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The effect of lead and zinc concentrations on the growth of four species of bryophytes

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ABSTRACT

Studies have been made on the morphological and physiological changes that occurred in four bryophyte taxa, namely, *Riccia crystalina*, *Timmiella barbuloides*, *Hyophila involuta* and *Physcomitrium repandum* in response to the various concentrations of two heavy metals, i.e. zinc and lead. Effect of different concentrations of lead nitrate and zinc sulphate on various parameters was studied at regular intervals on different plants since the amount, duration, intensity of precipitation and plant species affect accumulation of heavy metal. The plants showed reduced number of regenerants and retarded growth when exposed to >0.5 ppm concentration of metals. At higher concentration and exposure for longer duration, the plants lost the chlorophyll content. Effect of Lead nitrate on various parameters was found to be more drastic than Zinc sulphate in most of the cases except in *T. barbuloides.* The uptake efficiency of the most common heavy metals in mosses follows mostly the order: Pb > Co, Cr > Cu, Cd, Mo, Ni, V > Zn > As.

Keywords: Mosses, pollution monitoring, phytotoxicity, heavy metal uptake, cation exchange capacity.

INTRODUCTION

Biological response to environmental degradation is now being used as a tool for the restoration of heavy metal contaminated sites. The living organisms reflect the conditions and a change in some aspect of the biota implies a change of some sort in the environment in which the organisms live. Adaptation of plants to heavy metal contamination is a character that appears to have evolved in part in response to human disturbance. Local enrichment of heavy metals in soil is mainly contributed either by anthropogenic influences (mineral resource exploitation) or by natural processes (agrochemical anomalies). Heavy metals are cumulative environmental pollutants. Lead and Mercury possess neurotoxic characteristics producing short and long term health implications in animals and stunted growth and necrosis in plants (Pitcarin et al., 1995). During mining, transport, refining and manufacturing, heavy metals enter in the living organism through food chain either as inorganic salts or organometallic derivatives. The ions of some of these heavy metals readily form ligands with organic molecules, tearing up the organic molecules very badly. Heavy metals affect enzyme functions, cell membrane permeability and cell growth (Moriarty, 1999). The effect of pollutants can be studied through the observations on the change in the population, morphology and physiology of the individuals and element bioaccumulation. Heavy metals have been found to affect the permeability of the cell membranes of lichens. However, the detrimental effects of heavy metals on the occurrence of lichens usually only become apparent at very high concentrations (Tarhanen et al., 1996).

Mosses are successfully employed as a biomonitoring agent in a heavy metal contaminated zone. They are fast growing and have high ion exchange capacity. Widespread occurrence of these plants and ability to accumulate metals have lead to their use in environmental monitoring programmes, thus facilitate the assessment of overall environmental conditions (on the basis of stress analysis) along with the environmental concentration from tissue analysis (Puckett 1988). They entrap the elements on the surface of the cells, incorporating into the outer walls of the cells through ion exchange processes and metabolically controlled passage into the cells (Brown and Bates, 1990; Tyler, 1990). Heavy metals pass into mosses through the substratum can also be translocated from one part of the moss to another (Brown and Brumelis, 1996). The use of mosses as biomonitors for atmospheric pollution became common after suitable methods for sampling and analyzing mosses had been developed (Tyler, 1970; Ross 1990; Halleraker et al., 1998; Carballeira et al., 2002). Comparative analysis of mosses as deposition accumulators was carried out by Bargagli et al. (2002). In the present investigation, four bryophytes, namely, *Hyophila involuta* (Hook.) Jaeg., *Riccia crystalina* Kash, *Timmiella barbuloides* (Brid.) Moenk. and *Physcomitrium repandum* Mitt ex Bruehl. have chosen for heavy metal (lead and zinc) impact studies in in-vivo conditions. These studies can be integrated to establish parameters of biological monitoring programmes conducted under natural conditions.

MATERIALS AND METHODS

Four taxa of bryophytes, namely, *Riccia crystalina*, *Hyophila involuta*, *Timmiella barbuloides* and *Physcomitrium repandum* were selected to study the impact of variable concentrations of two heavy metals (lead and zinc) on various parameters. Rationale for taking a thalloid form (*Riccia crystalina*) was to assess whether its habit makes any difference to metal uptake.

