The definitive publisher version is available online at https://doi.org/10.1016/j.eti.2021.101629

Uptake and mobilization of heavy metals through phytoremediation process from native plants species growing on complex pollutants: Antioxidant enzymes and photosynthetic pigments response

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Keywords:
Metal detoxification strategy
Accumulation pattern
Antioxidants
Removal efficiency
Treatment technology

A B S T R A C T

The aim of this work was to study the metal removal efficiency of *Rumex dentatus*, *Ranunculus sceleratus*, and *Cammelina benghalensis* which thrive on wastewater containing complex co-pollutants. Physico-chemical characteristic of wastewater showed high levels of biological oxygen demand (7136 mg kg^{-1}), chemical oxygen demand (26324 mg kg^{-1}), electric conductivity (1531 μS cm^{-1}), along with metals (mg kg^{-1}) such as Fe (124.65 mg kg^{-1}), Zn (56.33 mg kg^{-1}), Cu (6.34 mg kg^{-1}), Cd (9.02 mg kg^{-1}), Mn (23.64 mg kg^{-1}), Ni (6.04 mg kg^{-1}), Pb (1.20 mg kg^{-1}), Hg (1.08 mg kg^{-1}), Cr (1.31 mg kg^{-1}) and As (1.43 mg kg^{-1}) along with complex co-pollutants. All three plants reduced more than <50% of all physicochemical parameters and metal concentrations in wastewater. The chlorophyll (Ch-a, Chl-b) contents was highest in *Rumex dentatus* (5.03–6.74 mg g^{-1} fw), followed by *Ranunculus sceleratus* (5.69.00–8.03 mg g^{-1} fw), and *Cammelina benghalensis* (4.65–7.08 mg g^{-1} fw), which showed the potentiality of plants. All the plants showed antioxidant activity (U/mL), i.e., superoxide dismutase (SOD), estimate peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), and hydrogen peroxidase (H_2O_2), bioaccumulation factor, and translocation factor, which demonstrated these plants' high translocation abilities. The results offered evidence to support the potential of using native plants for phytoremediation as a novel green process to be integrated into the treatment and management of hazardous industrial wastewater.

1. Introduction

Toxic metals are released and accumulate in the ecosystem due to continuous anthropogenic activity such as metal processing, fossil fuel combustion, uncontrolled use of pesticides, and artificial chemicals (Ali et al., 2019; Gao et al., 2020;

Park et al., 2021). The high concentration of different pollutants in the environment from industrial activities causes major changes in the climate and also responsible for global warming, which ultimately impacts human and animal health (McBride and Lacan, 2018). The annual temperature cycle is impacted by the gradual warming of the earth's atmosphere, which changes the temporal and spatial plants' activities (Duan et al., 2019). Toxic metals destroy the world's largest food web because they have such a high degree of persistence that removing them without the use of physical, chemical, and biological methods is nearly impossible.

The pulp and paper industry uses billions of water each year and generates huge quantities of highly contaminated wastewater. Pulp and paper processing has grown to become one of the world's largest industries, as well as one of the most water-intensive and energy-intensive (Toczylowska-Maminska, 2017). The amount of water used in pulp and paper mills varies from 5 to 100 m^3/1 tonne of paper produced, depending on the substrate and type of paper manufactured (Doble and Kumar, 2005). The world's third-largest pollution (42%) is caused by pulp and industry wastewater from other industrial wastewater (Ashrafi et al., 2015).

Copper (Cu), lead (Pb), mercury (Hg), aluminum (Al), chromium (Cr), zinc (Zn), As (arsenic), and cadmium (Cd) are some of the hazardous heavy metals and metalloids that are reported as highly toxic contaminants (Manoj et al., 2020; El Rasafi et al., 2020; Sharma and Rath, 2021; Sharma et al., 2021e). Mercury (Hg) and its metabolites are the most toxic contaminants in the ecosystem, and they can be found in soils at different levels all over the world, as well as in pulp and paper industry wastewater (Liu et al., 2020a; Sharma et al., 2020a). Restoration of polluted land is also critical for the recovery of ecosystems and ecosystem functions, as well as the accomplishment of the United Nations Sustainable Development Goals (UN-SDGs). Given the current awareness of sustainable protection, green technologies have a lot of promise to protect the soil for mitigation programs during the United Nations Decade of Ecosystem Restoration (2021–2030). Phytoremediation is a new, low-cost, technically feasible, and environmentally sustainable technology that involves microbes associated with plants to decrease, vaporize, extract, remediate, and metals are immobilized that are toxic in contaminated areas (Eid et al., 2020a; Sharma et al., 2021b). Plant-mediated techniques are primarily concerned with plants' ability to absorb a larger amount of metals root, shoot, and leaves (Liu et al. 2020).

Phytoremediation is considered a feasible plant-mediated technique that helps in the removal of a high concentration of metals from the contaminated soil/sludge *via* different strategies (Ashraf and Asghar, 2019). The most important factors in reducing the metal concentrations include bioavailability and mobility as well as their receptor binding with organic co-pollutants (Ke and Wang, 2018). Even though heavy metal uptake and accessibility in contaminated water are typically low, particularly the time of soil at alkaline pH and correlated with other pollutants (Zhang et al., 2018). In the polluted area, the abundant growth of many indigenous plants suggested the phytoextraction concentration of different toxic chemicals and the restoration of agricultural areas (Fernandez et al., 2017), Petelka et al. (2019). Since phytoremediation employs plants and associated soil microorganisms to reduce contamination levels and harmful effects in the ecosystem (Gouda et al., 2018; Sharma et al., 2021c). The phytoremediation method is regulated by nature and the concentration of emerging contaminants, which determine metal binding and bioavailability, as well as plant characteristics. Metal speciation and environmental conditions are two other factors that affect metal absorption in plants (Kang et al., 2017).

