spectrophotometry. The filtered sample is directly aspirated into the atomizer for dissolved copper. An acid digestion procedure is carried out prior to aspiration of the sample, the total recoverable copper, This method is applicable in the range 0·02 to 5 mg/l.

Interferences

Cadmium, lead, nickel, Zinc, cobalt, manganese and chromium up to 10mg/l do not interfere. Alkali and alkaline earth metals can be tolerated up to 5000 mg. Iron does not interfere upto 1000 mg/l.

Apparatus

Atomic Absorption Spectrophotometer - with air-acetylene flame and Copper Hollow Cathode Lamp (used at 324·7 nm) were the apparatus used.

Reagents

The reagents used were concentrated hydrochloric acid, concentrated nitric acid, diluted nitric acid in the ratio (1: 500), diluted sulphuric acid in the ratio (1:1), stock and standard solutions .

Preparation of Stock copper solution

1·0g of pure copper metal were dissolved in 30 ml of (1 : 1) nitric acid and then 4 ml of (1:1) sulphuric acid was added and heated until sulphur trioxide fumes get evolved. The solution was cooled and diluted to 1 litre with distilled water. 1ml = 1·0 milligramof copper.

Preparation of Standard copper solution

100 ml of copper stock solution was diluted to 1 litre with distilled water. 1 ml = 0.1 milligram of copper.

Procedure

5 ml of concentrated hydrochloric acid were added and evaporated the solution to 15 to 20 ml. The sample get filtered after cooling through acid washed filter paper. Then the sample made up to 100 ml in a volumetric flask, the solution was aspirated and the absorbance was measured at 324·7 nm using copper hollow - cathode lamp. Prior to sample aspiration, nitric acid (1 : 500) were aspirated. Then a reagent blank was prepared and series of standards containing 0,0.0.2, 0.1, 0.5, 1, 2, 5 mg/l of copper by diluted a suitable volume of the standard solution with 100 ml of nitric acid (1 : 500) and repeated as above. The solutions were aspirated and absorbance was measured.

Calculation

A standard calibration graph was constructed by plotting the absorbance versus copper concentration (mg/l) for each standard. After that the concentration of the sample from the graph were read out.

$$\text{Copper, mg/l} = \frac{M}{V} \times 1\,000$$

Where,

M = mass (in g) of copper in the sample, and

V = volume of sample in milli litre

- **Detection of Lead IS 3025(P) 47-1994 RA2014**

1.ATOMIC ABSORPTION SPECTROMETRY METHOD (DIRECT)

Principle

The lead content of the sample was determined by directly aspirating the sample into the flame of an atomic absorption spectrophotometer. This method is applicable in the range from 1.0 to 10.0milligram/litre of lead.

Interferences

Other metals usually do not interfere. But high concentrations of calcium do interfere and give high values for lead.

Apparatus

Atomic absorption spectrophotometer with air-acetylene flame and hollow-cathode lamps or electrodeless discharge lamps (used at 283.3 nm) were the apparatus used.

Reagents

The reagents used were concentrated hydrochloric acid, concentrated nitric acid, diluted nitric acid in the ratio (1:499), lead solutions- both stock and standard.

Preparation of Stock lead solution

1.5999 g of lead nitrate was dissolved in a mixture of 10 ml or concentrated nitric acid and then add 100 ml of water and diluted to 1 litre (1 ml = 1.0 milligram of Pb).

Preparation of Standard lead solution

100 ml of lead stock solution was diluted to 1 litre with dilute nitric acid (1 : 499)

(1 ml= 0.1 mg of lead).

Procedure

To 100 ml portion of the acidified sample, 0.5 ml of nitric acid and 5 ml of concentrated hydrochloric acid were added and heated to reduce the volume to 20 ml in a well-ventilated

hood. After cooling, the sample get filtered and made up to 100 ml in a standard flask. The sample solution was aspirated and the absorbance was measured at 283.3 nm. Prior to sample aspiration, nitric acid (1 : 499) get aspirated.

Then a reagent blank and sufficient standards containing 1.0, 2.5, 5.0, 7.5 and 10.0 mg/1 of lead by diluting suitable volume of the standard solution with nitric acid (1 : 499) were prepared and repeated as above. After aspirating the solutions, the absorbance was measured.

Calculation

A standard calibration grap was constructed by plotting the absorbance versus mg of lead concentration of each standard. The concentration of the sample was read out from the graph.

$$\text{Lead, (mg/l)} = \frac{M}{V} \times 1000$$

where ,

M = mass of lead present in mg in the sample, and

V = volume of sample in ml

- **Detection of Cadmium IS3025(P)41-1992 RA2014**

1.ATOMIC ABSORPTION SPECTROPHOTOMETRY METHOD (DIRECT)

Principle

The cadmium concentration in the sample is determined by directly aspirating the sample into the flame of an atomic absorption spectrophotometer. The absorbance is measured at 228.8 nm by using a cadmium hollow-cathode lamp. But, the concentration range will vary with the sensitivity of the instrument used.

Interferences

Metals like nickel, lead, copper zinc, cobalt and chromium do not interfere up to 10 mg/l. Alkali and alkaline earth metals ere able to tolerate up to 5.003 mg/l. Iron cannot interfere up to 4000 milligram/litre.

Apparatus

Atomic absorption spectrophotometer with air-acetylene flame and Cadmium hollow-cathode lamp or multielement hollow-cathode lamp (used at 228.8 nm) were the apparatus used.

Reagents

The reagents used were concentrated hydrochloric acid, nitric acid, diluted nitric acid (1:499), stock and standard cadmium solutions.

Preparation of Stock cadmium solution

1.0g of pure cadmium metal was dissolved in minimum quantity of concentrated nitric acid and diluted to 1 litre with distilled water (1 ml= 1 mg of Cd).

Preparation of Standard cadmium solution

1 ml of concentrated nitric acid was added to 100 ml of the cadmium stock solution (5.4.4.1) and diluted to 1 litre with distilled water.

Procedure

To 100 ml portion of the acidified sample, 5 ml of concentrated hydrochloric acid was added and evaporated to 20 ml. After cooling, filter the sample was filtered and made up to 100 ml in a standard flask. For the determination of dissolved cadmium, 100 ml of the sample was filtered and acidified with 0.1 ml of concentrated hydrochloric acid. Aspirated the sample solution and measured the absorbance at 228.8 nm. prior to sample aspiration, nitric acid (1 : 499) was aspirated.

Then a reagent blank and a series of 100 ml standards containing 0.0, 0.05, 0.1, 0.5, 1 and 2 mg/l of cadmium were prepared by diluting a suitable volume of the standard solution with dilute nitric acid and repeated as above. After aspiratng the solutions and the absorbance was measured.

Calculation

A standard calibration graph was constructed by plotting the absorbance versus cadmium concentration (mg/l) of each standard and the concentration of the sample was read out from the graph.

$$\text{Cadmium ,mg/l} = \frac{M}{V} \times 1000$$

where,

M= mass of cadmium in milligram in the sample, and

V=volume of the sample in milliliter.

- **Detection of Zinc IS 3025(P) 49- 1994RA2014**

1 .ATOMIC ABSORPTION SPECTROPHOTOMETRY METIHOD (DIRECT)

Principle

The concentration zinc in the sample is determined by atomic absorption spectrophotometry. The filtered sample is directly aspirated to the atomizer, if dissolved zinc is to be estimated. For total recoverable zinc, an acid digestion procedure is done before the aspiration of the sample. But the concentration range will vary with the sensitivity or the instrument used.

Interferences

Heavy metals such as cadmium, lead, copper, nickel, cobalt and chromium up to 10 mg/l do not interfere. Alkali and alkaline earth metals were tolerated up to 4000 mg/l.

Apparatus

The apparatus used were atomic absorption spectrophotometer with air-acetylene Flame and multi-element hollow-cathode lamps or electrodeless discharge lamps (used at 213-8 nm).

Reagents

The reagents used were concentrated hydrochloric acid, nitric acid, diluted nitric acid (1:499) and Zinc solutions- both stock and standard.

Preparation of Stock zinc solution

1 gram of acid washed and rinsed zinc granules were dissolved or 1·245 I of zinc oxide (ZnO) in 20 ml or 1 : 1 nitric acid. Diluted to 1:1 with water. 1 millilitre = 1.0 milligram of Zinc.

Preparation of Standard zinc solution

100 milli litre of zinc stock solution and 1 millilitre of nitric acid were diluted to 1 litre with water.

Procedure

0·5 milli litre of nitric acid was added to 100 millilitre of the sample (filtered or unfiltered). For the determination of total recoverable zinc, add 5 millilitre of concentrated hydrochloric acid and the sample was filtered through acid washed filter paper. Made up to 100 millilitre in a volumetric flask, the solution was aspirated and the absorbance was measured at 213·8 nm. Prior to sample aspiration, nitric acid (1 : 499) was aspirated.

Then a reagent blank and sufficient standards containing 0'01, 0.05, 0·1, O·5, 1·0 and 2·0 milligram/litre of zinc by diluting suitable volume of the standard solution with nitric acid (1 : 499) were prepared and repeated as above. After aspirating the solutions and absorbance was measured.

