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# Adaptation of Catharanthus roseus to heavy metal stress by altering total protein content

**V. Soumya[1], P. Kiranmayi[1]*, V. Saritha[2], K. Srinidhi[1]**

[1]Department of Biotechnology, Institute of Science, Gandhi Institute of Technology and Management, Visakhapatnam, Andhra Pradesh, India- 530045

[2]Department of Environmental Science, Institute of Science, Gandhi Institute of Technology and Management, Visakhapatnam, Andhra Pradesh, India- 530045

*Corresponding Author Email: kpatnala@gitam.edu

**Abstract**

The emission of pollutants into the environment is rampant due to rapid industrialization. Being immobile, plants are routinely confronted by a wide array of abiotic stresses including heavy metals. Plants growing in industrial areas are exposed to several heavy metals. These metals are thought to obstruct the biological functions of a protein by altering the native conformation. To study the effects of heavy metals on the plant protein content, the present study incorporated *Catharanthus roseus* as a plant of interest.

The primary objective of the study was to estimate the total protein content from these plants grown in varied industrially contaminated soils. The protein content was estimated by UV-Vis spectrophotometric technique using the conventional Lowry's method. Bovine Serum Albumin (BSA) was used as standard reagent against which unknown plant protein concentration was estimated. The results showed that plants grown in soil with highest metal concentration also had highest protein content, while plants grown in soil with least metal concentrations expressed lowest protein content. By analysing the alterations of total proteins in plants grown under metal stress, several biotechnological strategies can be devised for improving the tolerance of plants to heavy metals. Also, further studies involving biochemical and -omics analyses can provide in-depth study which will be necessary to exploit the great potential of the stress-responsive proteins.

**Keywords**: *Catharanthus roseus*, contaminated soil, heavy metals, protein, root-shoot ratio.

## Introduction

Proteins are functionally versatile macromolecules that are considered as vital toilers of living cells. They function in cellular signalling, regulation, metabolism, catalysis, intra and inter cellular movement of nutrients and other molecules, membrane fusion, structural support, and protection (Amm et al, 2014). Additionally in plants, they act as storage medium to meet the nutritional demands of developing seedlings, catalyzes the transfer of solar energy to chemical energy that can be used by the plant, through $CO_2$ fixation and aid in overall plant health and growth. The function of a protein is basically determined by its structure, which is acquired following ribosomal synthesis of its amino acid chain. In addition, the conformation of a protein largely depends on the physical and chemical conditions of the protein environment as affected by abiotic stresses like temperatures, reactive molecules and heavy metals that not only alter the folding process of a newly synthesized protein, but also induce changes in already existing proteins (Goldberg, 2003; Amm et al, 2014; Zhou et al, 2016).

Over the last several decades, the emission of pollutants into the environment has been increased tremendously due to rapid industrialization. Being immobile, plants are routinely confronted by a wide array of biotic and/or abiotic stresses including heavy metal stress (Al-Whaibi, 2011). Several industrial

areas are contaminated with heavy metals like cadmium, mercury, lead, chromium, copper, zinc, cobalt, and nickel. Heavy metals are thought to obstruct the biological functions of a protein by altering the native conformation through binding on it (Hossain and Komatsu, 2013). Based on the importance of proteins in plant survival, it can be hypothesized that soil heavy metal stress would result in altered total protein content within growing plants.

The present study incorporated *Catharanthus roseus* as a plant of interest to study the effects of heavy metals on plant protein content and root-shoot ratio. *Catharanthus roseus* is a renowned medicinal plant, belonging to the family Apocynaceae. The high added value of this plant is based on its enormous pharmaceutical interest, producing more than 130 TIAs, some of which exhibit strong pharmacological activities (Almagro et al, 2015). Our earlier studies on this plant showed enhanced alkaloids, flavonoids, and phenolic content within plant parts (Soumya et al, 2021). The most striking biological activity investigated has been the antitumour effect of dimeric alkaloids such as vinblastine and vincristine (Mekky et al, 2018). Intensive research on the biosynthesis of TIAs and the regulation of their pathways has been developed with the aim to increase by biotechnological approaches, the production of these high added value compounds. However, all these biochemical pathways have been known to involve protein interactions. Therefore, the current study aimed to identify alterations in total plant protein in *Catharanthus roseus* grown in different industrially contaminated soils.

