RESEARCH ARTICLE

Growth, Metal Accumulation Potential and Antioxidant Enzyme Responses of *Ricinus communis* L. Genotypes Exposed to Cement Industry Effluent

Ankit[1], Amit Kumar[2], Mohammad Amir[2], Kuldeep Bauddh[1*]

DOI: 10.18811/ijpen.v9i03.10

Abstract

The current study was carried out to delineate the effect of cement industry effluent on the two screened varieties of *R. communis* (Castor) viz. MSC-55 and Western-6. A number of analyses such as growth parameters, photosynthetic pigment, metal accumulation and antioxidant enzyme were performed on both the varieties. With respect to T0 (100% tap water), an increase of 86.84, 90.97 and 80.15% was noticed in the fresh weight (FW) of roots of MSC-55 variety at 30, 60 and 90 DAS, respectively, when irrigated with T2 (50% effluent+50% tap water). The results obtained significantly differed between T0 and T2 and T0 and T4 (at $p \leq 0.05$). MSC-55 variety was observed to contain an increased amount of total chlorophyll and carotenoids up to T2 concentration which declined at higher effluent concentration i.e., T4 (100% effluent). Increased concentration of Cement Industry Effluent (CIE) enhanced the generation of some antioxidant compounds like Guaiacol peroxidase (GPX) and Ascorbate peroxidase (APX) in both the varieties up to certain treatment concentrations viz. MSC-55 and Western-6. As the effluent concentration increased in both the varieties, the amount of malondialdehyde (MDA) in the leaves and roots also rose. With increasing effluent concentrations, Catalase (CAT) and Superoxide dismutase (SOD) also increased. MSC-55 variety of *R. communis* was observed to have a strong antioxidant defence system against CIE and could be recommended for cultivation during metal-contaminated industrial effluent condition.

Keywords: Wastewater Suitability, Industrial Effluents, Antioxidant Compounds, *Ricinus communis*, Heavy Metal, Metal Accumulation, Enzyme Response.

International Journal of Plant and Environment (2023); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

By 2025, climate change is expected to cause water shortages for two-thirds of the global population. With a population of 9 billion by 2050, there will be a need to increase global food production by at least 50% (Ungureanu *et al.*, 2020; Gaur and Verma, 2023; Soltani and Mellah, 2023). Reusing wastewater, such as treated effluent from industries and desalinated water, can offer a solution by alleviating freshwater scarcity and promoting circular economies (Ofori *et al.*, 2021). Furthermore, wastewater is a potential source of nutrients that can partially replace the need for fertilization, as it contains higher nutrient levels compared to regular water sources (Lahlou *et al.*, 2021). Reutilization of treated effluent as an alternative source of irrigation has been proposed in the past (Ibekwe *et al.*, 2018). However, concerns regarding health and environmental impacts arise because toxins may enter into the human food chain through edible crops such as *Raphanus sativus*, *Lycopersicon esculentum*, etc. (Gupta *et al.*, 2011; Kumbhakar *et al.*, 2023; Paschal 2023).

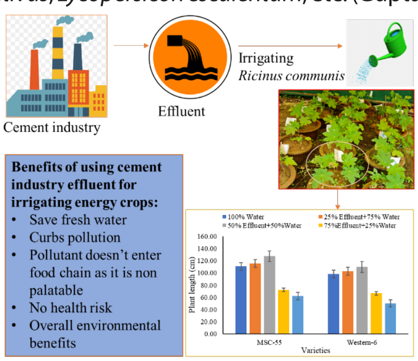

Fig. 1: Graphical abstract

Castor (*Ricinus communis*), a perennial shrub of the Euphorbiaceae family (Wang *et al.*, 2023; Cheikhyoussef and Cheikhyoussef, 2023), exhibits varying appearances and growth patterns (Alsubeie 2023). The fruit is a large, spherical, toxic seed with brown speckles. Castor is a tropical plant widely distributed worldwide, commonly known as castor bean, and predominantly cultivated in subtropical and tropical regions (Perdomo *et al.*, 2013). It can grow up to 12 m, but commercially cultivated cultivars typically reach 1 to 4 m in height. The plant has long-stalked leaves with deep lobes measuring 15 to 45 cm long, and terminal panicles holding inflorescences (Chakrabarty *et al.*, 2021).

Calcium carbonate, alumina, silica, and metals are present in cement industry effluent (CIE). Concrete production is a major source of pollution, and irrigating plants with CIE can

[1]Department of Environmental Sciences, Central University of Jharkhand, Ranchi, India

[2]Department of Botany, Lucknow University, Lucknow, India

***Corresponding author:** Kuldeep Bauddh, Department of Environmental Sciences, Central University of Jharkhand, Ranchi, India, Email: kuldeep.bauddh@cuj.ac.in

How to cite this article: Ankit, Kumar, A., Amir, M., Bauddh, K. (2023). Growth, Metal Accumulation Potential and Antioxidant Enzyme Responses of *Ricinus communis* L. Genotypes Subjected to Cement Industry Effluent. International Journal of Plant and Environment. 9(3), 268-276.

Submitted: 13/07/2023 **Accepted:** 27/08/2023 **Published:** 28/09/2023

cause oxidative stress due to heavy metals (HM) like lead, nickel, cadmium, and cobalt. Such stress damages membranes, pigments, proteins, and lipids in cells. Despite risks, limited studies suggest using CIE for irrigating bioenergy crops like *R. communis* may be a viable option to address freshwater scarcity, but further investigation is needed to understand the impact on the growth of plant, accumulation of metal, and antioxidant enzymes (Anjum *et al*., 2011). In light of the potential hazards posed by HMs, it is imperative to discern that CIE is ill-suited for the cultivation of edible crops. Conversely, it holds promise for the cultivation of robust bioenergy crops such as *R. communis*. In the wake of a scarcity of comprehensive investigations on industrial effluent utilization, this research endeavors to scrutinize the impact of CIE on the growth patterns, metal accumulation kinetics, and antioxidant enzyme activities in two distinct varieties of *R. communis*, MSC-55 and Western-6. Limited studies press for industrial effluent use, so this work aims to assess CIE impact on plant growth, metal accumulation, and antioxidant enzymes in *R. communis* varieties (MSC-55 and Western-6).

