Research Article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Investigation on Chromium Tolerance and Bioaccumulation in *Aerva lanata* (L.) Juss. ex Schult

Roshnara Mohanty[1,2], Manikkannan Thirunavoukkarasu[1]*and Thangavel Sekar[2]

[1]CSIR - National Environmental Engineering Research Institute, Chennai Zonal Laboratory, Chennai - 600113, India

[2] PG and Research Department of Botany, Pachaiyappa's College, Chennai - 600030, India

---

ABSTRACT

Plants are capable of accumulating heavy metals through their roots and translocate in the aerial biomass. However heavy metals in herbal plants are a threat to human beings, hence it is necessary to examine whether plants used in human consumption are capable of accumulating toxic metal and at what concentration. *Aerva lanata* (L.) Juss. ex Schult an important medicinal plant was taken as test material. In order to get uniform plants for chromium treatment, we followed *in vitro* micropropagation technique to avoid any prior contamination in the plant. *In vitro* plants of 5 cm length with healthy roots were cultured in Cr wastewater (Cr WW). The significantly highest Cr(VI) accumulation (53.7 mg/kg DW) was found at 75% of Cr WW treatment giving 71.6% reduction in Cr, where roots alone showed 52.2% reduction. Both BCF & TF were found in order of higher to lower from 5% >10% >25% >50% >100% of WW treatments respectively. The plant's biomass, photosynthetic pigments, total proteins and carbohydrates were found to show similar patterns of significant increase in their respective contents up to 50% of Cr WW treatment, but got reduced at higher concentrations (75% and 100%). Contradictorily, there was an increase of total antioxidant activity by 1.7 times in leaves (19.8 mg/g), 2.5 times in stems (6.1 mg/g) and 1.6 times in roots (5.9 mg/g) at 75% of WW treatments respectively. Therefore, the experimental plant system has potential in accumulating Cr(VI) if grown under contaminated areas, hence cautionary to be taken during pharmaceutical preparations.

KEY WORDS: *Aerva lanata*, chromium (VI), tissue culture, phyto-toxicity, medicinal plants

---

***Corresponding Author**

Dr. Manikkannan Thirunavoukkarasu

Chief Scientist & Head
CSIR - National Environmental Engineering Research Institute,
Chennai Zonal Laboratory, Taramani, Chennai 600113, India
E-mail address: mtarasu@yahoo.com, mt_arasu@neeri.res.in
Tel: +91- 44-22544660

1. INTRODUCTION

Heavy metals as the name suggests has relatively high density and is toxic even at low concentrations. They are naturally present in the environment as fractions in soil, water and air. But when their concentration reaches above the permissible limits, it leads to contamination of the environment having deleterious effects to both biotic and abiotic components. Heavy metal contamination is a worldwide issue which is dejectedly related to the anthropogenic activities. Chromium (Cr) in the valences from -2 to +6 is abundantly present in the earth's crust.[1] Out of all the oxidation forms, chromium (VI) (CrVI) shows higher toxicity because of its higher solubility and mobility in the solvent entity.[2] Both natural and manmade activities are responsible for chromium pollution in the environment where the latter is on the heavier part (more than 70%). Steel industries, leather tannery, fertilisers, pesticides, metal smelters, surface coatings, dyes, paints, and plastics are some of the unrewarding industrial uses of chromium which may look alluring to the modern society but literally destroys the biosphere with infiltrating the majority of the toxic Cr, hence poisoning the environment.[3, 4, 5] Tanning industries plays a major role in affecting the Cr content in the atmosphere where about 40% of the Cr contamination occurs due to leather industries when compared with others because of the use of $Cr_2(S0_4)_3$ as tanning agent. In India, over 32,000 tons of Cr passages annually from the tanning industries. In the developing countries like United States around 25.9 g/kg of Cr is found in the soil around the proximity of the chrome construction sites.[5]

The toxicity of Cr mainly depends on its oxidation states. Chromium (III) (CrIII) also called as chromium picolinate is a beneficial micronutrient required in very little amount whereas Cr(VI) is most toxic of all the oxidation states.[3, 6] Cr(III) helps in maintaining insulin production, also working as a receptor for proper functioning of the metabolic pathways in animal body. But excess amount of the trivalent chromium can cause detrimental effects with long term toxicity. On the other hand, Cr(VI) is carcinogenic in nature and causes kidney and liver diseases in humans. Its toxicity is because of the higher oxidation potential and permeability when compared to Cr(III).[7] It entering into the body by any ways is lethal as the toxic air fumes of Cr(VI) can cause asthma, skin cancer, gastrointestinal ulcers, reproductive problems, and allergic reactions when ingested.[4] Less than 2g of soluble hexavalent chromium can cause kidney and liver damage whereas 2-5 g of the same can be fatal to an adult human body.[1] Plants ability to acclimate in stressful environmental conditions benefits them to scrutinize the toxic substances from the soil, water, or air via various processes like phytoextraction, stabilisation, volatilization, degradation, and rhizofiltration. The effects of Cr on plants solely depends on its

oxidation states where Cr(III) is toxic when present in huge amount whereas Cr(VI) is highly toxic even in limited quantity.[2, 6] As per the reports, the authoritative mechanism taken by Cr or other metal hyperaccumulators is the effort of rhisopheric transporters in absorbing and translocating it to the shoots via xylem loading where chelation and segregation takes place forming less toxic forms of the metals.[6]

The plant *Aerva lanata* (L.) Juss. ex Schult, also known as mountain knot grass belongs to the family Amaranthaceae, native to Africa and Asia. The plant is recognized for having enormous biological activities and traditionally is used to treat cough, diabetes, kidney stones, diarrhea, dysentery and skin diseases. The whole plant extracts has antimicrobial, diuretic, antihepatotoxic, antitumor and antioxidant activities. The plant is also known for the antifertility, antiasthmatic and antiparasitic activities as found in the aerial parts. The valuable biochemical compounds present in the plant are sterols, phenols, alkaloids and flavonoids.[8] However there have been reports concerning the distress among traditional medicine consumers because of the possibility of pollutant being found in them.[9] This is because of the natural ability of certain plants to thrive in varied environments. To overcome this, proper understanding of the toxic metal concentrations in the medicinal plant parts after metal exposure should be executed, which could also further endorse for quantification of extent of phyto-toxicity. The plant tissue culture studies aids in providing a large number of uniform, healthy and true to the type plants in a short span while also promoting the conservation of the wild variety.[10] The use of *in vitro* assay allows uninterrupted investigation during metal tolerance studies while differentiating the plants response towards stress.