## Materials and Methods

### Soil collection

Soil samples were collected from five different contaminated sites viz., dockyard, Hindustan Petroleum Corporation Limited – HPCL, pharmaceutical industry, shipyard, steel plant and GITAM garden located in Visakhapatnam city of Andhra Pradesh. Physicochemical properties of soil samples like pH, electrical conductance, organic matter, particle size, moisture and heavy metals were analysed and reported earlier (Soumya et al, 2021).

### Plant growth

Seeds of *Catharanthus roseus* were germinated in collected soils under dark conditions. The plant was single seeded into plug trays and covered with moist paper to hold the humidity close to the seed. Seeded trays were misted until wet and then placed in a warm, dark room. The soil temperature was maintained at 77 $^{o}$F and 100% relative humidity with frequent misting of trays for a period of 7 days until the radicle emerged from the seeds. The plants were further allowed to grow for 60 days during which the relative humidity was kept as low as 40% to avoid air-borne diseases. Periodic scouting was done throughout the period of growth.

### Sample processing

The fresh leaves were collected from the 60 days old plants grown in different soils. Prior to sample processing, the uprooted plants were cleaned under running tap water, followed by double distilled water to ensure removal of dust. The plants were separated into roots and shoots for determining the ratio between them for two parameters viz., their biomass and height. The weights and heights of harvested biomass, shoots, and roots separately, of all the plants grown in control and contaminated soils were measured and recorded. The biomass of roots was compared to that of shoots and

expressed as root-shoot ratio. The root and shoot lengths were expressed as percentage increase in comparison to plants grown in garden soil.

**Protein isolation**

1 g of finely chopped shoot and root of grown plants was ground separately in cold conditions with freshly prepared phosphate buffer saline (pH 7.4) using a mortar and pestle. The solutions were centrifuged at 10000 rpm for 10 min. The final supernatants containing proteins were collected into tubes for estimation of total protein.

**Preparation of BSA standard curve**

Precisely measured aliquots (20, 40, 60, 80, and 100) µl of Bovine Serum Albumin (BSA) standard solution (1 mg/ml) was transferred to different tubes and volume was made up to 1 ml with distilled water. 5 ml of reaction buffer solution [2% (w/v) Sodium carbonate in 0.1N Sodium hydroxide added to 0.5% (w/v) Copper (II) sulphate in 1% Sodium potassium tartarate in the ratio 50:1] was added to each tube, vortexed and allowed to incubate at 37 $^{o}$C for 10 minutes. 0.5 ml of Folin- Ciocalteau (1N) reagent was added to all the tubes and allowed to stand for 30 minutes before measuring the absorbances at 650nm spectrophotometrically. (Lowry et al., 1951). Distilled water was used as blank. The standard curve was plotted with protein concentration as X-axis and absorbance as Y-axis.

**Protein quantification**

The unknown protein concentrations of supernatants were determined using standard curve by extrapolating their absorbances at 650 nm. The protein content in supernatants were calculated by the same method that was adopted for constructing the standard curve of BSA, and results were reported as BSA equivalents i.e., µg BSAE/ g of fresh weight based on the standard curve of BSA (mg/ml) (Shakir et al, 1994).

**Statistical analysis**

Data shown were mean values of triplicates from two separate experiments. The obtained results expressed as the average ± standard deviations and were evaluated based on the calibration curves.

**Results and Discussion**

The physicochemical properties of all the soils analysed were given in table 1. The soil sample collected from shipyard had high concentrations of all the tested heavy metals whereas, soils from garden had the lowest. Hence garden soil was considered as control soil and plants grown in this soil were considered as control plants.

The control plants exhibited highest root-shoot ratio (0.56). All other plants grown in contaminated soils had lower but similar root-shoot ratio ranging from 0.49 to 0.54, with lowest ratio in plants grown in shipyard. Table 2. However, the plants grown in contaminated soils showed striking changes in root and shoot length. Figure 1. The highest percent increase in shoot length was observed in plants grown in shipyard in comparison to control plants, whereas the highest percent increase in root length was observed in plants grown in pharmacy. Figure 2.

Root and shoot system maintain a dynamic balance in biomass which reflects relative abundance of root-zone resources (water and nutrients) compared with above-ground resources (light and $CO_2$). The root-shoot ratio is the amount of plant tissues that have supportive functions to the amount of those that have growth functions. It is a measure to assess the overall health of the plants. Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy (Upendra et al, 2017). Root biomass is influenced by below-ground conditions where low availability of either water or nutrients commonly leads to greater root-shoot ratio. Whole-plant growth rate and summary measures such as root-shoot ratio can thus be considered an outcome of developmental stage and of environmental influences.

