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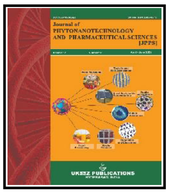

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Effect of heavy metals on *Boerhavia diffusa* L. and SDS-PAGE profiling of protein

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Abstract

Heavy metals are class of elements in which no biological role in plants, at the same time it is imparting toxicity in plants especially metabolic activities. Toxic effect of different concentrations of heavy metals such as cadmium, chromium, mercury and lead was studied by cultivating rooted propagules of *Boerhavia diffusa* L. for a period of twenty days in Hoagland nutrient medium, artificially contaminated with known concentration of those heavy metal ions. Toxic effect of these heavy metals are negatively influencing the metabolic activity of the plants. Protein profiling of root, stem and leaves of *B. diffusa* are traced by using the techniques SDS-PAGE. Protein profiles of cadmium, chromium, mercury and lead stressed proteins showed significant difference when compared each metals and tissues, respectively.

1. Introduction

In recent years, heavy metal (HM) toxicity has become a global concern which always imposing a severe threat to the environment and human health. In the case of plants, a higher concentration of heavy metals, above a threshold, adversely affects cellular metabolism because of the generation of reactive oxygen species which mark the key biological molecules. Moreover, some of the heavy metals such as mercury and arsenic, among others, can directly alter the protein/enzyme activities by targeting their – SH group to further impede the cellular metabolism (Noctor *et al.*,2012; Shahid *et al*., 2014; Riyazuddin *et al*., 2022). *B.diffusa* (Common name-Hogweed) belonging to the family of Nyctaginaceae, is a diffused perennial herbaceous medicinal plant growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds (known also under its traditional name as 'Punarnava' in sanskrit and "Chuvannathazhuthama" in malayalam). The plant was named in honour of Herman Boerhaave, a famous Dutch Physcian of the 18^{th} Century (Chopra, 1969). *B. diffusa* plant has a long history of uses in Ayurvedic or natural herbal medicines (Dhar *et al*., 1968). The major active principle present in the root is alkaloidal and is known as 'punarvavine'. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, 'Charaka Samhita', and 'Sushruta Samhita'. About 45 Ayurvedic preparations inclusive of 'Dhanvantaaristam', 'Chyavana parasam,'' Ashokarishtam', 'Punarnavasavam', 'Rasanadikasayam', 'Narasimharasayam', *etc*., contain the roots, leaves or entire plant of *B. diffusa* (Sivarajan and Balachandran, 1994). The roots, leaves or the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine in India, Nepal, Sri Lanka and China. The root is mainly used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, odema, jaundice, menstrual disorders, anaemia, liver-gallbladder and kidney disorders, enlargement of spleen, abdominal pain, *etc*. (Kirtikar and Basu, 1956). It was also demonstrated that the drug decreased the albumin urea, increased the serum protein and lowered serum cholesterol level (Ramabhimaiah *et al*., 1984). Singh and Udupa (1972) reported that the dried root powder showed curative efficiency for the treatment of helminth infection.

B. diffusa is a medicinal plant widely used as an important ingredient of many Ayurvedic preparations. These plants grow profusely as wild plants and are well adapted to polluted areas such as road side, railway track, banks of drainage, vicinities of public comfort station, *etc*. By trial and error experiments, the present author observed that *B. diffusa* plants grow well in Hoagland nutrient medium under hydroponic system. So, simulated experiments were set up to analyse the responses of *B.diffusa* by cultivating rooted propagules in Hoagland solution artificially contaminated with known quantities of cadmium chloride ($CdCl_2$), potassium dichromate ($K_2Cr_2O_7$), mercuric chloride ($HgCl_2$) and lead acetate $(CH_3\text{-}COO)_2Pb\ 3H_2O$.

Eventhough, effect of Cd, Cr, Hg and Pb have been investigated in a number of plants. Effect of these heavy metals on medicinal plants in general and *B. diffusa* in particular have not yet been

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elucidated. Similarly, investigations on the effect of different heavy metals in varying concentrations in one and the same plant are very scanty. Effect of heavy metals on medicinal plants and bioaccumulation potential in general and *B. diffusa* in particular has not yet been investigated. The objectives of the present study include standardisation of different concentrations of Cd, Cr, Hg and Pb on *B. diffusa* to impart more or less similar visible morphological symptoms of growth retardation. Study mainly aims to electrophoretic analysis of proteins in order to correlate synthesis/ degradation of proteins due to the impact of heavy metals.