Plants grow with the ability to withstand metals and metal(loid) stress. Their ability to resist metal stress varies depending on their biochemical pathways, which has an impact on their phytoremediation efficiency (Antoniadis et al. 2021). Photosynthetic pigments such as chlorophylls (Chl), carotenoids, and phycobilin, are important for plants and microorganisms because they activate photosynthesis, the transformation of solar energy into chemical energy (Tang et al., 2020). Hyperaccumulators plants that dissociate and accumulate the greatest amount of heavy metals are favored options for phytoremediation of polluted sites (Hou et al., 2020; Sharma et al., 2021d). A higher number of plant species are identified for metal accumulation as hyperaccumulator plants with high capacity and more than 300 Ni hyperaccumulators (Xu et al., 2020; Leong et al., 2018; Rambabu et al., 2019). Long-term phytoremediation is required to reduce the metal detoxification from the environment for sustainable development (Cameselle and Gouveia, 2019). This requires looking for hyperaccumulators plants with higher extraction yields and percentages (Bian et al., 2020). Although phytotechnology has been thoroughly researched and applied to reduce metals from ecosystems, the plants used have sometimes failed to remediate the soil due to abiotic stress caused by global warming (Nguyen-Sy et al., 2019). Furthermore, changes in the climates cause harm for crops as well as a major sustainability problem.

Processes to facilitate desorption and subsequent removal in the liquid stage are among the modern physical and chemical strategies for reducing total concentrations of hazardous heavy metals (Noyma et al., 2016; Cheng et al., 2019). Both of these can be effectively added to the water treatment process; furthermore, they necessitate a considerable amount of external capital and are, in most cases, prohibitively costly for broad application (Sarma et al., 2019). Since crystallization, drilling, and dumping of polluted soil are needed, currently integrated soil and heavy metal remediation techniques do not solve the pollution issue. Because of these factors, phytoremediation processes involving plants in the extraction of metals to clean up contaminated habitats and sites have gained more attention in recent decades. Phytoremediation is a procedure that includes the preservation or extraction of pollutants from polluted soils using a plant or its rhizosphere (Yang et al., 2020a; Sharma et al., 2021a). Since it is available at a low cost (about 5% of other alternatives) for restoring or decontaminating metal-polluted sites, Phytoremoval can be considered attractive and economically viable. As a result, discovering environmentally safe and effective wastewater treatment methods for the pulp and paper industry remains a major challenge. The use of *Rumex dentatus, Ranunculus sceleratus*, and *Cammelina benghalensis* using improving investigators to detoxify polluted water could be a more cost-effective option than traditional remediation methods. (Rodrigo and Carabal, 2020; Hidayati and Rini, 2020; Parihar et al., 2021). This study focused on the evaluation of potential selected native plants growing on the wastewater disposed site and prevention of river pollution. The information obtained will indicate the effectiveness of using native plants to remove heavy metals and its potential to use as a detoxification technology to develop a constructed wetland near the industry.

2. Material and methods

2.1. Geographical location and sample collection

For this study wastewater samples were collected from the K.R. Pulp and Paper Limited, Shahjahanpur-India. The wastewater discharged from industry form broomstick which ultimately confluence with Garra River of India. The discharge drains of pulp and paper industry wastewater samples were collected in 20-liter of sterile plastic jerrycan (Tarsons Production Pvt. Ltd., USA). Collected samples were preserved at 4 °C before analysis and during the experiments.

2.2. Selection of the plants

Some native plants grow near industrial wastewater sites, and they can resist heavy metal concentrations as well as other pollution parameters. Based on abundance and luxuriant growth three (03) native plant species were collected included *Rumex dentatus* (Plant-1)- (Polygonaceae), *Ranunculus sceleratus* (Plant-2) (Rannanculaceae), and *Cammelina benghalensis* (Plant-3) (Commelinaceae). To remove debris and adhering particles from collected plants were removed carefully and then with a 10 mmol L^{-1} calcium chloride solution.

2.3. Physico-chemical analysis

The samples were analyzed for their physico-chemical properties, including the total pollutant loads in the pulp and paper industry wastewater. A laboratory model pH meter and conductivity meter (Orion, India) were used to measuring the pH and electric conductivity (EC) of the wastewater samples, respectively. Total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen, total phenols, and ions (K^+, Na^+, and Cl^-) were measured according to the American Public Health Association's standard procedure (APHA AW. WEF., 2012) using standard analysis as described by Sharma and Rath (2021). The concentration of metals (Fe, Cu, Zn, Cd, Mn, Cr, Ni, Pb, As, and Hg) in wastewater was calculated using atomic absorption spectrophotometry (AAS-ZEEnit 700, Analytic Jena, Germany) as per the APHA standard method 3030H (APHA AW. WEF., 2012).

2.4. Estimation of photosynthetic parameters

Metal stresses have a significant impact on photosynthetic activity, the most basic and complex physiological process in all green plants, at all stages. Chl-a, Chl-b, and carotenoids are measured in plant-1, plant-2, and plant-3 from the leaf for photosynthetic pigments. 100 mg of fresh leaves were crushed in 5 ml of chilled % acetone with the help of a pestle and mortar (Arnon, 1949). After centrifuging the sample at 5,000 rpm for 10 min at 4 °C, the supernatant was collected, and the chlorophyll content was determined using a spectrophotometer. The carotenoids percentage was determined using the same procedure as above and reading analysis of the supernatants at 480 and 510 nm (Duxbury and Yentsch, 1956).

