Research Article

STUDIES ON THE EFFECT OF HEAVY METALS ON THE GROWTH OF SOME BRYOPHYTES-I (MOSSES)

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ABSTRACT

The effect of some heavy metals (eg. Cu,Zn,Fe,Hg,Cd,Co and Ni) regarding their toxic effect has been studied in seven taxa of mosses (eg. *Rhodobryum roseum*, *Pohilia elongata*, *Atrichum pallidum*, *Thudium recognitum*, *Fissidense taxifolius*, *Brachythecium kannounense* and *Funaria hygrometrica)* it is found that Zn, Cu, Fe are better tolerated than Hg, Cd, Ni, Zn is considered to be an essential element as in higher plants. Hg is found to be most toxic Co follower. Broadly speaking the taxa studied may follow one of the following sequence in their response to toxicity level

(1) Hg>Co>Cd>Ni>Cu>Fe>Zn.

(2)Hg>Co>Ni.>Cd>Cu.>Fe>Zn.

(3)Hg>Co>Cd=Ni>Cu>Fe>Zn

Keywords: Heavy metals, bryophytes, mosses.

INTRODUCTION

Experimental studies on Bryophytes were initiated as early as 1903 by Lohmann[1], however, some significant work was done by some Japanese workers (Asakawa and associates[2],1981), Swedish group (Bendz *et al*[3], 1962), English researchers, Connolly[4] (1973) and in Australia by Markham[5] (1978) etc. Most of the studies have been focused on the chemical contents, enzymes and their activity and growth patterns etc.

The data gleaned from various findings (*cf* Brownand and Whitehead[6], 1986) indicate that bryophytes as compared to the vascular plants mosses and liverworts have the ability to tolerate excessively high concentrations of heavy metals as compared to vascular plants. Inspite of this, sometimes, their growth is restricted and even stopped by very small concentrations of some heavy metals i.e. Pb, Cd, Co, Ni, Fe and Hg.

Heavy metals have long been known to have toxic effects on higher plants (Nobbe *et al*[7]*.,* 1884). Interestingly however, some moss species are even known to accumulate certain heavy metals without visible symptoms of injury (Martensson and Berggreu[8], 1954). But nevertheless, it is also known that heavy metals do influence the physiological traits of bryophytes (Pickering and Puia[9] 1969; Lepp and Roberts[10], 1977; Brown and Whitehead 1986).

The present study is undertaken to investigate the effect of Cu, Zn, Fe, Hg, Cd, Co and Ni on spore germination in some mosses.

MATERIALS AND METHODS

Materials were collected from various parts of Western Himalayas. Precise data concerning the locality, altitude and nature of the substratum for each collection was recorded on the spot. Carefully cleaned, purified and thoroughly washed plant materials were dried at room temperature between folds of blotting sheet and then crushed in pestle and mortar and then sieved for various studies.

The voucher specimens are deposited in the Herbarium, Department of Botany, Punjab University, Chandigarh under reference numbers (given in Table 1) assigned to each sample.

The methods given below were followed for various estimations. For the study of effect of heavy metals on the growth of mosses, apices with growing points/mature capsules were taken in both control and treated petri-dish.

For each study these apical portions were kept in two different petri-dish containing 1 gm of sterilized sand each. One was treated with different concentrations of heavy metals and the other was kept as control. Different sets were prepared for different concentrations. For each concentration two replications were taken. Soil was kept moist at all times by spraying sterilized water with atomizer. The treated material was sprayed with different concentrations of heavy metals after every second day. The growth was noted on alternate days till it was 'o' and then percentage of growth was calculated.

Table 1: The selected mosses, their families, locality, altitude of locality, substratum, date of collection and herbarium reference numbers are given in the table.

S. No.	Name of Taxon	Order Family	Locality	Altitude of locality	Substratum	Date of collection	Herbarium reference number
1.	*Rhodobryum roseum* (Hedw.) Limpr.	Eubryales Bryaceae	Way to Glen, Shimla.	1900 m	Wet soil on rocks	9.9.93	4481
2.	*Pohlia elongata* Hedw.	Eubryales Bryaceae	Way to Glen, Shimla.	1900 m	Soil on rocks	9.9.93	4482
3.	*Atrichum pallidium* Ren. et Card.	Polytrichales Polytrichaceae	Summer Hill, Shimla	2000 m	Soil on rocks	10.9.93	4483
4.	*Thuidium recognitum* (Hedw.) Lindb.	Hypnobryale, Thuidiaceae	Summer Hill, Shimla	2000 m	Soil on rocks	10.9.93	4484
5.	*Fissidens taxifolius* Dix.	Fissidentales Fissidentaceae	Way to Chewick Fall, Shimla	1586 m	On Soil	10.9.93	4485
6.	*Brachythecium kamounense* (Harv.) Jaeq	Hypnobayales Brachytheciaceae	Way to Glen, Shimla	1830 m	Soil on rocks	9.9.93	4486
7.	*Funaria hygrometrica* Hedw.	Funariales Funariaceae	Glen, Shimla	1830 m	Soil on rocks	9.9.93	4487

