PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(2): 601-611
https://doi.org/10.14719/pst.3071

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RESEARCH ARTICLE

Assessment of Heavy Metal Accumulation and its Effect on Phytochemical Profiling in *Jacobaea maritima* (L.) Pelser & Meijden

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OPEN ACCESS

ARTICLE HISTORY

Received: 06 November 2023
Accepted: 08 March 2024

Available online
Version 1.0 : 29 April 2024
Version 2.0 : 21 May 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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Satuluri WJK, Xavier J. Assessment of Heavy Metal Accumulation and its Effect on Phytochemical Profiling in *Jacobaea maritima* (L.) Pelser & Meijden. Plant Science Today. 2024; 11(2): 601-611. https://doi.org/10.14719/pst.3071

Abstract

Modernization and industrialization have been of great importance in the recent past, yet there are a few disadvantages, including the release of harmful effluents into the environment. The medicinal plants that are found in these heavy metal-polluted soils can have positive and negative effects as well. In this study, the plant *Jacobaea maritima* (L.) Pelser & Meijden, an important medicinal plant in the field of homeopathy was subjected to 3 different heavy metals: cadmium, chromium and lead at a concentration range of 50 ppm to 250 ppm. The morphological parameters show a clear effect on the growth of the plant and its development, which includes the shoot length and root length reducing by more than 50 % and 25 % in the case of shoot and root respectively. Phytochemical analysis shows significant variation, including chlorophyll. The highest protein is seen in Cr 100 ppm (7.47 mg) and the lowest was found in Cd 250 ppm (0.38 mg). Proline, which is a stress-induced compound, was found to be highest in Pb 200 ppm (1.242 mg/mL) and least in Cr 50 ppm (0.368 mg/mL). The Total Phenolic Content (TPC) was seen to be highest in Cr 250 ppm (3.229 mg/g) and least in control (0.57 mg/g) and the Total Flavonoid Content (TFC) was found to be highest in Cd 100 ppm and least in control (0.04 mg/g) plant which includes root, shoot and leaf.

Keywords

heavy metals; morphological parameters; phytochemicals; *Jacobaea maritime*; atomic absorption spectroscopy

Introduction

Heavy metals have become more prevalent in the environment in recent years due to many reasons. The impact of these heavy metals is not just on the plants in the area where they are present but also in various places where the soils are contaminated. In these soils, the growth of plants becomes problematic as it shows the impact on the complete growth and development of the plant. In this instance, the growth of medicinal plants, which are economically important and are key for the preparation of the medicines, also gets affected by heavy metals (1). The medicinal plant metabolites act as key factors in the preparation of medicines.

The plant *Jacobaea maritima* is a shrub that belongs to the family Asteraceae and is commonly called silver dust. This plant originates from the Capria Islands in Italy (2). The plant is used in the homeopathic system of medicine for the preparation of eye drops, which are also helpful in the treatment of ophthalmological disorders, including cataracts (3). The

extract of the plant leaf has been used as a crude drug to cure eye-related ailments in traditional systems of medicine.

Materials and Methods

Collection of Plants and Heavy Metal Treatment

Jacobaea maritima (L.) Pelser & Meijden has been purchased from the Center for Medicinal Plant Research Institute (CMPRI), CCRH, Ooty (3). This is grown in a polyhouse with appropriate requirements. This plant was grown for three months before the treatment started. The plants were potted in soil containing a 2:1 ratio of red soil and organic compost in mud pots (5). The plants, after getting adapted to the conditions and growth, were at a good pace; the treatment was given in 7 days each. Three different heavy metals were used and 5 concentrations - 50, 100, 150, 200 and 250 ppm of each heavy metal were used (6). The salts that are used as a source of heavy metals are $CdCl_2$ (cadmium chloride), $Cr_2(SO_4)_3$ (chromic sulfate) and $C_4H_6O_6Pb$ (lead acetate). These salts were calculated based on the amount of soil in the pots, which was 2 kg and accordingly, the heavy metal solutions of 50 mL were given as treatment weekly for 5 consecutive weeks.

To induce the effect of heavy metal stress, the plants are treated with heavy metal solutions at particular intervals. The heavy metals cadmium, chromium and lead were used for this study (4). Once the treatment cycle was finished, the plants were harvested and the physiochemical and phytochemical parameters, heavy metal accumulation and the change in phytochemicals were studied with the help of standard methods, AAS (Atomic Absorption Spectroscopy).

Heavy Metal Analysis

AAS, the atomic absorption spectroscopy was performed to determine the accumulation of heavy metals in the different plant tissues like the root, shoot and leaf (7). The sample was shade-dried and powdered. The acid digestion mixture was prepared by using aqua-regia, which is a 3:1 ratio of nitric acid and hydrochloric acid (8). 1 g of plant sample was digested using 12 mL of nitric acid and 4 mL of hydrochloric acid; this was kept on a hot plate at around 60 °C until the sample reached 30 % of its original volume. Later, it was cooled and made up to 25 mL with distilled water. This was filtered using Whatman, but there was no filter paper and the sample was used for analysis using an AAS (9) (atomic absorption spectrophotometer, SHIMADZU AA-6880).

Morphological Parameters

The morphological analysis of the plant was done based on the 5 main characteristics of the plant. They are root length, shoot length, number of leaves, fresh weight and dry weight for each heavy metal. For each concentration, triplicates of plants were maintained along with one control plant in order to check for differences in all the parameters (10).

Phytochemical Analysis

The phytochemical parameters of the plants, like carbohydrates, protein, proline, total phenolic content and total flavonoid content were measured using different methods (11). For all the analyses, a spectrophotometer (SHIMADZU UV-1800) was used (12). The carbohydrate analysis was done using the phenol sulphuric acid method, where 100 mg of the sample was taken in a boiling tube and 5 mL of 2.5N HCl was added and incubated in a water bath for 3 h. After the incubation, Na_2Co_3 was added until the effervescence ceased. This was made up to 10 mL with distilled water and then 1mL of phenol solution along with 5 mL of conc. H_2SO_4 was added. This was mixed and then kept in a water bath for 20 min at 20-30 °C. The absorbance was read at 490 nm (13, 14).

The protein analysis was done using Lowry's method, where 100 mg of plant sample was macerated using 5 mL of phosphate buffer and from this, 0.2 mL of the extract was taken and made up to 1 mL using distilled water. To this 5 mL of reagent C, which is a mixture of reagent A (2 % Na_2CO_3 in 0.1 N NaOH) and reagent B (0.5 $CuSO_4$ in 1 % potassium sodium tartrate) in a 48:2 ratio, 0.5 mL of FC reagent was added and mixed well. This was incubated in a dark environment for 30 min. The absorbance was read at 660 nm (15).

The proline analysis was done using a method with minor modifications (16); 100 mg of the sample was taken and homogenized with 3 % sulfosalicylic acid and centrifuged at 3000 rpm for 10 min, 2 mL supernatant was transferred into a different test tube and 2 mL of 6 M orthophosphoric acid, 2 mL acid ninhydrin and 2 mL of glacial acetic acid were added. It was incubated in a boiling water bath for 1 h. This was cooled rapidly by keeping it in ice water and 4 mL of toluene was added and vortexed to mix the contents well. The upper layer was separated using a glass pipette and the absorbance was read at 520 nm (16).

The phenolic and flavonoid content analysis was done using the methanol extract of the sample and a stock concentration of 10 mg/mL was prepared. The working standards were prepared prior to the analysis, which was a 1 mg/mL concentration (17).

