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Effect Of Heavy Metal Stress (Pb, Ni and Cd) On Protein And Fat Content Of The Moss *Thuidium cymbifolium* (Doz. and Molk.) Doz. And Molk

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Abstract

In the present study individual effect of Pb, Ni and Cd respectively was studied on protein and fat content of the moss *Thuidium cymbifolium*. A decrease in protein content was noticed in moss treated with Ni and Cd for 3 days, whereas for Pb an increase of 18 % was noticed at 0.1 M conc. However following an exposure period of 6 days, a significant increase in protein content was noticed for all metals, which again declined after 15 days of treatment. A significant increase in fat content of the moss (136 % and 269 % at 0.1 and 0.2 M Pb) was noted after 3 days. Conversely the increase in Cd ranged between 1-27 % during 72 hrs treatment that further increased to 77 % at 0.2 M Cd concentration following 6 days of exposure. After 15 days of Pb, Ni and Cd treatment a more or less decreasing trend was noticed with increasing concentration. Results revealed that *T. cymbifolium* shows heavy metal sensitivity as evidenced by change in protein and fat content.

Introduction

Bryophytes are an important part of many forest ecosystems that are very effective accumulators of heavy metals (Raeymaekers, 1970). Due to their high accumulation capacity these are used quite extensively in assessing atmospheric deposition rate of heavy metals (Tyler, 1990). However physiological and biochemical responses of bryophytes to heavy metal stress is not yet fully understood. In most studies decrease in growth rate (Margot, 1980; Klein and Bliss, 1984) and changes in photo-pigment content (Saxena *et al*., 2002) has been used as the measure of the toxic effect. However, the fate of protein and neutral lipids (fats) under heavy metal stress has received little attention to date.

Both protein and lipids are sensitive indicators of stress and act as a key substrate for metabolism (Peters, 1973). Therefore the aim of this study was to examine the potential toxicity of Pb, Ni and Cd on fat and protein content of the moss *Thuidium cymbifolium* (Doz. and Molk.) Doz. and Molk. *T. cymbifolium* is a pleurocarpous feather moss; so far no literature is available on the use of this moss as a test plant to study phyto-toxic effect of heavy metals. Therefore this species was chosen for the present work.

Material and Methods

Samples of moss *Thuidium cymbifolium* was collected from Mukteshwar (alt. ca. 2290 m) in polystyrene bags from a uniform area of 50 X 50 cm. After collection the samples were sorted in lab to remove extraneous material (adhering bark, other moss species, soil particles) as much as possible. It was then thoroughly cleaned and washed first with running tap water and finally with distilled water. Dilute solutions of heavy metals $CdCl_2$, $Pb(CHCOO_3)_2$ and $NiSO_4$ were used to determine plant sensitivities to metal toxicity.

Moss samples were treated with different concentrations (0.01, 0.1 and 0.2 M) of metals for 3, 6 and 15 days respectively. After treatment period plant tissues were used for physiological analyses. All the treatments had three replications.

Protein analysis: Oven dry samples were digested in digestion mixture (0.5 g of $CuSO_4$ and 9.5 g of Na_2SO_4) with 5 ml of conc. H_2SO_4 for 5 hrs. It was estimated by Kjeldahl method (Jackson, 1958).

Fat analysis: Oven dry samples were transferred on Whatman paper and percentage fat was estimated with the help of Soxhlet apparatus (Sankaran, 1965).

Results

The protein of the moss exposed to heavy metal stress (Pb, Ni and Cd) is shown in Fig 1. After 3 days of lead treatment, the crude protein in moss plants treated with 0.01 and 0.1 M Pb increased by 18 % and 5 % respectively in comparison to untreated plants while a drop follows to 19 % at 0.2 M concentration. The crude protein increased further to about 65 % and 22 % at 0.01 and 0.2 M concentrations respectively, whereas at 0.2 M Pb treatment the protein content was comparable to control plants. Again after 15 days of treatment the crude protein of the treated moss plants, was found to be well below the untreated plants.

