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EFFECTS OF HEAVY METALS ON MORPHOLOGY OF THE MOSS ANOECTANGIUM CLARUM

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ABSTRACT

The present study was conducted to investigate the toxic effects of cadmium and lead salts on growth parameters. 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. 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 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 and 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 Anoectangium 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 pollution.

MATERIAL AND METHODS

Sporophyte bearing moss plants were collected from north western parts of India in 2012-2013. From the gametophytic material, sporophytes with operculum intact 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.

ANOECTANGIUM CLARUM

Spores of this species germinated after planting on basal medium under ordinary cultural conditions. Germination was preceded by increase in diameter of spores and rupture of exine. This was followed by emergence of intine in the form of germ papilla, which by further growth and transverse divisions, developed into chloronema . Both unipolar and bipolar germination were observed**.** After two weeks chloronema differentiated into caulonema with oblique cross walls and elongated chloroplasts (Plate A). Protonema exhibited 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 Cadmium sulphate) and two salts of Lead (Lead acetate and Lead nitrate) was studied on the protonemal growth and bud formation. The responses of Anoectangium clarum are being presented under specific headings.

Cadmium acetate- Protonemal growth was inhibited and degree of inhibition increased with increase in its concentration (Fig. 1). Branching of protonema and growth of prostrate system were reduced considerably. Protonema turned brownish-green and showed various morphological aberrations. Length of cells decreased and their width increased. Many terminal and intercalary cells assumed spherical shape .These cells after detachment produced fresh protonema. Buds failed to appear on cadmium acetate – supplemented medium

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

Like cadmium acetate, cadmium sulphate also had no effect on the initiation of buds and protonema remained bud-free at all the levels of cadmium sulphate.

Cadmium nitrate- No appreciable change in Protonemal growth was observed at 10^{-8}M but with increase in concentration caused inhibition of protonemal growth (Fig.2). Degree of inhibition increased with increase in concentration of cadmium nitrate. At 10-4M 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 got detached and started producing protonema (Plate D). Bud formation was also not favoured by cadmium nitrate at any of the concentration of cadmium nitrate tried

Effect of Lead acetate and lead nitrate was studied by incorporating these salts individually to the basal medium in the concentration range 10^{-8} to 10^{-4}M.

Lead acetate – It proved inhibitory for protonemal growth. Inhibition increased with increase in concentration of lead acetate (Fig.4). Inoculum failed to regenerate at 10-5 and 10¯4M, whereas at lower levels, regeneration was delayed considerably. Inhibitory effect was more pronounced on prostrate system and its branching. Protonema turned brownish-green. Protonema exhibited morphological aberrations like swelling of tip and intercalary cells and thickening of cell walls resulting in the formation of brood cell like structures. These cell detached from parent protonema and grew independently **.**

Buds failed to appear on any concentration of lead acetate tried

Lead nitrate – Like lead acetate, lead nitrate also inhibited protonemal growth. At 10-5and 10^{-}4M inoculum failed to regenerate (Fig.5). At lower concentration protonema took more time to regenerate. 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. Considerable reduction in cell length and formation of brood cells like structures at tip and intercalary positions occurred on lead nitrate containing media (Plate E-F).
Bud formation was also not favoured by lead nitrate and buds failed to appear at any concentration of lead 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 bioindicator of environment pollution(Le Blanc, 1961; Gilbert, 1968; Grodzinska 1978; Tyler 1990 ; Uhlirova et al 1995; Pott & Turpin 1996; Bruns et al. 1997 & Gerdol et al. 2000). 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. Physcomitrium cyathicarpum and Barbula constricta accumulates Pb, Cd, Hg and Cu at lower concentration of these salts (Vats et al. 2010). Higher conc. of heavy metals adversely affected the morphology of protonema as shown in the present investigation where Cd salts (Cadmium acetate, Cadmium sulphate , Cadmium nitrate) and Pb salts (Lead acetate & Lead nitrate) caused retardation of protonemal growth as well as bud formation in the Anoectangium sp. Protonemata of mosses exhibited 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 spherical shape and are morphologically similar to brood cells as in Anoectangium bicolor, Bryum argenteum (Saini, 1994) Bryum capillare, Brachymenium bryoides (Chaturvedi, 2001), Funaria hygrometrica (Coombes & Lepp 1974, Timmiella anomala (Kapur & Chopra 1989) and in Anoectangium clarum (present investigation). Coombes and Lepp (1974) suggested that formation of such brood cells or capsule cells could be defence mechanism to reduce surface area of protonema over which uptake of heavy metals may occur