The present study was aimed to establish *in vitro* plantlets of *Aerva lanata* (L.) Juss. ex Schult to evaluate its potential in accumulating Cr from the wastewater. It will assist in understanding the response of the selected plant species during Cr stress, its metal accumulation ability, and thorough localization of the metal in the plant parts which eventually will facilitate its use in therapeutic medicines.

2. MATERIALS AND METHODS

2.1. Media preparation for tissue culture studies

Murashige and Skoog's (1962)[11] basal medium (MS) was used throughout the present investigation. According to the requirements the MS nutrient media was further supplemented with plant growth regulators (PGRs) in different ranges and combinations like BA (N^6-benzyl adenine, 0-3 mg/l), Kn (kinetin, 0-2 mg/l), IAA (indole-3-acetic acid, 0-2 mg/l), and IBA (indole butyric acid, 0-3 mg/l).

The glassware containing the medium (pH maintained at 5.8 ± 0.2) were then steam sterilized in an autoclave for 20 minutes at 121°C under 15 psi pressures.

2.2. Explant preparation and in-vitro culture

A. lanata maintained in the green house was used as explants source throughout the studies. Young shoots were defoliated and the first 2-3 nodes from the apical region as well as 1-2 nodes from the basal region of these shoots/branches were discarded. The stem node segments (*ca*.0.8-1.2 cm) were cut with the help of a clean razor blade/scalpel so that each contained a dormant axillary bud. Then they were soaked in a dilute surfactant solution (0.5%, Extran, E. Merck India Ltd.) for about 5 minutes and washed in running tap water for 8-10 minutes. Further the explants were surface sterilized in an aqueous solution of 0.1% (w/v) mercuric chloride ($HgCl_2$) for about 3-5 minutes.

Following surface sterilization all the explants were rinsed 3-4 times in sterile distilled water and finally inoculated on to the surface of sterilized nutrient agar media pre-packed in culture tubes or Erlenmeyer's conical flasks. Proliferated micro shoots were separated and those measuring 2-3 cm and above were individually planted onto the basal MS medium with or without the supplementation of auxins (IAA or IBA) for rooting at different concentration ranges (0.25-1 mg/l). Half strength MS media was also used for roots initiation from the *in-vitro* grown shoots. All the cultures were incubated in a growth room with a 16h photoperiod (cool, white fluorescent light (30 µmol m/s) and the temperature maintained at 25 ± 2°C, with 50-80% relative humidity.

2.3. Waste water sampling and treatment

Chromium wastewater (Cr WW) was collected from Ranipet, Tamilnadu, India from an abandoned chromium contaminated site. The wastewater (WW) was straw yellowish colored liquid, was collected in plastic barrels, brought to the lab and stored in room temperature. Before using it for the experiments it was filtered and was consigned for the heavy metal detection analysis. The general water parameters (Salinity, conductivity, total dissolved solids) were also calculated before and after the treatment by using a multi-parametric water quality meter (Labman, Scientific instruments, Chennai). Before using the Cr WW for the bioaccumulation studies with the *in vitro* plantlets the pH was maintained at 5.8 ± 0.2 and was autoclaved.

In vitro plantlets of *A. lanata* (L.) Juss. ex Schult with healthy root systems were washed free of the agar gelled medium (especially the root portions) with sterile water, and were used for the treatment

under hydroponic system. The plants were maintained in a growth room with a 16h photoperiod (cool, white fluorescent light (30 µmol m/s) and the temperature maintained at 25 ± 2°C, with 50-80% relative humidity as discussed earlier. The autoclaved Cr WW, was treated at different percentage concentrations starting from 0% to 100% with liquid MS media. For each concentration triplicates were maintained and plain MS media was used as control. The treatment was continued for 4 weeks and at the end of the treatment period the samples were tested for the presence of Cr in the plant parts individually. To study the effects of Cr stress on the plant, certain screening techniques such as physiological and biochemical analysis were also performed which are discussed below.

2.4. Determination of Cr concentration in plants

After the treatment, the *in vitro* plantlets were analyzed for the presence of Cr accumulated in each of its parts i.e. roots, stems and leaves individually. Before the analysis, the plant parts were dried in an oven at 80 ^{0}C for 24 hours, weighed and subsequently used to measure Cr concentration. The plant samples were acid digested in aqua regia (Hydrochloric acid: Nitric acid, 3:1), and were analyzed by AAS (Atomic Absorbtion Spectrophotometer).

2.5. Physiological and Biochemical Analysis

The effects of Cr on the treated plantlets were studied by analyzing the different aspects of plants metabolism. The effect on plants growth, photosynthetic pigments, antioxidant activities, total protein, total carbohydrates, bioconcentration and translocation factors (BCF and TF) were examined with each plant parts (leaves, stems, roots) individually also by exploring all the concentrations in which the plantlets were given treatments (from 0% to 100%). This was done to thoroughly understand the effect of each concentration of Cr WW given as treatment to the *in vitro* plantlets and their survival mechanism during Cr stress. All the analytical methods studied are given below.

2.5.1. Effects of Cr on plant growth

Some of the heavy metals like cobalt, manganese, iron, copper, nickel, zinc are essential for the plants growth. Toxic metal stress influences the plant's growth in a number of ways. Among all the parts, roots are likely to get in contact with the toxic environment first showing the early visible effects. Similarly, stems and leaves are also affected as a consequence of metal stress. In order to understand the

toxic metal effects, the physiological changes after the Cr WW treatment were analyzed by comparing the initial with final overall plant length, shoot length, root length and number of leaves.