Calculations

A standard graph was constructed by plotting the absorbance versus standard concentration for every standard. Then the concentration of the samples were read from the graph.

$$\text{Zinc, mg/l} = \frac{M}{V} \times 1\,000$$

where ,

M = mass of zinc present in milligram within the sample,

V = volume of sample in millilitre

B.Chlorophyll Content Analysis (Arnon, 1949)

One gram of finely cut/crushed fresh leaves was taken and transferred in a clean mortar. Ground the tissue to fine pulp with the addition of chilled 20ml 80% acetone in water. It had been then

centrifuged at 5000 rpm for 5minutes. The supernatant was transferred to in a dry volumetric flask. The procedure was replicated till the residue became colourless. Then the motor and pestle were washed thoroughly with acetone and picked the washings in volumetric flask. The absorbance of the solution was measured at 645nm and 663nm wavelength against the acetone(solvent) as blank (Arnon, 1949).

Calculation

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the subsequent equations :-

1. In µg/ml

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68\ (A_{663})$$

$$\text{Total chlorophyll} = 20.2(A645)+8.02(A663)$$

2.In mg/g tissue

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = 20.2(A_{645})+8.02(A_{663})) \times \frac{V}{1000 \times W}$$

Chapter 4

RESULTS

Heavy metal pollution is a global crisis, which can impart negative effects on both plants and animals. This pollution have the power to destruct the entire ecosystem. So it is very important to tackle the situation with modern science. It is the time to find new alternatives to cope up with the heavy metal contamination. Many scientists reported that Bryophytes can bioremediate heavymetals sucessfully from soil and also from water. Aquatic mosses, *Taxiphyllum barbeiri* and *Vesicularia montagnei* can be introduced to heavymetal contaminated

water to check the bioaccumulation property and to study the effects of heavy metals on chlorophyll content.

4.1. Analysis of water sample collected from Eloor region

Water sample were collected from Company padi, Eloor region near Ernakulam. Polluted sample were used for heavy metal analysis using standard protocols. The results shows the presence of following heavy metals such as Cadmium (Cd), Lead (Pb), Zinc (Zn) and Copper (Cu) at different concentration. The concentration of Cadmium, Lead , Zinc and Copper are shown in the table 1. Among the heavy metals analysed, concentration of Zinc was found to be in highest in polluted water sample, (88mg/l). It also contain 2 mg/l of Copper and 2.7 mg/l of Cadmium. When compared to Copper and Cadmium, the concentration of Lead is slightly higher than them (11.56 mg/l).

Fig:1. ***Vesicularia montagnei***

Fig:2 . ***Taxiphyllum barbieri***

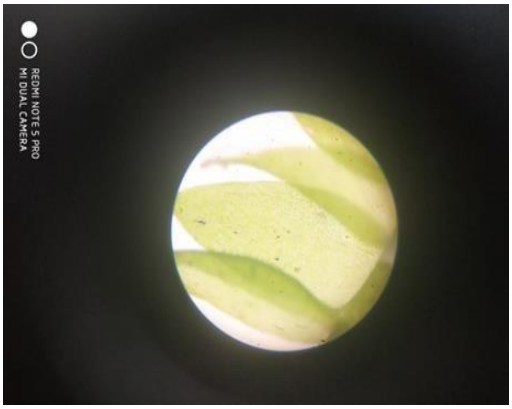

Fig:3.Leaf Apex of ***V.montagnei***

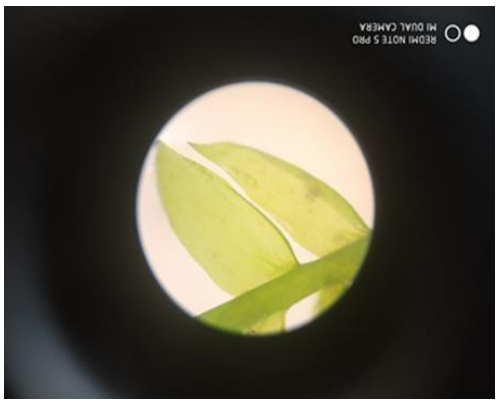

Fig:4.Leaf Apex of ***T.barbieri***

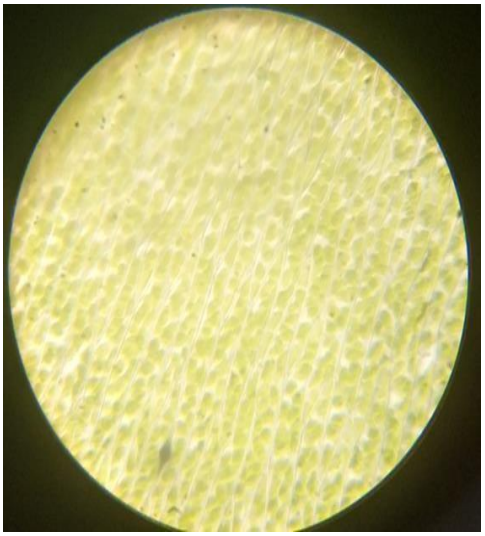

Fig:5.Internal leaf cells- *V.montagnei*

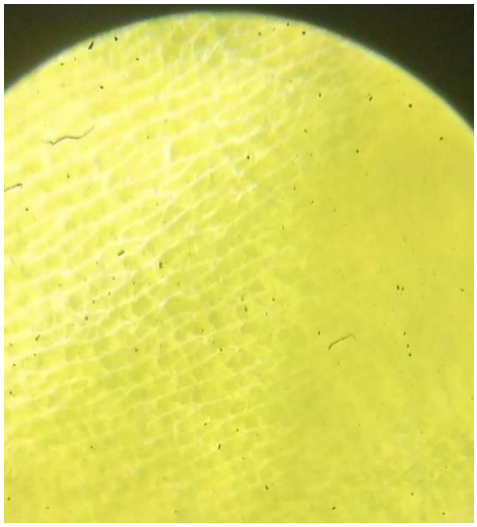

Fig:6.Internal leaf cells- *T.barbieri*

Fig:7.Thallus of *V.montagnei*

Fig:8.Thallus of *T.barbieri*

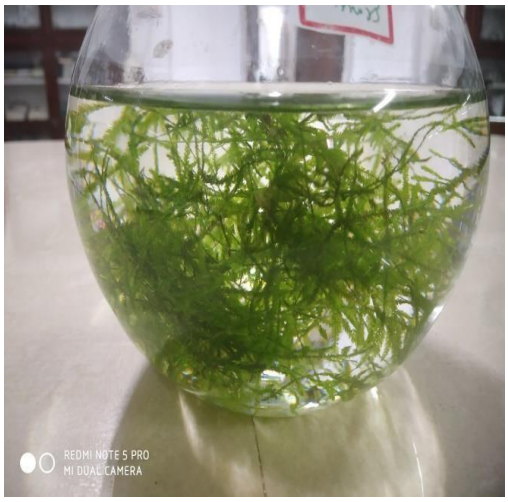

Fig:9.*V.montagnei* growing in uncontaminated water

Fig:10. *T.barbieri* growing in uncontaminated water

Fig:11.*V.montagnei* exposed to known concentration of heavy metals

Fig:12. *T.barbeiri* treated with known concentration of heavy metals

V.montagnei **before heavy metal treatment** **After Heavy metal treatment**

T.barbieri **before treatment** ***T.barbieri*** **after heavy metal treatment**

Fig:13.Indication of chlorosis before and after heavy metal treatment in both *V.montagnei* (Christmas Moss) and *T.barbieri* (Java moss)

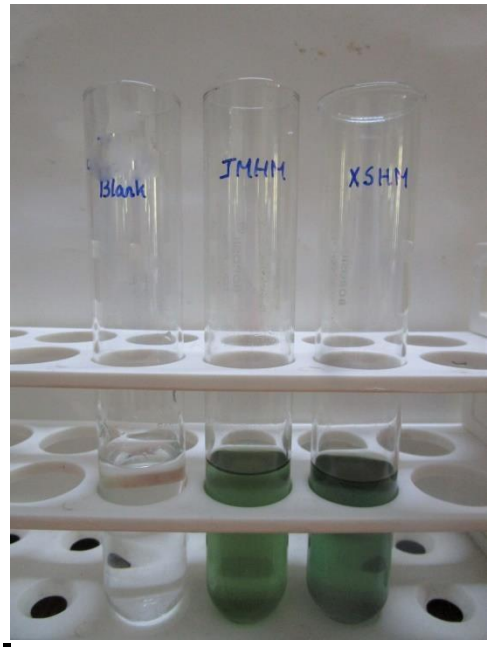

Fig:14.Estimation of chlorophyll before metal treatment

Fig:15.Estimation of chlorophyll after heavy heavy ment treatment

Fig:16. - Polluted Water collection from Companypadi , Eloor region

4.2.Estimation of Rate of absorption of heavymetals by T.barbieri and V. montagnei

Taxiphyllum barbieri and *Vesicularia montagnei* were grown in polluted water for one week and rate of heavy metals absorbed by two mosses were analysed. Table 1 showed that both *T.barbieri* and *V.montagnei* have the ability to absorb heavy metals like Copper, Cadmium, Zinc and Lead at different concentration. *Taxiphyllum Sps* have high rate of accumulation of Lead (9.339 mg/l) compared to *V. montagnei* (0.13 mg/l). Cadmium absorption rate is more or less similar in both *Taxiphyllum barbieri* (0.7 mg/l) and *Vesicularia montagnei* (0.22 mg/l) respectively. Rate of absorption of Copper is about 9.88 mg/l in *V. montagnei* that of 0.46 mg/l in *T. barbieri*. The heavy metal Zinc get more readily absorbed by *V.montagnei* (7.99 mg/l) than *T.barbieri* (0.13mg/l).