OBSERVATIONS

***Rhodobryum roseum* (Hedw.) Limpr. (PAN 4481)**

Experimental studies were conducted to see the effect of some heavy metals on the % spore germination of *Rhodobryum roseum* and the results are summarized in Table 2.

Table 2: Data showing % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50 % (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	69	30
	65	0	
Zn	100	90	300-350
	20	0	
Fe	20	83	50-55
	80	0	
Hg	0.05	0	
Cd	1.0	73	
	5.0	0	3.0 - 3.5
Co	0.01	81.1	0.06-0.07
	0.10	0	
Ni	0.5	94	2.5-3.0
	5.0	0	

These results show that:

1. *Rhodobryum roseum* reacts differently in its spore germination response to the various concentrations of Cu, Zn, Fe, Hg, Cd, Co & Ni.

2. Hg, Co. Cd & Ni inhibit spore germination of this taxon at very low concentration i.e. o.05 ppm, 0.10 ppm, 5.0 ppm and 5.0 ppm respectively as compared with Cu, Fe and Zn, which inhibit germination at relatively much higher concentration i.e. 65 ppm, 80 ppm and 500 ppm respectively.

3. The heavy metals in respect of their relative toxic effect on % spore germination of *Rhodobryum roseum* follow the below given sequential order:

Hg > Co > Cd = Ni > Cu > Fe > Zn

***Pohlia elongata* Hedw. (PAN 4482)**

Studies on the effect of various elements on spore germination are given in Table 3.

Table 3: Data showing % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50% (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	96	45-50
	75	0	
Zn	100	98	350-400
	500	0	
Fe	20	96	65
	90	0	
Hg	0.05	0	
Cd	1.0	57	1.0-1.5
	3.0	0	
Co	0.01	84	0.05-0.06
	0.08	0	
Ni	0.5	94	3.5-4.0
	4.5	0	

The data in the table reveals that:

1. Hg, Co. Cd & Ni inhibit spore germination of *Pohlia elongata* at very low concentration i.e. 0.05 ppm, 0.08 ppm, 3.0 ppm and 4.5 ppm respectively. Cu, Fe and Zn on the other hand, inhibit spore germination at relatively much higher concentration i.e. 75 ppm, 90 ppm and 500 ppm respectively.

2. The heavy metals in respect of their toxic effect on the spore germination of *Pohlia elongata* follow the below given sequential order:

Hg > Co > Cd > Ni > Cu > Fe > Zn

Atrichum pallidum Ren. et card. (PAN 4483)

The effect of various elements on the % spore germination of *Atrichum pallidum* is given in Table 4.

Table 4: Data showing % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50 % (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	94	40
	70	0	
Zn	100	91	300-350
	500	0	
Fe	20	92	60-65
	85	0	
Hg	0.05	0	
Cd	1.0	64	
	4.5	0	2.0 - 2.5
Co	0.01	86	0.06-0.07
	0.10	0	
Ni	0.5	96	4.0-4.5
	5.0	0	

Results show that:

1. *Atrichum pallidum* reacts differently in its spore germination response to the various concentrations of Cu, Zn, Fe, Hg, Cd, Co & Ni.

2. Hg, Co. Cd & Ni inhibit spore germination at very low concentration i.e. 0.05 ppm, 0.10 ppm, 4.5 ppm and 5.0 ppm respectively as compared with Cu, Fe and Zn, which inhibit germination at relatively much higher concentration i.e. 70 ppm, 85 ppm and 500 ppm respectively.

3. The heavy metals in respect of their relative toxic effect on the % spore germination of *Atrichum pallidum* follow the below given sequential order:

Hg > Co > Cd >Ni > Cu > Fe > Zn

Thuidium recognitum (Hedw.) Lindl. (PAN 4484)

Studies on the response of spore germination to various elements in this taxon are given in table 5.

