Effects of some Cadmium salts on Protonemal growth and Bud formation in The *in vitro grown* Moss. *Anoectangium clarum*

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Abstract

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

Keywords

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 the source of emission is a complex problem(Borut *et al*., 2002 & Tripathi & Gautam, 2007).

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

Since only a few studies on the effect of heavy metals on bryophytes have been carried out (Ghate & Chaphekar, 2000) and hence further studies need to be carried out for the 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 cadmium heavy metals on the moss *Anoectanium clarum* under *in vitro* conditions on various phases of development to assess more information on this little-known aspect of bryophyte i.e.biomonitoring of heavy metal pollution.

Material and Methods

Sporophyte beard moss plants were collected from northwestern parts of India in 2012-13. From the gametophyte material , sporophytes with operculum intact were detached and washed in running tap water for 2-3 hrs followed by surface sterilization with chlorine water for 1 minute and then by sterilized double distilled water 3-5 times . Capsules were punctured with the help of a sterile needle and spores were sown 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 conditions in a laminar airflow cabinet. After spore germination one of the cultures was selected and its protonema was subcultured for further experimentation a small amount of bud-free protonema was used as inoculum. This was inoculated in each test tube sterile nutrient medium containing heavy metals in different concentrations (ranging from 10^{-8}- 10^{-4}M) individually. Cultures were kept in continuous illumination of 3,500 to 4,500 Lux. For each experiment, 10 replicates were maintained along with a control culture without heavy metal salt. The experimental cultures were maintained for 60 days in a culture room at 25+-2°C for the purpose of observing morphological signs of toxicity.

Observations

Anoectanium Clarum

Spores of this species germinated after planting on basal medium under ordinary cultural conditions. Germination was preceded by an increase in the diameter of spores and

rupture of the exine . This was followed by the emergence of intine in the form of germ papilla, which by further growth and transverse divisions, developed into chloronema. Both unipolar and bipolar germination was observed. After two weeks chloronema differentiated into caulonema with oblique cross walls and elongated chloroplast. Protonema exhibit heterotrichous habit.

To study the effect of some salts of heavy metals on protonemal growth , cultures were subjected to varying concentrations of three salts of Cadmium (Cadmium acetate, Cadmium nitrate and Cadium sulfate) was studied on the protonemal growth and bud formation . The responses of *Anoectanium clarum* are being presented under specific headings.

Effects of Cadmium acetate, Cadmium sulfate and Cadmium nitrate were studied by incorporating these salts individually into the basal medium in the concentration range 10^{-8} – 10^{-4} M.

CADMIUM acetate- Protonemal growth was inhibited and the degree of inhibition increased with an increase in its conc. (Fig. -1). The branching of protonema and the growth of the prostrate system were reduced considerably . Protonema turned brownish–green and showed various morphological aberrations. The length of cells decreased and their width increased . Many terminal and intercalary cells assumed spherical shapes. These cells after detachment produced fresh protonema. buds failed to appear on Cadmium acetate supplemented medium.

Cadmium sulfate- Growth of protonema was inhibited markedly and inoculums failed to regenerate at 10^{-5} and 10^{-4} M(Fig. 3). Extant of branching and prostrate system decreased considerably . Protonema became pale-green on Cadmium sulfate supplemented media and it is exhibited many abnormalities. Terminal cells and intercalary cells of protonema developed into a narrow disc-shaped cell between parent filament and brood cells (Plate –B). These cells detached from the filaments after the degeneration of adjoining cells.

Cadmium nitrate- No appreciable change in protonemal growth was observed at 10^{-8}M but with increase in conc. caused inhibition of protonemal growth (Fig. 2) . Degree of inhibition increased with an increase in the concentration of Cadmium nitrate . At 10^{-4}M protonema failed to regenerate . Protonemal patch comprised of somewhat better developed aerial filaments and highly reduced sparingly branched prostrate filaments, protonema showed many abnormalities like swelling of tip and rounding of intercalary cells with reduction in length of cells . These spherical cells ot detached and started producing protonema (Plate –A). Bud formation was also not favored by Cadmium nitrate at any of the conc. of Cadmium nitrate.

Discussion

Bryophytes have great ion exchange capacity , absence of cuticle in the gametophyte and simple organization of tissue. So they were extensively used as bioindicators of environmental pollution(Tyler 1990 and Gerdol *et al*. 2000).

