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SCREENING OF Pb TOLERANCE IN *BRYUM CELLULARE* HOOK. & *PLAGIOCHASMA APPENDICULATUM* L. ET. L. UNDER GROWTH RESPONSE

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Comparative studies of growth responses based on metal treatment in lab was found useful to screen tolerance potential of species. Present paper discusses the effect of different concentrations of metal (Pb) under different treatment days on biomass, productivity and growth parameter of two bryophytes *Bryum cellulare* Hook. and *Plagiochasma appendiculatum* L. et. L. Higher concentration i.e 50 &100pmm treatment for prolong incubation period showed severe impact on both the plants physiology. Though, leafy liverworts are least resistant to pollutants, changes in photosynthetic pigment composition caused by different ecological factors can be considered as an indication in the change of photosynthetic apparatus.

Keywords: *Bryum cellulare* Hook.; Growth; Lead; *Plagiochasma appendiculatum* L.et.L.; Productivity.

Introduction

Bryophytes are important contributors to biomass and productivity in a wide variety of ecosystems and form an important part of hill cover in Kumaon region [1]. Majority of bryophyte species are very dominating. Therefore, their potential to use as bioindicator of heavy metal deposition in environment is enormous[2-9]. However some species disappeared from contaminated areas were used to indices the polluted area on the basis of their sensitivity. Therefore, species composition varies according to the intensity and composition of pollutant in the atmosphere. Several bryophyte species have a wide geographical and ecological distribution which is useful for comparative studies. The tolerant and sensitive bryophytes, both can be used in the biomonitoring program of atmospheric precipitations. Sensitive species react quickly, and the effect on their morphology, physiology and biochemistry have long been recognized as a tool of biomarker [10, 11]. There are reports of the reduction on the photosynthesis[12] and productivity parameter protein, in the field as well as in the laboratory conditions[1, 12-14]. As a response on physiology of bryophytes, the nonstructural carbohydrate and soluble sugar content are used to compensate the reduced production of photosynthates [15]. The knowledge of photosynthetic pigment content and composition is indispensable in photosynthetic physiology and is important to know the status of health of plant, including bryophytes. However, there are very few information available about status of metal 'Pb' and its presence in bryophytes of Kumaon hill, as very few studies were carried out on these interesting aspect [16]. Accordingly, the present work was aimed to explore the tolerance potential amongst bryophytes and experiments were planned to compare the effect of heavy metal Pb on growth efficiency and productivity of Bryum *cellulare* Hook. and *Plagiochasma appendiculatum* L. et. L and in screening the possible tolerance for metal Pb .The study will further useful for its potential application for biomonitoring in future in this region.

Material and Methods

Plant material and growth conditions - Bryophytes samples of *Bryum cellulare* Hook. and *Plagiochasma appendiculatum* L. et L. were collected in the month of December, 2003 from Ranikhet (Kumaon hill). The number of individuals of a species in each sampling unit was then counted for frequency and distribution of that species and finally brought to laboratory in polythene bags. Samples were carefully cleaned from all dead material and attached litter and finally washed with running tap water to remove soil and adhering dust particles and were then washed in distilled water. Only green or green-brown shoots were transferred to Petriplates containing various concentrations (0, 5, 10, 20, 50 and 100 ppm) of lead nitrate. The plates were transferred to B.O.D. chamber under continuous white light by two fluorescent white tubes (Philips 20 W TLD. India) with a photon flux density of 52 mE $m^{-2} S^{-1}$ (PAR) and kept at 22±2°C. After 24 hrs, 3 and 6 days of metal treatment, plant material was taken for various physiological experiments. No nutrient medium were provided exogenously as bryophytes have unique property to intake directly from their surrounding (air).

