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Physiological and biochemical responses of duckweed under heavy metal stress: Insights from chlorophyll, nutrient dynamics and SEM analysis

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Abstract

Heavy metal contamination in aquatic ecosystems poses a significant threat to plant health and environmental sustainability. The present study investigates the physiological and biochemical responses of two duckweed species, *Lemna minor* and *Lemna gibba*, under cadmium (Cd) and chromium (Cr) stress. Plants were exposed to varying concentrations (1–9 mg/L) of Cd and Cr for 7 and 15 days, and their responses were evaluated through chlorophyll analysis, nutrient profiling, and scanning electron microscopy coupled with energy dispersive X-ray (SEM-EDX) analysis. A concentration-dependent decline in chlorophyll *a*, chlorophyll *b*, and total chlorophyll content was observed, indicating inhibition of photosynthetic activity under heavy metal stress. Nutrient analysis revealed significant imbalances in essential macro- and micronutrients, including potassium (K), calcium (Ca), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), magnesium (Mg), and copper (Cu), suggesting disruption of nutrient uptake and metabolic processes. SEM analysis demonstrated pronounced structural damage in treated plants, while EDX confirmed the accumulation of Cd and Cr within plant tissues. Comparative analysis indicated that *Lemna minor* exhibited relatively higher tolerance, whereas *Lemna gibba* showed greater sensitivity to metal stress. Overall, the study highlights the integrated impact of heavy metals on plant physiology, linking chlorophyll degradation, nutrient imbalance, and structural alterations as key indicators of stress. These findings provide valuable insights into plant stress mechanisms and support the potential use of duckweed species as bioindicators for monitoring heavy metal pollution in aquatic environments.

1. Introduction

Heavy metal pollution in aquatic ecosystems has emerged as a serious environmental concern due to rapid industrialization, urbanization, and intensified agricultural activities. Among various heavy metals, cadmium (Cd) and chromium (Cr) are particularly hazardous because of their high toxicity, persistence, and non-biodegradable nature, which enable their accumulation in water bodies and living organisms over time (Baby *et al*., 2010; Charkiewicz *et al.,* 2023). These metals are introduced into aquatic systems through industrial effluents such as electroplating, leather tanning, textile dyeing, and chemical processing, eventually contaminating rivers, lakes, and groundwater (Murthy *et al*., 2022). Once present in aquatic environments, Cd and Cr can enter the food chain through bioaccumulation and biomagnification, posing severe risks to both ecosystems and human health.

Exposure to heavy metals such as Cd and Cr induces a wide range of physiological and biochemical alterations in aquatic plants, ultimately affecting their growth and survival (Charkiewicz *et al.*, 2023; Murthy *et al.,* 2022). One of the most prominent effects is the disruption of photosynthetic machinery, reflected by a decline in chlorophyll content, which leads to reduced photosynthetic efficiency and chlorosis (Glombitza & Reichel, 2013). Heavy metals also interfere with nutrient uptake and transport, resulting in significant imbalances in essential macro- and micronutrients such as potassium (K), calcium (Ca), iron (Fe), zinc (Zn), and manganese (Mn), thereby impairing metabolic processes and cellular functions (Siedlecka & Krupa, 1996; Wallace *et al.,* 1992). At the biochemical level, heavy metal stress triggers the generation of reactive oxygen species (ROS), leading to oxidative stress, lipid peroxidation, and damage to proteins, enzymes, and cellular membranes (Mahurpawar, 2015). Although plants activate defense mechanisms such as antioxidant systems to mitigate stress, prolonged exposure often overwhelms these protective responses, resulting in severe physiological damage.

Duckweeds (family Lemnaceae) are small, rapidly growing aquatic plants that have gained significant attention as cost-effective and eco-friendly tools for wastewater treatment and environmental remediation. Due to their high growth rate, simple structure, and remarkable ability to accumulate pollutants, duckweeds play an important role in maintaining the ecological balance of aquatic systems (Liu *et al*., 2021). In recent years, they have also emerged as model plants for studying fundamental biological processes, including photosynthesis and nutrient cycling. Additionally, their high biomass production and nutrient content make them valuable for applications such as animal feed and biofuel production. These unique characteristics highlight the potential of duckweeds as sustainable and efficient agents for the phytoremediation of heavy metal-contaminated wastewater (Zhou *et al*., 2023).

The assessment of heavy metal stress in aquatic plants requires an integrated approach that combines physiological, biochemical, and structural analyses. Chlorophyll estimation serves as a key indicator of photosynthetic efficiency and plant health, as heavy metal exposure often leads to pigment degradation and reduced photosynthetic activity. Nutrient profiling provides insights into disruptions in essential macro- and micronutrient balance, reflecting alterations in uptake and metabolic processes under stress

conditions. Furthermore, advanced imaging techniques such as scanning electron microscopy coupled with energy dispersive X-ray analysis (SEM-EDX) enable detailed visualization of surface morphology and elemental composition, revealing structural damage and metal accumulation at cellular levels. The integration of these techniques offers a comprehensive understanding of plant responses to heavy metal stress and allows for accurate evaluation of toxicity mechanisms.

Despite extensive research on the phytoremediation potential of duckweed species, most studies have primarily focused on metal removal efficiency and accumulation capacity, with limited emphasis on understanding the combined physiological, biochemical, and structural responses under heavy metal stress. In particular, studies integrating chlorophyll dynamics, nutrient imbalance, and microscopic structural alterations within a single experimental framework are scarce. Moreover, comparative analyses between different duckweed species under similar stress conditions remain insufficiently explored. Therefore, the present study aims to address this gap by investigating the physiological and biochemical responses of *Lemna minor* and *Lemna gibba* under cadmium and chromium stress using an integrated approach involving chlorophyll analysis, nutrient profiling, and SEM-EDX techniques. This comprehensive evaluation will provide deeper insights into plant stress mechanisms and support the development of efficient and sustainable phytoremediation strategies.

2. Material and Methods

2.1 Sample collection and its maintenance

Healthy fronds of *Lemna minor* and *Lemna gibba* were collected from village ponds located at Talwandi Rana (Hisar) and Dhand (Kaithal), respectively. The collected samples were thoroughly washed with tap water to remove surface impurities and subsequently surface-sterilized using 0.5% (v/v) ethanol followed by immersion in 0.5% sodium hypochlorite solution for 3 minutes. The plants were then cultured in Duckweed Nutrient Solution (DNS) prepared according to Uysal, 2010, containing essential macro- and micronutrients, and the stock solution was diluted in a 1:100 ratio with distilled water to obtain the working nutrient medium. The duckweed species were acclimatized in the nutrient medium for 2–3 days to ensure healthy growth before experimentation. All cultures were maintained under controlled laboratory conditions with a photoperiod of 16 h light and 8 h dark, at room

temperature, and pH maintained between 6.5 and 6.8.

Table 1: Composition of Duckweed Nutrient Solution (DNS)

Chemical components	mM
KMO_3	3.39
K_2HPO_4	0.073
KH_2PO_4	0.64
$CaCl_2 \cdot 2H_2O$	1.01
NH_4NO_3	0.99
$FeCl_3$	0.64
$MnCl_2$	1.97
$MgSO_4$	0.51
H_3BO_4	1.96
$Na_2MoO_4 \cdot 2H_2O$	0.030
$ZnCl_2$	0.025
$CoCl_2$	0.006
$CuCl_2$	0.0029

2.2 Experimental Design and Metal Treatment

Metal ion analysis of wastewater samples collected from different sites, including tap water, sewage from CSSRI (Karnal), sludge from CSSRI (Karnal), and the storm-drain corridor at Barwala Industrial Estate (500 m west of Delhi road junction), was performed using an Atomic Absorption Spectrophotometer (Shimadzu AA-6300, Japan) to detect metals such as Cr, Cd, Pb, Cu, Zn, and Ni. For experimental treatments, stock solutions of cadmium (Cd) and chromium (Cr) (1000 mg/L) were used to prepare five concentrations (1, 3, 5, 7, and 9 mg/L) in Duckweed Nutrient Solution (DNS). The experiments were conducted in pre-cleaned glass containers (4 L capacity) containing 2 L of nutrient medium. Fresh biomass (40 g) of *Lemna minor* and *Lemna gibba*, including both fronds and roots, was introduced into each treatment setup. The plants were monitored daily for physical changes, and water levels were maintained consistently, while the nutrient medium was renewed twice weekly to sustain optimal growth conditions. All treatments were performed in triplicates under batch mode, and average values were recorded for analysis. Samples were collected after 7 and 15 days of exposure. Control setups included containers with metal solutions but no plants, and containers with plants but without metal treatment.

2.3 Chlorophyll Estimation

Chlorophyll 'a' and 'b' contents and total chlorophyll were determined according to method given by **Arnon, 1949.**

(a) Extraction of chlorophyll: Fresh leaf tissues (100mg) were homogenized in 80% acetone in a vial and centrifuged at 5000 rpm for approximately 5min. The supernatant was transferred to a graduated tube and volume

was made 10ml with 80% acetone and it was assayed immediately.

(b) Estimation: Absorbance of supernatant was recorded at 645nm and 663nm in a UV-Visible spectrophotometer (BioMate 3S, Thermo scientific, India) against an 80% acetone (used as blank). Chlorophyll 'a' and 'b' were calculated using the equations given by Arnon, (1949).

Chl a (mg/l) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue (eq. 5)

Chl b (mg/l) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue (eq. 6)

Chl $_{Total}$ (mg/l) = Chl a + Chl b (eq. 7)

Where, A645= Absorbance at 645nm.

A663= Absorbance at 663nm.

Chl a= Chlorophyll a

Chl b= Chlorophyll b

2.4 Nutrient Analysis

2.4.1 Sample Digestion

Dried plant samples were subjected to acid digestion using a di-acid mixture of nitric acid and sulfuric acid (HNO_3:H_2SO_4; 9:1) following the method of Verma and Suthar (2015). Approximately 15 mL of the di-acid mixture was added to a known weight of oven-dried plant material and allowed to stand overnight. The samples were then heated on a hot plate with intermittent shaking until dense fumes appeared. The digestion process was repeated until a clear or nearly colourless solution was obtained. The final volume of the digested sample was reduced to approximately 2–3 mL, after which it was diluted to 50 mL with distilled water and filtered using Whatman No. 42 filter paper.

2.4.2 Micronutrient Estimation by Atomic Absorption Spectroscopy (AAS)

The concentrations of micronutrients such as magnesium (Mg), zinc (Zn), manganese (Mn), copper (Cu), and iron (Fe) were determined using an Atomic Absorption Spectrophotometer (Shimadzu AA-6300). Standard solutions (1–5 mg/L) were prepared from stock solutions, and the instrument was calibrated accordingly. The digested plant samples were analyzed, and the readings were recorded for each element. Nutrient concentrations were calculated using standard equations and expressed in mg/kg dry weight.

2.4.3 Macronutrient Estimation by Flame Photometry

The concentrations of macronutrients, including potassium (K), sodium (Na), and calcium (Ca), in plant samples were determined using a flame photometer (ELICO CL-378). Oven-dried plant samples

were digested using a di-acid mixture of nitric acid and sulfuric acid (HNO_3:H_2SO_4; 1:9, v/v). The digested samples were filtered through Whatman No. 1 filter paper and the final volume was adjusted to 50 mL with distilled water. Standard stock solutions (1000 ppm) of K, Na, and Ca were prepared using KCl, NaCl, and $CaCl_2$, respectively, and further diluted to obtain working standards in the range of 10–50 ppm for instrument calibration. The concentrations of K, Na, and Ca in the samples were then measured using the flame photometer based on the standard calibration curves.