Higher conc. of heavy metals adversely affected the morphology of protonema as shown in the present investigation where cd salts caused retardation of protonemal growth as well as bud formation in the *Anoectanium* sp. Protonemata of moss exhibit various abnormalities like reduction in cell length and change in shape , irregular branching , swelling of tip cells , rounding of intercalary cells , irregular branching of protonema and formation of gemma–like structure. These cells tend to assume a spherical shape and are morphologically similar to brood cells as in *Anoectangium bicolor* , *Bryum* argentium (Saini, 1994)*Bryum capillare* , *Brachymenium bryoides* (Chaturvedi , 2001), *Funaria hygrometric* (Coombes & Lepp 1974), *Timmiella Anomala* (Kapur & Chopra, 1989) an in *Anoectangium clarum*(present investigation).

Many workers reported the accumulation of cadmium in large quantities by *Sphagnum* sp. (Pakarinen & Tolonen, 1976, *Rhytidiadelphus squarossus* (brown and Beckett, 1985) *Hydrogonium ochraceum* (Carter & Porter 1997). On the other hand, some species are sensitive to Cd and these sps. at various stages of growth and development. are inhibited to various degrees . Inhibitory effects of protonema and bud formation have been observed in *Timmiella anomala* (Kapur & Chopra, 1989); *Pohlia elongate*, *Atrichum pallidium*, *Funaria hygrometrica*, *Fissidens taxifolium* (Kaur et al. 2010) and *Anoectangium bicolor* , *Barbula horricomis* (Saini , 1994).

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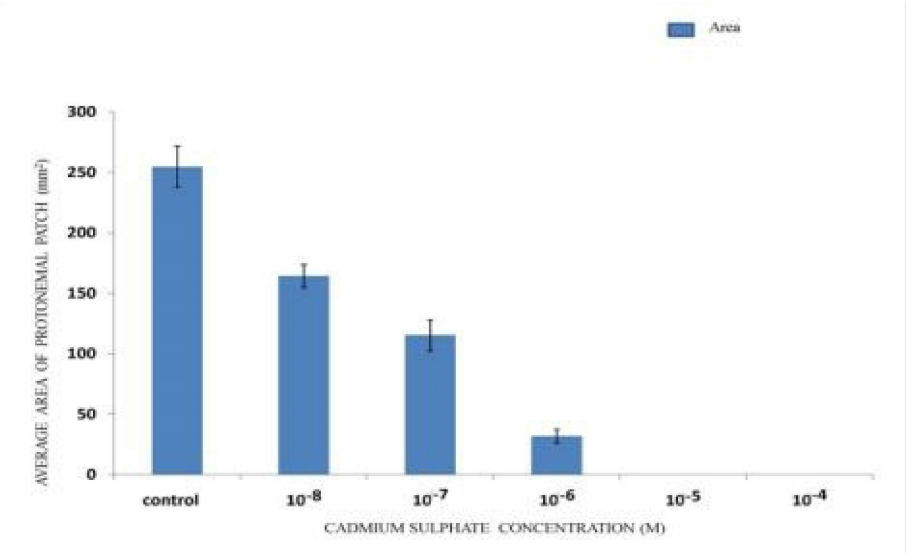

Fig. 1 Effect of Cadmium acetate onProtonemal growth and bud formation in *Anoectangium clarum*

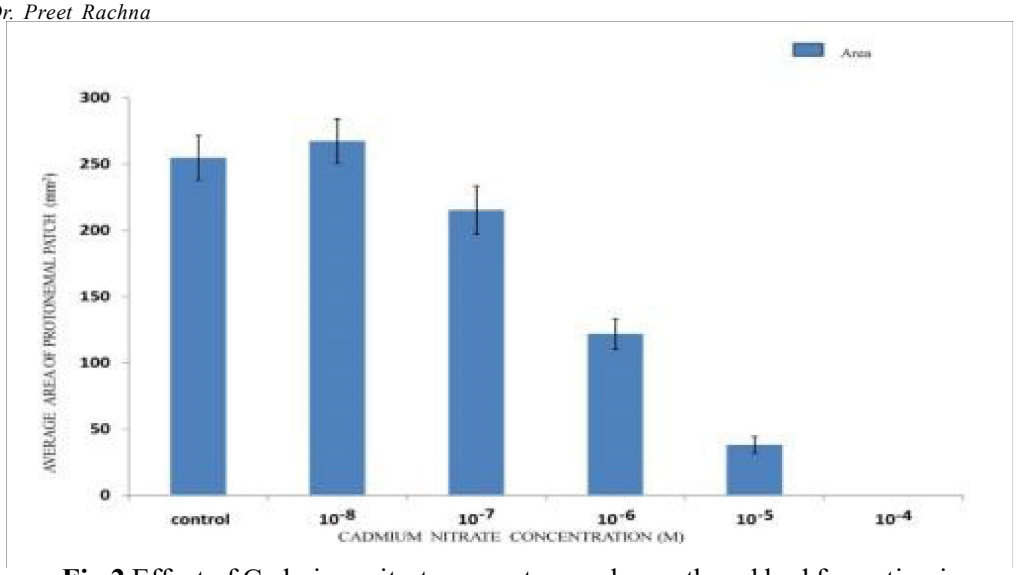

Fig.2 Effect of Cadmium nitrate on protonemal growth and bud formation in *Anoectangium clarum*