Assay of dry mass, carbohydrate and total soluble sugar - To determine the dry matter, plants were dried in oven at 70°C for 24 hours. The dried sample were then weighed. Total carbohydrate was estimated by the method of Hedge and Hofreiter[17]. The total soluble sugar were determined

by the method of Loewus [18] and Cerning and Beroard [19]. Samples of previously frozen tissue (approximately 100 mg fresh wt.) were ground with 3-4 ml of 60% ethanol (chilled below 0 °C). It was centrifuged at 2000 rpm for 4 min. The pellet was resuspended in 2 ml of ethanol and centrifuged again. The alcohol extract was evaporated on a water bath at 80-85 °C until most of the alcohol was removed. The residue was dissolved in 2 ml of 20% ethanol and volume was made to 10 ml with distilled water. Further 5 ml of sugar extract was transferred to a 100 ml volumetric flask and made up to volume with distilled water. To 1ml of this diluted sugar extract, 3ml of anthrone reagent (100 mg anthrone dissolved in 95% ice cold H_2SO_4) was added. The test tubes were put in a boiling water bath for exactly 7.5 minutes. Then these were immediately cooled in ice and the absorbance was measured at 630 nm. Sugar standards were obtained from a stock of 1 mg each of sucrose, glucose and fructose in 1ml ethanol. The amount of sugar were calculated in similar fashion as of total carbohydrate.

Assay for percent nitrogen, crude protein and photosynthetic pigments - Percent nitrogen and crude protein were estimated by Micro-Kjeldahl method [20]. Oven dry samples were digested in digestion mixture (0.5 g $CuS0_4$ and 9.5 g of Na2S04) with 5ml concentrated H_2SO_4 for 4 to 6 hours. Chlorophylls (a+b) and carotenoid (x+c) were determined with double-beam spectrophotometer (Systronic) in 100% acetone extract[21]. All data statistically represents mean of three separate experiments ± standard error (SE). The data were analyzed by student's t-test at P = 0.05 significance level.

Results and Discussion

It is apparent from all aspect of the study that the Pb had severe effect on the physiology of both bryophyte species *i.e. Bryum cellulare* and *Plagiochasma appendiculatum*.

Table 1 shows that carbohydrate content in *Bryum cellulare* was significantly reduced upon treatment of 50 ppm & 100 ppm, over control after 3 and 6 days treatment. Comparatively, *Plagiochasma appendiculatum* did not show such significant difference in the value of carbohydrate over that of control after 24 hrs. The decrease in carbohydrate content under metal stress was caused by substantial lower amounts of starch. This suggests that under environmental stress, starch is converted into soluble sugars probably as a source of energy. Present result is also consonance with earlier findings[15], that at higher doses of metals i.e 50ppm &100ppm, carbohydrate content decreases and sugar content increases. Sugar content was found to increase in 50 ppm & 100 ppm over control both in *Bryum cellulare* and in *Plagiochasma appendiculatum* in 3 days and 6 days while no such significant change was observed in 24 hrs treatment. Seemingly the starch reserves serves as a buffer to compensate the reduced production of photo synthates during stress.

Study of growth, both in *Bryum cellulare* and *Plagiochasma appendiculatum* with respect to percent nitrogen and protein content, revealed that 50 and 100 ppm treatment of Pb shows toxicity for both the plants and a significant decrease observed under long incubation period (i.e. 3 & 6 days). Both plants show an increase in percent nitrogen up to 10 ppm over control in 3 & 6 days treatment (Table 2). The total crude protein (CP) content of Pb treated sample of both plants showed same trend as was found in case of percent nitrogen in regard to the toxicity of Pb. However, the CP at 10 ppm was maximum than in other concentrations of Pb in both *Bryum cellulare* and *Plagiochasma appendiculatum* (Table 2). The 24 hr treatment of Pb in both plants did not show any marked difference in their dry wt. (Table 3) and therefore the study conducted on biomass, attributes that 24 hr is very short time to predict any result of metal response or plant is able to mitigate the toxicity of metal treatment of 24 hrs. Only difference observed was that *Plagiochasma appendiculatum* had more dry matter over *Bryum cellulare* with respect to 0.5 g Fresh wt, while the prolonged treatment data of 3 & 6 days gives significant decrease in dry weight at higher concentration i.e 100ppm of Pb. The reason for a significant increase in dry weight on 10ppm treatment for 6 days were not clear.