Materials and methods

Plant Materials and Experimental Design

Soil irrigation CIE was collected from Khalari cement factory in Khalari, Jharkhand, India. Garden soil was collected near CUJ campus, Brambe, Ranchi, Jharkhand, India. APHA (2005) standard methods were used to analyse physicochemical parameters and HM concentration. Earthen pots were procured from a local shop at Birsa chowk, Ranchi. Seeds of Western-6, MSC-55, Western-1515, Western-VJ 66, Western-27, Western sarpanch, Sai-33 and Western Mukhi varieties of *R. communis* were procured from Western Agri Seeds, Gujarat, India. Out of these 8 varieties, MSC-55 and Western-6 varieties were screened as tolerant and sensitive varieties, respectively. Chemicals were from E. Merck (India) Limited, Mumbai. Seeds were surface sterilized in 3% (v/v) formaldehyde for 5 minutes to prevent fungal infestation. For the purpose of experiment, a naturally illuminated green net house was constructed near the Department of Environmental Sciences, Central University of Jharkhand, eastern state of India. Five (T_0, T_1, T_2, T_3 and T_4) treatments were made; each comprised of three replicates. T_0 was taken as control in which tap water was used for irrigating the plants. For T_1, T_2, T_3 and T_4 different concentrations i.e., 25, 50, 75 and 100% of effluent, were used for irrigating the plants, respectively. In each replicate, 7 seeds/pot of 2 different screened varieties (MSC-55 and Western-6) of *R. communis* were sown. Seeds were irrigated routinely with different concentrations of effluent every third day. After seedling emergence, 3 plants per pot were maintained. The plants were extracted from the soil, along with their roots, and the length of both shoots and roots was measured in centimeters at 30, 60, and 90-day intervals. The FW of shoots and roots (g plant^{-1}) was quantified using electronic balance (Mettler Toledo (ME204/AO4)). The tests were performed in three replicates for each concentration along with control.

Plant Biomass Analysis

At 30, 60 and 90 DAS, the plants were taken out from pots and immersed in a bucket filled with water. Adhered soil particles were removed by thoroughly washing the plant roots. Roots and shoots were measured for their FW on electronic balance and the length (cm) of roots and shoots were also measured. Parts of the plant were subsequently kept in an oven set at 70°C till a constant weight was achieved (Bauddh and Singh, 2012a). Dried roots and shoots were then measured to determine their dry weight.

Estimation of Chlorophyll and Carotenoids

Estimation was done using the method given by Maclachlan and Zalik (1963). Using a pestle and mortar in dark, 1 g of fresh leaf was crushed in 10 ml of cold 80% (v/v) acetone/water. Leaf was then centrifuged at 10°C for 15 minutes at 5000 rpm. Chlorophyll was estimated 90 days after sowing (DAS). Upon obtaining the supernatant, OD was measured using a spectrophotometer (Shimadzu UV-1900 UV-VIS spectrophotometer). As a blank, 80% acetone was utilised.

Assay of MDA and Antioxidant Enzymes

Thiobarbituric acid (TBA) reactive substances test was used for the estimation of MDA. Homogenisation of plant material (500 mg) was done at 4°C in chilled potassium phosphate buffer (pH 7.0) containing 100 mM, along with 1% polyvinylpyrrolidone (w/v) and 0.1 mM Ethylenediaminetetraacetic acid (EDTA). The homogenate obtained was subsequently pressed via a four-layered cheesecloth, and the resulting part was centrifuged at 4°C for 15 minutes at 15,000g. The supernatant obtained from the centrifugation was used to measure the activity of various antioxidant enzymes.

Ascorbate Peroxidase (APX)

APX activity (EC 1.11.1.11) was quantified by evaluating the ascorbate oxidation rate (extinction coefficient 2.8 mM^{-1} cm^{-1}) at 290 nm. In 0.5mM sodium ascorbate, 0.1 mM EDTA, 0.1mM H_2O_2, 50 mM phosphate buffer (pH7.0) and an appropriate amount of enzyme extract were included in the 3 mL reaction mixture (Nakano and Asada 1981).

Guaiacol Peroxidase (GPX)

Activity of GPX (EC 1.11.1.7) was quantified by employing the method as per Hemeda and Klein (1990). The reaction mixture of 100 mL included 10 mL of 0.3% H_2O_2, 80 mL of 50 mM phosphate buffer with pH 6.6 and 10 mL of 1% guaiacol (v/v). Ultimately, 3 mL of the mixture of reaction included 75 mL of the enzyme extract. The rise in absorbance owing to guaiacol oxidation (extinction coefficient 26.6 mM^{-1} cm^{-1}) was observed at 470 nm.

Malondialdehyde (MDA)

Using the method by Heath and Packer (1968), the number of products of lipid peroxidation, or MDA content, in leaf samples were measured. Fresh leaves weighing 200 mg were crushed in a mortar and pestle with 0.25% 2-thiobarbituric acid (TBA) along with 10% trichloroacetic acid (TCA). After subjecting the mixture to 30 minutes of heating at 95˚C, it was swiftly cooled by an ice bath and centrifuged at 10,000 rpm for 10 minutes. The supernatant's absorbance at 532 nm was measured, and its turbidity was corrected by subtracting its absorbance obtained at 600 nm. The blank contained 0.25% TBA in a 10% TCA solution. Thus, the amount of MDA was used to quantify the quantity of lipid peroxides and oxidatively altered the plant proteins, employing an extinction coefficient of 155 $mM^{-1} cm^{-1}$ and expressing the result as m mol g^{-1} FW.

Super Oxide Dismutase (SOD)

The method by Beauchamp and Fridovich (1971) was employed to determine the SOD activity (EC 1.15.1.1). Amount of SOD was assessed by evaluating its capability to prevent the reduction of nitro blue tetrazolium (NBT) photochemically. Test tubes having 3 mL of reaction mixture (75 mM NBT, 13 mM methionine, 40 mM phosphate buffer with pH 7.8, 2 mM riboflavin, 0.1 mM EDTA and a suitable amount of enzyme extract) were kept beneath a light source and post 30 min, the absorbance was measured at 560 nm. The quantity of protein necessary to prevent a 50% initial drop in NBT under light is equal to one unit of activity.

Catalase

For quantifying the CAT (EC 1.11.1.6) activity, the process of extraction was accomplished in the buffer containing 0.3 g g^{-1} FW polyvinyl pyrrolidone (PVP), 0.1 mM EDTA, 50 mM Tris–HCl with pH 7.0 along with 1.0 mM phenylmethylsulfonyl fluoride. Method as per Aebi (1974) was used to measure the activity. 3 mL reaction mixture contained 20 mM H_2O_2, 50 mM sodium phosphate buffer with pH 7.0 and appropriate enzyme aliquot. Absorbance (240 nm) decline was recorded (molar extinction coefficient of H_2O_2 = 0.04 cm^2 mM^{-1}).

