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Seasonal pattern of metal bioaccumulation and their toxicity on *Sphagnum squarrosum*

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Abstract : *Present study was undertaken as an attempt to study the effect of pollutants on biological responses of Sphagnum growing at Kainchi, Kumaon hills (Uttranchal). Sphagnum plants of almost identical size, collected from the marked sites of Kainchi in different seasons viz., monsoon, winter, summer and again in monsoon, were analysed for chlorophyll, protein, shoot length and nitrate reductase and peroxidase activities. Maximum chlorophyll, protein, shoots length and nitrate reductase activities were observed during the monsoon while minimum in summers. The abundance of Sphagnum and two other bryophytes, Marchantia and Plagiochasma was also higher in monsoon than in other seasons. The study also indicated that Sphagnum has more bioaccumulation and tolerance potential for heavy metals than Marchantia and Plagiochasma.*

Key words : *Bryophytes, Metal pollution, Phytotoxicity*

Introduction

Bryophytes particularly the mosses have been known to accumulate atmospheric particulates and metals from dry and wet depositions (Weiss *et al.*, 1997; Vasconcelos and Taveres, 1998). This has made them popular for use in biomonitoring and phytoremediation studies of air pollutants. Valuable knowledge about input of anthropogenic metals from local and distinct emission sources could be obtained from the physiological studies and chemical analysis of mosses (Syso, 1998). *Sphagnum* moss has been used in pollution studies on several occasions (Glooschenko and Capobianco, 1976; Ho *et al.*, 1996a; Saxena, 1999) and metals have found to be accumulated readily by the shoots mainly by cation exchange at cell walls (Ho *et al.*, 1996b; Saxena *et al.*, 2003).

The suitability of mosses particularly *Sphagnum* as bioindicator is mainly due to one cell thick leaf structure, lack of any protective layer like cuticle or epidermis, absorption and accumulation of nutrients or pollutants directly from the atmosphere, absence of vascular supply for transportation of minerals within the plant, green and perennial growth in wide variety of habitat, ability to be stored for several years without any special care and easy chemical analysis (Callghan *et al.*, 1978; Baker, 1992).

The pollutants may have indirect effect on population caused by alteration in the habitat, which in turn is an indicator of intensity of pollutants in environment or in ecosystem that can be assessed by change in community structure or growth. Growth of an organism can be considered to be the integrated result of numerous physiological and biochemical responses of environmental parameters and therefore, it can also be used to judge the intensity of pollution.

Materials and Methods

Study sites: Kainchi (District Nainital) is located at an altitude of 1873 msl. (5900 feet) on Kumaon hills, situated along the road no. 37, connecting Almora and Ranikhet from Bareilly via Haldwani. Its climate varies through out the year. It is quite cold in winter and mild warm through May-June followed by monsoon rains till October. The average rainfall is 200 cm; minimum and maximum temperatures are 4 ºC and 36 ºC; relative humidity 60-85%, highest in the month of July-August and lowest in January.

Sampling and preparation of moss bags: *Sphagnum squarrosum* Crome. Samml plants were collected from undisturbed habitat of Muketswar (Kumaon hills of Uttranchal) located at 7500 feet and brought to the laboratory in polythene bags. Plants were washed several times and sorted for gametophytes only. Nine moss bags were prepared for transplantation at three sites in triplicate. Each nylon bag was of 1.8” X 1.8” quadrangular plastic frame, interwoven with polymer wire net. 20 gm (dry wt.) of *Sphagnum* was pressed between the folds of moss bag. Moss bags were transplanted at catchment sites for three successive periods (each of four months).