The total phenolic content analysis was done using the FC method (18); 0.5 mL of methanolic extract was taken and to it, 1.5 mL of FC reagent was added and incubated for 10 min. 1 mL of 7.5 % Na_2CO_3 was added and incubated in the dark for 30-60 min and the absorbance was read at 765 nm.

The total flavonoid content analysis was done using the aluminum chloride method (19). 0.5 mL of methanolic extract was taken and 1.5 mL of methanol, 0.1 mL of 10 aluminum chloride solution and 0.1 mL of 1 M potassium acetate were added. They were made to an equal volume of 5 mL with distilled water and incubated at room temperature for 30 min; the absorbance was read at 415 nm. These were calculated based on the gram-equivalent weights of gallic acid for phenols and quercetin for flavonoids.

The total chlorophyll was estimated using the method with slight modifications (20), in which the plant leaf sample was ground along with 80 % acetone and the solution was used to measure the absorbance spectrophotometrically at 645 nm and 663 nm, which gives the values of chlorophyll-a and chlorophyll-b. By calculating the values of chlorophyll a and b, the total chlorophyll was estimated.

All the phytochemical parameters were performed in triplicates and the mean values were taken for analysis. The obtained results were statistically analysed using SPSS software and DMRT to get the significance, which was found to be $p<0.05$, stating all the parameters had a significant variation with the treatment of heavy metals.

Results

The morphological parameters like root length, shoot length, number of leaves, fresh weight and dry weight of the plants after the treatment with heavy metals were measured and a significant variation was observed in all the parameters (Table 1). It was clearly observed that the root length was the maximum in Cd 100 ppm (42 cm) and the minimum in Cd 200 ppm (9.1cm). The shoot length was high in Pb 200 ppm (84 cm) and lowest in Cd 250 ppm (20 cm). The total number of leaves was high in Pb 50 ppm (55) and was the least in Cr 200 ppm (12). The fresh weight was found to be higher in Pb 50 ppm (51g) and lowest in Cd 200 ppm (8.38 g), whereas the dry weight after treatment was found to be high in Cd 50 ppm (9.279 g) and the least was found in Cd 250 ppm (2.62 g).

The AAS results showed a marked accumulation of all 3 metals in the plant tissues. The cadmium showed the highest accumulation in the Cd 200 ppm (3.33 ppm) leaf as well as the shoot (2.26 ppm) and the root showed the highest accumulation in the Cd 150 ppm (2.37 ppm) concentration. Chromium showed the highest accumulation in the root of Cr 200 ppm (12.25 ppm), a shoot of Cr 250 ppm (16.75 ppm) and a leaf of Cr 200 ppm (6.97 ppm) concentration. Lead showed the highest accumulation in the root (73.1 ppm), shoot (40.42 ppm) and leaf of Pb 250 ppm (12.5 ppm) concentrations as shown in Figs. 1-3.

The phytochemical analysis was performed with 6 major phytochemicals, which are protein, proline, total phenolic content (TPC), total flavonoid content (TFC), carbohydrates and chlorophyll. The protein analysis that was performed using Lowry's method showed that in the cadmium-treated plants, the maximum amount of protein was found in the leaf of Cd 100 ppm (6.98 mg/mL) and the minimum was found in the leaf of Cd 250 ppm (0.34 mg/mL). In the chromium-treated plants, the maximum protein content was found in the leaves of Cr 100 ppm (7.43 mg/mL) and the minimum protein content was found in the shoots of Cr 200 ppm (1.2 mg/mL) In the lead-treated plants, the maximum amount of protein was found in the Pb 150 ppm (5.2 mg/mL) leaf, whereas the least was found in the shoot of Pb 50 ppm (0.57 mg/mL) as shown in Fig. 5.

The highest amount of proline was found in the leaf of Pb 200 ppm (1.242 mg/mL) and was found in the least in Cr 50 ppm (0.368 mg/mL) concentration, as shown in Fig. 4.

The phenols and flavonoids are normally considered the precursors of many secondary metabolites in plants. The TPC was found to be the highest in Cd 200 ppm (2.7 mg/g) shoot, Cr 150 (2.01 mg/g) and 250 ppm (3.22 mg/g) root and Pb 200 ppm (2.4 mg/g) root and (2.41 mg/g) shoot. The lowest TPC was found in Cd 200 ppm (0.7 mg/g) root, Cr 200 ppm (1.5 mg/g) root and Pb 100 ppm (1.02 mg/g) shoot, as shown in Fig. 6. Whereas the TFC was found to be highest in Cd 100 ppm (0.08 mg/g) shoot, Cr 50 ppm leaf (0.06 mg/g), Pb 100 ppm leaf (0.07 mg/g) and Pb 150 ppm (0.07 mg/g) root. The TFC was the least in Cd 150 ppm (0.04 mg/g) shoot, Cr 200 ppm root (0.03 mg/g) and Pb 200 ppm (0.03 mg/g) shoot, as shown in Fig. 7.

Table 1. Result of morphological parameters of the plant *Jacobaea maritima*

Concentration (ppm)	Root length (cm)	Shoot length (cm)	No of Leaves	Fresh weight (g)	Dry Weight (g)
Control	15.4 ± 1.2	60 ± 4	43 ± 3	26.4 ± 0.3	9.683 ± 0.5
Cd 50	12.2 ± 0.8	59 ± 3.5	36 ± 2	29.8 ± 0.8	9.279 ± 0.5
Cd 100	42 ± 2.9	53 ± 3	26 ± 2	21.3 ± 0.6	8.636 ± 0.4
Cd 150	12.8 ± 0.8	43 ± 2	19 ± 1	10.1 ± 0.4	3.812 ± 0.2
Cd 200	9.1 ± 0.7	24 ± 1.5	14 ± 2	8.3 ± 0.9	3.638 ± 0.2
Cd 250	10.1 ± 0.75	20 ± 1	14 ± 1	7.2 ± 0.5	2.625 ± 0.3
Cr 50	28.5 ± 2.2	53 ± 2	40 ± 4	49 ± 1.2	8.225 ± 0.9
Cr 100	15 ± 1.3	54 ± 3	34 ± 4	22.8 ± 0.9	5.446 ± 0.8
Cr 150	11.8 ± 1.1	37 ± 2	32 ± 4	17.5 ± 0.7	3.67 ± 0.4
Cr 200	18 ± 1.9	48 ± 3	12 ± 1	19.2 ± 0.8	5.112 ± 0.5
Cr 250	27 ± 2.2	39 ± 3	24 ± 1	14.3 ± 0.6	4.698 ± 0.7
Pb 50	37 ± 2.8	74 ± 5	55 ± 5	51 ± 2.3	9.162 ± 1.1
Pb 100	16 ± 1.9	70 ± 4	50 ± 4	44.7 ± 1.9	8.442 ± 0.9
Pb 150	25 ± 2.3	49 ± 3.5	46 ± 4	41.9 ± 1.7	8.385± 0.8
Pb 200	26 ± 2.4	84 ± 5	39 ± 4	37.8 ± 1.3	6.818 ± 0.7
Pb 250	18 ± 1.9	46 ± 3	21 ± 3	21.8 ± 0.8	± 0.5

*Data represent mean values ± SE of three replicates; each experiment was repeated thrice