Samples	**Amount of heavy metals (mg/l)**			
	Cu	**Cd**	**Pb**	**Zn**
Water	2	2.7	11.56	88
T.barbieri	0.46	0.74	9.39	0.13
V.montagnei	9.88	0.233	0.13	7.99

Table 1:- Heavy metals present in water sample, *T.barbieri* and *V.montagnei*

4.3. Estimation of Chlorophyll content

T. barbieri and *V. montagnei* which were grown in polluted water are subjected to chlorophyll content analysis. The result have shown that there is a significant change in the chlorophyll content with respect to heavymetal bioaccumulation.

Table 2 shows the effect on chlorophyll content without absorption of heavy metals by the two mosses. The concentration of chlorophyll a in *T. barbeiri* is 13.79µg/ml and 12.84µg/l in *V.montagnei*. Chlorophyll b content is higher in *T. barbeiri* (21.54µg/l) to (16.02µg/l) *V.montagnei*. Total chlorophyll content in *T. barbeiri* is found to be 35.31µg/l and 28.87µg/l in *V.montagnei.*

Sample material	**Amount of Chlorophyll content ((µg/ml*)***		
	Chlorophyll a	**Chlorophyll b**	**Total Chlorophyll**
T.barbieri	13.79	21.54	35.31
V.montagnei	12.84	16.02	28.87

Table 2 :- Comparative amount of chlorophyll content

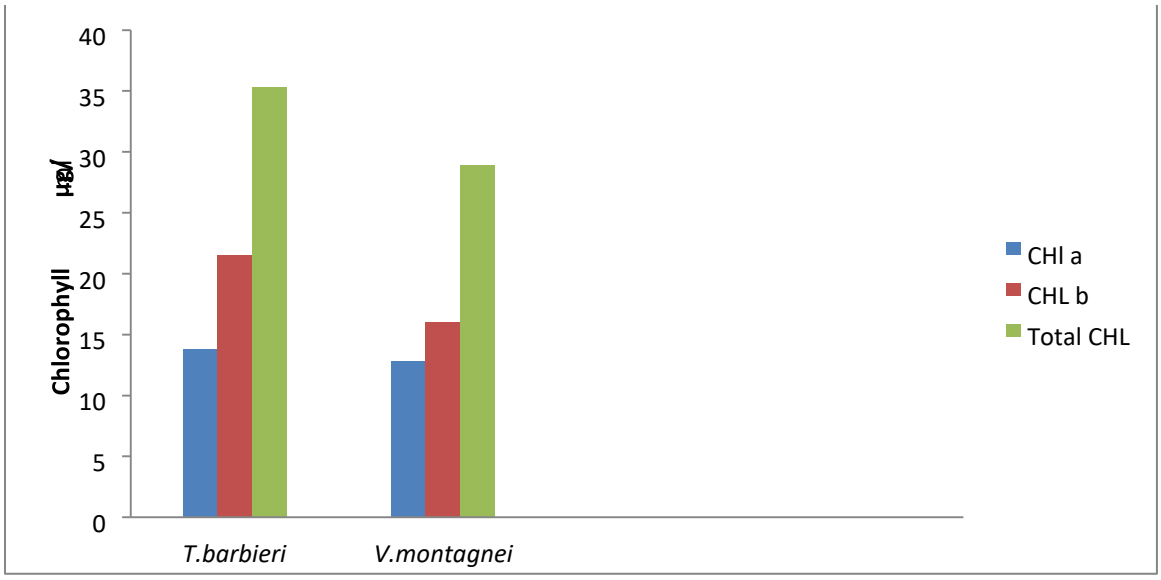

Fig :17. Comparative amount of chlorophyll content in both *T.barbieri* and *V.montagnei*

4.3.Estimation of chlorophyll content in *T.barbieri* and *V. montagnei* after treated with heavy metal in vitro

Both *T. barbieri* and *V. montagnei* have different heavymetal accumulation rate from 24 hrs to 96 hrs of period of time. It also indicate a marked decrease in chlorophyll content from day 1 to day 4.

When Java moss treated with known concentrtion of Cadmium Chloride($Cdcl_2$), concentration of chlorophyll a changed gradually from 22.21 mg/g tissue, 17.2mg/g tissue, 10.03 mg/g tissue and 6.60 mg/g tissue at 24hrs, 48hrs, 72hrs and 96hrs respectively.

Chlorophyll b also in the range from 38.81 mg/g tissue, 13.43mg/g tissue, 16mg/g tissue and 10.41 mg/gtissue and it is represented in figure 18.

It is found that the heavy metal Cu could induce more reduction in the chlorophyll content in both *T. barbieri* and *V.montagnei*. When *T .barbeiri* treated with Copper Sulphate ($CuSo_{4)}$, the total chlorophyll content vareird from 61.12mg/g tissue, 30.63mg/g tissue, 26.03mg/g tissue and 17.01 mg/gtissue with 24hrs, 48hrs, 72hrs and 96hrs of exposure. Whereas in *V. montagnei*, the total chlorophyll content declined from 34.79mg/g tissue, 26.5mg/g tissue. 25.8mg/gtissue and 16.3mg/gtissue.

Total chlorophyll content in *T. barbieri* get lessened when treated with Lead chloride ($(Pbcl_2$), in the range 39.0mg/g tissue, 29.67mg/g tissue, 2.01mg/g tissue, and 20.9 mg/g tissue and in *V. montagnei* it has characteristic variation from 38.53 mg/g tissue, 25.79mg/gtissue, 21.21 mg/g tissue and 25.78 mg/g tissue at 24hrs,48hrs, 72hrs and 96hrs.

After exposed to known concentration of Zinc chloride, chlorophyll content value in *T.barbieri* changed to 41.68 mg/g tissue, 30.85 mg/g tissue, 26.02 mg/g tissue, and 12.191mg/g tissue. Simillarly, in the case of *V.montagnei* chlorophyll value get declined from 38.5 mg/g tissue, 28.5 mg/g tissue, 16.47 mg/g tissue and 13.78 mg/g tissue.

V.montagnei have effects on chlorophyll content which were grown in in vivo Copper sulphate solution, i.e, the value get descended from 26.5 mg/g tissue,25.72 mg/g tissue, 12.3mg/g tissue and 4.7.mg/gtissue.

