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Effects of heavy metals on protonemal growth and bud formation in the moss *Hydrogonium arcuatum*

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ABSTRACT

The present study was conducted to investigate the toxic effects of cadmium and lead salts on protonemal growth and bud formation. For this purpose bryophyte tissue were acclimated to the laboratory conditions by culturing on Nitsch's basal medium supplemented with various salts of heavy metals (Cadmium and Lead) in the specific concentration range 10^{-8} – 10^{-4} M to assess physiological stress- response. Rounding of protonemal cells and formation of brood cells like structures were observed and Bud formation was adversely affected. The present study thus confirmed the morphological changes by mosses can be used to detect heavy metal pollution.

Key Words: Acrocarpous moss, Brood cells, Heavy metals, Protonema morphology.

INTRODUCTION

Pollution of air, water and soil, caused mainly by increasing industrialization, has become a matter of global concern. Pollution monitoring without knowing their source of emission is a complex problem (Borut *et al.* , 2002; Giordano *et al.,* 2005 & Tripathi & Gautam, 2007)

In last few years, the use of bryophytes as pollution monitors (Richardson, 1981) has been emphasized due to the potentiality of these plants to accumulate the toxic elements (Martin & Cougerty, 1982; Ruhling & Tyler, 1984). Bryophytes are suitable biomonitors of pollution as they have rapid absorption rate, lack of roots show absorption through plant surface , differential ability to accumulate wide range of metals etc.

Since only a few studies on the effect of heavy metals on bryophytes has been carried out (Kapur & Chopra, 1989; Ghate & Chaphekar, 2000) and hence further studies need to carried out for detection of heavy metal pollution by using morphological changes which bryophytes undergo. So the present study was carried out to understand the effect of some heavy metal on the moss *Hydrogonium arcuatum* under *in vitro* conditions on various phases of development to assess more information on this little known aspect of bryophyte i.e. biomonitoring of pollution.

MATERIALS AND METHODS

Sporophyte bearing moss plants were collected from north western parts of India in 2012-2013. From the gametophytic material, sporophytes with operculum intect were detached and washed in running tap water for 2-3 h followed by surface sterilization with chlorine water for 1 minute and than by sterilized double distilled water 3-5 times. Capsules were punctured with the help of sterile needle and spores were shown on the semi solid Nitsch's basal medium comprising Knop's major salts, Nitsch's trace elements, Ferric citrate and Sucrose, gelled with 0.8% agar under aseptic condition in a laminar air flow cabinet. After spore germination one of the cultures was selected and its protonemata were subcultured for further experimentation. A small amount of bud- free protonema was used as inoculums in each test tube. This was inoculated in sterile nutrient medium containing heavy metals in different concentrations (ranging from 10^{-8} to 10^{-4}M) individually. Cultures were kept in continuous illumination of 3,500 to 4,500 lux. for each experiment 10 replicates were maintained alongwith a control culture without heavy metal salt. The experimental cultures were maintained for 60 days in culture room at 25±2°C for the purpose of observing morphological sign of toxicity.

RESULTS AND DISCUSSION

The Effect of cadmium salts (cadmium acetate, cadmium nitrate & cadmium sulphate) and lead salts (lead acetate and lead nitrate) was studied individually in the concentration range 10^{-8} to 10^{-4}M.

Cadmium acetate- It had adverse effect on protonemal growth. At all the conc. of cadmium acetate tried, inoculum took more time to regenerate and protonemal growth was inhibited (Fig.1.). Degree of inhibition increased with increase in concentration. Growth of prostrate system and branching were considerably reduced especially at higher concentrations. Protonema exhibited many abnormalities. Many filaments showed enlarged terminal and intercalary

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cells. Gemmae -like structures were produced at all levels (Plate 1).

Like protonemal growth cadmium acetate also affect bud formation adversely (Fig.2). Their initiation was delayed considerably. Buds appeared after 35, 40, 46, 52 and 55 days at 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}M, respectively. Buds were highly stunted and possessed highly reduced leaf primordia.

Cadmium sulphate – Protonemal growth was adversely affected by cadmium sulphate (Fig.1). At all the levels, regeneration of inoculum was delayed considerably. Branching of protonema was reduced and protonema was pale-green on cadmium sulphate-supplemented media. At higher concentrations, protonema developed some morphological abnormalities like swelling of terminal and intercalary cells. Gemmae were formed at all levels (Plate 1).