Changes in chlorophyll and carotenoid content and pigment ratios are important indicators of environmental stress (Table 4) and describes about the tolerance status of the species, as earlier reported[22, 23]. A statistically significant increase in chlorophyll content was observed in moss plant after application of different concentrations of metal, showed minimum at 50 ppm and 100 ppm treatments in both plants under 3 and 6 days (Table 4). The increase in total chlorophyll content in *Bryum cellulare*, was either due to its increased production or due to protective action of intracellular substances, like carotenoids against the destruction of chlorophyll due to metal toxicity as supported by quotient value (Table 4). Interestingly, result also showed that *Plagiochasma appendiculatum* had more chlorophyll content than *Bryum cellulare* (Table 4). There were small but statistically significant changes observed in the chlorophyll / carotenoid ratio.

In tolerent mosses the total chlorophyll content is apparently not influenced much under short exposure [24], while direct impact of pollutants results in chlorophyll degradation in sensitive species [25] as evident in present study too. Changes in photosynthetic pigment composition caused by different ecological factors can be considered as an indication in the change of photosynthetic apparatus[23].

The increase in the chlorophyll / carotenoid quotient under long incubation period signifies rapid degradation of chlorophyll pigment and significantly decrease in the

Table 1. Effect of different concentrations (Control, 5ppm, 10ppm, 20ppm, 50ppm.and 100ppm) of Pb on total carbohydrate content (mg/ 100mg FW) and total soluble sugar (mg/g FW) . Each value is the mean of 3 replicates ± S. E. Significance (*) differs from control (P≤ 0.05).

Bryophyte sample under study	Time	Treatments in ppm					
		Control (0)	5	10	20	50	100
TOTAL CARBOHYDRATE CONTENT							
Bryum cellulare	24 hr	4.24 ±0.01	4.03 ±0.02	4.06 ±0.06	4.17 ±0.08	3.5* ±0.1	3.23*±0.11
Plagiochasma appendiculatum	24 hr	4.29±0.02	4.25 ±0.02	4.26±0.02	4.25 ±0.03	4.23 ±0.03	4.13 ±0.03
Bryum cellulare	3 days	4.75±0.04	4.62±0.03	4.58±0.112	3.89*±0.11	2.06*±0.235	2.76*±0.022
Plagiochasma appendiculatum	3 days	4.4±0.11	4.39±0.08	4.35±0.08	4.12±0.14	2.52*±0.5	2.73*±0.08
Bryum cellulare	6 days	4.89±0.25	4.92±0.5	4.95±0.55	3.42*±0.123	1.54*±0.55	1.89*±0.22
Plagiochasma appendiculatum	6 days	4.46±0.08	4.4±0.52	4.37±0.225	3.56*±0.08	2.96*±0.02	2.39*±0.12
TOTAL SOLUBLE SUGAR							
Bryum cellulare	24 hr	3.56 ±0.02	3.54 ±0.03	3.59 ±0.01	3.55 ±0.02	4.93 ±0.02	4.89*±0.13
Plagiochasma appendiculatum	24 hr	3.66 ±0.01	3.57 ±0.02	3.66 ±0.02	3.94 ±0.02	4.10 ±0.03	4.16* ±0.03
Bryum cellulare	3 days	3.72±0.35	3.82±0.02	3.9±0.21	4.25*±0.23	6.78*±0.11	6.96*±0.08
Plagiochasma appendiculatum	3 days	3.89±0.25	3.96±0.54	3.99±0.251	4.37*±0.5	6.52*±0.08	6.73*±0.12
Bryum cellulare	6 days	3.76±0.23	3.77±0.55	3.82±0.08	5.42*±0.554	7.92*±0.41	8.38*±0.08
Plagiochasma appendiculatum	6 days	3.92±0.65	3.96±0.5	3.99±0.55	5.76*±0.45	8.96*±0.44	7.39*±0.12

Table 2. Effect of different concentrations (Control, 5ppm, 10ppm, 20ppm, 50ppm.and 100ppm) of Pb on percent nitrogen content and total percent crude protein in percent . Each value is the mean of 3 replicates ± S. E. Significance (*) differs from control (P≤ 0.05).