2.5. Estimation of antioxidant contents

To estimate the antioxidants enzymes, 250 mg of fresh leaves were homogenized in 3.0 mL of a 100 μM potassium phosphate buffer (pH 7.5) containing 1 mM of ethylene diamine tetra-acetic acid (EDTA) and a pinch of polyvinylpolypyrrolidone (PVP). Optical density (OD) measurements at 420 and 560 nm were used to estimate peroxidase (POD) and superoxide dismutase (SOD) (Nishikimi et al., 1972; Onsa et al., 2004). The oxidation of ascorbate in the presence of H_2O_2 was observed at 250 nm in the analysis of ascorbate peroxidase (APX) where the decrease in absorbance was measured (Nakano and Asada, 1981). The determination of catalase (CAT) at 37 °C and 240 nm activity was determined using spectrophotometric analysis. Hydrogen peroxidase (H_2O_2) content, absorbance was measure at 350 nm (Velikova et al., 2000).

2.6. Metal accumulation

Total metals were estimated in the root, shoot, and leaves of plant-1, plant-2, and plant-3 growing on the pulp and paper industry wastewater. For the removal of attached soil and dust particle from the plant root, these were thoroughly rinsed with deionized water (three times). The plant was separated into leaves, shoots, and roots, and dried to a constant mass (Sharma et al., 2020a). Dry plant biomass was crushed and homogenized with a mortar and pestle to make a fine powder. A 250 mg dried samples was digested in 3.0 mL HNO_3 (2%) and after the release of the white fume, samples were filtered through a 0.45 μM glass fiber filter. The analyses of different metals was done using an atomic absorption spectrophotometer (AAS-ZEEnit 700, Analytic Jena, Germany).

2.7. *Biological factors and accumulation rate*

2.7.1. *Bioconcentration factor*

The bioconcentration factor (BCF) was calculated to assess the capacity of plants to accumulate the metals for *in-situ* phytoremediation. The BCF is the concentration of the metal in plant roots and wastewater. The calculation was done using the following formula (Yoon et al., 2006; Padmavathiamma and Li, 2012).

$BCF = [MCP]_{root}/[MCW]_{wastewater \times 100}$

where,

MCP-Metals concentration in the root

MCW-Metals concentration in wastewater

2.7.2. *Translocation factor*

The metal ratio between the plant shoot and the wastewater in which the plant root was growing was assessed using the translocation factor (TF), as shown in the formula below (Gupta and Sinha, 2008).

$TF=[MCP]_{shoot}/[MCPS]_{root} \times 100$

where,

MCP-Metals concentration in shoot

MCW-Metals concentration in the root

2.8. *Wastewater degradation rate (%)*

The in-situ phytoremediation studies were performed in pulp paper industry wastewater samples near the rhizospheric zone of *Rumex dentatus, Ranunculus sceleratus*, and *Cammelina benghalensis* is compared to control. The estimation of metals from different parts of plant selected plants and assessment the rate of degradation of pollution parameter period of plant growth.

2.9. *Quality control*

Standard reference content was used for quality assurance and calibration of the analytical determinations. A mixture of one analytical blank, one analytical repeat, and sample control for every three sample samples was used to establish quality control during the analysis.

2.10. *Statistical analysis*

The Student t-test (P 0.001) was used to compare the values before and after in-situ phytoremediation of metals accumulation by plants. The average metal concentrations were statistically analyzed using the SPSS statistical program (version 17.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. *Physico-chemical characteristic and metals contents*

The pulp and paper industry wastewater showed high pollution load by physico-chemical analysis and had an alkaline pH (8.4), total dissolve solid (2456 mg L^{-1}), total suspended solid (136 mg L^{-1}), total dissolve solid (1354 mg L^{-1}), total phenols (530 mg L^{-1}), total nitrogen (174 mg L^{-1}), sulfate (2160 mg L^{-1}), phosphorus (165 mg L^{-1}), electric conductivity (1531 μS cm^{-1}) and ions (Cl^- 5.03, Na^+ 326, K^+ 21.01) along with heavy metals, which were above the acceptable limit (Table 1). Abiotic influences in the aquatic environment, such as humidity, pH, total suspended solids, hardness, salinity, and others, have direct effects on chemical chemistry and have the ability to affect the degree of toxicity of substances in aquatic species (Canosa et al., 2005); Paul et al. (2020). Most plant growth was highest when the pH of the soil was between 5.5 and 8.5 (Neina, 2019). Inorganic nitrogen contamination in marine environments has both ecological and toxicological consequences (Camargo and Alonso, 2006). Metal bioavailability and phytouptake in soils are influenced by pH, metal content, and the presence of organic acids, carbonates, chlorides, sulfide, organic compounds, and other co-pollutants in the root region (Pinto et al., 2015). The high concentration of phenol is toxic to aquatic organisms and causes ecotoxicology (Duan et al., 2018; Sharma et al., 2021b). The decrease in EC in phytoremediation wastewater may be explained by the use of mineralized irons by rhizospheric species and the uptake by roots of plants. Results revealed that the decrease in all Physico-chemical parameters observed after phytoremediation of metals through *Rumex dentatus, Ranunculus sceleratus,* and *Cammelina benghalensis*, as well as their rhizospheric microbial communities from the rhizospheric zone. Selected plants accumulate high levels of heavy metals in both above and below ground parts because microorganisms are helping in the biotransformation, degradation of organometallic compounds, and increasing their bioavailability to plants. The reduction in EC also encourages microbial activity to grow in the rhizosphere of plant roots.

Table 1
Physico-chemical characteristics of discharged wastewater along with heavy metals content and their reduction (%) near the rhizospheric zone of Plant-1.