Bud formation was also inhibited and it decreased with increase in concentration of cadmium sulphate (Fig.2). Buds were initiated after 38, 42, 50, 52 and 49 days at 10^{-8}, 10^{-7},10^{-6}, 10^{-5} and 10^{-4}M, respectively**.** Buds failed to develop into normal shoots and formed stunted gametophores with reduced leaf primordia .

Cadmium nitrate- Cadmium nitrate had inhibitory effect on protonemal growth. The degree of inhibition increased with increase in concentration of cadmium nitrate (Fig.1). Protonema was brownish-green on cadmium nitrate-supplemented media. Prostrate system and branching were considerably reduced at higher levels. It also induced many morphological aberrations in the protonema. Many terminal and intercalary cells assumed spherical shape and developed thick walls. Buds appeared after 38, 42, 50 days at 10^{-8},10^{-7} and 10^{-6}M cadmium nitrate. Buds did not develop into normal leafy gametophores but remained stunted with highly reduced leaf primordia. At 10^{-5} and10^{-4}M bud formation was completely inhibited and only gemmae appeared on the protonema (Plate 1).

Lead acetate- Protonemal growth was retarded at all the levels and at 10^{-4}M inoculum failed to regenerate (Fig.1). Branching and growth of branches were also affected adversely. At higher concentration protonema became brownish-green and it exhibited some abnormalities. Swelling of terminal cells, irregular branching and death of intercalary cells occurred. Gemmae were produced in large numbers (Plate 2)

Bud formation was also affected adversely at all concentrations of lead acetate tried. Bud formation was completely suppressed at 10^{-5}M (Fig. 2)**.** Buds appeared after 41, 45, and 47 days at 10^{-8}, 10^{-7} and 10^{-6}M, respectively**.** On lead acetate –supplemented media buds developed into thin, pale-green gametophores.

Lead nitrate– Like lead acetate, lead nitrate also inhibited protonemal growth (Fig.1). At 10^{-5} and 10^{-4}M inoculum failed to regenerate . At other levels, regeneration of inoculum was

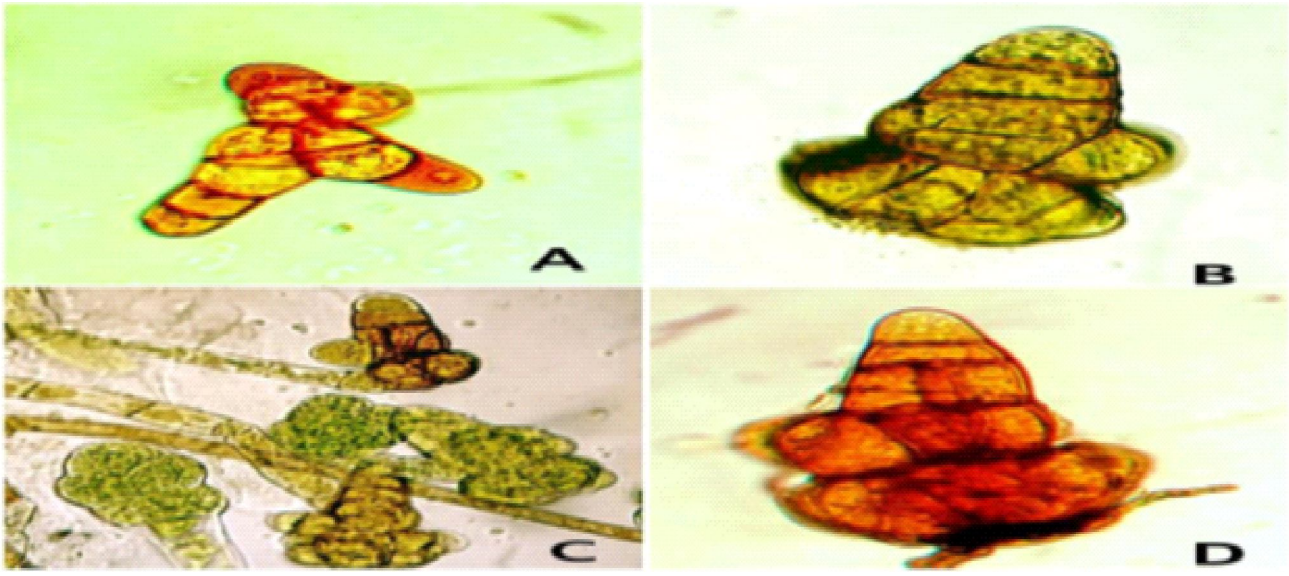

Plate 1

Hydrogonium arcuatum

Effect of heavy metals: Production of gemmae like structures.