Bryophyte sample under study	Time	Treatments in ppm					
		Control (0)	5	10	20	50	100
TOTAL NITROGEN CONTENT (%)							
Bryum cellulare	24 hr	1.52 ±0.03	1.56 ±0.02	1.58 ±0.01	1.49 ±0.06	1.42 ±0.01	1.40 *±0.06
Plagiochasma appendiculatum	24 hr	1.49 ±0.01	1.51 ±0.01	1.54 ±0.02	1.45 ±0.04	1.44 ±0.03	1.40 ±0.02
Bryum cellulare	3 days	1.63±0.11	1.83±0.02	1.92*±0.45	1.42±0.5	1.89*±0.44	1.24*±0.55
Plagiochasma appendiculatum	3 days	1.54±0.30	1.74±0.02	1.86*±0.54	1.36±0.44	1.9*±0.41	1.32*±0.55
Bryum cellulare	6 days	1.69±0.52	1.96*±0.05	2.06*±0.254	1.53±0.145	1.17*±0.10	1.22*±0.545
Plagiochasma appendiculatum	6 days	1.63±0.50	1.78±0.05	1.92*±0.25	1.5±0.25	1.26*±0.54	1.29*±0.65
TOTAL CRUDE PROTEIN (%)							
Bryum cellulare	24 hr	9.5 ±0.03	9.75 ±0.01	9.88 ±0.01	9.31 ±0.05	8.88* ±0.02	8.75 ±0.04
Plagiochasma appendiculatum	24 hr	9.31 ±0.02	9.43 ±0.01	9.63 ±0.01	9.06 ±0.04	9.00 ±0.03	8.75 ±0.01
Bryum cellulare	3 days	10.56±1.32	11.43*±0.04	12.0*±0.55	8.875±0.121	11.81*±0.25	7.75*±0.21
Plagiochasma appendiculatum	3 days	9.625±1.02	10.875±2.03	11.62*±0.23	8.5±0.0223	11.87*±0.005	8.25±0.55
Bryum cellulare	6 days	10.56±1.002	12.25*±0.02	12.87*±0.23	9.56±0.21	7.3*±0.5	7.6*±0.13
Plagiochasma appendiculatum	6 days	10.18±6.02	11.12*±0.054	12.0*±0.54	9.37±0.3125	7.8*±0.002	8.06*±0.22

Table 3. Effect of different concentrations (Control, 5ppm, 10ppm, 20ppm, 50ppm.and 100ppm) of Pb on dry weight content (g) with respect to 0.5 g fresh weight of experimental bryophytes. Each value is the mean of 3 replicates ± S. E. Significance (*) differs from control (P≤ 0.05).

Bryophyte sample under study	Time	Treatments in ppm					
		Control (0)	5	10	20	50	100
DRY WEIGHT CONTENT (g)							
Bryum cellulare	24 hr	0.25 ±0.11	0.26 ±0.12	0.26 ±0.16	0.25 ±0.13	0.26±0.15	0.26 ±0.12
Plagiochasma appendiculatum	24 hr	0.31±0.12	0.30 ±0.12	0.31±0.12	0.30 ±0.03	0.30 ±0.13	0.31 ±0.13
Bryum cellulare	3 days	0.24±0.002	0.47±0.05	0.49±0.22	0.23±0.01	0.32±0.002	0.19*±0.005
Plagiochasma appendiculatum	3 days	0.33±0.002	0.54±0.02	0.58*±0.23	0.32±0.2	0.38±0.02	0.24*±0.004
Bryum cellulare	6 days	0.32±0.02	0.58±0.002	0.63*±0.51	0.25±0.02	0.19±0.002	0.15*±0.002
Plagiochasma appendiculatum	6 days	0.45±0.21	0.67±0.02	0.72±0.02	0.32±0.36	0.18*±0.025	0.18*±0.002