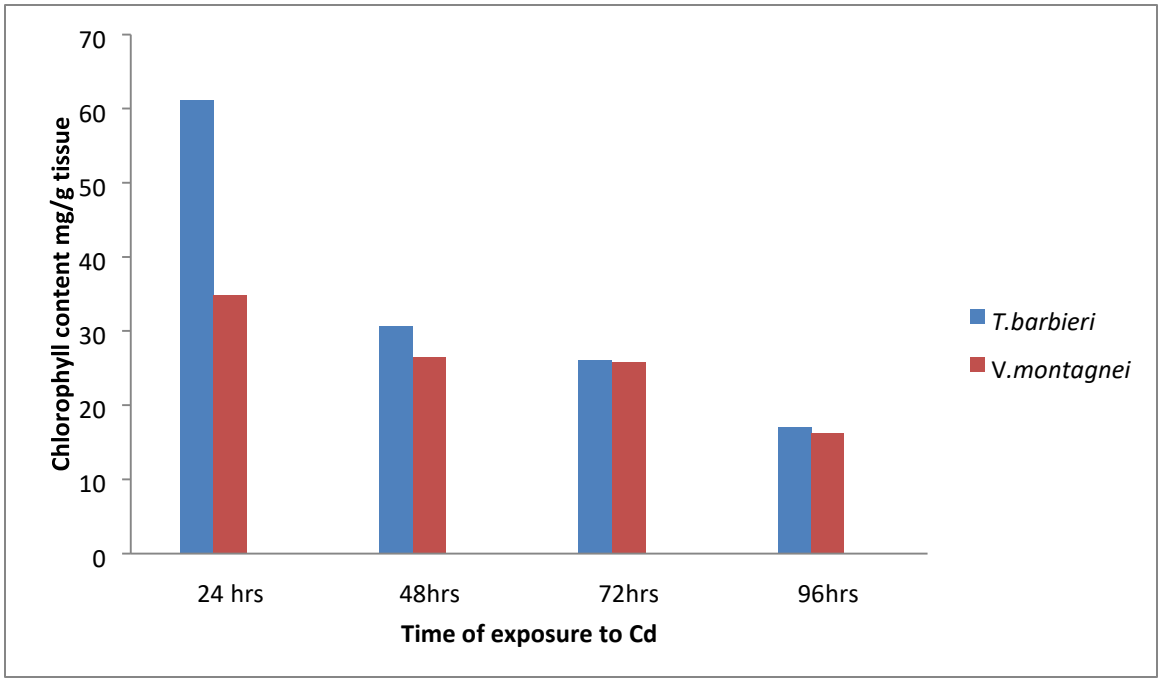

Fig:18.Chlorophyll content variation in response to Cadmium chloride exposure

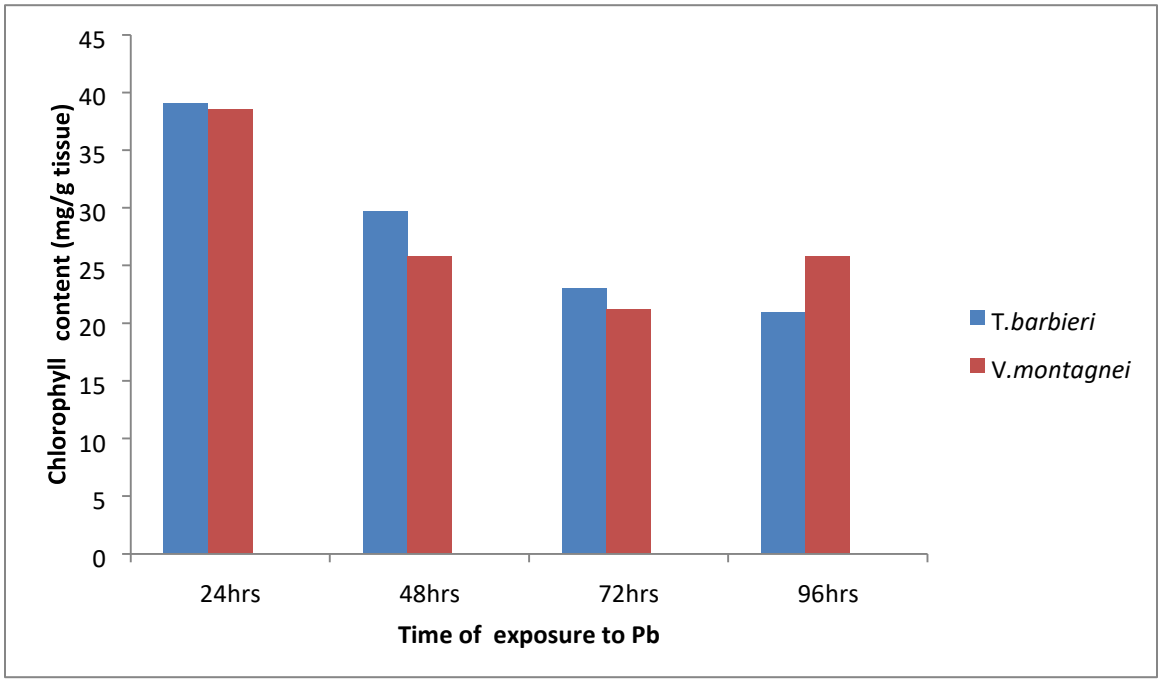

Fig:19.depicts the effects on chlorophyll content when treated with Lead chloride ($Pbcl_2$)

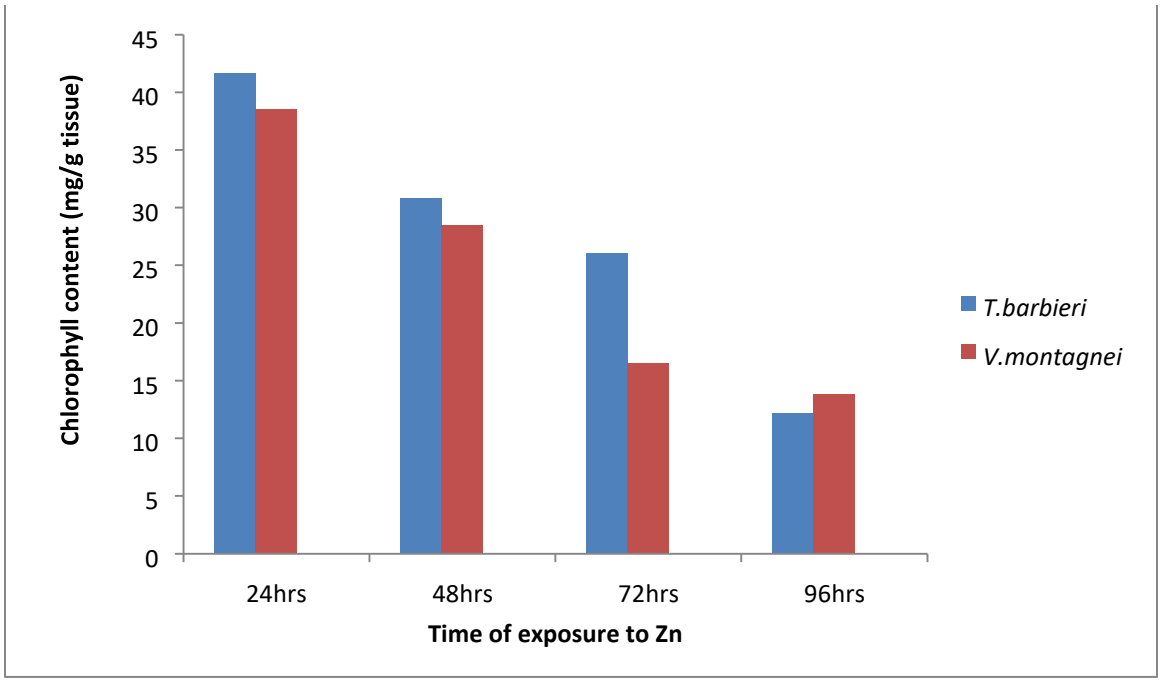

Fig:20.Chlorophyll content analysis after exposure to Zinc Chloride ($ZnCl_2$)

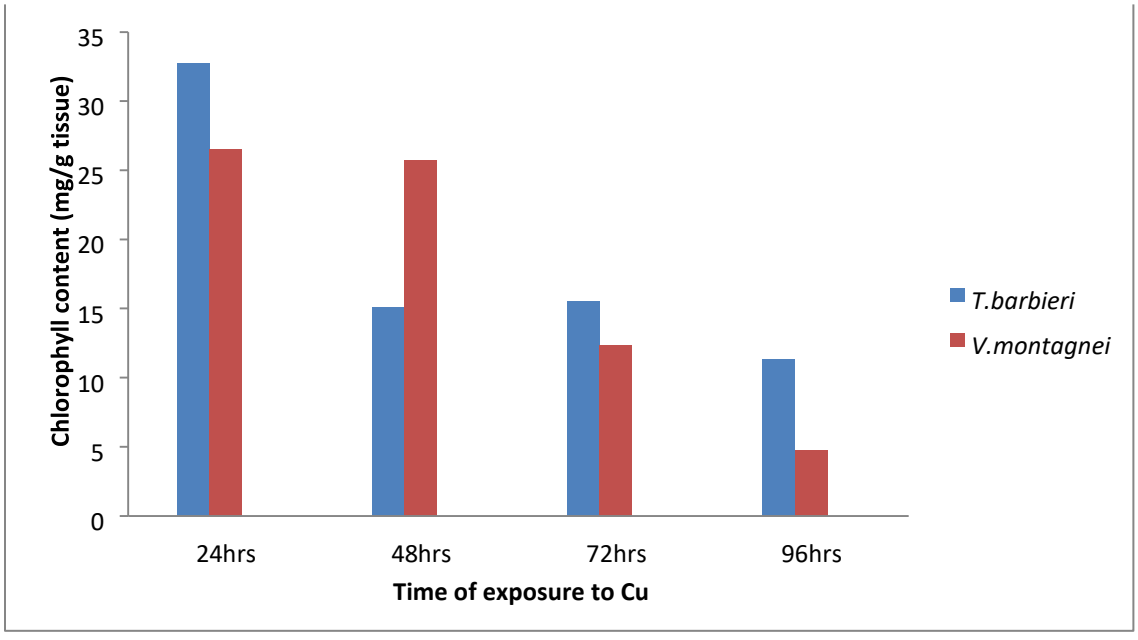

Figure:21. represents Estimation of chlorophyll content after treated with Copper Sulphate (Cuso4)

Correlation between Cadmium (Cd) accumulation and chlorophyll content in *T.barbieri*

Cadmium(Cd) accumulation in *T.barbeiri*	Chlorophyll content(mg/g tissue)	
11	14.3	***r* =**
20.7	29.67	**-0.019**
31.26	30.87	
44.39	15.08	

Table:3.Correlation between Cd and chlorophyll content in *T.barbieri*

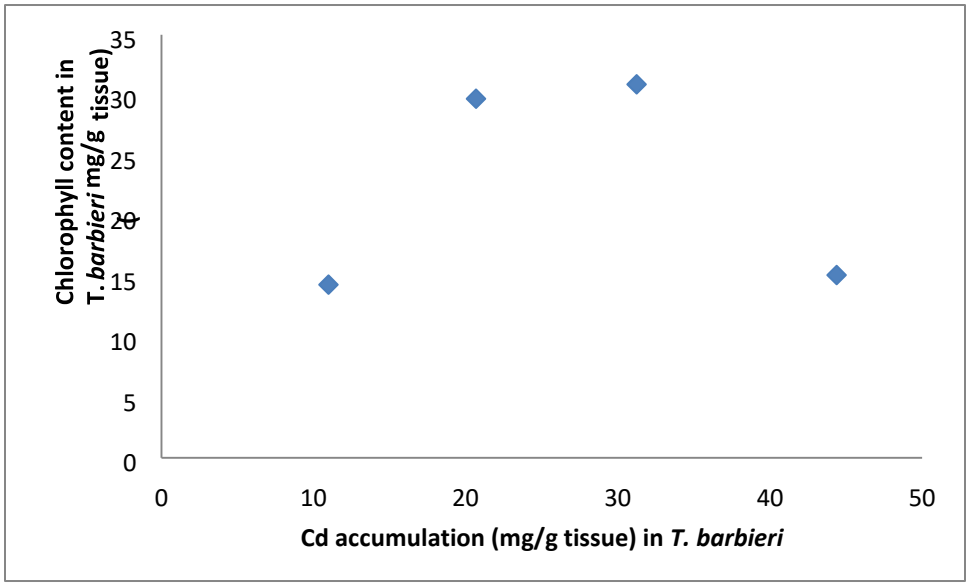

Fig:21.Scattered diagram depicting negative correlation between Cd accumulation and chlorophll content

Correlation between Cadmium accumulation and chlorophyll content by *Vesicularia montagnei*

Cadmium accumulation by *V.montagnei* (mg/g tissue)	Chlorophyll content (mg/g tissue)	
11.27	*26.59*	***r*** **=**
18.34	*18.34*	-0.05269
25.12	*25.12*	
16.89	*16.89*	