The estimated protein content within plants grown in contaminated soils were calculated based on standard curve of BSA. Figure 3. In shoots, the highest estimated protein (86.1 µg/ml) was observed in plants grown in shipyard soil. Shoots of plants grown in steel plant, pharmacy, HPCL and dockyard had 73.6 µg/ml, 76.6 µg/ml, 76.2 µg/ml and 79.9 µg/ml in order. The least protein content (53.4 µg/ml) was observed in garden grown plants. Similarly, estimated protein content in roots of plants grown in garden, steel plant, pharmacy, HPCL, dockyard and shipyard soils were 57.6 µg/ml, 75.3 µg/ml, 78.1 µg/ml, 79.0 µg/ml, 83.6 µg/ml and 90.2 µg/ml in order. Figure 4. The Lowry method (Lowry et al, 1951) of protein estimation used in this study was known to remain constant from protein to protein in almost all circumstances in which protein mixtures or crude extracts were involved. This assay was based on the biuret reaction and Folin- Ciocalteau reaction. Briefly, the peptide bonds of proteins react with copper under alkaline conditions to produce Copper ions. These copper ions on reacting with Folin-Ciocalteau phenol reagent reduced phosphomolybdic-phosphotungstate to a coloured complex by the copper-catalyzed oxidation of aromatic amino acids. The reaction resulted in a strong blue colour, which depends partly on the tyrosine and tryptophan content that was detected at 650 nm (Peterson, 1983).

Briefly, the overall results exhibited a direct correlation among the three tested parameters: heavy metal stress, protein content and plant growth in terms of shoot-root elongation. Plants growing in highest contaminated soil (i.e., shipyard) exhibited highest protein content and tallest shoot system when compared to plants grown in other soils. However, the root length decreased in shipyard plants probably due to surrounding metal stress. As plants are static, they cannot escape from unfavourable environmental conditions. The exposure of plants to these toxic metals triggers various biochemical and physiological processes, which eventually lead to adaptation and ability to survive under adverse conditions (Singh et al, 2016). Different mechanisms are involved in the plant responses to heavy metal toxicity. They have innate mechanisms to tolerate toxicity by metal ions, notably via the up regulation of some genes which provide resistance and acclimatize plants under abiotic stress conditions. Proteins involved in plant metal stress response act as initiators or precursors and continue the signalling pathway; for example, increase in antioxidants to lower the levels of free radicals under stress conditions (Maksymiec, 2007). Some of the proteins act as transporters which help in the translocation of toxic metal ions like cadmium, copper, zinc and release them out from the plants. Proteins also have chelating properties which chelate the toxic ions by breaking down complex compounds. Some of the other types of proteins also maintains the redox potential of plant cell during metal toxicity. Due to the presence of toxic metal ions like Ni, Cd in plants, the proteins unfold and become non-functional (Liu and Howell, 2016). In such cases, plant chaperones facilitate the correct folding of proteins. Hence, these plant proteins hold a central role in providing protection during abiotic stress by metals.

**Table 1.** Physiochemical properties of contaminated soil samples.

| Metals(ppm) | Garden | Dockyard | HPCL | Pharmacy | Shipyard | Steel plant |
|---|---|---|---|---|---|---|
| ***pH*** | 7.78 | 7.53 | 7.73 | 7.69 | 7.47 | 7.59 |
| ***Electrical conductivity(EC)*** | 0.29 | 0.14 | 0.24 | 0.20 | 0.11 | 0.19 |
| ***Organic matter (OM-LOI %)*** | 17.6 | 6.95 | 15.55 | 13.35 | 5.9 | 9.95 |
| ***Sand %*** | 13 | 18 | 13 | 28 | 33 | 28 |
| ***Silt %*** | 69 | 60 | 71 | 55 | 58 | 45 |
| ***Clay %*** | 12 | 10 | 10 | 5 | 3 | 23 |
| ***Gravel %*** | 6 | 12 | 6 | 12 | 16 | 4 |
| ***Moisture (MC%, MF)*** | 17.508, 1.175 | 9.829, 1.098 | 15.074, 1.150 | 12.296, 1.122 | 7.009, 1.070 | 11.607, 1.116 |
| ***Lead*** | 9.8 | 31.51 | 19.95 | 17.8 | 33.41 | 20.6 |
| ***Nickel*** | 11.0 | 24.16 | 33.72 | 21.36 | 348.78 | 23.11 |
| ***Chromium*** | 12.4 | 29.96 | 29.53 | 24.57 | 142.73 | 16.45 |
| ***Cadmium*** | 1.4 | 2.3 | 2.2 | 3.0 | 6.5 | 2.5 |
| ***Copper*** | 17.8 | 47.94 | 32.72 | 24.57 | 3187 | 21.74 |
| ***Zinc*** | 14.0 | 84.0 | 82.0 | 81.0 | 2218 | 94.0 |
| ***Iron*** | 16.1 | 36.6 | 28.7 | 24.3 | 249.1 | 20.7 |
| ***Mercury*** | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |