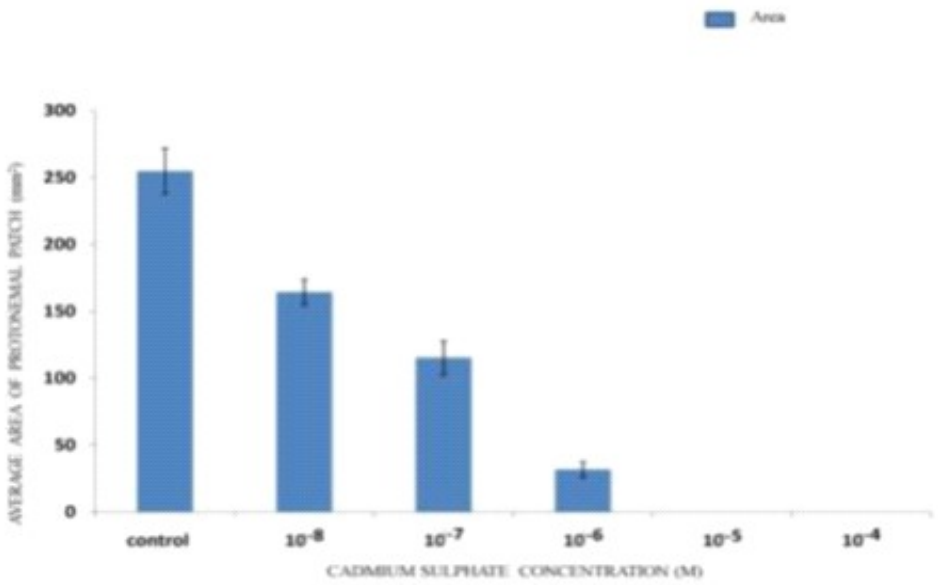

Fig. 3 Effect of Cadmium sulfate on Protonemal growth and bud formation in *Anoectangium clarum*

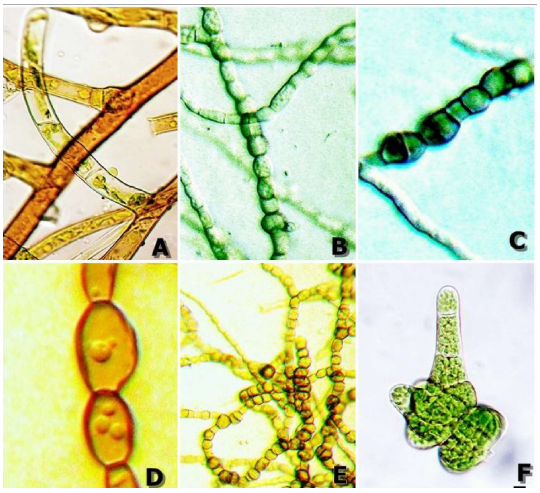

PLATE- A

Anoectangium clarum

Effect of Cadmium nitrate: **A.** Normal protonema **B-E.** Formation of spherical cells at higher concentrations of Cadmium nitrate **F.** Brood cell producing fresh protonema on basal medium.

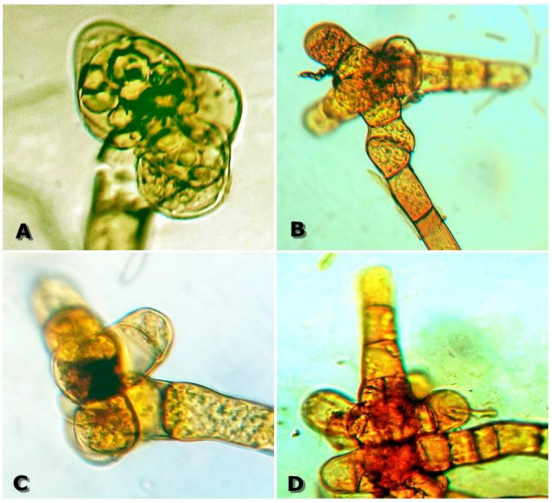

Plate B

Anoectangium clarum

Effect of Cadmium sulfate: **(A-D)** Brood cell formation at lower concentrations of Cadmium sulfate.