Table :4. Showing correlation between Cd accumulation and chlorophyll content in *V.montagnei*

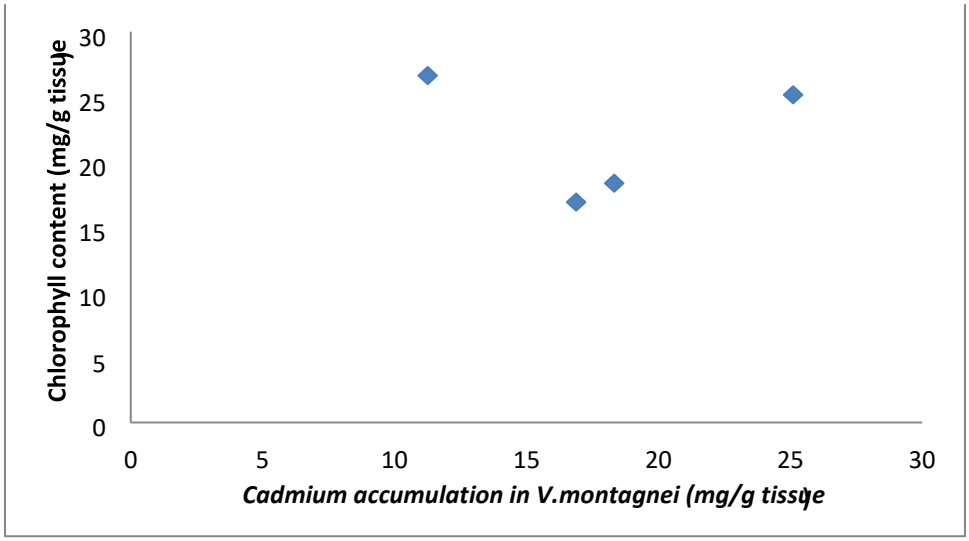

Fig:22.diagram Showing negative correlation between Cd accumulation and chlorophyll content in *V.montagnei*

Correlation between Pb accumulation and chlorophyll content in *T.barbieri*

Pb accumulation in *T. barbieri* (mg/g tissue)	Chlorophyll content (mg/g tissue)	
14.64	29.67	***r*** **= -** *0.81773*
12.16	21.7	
22.1	17.45	
28.7	12.9	

Table :5.Showing correlation between Pb accumulation and chlorophyll content in *T.barbieri*

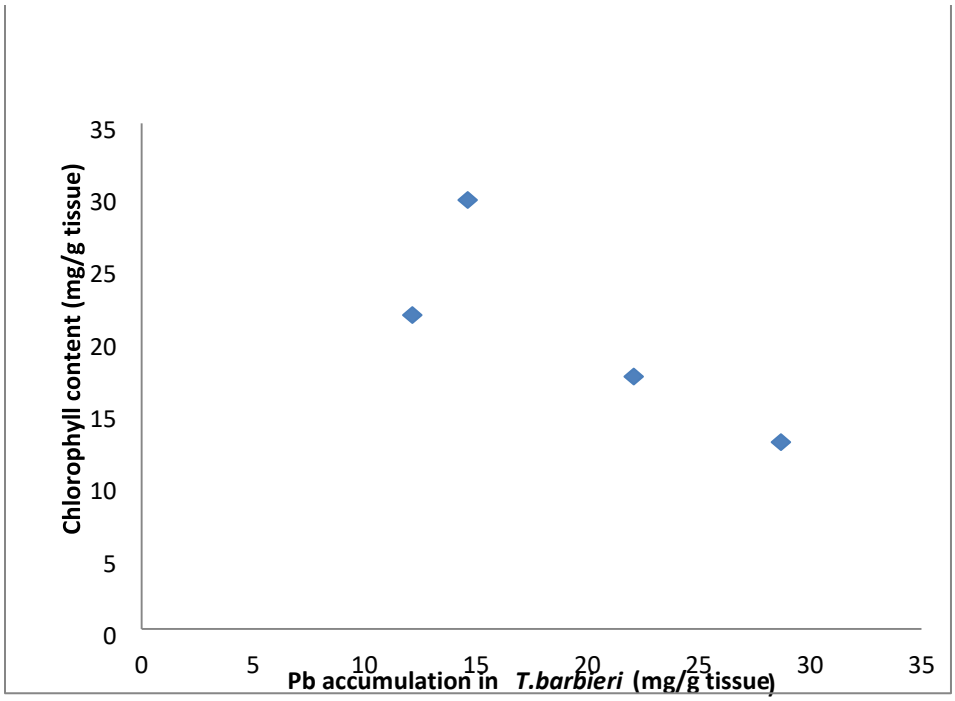

Fig:23. diagram Showing negative correlation between Pb accumulation and chlorophyll content in *T.barbieri*

Correlation between Lead absorption and chlorophyll content in *V.montagnei*

Pb accumulation in *V.montagnei* (mg/g tissue)	Chlorophyll content (mg/g tissue)	
15.17	38.53	
21.6	23.79	***r*=**
19.25	21.2	-0.76179
23.14	25.4	

Table :6.showing correlation between Lead absorption and chlorophyll content in *V.montagnei*

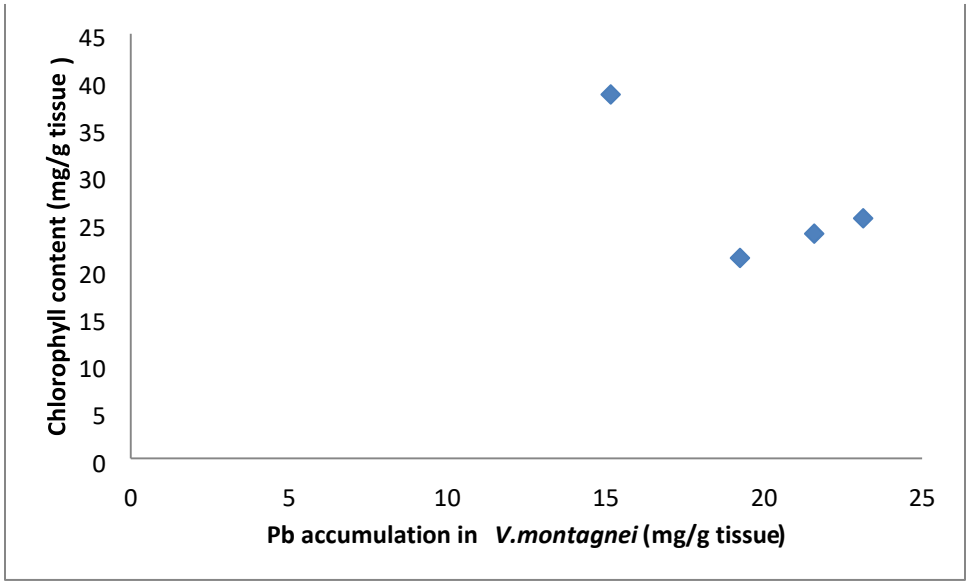

Fig:24.diagram showing negative correlation between Lead absorption and chlorophyll content in *V.montagnei*

Correlation between Zn accumulation and chlorophyll content in *T.barbieri*

Zn accumulation in T.barbieri (mg/g tissue)	Chlorophyll content (mg/g tissue)	
9.14	41.68	***r* = -**
31.67	30.895	***0.88837***
24.53	26.02	

38.76	12.191	

Table :*7.showing correlation between Zn accumulation and chlorophyll content in *T.barbieri

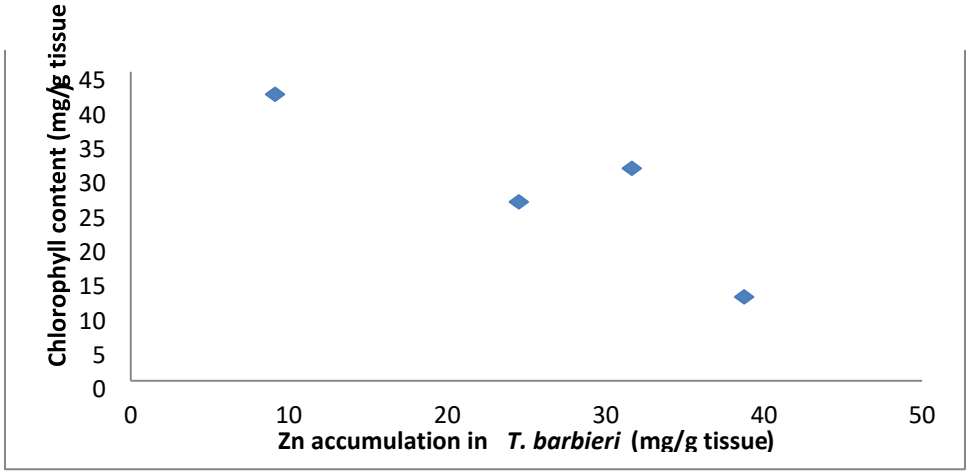

Fig:25.diagram showing negative correlation between Zn accumulation and chlorophyll content in *T.barbieri*

Correlation between Zn accumulation and chlorophyll content in *V.montagnei*

Zn accumulation in *V.montagnei* (mg/g tissue)	Chlorophyll content (mg/g tissue)	
7.15	33.44	**r = -**

16.3	23.79	**0.97122**
24.8	16.47	
22.6	13.78	

Table:8.Showing correlation between Zn acccumulation and chlorophyll content in *V.montagnei*