The samples were slightly basic in nature, with electrical conductivity between 0.1 to 0.3. Organic matter is expressed in terms of 'Loss on Ignition' percentage. The texture of the collected soils was determined based on the percentage of its sand, silt, clay and gavel components. Moisture was calculated as percentage moisture content (ratio of weight of water to the weight of solids in soil) and moisture content factor (ratio of moist soil to dried soil). The heavy metals were analysed by Atomic Absorption Spectrophotometric method (Soumya et al, 2021)

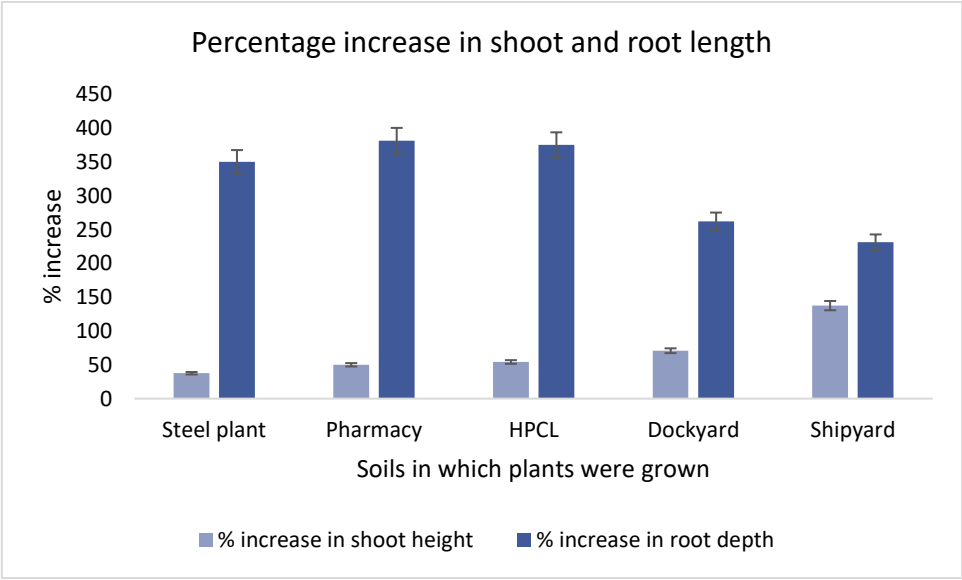
**Table 2.** Root-shoot ratio of biomass. It is a measure to assess the overall health of the plants.

| Soils in which plants were grown | Root-shoot ratio |
|---|---|
| Garden | 0.56 ± 0.04 |
| Steelplant | 0.53 ± 0.01 |
| Pharmacy | 0.54 ± 0.02 |
| HPCL | 0.52 ± 0.01 |
| Dockyard | 0.51 ± 0.02 |
| Shipyard | 0.49 ± 0.04 |