Many species of bryophytes have been shown to be tolerant to very high concentration of lead which is 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, Marchantia polymorpha (Briggs, 1972), Hypnum cupressiforme (Thomus, 1979), Fontinalis antipyretica (Siebert et al. 1996) , Isothecium stoloniformis (Pott & Turpin, 1998). In contrast many species are sensitive to higher conc. of lead with adverse effects on growth and development and formation of spherical cells as seen in Anoectangium clarum. Other species in which growth and development are adversely affected are Timmiella anomala (Kapur& Chopra, 1989), Barbula horricomis (Saini,1994) and in A clarum **(**present investigation). Coombes & Lepp (1974) reported that in Funaria hygrometrica protonemal growth was inhibited at higher levels of lead , whereas in the liverwort M. polymorpha lead retarded development of gemmalings. In Semibarbula orientalis lead has inhibitory effect on protonemal growth and bud formation and order of toxicity was lead nitrate> lead acetate> lead nitrate+lead acetate(Sharma & Chopra, 1987). In Timmiella anomala (Kapur & Chopra, 1989); Anoectangium bicolor and Bryum argenteum(Saini, 1994) lead adversely affected protonemmal growth & bud formation . In Bryum capillare and Brachymenium bryoides lead at all levels caused inhibition of protonemal growth and bud initiation and at higher conc. Protonema showed formation of brood cell like spherical structuires(Chaturvedi, 2001**)**and in the present investigation .
Many workers reported accumulation of Cd in large quantities by Sphagnum sp. (Pakariven&

Tolonen, 1976; Rhytidiadelphus squarossus(Brown and Beckettt, 1985,Hygrohypnum ochraceum (Carter & Porter 1997; Isothecium stoloniforrm (Pott &Turpin 1998). On the other hand some sps. are sensitive to Cd and these sps. at various stages of growth and dev. are inhibited to various degrees. Inhibitory effect of protonema and bud formation has been observed in Timmiella anomala (Kapur & Chopra , 1989). Cadmium higher conc. plays inhibitory role in Pohlia elongate, Atrichum pallidum, Funaria hygrometrica, Fissidens taxifolium (Kaur et al.2010) and in Anoectangium (present investigation). In Funaria hygrometrica Cd at higher conc. inhibited spore germination (Lepp & Roberts, 1977) .In Polytrichum commune, Cadmium proved more toxic than Zinc for spore germination and protonemal growth (Francis & Peterson, 1989). Inhibitory effect on protonemal growth and bud formation has also been observed in A. bicolor(Saini, 1994), Timmiella anomala (Kapur and Chopra 1989) and Barbula horricomis (Saini, 1994).

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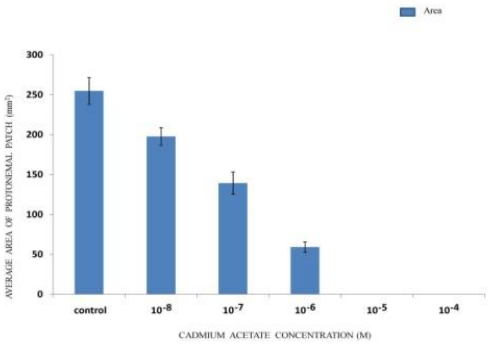


Fig. 1 Effect of Cadmium acetate on Protonemal growth and bud formation in formation in Anoectangium clarum

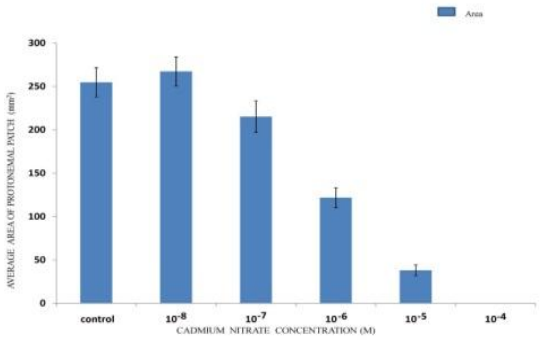


Fig.2 Effect of cadmium nitrate on protonemal growth and bud Anoectangium clarum

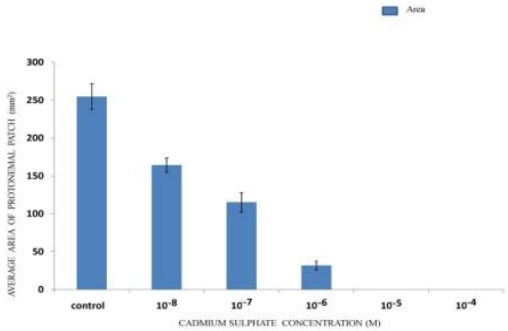


Fig. 3 Effect of Cadmium sulphate on Protonemal growth and bud formation in Anoectangium clarum