2.5.2. Bioconcentration factors (BCF) & Translocation factors (TF)

Both the factors (BCF & TF) are important while studying the potential of any plant employed for tolerance studies. To study the competence of the plant to accumulate toxic metals in its tissue from the dissolved state, the bioconcentration factor was calculated. It can be calculated from each part of the plant like leaves, stems, roots which eventually helps in interpreting the symmetry between the contaminant and plants. Whereas, TF provides the shoot-root quotient illustrating the plant's ability to translocate the contaminant or the metal studied from roots to the aerial parts. Plants are categorized as phytostabilizers, phytoextractors on the basis of TF & BCF values which are calculated by the equation given below:

$$\text{BCF} = \frac{\text{Concentration of metal present in plant part}}{\text{Initial concentration of metal present in substrate}} \quad (1)$$

$$\text{TF} = \frac{\text{Concentration of metal present in shoot}}{\text{Concentration of metal present in root}} \quad (2)$$

2.5.3. Photosynthetic pigments

The process of photosynthesis also gets affected during metal stress where the enzyme responsible for the electron transport mechanism gets inhibited. The photosynthetic pigments (Chl a, Chl b, Total carotenoids) were calculated spectrophotometrically via method given by Arnon.[12] Fresh plant tissue (leaves) was homogenized by using 80% acetone and was centrifuged at 13,000 rpm for 20 minutes at 4°C. The plant extract's supernatant was spectrophotometrically analyzed at different wavelengths and calculated as mentioned below:

$$Chl\ a\ = 12.72(A663) - 2.59(A645)\left(\frac{V}{1000} \times W\right) \quad (3)$$

$$Chl\ b\ = 22.88(A645) - 4.67(A663)\left(\frac{V}{1000} \times W\right) \quad (4)$$

$$Total\ Chl\ (mg/g)\ =\ Chl\ a\ +\ Chl\ b \quad (5)$$

$$\text{Car} = 7.6(\text{A480}) - 1.49(\text{A510})\left(\frac{\text{V}}{\text{d}} \times \text{W} \times 1000\right) \quad (6)$$

Where,

V= volume of the sample

W= weight of the plant's part taken

d = length of light

2.5.4. Total antioxidant capacity (TAC)

Phosphomolybdenum method was employed for determining the total antioxidant capacity (TAC).[13] Each plant part (leaves, stems, roots) from each Cr WW treatment studies was homogenized using a reducing agent (ethanol) and was further mixed with phosphomolybdenum buffer (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Afterwards it was incubated for 100 minutes at 95 ^{0}C. The samples were cooled and were analyzed spectrophotometrically at 695 nm for the presence of TAC.

2.5.5. Total protein

Proteins are also considered important in plant's survival mechanisms. Toxic metals are found to be altering the fundamental conformation of proteins by binding to it. Therefore, in order to understand the toxic effects of Cr waste water on plants protein content, total protein estimation was done by using Lowry's method.[14] Each plant part was grounded separately with freshly prepared phosphate buffer (pH-7.4) in cold conditions and centrifuged at 10,000 rpm for 10 minutes. To each sample around 5ml of reaction buffer (2% sodium carbonate in 0.1 N sodium hydroxide added to 0.5% Copper sulphate in 1% sodium potassium tartarate in 5:1) was added, vortexed and incubated for 10 minutes. Folin-ciocalteu reagent was added to the samples and again incubated for 30 minutes. Absorbance of aqueous phase was measured at 660 nm and the amount of protein was expressed as mg BSAE/g of fresh weight based on the standard curve plotted against known concentrations of bovine serum albumin (BSA).

2.5.6. Total carbohydrates

Carbohydrates are responsible for the storage and structural functions in plants. Estimation of total carbohydrates was done using the procedure given by Hedge, et al.[15] Plant tissues from each plant parts of each concentration were kept in boiling water bath with 5ml of 2.5 N HCL for around 3 hours. After

bringing the samples to room temperature Na_2CO_3 was added to give a neutralizing effect. The samples were diluted to 100 ml, from which 1 ml was mixed with 4 ml of anthrone reagent followed by 10-15 minutes of heating. D-Glucose was used as standard and the absorbance was measured at 630 nm to obtain the amount of carbohydrates present in the samples.

2.6. STATISTICAL ANALYSIS

For statistical efficacy, each treatment was made into three replicates, and the results were expressed as ± standard deviation (SD) during all the parameters tested. Significance *($p < 0.05$)* was estimated by analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS software.

3. RESULTS AND DISCUSSIONS

3.1. In vitro micropropagation

The inter-nodal segments in MS media along with different concentrations of plant growth regulators were used to generate *in vitro* plantlets of *A. lanata* (L.) Juss. ex Schult. Out of all the hormones tested, the optimum observation was achieved in media containing BA (1.5 mg/l) + IAA (0.25 mg/l), which gave around 30-36 shoots per explants in 30 days (Fig. 1). However, BA alone was also able to generate multiple shoots (25-29) when supplied alone in the concentration of 1.5 mg/l. MS media without growth regulators (MS0) were unable to elucidate any morphogenetic responses. Proper elongation of the micro shoots obtained earlier were attained in MS media enriched with BA (1.5 mg/l) + Kn (0.5 mg/l). The average time for the shoots to elongate was around 25-30 days. The next and important step in the micropropagation study was the rooting phase. Although both the auxins (IBA and IAA) were able to induce *in vitro* roots at all concentrations studied (data not given), but the best rooting response was accomplished using ½ MS along with IAA (0.25 mg/l). *In vitro* plant regeneration using cytokinins and auxins were also studied by a number of researchers.[10, 16]