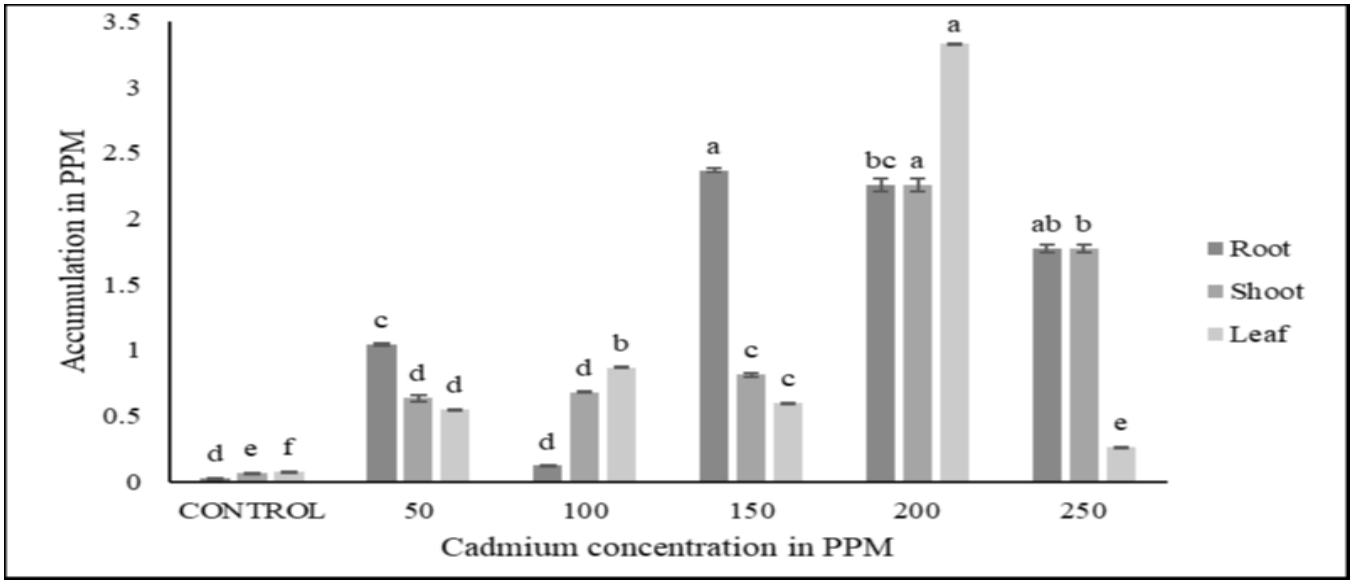


Figure 1. Graph showing the accumulation pattern in Cadmium treated *J. maritima*

*a – represents the maximum value, f – represents the minimum value in DMRT analysis

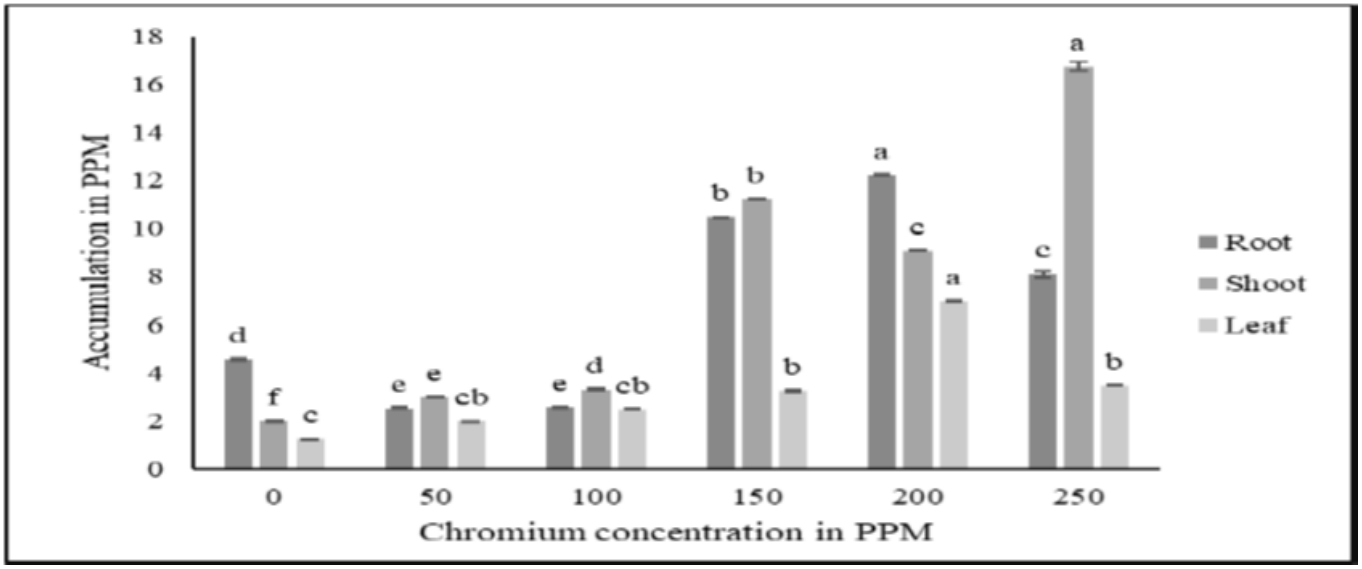


Figure 2. Graph showing the accumulation pattern in Chromium treated J. maritima

*a – represents the maximum value, f – represents the minimum value in DMRT analysis

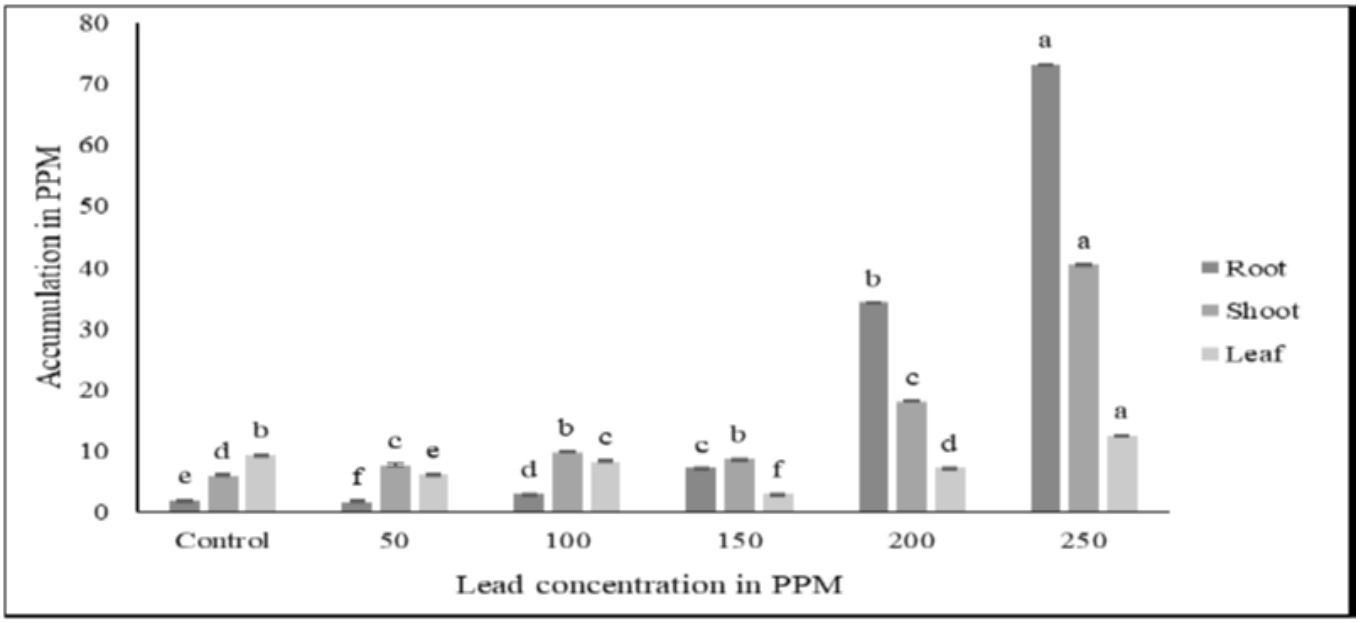
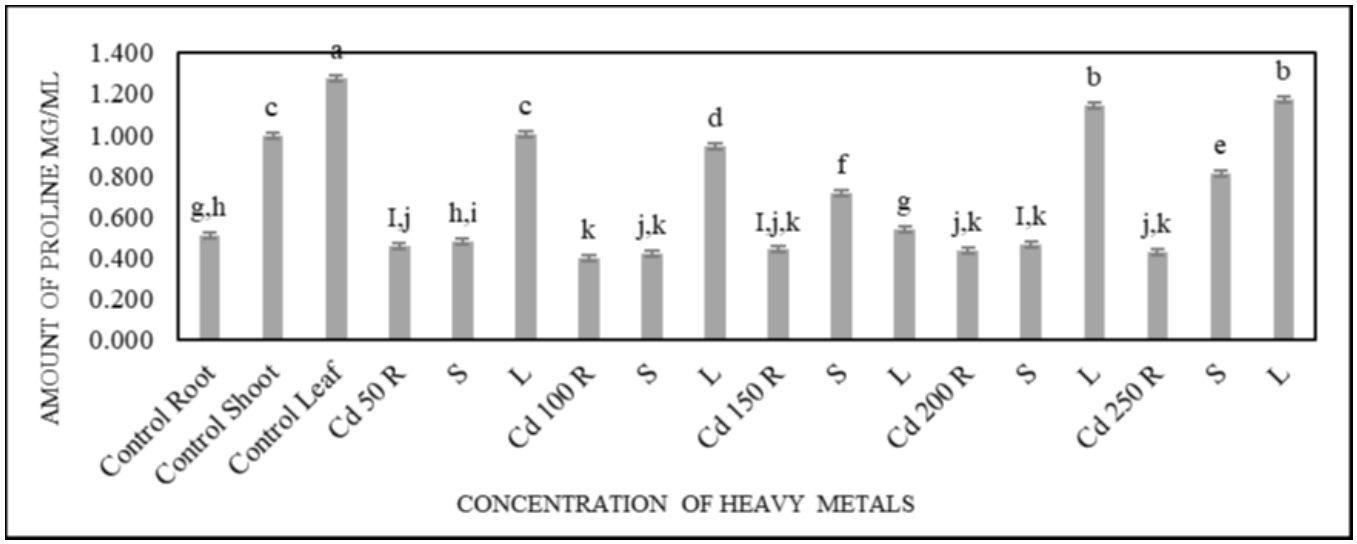