Biological studies: *Sphagnum squarrosum*, *Marchantia polymorpha* and *Plagiochasma appendiculatum* collected from Mukteswar and Kainchi in different seasons were analysed for biochemical and physiological parameters. *In vivo* nitrate reductase activity was measured in accordance to the method of Srivastava (1975) by spectrophotometrically quantifying the nitrite released into the incubation medium. The colour is developed due to the formation of diazo compound with sulphanilamide and nitrite, which is coupled with NED. For peroxidase estimation the method of Putter (1974) was followed using guaiacol as a dye. Protein was estimated following the Folin-phenol method of Lowry *et al.* (1951) using Bovin Serum albumin as standard. Chlorophyll was estimated according to modified Arnon (1949) method by extracting the pigment in 80% acetone. Method of Shimwell and Laurie (1972) was adopted for metal analysis by digesting 1 g of oven dried material (80 ±2 ºC for 6 hrs) in concentrated (3 : 1) $HClO_4$: HNO_3. The metal content was estimated by atomic absorption spectrophotometer (Perkin-Elmer model).

The data presented are average of three replicates with SE. For statistical analysis of data Dunken Multiple Range Test (mean separation test) was applied.

Results and Discussion

Seasonal oscillation in biological parameters of *Sphagnum*: Higher chlorophyll, total protein, dry matter, shoot density, shoot length and nitrate reductase activity were recorded during the monsoon followed by winter, whereas a sharp decline in above parameters was observed during the summer in the moss samples collected from Kainchi. However, peroxidase activity followed a reverse trend (Table 1). A very minor seasonal variation was observed in above parameters in the *Sphagnum* collected from Mukteswar (Table 2).

Experiments demonstrate that the October is the best month for the overall growth and metabolic state of *Sphagnum* and other bryophytes. Low leaf area indices were observed during summers (data not shown), which is particularly due to low shoot density or number of leaves per unit area. The shoot density depends upon light, water and growing seasons (Proctor, 1982; Bates, 1988; Van der Hoeven and During, 1997). But here seasonal variations in shoot density and leaf area indices were observed, which is strongly correlated with seasons and metal accumulation pattern. This needs a further investigated.

Certain physiological and biochemical effects of metals on higher plants have been reviewed by several workers (Thapa *et al.*, 1988; Pahlsson, 1989). Decline in chlorophyll content has been shown to be a reliable criterion in many studies with environmental stresses, including heavy metals. Metal induced decline in chlorophyll content has been demonstrated in some higher plants (Burzynski, 1985; Sinha *et al.*, 1988). The decline is apparently due to reduced chlorophyll synthesis (Nag *et al.*, 1981), which is the consequence of interaction of metal with some vital enzymes involved in the synthesis of chlorophyll (Stobart *et al.*, 1985). The decreased chlorophyll content may lead to reduce photosynthesis and ultimately to reduce biomass. The present study suggests that seasonal variation in chlorophyll content can be correlated with metal precipitation pattern of atmosphere.

Inhibition of nitrogenous parameters such as protein, total nitrogen and nitrate reductase activity under metal stress has been observed in many higher plants (Bhandal and Kaur,

Table – 1: Physiological parameters of *Sphagnum* harvested in different seasons from marked sites of Kainchi.

Parameters	Seasons			
	October, 1997 (Monsoon)	February, 1998 (Winter)	June, 1998 (Summer)	October, 1998 (Monsoon)
Chlorophyll (mg g^{-1} FW)	3.08 ± 0.07^a	2.87 ± 0.09^a	2.33 ± 0.06^b	2.95 ± 0.07^a
Nitrate reductase (n mol NO_2 h^{-1} g^{-1} FW)	5017 ± 3.37^a	4968 ± 5.29^a	4266 ± 2.56^b	5021 ± 3.47^a
Protein (mg g^{-1} FW)	17.64 ± 0.13^a	16.85 ± 0.11^b	13.92 ± 0.13^c	17.58 ± 0.08^a
Peroxidase (Δ OD min^{-1} g^{-1} FW)	0.185 ± 0.06^a	0.265 ± 0.07^b	0.495 ± 0.09^c	0.197 ± 0.05^a
Moisture content (percent)	68 ± 1.81^a	65 ± 0.74^a	59 ± 0.88^b	66 ± 0.05^a
Dry matter (g cm^{-2})	0.37 ± 0.02^a	0.29 ± 0.01^b	0.13 ± 0.01^c	0.38 ± 0.02^a
Shoot length (mm)	65 ± 2.0^a	56 ± 2.08^b	37 ± 2.0^c	67 ± 1.73^a
Dry weight (mg per plant)	8.0 ± 0.15^a	7.6 ± 0.20^a	4.6 ± 0.11^b	7.8 ± 0.21^a

**Sphagnum* samples were analysed in the laboratory within 24 hrs of harvesting from their natural habitat (Kainchi). Values with the same superscript are statistically same.