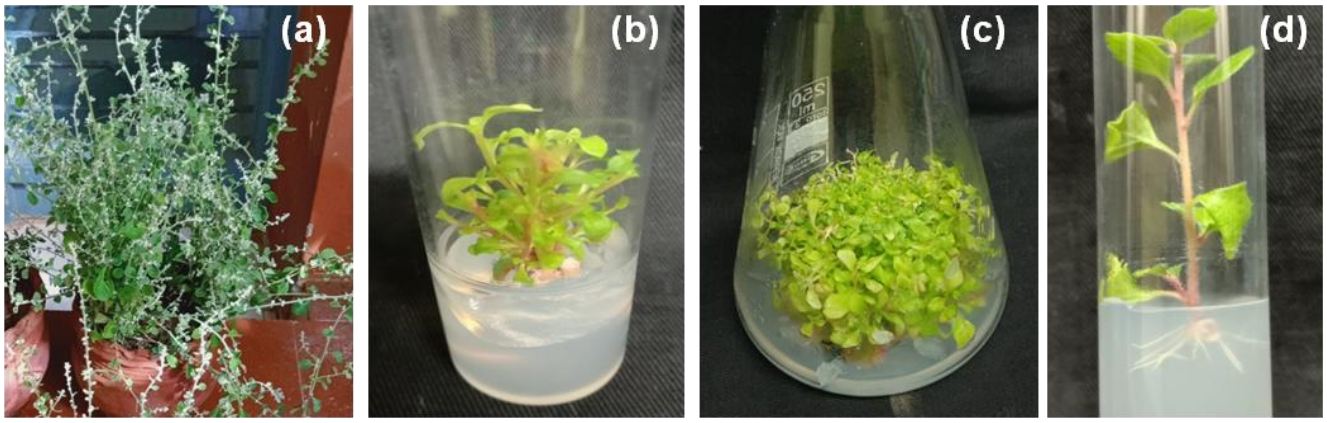

Fig. 1. Micropropagation Studies of *A. lanata* (L.) Juss. ex Schult. (a) The green house plant taken as explants source, (b) Initiation of multiple shoots via axillary bud proliferation, (c) Mass of multiple shoots, (d) Micro shoot in rooting media showing root initiation.

3.2. Investigating Cr content and tolerance capacity

The AAS analysis was performed to detect the amount of Cr(VI) present in the WW initially and also accumulated in each plant part (leaves, stem, and roots) after the metal tolerance studies. The initial Cr(VI) content in the ww was found to be 100 ppm. The other basic water parameters calculated before and after the treatments are shown in table 1. The significantly highest Cr(VI) accumulation (53.7 mg/kg) on dry weight (DW) basis was found at 75% (which is 75 ppm, obtained after finding the WW initial Cr content via AAS) treatment where the roots were the major organs to accumulate around 39.2 mg/kg (DW) of Cr(VI). The *in vitro* plantlets kept as control didn't show any presence of Cr(VI) in it (data not shown). There was also maximum accumulation found in the leaves (10.1 mg/kg, DW) and stem (5.4 mg/kg, DW) of plants treated at 75% of Cr WW respectively (Fig.2).

Table. 1. Basic water parameters tested before and after the treatment at 75% of Cr WW treatment studies.

Cr WW parameters	Initial concentration	After Treatment
TDS	801 ppm	528 ppm
Salinity	0.76 ppt	0.49 ppt
Conductance	1592 µs/cm	998 µs/cm

Note: The optimum reduction in the water parameters were found at 75% of Cr WW treatment studies.

Another notable outcome observed in the present study was the accumulation of Cr(VI) more in leaves as compared to the other aerial part (stem). Similarly, in *Vallisneria spiralis* L. after 72 hrs of treatment with 10 ug/ml of Cr, the maximum accumulation was found in leaves (697 μg/g DW) followed by rhizomes (437 μg/g DW).[3] The efficiency of Cr(VI) uptake by the plants was recorded to be more with increase in Cr WW concentrations, as Cr accumulation from any liquid substrate solely depends on the its concentration, pH, and presence of nutrient salts.[17] The Cr(VI) accumulation competence was found to be in order of higher to lower from roots >leaves >stem at every Cr WW treatment studies.

After the treatment, the BCF and TF values (Fig. 3) were also evaluated to understand the attributes of the experimental plant during Cr(VI) metal stress in translocating the metals in its tissues. Both the values were calculated by using the equation 1 & 2 noted in earlier sections. The highest BCF value was recorded in plant treated at 5% (0.94) of Cr WW, whereas the lowest was at 100% (0.2).

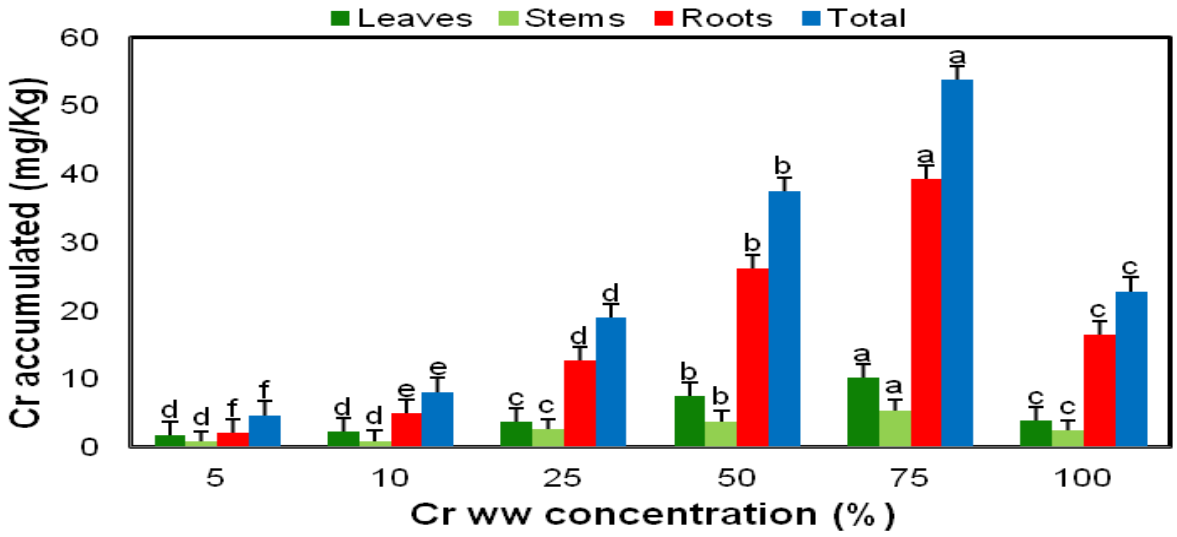

Fig. 2. Cr accumulated by *A. lanata* (L.) Juss. ex Schult. in its various parts at different ww concentrations. All the values are expressed as mean ± SD, n=3. Bars with different letters indicate significant differences at $p \leq 0.05$, determined within the ww concentrations by using Tukey's post hoc test.