Metal solutions were prepared in different concentrations of metals in parts per million. Stock solution was made of 100 ppm and then respective concentrations of metals (0.5 ppm to 1.0 ppm) were made (see tables). The plant material was thoroughly washed to remove the bound soil and contaminants. These plants were used for transplantation.

Plant material of four specimens having patch diameter (2cm × 2cm) were transplanted in earthen pots (10 cm diameter) in autoclaved soil. All pots were kept in shady and humid portion of the garden for proper growth of plants. Ten ml of distilled water was poured in each pot to make the soil moist after transplantation and 5 ml each of respective concentrations of metal solutions was immediately added to all the labelled transplanted plants. The respective solutions were added to the plants at regular interval. The numbers of new regenerants were counted after 7, 14, 27 and 35 days intervals. Significant morphological changes were recorded in the plants after metal solution application.

RESULTS

Owing to the efficient nutrient absorbing capabilities, bryophytes commonly accumulate metals and metalloid elements (Onionwa, 2001; Zechmeister et al., 2003). Lead is not essential for growth and is rather toxic even at low concentration. Zinc is an essential nutrient but at higher levels in divalent forms is considered to be toxic to plants. Lead and zinc bind to nitrogen and sulphur-containing molecules, and are extremely toxic to plants even at low concentrations (Nriagu, 1989). Substratum and wet and dry deposition are likely sources of metal uptake in terrestrial bryophytes in nature.

Wet deposition method was adopted in the present study since in aqueous solution, metals are available in ionic form facilitating rapid uptake. Uptake efficiency of metal is affected by competition for free ion exchange sites (Gjengendal and Steinnes, 1990). Uptake appears to involve three separate processes:

- There is a passive absorption on to bryophyte cation exchanger (Clymo, 1967). Cell walls of most plants possess net negative charge owing to the ionization of weak acid built into their structure. Metals and other cations permeating the cell wall easily displace the proton from ionized weak acids and may become held by negative charge. Exchangably bound easily displaced by other cations in the external medium (Richter and Dainty, 1989).
- Some metals are capable of entering the cell (Brown and Beckett 1985; Brown and Sidhu 1992; Basile et al., 1994).
- Numerous small leaves offer many possibilities for entrapment of metal

containing soil particles (Ceburnis and Valiulis, 1999).

The present study is therefore focusing on the ability of bryophytes to show remarkable morphological and physiological changes in response to heavy metal stress. Increased level of both essential (zinc) as well as non essential elements (lead) can result in growth inhibition and toxicity symptoms. At low concentrations of metal solutions (0.15 ppm to 0.65 ppm), no significant changes in morphology were observed. Therefore, taking 0.55 ppm and 0.65 ppm as reference, experiment was performed for higher concentrations up to 1.0 ppm.

Remarkable changes were observed in number of new regenerants, size of thallus/ leafy stem and survivability. In *Riccia crystalina*, in response to both metal solutions, the number of new regenerants relatively increased at 0.65 ppm (Table 1). A sharp reduction was observed at 0.85 ppm on 14th day of metal treatment in both cases (Table 1). As compared to the thallus size in control pots, metal-treated thalli were significantly smaller. Normally, they are 1cm long and 5mm wide. Margins of the thalli shrink after 14th day of metal solution treatment. Plant turned brown at 0.85 ppm on 27th day of metal solution treatment in both cases. Plants that turned brown after metal treatment ultimately lost their identity in soil at 1.0 ppm on 27th day of metal solution application in both cases (Table 1).

In *Timmiella barbuloides* a sharp reduction was observed at 0.85 ppm on 14th day of metal treatment in both the cases (Table 2). But no appreciable variation in stem size was found in spite of metal treatment. After two weeks of metal solution application, new green regenerants were observed (Table 3). Zinc sulphate had more drastic effect on plants than lead nitrate. After turning brown, all plants lost their identity in soil at 1.0 ppm on 14th day of metal solution treatment, in case of zinc sulphate while many plants survived lead nitrate treatment (Table 3).