Calculation of Nutrient Concentration

The concentration of micronutrients in plant samples was calculated using the following formula:

$$\text{Concentration (mg/kg)} = \frac{\text{Metal reading (mg/L)}}{\text{Dry weight of sample (g)}} \times \text{Final volume (mL)}$$

All reagents and chemicals used during the analysis were of analytical grade, and water samples from the nutrient medium were filtered and analyzed directly for metal content.

2.5 Scanning Electron Microscopy and EDX Analysis

Surface morphology and elemental composition of control and metal-treated plant samples were analyzed using Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray (EDX) analysis. Plant samples were washed, air-dried, and mounted on specimen stubs. The samples were then examined under SEM to observe structural changes, while EDX analysis was used to determine the elemental composition and confirm the presence of heavy metals in plant tissues.

2.6 Statistical Analysis

All experiments were conducted in triplicates, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software (version XX, IBM Corp., USA). A two-way analysis of variance (ANOVA) was applied to evaluate the effects of two independent factors, namely heavy metal concentration (1–9 mg/L) and exposure duration (7 and 15 days), on the measured physiological and biochemical parameters, including chlorophyll content and nutrient levels in *Lemna minor* and *Lemna gibba*. The interaction effects between concentration and exposure time were also assessed. Differences among treatment means were considered statistically significant at $p < 0.05$. Post hoc comparisons were performed using Tukey's test to identify significant differences between individual treatments.

3. Results and Discussion

3.1 Total chlorophyll analysis

Most remarkable physiological indication of metal toxicity is chlorosis. Chromium metal affects the photosynthetic pigments to a large extent when *Lemna minor* was treated with Cr. It was observed that concentration of chlorophyll pigments decreased with increasing metal dose and time of treatment. Chl *a,* Chl *b* and Total Chl concentration declined to 51.8 mg/g, 23.9 mg/g and 79.2 mg/g respectively for 9 mg/l of Cr after 7th day of treatment, when compared to control. In the same way Chl *a*, Chl *b* and Total Chl decreased to 38.3 mg/g, 10 mg/g and 47.8 mg/g, respectively for 9 mg/l of Cr after 15 days of treatment when compared to control. In the similar research conducted by Susplugas et al. (2000), duckweed was observed to be affected by chromate concentration when incubated for 14 days and change in chlorophyll contents was measured after 7, 10 and 14 days. During their study, they found that chromate expressed its ill effects on chlorophyll even after 7th day and pigment decreased to 75% in comparison with control plant which was nearly comparable with our results. Reale et al., (2016), also studied the effect of chromium on *Lemna minor* after 7 days of incubation with Cr and their research results were similar to current results. There was significant decrease of Chl *b* at 6 mg/l of chromium but current results also observed decrease in Chl *a* at the same Cr dose.

Table 1: Effect of Cr metal concentration on Chlorophyll content of *Lemna minor* species after 7th and 15th days of treatment

Cr Concentration (mg/l)	Chl a (After 7 days)	Chl a (after15 days)	Chl b (After 7 days)	Chl b (after15 days)	Total Chlorophyll after 7 days of experiment	Total Chlorophyll after15 days of experiment
Control	361.4	97.7	126.6	39.9	481.8±5.7	137.3±4.3
1	322.1	92.5	112.6	38.3	438.9±4.2	132.3±4.9
3	206.7	82.5	74.4	34.6	282.0±6.8	118.3±5.4
5	124.2	41.5	46.8	20.7	177.0±5.5	67.6±4.9
7	75.9	43.0	36.5	17.5	118.7±5.9	64.8±4.3
9	51.8	38.3	23.9	10.0	79.2±3.4	47.8±5.2

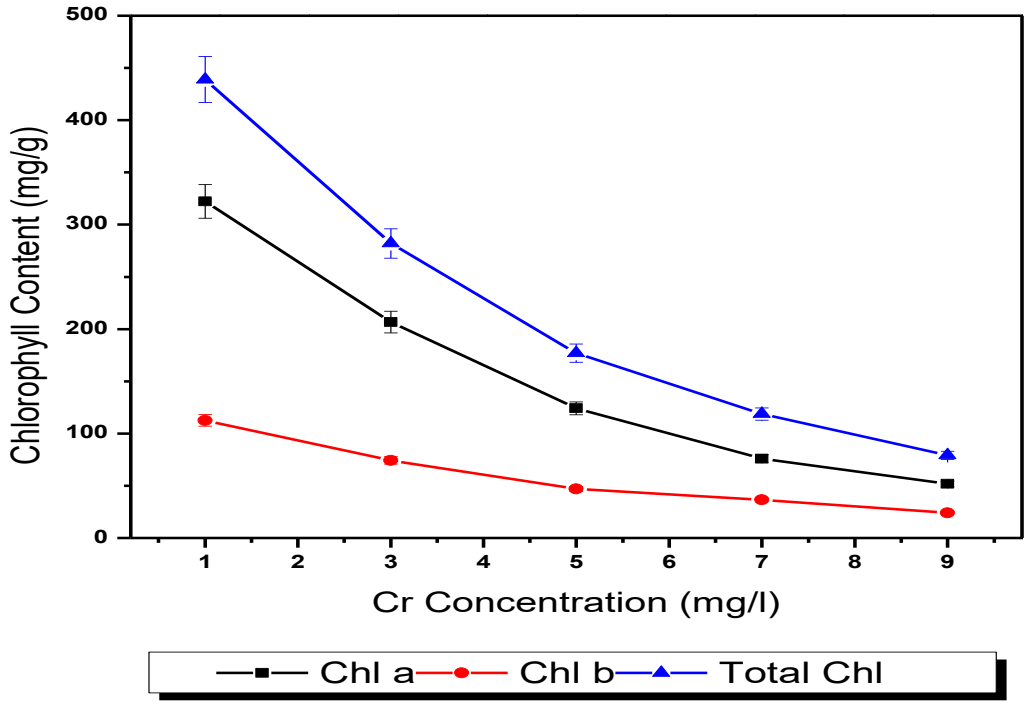


Figure 1: Effect of Cr metal concentration on Chlorophyll content of *Lemna minor* species after 7th day of treatment

3.2 Effects on macro-micronutrients of *Lemna minor and Lemna gibba* on treatment with Chromium and Cadmium metal

3.2.1 Effects on macro-micronutrients

When *Lemna minor* was treated with Cr metal (Table 2 & Table 3, Fig. 2 & Fig. 3) for 15 days of incubation period, it was noticed that Zn, Cu and Mn concentration in plant tissues was found elevated after 15 days of treatment when compared with 7 days of inoculation. On contrary to this, Fe, Na, K and Ca were observed to decrease with increase in metal exposure period. As far as effect of metal concentration was concerned, Zn concentration was maximum at 9 mg/l of Cr concentration after 7th day of treatment and similar results were noticed after 15th day of treatment. Cu concentration decreased gradually with increasing Cr concentration (except for 9 mg/l of Cr). Mn concentration in plant tissues was at its maximum of 1555 mg/kg after 7th day at 7 mg/l of concentration and similarly with maximum value i.e., 1875 mg/kg at 7 mg/l of concentration in comparison to control after 15 days. Elevated level of Fe (95 mg/kg for 7th day and 103.3 for 15th day) was also noticed at

higher Cr concentration of 9 mg/l. Initially Cr appeared to affect the sodium content but with further increase in Cr doses, there was an increase in sodium concentration with maximum value of 9733.3 mg/kg (3 mg/l) after 7 days of treatment and 5250 mg/kg (5 mg/l) after 15 days of treatment, it further declined to 1283.3 mg/kg (9 mg/l) and 1150 mg/kg (9mg/l) at high metal stress after 7th and 15th day of treatment, respectively. K concentration in plant tissues increased gradually with increased Cr dose during the experiment and attained its highest value of 15766.6 mg/kg at 3 mg/l of Cr after 7 days of treatment and 8800 mg/kg for the same dose after 15 days of treatment. Similar observation was noticed for Ca concentration as it also risen up with increased metal dose. Maximum values of 15766.6 mg/kg and 32250 mg/kg were achieved at 3 mg/l of Cr concentration after 7 days and 15 days of inoculation, respectively.

Table 2: Effects of Cr on macro and micronutrients of *Lemna minor* species after 7 days of treatment

Concentration Cr (mg/l)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	935	25	990	70	8283.33	7733.33	6233.33
1	1105.00	15.00	1130.00	30.00	5766.67	11750.00	22766.67
3	990.00	15.00	570.00	55.00	9733.33	15766.67	34216.67
5	1175.00	10.00	1105.00	25.00	8683.33	13300.00	10183.33
7	1190.00	15.00	1555.00	35.00	4233.33	12783.33	18216.67
9	1325.00	20.00	1185.00	95.00	1283.33	8250.00	18750

Table 3: Effects of Cr on macro and micronutrients of *Lemna minor* species after 15 days of treatment

Concentration Cr (mg/l)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	935	25	990	70	8283.33	7733.33	6233.33

1	1258.33	23.33	1420.00	73.33	2683.33	8700.00	20250
3	1181.67	21.67	636.67	83.33	3300.00	8800.00	32250
5	1323.33	15.00	1475.00	76.67	5250.00	5766.67	7683.333
7	1468.33	16.67	1875.00	81.67	2700.00	5733.33	13716.67
9	1421.67	25.00	1271.67	103.33	1150.00	5700.00	13733.33

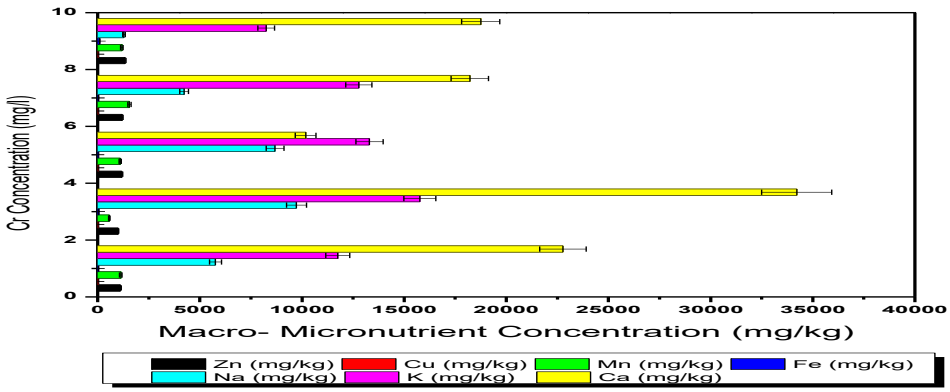


Figure 2: Effects of Cr on macro and micronutrients of *Lemna minor* species after 7 days of treatment

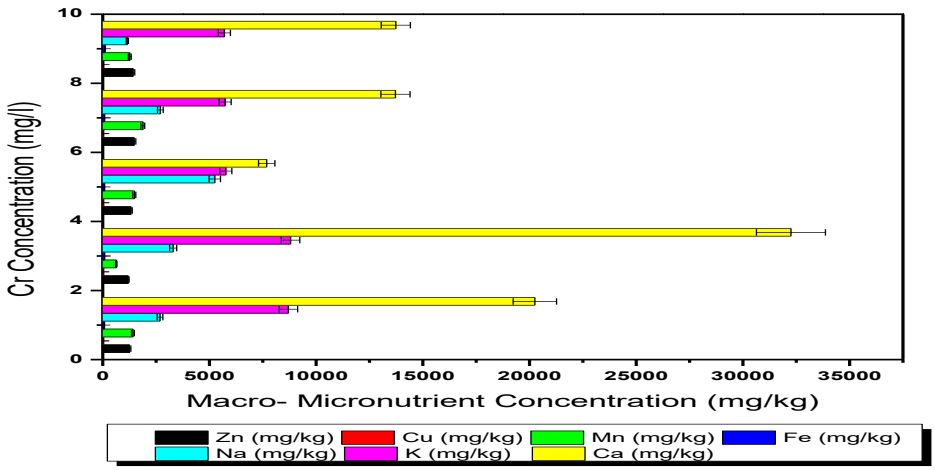


Figure 3: Effects of Cr on macro and micronutrients of *Lemna minor* species after 15 days of treatment