Table – 2: Physiological parameters of *Sphagnum* harvested in different seasons from marked sites of Mukteswar.

Parameters	Seasons			
	October, 1997 (Monsoon)	February, 1998 (Winter)	June, 1998 (Summer)	October, 1998 (Monsoon)
Chlorophyll (mg g^{-1} FW)	3.32 ± 0.05^a	3.27 ± 0.02^a	3.18 ± 0.02^a	3.29 ± 0.02^a
Nitrate reductase (n mol NO_2 h^{-1} g^{-1} FW)	5056 ± 3.87^a	4982 ± 2.20^b	$5036 \pm 2.53^{a,b}$	5066 ± 4.67^a
Protein (mg g^{-1} FW)	18.24 ± 0.07^a	17.71 ± 0.02^b	15.51 ± 0.03^c	17.86 ± 0.03^b
Peroxidase (Δ OD min^{-1} g^{-1} FW)	0.135 ± 0.07^a	0.124 ± 0.01^b	0.138 ± 0.01^c	0.115 ± 0.01^b
Moisture content (percent)	70 ± 0.30^a	67 ± 0.19^b	61 ± 0.15^c	69 ± 0.30^a
Dry matter (g cm^{-2})	0.42 ± 0.02^a	0.39 ± 0.02^a	0.36 ± 0.01^b	0.41 ± 0.02^a
Shoot length (mm)	68.8 ± 0.32^a	61.93 ± 0.32^b	60.13 ± 0.12^c	70.73 ± 0.06^a
Dry weight (mg per plant)	8.3 ± 0.09^a	7.9 ± 0.04^a	7.6 ± 0.06^b	$8.1 \pm 0.08^{a,b}$

**Sphagnum* samples were analysed in the laboratory within 24 hrs of harvesting from their natural habitat (Mukteswar). Values with the same superscript are statistically same.

Table – 3: Distribution of bryophytes in natural habitat at Kainchi and Mukteswar in different seasons.

Seasons	Species	Abundance*	
		Kainchi	Mukteswar
March to June (Summer)	*Sphagnum sp.*	+ + +	+ +
	Marchantia sp.	+	+ + +
	Plagiochasma sp.	+ +	+ + + +
July to October (Monsoon)	*Sphagnum sp.*	+ + + +	+ + +
	Marchantia sp.	+ + +	+ + + + +
	Plagiochasma sp.	+ + + +	+ + + + + +
November to February (Winter)	*Sphagnum sp.*	+ + + +	+ + +
	Marchantia sp.	+ + +	+ + + + +
	Plagiochasma sp.	+ + +	+ + + + +

* Each + mark represent population at three sites.

Table – 4: Determination of metal content (μg g^{-1} dw) at Kainchi during 1998-99 in native bryophytes.