In response to both metal solutions in *Hyophila involuta*, the number of new regenerants relatively increased at 0.65 ppm (Table 4). Retarded growth of stem was found and the stems remained 0.5 cm long which is less than the normal size i.e. 1 cm at 0.75 ppm onwards in case of zinc sulphate while normal stem size was observed after lead nitrate solution application. Plant turned brown at 0.75 ppm on 14th day of zinc sulphate solution treatment. In case of lead nitrate, plants turned brown at 0.65 ppm on 14th day. Plants that turned brown after metal treatment ultimately lost their identity in soil at 1.0 ppm on 35th day of zinc sulphate solution application. In case of lead nitrate, similar effect was observed at 1.0 ppm on 27th day of metal treatment.

In case of *Physcomitrium repandum*, the number of new regenerants relatively increased at 0.65 ppm, in response to both metal solutions (Table 5). A sharp reduction was observed at 0.75 ppm on 14th of metal treatment in both cases (Table 5). Initiation of green filaments (protonema) from the basal part of the stem was observed at 0.75 ppm onwards on 14th day of metal solution treatment in both cases and remained there until 27th day of metal treatment. These protonemata remain for a short period and the browning of plants occurred at 0.75 ppm on 27th day of metal treatment in both cases. Later, they lost their identity in soil on 27th day of metal solution application in both cases.

DISCUSSION

According to Baker and Walker (1990) plants have developed three ways of adapting to heavy metal polluted ecosystems:

- Metal exclusion: prevents metals from entering the above ground parts still containing large amount of metals in their roots.
- Metal indication: accumulates metal in their aerial parts and metal level generally corresponds to metal level in soil.
- Metal accumulation: can concentrate metal in aerial parts to level higher than those present in soil.

Effect of different concentrations of lead nitrate and zinc sulphate on various parameters was studied at regular intervals on different plants since the amount, duration, intensity of precipitation and plant species affect accumulation of heavy metal. Effect of lead nitrate on various parameters was found

Table 1: Effect of different concentrations of lead nitrate and zinc sulphate on number of new regenerants in *Riccia crystalina* after 7, 14, 27 and 35 days intervals.

Concentration (ppm)		Number of regenerants			
		7th day	14th day	27th day	35th day
Zinc sulphate	Control	29 ± 0.5	31 ± 0.5	35.5 ± 1.5	22.5 ± 1.5
	0.55	25 ± 1.5	20 ± 1	22 ± 2	15 ± 0.5
	0.65	26 ± 1	24 ± 0.5	25 ± 1	10 ± 2
	0.75	23.5 ± 1.5	19 ± 3	21.5 ± 0.5	7 ± 1.5
	0.85	20 ± 1	7 ± 1	5.5 ± 0.5	5 ± 0.5
	0.95	21 ± 2.5	6 ± 1	3.5 ± 0.5	3 ± 0.5
	1.00	20 ± 1	3.5 ± 0.5	0	0
Lead nitrate	Control	29 ± 0.5	31 ± 0.5	35.5 ± 1.5	22.5 ± 1.5
	0.55	25.5 ± 3.5	24.5 ± 1	25.3 ± 0.5	15 ± 0.5
	0.65	28 ± 1	27.5 ± 0.5	24 ± 1.5	18 ± 1
	0.75	26 ± 1	25 ± 2	20.5 ± 0.5	9 ± 1.5
	0.85	24 ± 0.5	11 ± 1.5	3.5 ± 0.5	2 ± 0.5
	0.95	23 ± 0.5	5 ± 0.5	2.5 ± 0.5	1 ± 0.5
	1.00	21 ± 1.5	1.5 ± 0.5	0	0

Table 2: Effect of different concentrations of lead nitrate and zinc sulphate on number of new regenerants observed in *Timmiella barbuloides* after 7, 14, 27 and 35 days intervals.