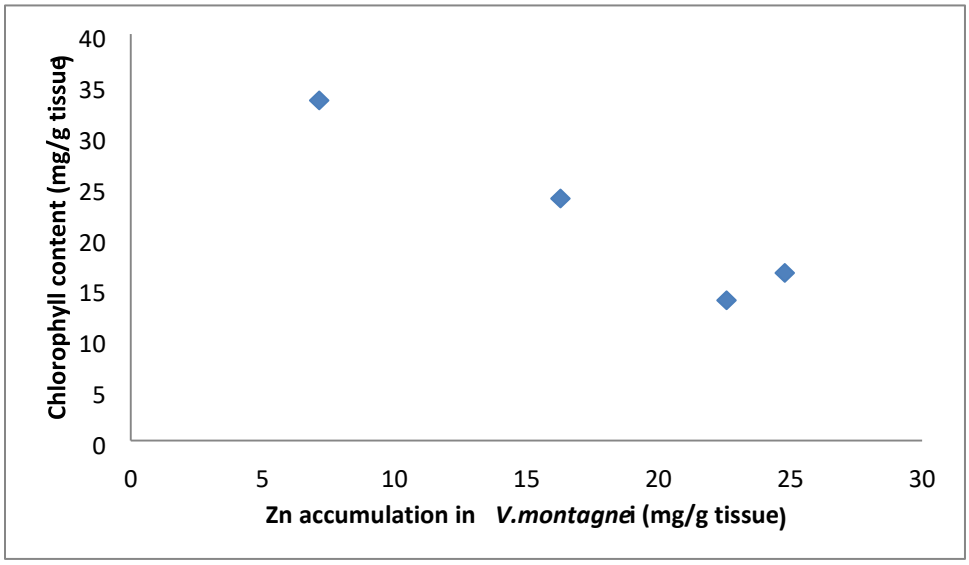

Fig:26.diagram showing negative correlation between Zn acccumulation and chlorophyll content in *V.montagnei*

Correlation between Copper (Cu) accumulation and chlorophyll content in *T.barbieri*

Cu acumulation in *T.barbieri* (mg/g tissue)	Chlorophyll content (mg/g tissue)	
11.21	32.7	***r* = -**
18.93	15.08	**0.87651**
17.12	15.49	
26.47	11.31	

Table:9.showing correlation between Cu accumulation and chlorophyll content in *T.barbieri*

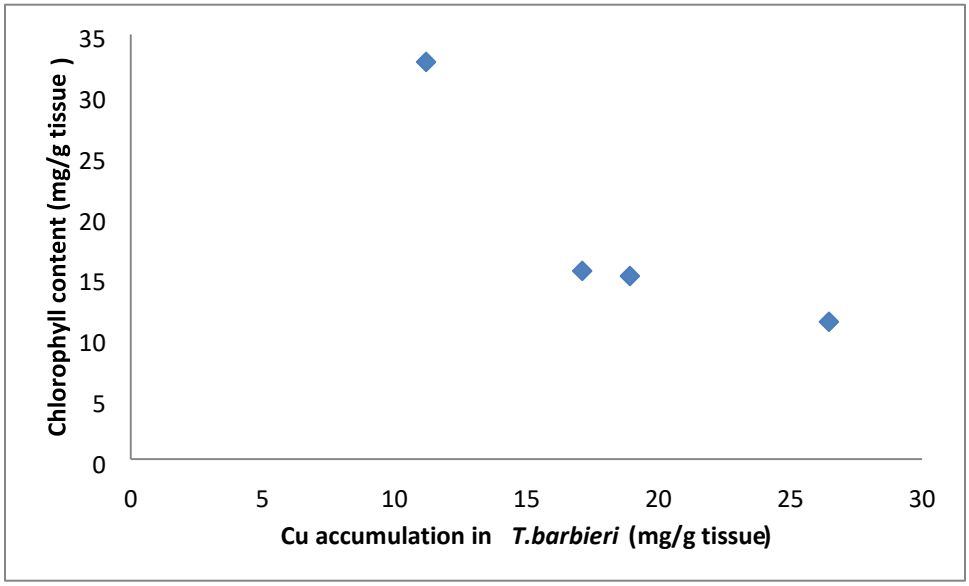

Fig:27.diagram showing negative correlation between Cu accumulation and chlorophyll content in *T.barbieri*

Correlation between Copper (Cu) accumulation and chlorophyll content in *V.montagnei*

Cu accumulation in *V.montagnei* (mg/g tissue)	Chlorophyll content (mg/g tissue)	
12.73	29.353	***r* = -**
17.27	20.182	***0.98433***
27.53	12.3	
33.56	5.41	

Table :10.Showing correlation between Cu accumulation and Chlorophyll content in *V.montagnei*

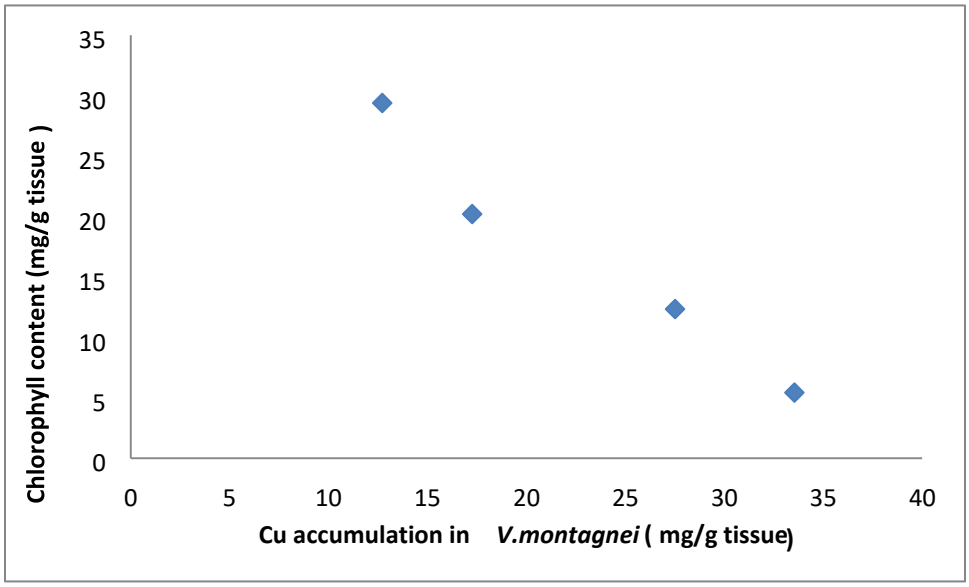

Fig:28.Showing negative correlation between Cu accumulation and Chlorophyll content in *V.montagnei*

DISCUSSION

Heavy metals are naturally occurring elements with high relative atomic mass and a density a minimum of five times bigger than that of water. They have numerous industrial, domestic, agricultural, medical and technological applications which led to their wide distribution in the environment, raising perturbs over their potential role on human health and the environment. Their toxicity relies on factors including the dose, route of exposure, chemical species, age, gender, genetics, and nutritional status of exposed individuals (Tchounwou *et al*., 2012). Some heavy metals are essential and others nonessential. Nonessential heavy metals comprise cadmium, chromium, mercury, lead, arsenic, and antimony. These can alter biochemical and physiological processes in organisms (Edelstein *et al* ., 2018).

Due to the absence of true roots and thick cuticle and with high surface to volume ratio and a high cation-exchange capacity, allowing capillary action to transport accessible minerals and water to entire surface (Viviana maresca *et al*., 2020).

Moreover, phyllids or the leaf-like sructures and thalli of bryophytes have extreme absorbent surfaces and an lack of waxy cuticle over the laminal surfaces. Therefore, cell walls can freely absorb moisture and a wide range of minerals and metal ions from water that flows over the plant (Koz and Cevik, 2014).

Mechanism of uptake of heavy metals

One mechanism which led to the uptake of metals include Calcium Calmodulin model. When plants absorbed excess heavy metals, led to the alteration of calcium channel activity and resulted in the increase of calcium flux occurs in the cell. Within the cell, calcium acts as a second messenger and activates calmodulin which in turn boost up the uptake of heavy metals, transport and metabolism (Ghori *et al*., 2019). Some functional groups present in the cell wall of bryophytes acts as binding site of heavy metals. These comprise of phosphodiester, carboxyl, phosphoryl, amine and polyphenol functional groups (González and Pokrovsky, 2014).

Bryophytes have distinctive process for uptake and translocation of minerals and heavy metals. The range of known transport mechanisms or specialized proteins embedded within the

plant cell plasma membrane concerned in ion- uptake and translocation include (1) Proton pumps, (ATPases that absorbs energy and generate electrochemical gradients), (2) co- and antitransporters (proteins which use that electrochemical gradients generated by ATPases to carry out the active uptake of ions), and (3) Channels (Proteins that promote the transport of ions in to the cell). Every transport mechanism require a range of ions.

Both *Taxiphyllum barbieri* and *Vesicularia montagnei* have shown wide response to heavy metal accumulation. As a result of the deposition of heavymetals like Cd, Cu, Pb and Zn ,the chlorophyll content get reduced. It indicate the inverse relationship between heavy metal bioaccumulation and chlorophyll content.

During first day of exposure to Cadmium Chloride, reduced chlorophyll content is observed in *Vesicularia montagnei* compared to *Taxiphyllum barbieri*. Afer 48hrs, both Species *Taxiphyllum barbeiri* and *Vesicularia montagnei* shows more or less similar cadmium accumulation rate. It indicate that chlorophyll content in both mosses get declined in a similar pattern when treated with cadmium chloride.