Above data shows:

1. The spore germination of *Thuidium recognitum* is inhibited by Hg, Co. Ni & Cd in very low concentrations of these i.e. 0.05 ppm, 0.15 ppm, 7.5 ppm and 8.0 ppm respectively. Cu, Fe and Zn, on the other hand also inhibit spore germination but in comparatively higher concentration i.e. 70 ppm, 80 ppm and 450 ppm respectively.

2. The various heavy metals in respect of their relative toxic action on the % spore germination of *Thuidium recognitum*, are observed to act in the below given descending order:

Hg > Co > Ni >Cd> Cu > Fe > Zn

Table 5: Data showing % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50 % (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	95	35-40
	70	0	
Zn	100	96	350
	450	0	
Fe	20	98	50-55
	80	0	
Hg	0.05	0	
Cd	1.0	97.1	5.5-6.0
	8.0	0	
Co	0.01	98	0.09-0.10
	0.15	0	
Ni	0.5	92	4.5
	7.5	0	

Fissidens taxifolius Dix. (PAN 4485)

The effect of some elements on the % spore germination of *Fissidens taxifolius* is given below in table 6.

Table 6: The data on % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50 % (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	98	50-55
	80	0	
Zn	100	98	200-250
	300	0	
Fe	20	95	65-70
	90	0	
Hg	0.05	1	
	0.1	0	
Cd	1.0	95	5.5
	7.5	0	
Co	0.01	92	0.08
	0.14	0	
Ni	0.5	90	4.5
	7.0	0	

The study shows that:

1. *Fissidens taxifolius* reacts differently in its spore germination response to Cu, Zn, Fe, Hg, Cd, Co & Ni. and also to their various concentrations .

2. Hg, Co. Ni & Cd inhibit spore germination at very low concentration i.e. 0.1 ppm, 0.14 ppm, 7.0 ppm and 7.5 ppm respectively as compared with Cu, Fe and Zn, which inhibit growth at relatively much higher concentration i.e. 80 ppm, 90 ppm and 300 ppm respectively.

In respect of their relative toxic effect on the % spore germination of *F. taxifolius*, the tested heavy metals were observed to follow the below given sequential:

Hg > Co > Ni > Cd > Cu > Fe > Zn.

Brachythecium kamounense **(Harv.) Jacq. (PAN 4486)**

Study on the effect of various elements on spore germination in this taxon are given in table 7 below.

Table 7: Data showing % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50% (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	88	60
	80	0	
Zn	100	96	200-250
	350	0	
Fe	20	88	65-70
	95	0	
Hg	0.05	0	
Cd	1.0	92	4.5-5.0
	7.5	0	2.0 - 2.5
Co	0.01	95	0.09
	0.14	0	
Ni	0.5	96	4.5-5.0
	7.5	0	

Data in the table reveals that:

1. Spore germination of *B. kamounense* is inhibited by Hg, Co, Cd & Ni at low concentration i.e. 0.05 ppm, 0.14 ppm, 7.5 ppm and 7.5 ppm respectively. Cu, Fe and Zn, on the other hand, inhibit spore germination of this taxon at comparatively much higher concentration i.e. 80 ppm, 95 ppm and 350 ppm respectively.

2. The various heavy metals, in respect of their relative toxic action on the % spore germination of *B. kamounense*, display the below given sequential order:

Hg > Co > Cd = Ni > Cu > Fe > Zn.

Funaria hygrometrica **Hedw. (PAN 4487)**

Studies on the response of spore germination against various elements are shown in table 8.

This study shows that:

1. *Funaria hygrometrica* reacts differently in its spore germination response to the various concentrations of Cu, Zn, Fe, Hg, Cd, Co & Ni.

2. Hg, Co. Cd & Ni inhibit spore germination at very low concentration i.e. 0.1 ppm, 0.10 ppm, 4.5 ppm and 7.0 ppm respectively, as compared with Cu, Fe and Zn, which inhibit germination at relatively much higher concentration i.e. 80 ppm, 90 ppm and 500 ppm respectively.

3. The various heavy metals in respect of their relative toxic action on the % spore germination of *F. hygrometria* are observed to follow the below given sequential order:

Hg > Co > Cd >Ni > Cu > Fe > Zn.

Table 8: Data showing % spore germination at initial and final concentrations of various elements and the concentration at which the spore germination is 50 % (LC_{50}).