Parameters	Values (Mean ± SD) Wastewater sample	Values (Mean ± SD) Wastewater near plant 1	Reduction (%)	Permissible limit (EPA, 2002)
pH	8.02 ± 0.04	7.01 ± 0.01[b]		5-9
Solids				
I. Total solid	2456 ± 142	738 ± 19.04[c]	<80%	–
II. Total dissolved solid	1354 ± 29.46	5.26 ± 11.06[a]	<80%	–
III. Total suspended solid	136 ± 1.02	37 ± 3.21[c]	<80%	35
Chemical oxygen demand	26324 ± 263	12654 ± 123.07[a]	<70%	120
Biological oxygen demand	7136 ± 173	3256 ± 0.66[a]	<70%	40
Electrical conductivity (–S cm^1)	1531 ± 96.07	631 ± 55.28[b]	<80%	1000
Total Phenols	530 ± 21.54	294 ± 21.80[b]	<70%	0.50
Total nitrogen	174 ± 4.33	81 ± 2.67[b]	<70%	143
Sulfate	2160 ± 9.30	965 ± 06[b]	<70%	250
Phosphorus	165 ± 4.06	51.01 ± 3.14[b]	<70%	200
Cl^-	5.03 ± 0. 04	2.98 ± 1.11[NS]	<70%	1500
Na^+	326 ± 13.21	156 ± 1.65[b]	<70%	200
K^+	21.01 ± 0.10	8.36 ± 0.3[b]	<70%	–
Heavy metals (mg L^{-1})				
Iron	124.65 ± 1.23	64.02 ± 1.20	<50%	2.00 mg L
Zinc	56.33 ± 0.34	23.16 ± 1.51[a]	<50%	2.00 mg L
Copper	6.34 ± 0.01	1.32 ± 0.02[NS]	<50%	0.50 mg L
Cadmium	9.02 ± 0.05	4.39 ± 0.01[a]	<50%	0.01 mg L
Manganese	23.64 ± 0.20	11.08 ± 0.02[a]	<50%	0.20 mg L
Nickel	6.04 ± 0.01	2.01 ± 0.10[b]	<50%	0.10 mg L
Lead	1.20 ± 0.05	0.59 ± 0.07[c]	<50%	0.05 mg L
Mercury	1.08 ± 0.05	0.32 ± 0.29	<50%	
Chromium	1.31 ± 0.09	0.26 ± 0.02[b]	<50%	0.10 mg L
Arsenic	1.43 ± 0.03	0.47 ± 0.01[NS]	<50%	0.1 g m^3

All the values are means of triplicate ($n = 3$) ±SD. Students t test (two tailed as compared to pre-treated sludge).
[a]Highly significant at $p < 0.001$.
[b]Significant at $p < 0.01$.
[c]Less significant at $p < 0.05$.
[NS]Non-significant at $p > 0.05$.

Besides, after phytoremediation, the concentration of all heavy metals (mg L^{-1}) in the wastewater was reduced: Fe (124.45> 50%), Zn (56.33> 50%), Cu (6.34> 50%), Cd (9.02> 50%), Mn (23.64 > 50%), Ni (6.04> 50%), Pb (1.20> 50%), Hg (1.08> 50%), Cr (1.31> 50%) and As (1.43> 50%) was above the 50%. Heavy metals can be present due to the industry's alkali pulping and bleaching methods, as well as bioaccumulation by plants for use as raw materials. Metals have a remarkable tolerance for lignocellulosic compounds, resulting in cationic molecules. This study reveals important details about the output of these native plants in a field environment, as well as their potential to improve the quality of wastewater streams.

The high concentration of metals present in the wastewater can directly affect aquatic life and damage cell organelles (Hong et al., 2020). The studies showed that the high concentrations of Pb, Hg, and Cd caused toxicity to fish organs (kidney); most of the organs were affected by chronic toxicity of metals due to the presence of low molecular weight proteins (LMWP), 2-microglobulin (β2-MG) (Bernard et al., 1997; Tamele and Loureiro, 2020). Hg is more toxic due to its volatility, versatility, and strong propensity to bioaccumulate. Pb molecular toxicity can occur at blood concentrations as low as 5 μg/dl. Delta-amino levulinic acid synthase, delta-aminolevulinic acid dehydratase, and ferrochelatase are three important enzymes for human body function. Nickel impacts reproductive toxicity, phytotoxicity, neurotoxicity, hemotoxicity, hepatotoxicity, carcinogenicity, pulmonary toxicity, and nephrotoxicity. Contamination of water and soil, which ultimately poses serious risks to human health and aquatic life is among the negative ecological effects of excessive heavy metals (Sajad et al., 2020).

3.2. Response of photosynthetic pigments

Photosynthetic pigments such as chlorophyll (chl-a, chl-b) and carotenoids are important in the plants for converting solar energy into chemical energy (Rai et al., 2016; Yang et al., 2020b). The result of this study showed that the carotenoids contents were highest in *Rumex dentatus* (1.69 mg g^{-1} fw), followed by *Ranunculus sceleratus* (1.06 mg g^{-1} fw), and *Cammelina benghalensis* (1.56 mg g^{-1} fw). The highest chlorophyll contents were in *Rumex dentatus* (5.03–6.74 mg g^{-1} fw), followed by *Ranunculus sceleratus* (5.69.00–8.03 mg g^{-1} fw), and *Cammelina benghalensis* (4.65–7.08 mg g^{-1} fw), which showed the potentiality of the plants. Plants' chlorophyll response is one of the most significant stress responses. Furthermore, carotenoids were thought to act as antioxidants by scavenging free radicals, transferring electrons to dual bond structures, and eliminating light damage, cell damage, chloroplast membrane destruction, and genetic material