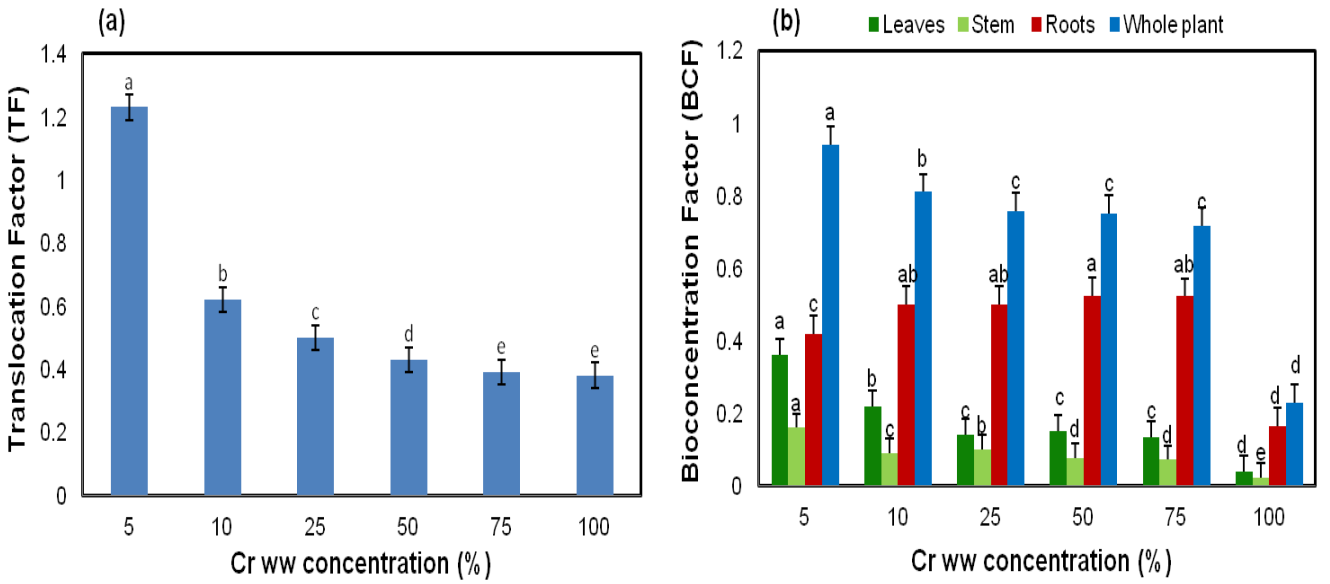

Fig. 3. The TF and BCF values calculated after the Cr WW treatments. (a) TF values at different Cr WW concentrations, (b) BCF values recorded in each plant part at each Cr ww concentration. All the values are expressed as mean ± SD, n=3. Bars indicating different letters are significantly different (among WW concentrations) at $p \leq 0.05$ by Tukey's post hoc test.

When considering the individual plant parts, the highest significant BCF value in all the treatments were seen in roots followed by leaves and then in stem ($p \leq 0.05$) (Fig. 3b). The BCF values of roots were found to be increasing with increase in Cr WW concentrations. Hence, summarizing the fact that almost all the Cr(VI) metals taken up by the plant system was retained in the roots at higher Cr WW concentrations. This process leads to reinforcement of a metal concentration gradient between the root tissues and the aerial parts of the plant.[18] Similarly the TF values displayed the similar pattern as that of BCF and were recorded more in plant treated at 5% (1.23), while lowest at 100% (0.38) of Cr WW (Fig. 3a). A similar aspect was seen in *L. hexandra* when a higher TF value (0.21) got decreased to a lower value (0.009) when the Cr(VI) concentration was increased from 30 mg/l to 60 mg/l respectively.[19] Both the BCF & TF were found in order of higher to lower from 5% >10% >25% >50% >100% of Cr WW treatments respectively. Taking all these values into deliberation it was concluded that the plant system studied here exhibited phytostabilising nature at higher Cr(VI) concentrations. Considering in broader sense, when all plants are made vulnerable to stress, each have distinct reactions either being stress avoiders or stress tolerators. The former has developed technique to confine their cells from the traumatic circumstances while the latter modify their mechanism in a way to avoid injuries.[20]

3.3. Physiological changes in plant system after Cr ww treatment

The usually visible but indefinite symptoms found after toxicity in plants is its changes in growth patterns. The phytotoxic effects of Cr(VI) on *A. lanata* were seen as chlorosis in leaves, wilting and stunted overall appearance (Fig. 4).

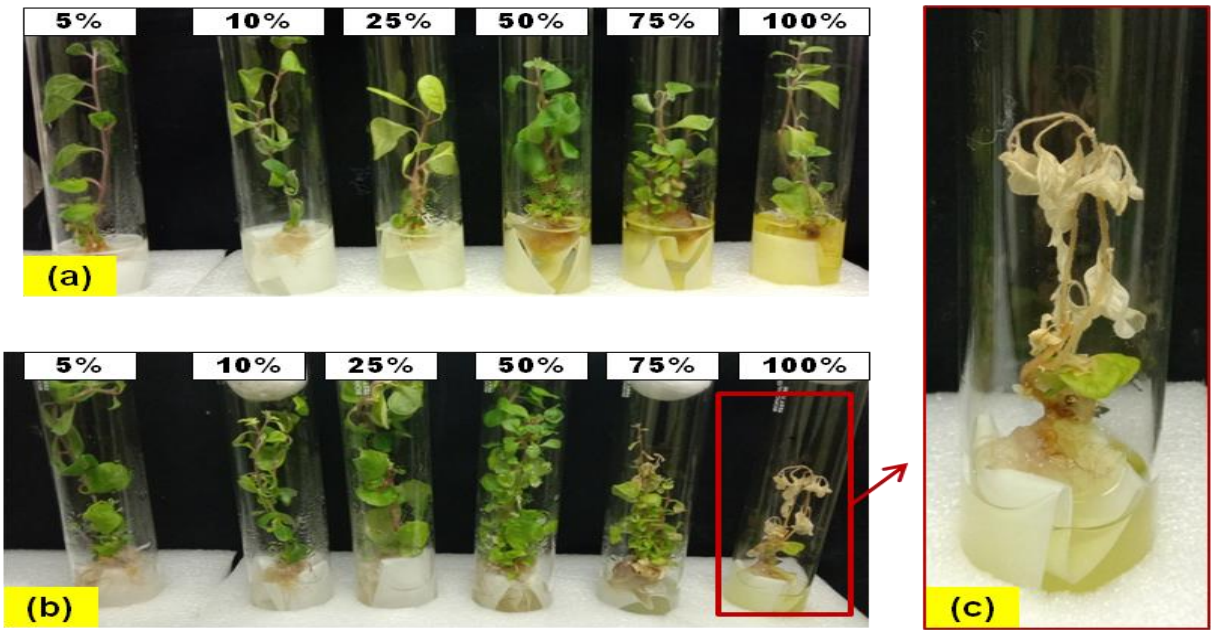

Fig. 4. Physiological changes seen in *A. lanata* (L.) Juss. ex Schult. during Cr WW treatment. (a) Plantlets at different Cr WW concentrations on 1st day of treatment, (b) after 4th week, (c) Plantlet at 100% showing phyto-toxic effects.