Excess Lead chloride exposure negatively affect the chlorophyll concentration in *T.barbieri* than *V.montagnei*. Concentration of chlorophyll is gradually decends from 24hrs to 96 hrs of treatment. So, *Taxiphyllum barbieri* can sucessfully used to biomonitor the heavymetal Lead.

Chlorophyll content in the *V. montagnei*, get decreased due to 72 hours of Zinc chloride treatment. That means *V.montagnei* is fruitful for the phytoremediation of Zinc in the living environment. But as the period of exposure progressed, both mosses have proved to be effectful for the heavymetal Zinc.

T.barbieri can tolerate the effect of the heavy metal copper as compared to *V.montagnei.* As the accumulation of copper increased, the chlorophyll content get reduced. This will disrupt the entire physiological activity of *V. montagnei.*

Baek et al,(2022) have worked on *Arabdiopsis thaliana* and explained that as the concentrations of the metals increased, a gradual decrease in root and shoot lengths, a chlorophylls. It also caused rise in anthocyanin content and variations in carotenoid content

depending on the type of metals.. The harmful effect of the metals decreased in the following pattern: Copper> Mercury > Lead > Zinc > Manganese.

The inhibitory effects of Cadmium, Copper, Zinc, Lead, and Iron on root elongation, concentraton of photosynthetic pigments, and metal accumulation in the roots and shoots of, *Sinapis alba* were examined. Metals can be arranged on the basis of inhibition of growth in a order Copper > Cadmium > Iron = Zinc > Lead. All the metals, except Iron, were bioaccumulated in a quite large amount in the roots than in the shoots. Chlorophyll a and Chlorophyll b content get reduced due to the accumulation of Cadmium, Zinc, Copper and lead. Besides these, Zinc and lead decreased the carotenoid content, but it is less than that of total chlorophyll content (Fargasova, 2001).

Bruns *et al.*, (1997) studied *Fontinalis antipyretica* as biomonitoring agent of heavy metals. In his study he stated that *F.antipyretica* can accumulate Cadmium, Copper, Lead, and Zinc. It putforward a positive correlation between phytochelatins and metal absorption. He reported that reduction in the chlorophyll content in the ratio chlorophyll a: chlorophyll b as 10% -15%. This loss is due to the activity of selected heavy metals on the photosynthetic system.

When Mosses get exposed to polluted water, which contain 2 mg/l Cu, 2.7 mg/l Cd, 11.56 mg/l Pb and 88 mg/l Zn, in which *T.barbieri* get accumulated 9.88mg/l Cu, 0.74mg/l Cd, 9.33 mg/lPb and 0.13 mg/l Zn and *V.montagnei* absorbed 9.88 mg/l Cu, 0.22 mg/l Cd, 0.1 mg/l Pb, 7.99mg/l Zn respectively. The total chlorophyll content also changed to 35.31 mg/gtissue (*T.barbieri*) and 28.87mg/gtissue *(V.montagnei)* from 47.23 and 32.17mg/g tissue.

Maresca *et al*., (2020) have reported that *Bryum radiculosum* Brid. (Bryaceae) grown in industrial areas of Portoscuso (Sardinia, Italy) and it was succeeded and recommended as a bioindicator for trace elements such as Lead, Cadmium and Zinc, with accumulation rates at 61 to 2141 mg kg^{-1}, 3 to 40.6 mg kg^{-1} and 32 to 2360 mg kg^{-1}, respectively.

Chettri *et al*., (1997) have noted the effect of Cu, Pb, and Zn on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis.* Study revealed that as 40%. chlorophyll content declined as the Copper accumulation exceeds 1560 µg/g. Like wise, 15% to 30% chlorophyll get degraded due to Zinc and Lead. There was an remarkable decrease in the

ratio of chlorophyll a:b from 3.0 to 0.4 for *C. convoluta* because of Copper content exceeded 175 mg g−1 DW and 200 mg g−1 DW copper led to 3.2 to 0.8 reduction for *C. rangiformis* respectively. Zinc and lead caused a 10 to 15% decrease in the ratio of chlorophyll a:b for *C. convoluta* at high concentrations greator than140 and 20 mg g−1 DW, respectively.

Studies in *Sphagnum squarrosum* discovered that after heavy metal stress, the chlorophyll content varied from 3.08 - 0.07a mg/g, 2.87 - 0.09a mg/g, 2.33 - 0.06b mg/g, 2.95 - 0.07a mg/g (Anuj Saxena, 2006).

Copper is an essential metal which is required for growth and development of plants. It plays a key role in regulating physiological functions such as the photosynthetic and respiratory electron transport chains, nitrogen fixation, protein metabolism, antioxidant production, the reactive oxygen species defense system, cell wall metabolism, and hormone perception. They acts as an vital cofactor for many metalloproteins.

Excessive Copper concentrations are harmful to plants at the cellular level because it may lead to inactivation and destruction of enzyme activity or protein functions. Hence copper accumulation may affect the whole physiological activities of the plants (Yruela , 2005).

Mosses can absorb excessive copper. *Taxiphyllum barbieri* filtered 0.46mg/l whereas *Vesicularia montagnei* accumulated 9.88mg/l of Copper. Printarakul *et al*., (2022) identified that *P. acutifolia* var. *birmanica* gametophyte absorbed 8.5 mg kg^{-1} to *S. cataractae* protonema absorb 530.1 mg kg^{-1} copper.

Lopez *et al*., (1989) work revealed that the ratio of chlorophyll a : chlorophyll b in moss *F.antipyretica* treated with copper and cadmium solutions. It changed from 0.8 to 2.4 and from 0.6 to 1.7 for Cadmium solutions respectively, mean ratio value remains 1.3. Likewise, mean chlorophyll a:b ratio had lowered with lead moss – 1.5, whereas a close margin between maximum and minimum values 1.2 – 1.9 was obtained.

Results also showed that 47% chlorophyll loss takes place when treated with Cu solution at 48-hour which can correlate with about 43% chlorophyll decline in the same species by (Lopez *et al*., 1989).

It is reported that *Fontinalis antipyretica* at its orginal site between the upstream of the Maritsa River and industrial and urban area of Plovdiv district, 25% of chlorophyll pigment get lessened (Yurukoval and Gecheva, 2004). This implied that *F. antipyretica* can withstand Copper, Cadmium and Lead pollution and has the capacity to endure a serious chlorophyll loss under stress.

It is obvious that when both Java and Christmas were grown in polluted water from Eloor region, there was a decrease in chlorophyll content. It specifies a positive sign of using *T. barbieri* and *V.montagnei* as biomonitors of heavy metal pollution in Eloor region.

When mosses were treated with controlled concentrations of heavy metals, there is a gradually decline in chlorophyll content from 24 hrs to 96 hrs. It is now clear that heavy metal assemblage have effects on chlorophyll. Degradation in chlorophyll concentrations generally speculates increasing stress. But long lasting effects on pigment ratio could be coincides with well defined effects on photosynthesis and the long-term survival (Gana *et al*., 2008).

The study of Claver *et al*., (1994) confirmed that heavy metal accumulation in bryophytes and mobility of metals relies, on their living environment and the source of minerals. The quantity, intensity, and the duration of the precipitation regulate the amount of accumulated and leached heavy metals from the earthly bryophytes (Chakrabortty and Paratkar, 2006).

Heavy metal accumulation rest on the competition between cell wall binding materials and heavy metals. Heavy metals have different binding site on the cell wall of bryophytes, so each one of them will compete with other for intracellular absorption.

Copper, Cadmium and lead are harmful and extensive heavy metals released into the aquatic ecosystem by several means. Cadmium is somewhat mobile in freshwater ecosystems and can be easily absorbed by freshwater biota. The dominant effects of Copper, Cadmium and Lead toxicity in higher plants include chlorosis and reduced growth. Particularly, Cadmium can disrupt the water balance, damage the photosynthetic apparatus, lowers chlorophyll and carotenoid content. It can also affects the activity of various enzymes through the substitution of other metal ions (Sanit A Di Toppi & Gabbrielli, 1999).

There are many assumptions considering the mechanisms which cause inhibition of enzymes e.g. protochlorophyllide reductase by heavy metals (Stobart *et al*, 1985). Copper is one of the heavy metal which can inhibit photosynthesis and reproductive processes, and eventually plant growth becomes restricted or impossible. Uptake of heavy metals resulted in the miscellaneous damage in cells of a single tissue.

It might represent a systematic distribution of heavy metal resistance which manifests phenotypic diversity of cells within a tissue. But it may be a clue, which direct towards an active stress response reaction of the plants and then these cells which acumulate heavy metals will die, but this proves the abidance of the remaining cells, which were maintained a low intracellular heavy metal concentrations.

The most important reason for the inhibition is due to its action on enzymes, which inturn affect all the metabolic processes of plants. Heavy metal stress and degradation in chlorophyll content are related to one another. This reduction is as a result of inhibition of the enzymes responsible for chlorophyll biosynthesis .

Copper toxicity inhibit metabolic processes by restricting the action of enzymes for chlorophyll biosynthesis, and this may be the chief cause of inhibition and decreased chlorophyll content. On the contrary ion toxicity affects photosynthesis by causing alterations in chloroplast ultrastructure, preventing the synthesis of photosynthetic pigment, chlorophyll and enzymes of the Calvin cycle.

In higher plants and green algae, the mechanism of heavy metal-induced damage that cause inhibition of photosynthesis, was evaluated under naturally applicable metal concentrations down to the sub-micromolar range (Küpper *et al*., 1996), which involves the in vivo substitution of Mg^{2+} in the Chlorophyll molecule by heavy metal ions; that is, [Hms]Chl formation (Küpper *et al.*, 1996, 1998, 2002b).