Estimation of Metals in Soil and Plants

For metal analysis, plant and soil samples were oven-dried until the weight became constant and were digested in conc. HNO_3 and $HClO_4$ in 3:1 for plants and 5:1 for soil (v/v) ratio at 70–80˚C. Increasing the temperature to 105˚C allowed the solution to evaporate until it became clear. Double distilled water was employed to maintain final known volume (Monni *et al.*, 2000; Ali *et al.*, 2002). The samples were analyzed using an atomic absorption spectrophotometer (Thermo AAS 301).

Calculation of Translocation Factor (TF)

The outcomes of this investigation were discussed using translocation factor (TF), which was calculated according to treatment concentrations (Mattina *et al.*, 2003).

$$\text{Translocation factor} = \frac{\text{C aerial}}{C\ root}$$

Where, C= metal concentration in µg/kg, in aerial part i.e., shoot.

Statistical Analysis

Data (n = 3) were statistically analyzed using one-way ANOVA and DMRT (MS Excel and SPSS) to compare the treatment and control means. The difference between treatments were considered significant at $p < 0.05$ and 0.01.

Table 1: Physicochemical analysis of CIE and Tap water (control).

Parameter	*Tap water*	*CIE*	*ISI standards (limits for discharges into inland surface waters) 1974*	*Standards for potable and irrigat ion water ISI (1983) for HMs*
pH	7.12 ± 0.71	8.09 ± 0.52	5.5-9.0	-
Odor	Odorless	Odorless	Odorless	-
Color	Colorless	Turbid	Colorless	-
TDS	59.8 ± 4.22	119.2 ± 7.24	2100.0	-
TSS	149.0 ± 12.1	222.0 ± 17.3	100	-
TS	208.8 ± 16.3	341.2 ± 22.7	-	-
DO	4.7 ± 0.19	3.1 ± 0.12	6.0	-
BOD	1.8 ± 0.21	15.6 ± 1.12	30	-
COD	7.9 ± 0.56	86 ± 2.54	-	-
Total hardness (CaCO3)	53 ± 4.21	215 ± 19.54	600	-
Ca	16 ± 0.72	49 ± 0.23	-	-
Mg	2.6 ± 0.11	20.1 ± 0.13	-	-
Alkalinity (CaCO3)	46 ± 3.12	116 ± 11.13	-	-
Cl	10 ± 0.15	18 ± 2.52	600	-
Pb	ND	0.533 ± 0.02	-	0.1
Ni	ND	0.231 ± 0.06	-	-
Cd	ND	0.161 ± 0.04	-	0.01
Co	ND	0.229 ± 0.07	-	-

The unit for each parameter is the same, mg/l except pH, color (visual) and odor (olfactory). COD= Chemical Oxygen Demand, TDS= Total dissolved solid, TS= Total solids, DO= Dissolved oxygen, TSS= Total suspended solid, BOD= Biochemical Oxygen Demand, ND = Not Detectable. The values are means of three replicates ± SD.

Table 2: Physicochemical characteristics of experimental soil

Parameter	*Value*
pH	6.02 ± 0.03
EC (mS/m)	113 ± 10.31
C organic (%)	2.51 ± 0.07
N (Kg/ha)	432 ± 32.06
P (Kg/ha)	39.3 ± 2.97
K(Kg/ha)	472 ± 30.34
Ca (meq/100g)	17.6 ± 2.52
Na (Kg/ha)	164 ± 12.53
S (mg/Kg)	13.5 ± 2.45
Zn (mg/Kg)	1.34 ± 0.06
Fe (mg/Kg)	7.0 ± 0.19
Cu (mg/Kg)	<1
Mn (mg/Kg)	13.6 ± 1.96
Cd	ND
Ni	ND
Hg	ND
Pb	ND

The values are means of three replicates ± SD. Where, ND = Not Detectable

Results and Discussion

Cement Industry Effluent Properties

The effluent's physicochemical properties indicate that all parameters, except for dissolved oxygen, were notably higher than tap water. HM concentration was found in the following order: Cd < Co < Ni < Pb, whereas, HMs were not detected in tap water (Table 1). Lower level of HM in the effluent may prove to be beneficial for the plant growth and development as some of these metals are required by plants in traces. Some metals such as Cu and Zn play crucial roles in several physiological processes, which include respiration and photosynthesis (Alloway 2012). Moreover, plants may exhibit stress responses against small amounts of HMs, which might improve their tolerance to HM pollution (Alloway 2012).

Soil Properties

Garden soil are slightly acidic (Sarkar *et al.*, 2017) with a pH of 6.02. Lower EC levels indicate low available nutrients (Robles-Aguilar et al., 2022), and very high EC levels indicate an excess of nutrients. Micronutrients such as zinc, iron, copper, manganese are vital to plants (Siddiqui *et al.*, 2022), they are well present in the soil. HMs like Cd, Ni, Hg, Pd were not detected in the soil sample (Table 2).

Effect on Root and Shoot Biomass in Response to Different Dosage of CIE

Both the roots and shoots were significantly affected due the application of CIE. At T_4 (100% effluent), the plants showed a significant decrease in the FW, whereas, at T_2 (50% effluent + 50% tap water), the plants exhibited notable increase in the fresh and dry weight (Table 3).

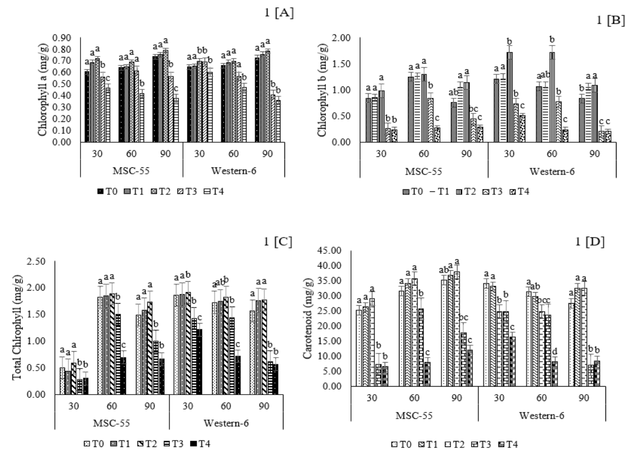

Fig. 1: Effect of different dosage of CIE on chlorophyll a [A], chlorophyll b [B], total chlorophyll [C] and carotenoid pigments [D] post 30, 60 and 90 days of sowing in MSC-55 and Western-6 varieties of R. *communis*.