On treatment with Cd metal, similar results were observed for the effect of treatment period on plant nutrients as were noticed on treatment with Cr metal. Plant nutrient like Zn, Cu, Mn and Fe were increased with the increase in treatment time whereas Na, K and Ca concentration found to be decreased with increasing incubation period. Maximum Zn concentration was 1123.3 mg/kg and 1460 mg/kg at 1 mg/l of Cd

concentration for 7th and 15th day of treatment respectively. Change in Cu concentration was not gradual with increasing metal dose but showed highest value of 40 mg/kg at initial metal dose after 15 days of treatment. Mn concentration was found high (1231 mg/kg and 1276 mg/kg for 7th and 15th day, respectively) at 3 mg/l of Cd concentration which further declined with increased metal stress. Fe was found sensitive for Cd treatment as it was continuously decreasing with increasing Cd concentration in plant tissue for initial time phase (7 days) and showed its maximum concentration (98.3 mg/kg) under high metal stress after 15 days of exposure. As mentioned earlier that Cadmium, being most toxic heavy metals, is well known as the opponent of Fe. Many previous researches documented Fe deficiency with increased metal content in plant tissues (Wallace et al. 1992, Siedlecka. 1995 and Siedlecka and Krupa 1996). Besides Fe deficiency, Cd also affects growth and chlorophyll synthesis because photosynthetic electron transport mechanism possesses many Fe-containing constituents (Siedlecka and Baszynski, 1993). Maximum value (12166.7 mg/kg) of Na was documented at 7 mg/l of Cd after 7 days of treatment but it gradually started decreasing with increasing metal dose and treatment period when compared with control. K concentration was maximum (10833.3 mg/kg and 9733.3 mg/kg for 7th and 15th days of inoculation respectively) at 1 mg/l of Cd but afterwards it was found to be decreasing with increase in metal dose and treatment period. Cd treatment caused elevation in Ca concentration in plant tissues which was affected at high metal dose of 9 mg/l of Cd during the experimental period.

Table 4: Effects of Cd on macro and micronutrients of *Lemna minor* species after 7 days of treatment

Concentration (Cd) mg/l	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	935	25	990	70	8283.3	7733.3	6233.3
1	1123.3	25	958.3	23.3	6150.0	10833.3	26266.6
3	1068.3	11.6	1231.6	25	4333.3	8733.3	25600
5	925	18.3	1120	15	8766.7	7733.3	24266.6
7	951.6	11.6	1123.3	25	12166.7	7183.3	26766.6

9	1013.3	23.3	1126.6	23.3	10700.0	5666.7	7766.6

Table 5: Effects of Cd on macro and micronutrients of *Lemna minor* species after 15 days of treatment

Concentration Cd (mg/l)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	935	25	990	70	8283.3	7733.3	6233.3
1	1460	40	1171.6	40	2703.3	9733.3	25200
3	1316.6	21.6	1276.6	75	1216.7	7266.7	24683.3
5	1113.3	25	1121.6	73.3	3666.7	5700.0	22750
7	1231.6	23.3	1221.6	71.6	5233.3	6133.3	24716.6
9	351.6	25	75	98.3	5200.0	5783.3	6733.3

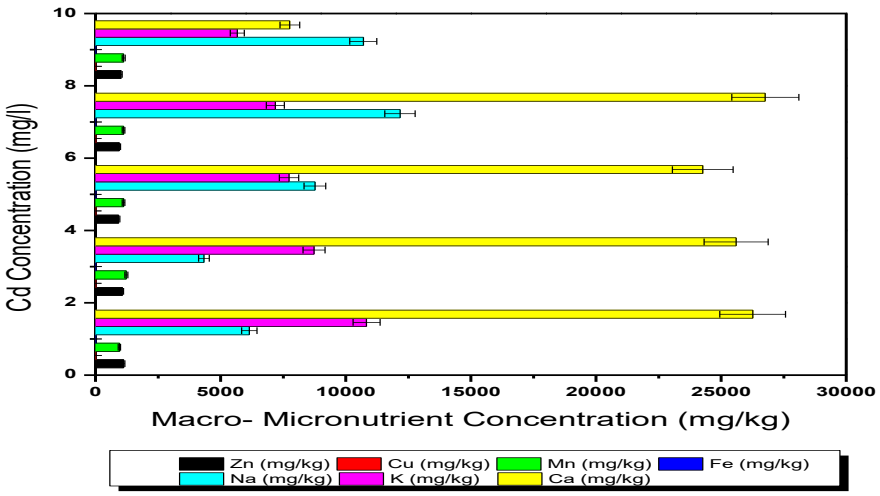


Figure 4: Effects of Cd on macro and micronutrients of *Lemna minor* species after 7 days of treatment

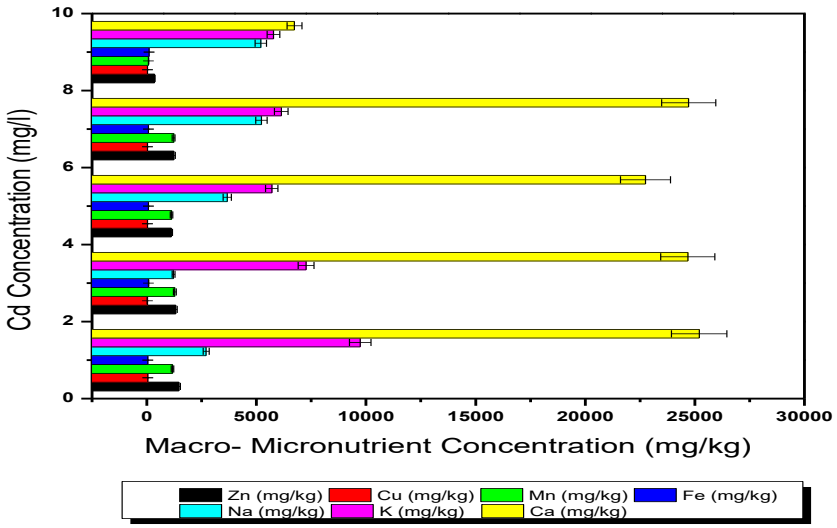


Figure 5: Effects of Cd on macro and micronutrients of *Lemna minor* species after 15 days of treatment

Lemna gibba showed decrease in Zn, Na and Mn content with increasing incubation period with Cr metal whereas

Cu, Fe, K and Ca concentrations increases with the increasing treatment period. As compared with *Lemna minor*, nutrient concentration in *Lemna gibba* was observed in lesser amount except for Ca which was nearly equal to *Lemna minor* in control treatment. Zn concentration in *Lemna gibba* was decreased to 78% and 73% of control concentration after 7th and 15th day of treatment. While Cu concentration was found maximum i.e., 25 mg/kg with increasing Cr dose at 7 mg/l after 7 days of Cr treatment and 20 mg/kg at 1 mg/l of Cr after 15 days of incubation. Mn was observed to increase many folds (12500 mg/kg at 7 mg/l) with rising Cr concentration when compared with control (131.7 mg/kg) after 7 days of treatment. However, with increase in incubation period it decreased to 28.3 mg/kg at 9 mg/l of Cr concentration. Fe concentration also risen up to 86.7 mg/kg at 7 mg/l of Cr after 7 days which further increased to 140 mg/kg at 5 mg/l of Cr concentration after 15 days of treatment. With increasing Cr concentration, Na content also risen up to 29233.3 mg/kg at 3 mg/l of Cr and it started decreasing afterwards with increasing metal stress in initial exposure time but as incubation time increased to 15 days, Na concentration started decreasing with rising Cr concentration. High K concentration of 18833.3 mg/kg and 15283.3 mg/kg was noticed at 1mg/l and 5 mg/l of Cr, respectively, which increased to 21750 mg/kg at 1 mg/l of Cr concentration after 15 days of treatment. It was noted that K content was found high in *Lemna gibba* in

comparison to *Lemna minor* for similar Cr dose. Ca concentration was observed maximum with 19300 mg/kg and 20166.7 mg/kg at 5 mg/l of Cr after 7th and 15th days respectively when compared with control.

Table 6: Effects of Cr on macro and micronutrients of *Lemna gibba* species after 7 days of treatment

Concentration Cr (mg/l)	Zn (mg/kg)	Cu (mg/kg	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	976.7	6.7	131.7	6.7	9050.0	11100.0	5750.0
1	306.7	11.7	5833.3	15.0	9700.0	18833.3	15166.7
3	368.3	10.0	5000.0	30.0	29233.3	5816.7	6766.7
5	451.7	15.0	7500.0	63.3	8650.0	15283.3	19300.0
7	465.0	25.0	12500.0	86.7	5183.3	5766.7	10716.7
9	215.0	16.7	8333.3	40.0	2283.3	5800.0	6283.3

Table 7: Effects of Cr on macro and micronutrients of *Lemna gibba* species after 15 days of treatment

Concentration Cr (mg/l)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	976.7	6.7	131.7	6.7	9050.0	11100.0	5750.0
1	406.7	20.0	826.7	75.0	9183.3	21750.0	15750.0
3	361.7	11.7	623.3	135.0	7250.0	18783.3	13800.0
5	366.7	15.0	916.7	140.0	3116.7	19166.7	20166.7
7	366.7	8.3	125.0	86.7	1333.3	5616.7	8800.0
9	270.0	6.7	28.3	85.0	450.0	5700.0	7766.7

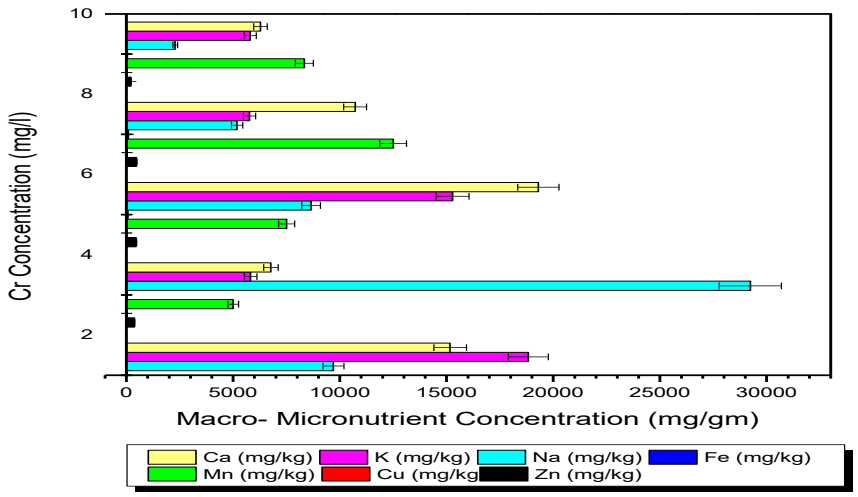


Figure 6: Effects of Cr on macro and micronutrients of *Lemna gibba* species after 7 days of treatment