Cadmium is a noxious transition metal. It have negative effects on plant's health, at morphological, (ultra) structural, molecular and functional level. Its competition with other divalent metals may lead to binding ligands that can bind other metals. The photosynthetic system also get damaged.

In actual fact, it is well known that cadmium, (Cd^{2+}) can substitute magnesium (Mg^{2+}) both in the RuBisco catalytic center and in chlorophyll (Chl) porphyrin ring (Kupper and Andresen, 2016). In the last, cadmium binding induces bleaching and subsequent degradation, thereby causing decreases in photosynthetic activity, which leads to serious impairments (Grimm *et al*., 2007).

Marchantia polymorpha and *Brachythecium populeum* were applicable to monitor heavy metal pollution in polluted areas of Mussorie. Goodman and Robert observed that *Hypnum cupressiforme* have the potential to accumulate heavy metals even though they are dead (Vinay Sahul *et al*., 2007).

The loss in total chlorophyll content in bryophytes under heavy metal stress might be a result of the heavy metal intrusion with chlorophyll synthesis either through the direct mechanism of inhibiting an enzymatic step or by bring about the deficiency of an essential nutrient (Zengin and Munzuroglu, 2005).

Cadmium is a perlious metal. Exposure to cadmium can inhibit photosynthesis and induces nutrient deficiencies, which leads to chlorosis in gametophytic tissues of *Physcomitrium patens* of Funariaceae and aquatic moss, *Fontinalis antipyretica* of family Fontinalaceae (Bellini *et al*.,2021).

Zinc and Iron are major components of various enzymes and proteins in plants and so they become essential micronutrients needed for biota. High concentrations of Zinc and Fe, on the contrary, can be noxious to moss cells, affecting the whole plant by decreasing moss growth and development ((Dos Santos *et al* ., 2017).

Paride Papadia confirmed that *Taxiphyllum barbieri* can efficiently used to decontaminate heavy metal polluted water. *T. barbieri* have shown wide response to heavy metals like Cadmium, Copper, Arsensic, Lead etc. (Paride Papadia *et al*., 2020).

Degradation in the chlorophyll content with respect to accumulation of heavy metals due to the modifications on the chloroplast. In the chloroplast, heavy metals alter the structure of thylakoid membranes, which in turn, affect light reaction processes at first hand, mostly those associated with PSII. Excess Copper has a potent effect on chloroplast's fine structure, resulting

in deterioration of grana stacks and stroma lamellae and an elevation in the number, size of plastoglobules and intrathylakoidal inclusions (Baszynski *et al*., 1988).

The thylakoid membranes which contain proteins and lipids in embedded form are directly involved in photosynthesis. Metals which induces oxidative damage to these essential protein and lipids, are the primary reason for the inhibition of electron flow. De Filippis *et al*., (1981) advocated that heavy metals can hinger the activity of the enzyme NADP reductase. It can also affect photosystems. PSII are the main site of action for heavy metal. It may distrub the reducing capacity of PSII (Sinha *et al*., 2021).

In Copper (Cu^{2+})inhibited thylakoids, diphenyl carbazide addition does not reveal the loss of Hill activity. The maximum yield of fluorescence induction revived by hydroxylamine in trisinactivated thylakoids is remarkably reduced by Cu^{2+}. This indicate that Cu^{2+} does not affect the donor side of PSII but on the reaction center of PSII or on components ahead.

Thermoluminescence and delayed luminescence studies revealed that charge recombination between the positively (+) charged intermediate in water oxidation cycle (S_2) and negatively (-) charged primary quinone acceptor of pSII (Q_A^-) is not much influenced by Cu^{2+}. But he $S_2Q_B^-$ charge recombination is considerably inhibited which counterpart the loss of Hill activity. This implies that Cu^{2+} disrupt photosystem II photochemistry, which primarily impinge the function of the secondary quinone electron acceptor, Q_B. It indicate that copper (Cu^{2+}) does not block electron flow between the primary and secondary quinone acceptor but modifies the Q_B site in such a way, that it becomes inappropriate for further photosystem II photochemistry (Mohanty *et al*., 1989).

Heavy metals often directly affect the structure of thylakoid membranes , chloroplast and also on photosynthetic proteins. Thus they perniciously affect the efficacy of photochemical properties in dark-adapted leaves and photosystem II (Yang-Er Chen *et al*., 2015).

The major damages caused by heavy metals include inhibition of ATPsynthase/ATPase, uncoupling of electron transport, inhibition of electron transport by inhibition of the water-splitting system and finally irreversible damage of the electron transport system. Comparing the

toxicity of Lead(Pb^{2+}), Cadmium(Cd^{2+}) and Zinc(Zn^{2+}), the heavy metal that can inhibit ATPsynthase/ATPase and destruct the membranes is Pb^{2+} but Zn^{2+} can disturb the water-splitting system (Teige *et al*., 1990).

Cadmium have the capability to disturb the structure and function of the most important chlorophyll-protein complexes - light-harvesting chlorophyll a/b protein complex II (LHCII) and light harvesting antenna molecules in photosynthesis. At first, cadmium indirectly change the oligomeric structure of this complex, which is of most important for the efficient collection of light energy. Metal leads to a reduction in the level of fra/75A3-hexadecenoic fatty acid, particularly bound in sn-2 position of the chloroplastic phosphatidylglycerol (PG) molecules (Krupa, 1988).

Heavy metal localization in bryophyte tissues suggets the suitability of using them for biomonitoring fresh water pollution. The accumulation of heavy metals in bryophytes, specifically their cell walls can be correlated to the degree of tolerance and resistance to heavy metals. The considerable heavy metal concentraations that bind to pectin is regarded as an effective defensive mechanism against divalent metal cations such as copper (Cu^{2+}) and (Pb^{2+}), allowing for successful adaptation to heavy metal fortified substrate (Krzeslowska, 2011).

In addition, some metals like Iron (Fe), Copper (Cu), Molybdenum (Mo), etc. which can interact with cellular oxygen to form reactive oxygen species (ROS), alter the cell redox status and ultimately they replace metallic cofactors in enzymes inhibiting their functions. (Baek *et al*., 2012).

Heavy metals Cadmium and Lead together afflicted the chlorophyll content of T.*barbieri* than V.*montagnei*. As the bioaccumulation of Zinc and Copper in the thallus of V.*montagne*i elevated, chlorophyll content get narrowed than *T. barbieri*. Eventually it is clear that there exist a negative corelation between chlorophyll content and bioaccumulation capacity of heavymetals in aquatic bryophytes *Taxiphyllum barbieri* and *Vesicularia montagnei*. It also suggest the potential of using both *T.barbieri* and *V.montagnei* as biomonitoring agents.

SUMMARY

Heavy metal pollution is a serious global concern. It not only deletorous to human being but the entire ecosystem. Phytoremediation is one of the cheapest and better option for the biofiltration of heavy metals from polluted water. Due to the larger surface area, rootless plant body and ease of propagation bryophytes can used as both biomarkers and bioaccumulators of heavy metals. *Taxiphyllum barbeiri* and *Vesicularia montagnei* are two commonly found aquarium mosses, which can make the polluted water with reduced lethal effect of metals.

In the present study, the effect of heavymetals (Cd, Cu, Pb, and Zn) on chlorophyll content of *T.barbieri* and *V.montagnei* due to metal absorption were studied. Both Java and christmas moss were grown in polluted water and chlorophyll content were analysed. Then after, these two mosses were treated and grown in known concentration for 4 days and chlorophyll content was estimated for 24 hrs, 48hrs, 72hrs, and finally at 96 hrs.

The heavy metal analysis of polluted water shown that they contain heavy metals such as Cadmium (Cd), Copper (Cu), Lead (pb), and Zinc (Zn). Both the leaves of *T. barbieri* and *V.montagnei* contain more or less same amount of total chlorophyll content. After exposed with polluted water, the results shown that there is a marked change in the chlorophyll content of both mosses with respect to its absorption rate of heavy metals by them.

Invivo grown *T.barbeiri* and *V. montagnei* in known concentrations of Cd, Cu, Pb and Zn, have characteristic change in their thallus colour. This colour change is due to the presence or accumulation of heavy metals and degradation of chlorophyll content. Competency among heavy metals to bind with cell membrane and replacement of Mg^{2+} from photosynthetic system, causes chlorophyll declination.

The results obtained from the study revealed that both *Taxiphyllum barbieri and Vesicularia montagnei* have high heavy metal bioaccumulation capacity and also heavymetals such as Cd, Cu, Pb and Zn can effect damage on photosynthetic systems. Hence change in chlorophyll content.

CONCLUSION

The present study, revealed that there is a negative correlation between heavy metal bioaccumulation and chlorophyll content of the two aquatic mosses *Taxiphyllum barbieri* and *Vesicularia montagnei*. When the thallus of these two mosses absorbed more and more heavy metals such as Cd, Cu, Pb and Zn, the total chlorophyll content also get varied. ie., it indicate that heavy metal accumulation can directly or indirectly pose effects on photosynthetic systems. This chlorophyll content variation with respect heavy metal accumulation can be used as parameter to study the effectiveness of *T.barbieri* and *V.montagnei* as phytoremediating agents or biofiltering agents. Hence it is concluded that studied heavy metals –Cu, Pb, Zn, and Cd posses effect on chlorophyll content of the two aquatic bryophytes *Taxiphyllum barbieri* and *Vesicularia montagnei*. Among the heavy metals analysed accumulation of Copper (Cu) by *V.montagnei* have high impact on declination of chlorophyll content .

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