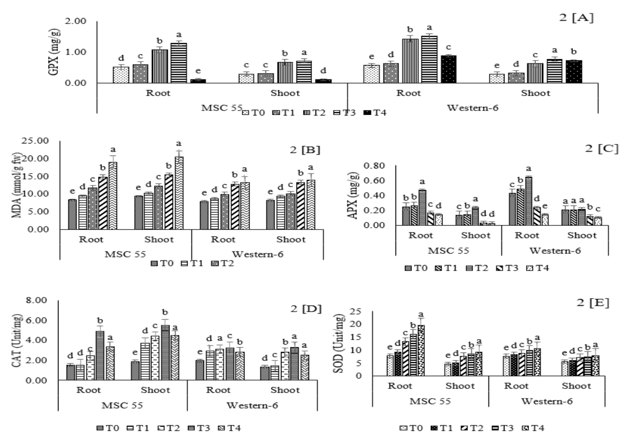

Fig. 2: Effect of different dosage of CIE on GPX [A], MDA [B], APX [C], CAT [D] and SOD [E] in MSC-55 and Western-6 varieties of R. *communis*.

Effect of CIE on Chlorophyll and Carotenoid Content

The photosynthetic pigments (total chlorophyll, chlorophyll a, b and carotenoids) increased in the leaves of MSC-55 variety plants treated with up to T_2 treatment, and declined at higher effluent concentrations. Increase in chlorophyll a was observed up to T_2 treatment; nevertheless, it declined at T_4 (Fig. 1A). The amount of chlorophyll b increased up to T_2 (Fig. 1B), maximum decline in chlorophyll b was observed at T_4. The amount of total chlorophyll in leaves also elevated up to T_2 treatment; nevertheless, it decreased at higher concentrations of effluent. The carotenoid content increased up to T2 treatment in the leaves compared to the control (Fig. 1C). The amount of carotenoid decreased in leaves of plants treated with T_4 treatment. The carotenoid content was significantly higher than chlorophyll a, b, and total chlorophyll (Fig. 1D).

Effect of CIE on MDA and Antioxidant Defence System

Antioxidant compounds such as CAT, APX, GPX, SOD, MDA contents were measured for both the varieties of *R. communis* at different concentrations of CIE (Fig. 2). Increased concentration of CIE enhanced the production of some antioxidant compounds like APX and GPX in both the varieties up to certain treatment

Table 3: Effect of different dosage of CIE on fresh weight and dry weight of MSC-55 and Western-6 variety of *R. communis* at 30. 60 and 90 days of sowing (DAS). Data are mean of three replicates ± SD. Data was analysed using one-way analysis of variance (DMRT) at $p < 0.05$. Different alphabets show significant differences between the treatments. Where, T_0 = 100% tap water; T_1= 25% effluent+75% tap water; T_2 = 50% effluent+50% tap water; T_3 = 75% effluent+25% tap water; T_4 = 100% effluent.

Treatments	*Roots*			*Shoots*		
	30 DAS	*60 DAS*	*90 DAS*	*30 DAS*	*60 DAS*	*90 DAS*
MSC-55 Fresh biomass (mg)						
T0	0.38 ± 0.02b	1.44 ± 0.52c	2.52 ± 0.54c	6.32 ± 0.48c	22.46 ± 4.29c	38.05 ± 4.07c
T1	0.60 ± 0.10c	1.37 ± 0.32c	2.59 ± 0.95c	6.97 ± 0.30c	24.56 ± 1.92d	39.83 ± 1.52d
T2	0.71 ± 0.09d	2.75 ± 0.50d	4.54 ± 0.51d	11.89 ± 0.49d	40.89 ± 2.34e	65.82 ± 2.78e
T3	0.26 ± 0.03a	0.72 ± 0.09b	1.37 ± 0.45b	4.88 ± 0.30b	16.46 ± 1.9b	28.53 ± 2.2b
T4	0.25 ± 0.02a	0.56 ± 0.11a	0.84 ± 0.21a	3.81 ± 0.13a	14.26 ± 0.57a	25.55 ± 2.06a
Western-6 Fresh biomass (mg)						
T0	0.36 ± 0.04c	1.06 ± 0.03c	2.39 ± 0.57c	5.30 ± 0.49c	15.81 ± 3.98c	23.18 ± 4.07c
T1	0.57 ± 0.05d	1.23 ± 0.35c	2.49 ± 0.95c	5.93 ± 0.27c	22.33 ± 1.88d	29.19 ± 1.11d
T2	0.64 ± 0.32e	1.97 ± 0.50d	3.98 ± 0.51d	9.63 ± 0.83d	27.92 ± 2.34e	41.90 ± 2.78e
T3	0.23 ± 0.03b	0.62 ± 0.09b	1.27 ± 0.35b	3.87 ± 0.25b	9.14 ± 1.89b	16.63 ± 2.1b
T4	0.21 ± 0.03a	0.48 ± 0.10a	0.77 ± 0.26a	2.85 ± 0.13a	6.14 ± 0.64a	12.62 ± 2.02a

Treatments	Roots			Shoots		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
MSC-55 Dry biomass (mg)						
T0	0.18 ± 0.02b	0.29 ± 0.07a	0.41 ± 0.13b	4.23 ± 0.37c	5.11 ± 0.17c	5.99 ± 0.18c
T1	0.24 ± 0.04c	0.38 ± 0.06b	0.52 ± 0.09c	5.43 ± 0.33d	6.27 ± 0.52d	7.11 ± 0.73d
T2	0.34 ± 0.04d	0.48 ± 0.08c	0.79 ± 0.13d	5.66 ± 0.19d	8.54 ± 0.78e	9.37 ± 1.41e
T3	0.11 ± 0.01b	0.26 ± 0.04a	0.41 ± 0.08b	3.09 ± 0.45b	3.80 ± 0.61b	4.51 ± 0.88b
T4	0.06 ± 0.03a	0.21 ± 0.02a	0.36 ± 0.02a	2.17 ± 0.43a	2.42 ± 0.49a	2.68 ± 0.66a
Western-6 Dry biomass (mg)						
T0	0.17 ± 0.02c	0.28 ± 0.08c	0.40 ± 0.13c	4.17 ± 0.41c	4.43 ± 0.65c	5.93 ± 0.16c
T1	0.23 ± 0.04d	0.37 ± 0.06d	0.50 ± 0.09d	5.18 ± 0.24e	6.18 ± 0.52d	6.72 ± 0.34d
T2	0.31 ± 0.04e	0.39 ± 0.08d	0.58 ± 0.10d	4.60 ± 0.26d	7.91 ± 0.78e	8.26 ± 0.87e
T3	0.10 ± 0.01b	0.25 ± 0.04b	0.40 ± 0.08b	2.96 ± 0.40b	3.61 ± 0.52b	4.48 ± 0.93b
T4	0.05 ± 0.03a	0.20 ± 0.02a	0.31 ± 0.02a	1.16 ± 0.52a	2.37 ± 0.42a	2.11 ± 0.66a

concentration viz. MSC-55 and Western-6. GPX levels in roots and shoots of MSC-55 increased up to T_3 but decreased thereafter. Similar trend was observed in Western-6 variety as well. The content of MDA and SOD in roots and leaves increased with increasing concentrations of effluent in both the varieties. CAT content increased with increasing concentration of effluent while it declined at highest effluent concentration.