Concentration (ppm)		Number of regenerants		
		7th day	14th day	27th day
Zinc sulphate	Control	24 ± 1	25 ± 0.5	18 ± 0.5
	0.55	20 ± 0.5	15 ± 1.5	13 ± 1
	0.65	21 ± 1.5	17± 0.5	14 ± 1.5
	0.75	18 ± 1	11 ± 0.5	7 ± 0.5
	0.85	16± 0.5	5 ± 0.5	4 ± 0.5
	0.95	15 ± 0.5	3.5 ± 0.5	1 ± 0.5
	1.00	15± 0.5	0	0
Lead nitrate	Control	24 ± 1	27.5 ± 0.5	25.5 ± 0.5
	0.55	22.5 ± 1.5	25 ± 0.5	19 ± 1.5
	0.65	27.5 ± 2	31 ± 1.5	16 ± 1
	0.75	24.5 ± 0.5	25 ± 0.5	12.5 ± 0.5
	0.85	21.5 ± 1	16 ± 0.5	5.5 ± 0.5
	0.95	18 ± 0.5	7 ± 1.5	5 ± 1
	1.00	15 ± 0.5	4 ± 1	3 ± 1.5

Table 3: Effect of different concentrations of lead nitrate and zinc sulphate on three parameters in *Timmiella barbuloides* after 7, 14 and 27 days interval.

	Concentration (ppm)	Leafy stem size			Survivability			Morphological features		
		7th day	14th day	27th day	7th day	14th day	27th day	7th day	14th day	27th day
Zinc sulphate	Control	N	N	N	+	+	+	B	G	B
	0.55	N	N	N	+	+	+	B	G	B
	0.65	N	N	N	+	+	+	B	G	B
	0.75	N	N	N	+	+	+	B	G	B
	0.85	N	N	N	+	+	+	B	G	B
	0.90	N	N	N	+	+	+	B	G	B
	0.95	N	-	-	+	-	-	B	-	-
Lead nitrate	Control	N	N	N	+	+	+	B	G	G
	0.55	N	N	N	+	+	+	B	G	G
	0.65	N	N	N	+	+	+	B	G	G
	0.75	N	N	N	+	+	+	B	G	G
	0.85	N	N	N	+	+	+	B	G	G
	0.95	N	N	N	+	+	+	B	G	B
	1.0	N	N	N	+	+	+	B	G	B

N: Normal; +: Plant survived; -: Plant dead; G: Green; B: Brown

to be more drastic than zinc sulphate in most of the cases except *Timmiella barbuloides.* The uptake efficiency of the most common heavy metals in mosses follows mostly the order (Zechmeister et al., 2003):

Pb > Co, Cr > Cu, Cd, Mo, Ni, V > Zn > As.

Also lead being a large polyvalent cation combines strongly with and "condenses" the fixed anions to varying extents (Richter and Dainty 1989).

In case of *Timmiella barbuloides,* zinc sulphate was found to have more drastic effects. No effect was observed initially at low concentrations of metal solution. Even at higher concentrations, plants showed significant change only after 14th day of metal solution treatment.

Metal absorption shows a phase of rapid uptake during early period of treatment and slower phase later. Rapid uptake represents passive absorption of metal ions on extracellular cation exchange sites in bryophyte tissues whereas slower phase is the true uptake in the cell (Brown, 1984; Pickering and Puia, 1969). Only when the metal accumulates inside the cell, it showed significant morphological and physiological changes.

Filamentous protonema was observed for a short period in *Physcomitrium repandum*. This may be due to the germination of brood cells that got detached from leaf tips of gametophytes and grew on soil. Switching to protonema can be accounted for a heavy metal stress tolerance by plant. In *Timmiella barbuloides,* green regenerants observed in otherwise dry brown plant material may be due to the germination of buds already present in the rhizonemal patch which on getting suitable atmosphere gave rise to green erect gametophores.

Stunted growth was observed due to metal toxicity. Sharma and Chopra (1987) reported the similar toxic effect of lead on growth of *Semibarbula orientalis*. Change in the plant colour was observed due to reduction

Table 4: Effect of different concentrations of lead nitrate and zinc sulphate on number of new regenerants observed in *Hyophila involuta* after 7, 14, 27 and 35 days interval.