On the contrary, if we focus on the impact of Ni treatment after 72 hrs on the moss plants an overall reduction in crude protein of the plant at all concentrations was noticed. However following an exposure period of 6 days, a significant increase in protein content (30-45 %) compared to control plants was detected, whereas a noteworthy decrease was noticed after 15 days. Likewise Ni, short-term treatment of Cd, caused a significant reduction at all treatments in crude protein compared to control. While after 6 days of treatment a very slight increase in crude protein at 0.01 and 0.1 M Cd concentrations was observed. After 15 days of Cd treatment in 0.2 M concentration a remarkable increase of 49 % relative to control plants was noticed, whereas at 0.01 and 0.1 M concentrations significant lower values were measured.

It is clearly apparent that three days of Pb treatment caused a significant increase in fat content (136 % and 269 % at 0.1 and 0.2 M Pb) of the moss with respect to control (Fig 2.). After 6 days of treatment, the decline in fat proportion ranged between 27-45 % relative to control that further decreased after 15 days of treatment. A slightly different trend was noted for Ni treatment, a more or less equivalent fat content was observed in moss plants in comparison to control after 72 hrs. However, after 6 days, a linear increase in fat content was noticed with increasing metal concentration. After 15 days an increase of almost 22 % was observed at 0.01 M Ni concentration relative to control that decreased gradually to 16 % at highest Ni concentration (0.2 M).

Fig 2 indicates that Cd treatment during 72 hrs of treatment induced an alteration in fat content. The percentage increase in fat ranged between 1-27 % with respect to control. A further increase in fat composition of moss plants in comparison to untreated plants was noticed, after 6 days of treatment. The maximum increase recorded was 77 % at 0.2 M concentration of Cd. However after 15 days, phytotoxicity of Cd was severe, as evident by decrease of fat content.

Discussion

The total protein content of moss *Thuidium cymbifolium* was found varying with metal supplied, concentration of metal and duration of exposure period. A striking increase in the protein content of the moss plants was detected after 6 days of metal (Pb, Ni and Cd) treatment. A comparable crude protein relative to control or slight increase was reported by several workers after metal exposure in a range of plants (Vogeli-Lange and Wagner, 1990; Srivastava *et al*., 2002; Gupta *et al*., 2003). The increase in protein content could be due to the direct increase of mRNA and protein synthesis (Singh *et al*., 1987; Hirt *et al*., 1989). Further this relative increase in the protein content could also be due to recycling of nitrogen from porphyrins and apoproteins of pigment-protein complexes in the synthesis of new protein molecules (Matile *et al*., 1990). A common feature of many environmental stresses is the cellular dehydration, which serves as the signal for the accumulation of stress induced proteins such as dehydrins in a wide variety of plants, in response to a wide variety of environmental stresses (Wisniewski *et al*., 1996).

Long term treatment of Cd, Ni and Pb caused a decrease in protein content may be due to the breakdown of soluble proteins or due to the increased activity of proteins or other catabolic enzymes which were activated and destroyed the proteins (Rai *et al*., 1998). It is well known that metals interfere with number of metabolic processes associated with normal development, especially synthesis of proteins (Vierstra, 1993). Reduced protein content has also been reported by Saxena *et al*. (2002) and Singh and Tewari (2003).

In present study a considerable increase in fat content of the moss plants were noted, when exposed to Pb and Cd levels. Ni exposure, on the other hand, caused small alterations in the fat content of the moss. This result is in consonance with Pham Thi *et al*., (1985) and Sakaki *et al*., (1994), who reported that storage lipids present in low levels in leaf tissues, increase within plants that undergo some environmental stresses. It is therefore, probable that this significant increase in fat content may have an adaptive character in heavy metal pollution. Moreover, acute exposure to Pb, Ni and Cd caused significant decrease in the fat content. Reduction in the fat level that serves as depots of metabolic fuel may be attributed to increased hydrolysis of stored fat during stress. At high external metal concentration, the energy requirement for active metal extrusion increases the ATP demand by 10-30 % of the normal ATP turnover (Losch and Kohl, 1999). Therefore under sub lethal toxic ion stress, this increased ATP demand is generally met by an increased supply from enhanced catabolic process. This might explain the decrease in the fat content of the moss under severe metal stress.