Metal Accumulation in Root and Shoot of MSC-55 and Western-6

Accumulation of HMs (Pb, Ni, Cd and Co µg/kg dry weight) in shoots and roots of plants irrigated with different effluent concentrations were found below detection limits in control (T_0); Accumulation of metals in MSC-55 variety was found to be higher in roots as compared to the shoot, also the metal accumulation in root and shoot increased with increasing concentration of effluent (Fig. 3A), a similar trend was observed in Western-6 variety (Fig. 3B).

Translocation factor

The translocation factor was <1 for all metals (Pb, Ni, Cd and Co) except Co (1.22) in Western-6 variety, at all effluent concentrations. The translocation factor for all the metals had contrasting difference at T_1 and T_4 treatments (Fig. 4).

Applying CIE to the soil caused decline in the development of both the varieties of *R. communis* viz. MSC-55 and Western-6 measured as dry and fresh weight. The decline in dry and fresh biomass was observed to be more noticeable in Western-6 as compared to MSC-55. *R. communis*'s high tolerance to HMs and its potential for phytoremediation are some of the factors responsible for extensive reporting on HM toxicity as well as the mechanism of its tolerance (Bauddh & Singh 2012a, 2012b, 2014).

The amount of metal accumulated by the plants was significantly higher in MSC-55 than that of Western-6 in both shoots and roots. The plants' capacity to accumulate Cd in different tissues varies (Kato *et al*., 2010). The accumulation

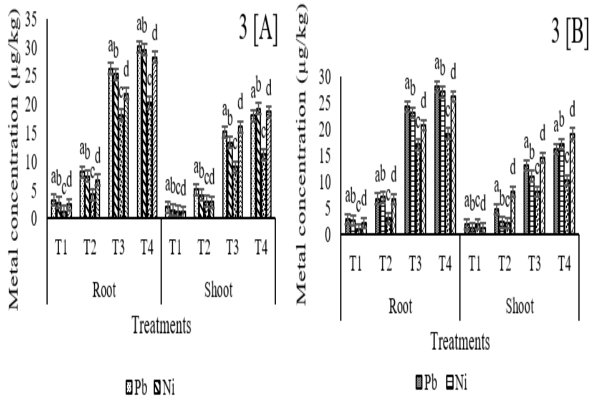

Fig. 3: Total metal accumulated in root and shoot of MSC-55 [A], Western-6 3[B] [90 DAS]. Data were analysed using one-way analysis of variance (DMRT) at $p < 0.05$. Different alphabets over the bars denote the significance of difference between the treatments

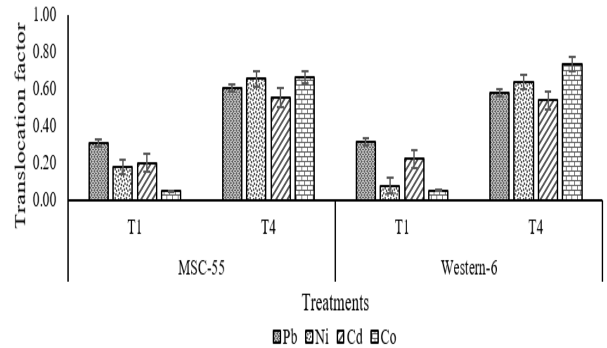

Fig. 4: Translocation factor for different metals in MSC-55 and Western-6 at T1 (25% Effluent+75% Tap water) and T4 (100% Effluent)

of metals in plants is influenced by various factors, including metal species, plant species, the genetic makeup of the plant species, the bioavailability of metals, and the prevailing climatic conditions in the cultivation area, microbial diversity in the soil (Částová *et al.*, 2009; Bauddh and Singh, 2015; Bauddh *et al.*, 2016; Saha *et al.*, 2021; Ankit *et al.*, 2022).

Application of industrial effluent for irrigation of crops is known to affect several physiological systems in plants (Chowdhry *et al.*, 2020; Tripathy *et al.*, 2022). However, few responses are indicative of the fact that if effluents are diluted sufficiently, it can bring some benefit to crop physiology (Kumar *et al.*, 2015). At decreased effluent concentrations, photosynthetic pigments were shown to be improved. When *Phaseolus radiatus* L. was subjected to tannery effluent, similar outcomes were reported (Verma and Verma, 1995), *Sonalum lycopersicum* L. plants subjected to fly ash (Khan and Khan, 1996), and tomato subjected to effluent obtained from tannery industry (Singh *et al.*, 2004). This demonstrates that response of the plant can be consistent across different effluent types. This value can be due to high uptake of nutrients, facilitated by the bioavailability of Mg and Fe and due to decrease in phenolic content in the effluent (Pandey *et al.*, 2008). At comparatively higher effluent concentrations, a decline in the photosynthetic content can be caused by the suppression of enzymatic strength involved in the generation of chlorophyll may be because of HM content in the effluents (Assche and Clijsters, 1990). Non-enzymatic antioxidants such as carotenoids guard chlorophyll pigments when plants are stressed (Kenneth *et al.*, 2000). As a defence mechanism of plants against metal stress in the current study, an increase in the amount of carotenoid at lower levels of effluent treatments may be taken into account (Singh *et al.*, 2004). Carotenoid levels decreased at higher effluent concentrations; similar outcomes were documented by Sinha *et al.*, (2007) and Singh *et al.*, (2006). This can be because of defence mechanisms being overburdened by high concentration of effluent.

One of the enzymes that can withstand stress is SOD, which may help two O_2^{-1} radicals split into O_2 and H_2O_2. H_2O_2 is also hazardous to plant cells, but CAT can eliminate it by converting it to H_2O and O_2. Hence, the interplay of SOD and CAT is essential for a plant's ability to survive environmental stress (Tripathi *et al.*, 2013). To withstand hazardous metal pollution, one of the plant's defence strategies is to produce more antioxidant enzymes (El-Beltagi *et al.*, 2010). In plant *R. communis*, the authors have already reported an elevated level of antioxidative compounds produced due to metal toxicity (Bauddh *et al.*, 2015). Metals present in the effluent led to rise in the total SOD activity of both roots and shoots of both the investigated plants, that has been documented in prior investigations (Yu *et al.*, 2013).