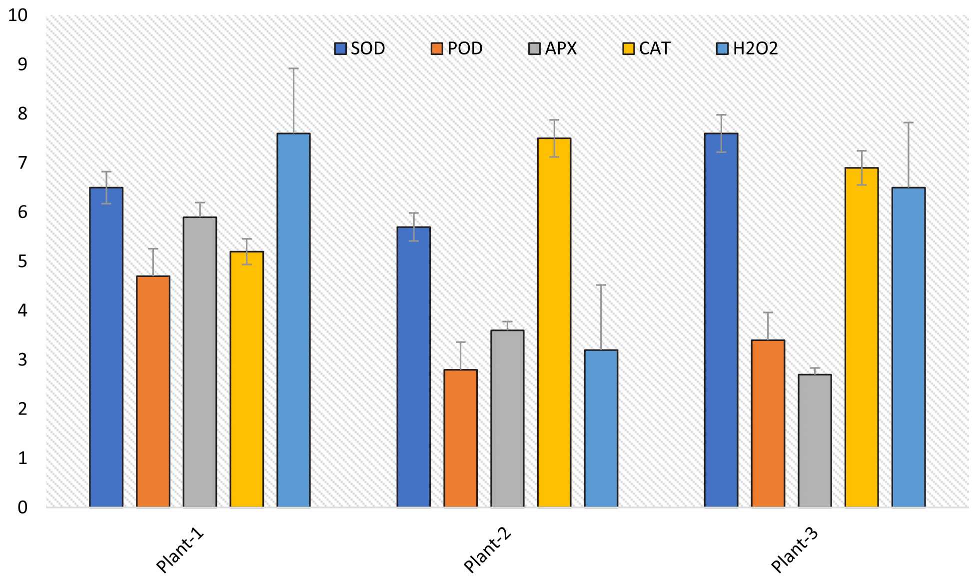

Fig. 1. Comparative analysis of SOD, POD, APX, CAT and H_2O_2 antioxidants activity in Plant-1, Plant-2, and Plant-3 from the contaminated site of wastewater.

through photodynamic reaction, quenching, and membrane collapse. Peroxidation is replaced by an increase in the accumulation of hazardous elements and meta-metals, as well as a reduction in the uptake of hazardous metals (Czerpak et al., 2006). The ratio of chl-a, chl-b, and carotenoids is an important parameter that has been linked to lower light chlorophyll protein harvesting rates (LHCP) (Mobin and Khan, 2007). Carotenoids are important in a variety of plant processes and can act as antioxidants while plants are stressed (Uarrota et al., 2018).

3.3. Response of antioxidants enzymes

When metal ions are present, antioxidants are thought to be essential in the detoxification of toxic oxygen species produced. The selected plants i.e. *Rumex dentatus*, *Ranunculus sceleratus*, and *Cammelina benghalensis* presented higher antioxidants enzymes such as SOD, POD, APX, CAT, and H_2O_2 activity compared to control plants growing at wastewater containing organometallics from the pulp and paper industry. The highest SOD activity was shown in *Rumex dentatus* (230.14 U/mL), followed by *Ranunculus sceleratus* (219.14 U/mL) and *Cammelina benghalensis* (214.14 U/mL) (Fig. 1). Plants tolerate a variety of non-enzymatic cellular entities such as cysteine, nonprotein thiols, and ascorbic acid to protect themselves from oxidative stress caused by the free radicals (Sinha and Saxena, 2006; MacFarlane et al., 2007). Decomposition and detoxification of SOD and CAT. For example, anions of hydrogen peroxide can be converted to radical oxygen and then to O_2 and H_2O at ground level. The highest concentration of POD was found in *Ranunculus sceleratus* (125.03 U/mL), followed by *Rumex dentatus* (103.01 U/mL) and *Cammelina benghalensis* (83.07 U/mL). Furthermore, the POD enzyme is involved in a variety of processes such as cell formation, auxin catabolism, lignification (Hassan et al., 2008). The POD enzyme activity was increased in the hydroponic study in soybean and rice plants after exposure to Cd. The concentrations of APX and CAT were the highest in *Ranunculus sceleratus* (156.12 U/mL), followed by *Rumex dentatus* (136.09 U/mL) and *Cammelina benghalensis* (127.01 U/mL)., respectively. The increased amount of APX under metal stress has demonstrated the inconsistent position of the mechanism of detoxification of H_2O_2. For photosynthetic machinery maintenance and another injury, the APX uses ascorbate to scavenge the peroxide molecule. Furthermore, *Rumex dentatus* had the highest H_2O_2 content (169.37 U/mL), which was supported by the presence of toxic heavy metals that neutralize ROS and inhibit lipid peroxidation. The affected plants showed toxic effects such as damage of membrane, plasmolysis, and electrolytic leakage membrane damage due to a high level of H_2O_2. Metal toxicity in plants causes DNA inhibition, oxidative damage, and toxicity in mitochondria due to reactive oxygen species (ROS) (Jiang et al. 2010). SOD, APX, and CAT are antioxidant enzymes that protect cells from the toxicity of reactive oxygen species (ROS). They can protect plants from several problems. Some antioxidants also serve as substrates for the synthesis of phytochelatins, which are essential for the detoxification of dangerous heavy metals like Ni and Cd. In studies of plant protection mechanisms against oxidative stress induced by heavy metals, antioxidant activity was discovered to be higher in the leaves and stems of wild plants. These native plants demonstrated a high tolerance ability for organometallic-containing wastewater (Sharma et al. 2020). To organometallic pollutants from the disposal site of industry, cytokinins and auxins have been shown to regulate stomata activity, which regulates open and close mechanisms. The findings revealed a high degree of native plant tolerance as well as a rapid increase in transpiration rate.