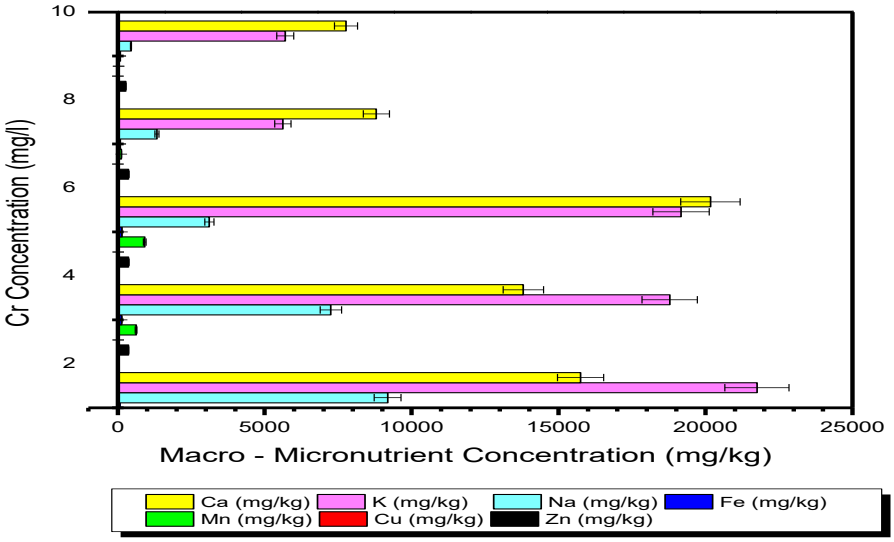


Figure 7: Effects of Cr on macro and micronutrients of *Lemna gibba* species after 15 days of treatment

While *Lemna gibba* was treated with Cadmium (Table 4.23 & Table 4.24, Fig. 4.23 & Fig. 4.24) for 15 days, it was observed that concentration of Zn, Cu, Mn, Fe and Ca content increased with increase in inoculation period in contrast to Na and K which declined in concentration with increasing treatment time. Maximum concentration of Zn (1460 mg/kg) was found at 1mg/l of Cd which risen up to 1621.7 mg/kg at 7 mg/l after 15 days. After attaining its maximum concentration (28.3 mg/kg), Cu content declined to 6.7 mg/kg at 9 mg/l after 15 days of treatment. Mn

concentration ranged between 173.3-80 mg/kg and 1081.7- 411.7 mg/kg for 7th and 15th days of treatment. Earlier research (Harrisson et al. 1983) documented that Cd uptake was inhibited by the presence of Mn. Fe concentration was noticed between 31.7 mg/kg to 8.3 mg/kg for 7 days and between 18.3 mg/kg to 63.3 mg/kg after 15 days of treatment. There was no significant difference was observed in Na concentration after 7 days of treatment but it reduced to 1233.3 mg/kg at 1 mg/l of Cd when compared with control (9050 mg/kg) after 15 days of inoculation. K concentration increased at initial Cd dose but as metal concentration was increased further during experiment, there was continuous reduction in K content. Ca concentration increases under Cd metal stress till 5 mg/l of concentration and then it showed decreasing pattern with increasing metal dose during initial treatment phase which started increasing after 7th day of inoculation period until it reached maximum concentration of 19300 mg/kg at 7 mg/l of Cd.

Table 8: Effects of Cd on macro and micronutrients of *Lemna gibba* species after 7 days of treatment

Concentration Cd (mg/l)	Zn (mg/kg)	Cu (mg/kg	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	976.7	6.7	131.7	6.7	9050.0	11100.0	5750.0
1	1460.0	11.7	173.3	8.3	8200.0	15216.7	13183.3
3	1280.0	13.3	80.0	20.0	9300.0	7766.7	13283.3
5	1278.3	15.0	171.7	15.0	9200.0	5750.0	12250.0
7	1180.0	28.3	130.0	31.7	7766.7	5766.7	11800.0
9	618.3	21.7	123.3	23.3	7283.3	5783.3	11716.7

Table 9: Effects of Cd on macro and micronutrients of *Lemna gibba* species after 15 days of treatment

Concentration Cd (mg/l)	Zn (mg/kg)	Cu (mg/kg	Mn (mg/kg)	Fe (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)
Control	976.7	6.7	131.7	6.7	9050.0	11100.0	5750.0

1	1510.0	23.3	411.7	20.0	1233.3	8683.3	13216.7
3	1520.0	20.0	621.7	30.0	1266.7	5750.0	14683.3
5	1575.0	21.7	956.7	18.3	1750.0	5850.0	14683.3
7	1621.7	8.3	1021.7	63.3	3216.7	5783.3	19300.0
9	1521.7	6.7	1081.7	21.7	1766.7	5800.0	15266.7

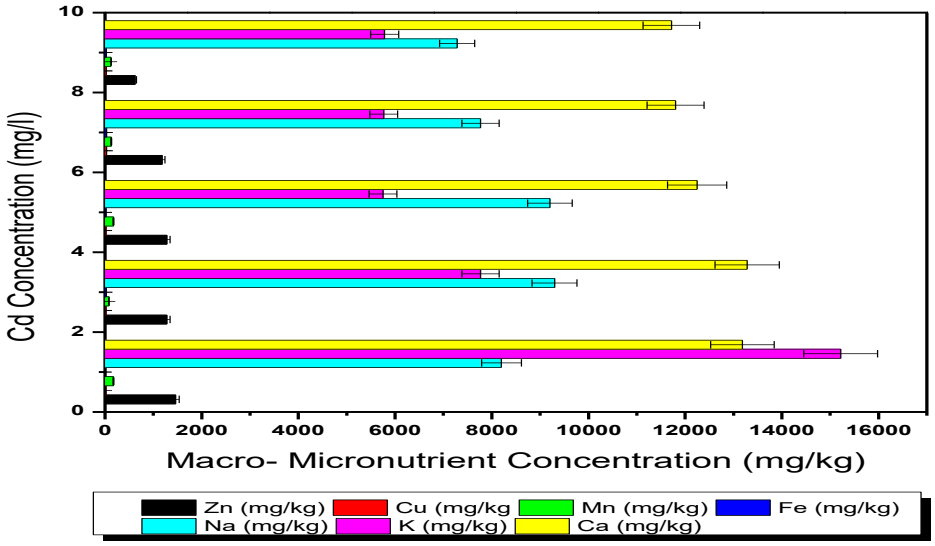


Figure 8: Effects of Cd on macro and micronutrients of *Lemna gibba* species after 7 days of treatment

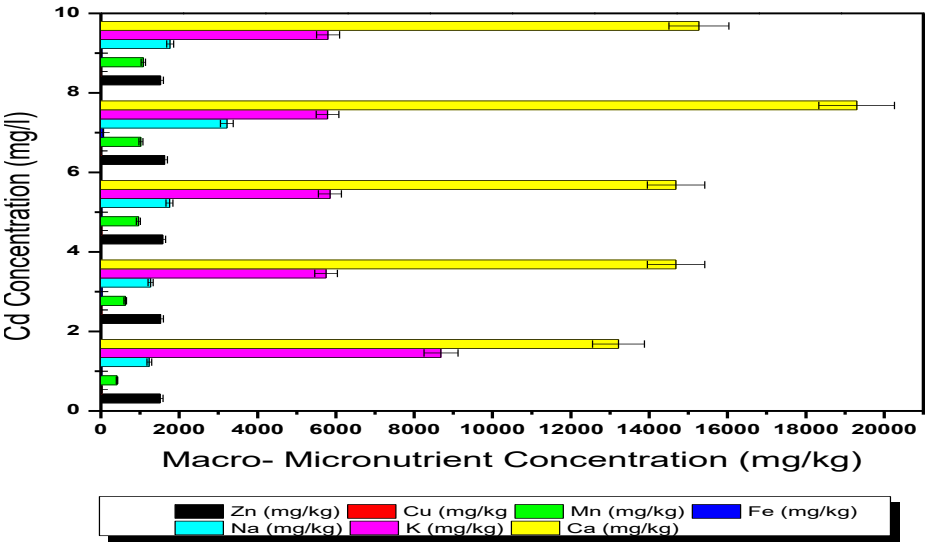
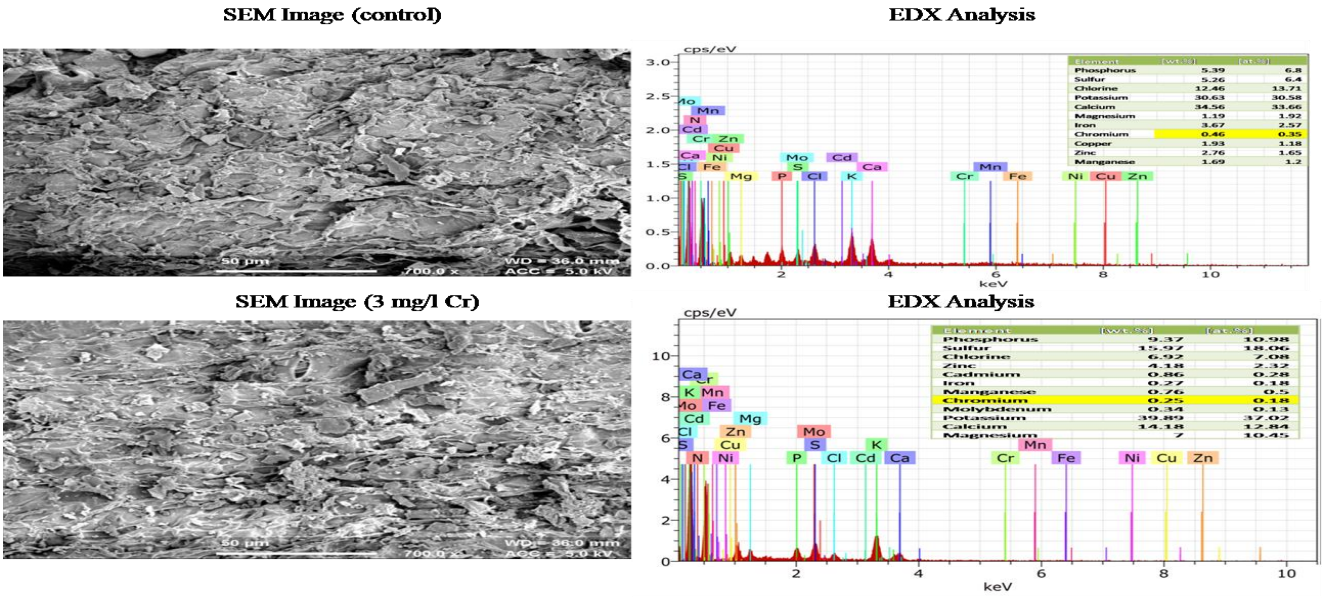


Figure 9: Effects of Cd on macro and micronutrients of *Lemna gibba* species after 15 days of treatment

3.3 Elemental analysis of Duckweed from Chromium and Cadmium metal using Scanning Electron Microscopy (SEM) - Energy Dispersive X-Ray (EDX)

Scanning Electron Microscopy (SEM) equipped with Energy Dispersive X-Ray (SEM-EDX) analysis was used to investigate the bioaccumulation of heavy metals at cellular and sub-cellular levels in plant surface at high resolution before and after incubation of *Lemna minor and Lemna gibba* with various concentrations of Cd and Cr metal.