The experiments reported here reveal that the moss *T. cymbifolium* shows heavy metal sensitivity. Both protein and fat content of the moss varied with supply of metal, its concentration and duration of exposure. Furthermore, such in vitro experiments are indeed necessary to investigate standard responses of plants thought to be bio-indicators.

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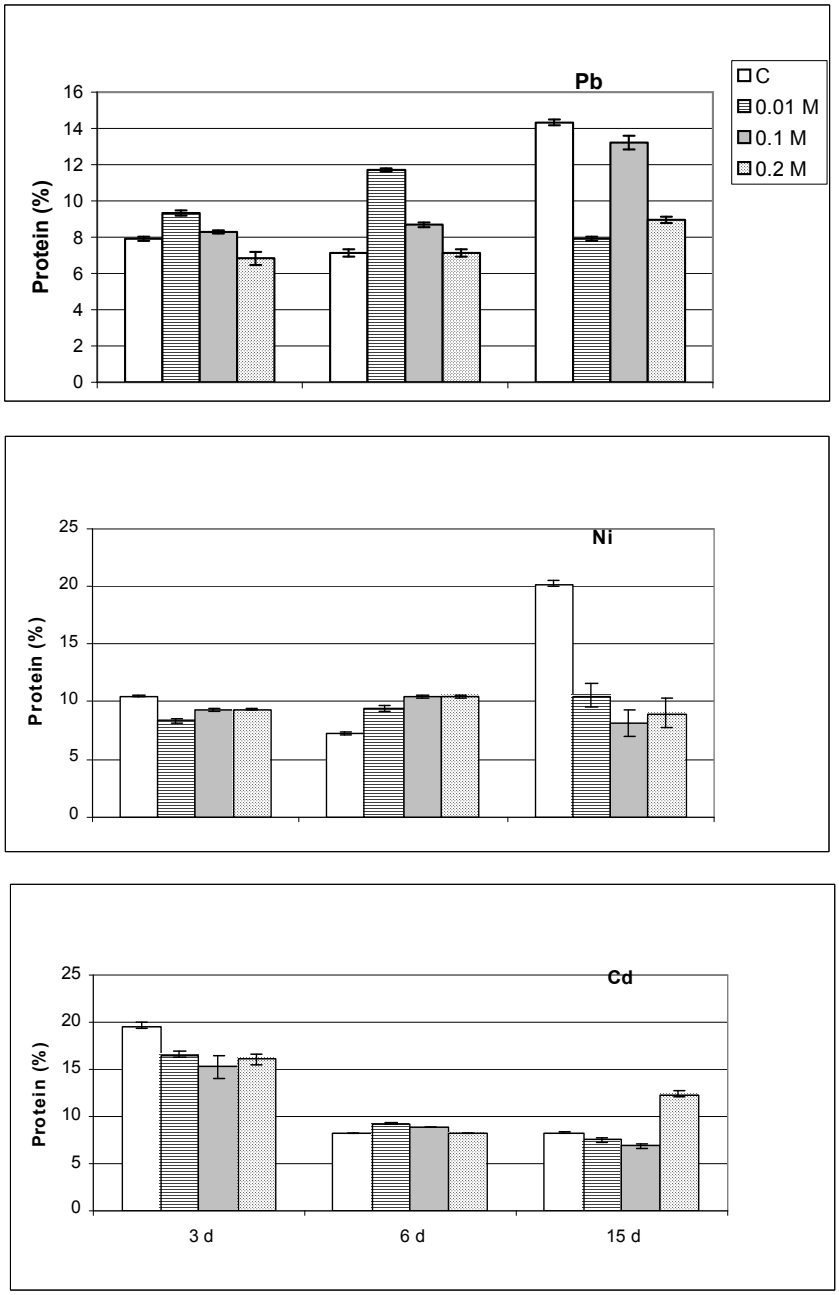

Fig 1. Effect of different metals (Pb, Ni, Cd) concentrations on Protein (%) content of moss *Thuidium cymbifolium* during different days of exposure (data were means of three replications ± S. E).