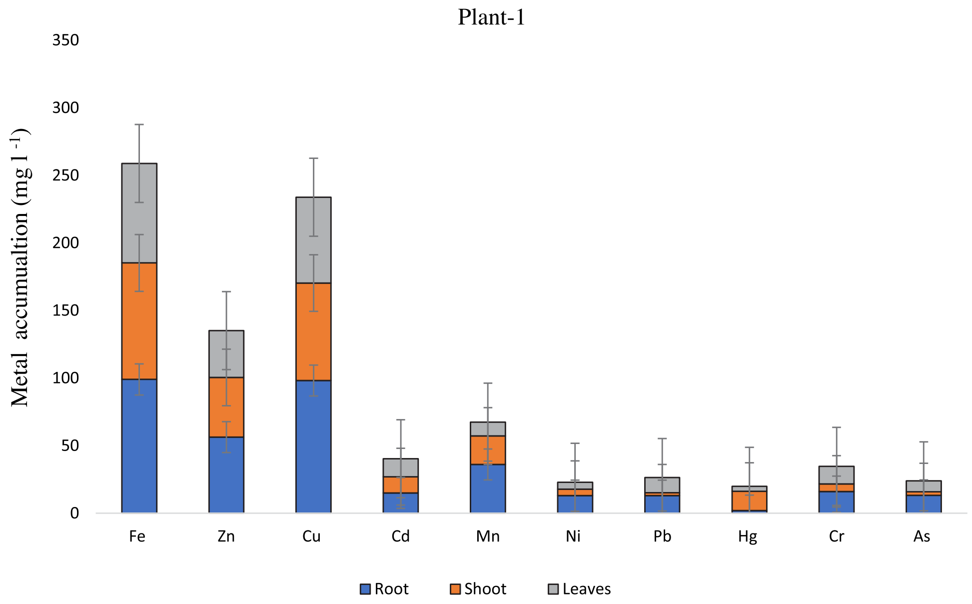

Fig. 2. Accumulation pattern of different heavy metals in root, shoot and leaves of Plant-1 grown on wastewater dumping site (Data are shown as mean ± SD, $n = 3$).

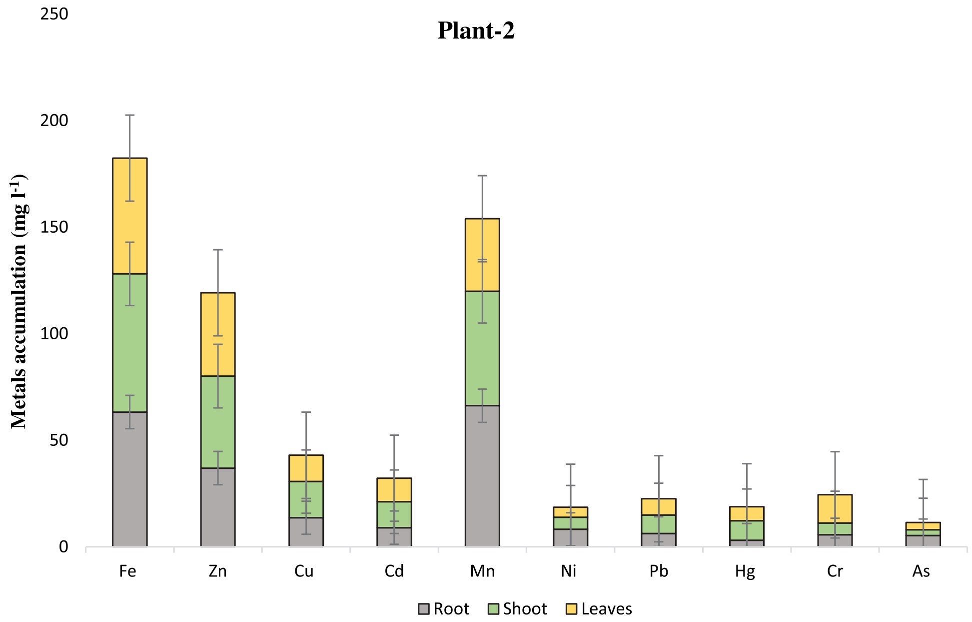

Fig. 3. Accumulation pattern of different heavy metals in root, shoot and leaves of Plant-2 grown on wastewater dumping site (Data are shown as mean ± SD, $n = 3$).

3.4. Translocation of metals root to leaves

In assessing the role of plants at a polluted site, the distribution and storage of metals in different plant components are important parameters to evaluate. The ability of *Rumex dentatus*, *Ranunculus sceleratus*, and *Cammelina benghalensis* to accumulate Fe, Zn, Cu, Cd, Mn, Ni, Pb, Hg, Cr, and As indicated a sporadic pattern of accumulation. The data revealed the contents of metals in the tissue of plant species at contaminated sites, suggesting their different metal absorption capacities. In our study, Fe concentration in plants samples ranged from 322.04 mg kg^{-1} (Plant-1) to 463.25 mg kg^{-1} (Plant-2), to 263.47 mg kg^{-1} (Plant-3) (Figs. 2, 3, 4 and Table 4). The uptake of Fe in the leaves of plant-2 was highest (254.33 mg kg^{-1}), followed by plant-3 (231.49 mg kg^{-1}) and plant-1 (186.53 mg kg^{-1}). The accumulation of Fe was highest