Lemna minor analysis after treatment with chromium (Fig. 10) revealed an increase from 0.46 (wt %) and 0.35% (at %) in control to 14.23 (wt %) and 11.43 (at %) at 9 mg/l of Cr after 15 days of treatment. SEM analysis of *Lemna minor* biomass clearly displayed the morphological changes (showed by arrows) after accumulation of heavy metal ions. A homogeneous surface area was observed before adsorption of heavy metal. This plant surface appeared abnormal with a large number of open pores expressing action due to metal-stress and the edge seemed damaged (Halaimia et al 2014).



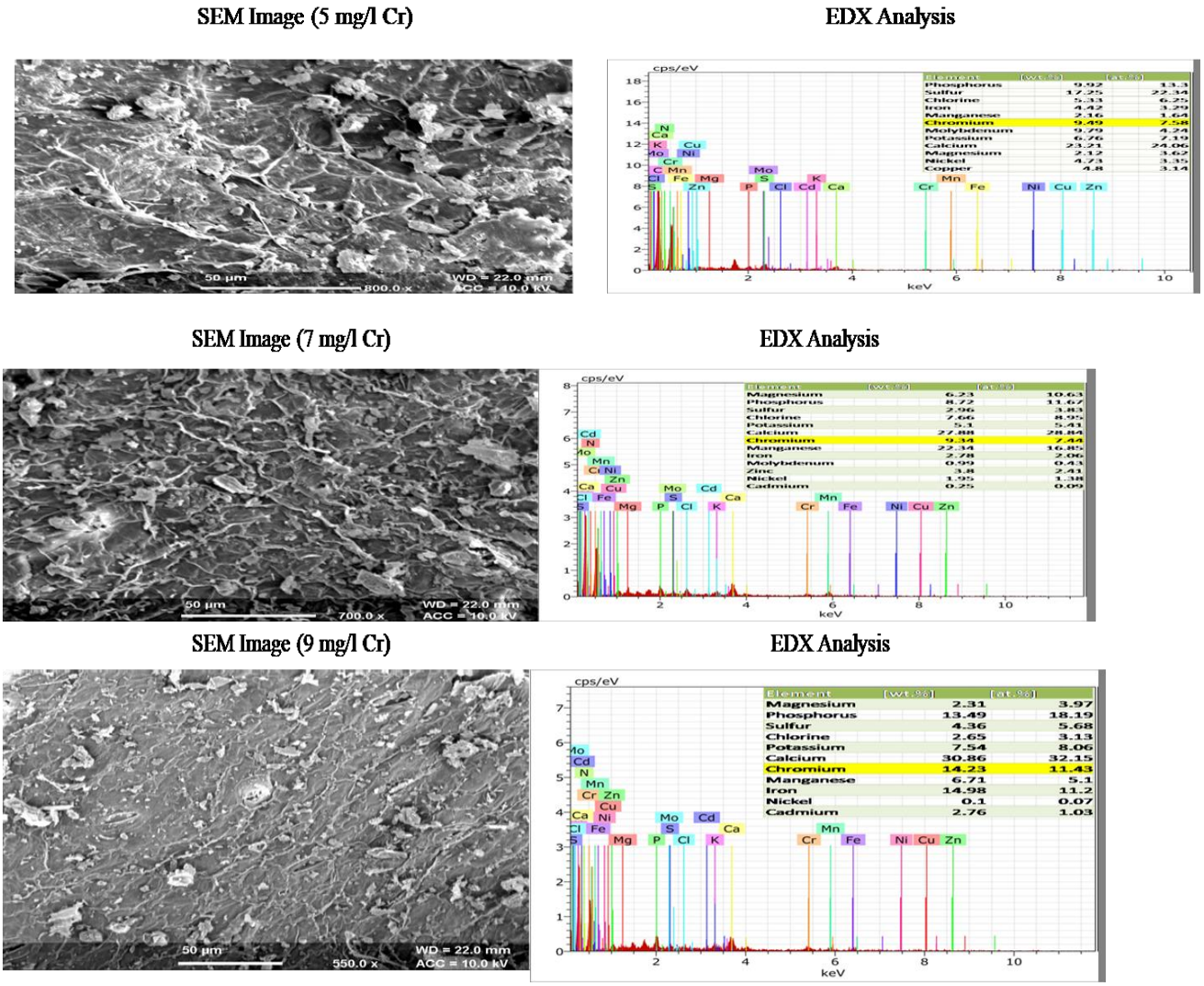
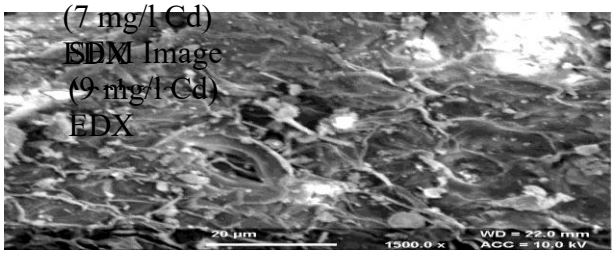
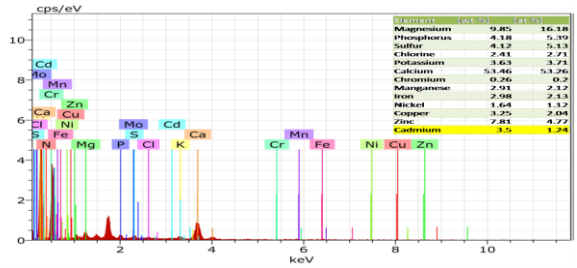


Figure 10: Effects of Cr on structural attributes of *Lemna minor* species after 15 days of treatment

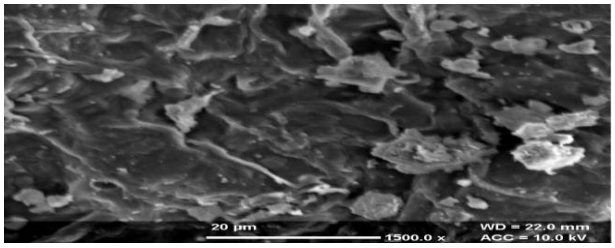
On the other hand, when this plant was experimented with Cd metal, (Fig. 11) biomass was found with 1.64 (wt %) and 0.57 (at %), 5 (wt %) and 1.63 (at %), 3.5 (wt %) and 1.24 (at %), 4.95 (wt %) and 1.64 (at %) for 3 mg/l, 5 mg/l, 7 mg/l and 9 mg/l respectively. These findings revealed the toxicity of Cd towards plant as after attainting maximum absorption at 5 mg/l of Cd, gradual decrease in Cd absorption was observed. These results also correlated with other studies (Reale *et al.,* 2016), suggesting that absorption of heavy metal like chromium and cadmium damages the plasma membrane and the proplastids failed to develop in the normal chloroplasts resulting in the ultrastructural alterations.

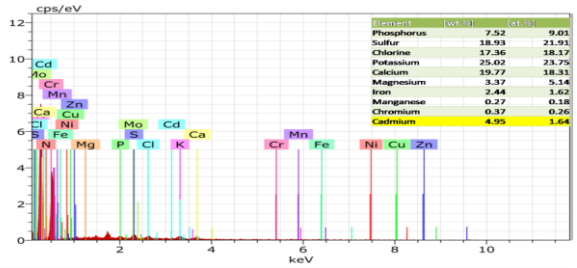
SEM Image (7 mg/l Cd)
EDX



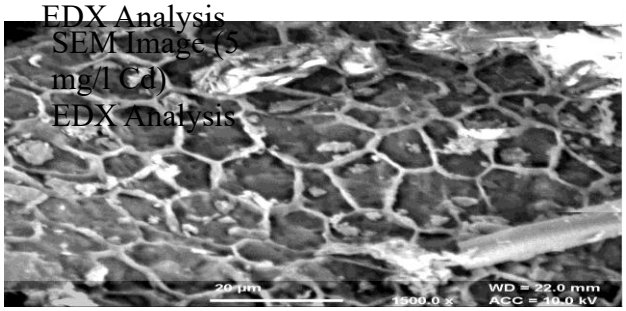


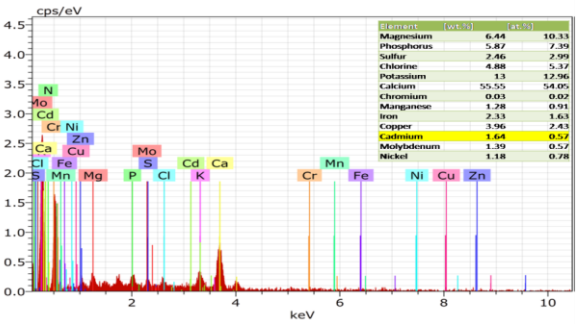
SEM Image (9 mg/l Cd)
EDX



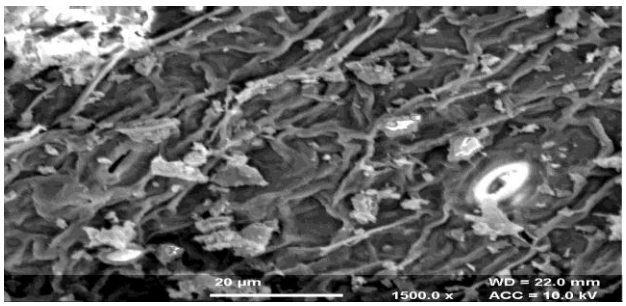


SEM Image (3 mg/l Cd)
EDX Analysis





SEM Image (5 mg/l Cd)
EDX Analysis



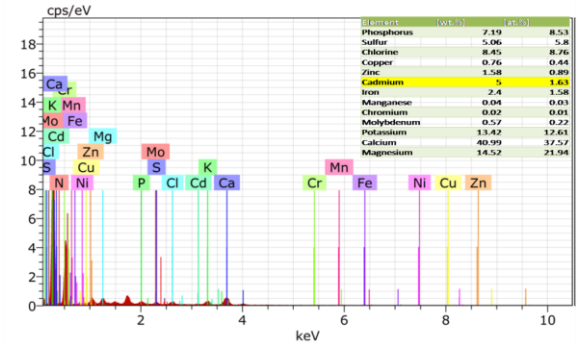
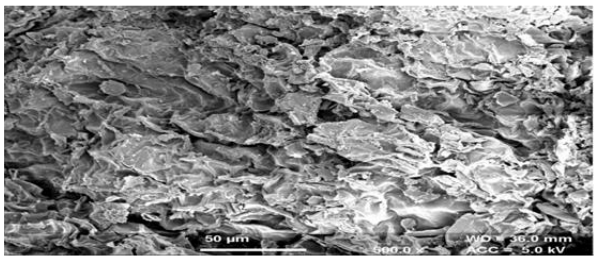


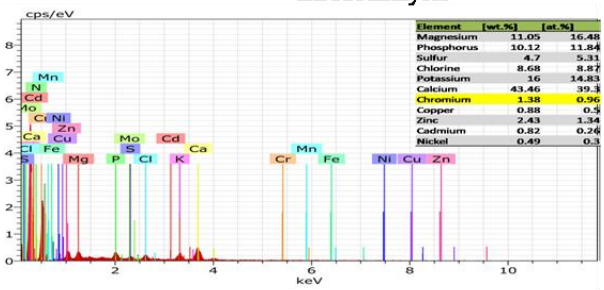
Figure 11: Effects of Cd on structural attributes of *Lemna minor* species after 15 days of treatment