However, the effect of Cr(VI) on roots was found to be more prominent as seen in all the concentrations tested, where with the increase in Cr WW concentration there was significant reduction in root increment (Fig. 5). After four weeks treatment, the significantly higher root increment was found in plant kept as control (Fig. 4). However, the maximum shoot increment (10.3 cm) and leaf numbers (15) were recorded in plants treated at 50% (50 ppm) of Cr WW respectively. It was also observed that up to 50% of Cr WW treatment there was an increase in root, shoot length as well as number of leaves, but at above 75% there was a gradual decrease in overall plants growth (Data not shown). Similar effects were also seen in a study conducted on *Allium cepa* cv. Hybrid plant, where at Cr(VI) concentration more than 150 mg/l only caused reduction in morpho-physiological parameters tested.[21] In another study on *Myriophyllum spicatum*, Cr(VI) at 1 mg/l was enough to induce phytotoxic effects by reducing the biomass and shoot lengths.[22] The effects of Cr on the seed germination, shoot length, root length and other physiological studies showed that the higher concentration exhibited growth inhibition by limiting

the seed germination rate, decline in shoot and root length, also degrading of the photosynthetic pigments.[23] Although in some plants the lower concentrations of Cr was found to be beneficial as seen in *Myriophyllum spicatum* and *Pisum sativum* promoting increase in shoot length at 0.5 mg/l Cr(IV), root length at 0.25 mg/l Cr respectively.[2]

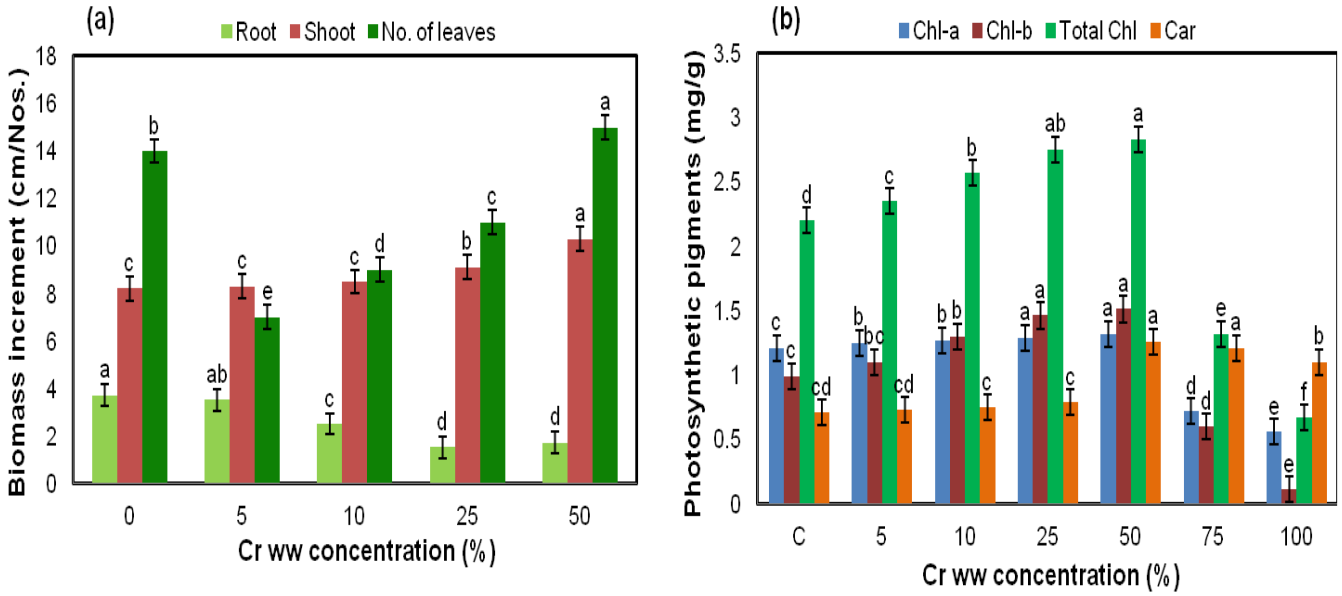

Fig. 5. Effects of Cr WW on *A. lanata* (L.) Juss. ex Schult. (a) on biomass increment at different ww concentrations, (b) on photosynthetic performances. All the values are expressed as mean ± SD, n=3. Bars indicating different letters are significantly different (among ww concentrations) at $p \leq 0.05$ by Tukey's post hoc test. Chl-a= Chlorophyll a, Chl-b= Chlorophyll-b, Total Chl= Total Chlorophyll, Car= Carotenoids.

3.4. Biochemical profiling

3.4.1. Effects of Cr ww on photosynthetic performances

The effects of Cr WW on the photosynthetic pigments (chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids) are shown in Fig 5b. As per the data, there was a significant increase found in the chlorophyll (a, b and total chlorophyll) and carotenoid pigment accumulation with increase in Cr WW concentrations. However at much higher Cr concentration levels (75%), there was a substantial decrease in the photosynthetic pigment contents. The highest chl-a (1.32 mg/g), chl-b (1.51 mg/g), total chl (2.83 mg/g) and carotenoid (1.26 mg/g) values were recorded at 50% of Cr WW treatment respectively. The process of pigment rejuvenation and construction can be considered as an approach towards the plant's defence mechanism towards Cr stress. Similar response was also found in *P. ovata* seedlings, where the photosynthetic pigments got increase at 1.5 mM Cr concentrations, but got decreased at higher concentrations (1.8 mM).[23] However there are reports validating the negative

impacts of Cr on the photosynthetic apparatus as the metal ion exerts harmful effects on the enzyme involved in chlorophyll biosynthesis.[3, 24]