CAT is mostly present in peroxisomes in plants and also in glyoxysomes, whose main purpose is to eliminate H_2O_2 produced during photorespiration or fatty acid oxidation in glyoxysomes. Lowering H_2O_2 levels during cell metabolism increases CAT activity, helping overcome tissue damage. Reduced CAT activity in plant leaves suggests enzyme inactivation, degradation, or inhibition at high metal concentrations, which may impair free radical neutralization during HM exposure. Several researches (Saygideger *et al.*, 2013) also observed a decline in activity of CAT when exposure of metal to the plants was sustained. According to Pandey and Sharma (2002) decrease in CAT activity might be due to the inhibition of the production of this iron-porphyrin enzyme.

It is widely known that the Halliwell-Asada enzyme pathway in the cells of plant uses APX to scavenge H_2O_2. It uses ascorbate as an electron donor to neutralize H_2O_2 and is mostly present in cytosol, chloroplast and mitochondria. In our study, APX levels were more in the roots than in the shoots. Different authors documented an enhancement in the activity of APX in plants subjected to stress due to Cd (Yu *et al.*, 2013). Lack of Fe in the APX metalloprotein complex may explain the reduction in APX activity seen in the leaves of *R. communis* (Pandey and Sharma 2002). Scavenging phospholipid hydroperoxides and shielding cell membranes from oxidative damage appear to be the primary roles of GPXs in plants (Gueta-Dahan *et al.*, 1997). The expression of numerous GPXs is increased due to abiotic and biotic stressors, toxicity due to HM, and infection with viral pathogens or bacteria, which is consistent with these two activities (Avsian-Kretchmer *et al.*, 2004). The metal stress prompted the plants' synthesis of GPX (Gupta and Sinha 2009).

Increased effluent concentrations raised MDA levels in roots and shoots of both plant varieties, possibly due to enhanced lipid peroxidation from reactive oxygen free radicals (Shanker *et al.*, 2005; Nwaogu *et al.*, 2011). Similar results were observed in sorghum leaves (Shanker *et al.*, 2005) and tomatoes (Pandey 2007) exposed to industrial effluent. Both varieties of *R. communis* may have developed cellular and non-enzymatic antioxidant mechanisms, such as carotenoids, to cope with metal stress and toxicity. Metal toxicity-induced ROS production can disrupt plant physiological processes, leading to increased MDA generation and cell membrane damage, as observed in *B. juncea* compared to *R. communis* (Muneer *et al.*, 2011; Tripathi *et al.*, 2013).

The Pb, Ni, Cd, Co were taken up in plant shoots and roots of both the varieties of *R. communis*. Pb is a significant contaminant that is hazardous to plants at even low amounts (Kabata-Pendias and Pendias 2001). Normal plants have a maximum Pb content of 20 µg/g (Bowen 1979). The HM Cd is recognized for its ability to induce oxidative stress through the generation of free radicals (Hegedus *et al.*, 2001). With higher effluent concentrations, *R. communis* roots and shoots accumulated more Cd. The observation of cadmium (Cd) inducing oxidative stress aligns with findings from previous studies conducted by other researchers (Radotic *et al.*, 2000; Singh *et al.*, 2006). Ni in the wastewater had an impact on plant growth and chlorophyll content. According to biochemical indicators, excessive Ni concentrations adversely impacted the plant's antioxidative defence mechanism, especially in the roots (Helaoui *et al.*, 2022), which also supports our study. Exposure of plants to Co leads to cellular and subcellular damage (Mahey *et al.*, 2020). Increased ascorbate peroxidase superoxide dismutase peroxidase activities were measured in roots and leaves in response to excess Co supply in mustard plants (Sinha *et al.*, 2012).

Conclusion

Previous studies have demonstrated the proficient metal phytoremediation capabilities of *R. communis* in environments contaminated with HMs. The present investigation undertakes a comparative analysis of two distinct *R. communis* varieties, namely MSC-55 and Western-6, with respect to their physiological responses encompassing growth patterns, metal accumulation kinetics, and antioxidant enzyme activities. MSC-55 displays superior enzymatic activity in comparison to Western-6, evident across all treatment regimens. Furthermore, exposure to CIE instigates a marked surge in enzymatic activities in the roots and shoots of both varieties. MSC-55 demonstrates a pronounced propensity for enhanced metal accumulation, affirming its suitability over Western-6 for HM uptake. Although both varieties demonstrate suitability for irrigation with CIE, MSC-55 emerges as the more tolerant variety. This revelation augurs, well for prospective investigations, warranting a comprehensive assessment of CIE's impact on MSC-55's seed and oil yield potential. This avenue of research promises to unveil new dimensions in the utilization of *R. communis* for phytoremediation and agricultural applications. The work undertaken expands our understanding of plant-based remediation methods and highlights the need for continued exploration in optimizing and harnessing the phytoremediation potential of *R. communis* in future environmental conservation efforts. This work paves the way for a deeper exploration of the genetic and molecular mechanisms underlying the differential enzymatic responses observed between MSC-55 and Western-6. Additionally, investigating the potential synergistic effects of combining these varieties with other phytoremediation techniques holds promise for even more effective metal reclamation strategies.

Acknowledgments

Author Ankit is obliged to UGC, New Delhi, for awarding and financial help in form of SRF. The authors are grateful to the Department of Science and Technology-Fund for Improvement of S&T Infrastructure (DST-FIST), GoI.

Statements and Declarations

Funding All the authors disclose that there are no any personal relationships that may be perceived as influencing this work.

Conflict of Interest

None

Author contributions

Ankit conceptualised the entire research, collected data and drafted the manuscript. Amit Kumar and Mohammad Amir edited the draft. Kuldeep Bauddh supervised and critically revised the draft.

References

Aebi, H. (1974). Catalase. In: Bergmeyer H.V., Editor. In: Methods in enzymatic analysis. Vol. 2. New York (NY): Acadamic press. 673–684.

Ali, N.A., Bernal, M.P., & Ater, M. (2002). Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. *Plant Soil, 239*, 103–111.

Alloway, B.J. (2012). Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. Springer Science & Business Media, (Vol. 22), 11-50.

Alsubeie, M.S. (2023). Morphological, Genetic Characterization, and Chemical Analysis of Castor Bean (*Ricinus communis*) Growing in Riyadh Saudi Arabia, *Entomology and Applied Science Letters, 10(1)*, 38-44.

Assche, F.V., & Clijsters, H. (1990). Effects of metals on enzyme activity in plants. *Plant Cell and Environment, 13*(3), 195–206.

Avsian-Kretchmer, O., Gueta-Dahan, Y., Lev-Yadun, S., Gollop, R., & Ben-Hayyim, G. (2004). The salt-stress signal transduction pathway that activates the GPX1 promoter is mediated by intracellular H_2O_2, different from the pathway induced by extracellular H_2O_2. *Plant Physiology, 135*(3), 1685–1696.