Figure 3. Graph showing the accumulation pattern in Lead treated J. maritima

*a – represents the maximum value, f – represents the minimum value in DMRT analysis



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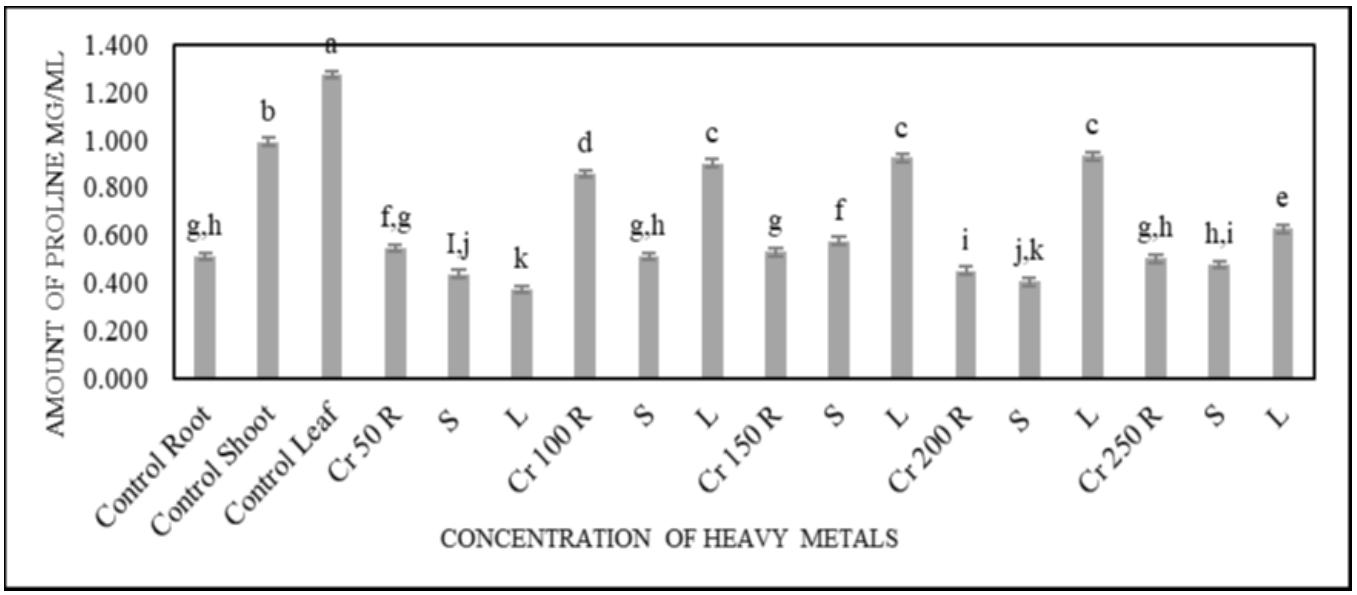

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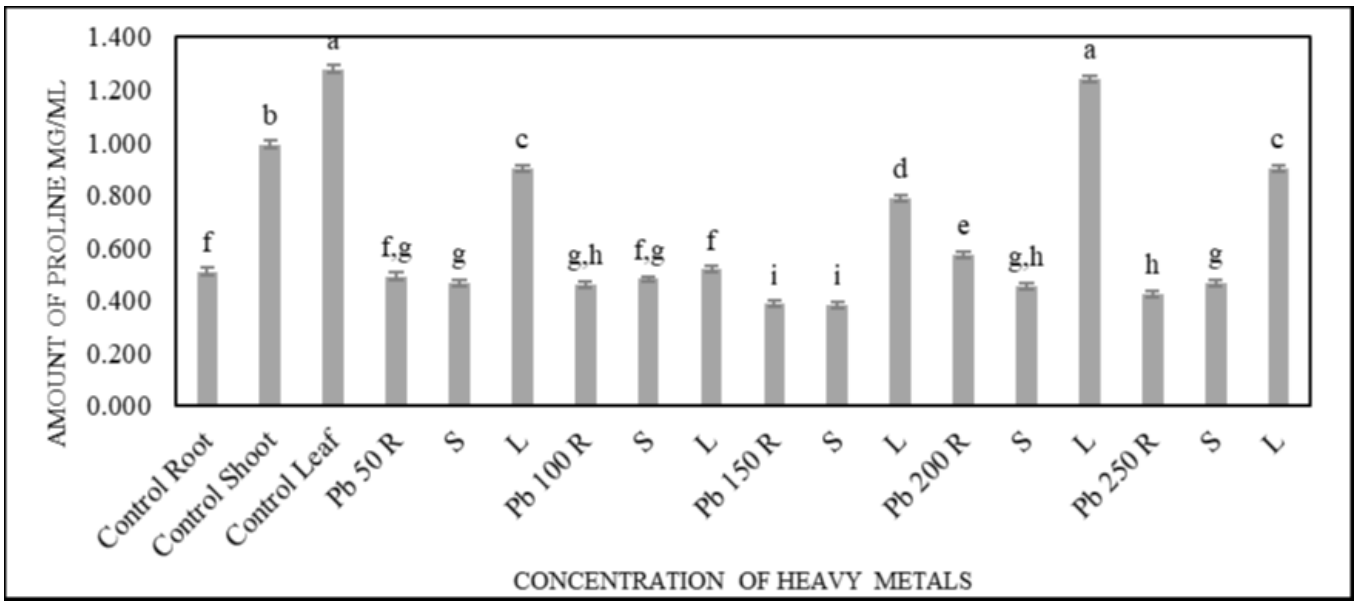