2. Materials and Methods

Boerhavia diffusa L. cuttings were collected from Sir Syed College Botanical Garden. Healthy and profusely growing plants were selected for experiments. Healthy cuttings of 10-15 cm length consisting of 3-4 nodes were selected for culture studies. Screening experiments on the effect of treatments of *B. diffusa* cuttings with cadmium chloride ($CdCl_2$), potassium dichromate ($K_2Cr_2O_7$), mercuric chloride ($HgCl_2$) and lead acetate $(CH_3\text{- }COO)_2Pb\ 3H_2O$ showed that tolerance of *B. diffusa* towards cadmium, chromium, mercury and lead varied widely. Hence, the concentrations in which seedlings survived but exhibited approximately 50% growth retardation were selected for the experiment. Rooted cuttings (3 numbers) were planted in one bottle containing 100 ml of Hoagland solution to which the heavy metal solutions were added to obtain the final concentration. Minimum 25 bottles were used for each treatment, so as to get sufficient tissues for experiments. The hydroponic system was maintained under green house conditions. Plants cultivated in Hoagl and solution without any heavy metal, salt served as the control. Samples of treatments and control were collected at comparable interval of four days up to 20 days of growth. At each interval, plants were harvested from each treatment, washed thoroughly in distilled water and blotted to dryness. A minimum of 5 plants of each treatment were separately cut into pieces, randomized and sampled in duplicates for each analysis. Protein profile of the treatments and control (root, stem and leaves) of *B. diffusa* were analysed by SDS poly acrylamide gel electrophoresis (Gaal *et al*., 1980), (Figure 1 and Figure 2).

Figure 1: *Boerhavia diffusa* L.

Figure 2: Cultivation of *B.diffusa* by hoagland solution method.

3. Results

SDS-PAGE profile analysis showed only two distinct bands with molecular weight 14.8 KDa and 18.5 KDa in the root of control plants whereas in root tissue of plants treated with cadmium, two new bands (8.85 KD and 18.71KDa) were present (Figure 3). Similarly in chromium treatment also, two new bands with different molecular weight were present. Due to mercury treatment, two bands more or less similar to that of chromium treatment were observed. Lead treated root tissue showed only a single additional band that was absent in the control. Stem tissue of plants treated with cadmium, the range of molecular weight was 5.18 KDa to 151.23 KDa compared to the control (Figure 4). Six new bands which were absent in the control. In chromium treated plants, only five new bands were present with different molecular weight which were not present in the control. More or less similar bands were present in mercury treatment, but molecular weight range was 4.26 KDa to 80.85 KDa. The protein profile of treated tissue showed very thick band with molecular weight 26.78 KDa in addition to 26.78 molecular weight band. Six more bands ranges from 3.63KDa to 80.34KDa.

Protein profile of leaf of plants treated with cadmium showed number of protein bands having molecular weight ranges from 2.81KDa to 62.59KDa compared to the control (Figure 5). Many bands were newly appeared particularly proteins with high molecular weight range. Plants treated with chromium exhibited lesser number of protein bands which were stained feebly compared to the control as well as the cadmium treated plants. Maximum number of protein bands 1.188 KDa to 65.15 KDa shown by leaf tissue of plants treated with mercury in which many protein were new which were absent both in the control and other treatments. Comparatively lesser number of bands were present in the leaf protein profile of plants treated with lead and the staining intensity of bands also was very high (Figure 3).