Bauddh, K., & Singh, R.P. (2012a). Cadmium tolerance and its phytoremediation by two oil yielding plants *Ricinus communis* (L.) and *Brassica juncea* (L.) from the contaminated soil. *International Journal of Phytoremediation, 14*(8), 772–785.

Bauddh, K., & Singh, R.P. (2012b). Growth, tolerance efficiency and phytoremediation potential of *Ricinus communis* (L.) and *Brassica juncea* (L.) in salinity and drought affected cadmium contaminated soil. *Ecotoxicology and Environmental Safety, 85*, 13–22.

Bauddh, K., & Singh, R.P. (2014). Studies on bio-accumulation and partitioning of Cd in *Brassica juncea* and *Ricinus communis* in presence of vermicompost, chemical fertilizers, biofertilizers and customized fertilizers. *Ecological Engineering, 74*, 93–100.

Bauddh, K., Kumar, A., Srivastava, S., Singh, R.P., & Tripathi, R.D. (2015). A study on the effect of cadmium on the antioxidative defense system and alteration in different functional groups in castor bean and Indian mustard. *Achieves of Agronomy and Soil Science, 62*, 877-891.

Bauddh, K., Singh, K., & Singh R.P. (2016). *Ricinus communis* L. A value added crop for remediation of cadmium contaminated soil. *Bulletin of Environmental Contamination and Toxicology, 96*, 265–269.

Bauddh, K., & Singh, R.P. (2015). Assessment of metal uptake capacity of castor bean and mustard for phytoremediation of nickel containing soil. *Bioremediation Journal, 19*(2), 124–138.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry, 44*(1), 151–155.

Bowen, H.J.M. (1979). Environmental chemistry of the elements. Academic Press, New York.

Čásová, K., Černý, J., Száková, J., Balík, J., & Tlustoš, P. (2009). Cadmium balance in soils under different fertilization managements including sewage sludge application. *Plant, Soil and Environment, 55*(8), 353–361.

Chakrabarty, S., Islam, A.K.M.A., Yaakob, Z., & Islam, A.K.M.M. (2021). Castor (*Ricinus communis*): An underutilized oil crop in the South East Asia. Agroecosystems—Very Complex Environmental Systems; IntechOpen: London, UK, 61-74.

Cheikhyoussef, N., & Cheikhyoussef, A. (2023). Bioactive phytochemicals from castor (*Ricinus communis* Linneo) seed oil processing by-products. In: Ramadan Hassanien, M.F. (eds) Bioactive Phytochemicals from Vegetable Oil and Oilseed Processing By-products. Reference Series in Phytochemistry. Springer, Cham, 703-722.

Chowdhary, P., Bharagava, R.N., Mishra, S., & Khan, N. (2020). Role of industries in water scarcity and its adverse effects on environment and human health. In: Shukla, V., Kumar, N. (eds) Environmental Concerns and Sustainable Development. Springer, Singapore, 235-256.

Demiral, T., & Turkan, I. (2005). Comparative lipid peroxidation antioxidant defence systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany, 53*(3), 247–257.

El-Beltagi, H.S., Mohamed, A.A., & Rashed, M.M. (2010). Response of antioxidative enzymes to cadmium stress in leaves and roots of radish (*Raphanus sativus* L). *Notulae Scientia Biologicae, 2*(4), 76–82.

Gaur, A., & Verma, S. (2023). Impact of Climate Change on Agriculture. The Impact of Climate Change and Sustainability Standards on the Insurance Market, 193-209.

Gueta-Dahan, Y., Yaniv, Z., Zilinskas, B.A., & Ben-Hayyim, G. (1997). Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. *Planta, 203*, 460–469.

Gupta, A.K., & Sinha, S. (2009). Antioxidant response in sesame plants grown on industrially contaminated soil: effect on oil yield and tolerance to lipid peroxidation. *Bioresource technology, 100*(1), 179–185.

Gupta, N., Khan, D.K., & Santra, S.C. (2011). Heavy metal accumulation in vegetables grown in a long-term wastewater-irrigated agricultural land of tropical India. *Environmental Monitoring and Assessment, 184*(11), 6673-6682.

Heath, R.L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetic and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics, 125*(1), 189–198.

Hegedus, A., Erdei, S., & Horvath. G. (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Science, 160*(6), 1085–1093.

Helaoui, S., Hattab, S., Mkhinini, M., Boughattas, I., Majdoub, A., & Banni, M. (2022). The Effect of Nickel Exposure on Oxidative Stress of *Vicia faba* Plants. *Bulletin of Environmental Contamination and Toxicology, 108*(6), 1074-1080.

Hemeda, H.M., & Klein, B.P. (1990). Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *Journal of food Science, 55*(1), 184-185.

Ibekwe, A.M., Gonzalez-Rubio, A., & Suarez, D.L. (2018). Impact of treated wastewater for irrigation on soil microbial communities. *Science of The Total Environment, 622*, 1603–1610.

Kabata-Pendias, A., & Pendias, H. (2001). Trace elements in soils and plants. CRC Press, Washington, D.C.

Kato, M., Ishikawa, S., Inagaki, K., Chiba, K., Hayashi, H., Yanagisawa, S., & Yoneyama, T. (2010). Possible chemical forms of cadmium and varietal differences in cadmium concentrations in the phloem sap of rice plants. *Soil Science and Plant Nutrition, 56*(6), 839-847.

Kenneth, E., Pallet, K.E., & Young, J. (2000). Carotenoids. In: Antioxidants in higher plants (eds.) G.A. Ruth and J.L. Hess, CRC Press, Boca Raton. 30–81.

Khan, M.R., & Khan. M.W. (1996). The effect of fly-ash on plant growth and yield of tomato. *Environmental Pollution, 92*(2), 105–111.

Kumar, N., Kumar, S., Bauddh, K., Dwivedi, N., Shukla, P., Singh, D.P., & Barman, S.C. (2015). Toxicity Assessment and Accumulation of Metals in Radish Irrigated with Battery Manufacturing Industry Effluent *International Journal of Vegetable Science, 21*(4), 373-385.

Kumbhakar, S.K., Chauhan, R., Jadhav, S.K., & Quraishi, A. (2023). Lead induced-toxicity in vegetables, its mitigation strategies, and potential health risk assessment: a review. *International Journal of Environmental Science and Technology, 20*(5), 5773-5798.

Lahlou, F.Z., Mackey, H.R., & Al-Ansari, T. (2021). Wastewater reuse for livestock feed irrigation as a sustainable practice: A socio-environmental-economic review. *Journal of Cleaner Production, 294*, 126331.

Ma, Y., Ankit, Tiwari, J., & Bauddh, K. (2022). Plant-mycorrhizal fungi interactions in phytoremediation of geogenic contaminated soils. *Frontiers in Microbiology, 13*, 843415.