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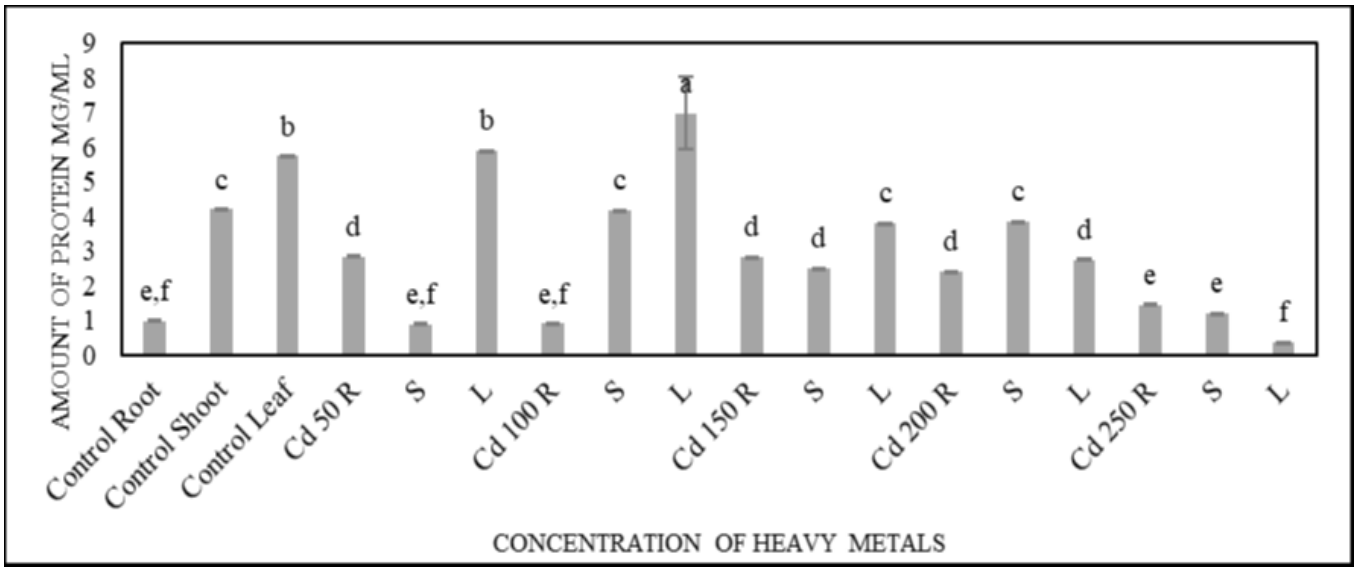
Figure 4: Amount of Proline in (A) Cadmium treated, (B) Chromium treated, (C) Lead treated *Jacobaea maritima*. Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)



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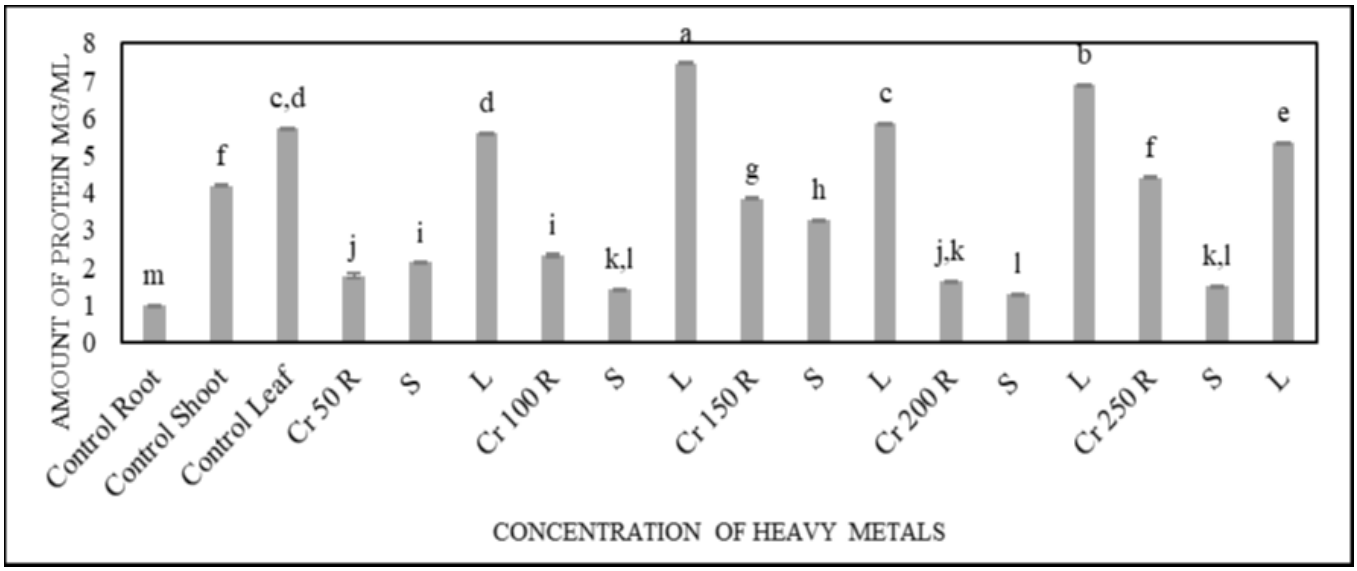

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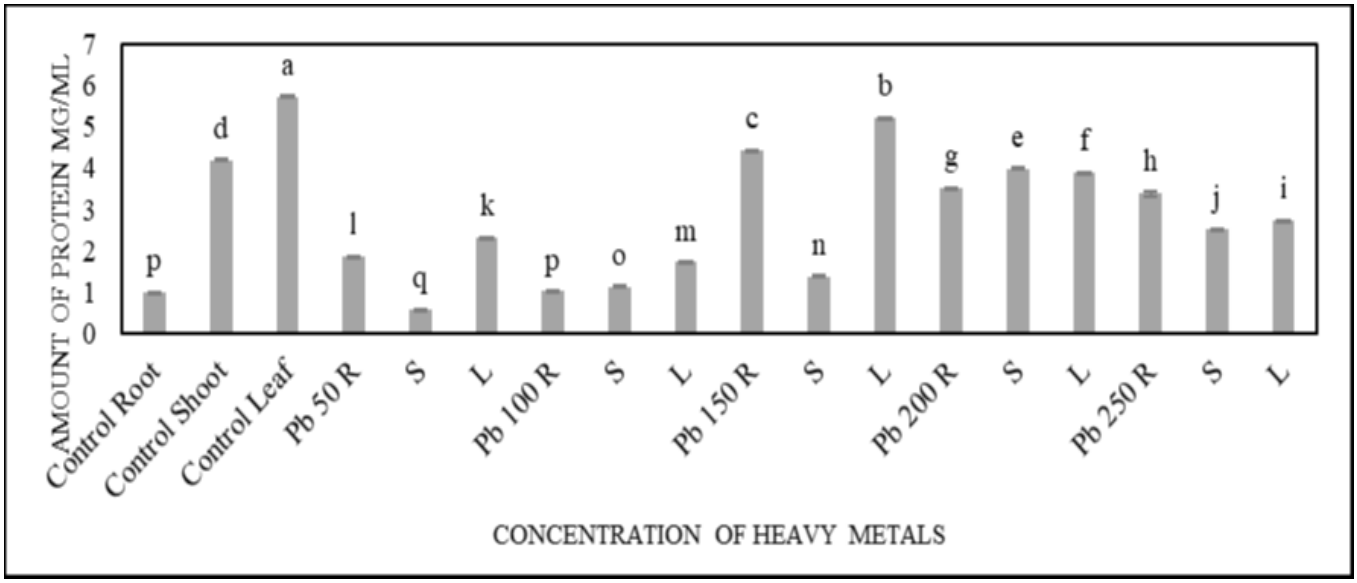