3.4.2. Effects of Cr WW on protein, carbohydrates and antioxidant contents

The protein, carbohydrate and total antioxidant contents calculated are given in Table 2. A constant and significant increase was observed in the protein contents when compared with the control plant values. However a drastic decrease was seen at higher Cr WW concentration (75% and 100 %). The highest protein content was found at 50% treatment in leaves (0.78 mg BSAE/g) followed by stem (0.68 mg BSAE/g) and then in roots (0.37 mg BSAE/g) respectively (Table 2a). The other primary metabolite carbohydrates were also seen to be following the same pattern as that of proteins. Although the significantly highest carbohydrate content was found in stem (0.79 mg glucose/g) followed by leaves (0.61 mg glucose/g) at 50% of Cr WW treatment. Contrary to the protein values present in roots, the highest carbohydrate content in roots was reported in control plant (0.73 mg glucose/g), which gradually got decreased with increase in Cr concentrations (Table 2b). Similarly in another study with the same plant species *A. lanata* grown under polluted environment exhibited increase in protein (271 μg/g DW) and carbohydrate (432.7 μg/g DW) content respectively.[20]

The total antioxidant activity of *A. lanata* treated at 75% of Cr WW was increased by 1.7 times in leaves (19.8 mg/g), 2.5 times in stems (6.1 mg/g) and 1.6 times in roots (5.9 mg/g) as compared to the plant kept as control (Table 2c). The degree of antioxidants gets increase or decrease depending on the stress intensity as also found in *P. ovate* where the total antioxidant activity was upgraded by 3.2 times as that of the control plant at 1.5 mM Cr(VI) treatment, whereas the same got reduced at higher concentrations (1.8 mM).[23] Both enzymatic and non-enzymatic antioxidants are plants defense systems which are biosynthesized either under normal metabolism or during stresses. Also the stress proteins produced during biotic or abiotic stresses are made up of antioxidant enzymes.[25]

Table 2: Biochemical responses of *A. lanata* towards Cr metal stress. (a) Total protein contents, (b) Total carbohydrates, and (c) Total antioxidant activity.

(a) Cr WW conc.	Proteins (BSAE mg/g) ±SD		
	Leaves	**Stems**	**Roots**
0%	0.45 ± 0.21^{Ad}	0.38 ± 0.23^{Bd}	0.23 ± 0.2^{Cd}
5%	0.47 ± 0.24^{Ad}	0.39 ± 0.24^{Bd}	0.25 ± 0.22^{Cd}
10%	0.51 ± 0.32^{Ac}	0.41 ± 0.38^{Bc}	0.29 ± 0.34^{Cc}
25%	0.61 ± 0.34^{Ab}	0.54 ± 0.14^{Bb}	0.32 ± 0.44^{Cb}
50%	0.78 ± 0.28^{Aa}	0.68 ± 0.29^{Ba}	0.37 ± 0.27^{Ca}
75%	0.32 ± 0.12^{Ae}	0.27 ± 0.31^{Be}	0.19 ± 0.07^{Ce}
100%	0.02 ± 0.15^{Af}	0.01 ± 0.21^{Bf}	0.01 ± 0.04^{Cf}

(b) Cr WW conc.	Carbohydrates (Glucose mg/g) ±SD		
	Leaves	**Stems**	**Roots**
0%	0.45 ± 0.21^{Cc}	0.58 ± 0.23^{Bd}	0.73 ± 0.2^{Aa}
5%	0.47 ± 0.24^{Bc}	0.59 ± 0.24^{Acd}	0.47 ± 0.22^{Bb}
10%	0.48 ± 0.32^{Bc}	0.62 ± 0.38^{Ac}	0.45 ± 0.34^{Cb}
25%	0.55 ± 0.34^{Bb}	0.74 ± 0.14^{Ab}	0.43 ± 0.44^{Cc}
50%	0.61 ± 0.28^{Ba}	0.79 ± 0.29^{Aa}	0.43 ± 0.27^{Cc}
75%	0.19 ± 0.12^{Bd}	0.27 ± 0.3^{Ae}	0.25 ± 0.07^{ABd}
100%	0.02 ± 0.15^{Ae}	0.01 ± 0.21^{Bf}	0.01 ± 0.14^{Be}

(c) Cr WW conc.	Antioxidants (Ascorbic acid mg/g) ±SD		
	Leaves	**Stems**	**Roots**
0%	11.4 ± 0.36^{Ae}	2.5 ± 0.28^{Cd}	3.7 ± 0.52^{Bd}
5%	12.5 ± 0.30^{Ad}	3.6 ± 0.34^{Cc}	3.9 ± 0.32^{Bd}
10%	12.7 ± 0.40^{Ad}	3.8 ± 0.48^{Cc}	4.1 ± 0.54^{Bc}
25%	13.4 ± 0.43^{Ac}	5.4 ± 0.24^{Bb}	4.5 ± 0.24^{Cc}
50%	18.0 ± 0.18^{Ab}	5.7 ± 0.49^{Bb}	5.2 ± 0.17^{Cb}
75%	19.8 ± 0.82^{Aa}	6.1 ± 0.20^{Ba}	5.9 ± 0.09^{Ca}
100%	1.2 ± 0.25^{Af}	0.6 ± 0.30^{Be}	0.3 ± 0.18^{Ce}

All the values are expressed as mean ± SD, n=3. Different letters indicate significant differences between Cr WW concentrations (small letters) and among plant parts (capital letters) at $p \leq 0.05$ by Tukey's post hoc test.