Seasons	Species	Pb	Fe	Cu	Cd	Ni	Zn	Total
June	*S. squarrosum*	32.83 ± 1.73	91.3 ± 1.86	6.6 ± 0.28	0.63 ± 0.03	5.4 ± 0.02	25.7 ± 1.33	162.46 ± 3.22
	M. polymorpha	14.17 ± 0.36	41.38 ± 0.53	2.84 ± 0.13	0.23 ± 0.01	2.59 ± 0.06	11.58 ± 0.12	72.79 ± 1.86
	P. appendiculatum	21.84 ± 0.48	48.18 ± 0.79	4.09 ± 0.21	0.38 ± 0.02	3.79 ± 0.08	16.31 ± 0.83	94.59 ± 1.79
October	*S. squarrosum*	18.37 ± 0.27	38.24 ± 0.58	3.37 ± 0.24	036 ± 0.02	3.36 ± 0.13	22.47 ± 1.37	86.17 ± 0.96
	M. polymorpha	8.84 ± 0.08	9.47 ± 0.65	1.97 ± 0.16	9.13 ± 0.01	1.41 ± 0.09	7.67 ± 0.09	38.49 ± 0.74
	P. appendiculatum	11.39 ± 0.12	17.76 ± 0.39	2.89 ± 0.17	0.24 ± 0.01	2.27 ± 0.11	11.73 ± 0.04	46.28 ± 0.93
February	*S. squarrosum*	16.64 ± 0.86	42.37 ± 1.16	5.14 ± 0.23	9.28 ± 0.30	2.95 ± 0.13	28.86 ± 0.67	105.24 ± 0.59
	M. polymorpha	7.48 ± 0.32	15.91 ± 0.67	1.87 ± 0.15	9.10 ± 0.13	1.03 ± 0.09	7.49 ± 0.38	42.88 ± 0.87
	P. appendiculatum	10.75 ± 0.74	26.68 ± 0.72	3.28 ± 0.12	0.17 ±0.01	1.67 ± 0.08	12.63 ± 0.76	55.18 ± 1.36

Table – 5: Determination of metal content (μg g^{-1} dw) at Mukteswar during 1998-99 in native bryophytes.

Seasons	Species	Pb	Fe	Cu	Cd	Ni	Zn	Total
June	*S. squarrosum*	7.46 ± 0.87	43.05 ± 1.35	3.97 ± 0.48	0.37 ± 0.04	1.15 ± 0.19	11.37 ± 0.81	66.32 ± 0.94
	M. polymorpha	3.14 ± 0.53	24.41 ± 0.87	1.26 ± 0.19	0.14 ± 0.01	0.41 ± 0.09	5.49 ± 0.32	34.84 ± 0.53
	P. appendiculatum	4.97 ± 0.48	32.74 ± 0.76	2.28 ± 0.23	0.17 ± 0.02	0.76 ± 0.12	7.31 ± 0.43	48.25 ± 0.37
October	*S. squarrosum*	6.63 ± 0.47	34.13 ± 0.52	3.15 ± 0.47	0.27 ± 0.02	0.83 ± 0.14	13.63 ± 0.57	60.60 ± 0.47
	M. polymorpha	2.47 ± 0.47	19.38 ± 0.43	1.73 ± 0.39	0.11 ± 0.01	0.31 ± 0.13	6.41 ± 0.09	30.69 ± 0.23
	P. appendiculatum	3.98 v 0.51	23.89 ± 0.27	2.14 ± 0.17	0.23 ± 0.01	0.59 ± 0.08	9.53 ± 0.14	40.55 ± 0.39
Feburary	*S. squarrosum*	6.59 ± 0.43	39.46 ± 0.56	2.93 ± 0.43	0.31 ± 0.02	0.74 ± 0.18	16.57 ± 0.48	66.52 ± 0.57
	M. polymorpha	2.75 ± 0.34	19.78 ± 0.32	1.21 ± 0.54	0.11 ± 0.01	0.27 ± 0.36	6.97 ± 0.53	30.79 ± 0.38
	P. appendiculatum	4.17 ± 0.56	25.17 ± 0.45	1.87 v 0.32	0.19 ± 0.01	0.53 ± 0.43	10.58 ± 0.34	42.26 ± 0.43

1992; Kevresan *et al.*, 1998). Inhibition of NR activity in *Sphagnum* has been demonstrated in earlier study also which is apparently due to the interaction of the metal with –SH groups of enzyme (Saxena *et al.*, 1999).