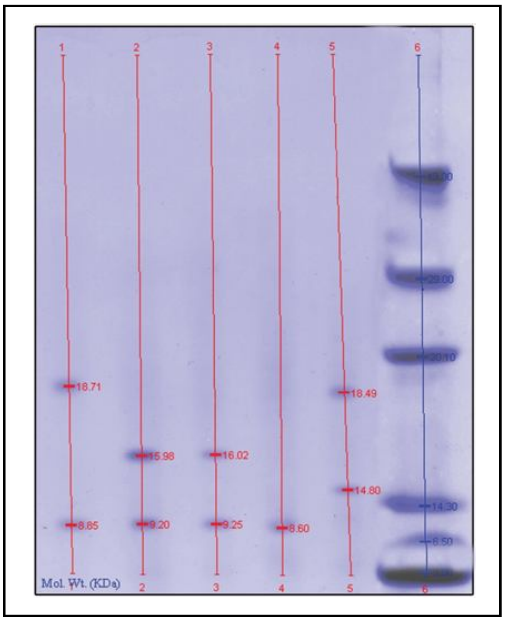

Figure 3: SDS-PAGE protein profile of *B. diffusa* root.
1. cadmium, 2. chromium, 3. mercury,
4. lead, 5. control, 6. marker protein

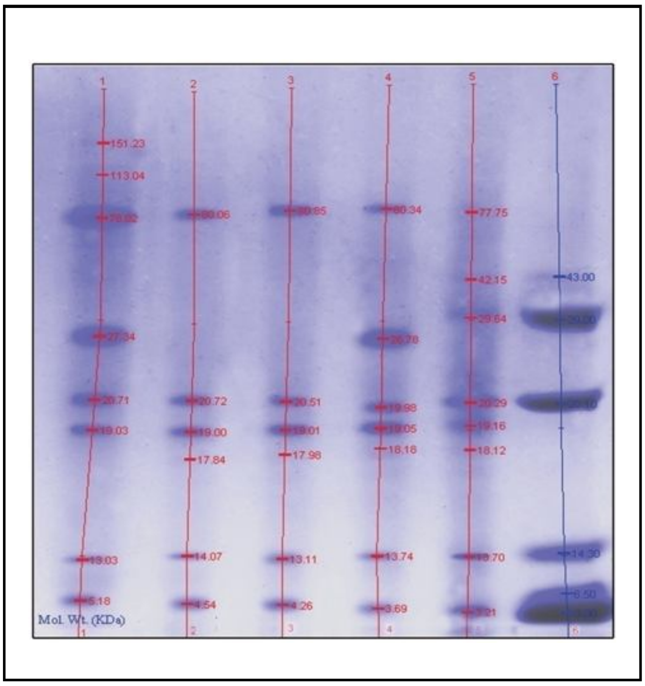

Figure 4: SDS-PAGE protein profile of *B. diffusa* stem.
1. cadmium, 2. chromium, 3. mercury,
4. lead 5. control, 6. marker protein

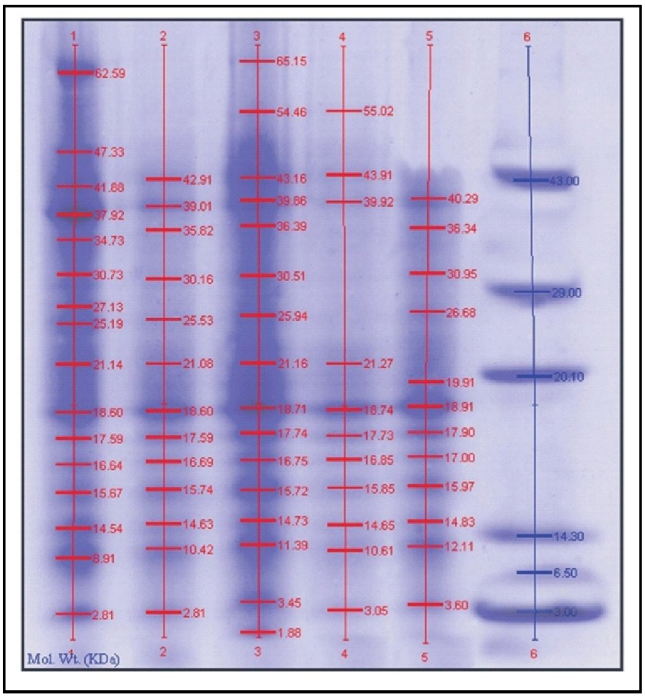

Figure 5: SDS-PAGE protein profile of *B.diffusa* leaf.
1. cadmium, 2. chromium, 3. Mercury,
4. lead, 5. control, 6. marker protein