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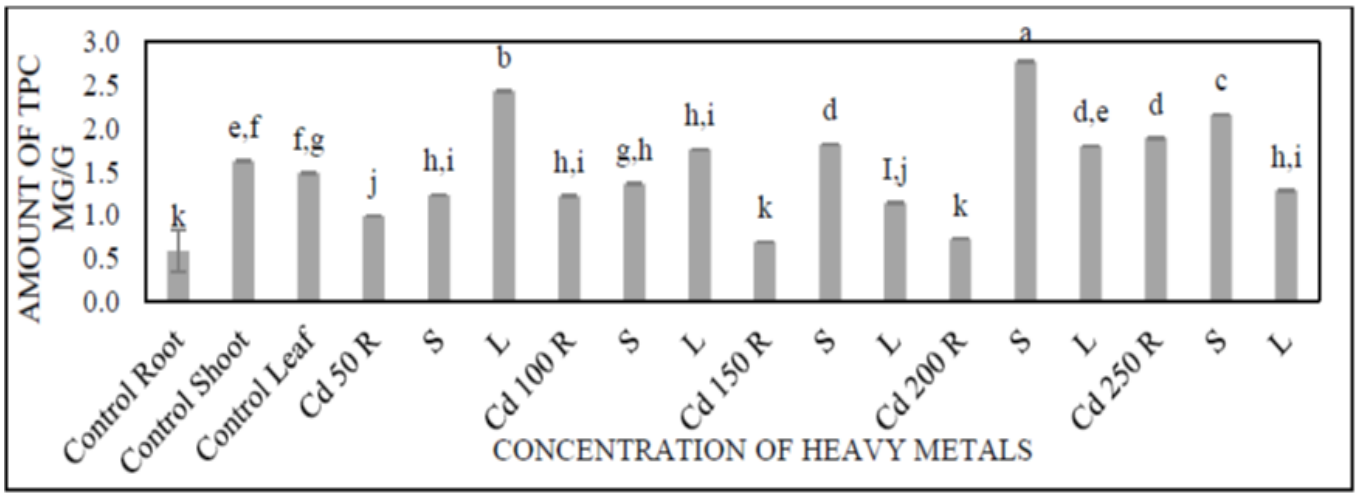
Figure 5. Amount of Protein in (A) Cadmium treated, (B) Chromium treated, (C) Lead treated *Jacobaea maritima*. Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)



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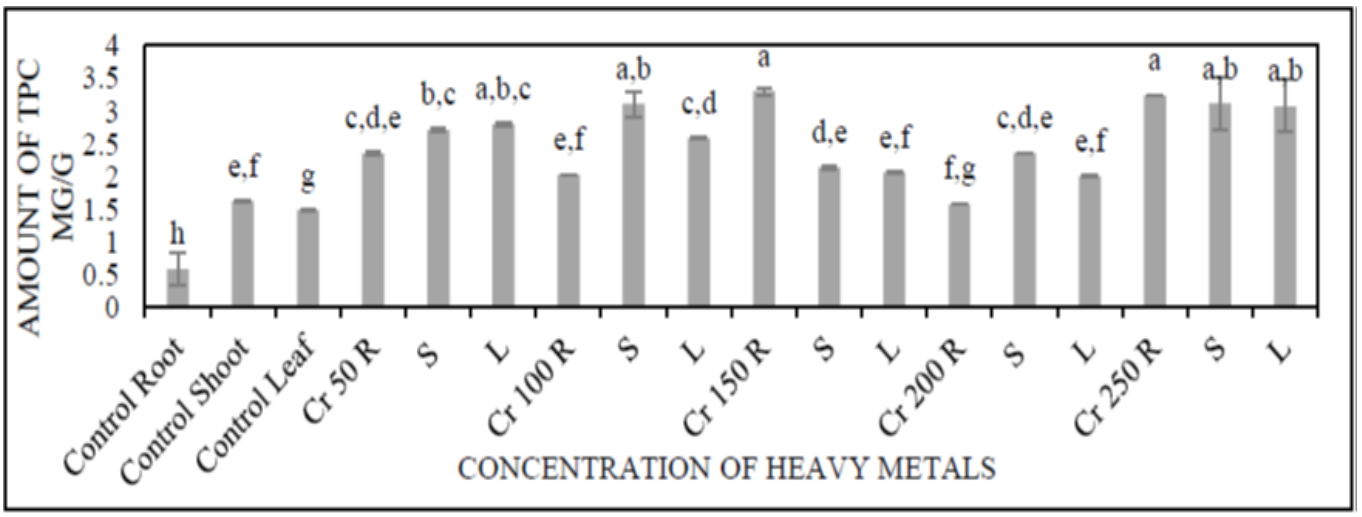

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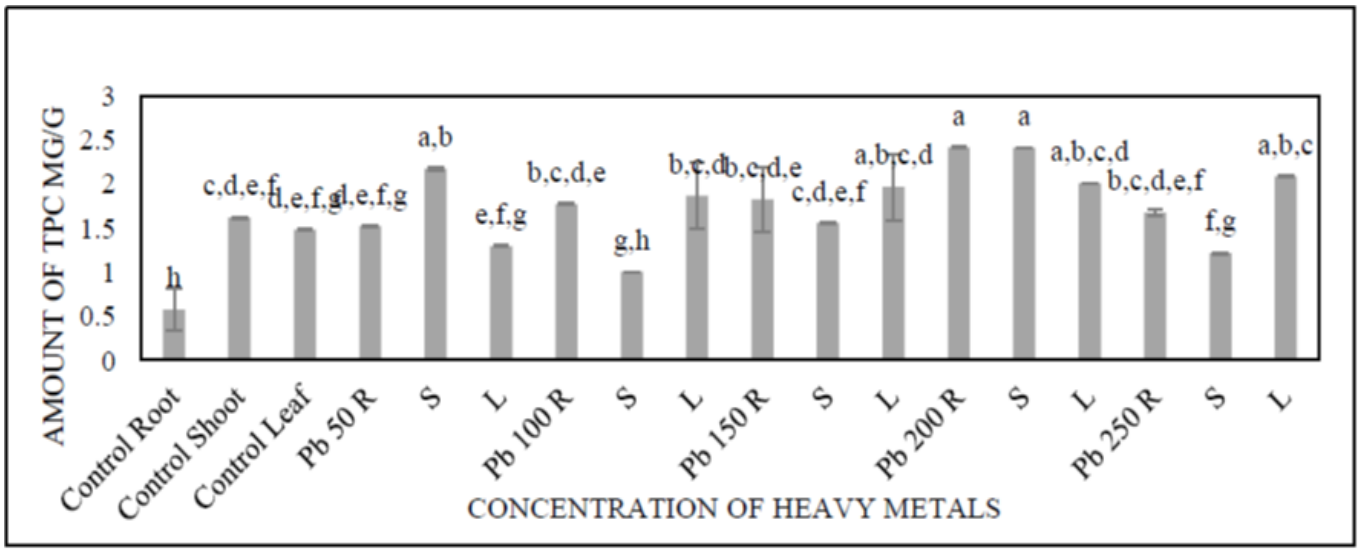