A.Gemma from cultures supplemented with 10^{-8}M cadmium acetate; **B.** Gemma from cultures supplemented with 10^{-8}M cadmium sulphate; **C.** Gemmae from cultures supplemented with 10^{-5}M cadmium nitrate; **D.** Gemma from cultures supplemented with 10^{-6}M lead acetate.

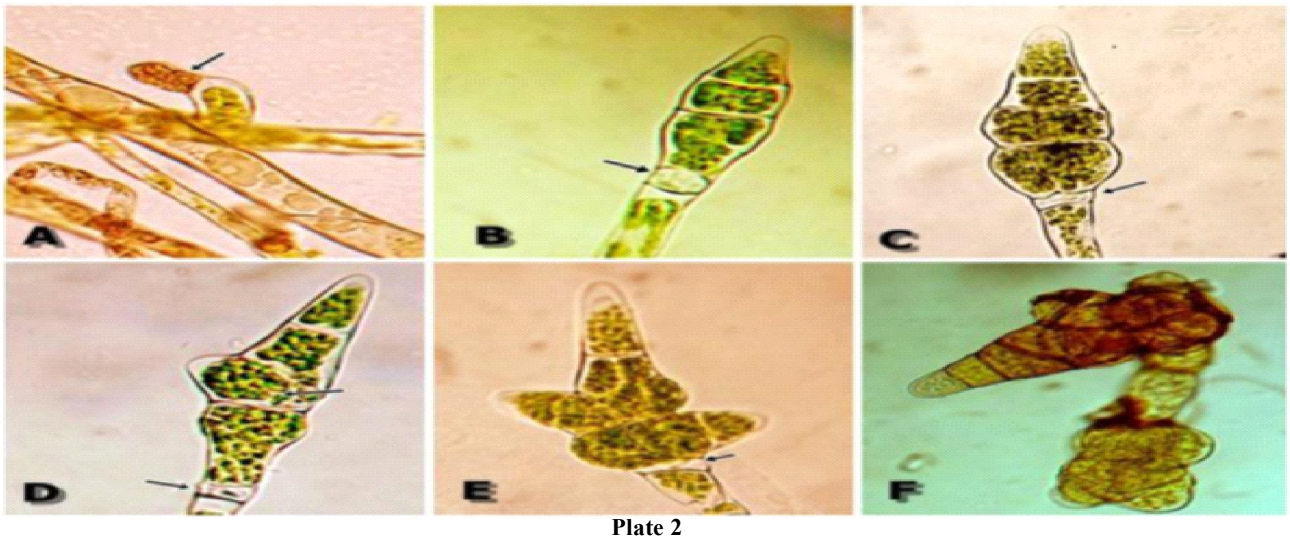

Plate 2

Hydrogonium arcuatum

Developmental stages in gemma formation : **A.** Gemma initial; **B.** Swollen apical cell showing transverse division; **C-D.** Vertical divisions and basal hyaline cell for detachment of gemmae at maturity; **E.** Multicellular gemma showing hyaline basal cell; **F.** Mature gemmae.

considerably delayed. Protonema branching was reduced considerably, especially at higher levels. Length of protonemal cells decreased and many cells became spherical. Growth of aerial system was better and there was a marked reduction in extent of prostrate system and its branching. Protonema was brownish-green at 10^{-6} M, whereas at lower levels, it was pale-green. Formation of brood cells like structures at tip and intercalary positions occurred on lead nitrate containing media.

Buds failed to appear at 10^{-5} and 10^{-4}M. Buds number was drastically reduced at 10^{-7} and 10^{-6}M (Fig. 2). Buds appeared after 31,35 and 39 days at 10^{-8},10^{-7} and 10^{-6}M, respectively**.** Buds developed into pale-green gametophores with reduced leaves and produced brownish filaments all over the surface.