4. Discussion

Polyacrylamide gel electrophoretic profile of root tissue showed two new bands in *B. diffusa* treated with cadmium, chromium and mercury. But, these two bands are dissimilar in molecular weight

(Figure 3). A number of new bands are appeared in the stem tissue of plants treated with cadmium, chromium and mercury (Figure 4). Only one feeble band is shown by lead treatment. The new bands appeared in the plants treated with heavy metals are absent in the control and hence can be presumed as stress-induced proteins *i,e*., phytochelatins, which have already been reported to be synthesised in plants under heavy metals such as cadmium, chromium, mercury, lead, *etc*. (Grill *et al*., 1985; Verkleij *et al*., 1990; Salt *et al*., 1998; Choudhury and Panda, 2005). According to Grill *et al*., (1989) phytochelatins are synthesised due to heavy metals and the role of phytochelatin synthase activity differs from metal-to-metal. Phytochelatin synthesis for the sequestration of heavy metal toxicity has been reported in plants (Rauser, 1987; Reddy and Prasad, 1990; Kubota *et al*., 2000; Cobbet and Goldsbrough, 2002). The protein bands observed under individual metals exhibit difference in number and molecular weight probably either due to the differences in their metal binding property or differences in tolerance potential of *B. diffusa* towards each metal.

Since the PAGE analyses of protein was done only in the samples of the first interval, the role of the phytochelatin in the sequestration of heavy metals is ambiguous in *B. diffusa* because phytochelatin synthesis has been reported to start during some hours after the heavy metal treatment (Rauser, 1995). Nevertheless, unlike the root and stem, maximum number of new protein bands are observed in the leaf tissue (Figure 3) probably revealing the sequestration, and hence the leaf metabolism is not adversely affected. It is also worth mentioning that leaf tissues exhibit accumulation of all heavy metals in the order Pb>Hg>Cr>Cd. So, their sequestration is presumed to take place by the formation of phytochelatins which occur in abundance in the leaf (Figure 4). Induction of new protein synthesis has been reported in wheat and mustard plants irrigated with tannerary effluents containing many heavy metals inclusive of cadmium (Chandra *et al*., 2009). Zhang *et al*. (2002) suggested that cadmium tolerance is enhanced by over-expressing the enzyme, γ-glutamyl cysteine synthetase which is the key enzyme involved in the synthesis of phytochelatins. In *Solanum tuberosum*, phytochelatin synthase activity increases proportional to the concentration of $CdCl_2$ and thereby the tissues possess more efficient cadmium detoxification mechanism (De Vos *et al*., 1992; Stroinski and Zielezinska, 2001).

Leaf protein profile shows many bands and significant variation is observed between treatments. Maximum new protein bands are present in the leaves of plants treated with mercury, whereas the plants treated with lead exhibit very few protein bands (Figure 5) revealing different responses of *B. diffusa* towards different metals and / or different concentrations. In *Potomogeton pectinatus*, protein synthesis is induced under cadmium stress up to 50 µM concentration, whereas in *B. diffusa* protein content is reduced at the concentration 30 µM cadmium. This disparity may be due to genetic variations of plants towards the sensitivity/tolerance to heavy metals as suggested by Angelova *et al*. (2006) and Meers *et al*. (2007).

5. Conclusion

Non-essential heavy metals such as cadmium, chromium, mercury and lead are highly reactive and interfere the normal metabolism and become toxic to plants, generating morphological and physiological alterations and modifications. More or less consistent growth performance was exposed by the plants irrespective of the difference of concentration of the heavy metals. Most of the medicinal plants are herbs which are cultivated or naturally growing in soil, contaminated with heavy metals by natural and anthropogenic activities and the plants accumulate considerable quantities of toxic heavy metals. PAGE studies showed new protein bands in experimental plants compared to control. Appearance of new protein bands varied among the four metals. Maximum bands of protein profile analysed by PAGE shown by leaf tissue are presumably phytochelatin which are involved in the sequestration of metals accumulated in the leaf tissue.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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