When *Lemna gibba* was treated with Cr metal (Figure 12) for 15 days, fixed shape and evenness in the cell structure was found to be maintained throughout the observed surface in control and 3 mg/l Cr treated plant whereas at 9 mg/l of Cr treatment, SEM images revealed shiny white surface with damaged edge and porous structure which is an evidence that chromium metal ion have got absorbed on the surface. EDX analysis was performed to determine the elemental composition of the plant before and after the Cr metal deposition. The quantitative analysis using EDX of control plant showed 1.38 (wt %) and 1.96 (at %) of chromium which get reduced to 0.35 (wt %) and 0.25 (at %) when treated with 3 mg/l of Cr which might be due to utilization of Cr metal in various metabolic processes as explained earlier but as the Cr concentration increased, there was an increase in metal composition in plant at 9 mg/l of Cr (7.71 % and 7.15 %) as plant was almost dead at high metal stress and could not utilize the absorbed chromium

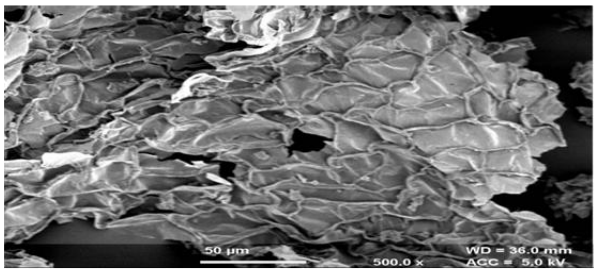
SEM Image (Control)



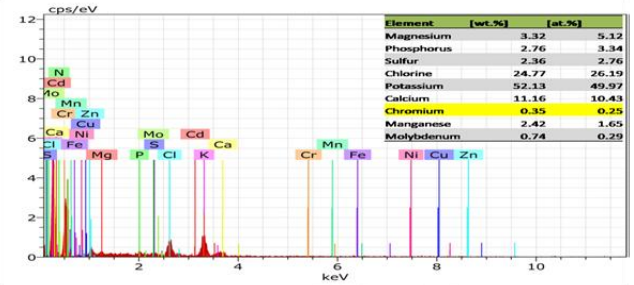
EDX Analysis



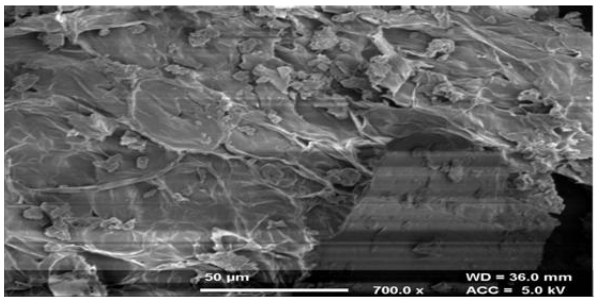
SEM Image (3 mg/l Cr)



EDX Analysis



SEM Image (9 mg/l Cr)



EDX Analysis

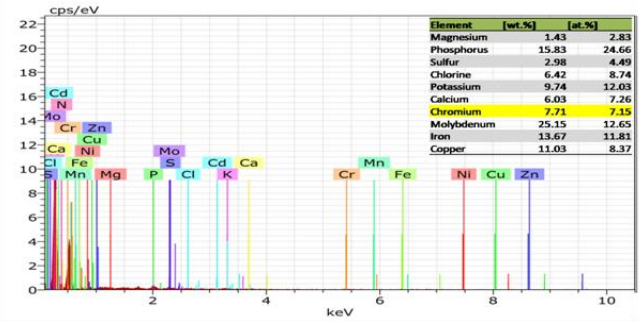


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Figure 12: Effects of Cr on structural attributes of *Lemna gibba* species after 15 days of treatment

After treatment with Cd metal, *Lemna gibba* (Fig. 13) showed a clear difference in the surface of control when compared to metal-loaded biomass samples. During the research it was observed that when plant was exposed to Cd concentration of 9 mg/l, it caused alterations in surface structure. After Cd deposition, surface was noticed heterogeneous and rough that proves, *Lemna gibba* is possessing promising metal adsorbing characteristics. EDX analysis stated that in control plant Cd was found to be 0.82 (wt %) and 0.26 (at %) which on Cd treatment of 1 mg/l and 9 mg/l, increased to 0.92 (wt %) and 0.3 (at %) to 5.23 (wt %) and 1.55 (at %) respectively. EDX also confirmed the accumulation capability of *Lemna gibba* towards cadmium metal ions.

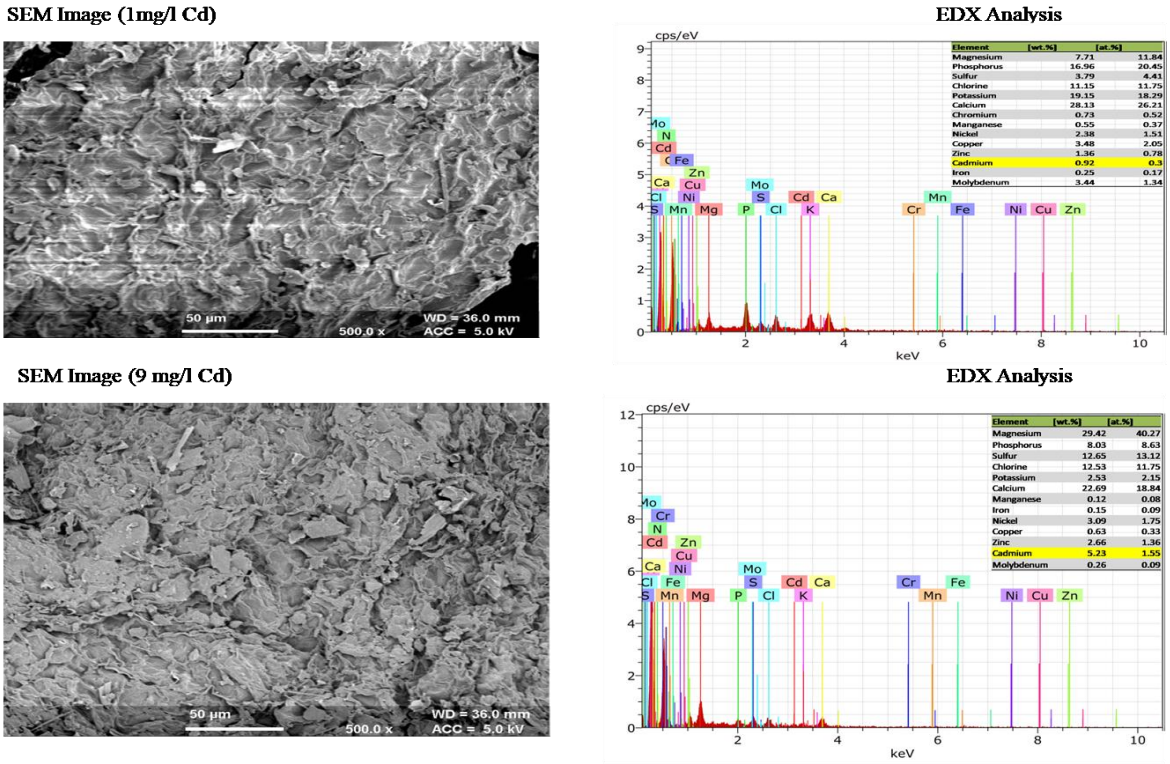

Figure 13: Effects of Cd on structural attributes of *Lemna gibba* species after 15 days of treatment

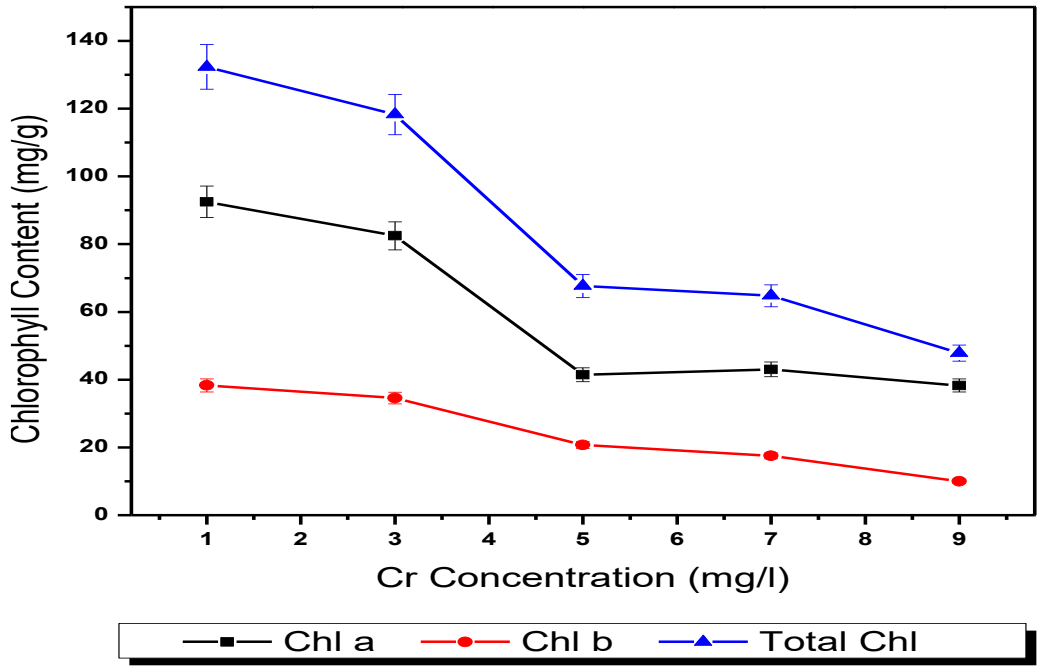

Figure 14: Effect of Cr metal concentration on Chlorophyll content of *Lemna minor* species after 15th day of treatment

On treatment with cadmium Chl *a* was increased to 394.8 mg/g at 5 mg/l of concentration after 7 days of treatment and 276.9 mg/g at 1 mg/l after 15 days of exposure. This increase in chlorophyll contents at some concentration of metal could be considered as a metal specific response which results in chlorophyll increase and synthesis of photo-synthates (Davies, 2003), while Chl *b* decreases continuously with increasing Cd concentration. Total Chl showed its maximum value of 461.6 mg/g at 5 mg/l of Cd concentration after 7 days of treatment and 375 mg/g at initial Cd concentration of experiment after 15 days of time period, which declined to 63.9 at 9 mg/l of Cd concentration. The decrease in total chlorophyll contents in high Cd levels could be considered as general responses due to elevated metal toxicity (Boswell *et al*., 2002; Vazquez *et al.*, 1987).