**Figure 1. Comparative growth of plants grown in different soils.**

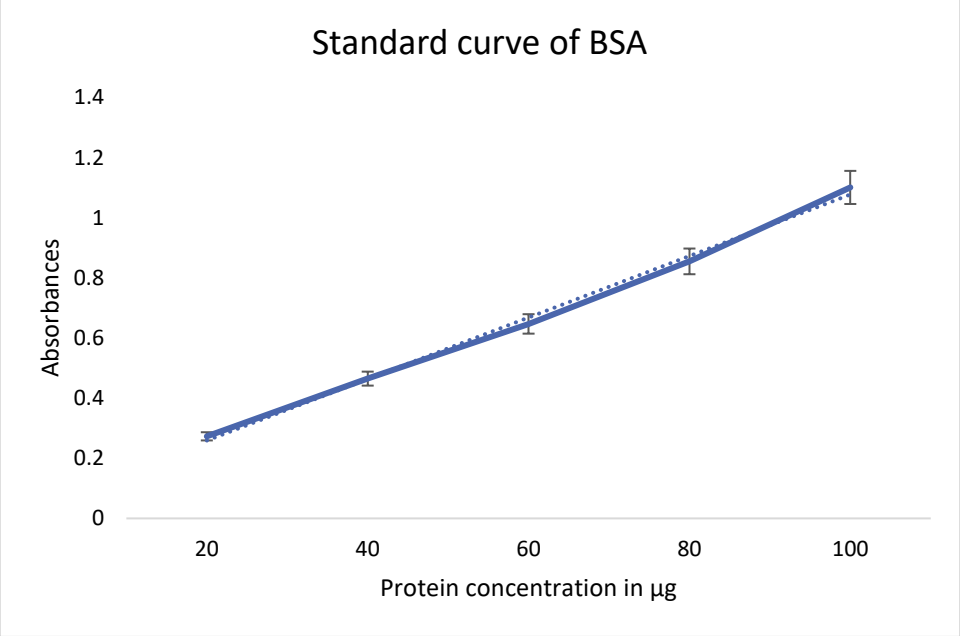

Plants grown in shipyard showed healthy growth.

**Figure 2. Percentage increase in the root-shoot length.**



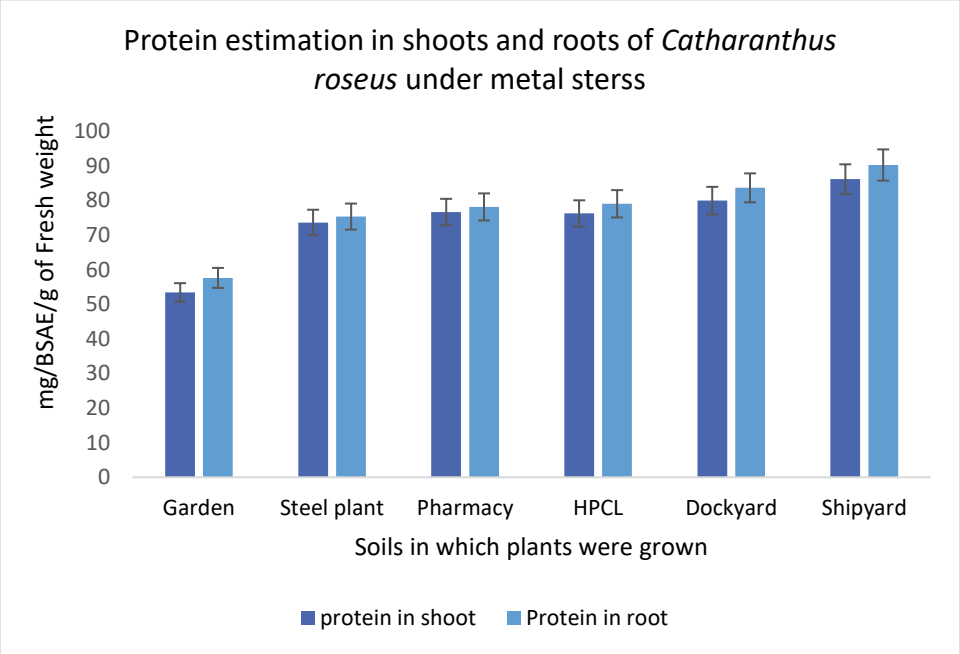

The lengths of shoots and roots of plants grown in contaminated soils were compared with those of control plants.

**Figure 3.** Standard curve of Bovine Albumin Serum.




Precisely measured aliquots of BSA standard solution (1 mg/ml) were used in preparing the standard graph for measuring the protein content.

**Figure 4.** Graphical representation of estimated protein content of in shoots and roots of plants grown in different contaminated soils.




The total protein content was calculated as BSA equivalents BSAE/ g of fresh plant material based on a standard curve of BSA.

## Conclusion

Heavy metals play a major role in environmental pollution. These metal ions are discharged through various industries which in due course of time accumulate in plants growing in these soils. By analysing the alterations in total protein and plant growth under metal stress we can devise biotechnological strategies for improving the tolerance of plants to heavy metals. Further studies involving biochemical and -omics analyses can provide in-depth study which will be necessary to exploit the great potential of the stress-responsive proteins.

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## CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

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