Development of gemma started with reduction in length of terminal cell of a filament. It became swollen and

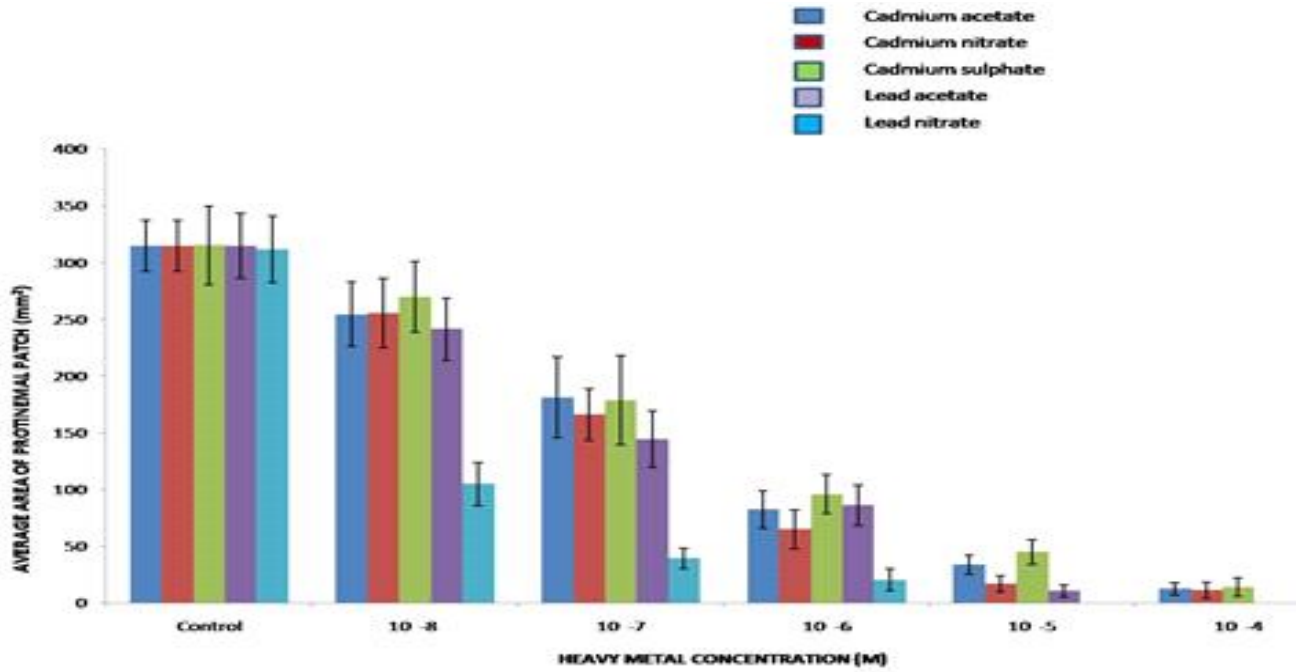

Fig.. 1

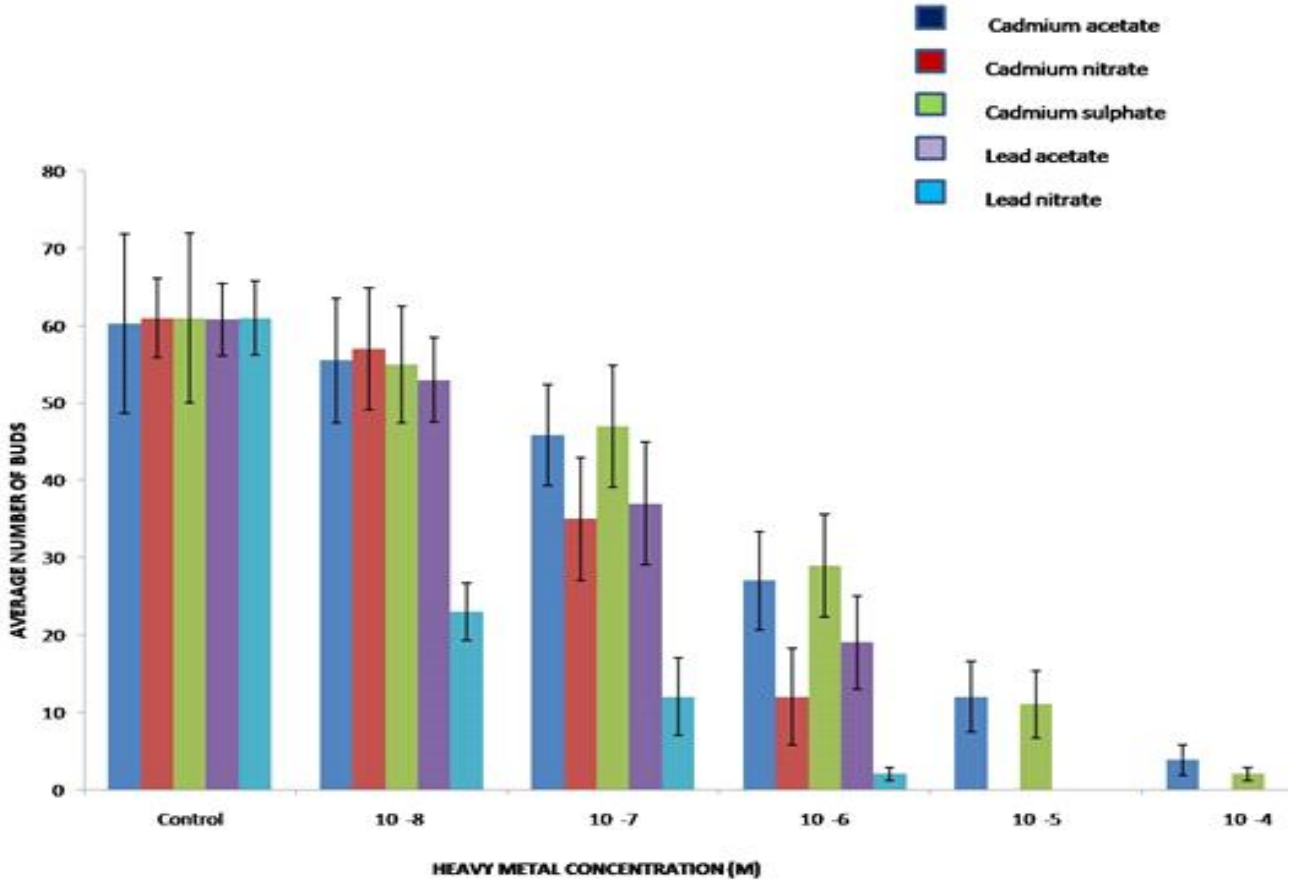

Fig 2

divided by transverse divisions which were further divided by vertical divisions resulting in the formation of multicellular structures of various shapes. Below the gemmae a narrow hyaline cell also appeared which helped in development of gemma from parent filament (Plate 2).

Various studies indicate that the presence of Cu, Zn, Pb, Ni, Cd and Cr elements may seriously retard the potential colonization of bryophytes at polluted sites. Cadmium, Copper, Lead and Uranium are heavy metals act as major atmospheric pollutants. At lower concentration, *Physcomitrium cyathicarpum* and *Barbula constricta* accumulates Pb, Cd, Hg, Cu etc. (Vats *et al*. 2010)

Lead is a metal that cannot be detoxified so it is supported to be more toxic and reactive. It is introduced into the atmosphere by exhaust fumes from vehicles, metal production and mining. Many species of bryophytes have been shown to be tolerant to very high concentration of lead which are toxic to other group of plants. Pb is accumulated by *Physcomitrium pyriforme* (Misra, 2006 cited in sahu *et al.* 2007), *Hydrogonium gracilentum* (Sharma & Kapila, 2007 cited in sahu *et al*., 2007). In contrast many species are sensitive to higher concentration of lead with adverse effects on growth and development and formation of spherical cells. Coombes and Lepp (1974) reported that in *Funaria hygrometrica* protonemal