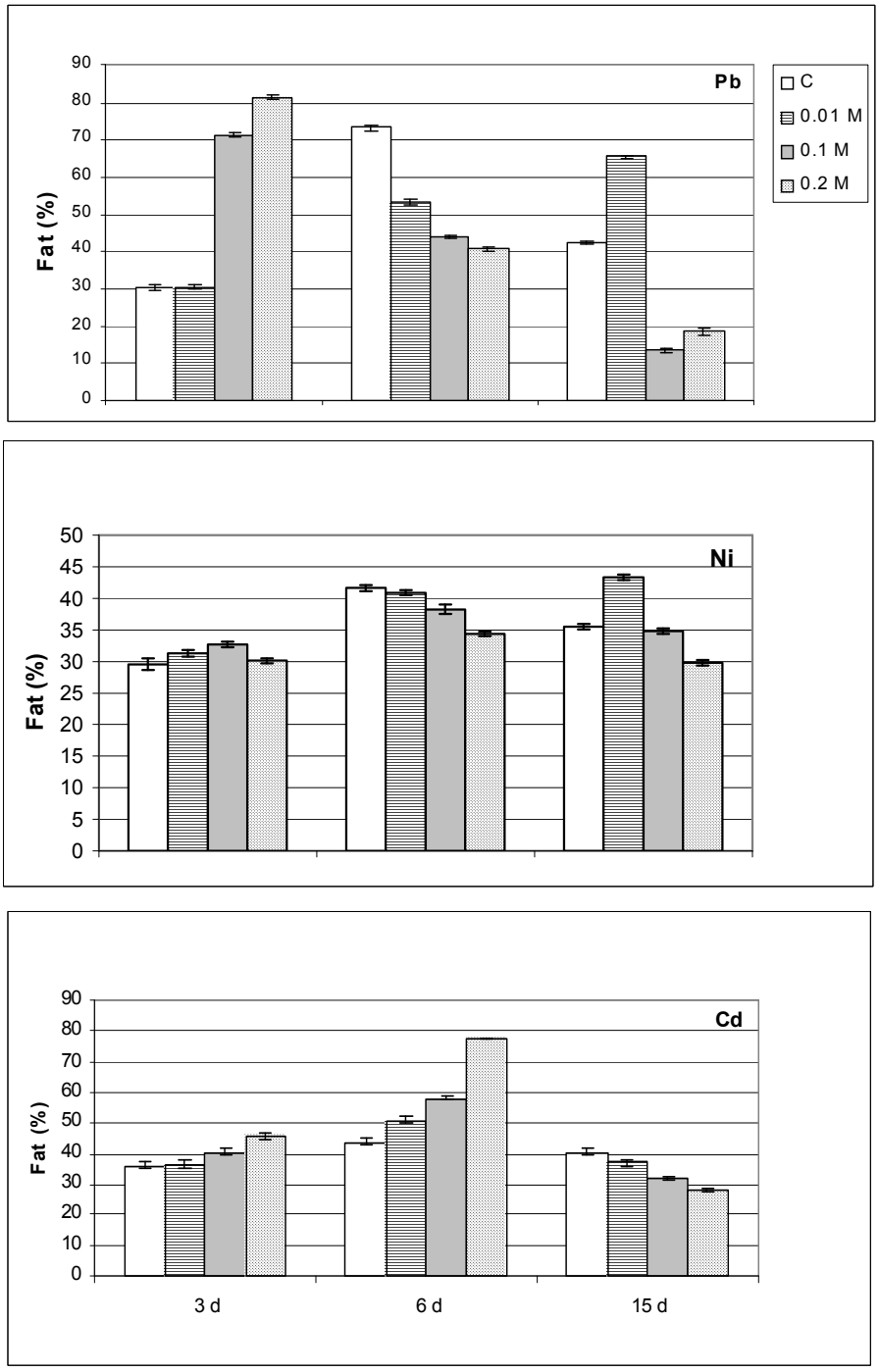

Fig 2. Effect of different metals (Pb, Ni, Cd) concentrations on Fat (%) content of moss *Thuidium cymbifolium* during different days of exposure (data were means of three replications ± S. E).