Table 10: Effect of Cd metal concentration on Chlorophyll content of *Lemna minor* species after 7th and 15th days of treatment

Concentration Cd (mg/l)	Chl a (after 7 days)	Chl a (after 15 days)	Chl b (after 7 days)	Chl b (after 15 days)	Total Chlorophyll after 7 days of experiment	Total Chlorophyll after15 days of experiment

Control	361.4	97.7	126.6	1953.7	135.3±3.6	108.2±3.2
1	322.8	276.9	110.6	101.1	438.0±4.3	375.0±3.1
3	298.7	154.1	110.7	46.8	406.1±3.3	205.1±4.2
5	394.8	111.0	79.2	38.1	461.6±19.3	144.9±3.7
7	129.7	56.4	49.2	19.6	176.0±3.0	74.1±1.8
9	139.4	46.6	36.8	14.0	172.4	63.9±4.0

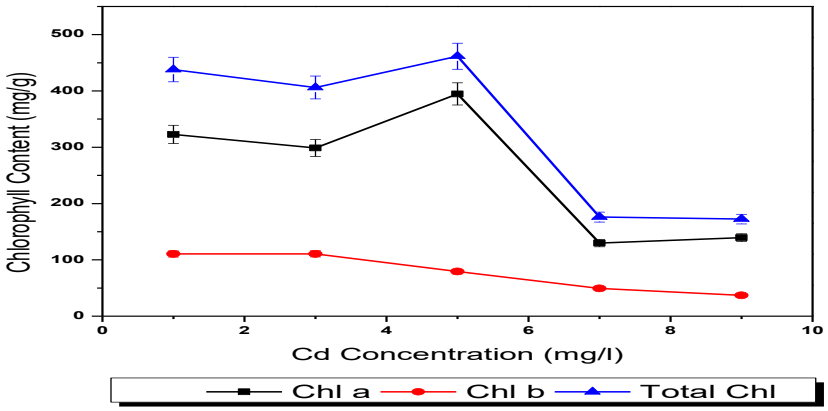

Figure 15: Effect of Cd metal concentration on Chlorophyll content of *Lemna minor* species after 7th day of treatment

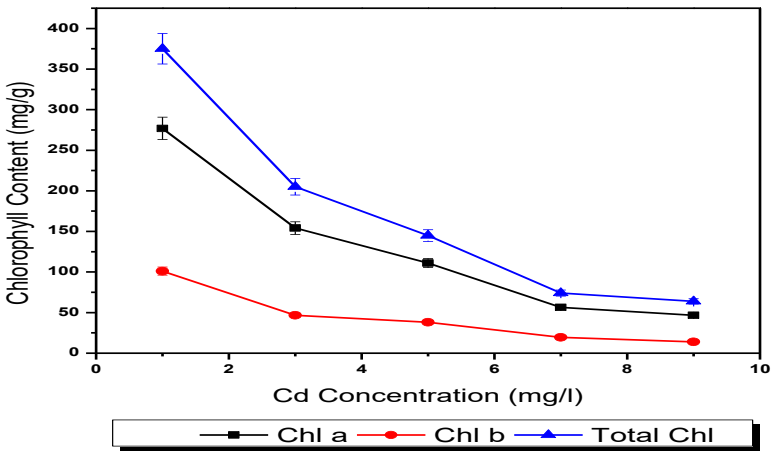

Figure 16: Effect of Cd metal concentration on Chlorophyll content of *Lemna minor* species after 15th day of treatment

On treatment with chromium metal (Table 11, Fig. 17 & Fig. 18), *Lemna gibba* expressed increase in Chl *a* till 3 mg/l of Cr concentration as compared with control after 7th day of incubation and this trend was continued after 15 days for 1mg/l of Cr concentration. As metal dose increased from 3mg/l and 1 mg/l after 7th and 15th day

respectively, Chl *a* showed a decreasing pattern. Total chlorophyll was found maximum with 252.3 mg/g value at 3 mg/l of Cr concentration after 7 days of time period which decreased to 60.5 mg/g for 9 mg/l of Cr. After 15 days of inoculation, Total chlorophyll was found to be 332 mg/g (highest value) at 1 mg/l of chromium concentration which declined to 41mg/g with increase in metal dose.

Table 11: Effect of Cr metal concentration on Chlorophyll content of *Lemna gibba* species after 7th and 15th days of treatment

Concentration Cr (mg/l)	Chl a (after 7 days)	Chl a (after 15 days)	Chl b (after 7 days)	Chl b (after 15 days)	Total Chlorophyll after 7 days of experiment	Total Chlorophyll after 15 days of experiment
Control	75.1	113.4	24.1	40.7	95.3±3.7	158.6±4.2
1	66.3	239.8	22.5	87.6	88.2±4.7	332.0±5.2
3	193.6	163.9	65.6	61.6	252.3±6.4	234.3±9.0
5	113.8	117.8	30.5	42.6	146.6±6.3	158.8±5.0
7	47.5	83.1	10.6	18.1	60.8±3.2	111.1±8.9
9	47.3	15.1	5.8	16.8	60.5±7.1	41.0±8.6

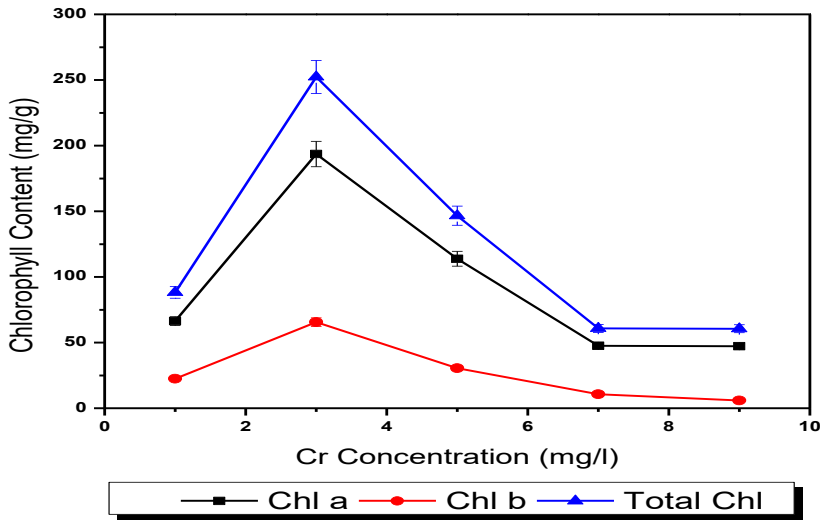

Figure 17: Effect of Cr metal concentration on Chlorophyll content of *Lemna gibba* species after 7th day of treatment

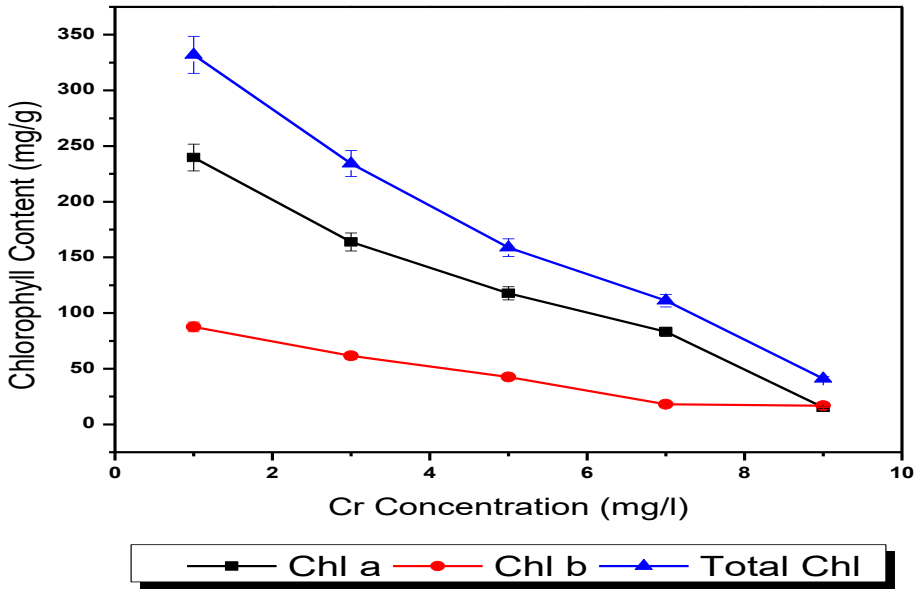

Figure 18: Effect of Cr metal concentration on Chlorophyll content of *Lemna gibba* species after 15th day of treatment

Cadmium showed detrimental effects on chlorophyll pigments of *Lemna gibba* (Table 12, & Fig. 19) when incubated for 7 days with Cd but after 15 days plant was observed with increase in Chl *a*, Chl *b* and Total Chlorophyll content for initial concentration of 1 mg/l of Cd. After attaining its maximum value, Chlorophyll content sharply decreased with increase in metal dose. Parlak & Yilmaz (2013) also noticed the decline in chlorophyll content of *Lemna gibba* after 10 mg/l of Cd. This might be due to cadmium- induced inhibition of chlorophyll biosynthesis possibly caused by induced nutrient deficiency. During their research effect on chlorophyll was found to be concentration dependent as observed in my research. A slight increase in chlorophyll content was noticed till 5 mg/l of Cd when incubated with 7 days of Cd treatment.

Table 12: Effect of Cd metal concentration on Chlorophyll content of *Lemna gibba* species after 7th and 15th days of treatment

Concentration Cd (mg/l)	Chl a (after 7 days)	Chl a (after 15 days)	Chl b (after 7 days)	Chl b (after 15 days)	Total Chlorophyll after 7 days of experiment	Total Chlorophyll after 15 days of experiment
Control	147.3	113.4	59.3	40.7	206.0±4.3	153.5±3.9
1	33.7	139.3	52.0	61.0	85.0±4.4	204.9±4.4

3	37.8	149.9	15.8	-32.0	58.5±4.4	114.9±2.7
5	32.7	100.5	13.8	-21.2	45.3±4.7	76.8±3.5
7	25.6	12.6	16.4	13.3	47.3±4.7	25.4±2.9
9	26.0	10.6	-0.3	16.3	24.9±4.4	25.8±2.4

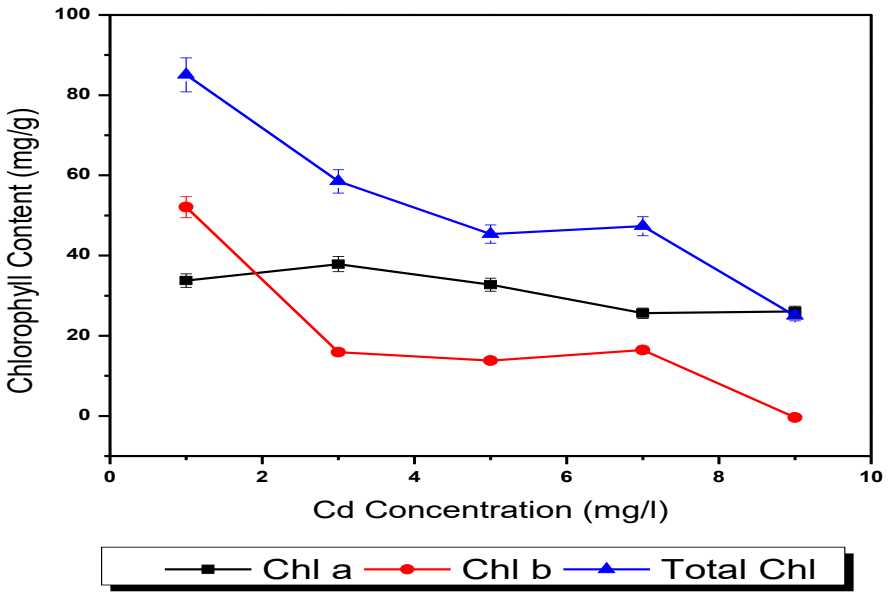

Figure 19: Effect of Cd metal concentration on Chlorophyll content of *Lemna gibba* species after 7th day of treatment

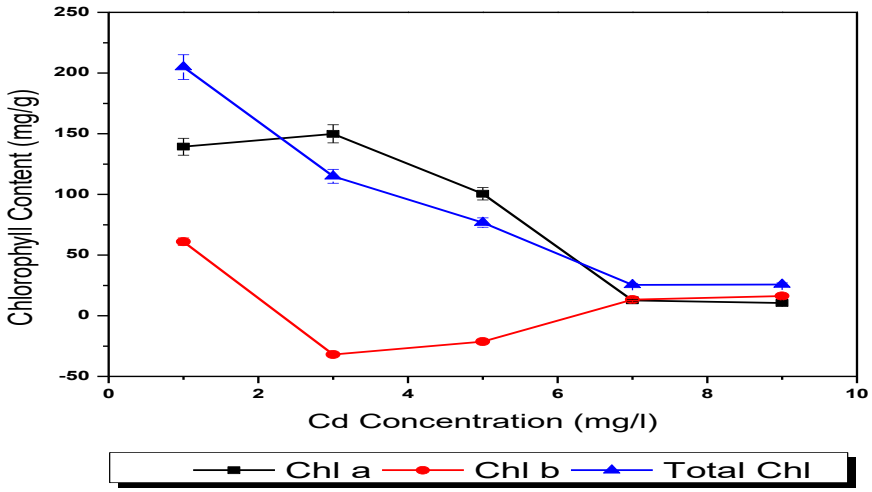

Figure 20: Effect of Cd metal concentration on Chlorophyll content of *Lemna gibba* species after 15th day of treatment

3.4 Statistical Analysis

(a) Total Chlorophyll

Total chlorophyll concentration in *Lemna minor* was affected by Cr concentration and treatment period which was clearly stated by Two- way ANOVA analysis (Table 13). Total chlorophyll concentration was found to be decreasing with increasing Cr concentration and time interval. Significant effects of Cr concentration (F value = 1851), time interval (F value = 5033) and of both factors cumulatively (F value = 686.1) were presented in Table 13.