growth was inhibited at higher levels of lead, whereas in the liverwort, *Marchantia polymorpha* lead retarded development of gemmalings. In *Timmiella anomala* (Kapur and Chopra, 1989) and *Anoectangium bicolor* and *Bryum argenteum* (Saini, 1994) lead adversely affected protonemal growth and bud formation. Inhibitory role of Pb can be seen in *Timmiella anomala*(Kapur & Chopra,1989), *Barbula horricomis* (Saini, 1994) and *in Hydrogonium arcuatum* (present investigation). Bioremediation using mosses is effective method of control of Soil pollution created by heavy metals. Toxic symptoms on plants were proved to be helpful in identifying the dominant metal of the area and eradicating it using suitable remedial method.

CONCLUSION

Many bryophytes are known to accumulate cadmium in large quantities includes *Sphagnum* sp.(Pakarinen & Tolonen, 1976), *Rhytidiadelphus squarrosus*(Brown & Beckett, 1985). On the other hand, NBsome species are sensitive to cadmium and these species at various stages of growth and development are inhibited

to various degrees. In *Funaria hygrometrica* cadmium at higher concentrations inhibited spore germination (Lepp and Roberts, 1977). Inhibitory effect of protonema and bud formation has been observed in *Timmiella anomala* (Kapur & Chopra, 1989). Cadmium higher concentration plays inhibitory role in *Pohlia elongata*, *Atrichum pallidum*, *Funaria hygrometrica* , *Fissidens taxifolium* (Kaur *et al.* 2010) and in *Hydrogonium arcuatum* (present investigation).

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