4. CONCLUSIONS

The best regenerative observations in favour of multiple shoot induction were found in MS media enriched with BA (1.5 mg/l) + IAA (0.25 mg/l) delivering 30-35 shoots per explants in 30 days time frame. Also half strength MS (½ MS) along with IAA 0.25 mg/l was found optimum to generate more number of roots. MS media supplemented with BA (1.5 mg/l) + Kn (0.5 mg/l) was observed to be beneficial for shoot elongation studies. The AAS results obtained demonstrated the plants response in accumulating or avoiding the toxic Cr(VI) induced stresses. There was around 94%, 81%, 76%, 75%, 71% and 22.8% reduction in the Cr content when the treatment was given at 5%, 10%, 25%, 50%, 75% and 100% of Cr WW respectively. The Cr(VI) accumulation competence was found to be in order of higher to lower from roots > leaves > stem at every Cr WW treatment studies. The basic water parameters tested also exhibited lower TDS, salinity and conductance values when compared with the initial. The BCF data of roots were found to be enhancing with increase in Cr WW concentrations. Hence, summarizing the fact that at higher Cr WW concentrations most of the Cr(VI) metals taken up by the plant system was retained in the roots. However both the total BCF & TF values were decreased with increase in Cr WW concentrations. Under physiological observation, the onset of phyto-toxicity was observed at 75% of WW treatment, otherwise there was increase in plant biomass until 50%. The photosynthetic pigments, total proteins and carbohydrates were found to show similar patterns as there was increase in the respective contents up to 50% of Cr WW treatment, but got reduced at higher concentrations (75% and 100%). However the total antioxidant activity was found to be significantly higher at 75% of Cr WW studies. Therefore, the Cr(VI) bioaccumulation results obtained here gave us a thorough insight into the response of the plant *A. lanata* during Cr stress and constrained us to be careful before utilising each of the respective plant parts in therapeutic medicines.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, CSIR-NEERI for their support to carry out this research work at CSIR-NEERI (Chennai Zonal Laboratory).

REFERENCES

1. Tumolo M, Ancona V, De Paola D, Losacco D, Campanale C, Massarelli C, Uricchio VF. Chromium pollution in European water, sources, health risk, and remediation strategies: An overview. Int. J. Environ. Res. Public Health. 2020; 17(15): 5438.

2. Srivastava D, Tiwari M, Dutta P, Singh P, Chawda K, Kumari M, Chakrabarty D. Chromium stress in plants: Toxicity, tolerance and phytoremediation. Sustainability. 2021; 13(9): 4629.
3. Vajpayee P, Rai UN, Ali MB, Tripathi RD, Yadav V, Sinha SN. Chromium-induced physiologic changes in *Vallisneria spiralis* L. and its role in phytoremediation of tannery effluent. Bull. Environ. Contam. Toxicol. 2001; 67: 246–256.
4. Ramírez V, Baez A, López P, Bustillos R, Villalobos MÁ, Carreño R, Contreras JL, Muñoz-Rojas J, Fuentes LE, Martínez J and Munive JA. Chromium hyper-tolerant *Bacillus sp*. MH778713 assists phytoremediation of heavy metals by mesquite trees (*Prosopis laevigata*). Front. Microbiol. 2019; 10: 1833.
5. Shanker A, Venkateswarlu B. Chromium: Environmental pollution, health effects and mode of action. In-Chief: Jerome ON (ed) Encyclopedia of environmental health. Elsevier, Burlington. 2011; 65: 650-659.
6. Saha P, Shinde O, Sarkar S. Phytoremediation of industrial mines wastewater using water Hyacinth. Int. J. Phytoremediation. 2017; 19: 87-96.
7. Gautam A, Kushwaha A, Rani R. Microbial remediation of hexavalent chromium: An eco-friendly strategy for the remediation of chromium-contaminated wastewater. In The Future of Effluent Treatment Plants; Elsevier: Amsterdam, The Netherlands, 2021; 361–384.
8. Goyal M, Pareek A, Nagori BP, Sasmal D. *Aerva lanata*: A review on phytochemistry and pharmacological aspects. Pharmacogn Rev. 2011; 5(10): 195-8.
9. Manan FA, Chai TT, Samad AA. Environmental Pollution in Malaysia: Are Medicinal Plants Potential Phytoremediation Agents?. Maejo Int. J. Sci. Technol. 2015; 9: 288–300.
10. Thirunavoukkarasu M, Panda PK, Nayak P, Behera PR, Satpathy GB. Effect of media type and explant source on micropropagation of *Dalbergia sissoo* Roxb. – An important multipurpose forest tree. Int. Res. J. Plant. Sci. 2010; 1155-1162.
11. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Plant Physiol. 1962; 15: 473–497.
12. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta Vulgaris*. Plant Physiol. 1949; 24(1): 1–15.
13. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 1999; 269: 337-41.

14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193(1): 265–275.
15. Hedge JE, Hofreiter BT. In: Carbohydrate Chemistry **17** (Eds Whistler R L and Be Miller, J N) Academic Press New York. 1962.
16. Al-Aradi H, Al-Maliki G, Al-Shmary A, Alhello A. Phytoremediation of wastewater by *Bacopa monnieri* plant growth *in vitro*. Eco. Env. & Cons. 2021; 26(1): 256-261.
17. Babula P, Adam V, Opatrilova R. et al. Uncommon heavy metals, metalloids and their plant toxicity: A review. Environ. Chem. Lett. 2008; 6: 189–213.
18. Sinha S, Basant A, Malik A, Singh KP. Multivariate modeling of chromium-induced oxidative stress and biochemical changes in plants of *Pistia stratiotes* L. Ecotoxicology. 2009; 18: 555–566.
19. Zhang X, Liu J, Wang D, Zhu Y, Hu C, Sun J. Bioaccumulation and chemical form of chromium in *Leersia hexandra* Swartz. Bull. Environ. Contam. Toxicol. 2009; 82(3): 358–362.
20. Neema PM, Jisha KC. Physiological and biochemical responses of *Aerva lanata* (L.) Juss. ex Schult. under heavy metal stress. J. Stress Physiol. Biochem. 2020; 16*:* 67-73.
21. Nematshahi N, Lahouti M, Ganjeali A. Accumulation of chromium and its effect on growth of (*Allium cepa* cv. Hybrid). Eur. J. Exp. Biol. 2012; 2: 969–974.
22. Chandra P, Kulshreshtha K. Chromium accumulation and toxicity in aquatic vascular plants. *Bot. Rev*. 2004; 70: 313-327.
23. Kundu D, Dey S, Raychaudhuri SS. Chromium (VI) - induced stress response in the plant *Plantago ovata* Forsk *in vitro*. Genes Environ. 2018; 40: 21.
24. Muslu A, Ergün N. Effects of copper and chromium and high temperature on growth, proline and protein content in wheat seedlings. Bangladesh J. Bot. 2013; 42: 105–112.
25. Lamhamdi M, Bakrim A, Aarab A, Lafont R, Sayah F. A comparison of lead toxicity using physiological and enzymatic parameters on spinach (*Spinacia oleracea*) and wheat (*Triticum aestivum*) growth. Moroccan J. Biol. 2010; 6–7: 64–73.