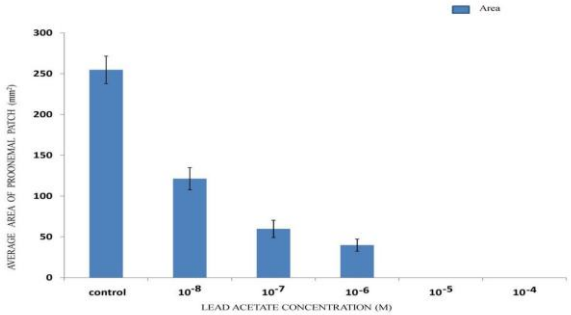


Fig. 4 Effect of Lead acetate on protonemal growth and bud formation in Anoectangium clarum

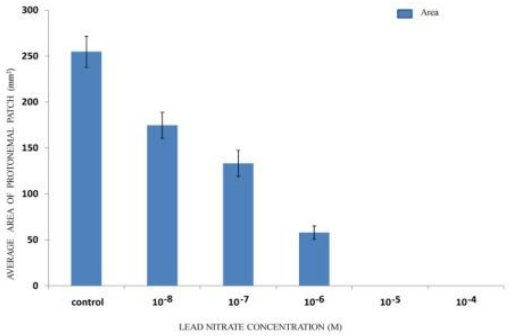
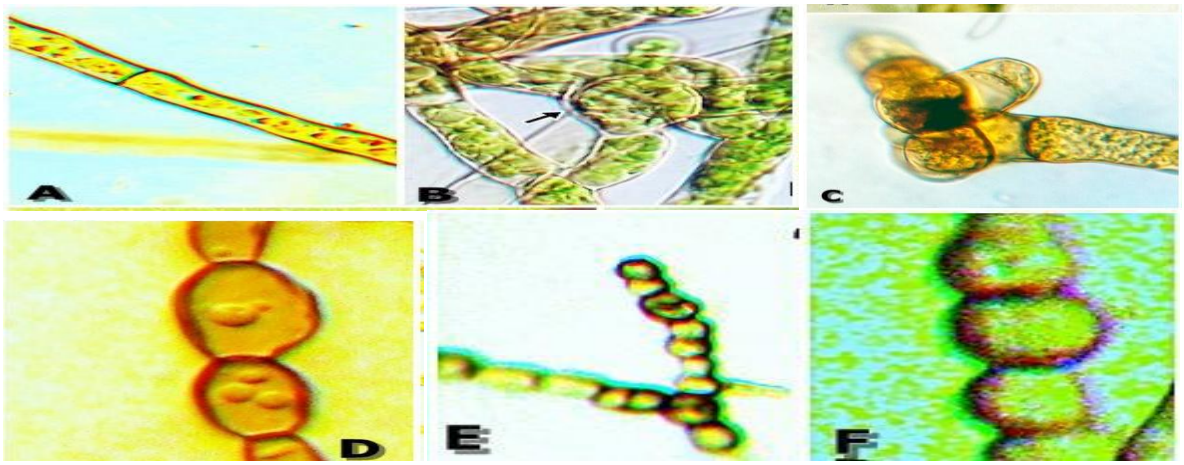


Fig. 5 Effect of Lead nitrate on protonemal growth and bud formation in Anoectangium clarum



Anoectangium clarum

Effect of heavy metals : **A.** Normal caulonemal filament; **B.** Cultures showing swollen terminal cell;

Effect of Cadmium sulphate : **C.** Brood cell formation at lower concentrations of cadmium sulphate;

Effect of Cadmium nitrate : **D.** Formation of spherical cells at higher concentrations of cadmium nitrate;

Effect of Lead nitrate : **E, F.** Formation of round cells at higher concentration of lead nitrate.