Maclachlan, S., & Zalik, S. (1963). Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany, 41*(7), 1053-1062.

Mahey, S., Kumar, R., Sharma, M., Kumar, V., & Bhardwaj, R. (2020). A critical review on toxicity of cobalt and its bioremediation strategies. SN Applied Sciences 2(7), 1-12.

Monni, S., Salemaa, M., & Millar, N. (2000). Thetolerance of *Empetrum nigrum* to copper and nickel. *Environmental Pollution, 109*(2), 221–229.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology, 22*(5), 867-880.

Nwaogu, L.A., Agha, N.C., Alisi, C.S. & Ihejirika, C.E. (2011). Investigation on the effect of cassava effluent-polluted soil on germination, emergence and oxidative stress parameters of *Telferia occidentalis*. *Journal of Biological & Environmental Sciences, 1*(6), 104–111.

Ofori, S., Puškáčová, A., Růžičková, I., & Wanner, J. (2021). Treated wastewater reuse for irrigation: Pros and cons. *Science of The Total Environment, 760*, 144026.

Pandey, N., & Sharma, C.P. (2002). Effect of heavy metals CO^{2+}, Ni^{2+}, and Cd^{2+} on growth and metabolism of cabbage. *Plant Science, 163*(4), 753-758.

Pandey, R. (2007). Some effects of untreated wastewater of Bombay (India) on *Brassica* and *Spinacia oleracea*. *Agricultural Research, 12*(2), 34–41.

Pandey, S.N., Nautiyal, B.D., & Sharma, C.P. (2008). Pollution level in distillery effluent and its phytotoxic effect on seed germination and early growth of maize and rice. *Journal of Environmental Biology, 29*(2), 267.

Paschal, A. (2023). Wastewater reuse for irrigation: Investigation of heavy metals and pharmaceuticals impact on crop irrigation. 59.

Perdomo, F.A., Acosta-Osorio, A.A., Herrera, G., Vasco-Leal, J.F., Mosquera Artamonov, J.D., Millan-Malo, B., & Rodriguez-Garcia, M.E. (2013). Physicochemical characterization of seven Mexican *Ricinus communis* L. seeds and oil contents. *Biomass and Bioenergy, 48*, 17–24.

Radotic, K., Ducic, T. & Mutavdzic, D. (2000). Changes in peroxidase activity and sisozymes in spruce needless after exposure to different concentrations of cadmium. *Environmental and Experimental Botany, 44*(2), 105–113.

Robles-Aguilar, A.A., Grunert, O., Meers, E., & Jablonowski, N.D. (2022). Evaluating the Fertilising Potential of Blended Recovered Nutrients in Horticultural Growing Medium on *Viola x wittrockiana* L. *Agronomy, 12*(1), 182.

Saha, L., Tiwari, J., Bauddh, K., & Ma, Y. (2021). Recent development in microbe-plant-based bioremediation for tackling metal-polluted soils. *Frontiers in Microbiology, 12* (2021), 731723.

Saygideger, S.D., Keser, G., & Dogan, M. (2013). Effects of lead on chlorophyll content, total nitrogen, and antioxidant enzyme activities in duckweed (*Lemna minor*). *International Journal of Agriculture & Biology, 15*(1), 145–148.

Shakeel, A.A., Xiao-yu, X., Long-chang, W., Muhammad, F.S., Chen, M., & Wang, L. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research, 6*(9), 2026-2032.

Shanker, A.K., Cervantes, C. Herminia, L. & Avudainayagaam. S. (2005). Chromium toxicity of plants. *Environment international, 31*(5), 739–753.

Shanmugaraj, B.M., Chandra, H.M., Srinivasan, B., & Ramalingam, S. (2013). Cadmium induced physio-biochemical and molecular response in *Brassica juncea*. *International Journal of Phytoremediation, 15*(3), 206–218.

Siddiqui, H., Singh, P., Arif, Y., Sami, F., Naaz, R., & Hayat, S. (2022). Role of Micronutrients in Providing Abiotic Stress Tolerance. In: Khan, S.T., Malik, A. (eds) Microbial Biofertilizers and Micronutrient Availability. Springer, Cham, 115-136.

Singh, K.P., Mohan, D., Sinha, S., & Dalwani R. (2004). Impact assessment of treated/untreated waste water toxicants discharged by sewage treatment plants on health, agricultural and environmental quality in the waste water disposal area. *Chemosphere, 55*(2), 227–255.

Singh, S., Eapen, S. & D'Souza, S.F. (2006). Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. *Chemosphere, 62*(2), 233–246.

Sinha, P., Khurana, N., & Nautiyal, N. (2012). Induction of oxidative stress and antioxidant enzymes by excess cobalt in mustard. *Journal of plant nutrition, 35*(6), 952-960.

Sinha, S., Gupta, A.K., & Bhatt, K. (2007). Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: Involvement of antioxidants. *Ecotoxicology and Environmental Safety, 67*(2), 267–277.

Soltani, L., & Mellah, T. (2023). Exploring farmers' adaptation strategies to water shortage under climate change in the Tunisian semi-arid region. *Environmental Management, 71*(1), 74-86.

Tripathi, P., Tripathi, R.D., Singh, R.P., Dwivedi, S., Chakrabarty, D., Trivedi, P.K., & Adhikari, B. (2013). Arsenite tolerance in rice (*Oryza sativa* L.) involves coordinated role of metabolic pathways of thiols and amino acids. *Environmental Science and Pollution Research, 20*, 884–896.

Tripathy, A.P., Dixit, P.K., & Panigrahi, A.K. (2022). Impact of effluent of Pulp & Paper industry on the flora of river basin at Jaykaypur, Odisha, India and its ecological implications. *Environmental Research, 204*, 111769.

Ungureanu, N., Vlăduț, V., & Voicu, G. (2020). Water Scarcity and Wastewater Reuse in Crop Irrigation. *Sustainability, 12*(21), 9055.

Verma, A., & Verma, A.P. (1995). Effect of tannery effluents on seed germination and chlorophyll content in *Phaseolus radiates* L. *Journal of Industrial Pollution Control, 11*(1), 63–66.

Wang, Y., Su, S., & Song, G. (2023). Lignin Extracted from Various Parts of Castor *(Ricinus communis* L.) Plant: Structural Characterization and Catalytic Depolymerization. *Polymers, 15*(12), 2732.

Yu, F., Liu, K., Li, M., Zhou, Z., Deng, H., & Chen, B. (2013). Effects of cadmium on enzymatic and non-enzymatic antioxidative defences of rice (*Oryza Sativa* L.). *International journal of phytoremediation, 15*(6), 513–521.