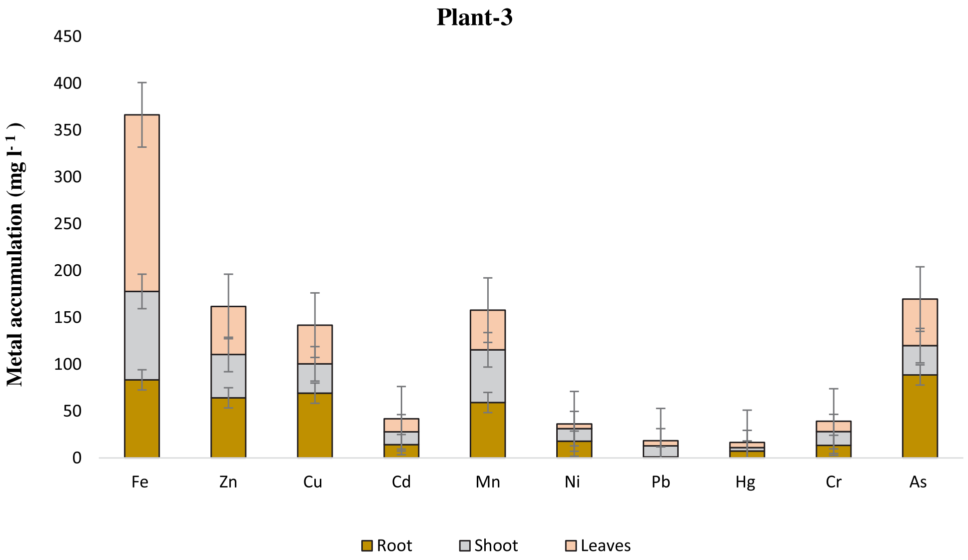

Fig. 4. Accumulation pattern of different heavy metals in root, shoot and leaves of Plant-3 grown on wastewater dumping site (Data are shown as mean ± SD, $n = 3$).

Table 2
Physico-chemical characteristics of discharged wastewater along with heavy metals content and their reduction (%) near the rhizospheric zone of Plant-2.

Parameters	Values (Mean ± SD) wastewater sample	Values (Mean ± SD) Wastewater near plant 2	Reduction (%)	Permissible limit (EPA, 2002)
pH	8.02 ± 0.04	7.0 ± 0.10[b]	<80%	5-9
Solids			<80%	
I. Total solid	2456 ± 142	1627 ± 53.0[c]	<80%	–
II. Total dissolved solid	1354 ± 29.46	613 ± 10.08[a]	<70%	–
III. Total suspended solid	136 ± 1.02	62 ± 1.05[c]	<70%	35
Chemical oxygen demand	26324 ± 263	12641 ± 149.06[a]	<80%	120
Biological oxygen demand	7136 ± 173	3314 ± 1.30[a]	<70%	40
Electrical conductivity (−S cm^1)	1531 ± 96.07	635 ± 44.02[b]	<70%	1000
Total Phenols	530 ± 21.54	241 ± 14.62[b]	<70%	0.50
Total nitrogen	174 ± 4.33	73.02 ± 5.28[b]	<70%	143
Sulfate	2160 ± 9.30	962 ± 0.02[b]	<70%	250
Phosphorus	165 ± 4.06	68.03 ± 1.06[b]	<70%	200
Cl^-	5.03 ± 0. 04	2.06 ± 1.01[NS]	<70%	1500
Na^+	326 ± 13.21	163 ± 0.07[b]		200
K^+	21.01 ± 0.10	9.04 ± 0.5[b]	<50%	–
Heavy metals (mg L^{-1})			<50%	
Iron	124.65 ± 1.23	61.77 ± 2.04[a]	<50%	2.00 mg/L
Zinc	56.33 ± 0.34	27.36 ± 1.06[a]	<50%	2.00 mg/L
Copper	6.34 ± 0.01	2.64 ± 0.31[NS]	<50%	0.50 mg/L
Cadmium	9.02 ± 0.05	4.23 ± 0.11[a]	<50%	0.01 mg/L
Manganese	23.64 ± 0.20	7.06 ± 1.00[a]	<50%	0.20 mg/L
Nickel	6.04 ± 0.01	2.1 ± 0.26[b]	<50%	0.10 mg/L
Lead	1.20 ± 0.05	0.21 ± 0.05[c]	<50%	0.05 mg/L
Mercury	1.08 ± 0.05	0.21 ± 0.01	<50%	
Chromium	1.31 ± 0.09	0.73 ± 0.02[b]	<50%	0.10 mg/L
Arsenic	1.43 ± 0.03	0.70 ± 0.03[NS]	<50%	0.1 g/m^3

All the values are means of triplicate ($n = 3$) ±SD. Students t test (two tailed as compared to pre-treated sludge).
[a]Highly significant at $p < 0.001$.
[b]Significant at $p < 0.01$.
[c]Less significant at $p < 0.05$.
[NS]Non-significant at $p > 0.05$.

in the shoots of plant-2 (364.86 mg kg^{-1}), followed by plant-1 (333.23 mg kg^{-1}) and plant-3 (231.49 mg kg^{-1}). Fe is an important co-factor for many enzymes and a key component of electron chains. For plants to resolve soil iron's often restricted availability by using strategies that increase its mobility while restricting its absorption when it is present in abundance (Schmidt et al., 2020). Since iron is needed for respiration, DNA synthesis, and photosynthesis are examples of

Table 3
Physico-chemical characteristics of discharged wastewater along with heavy metals content and their reduction (%) near the rhizospheric zone of Plant-3.