Elements	Conc. (ppm)	% Spore germination	LC_{50} (ppm)
Cu	10	98	45-50
	80	0	
Zn	100	97	350
	500	0	
Fe	20	96	60-65
	90	0	
Hg	0.05	2	
	0.1	0	
Cd	1.0	72	2.5-3.5
	4.5	0	
Co	0.01	96	0.09
	0.10	0	
Ni	0.5	97	4.0
	7.0	0	

DISCUSSION

A perusal of the data on the effect of Zn, Cu, Fe, Hg, Cd, Co & Ni on spore germination of mosses suggests that Zn, Cu and Fe are better tolerated than Hg, Cd, Co & Ni. The growth and germination of spores in soil cultures with different concentration of 'Zn' indicate that as in higher plants, it is an essential element for the growth of bryophytes as well. Being an essential component of chlorophyll 'a' (Rai and Dey[11], 1980), it effects the development and consequent photosynthetic efficiency of the plants.

'Zn' inhibited growth completely at very high concentration i.e 300-850 ppm in all the studied taxa, while 'Cu' checked the growth completely at 70-90 ppm. Presently it was found that in *Funaria* spore germination could occur in 150 ppm of 'Zn' and 15 ppm of 'Cu'. Beyond the mentioned concentrations, there was complete cessation of growth. These results are in line with earlier findings (Coombes and Lepp[12], 1974) in *Funaria*.

The greater toleration of 'Zn' compared with the other elements studied here may be due to the relatively very slow uptake of 'Zn' into the cytoplasm, as observed in *Fontinalis* (Pickering and Puia, 1969).

Of these two elements 'Cu' and 'Zn' we have more knowledge of the role of 'Cu' in biological systems (Williams[13], 1967).

The toxicity effects of copper on biological systems may be because of it being a co-factor of various oxidase enzymes, notably peroxides (Williams, 1967). Mukherji and Gupta[14], 1972 reported that it is quite probable that in bryophytes, as in higher plants 'Cu' may be stimulating the activity of peroxidase enzyme with a subsequent disruptive effect on cellular and hence whole organism metabolic patterns. Further the conversion of the active form to the inactive form of Cu/Zn-SOD by releasing copper (which is the first step in the process of degradation) may be increasing the activity of enzyme Cu/Zn-supuroxide dismutase as pointed out by Tanaka *et a1*[15]. (1995).

'Fe' stopped the growth completely at 70-90 ppm concentration in all the studied taxa. It appears that 'Fe' may be required in lower concentrations for the growth of bryophytes (Jackson *et al*[16], 1994). In mosses it plays a crucial role in the development of gametophytes by inducing a 3-dimentional growth in a single dimentionaly growing caulonema cells as observed in experimental studies on *Bryum pallescens* (Rahbar and Chopra[17], 1983, Bhatia and Chopra[18], 1983).

Of the studied element, 'Hg' was found to be the most toxic. It inhibited growth completely at 0.05-0.1 ppm levels. This toxicity may be caused by the inhibition of photosynthesis, temporarily stimulated respiration resulting in the degradation of chlorophyll and induction of K^+ loss as observed in *Rhytidiadelphus squarrosus* (Brown and Whitehead, 1986).

Generally compared to other plant groups, bryophytes appear to be more sensitive to mercury. In this connection it would be pertinent to cite an earlier study on some bryophytes, when a moss, as compared with some ferms, was found to be ten times more sensitive to 'Hg' (Patterson and Francis[19], 1980).

Of the remaining elements 'Co' follows 'Hg' in its toxic effect. It stopped the growth at 0.1-0.14 ppm level in all the studied taxa.

'Cd' and 'Ni' are nearly equally toxic. The former was found to affect growth remarkably beyond 1.5 ppm level and arresting it completely at 3.0-7.0 ppm concentration. The latter element affected the growth adversely beyond 2.0 ppm level. At 4.5-8.0 ppm concentrations, it completely arrested spore germination in all the studied taxa.

The present study indicates that the studied elements in respect of their toxic/tolerance levels, follow any of the below given three sequential orders:

(i) Hg > Co > Cd> Ni > Cu > Fe > Zn

(ii) Hg > Co > Ni > Cd > Cu> Fe> Zn

(iii) Hg > Co > Cd = Ni > Cu> Fe> Zn

Of these, sequence (i) is found to be most prevalent and found in *Pohlia elongata*, *Atrichum pallidum* and *Furnaria hygrometrica*. It is followed by sequence (ii) which is recorded in *Fissidens taxifolius* and *Thudium recognitum.* Sequence (iii) is also found in two taxa i.e. in *Brachythecium kamounense* and *Rhodobryum roseum*.

The differential response of the various taxa, in respect of regeneration and spore germination, to the different concentrations of the studied elements appears to have resulted from the different toleration levels, in which the various genotypes have evolved during the course of evolution.

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