In contrast to Cr treatment, Total chlorophyll concentration was declined with increase in time interval but risen up with increase in Cd concentration. Two-way ANOVA results also suggested the significant effect of Cd concentration (F value = 1974) and treatment period (F value = 3898) separately as well as in combined way (F value = 324.4).

Table 13: Two–Way ANOVA results for considering Cr & Cd concentration and Time interval as effect on Total Chlorophyll

ANOVA (two way)[a] P value	df[b]	F-value	P value	ANOVA (two way)[a]	df[b]	F-value
Cr concentration (*C*) ≤ 0.05	4	1851	≤ 0.05	Cd concentration (C)	4	1974
Time interval (T) ≤ 0.05	1	5033	≤ 0.05	Time interval (T)	1	3898
C × T ≤ 0.05	4	686.1	≤ 0.05	C × T	4	324.4
Total	30			Total	30	

[a] Considering Cr and Cd concentration and Time as factors in Total Chlorophyll.

[b] Error of df=29

Total chlorophyll concentration was increasing with increase in Cr concentration and time interval. Both Cr concentration (C) (F value = 157612.6) and time interval

(T) (F value = 10438.4) showed significant effect on total chlorophyll of *Lemna gibba* individually and cumulatively (C×T) (F value = 18066.8) as presented by the results of Two-way ANOVA test.

Increasing cadmium concentration was observed to be inversely proportional to total chlorophyll concentration but directly proportional to increasing time interval. Similar findings were given by Garnczarska and Ratajczak (2000) for duckweed in the form of chlorosis when treated with culture media having concentration of Pb> 1.12 mg/L. Two-way ANOVA results (Table 14) also proved the significant effect of Cd concentration (F value = 54583.8), time interval (F value = 10438.4) and combined effect of both factor (F value = 18066.8) on total chlorophyll concentration.

Table 14: Two–Way ANOVA results for considering Cr & Cd concentration and Time interval as effect on Total Chlorophyll.

ANOVA (two way)[a]	d*f*[b]	F-value	P value	ANOVA (two way)[a]	d*f*[b]	F-value	P value
Cr concentration (*C*)	4	157612.63	≤ 0.05	Cd concentration (C)	4	54583.89	≤ 0.05
Time interval (T)	1	21670.65	≤ 0.05	Time interval (T)	1	10438.40	≤ 0.05
C × T	4	72550.56	≤ 0.05	C × T	4	18066.83	≤ 0.05
Total	30			Total	30		

[a] Considering Cr and Cd concentration and Time as factors in Total Chlorophyll.

[b] Error of df=29

Both Cr concentration (Table 15) (F value = 41505.2, P value ≤ 0.05), time interval (F value = 9423.4, P value ≤ 0.05) and cumulative effect of both factor (F value = 22347.1, P value ≤ 0.05) affects Total chlorophyll concentration of *Lemna turionifera* significantly.

Similarly, Cd concentration and time interval showed significant effects on Total chlorophyll of *Lemna turionifera*. Two-way ANOVA results confirmed the effects of metal concentration (F value = 91589.6) and treatment period (F value = 523.3) as

well as collective effects of both factor (F value = 4351.8) on Total chlorophyll.

Table 15: Two–Way ANOVA results for considering Cr & Cd concentration and Time interval as effect on Total Chlorophyll.

ANOVA (two way)[a]	df [b]	F-value	P value	ANOVA (two way)[a]	df [b]	F-value	P value
Cr concentration (*C*)	4	41505.272	≤ 0.05	Cd concentration (C)	4	91589.615	≤ 0.05
Time interval (T)	1	9423.496	≤ 0.05	Time interval (T)	1	523.336	≤ 0.05
C × T	4	22347.112	≤ 0.05	C × T	4	4351.829	≤ 0.05
Total	30			Total	30		

[a] Considering Cr and Cd concentration and Time as factors in Total Chlorophyll.

[b] Error of df=29

(b) Chromium and Cadmium effect on macro and micro nutrients

Two-way ANOVA analysis of *Lemna minor* (Table 16) revealed that all the nutrient except Cu (Cr treatment) and Fe (Cd treatment) affected significantly with increase in chromium and cadmium metal concentration. Time interval showed significant effect (except for Cu with Cr treatment and Zn and Mn with Cd treatment) on macro- micronutrient concentration of *Lemna minor*. Cumulative effects of both factor metal concentration and time interval, also significantly affected the concentration of all nutrients except Cu, Fe and Ca with Cr metal treatment and Cu, Mn, Fe and Na with Cd metal treatment.

Table 16: ANOVA (Two Way) of *Lemna minor* with treatment of chromium and Cadmium

Multivariate ANOVA (Two Way) [a]	df [b]	F Value (Cr)	Sig.	F Value (Cd)	Sig.
Zn Concentration (*c*)	4	158.88	0.00	13.36	0.00
Cu	4	1.68	0.19	12.21	0.00
Mn	4	1631.01	0.00	5.25	0.00

Fe	4	16.39	0.00	1.32	0.29
Na	4	1153.69	0.00	28.22	0.00
K	4	61.67	0.00	665.69	0.00
Ca	4	462.41	0.00	4187.81	0.00
Zn Time (*t*)	1	491.65	0.00	5.44	0.03
Cu	1	2.78	0.11	31.07	0.00
Mn	1	599.44	0.00	1.24	0.28
Fe	1	80.71	0.00	48.04	0.00
Na	1	2364.59	0.00	106.93	0.00
K	1	677.66	0.00	332.05	0.00
Ca	1	63.67	0.00	135.70	0.00
Zn (*c*) × (*t*)	4	17.97	0.00	9.60	0.00
Cu	4	0.48	0.75	1.64	0.20
Mn	4	48.55	0.00	5.10	0.01
Fe	4	4.76	0.01	1.29	0.31
Na	4	292.43	0.00	2.57	0.07
K	4	33.34	0.00	30.48	0.00
Ca	4	1.48	0.25	5.40	0.00
Total	30				

[a] Considering Cr and Cd concentration and Time as factors in Effect on nutrients.

[b] Error of df=29

Increasing Cr and Cd metal concentration (Table 17) affects concentration of macro-micronutrients of *Lemna gibba* except Cu for Cr treatment and Cu, Mn and Na concentration for Cd treatment as suggested by the results of Two-way ANOVA analysis. Increasing Time interval also significantly affects the concentration of all nutrients except Zn and Cu for Cr treatment and Cu for Cd treatment. Combined effects of both factors (metal concentration and Time interval) also showed significant effects on studied nutrients except Zn, Mn and Na concentration for cadmium treatment.

Table 17: ANOVA (Two Way) of *Lemna gibba* with treatment of chromium and Cadmium

Multivariate ANOVA (Two Way) [a]	df [b]	F Value (Cr)	Sig.	F Value (Cd)	Sig.
Zn	4	180.50	0.00	8.47	0.00

Concentration (*c*)					
Cu	4	2.42	0.08	0.23	0.92
Mn	4	4242.04	0.00	2.75	0.06
Fe	4	83.89	0.00	26.05	0.00
Na	4	13464.37	0.00	0.48	0.75
K	4	9764.90	0.00	1663.01	0.00
Ca	4	5856.63	0.00	12.37	0.00
Zn Time (*t*)	1	2.28	0.15	29.13	0.00
Cu	1	5.00	0.04	0.00	1.00
Mn	1	5843.51	0.00	83.52	0.00
Fe	1	704.38	0.00	23.37	0.00
Na	1	17268.05	0.00	226.75	0.00
K	1	4258.51	0.00	781.92	0.00
Ca	1	733.61	0.00	117.62	0.00
Zn (*c*) × (*t*)	4	69.29	0.00	2.72	0.06
Cu	4	8.87	0.00	5.18	0.00
Mn	4	1291.62	0.00	2.77	0.06
Fe	4	65.27	0.00	5.53	0.00
Na	4	5790.62	0.00	3.77	0.02
K	4	1615.17	0.00	475.29	0.00
Ca	4	615.18	0.00	24.30	0.00
Total	30				

[a] Considering Cr and Cd concentration and Time as factors in Effect on nutrients.

[b] Error of df=29

4. Conclusion

The present study demonstrates that exposure to cadmium (Cd) and chromium (Cr) induces significant physiological, biochemical, and structural alterations in duckweed species (*Lemna minor* and *Lemna gibba*). A concentration-dependent decline in chlorophyll *a*, chlorophyll *b*, and total chlorophyll content was observed in both species, indicating severe impairment of the photosynthetic apparatus under heavy metal stress. The reduction was more pronounced at higher concentrations and longer exposure periods (15 days), suggesting cumulative toxicity effects on

pigment synthesis and photosynthetic efficiency.

Nutrient analysis revealed substantial imbalances in both macro- and micronutrients. Essential macronutrients such as potassium (K), calcium (Ca), and sodium (Na) showed a decreasing trend with increasing metal concentration, reflecting disruption in nutrient uptake and transport mechanisms. Similarly, variations in micronutrients such as iron (Fe), zinc (Zn), manganese (Mn), magnesium (Mg), and copper (Cu) indicated interference of heavy metals with metabolic and enzymatic processes. These nutrient imbalances further contributed to physiological stress and reduced plant vitality.

SEM analysis provided clear evidence of structural damage in treated plants, including surface irregularities, tissue distortion, and loss of cellular integrity compared to control samples. The EDX analysis confirmed the accumulation of Cd and Cr within plant tissues, validating the uptake and internalization of these metals. The combined structural and elemental findings strongly support the toxic impact of heavy metals at the cellular level.

Comparative evaluation between the two species indicated differential tolerance to heavy metal stress. *Lemna minor* exhibited relatively better tolerance, maintaining higher chlorophyll content and more stable nutrient levels compared to *Lemna gibba*, which showed greater sensitivity and pronounced damage under similar conditions. This suggests species-specific adaptive mechanisms and highlights the importance of selecting suitable species for phytoremediation applications.

Overall, the study provides integrated insights into the physiological and biochemical responses of duckweed under heavy metal stress, linking chlorophyll degradation, nutrient imbalance, and structural damage as key indicators of toxicity. These findings not only enhance the understanding of plant stress mechanisms but also support the potential application of duckweed species as bioindicators and effective agents for monitoring and managing heavy metal pollution in aquatic ecosystems.

5. References

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