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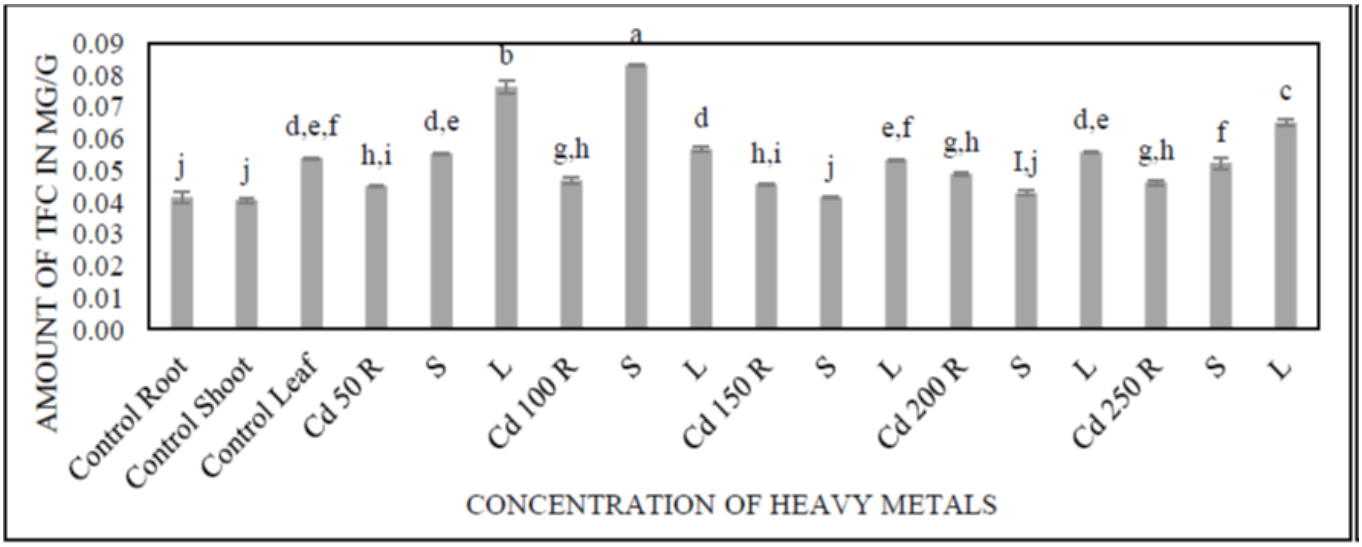
Figure 6. Total Phenol Content in (A) Cadmium treated, (B) Chromium treated, (C) Lead treated Jacobaea maritima. Data represent mean values } SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P . 0.05 according to Duncanfs multiple range test (DMRT)



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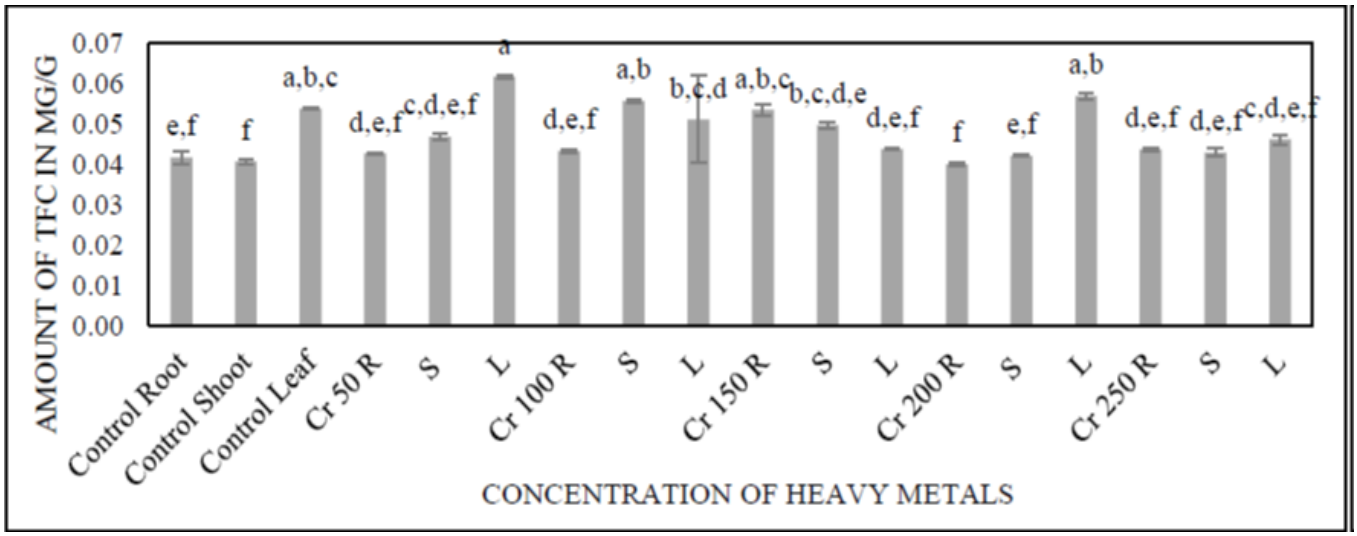

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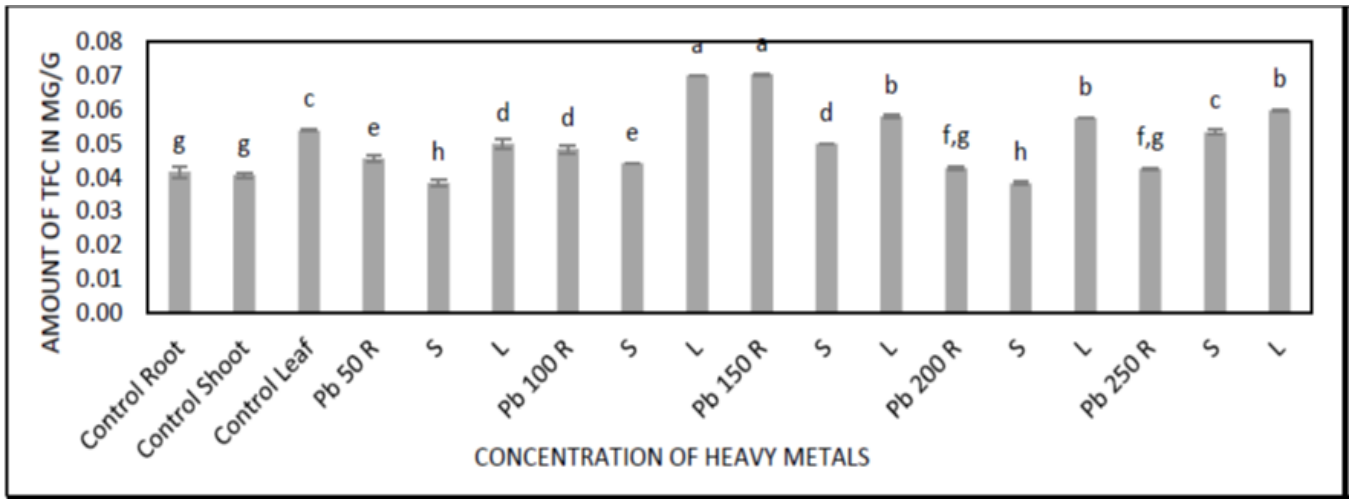