Variation in peroxidase activity may be due to seasonal variation in metal accumulation by *Sphagnum*, as reported here. It might be an indication of the induction of free radical scavenging metabolism, which is often seen under different environmental stresses (Van Assche and Clijster, 1990). Increased peroxidase activity has also reported in response to metals in barley and in sunflower (Gallego *et al.*, 1996; Jita *et al.*, 1998).

Seasonal variation in abundance of bryophytes: Although *Sphagnum* is more frequently distributed at Kainchi than at Mukteswar, its abundance varies from season to season at Kainchi. Whereas at Mukteswar *Sphagnum* is more or less uniformly distributed throughout the year. Seasonal variation in abundance of *Marchantia* and *Plagiochasma* was also observed specially at Kainchi (Table 3).

Metal content in native and transplanted bryophytes: A clear-cut difference was observed in metal content of all three studied bryophytes harvested from Kainchi and Mukteswar in different seasons. Mukteswar represented a pure, undisturbed and ideal niche for the luxuriant growth of most of the bryophytes as very low level of metals was detected with a very insignificant seasonal variation. (Table 4, 5 and 6). It was however observed that overall metal concentration was higher during March to June (*i.e.* summers), while lowest during July

Table – 6: Determination of metal content (µg g^{-1} dw) at Kainchi during 1998-99 in *Sphagnum squarrosum* transplanted at Kainchi.

Seasons	Site	Pb	Fe	Cu	Cd	Ni	Zn	Total
Pretransplanted *Sphagnum*		6.63 ±0.32	39.46 ± 0.56	2.93 ± 0.30	0.19 ± 0.03	0.74 ± 0.03	16.57 ± 0.17	66.52 ± 1.21
July to October	Post office	31.47 ± 0.47	138.01 ± 0.96	18.31 ± 0.8	0.56 ± 0.04	3.43 ± 0.93	54.63 ± 0.91	246.41 ± 3.17
	Plantiss agrotech	15.93 ± 0.57	94.16 ± 1.53	26.83 ± 1.21	0.34 ± 0.02	2.17 ± 0.85	69.51 ± 1.17	208.94 ± 3.43
	Kainchi temple	24.56 ± 0.75	119.17 ± 2.63	20.41 ± 0.52	0.45 ± 0.03	3.23 ± 0.77	48.47 ± 0.81	216.29 ± 4.22
November to February	Post office	38.12 ± 0.39	131.95 ± 2.91	27.63 ± 0.73	0.36 ± 0.02	1.93 ± 0.53	69.21 ±1.22	269.20 ± 1.97
	Plantiss agrotech	18.57 ± 0.34	119.80 ± 1.18	27.63 ± 0.61	0.29 ± 0.01	1.93 ± 0.87	83.65 ± 1.05	251.87 ± 1.34
	Kainchi temple	31.79 ± 0.53	186.35 ± 2.54	16.64 ± 0.47	0.31 ± 0.02	2.27 ± 0.59	61.35 ± 2.36	298.71 ± 1.62
March to June	Post office	69.41 ± 1.21	212.58 ± 3.87	15.92 ± 0.9	0.93 ± 0.04	4.36 ± 0.78	61.35 ± 0.57	364.55 ± 3.67
	Plantiss agrotech	38.84 ± 0.67	91.21 ± 1.14	18.43 ± 1.13	0.66 ± 0.02	3.18 ± 0.94	86.07 ± 1.87	238.39 ± 2.94
	Kainchi temple	55.83 ± 0.78	116.39 ± 1.11	14.37 ± 0.53	0.81 ± 0.05	3.9 ± 1.07	52.24 ± 0.96	243.54 ± 2.72

to October. Lower value of metals could be attributed to the (i) decrease in the amount of metal available due to lower number of tourist during rainy seasons, (ii) leaching of pollutants with rain water, (iii) increase in biomass and growth more rapidly in rains due to favorable conditions that might decrease the percentage of metal in proportion to biomass.

The study fairly demonstrates that moss *Sphagnum* can accumulate and tolerate elevated levels of metals without showing any toxic symptoms. Therefore, it can be used as an ideal tool for biomonitoring and phytoremediation studies.

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