Table 4. Effect of different concentrations (control, 5ppm, 10ppm, 20ppm, 50ppm and 100ppm) of Pb on photosynthetic pigment content (mg / g F W). Each value is the mean of 3 replicates ± S. E. Significance (*) differs from control ($P \leq 0.05$).

Bryophyte sample under study	Time	Treatments in ppm					
		Control (0)	5	10	20	50	100
CHLOROPHYLL (a+b)							
Bryum cellulare	24 hr	2.692±0.01	2.682±0.02	2.690±0.06	2.705±0.08	2.703*±0.15	2.703*±0.11
Plagiochasma appendiculatum	24 hr	3.29±0.02	3.25±0.02	3.26±0.02	3.25±0.03	3.23±0.03	3.13±0.03
Bryum cellulare	3 days	2.75±0.04	2.62±0.03	2.58*±0.112	2.89*±0.11	1.06*±0.235	1.76*±0.022
Plagiochasma appendiculatum	3 days	3.4±0.11	3.39±0.08	3.35±0.08	3.12±0.14	1.52*±0.5	1.73*±0.08
Bryum cellulare	6 days	2.89±0.25	2.92±0.5	2.95±0.55	1.42*±0.123	0.54*±0.55	0.89*±0.22
Plagiochasma appendiculatum	6 days	3.46±0.08	3.4±0.52	3.37±0.225	2.56*±0.08	1.96*±0.02	1.39*±0.12
CAROTENOIDS (x+c)							
Bryum cellulare	24 hr	1.56±0.02	1.54±0.03	1.59±0.01	1.55±0.02	1.93*±0.02	1.89±0.13
Plagiochasma appendiculatum	24 hr	2.66±0.01	2.57±0.02	2.66±0.02	2.94*±0.02	2.10*±0.03	2.16*±0.03
Bryum cellulare	3 days	1.72±0.35	1.82±0.02	1.9±0.21	1.25*±0.23	0.78*±0.11	0.96*±0.08
Plagiochasma appendiculatum	3 days	1.89±0.25	1.96±0.54	1.99±0.251	2.37*±0.5	0.52*±0.08	0.73*±0.12
Bryum cellulare	6 days	1.76±0.23	1.77±0.55	1.82±0.08	0.42*±0.554	0.092*±0.41	0.038*±0.08
Plagiochasma appendiculatum	6 days	1.92±0.65	1.96±0.5	1.99±0.55	0.76*±0.45	0.96*±0.44	0.30*±0.12
QUOTIENT (a+b) / (x+c)							
Bryum cellulare	24 hr	1.725±0.54	1.745*±0.39	1.6982±0.35	1.745±0.21	1.400±0.66	1.4301±0.54
Plagiochasma appendiculatum	24 hr	1.236±0.21	1.264±0.025	1.225±0.02	1.105±0.02	1.538±0.23	1.4490±0.23
Bryum cellulare	3 days	1.598±0.23	1.4395±0.23	1.357±0.54	2.312*±0.62	1.358±0.35	1.8333*±0.54
Plagiochasma appendiculatum	3 days	1.798±0.13	1.7295±0.23	1.68±0.23	1.3164±0.02	2.923*±0.35	2.369*±0.21
Bryum cellulare	6 days	1.642±0.54	1.6497±0.13	1.620±0.54	3.380*±0.24	5.869*±0.54	23.421*±0.02
Plagiochasma appendiculatum	6 days	1.802±0.02	1.7346±0.14	1.6934±0.32	3.3684*±0.13	2.041*±0.21	4.633*±0.54

carotenoid imply decrease in protection action of carotenoid.

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