C

Figure 7. Total Phenol Content in (A) Cadmium treated, (B) Chromium treated, (C) Lead treated Jacobaea maritima. Data represent mean values ± SE of 3 replicates; each experiment was repeated thrice. Means with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)

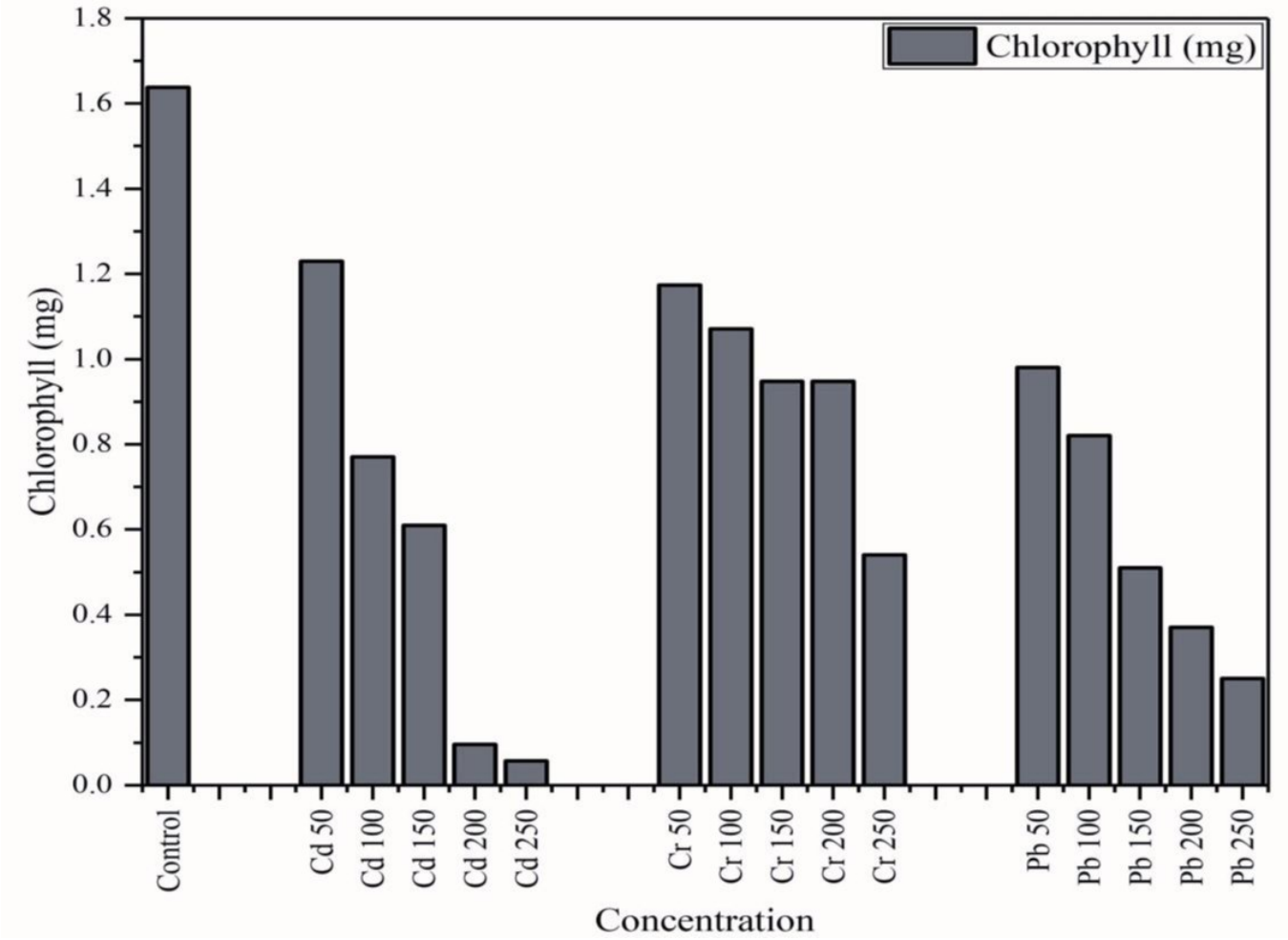

Figure 8. Graph showing the amount of chlorophyll in *Jacobaea maritima* treated with different heavy metals

The chlorophyll content was reduced as an increase in the heavy metal concentration was noticed. The control plant had the highest amount of chlorophyll content (1.62 mg/mL). In the treated plants, the highest amount of chlorophyll was found in Cd 50 ppm (1.23 mg/mL) and the least was found in Cd 250 ppm, which was less than 0.1 mg/mL.

The carbohydrates have shown a gradual decrease in their amount with the increase in the heavy metal concentration. All the leaf parts have the maximum carbohydrate content and the control had 23.630 mg/L whereas the treated plants at the highest concentrations had shown 10.963 mg/L in Cd 250 ppm, 13.690 mg/L in Cr 250 ppm and 10.145 mg/L in Pb 250 ppm respectively.

Discussion

The current work with heavy metals on the plant *Jacobaea maritima* has yet to be reported in the literature. The impact of the heavy metals on the various phytochemicals and the morphology can be seen in some of the works done in the same family and in the genus *Senecio* (21). The Asteraceae family plants were found to accumulate more heavy metals than Poaceae family members and the accumulation of Zn and Cd was found to be higher (22). The morphology was affected by lead and cadmium, it was seen that the biomass increased with the treatment of heavy metals (23). *Xanthium strumarium* L. (Asteraceae) has comparatively less ability to absorb Cd but greater ability to absorb aluminum (24), but in the current study, lead showed greater accumulation capacity, followed by cadmium and chromium.

The plant *Sonchus transcapicus* showed a greater accumulation in shoots and also a greater capacity to adapt to metal-induced stress, depending on a more effective antioxidative defense mechanism (25). *Trifolium pratense* has been observed to have the capability of accumulating heavy metals and is also used as a bio-accumulator of heavy metals (26). *M. chamomilla* is a suitable species for the bioremediation of soils polluted with Cd and Pb; with increasing Cd and Pb concentrations, plant pigment contents, including chla, chlb, chlT and carotenoid, significantly decreased (27).

In a study, the protein content was drastically reduced by approximately 60 % from the control plants to the treated plants (28). Another study reported that, *Parthenium*, *Cannabis*, *Euphorbia* and *Rumex* species showed a positive correlation with Pb accumulation with endogenous free proline and phenolic contents (29). There's a drastic variation in the TPC and TFC in the treated plants. According to a study, the TPC varied in a range of 0.9 to 4.7 mg/g of dry extract, but in the current study, the TPC ranged from 0.7 to 3.22 mg/g of dry weight (30). According to a study, *Mentha* sps. has shown a phototoxic effect and chlorosis due to the treatment of heavy metals, but it also has a good metal accumulating capacity (31).

Conclusion

The work done by treating the *Jacobaea maritima* plant with heavy metals has not been reported in any previous research. This current work gives us an idea and detailed information on the complete plant's physiology, phytochemistry and ability to accumulate heavy metals and proves that *Jacobaea maritima* is a good accumulator of heavy metals, changing the composition of the compounds in the plant. All these factors ultimately affect the medicinal properties and the preparation of medicine. The future perspective can be to identify the specific secondary metabolite and quantify it with advanced techniques like High-Performance Liquid Chromatography (HPLC), which will help in the mass production of the metabolite within the specific timeline with no or minimal effects.

Acknowledgements

The authors would like to thank the management of the Department of Life Sciences, CHRIST (deemed to be University), for providing the lab facilities and required support.

Authors' contributions

WJKS did all the lab work and the analysed the results; JX provided the necessary guidance and helped in formulating the work.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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