Concentration (ppm)		Number of new regenerants			
		7th day	14th day	27th day	35th day
Zinc sulphate	Control	45 ± 1.0	40 ± 0.5	45 ± 0.5	27 ± 1.5
	0.55	43 ± 2.5	41.5 ± 2	35 ± 1.5	20 ± 0.5
	0.65	52.5 ± 1	48.5 ± 0.5	36 ±0.5	26 ± 2
	0.75	40 ± 1.5	30 ± 1.5	22 ± 2	17 ± 0.5
	0.85	38 ± 0.5	23.5 ± 0.5	17 ± 2	14 ± 1.5
	0.95	35 ± 2.5	11.5 ± 1	6.5 ± 0.5	7 ± 0.5
	1.00	32 ± 0.5	8 ± 0.5	3 ± 0.5	0
Lead nitrate	Control	45 ± 1.0	40 ± 0.5	45 ± 0.5	27 ± 1.5
	0.55	42 ± 1.5	33 ± 1.5	30 ± 2	23 ± 0.5
	0.65	46 ± 2.5	35 ± 2.5	34.5 ± 1.5	19 ± 1.5
	0.75	42 ± 0.5	17 ± 1	19 ± 2	10 ± 0.5
	0.85	38 ± 2.5	14 ± 1.5	12 ± 1.5	6 ± 1
	0.95	35 ± 0.5	8.5 ± 0.5	3.5 ± 0.5	2± 0.5
	1.00	34 ± 1	2.5 ± 1.5	0	0

Table 5: Effect of different concentrations of lead nitrate and zinc sulphate on number of new regenerants observed in *Physcomitrium repandum* after 7, 14, 27 and 35 days interval.

Concentration (ppm)		Number of new regenerants			
		7th Day	14th Day	27th Day	35th Day
Zinc sulphate	Control	47 ± 2.5	50 ± 1	55 ± 0.5	25.5 ± 1
	0.55	43.5 ± 1.5	44 ±0.5	42 ± 0.5	23 ± 0.5
	0.65	48.5 ± 0.5	45 ± 1	40 ± 1	26 ± 1.5
	0.75	45.5 ± 1	25 ± 2.5	22.5 ± 1.5	17 ± 0.5
	0.85	42 ±1.5	17 ± 1.5	11 ± 2	7 ± 1.5
	0.95	41± 2	7.5 ± 0.5	6 ± 0.5	2 ± 0.5
	1.00	39 ± 1	4 ± 0.5	1± 0.5	0
Lead nitrate	Control	47 ± 2.5	50 ± 1	54 ± 0.5	25.5 ± 1
	0.55	40 ± 1	34 ± 0.5	33.5 ± 1.5	22 ± 0.5
	0.65	46.5 ± .5	42 ± 0.5	39.5 ± 0.5	29 ± 0.5
	0.75	45 ± 2	23.5 ± 0.5	20 ± 2	10 ± 1.5
	0.85	40 ± 1	13 ± 1.5	8 ± 1.5	5 ± 0.5
	0.95	38 ± 0.5	5.5 ± 0.5	2.5 ± 1	2 ± 1.5
	1.00	37 ± 0.5	2 ± 1	0	0

in chlorophyll contents as a result of heavy metal toxicity. Heavy metal treatment inhibits photosynthesis, increases respiration and reduces the chlorophyll level (Brown and Whitehead 1986). Excessive zinc also causes chlorosis. Skaar et al. (1973) observed electron dense inclusions in the nuclei and severe damage in nuclear envelope of polluted leaf cells of *Rhytidiadelphus squaqrrosus*. Survivability ceased at 1.0 ppm of metal solution due to reduction in photosynthesis. In *Rhytidiadelphus squarrosus*, Brown and Sidhu (1992) found a linear relationship between concentration of intercellular zinc and inhibition of photosynthetic activity suggesting that membranes may be poisoned when heavy metals gain access to cell interior.

From this study the following conclusions can be drawn:

- Heavy metal stress triggers morphological and physiological changes in bryophytes.
- No apparent change could be observed in the plant species under investigation at metal concentrations below 0.65 ppm for either lead nitrate or zinc sulphate. However, concentrations within the range of 0.65-1.0 ppm showed significant morphological changes.
- Heavy metal toxicity causes reduction in shoot length, chlorophyll level and photosynthetic activity.
- Effect of lead nitrate on various parameters is more drastic than zinc sulphate.
- Zinc sulphate has shown more drastic effects in *Timmiella barbuloides* as compared to other three species under investigation.

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