Parameters	Values (Mean ± SD) wastewater sample	Values (Mean ± SD) Wastewater near plant 3	Reduction (%)	Permissible limit (EPA, 2002)
pH	8.02 ± 0.04	7.03 ± 0.01[b]	<80%	5-9
Solids			<80%	
I. Total solid	2456 ± 142	1185 ± 33.01[c]	<80%	–
II. Total dissolved solid	1354 ± 29.46	756 ± 30.00[a]	<70%	–
III. Total suspended solid	136 ± 1.02	56 ± 0.01[c]	<70%	35
Chemical oxygen demand	26324 ± 263	12531 ± 132.04[a]	<80%	120
Biological oxygen demand	7136 ± 173	3024 ± 05[a]	<70%	40
Electrical conductivity (−S cm^1)	1531 ± 96.07	869 ± 05.11[b]	<70%	1000
Total Phenols	530 ± 21.54	247 ± 08.02[b]	<70%	0.50
Total nitrogen	174 ± 4.33	72 ± 3.14[b]	<70%	143
Sulfate	2160 ± 9.30	1324 ± 06[b]	<70%	250
Phosphorus	165 ± 4.06	117 ± 5.04[b]	<70%	200
Cl^-	5.03 ± 0. 04	2.1 ± 1.01[NS]	<70%	1500
Na^+	326 ± 13.21	146 ± 02.00[b]		200
K^+	21.01 ± 0.10	11.06 ± 1.1[b]	<50%	–
Heavy metals (mg L^{-1})			<50%	
Iron	124.65 ± 1.23	61.02 ± 1.01[a]	<50%	2.00 mg L
Zinc	56.33 ± 0.34	21.36 ± 1.60[a]	<50%	2.00 mg L
Copper	6.34 ± 0.01	2.01 ± 0.02[NS]	<50%	0.50 mg L
Cadmium	9.02 ± 0.05	4.52 ± 0.25[a]	<50%	0.01 mg L
Manganese	23.64 ± 0.20	11.03 ± 2.00[a]	<50%	0.20 mg L
Nickel	6.04 ± 0.01	2.01 ± 0.20[b]	<50%	0.10 mg L
Lead	1.20 ± 0.05	0.15 ± 0.07[c]	<50%	0.05 mg L
Mercury	1.08 ± 0.05	0.39 ± 0.03	<50%	
Chromium	1.31 ± 0.09	0.29 ± 0.03[b]	<50%	0.10 mg L
Arsenic	1.43 ± 0.03	0.18 ± 0.01[NS]	<50%	0.1 g m^3

All the values are means of triplicate ($n = 3$) ±SD. Students t test (two tailed as compared to pre-treated sludge).
[a]Highly significant at $p < 0.001$,
[b]Significant at $p < 0.01$,
[c]Less significant at $p < 0.05$,
[NS]Non-significant at $p > 0.05$

metabolic processes. it is an important micronutrient for almost all living organisms. Iron also activates many metabolic pathways and is a component of many enzymes' prosthetic groups (Rout and Sahoo, 2015). Although Fe is an important micronutrient found in high concentrations in pulp and paper industry wastewater, it improves the plant's ability to resist metal stress and also thus phytoremediation capacity.

The highest concentration of Cu and Zn was observed in the roots of plant-1 (98.25 and 68.33 mg kg^{-1}, respectively), followed by shoots of plant-1 for Cu (72.14 mg kg^{-1}) and shoots of plant-3 for Zn (46.25 mg kg^{-1}). The minimum concentration of Zn and Cu was in the leaves of plant-3 (51.24 and 41.36 mg kg^{-1}, respectively). Drought stress reduces plant growth by altering the biochemical pathways. Zn is an essential micronutrient that regulates a variety of physiological and molecular mechanisms in plants to help them tolerate drought conditions. Zn improves seed germination, plant water ties, cell membrane stability, osmolyte accumulation, stomatal control, water use efficiency, and photosynthesis in drought-stressed plants, resulting in significantly improved plant production (Umair Hassan et al., 2020). Metal biosynthesis and accumulation in plant tissues are influenced by the presence of metals, metal speciation, pH, salinity, total organic carbon (TOC), soil type, cation exchange potential (CEC), and soil mineralogy. One of the most important factors influencing metal bioavailability and thus concentrated in the surrounding media is soil pH variation.

The present study also revealed a high concentration of metals in the plant's aerial portion, indicating this could be applied for restoration and phytoremediation of areas polluted by toxic waste. This variable metal accumulation pattern in the roots, shoots, and leaves has the highest metal accumulation. The capacity of different parts of plant-1, plant-2, and plant-3 to accumulate metals indicated that different biochemical mechanisms of metal bioaccumulation regulated their separation and efflux in the plant. As a result, some plants can tolerate high levels of essential and toxic elements in their tissues without experiencing toxic effects. Many authors have reported the heavy metals translocation strategies adopted by the plants (Pehoiu et al., 2020). Metal ions may form mixed with multiple chelators, such as organic acids, after entering root cells. Carbonate, sulfate, and phosphate form complexes in the extracellular and intracellular spaces that become immobilized. Metal ions captured in vacuoles may pass through the root symplasm and into the stele and xylem stream, where they are transferred to the shoots via xylem vessels. The apoplast transports and distributes them in the leaves, extracellular compartments (cell walls) or the plant vacuole sequester the ions, preventing free metal ions from accumulating in the cytoplasm. The identification and characterization of the genes that regulate heavy metal absorption should be a major research goal in the future. The translocation in a plant's in aerial parts (i) stem and leaves in metals are effectively moved by biosorption plants (ii) plants for human nutrition that only transport trace amounts of heavy metals to seed or fruits (Aprile and De Bellis, 2020).

Table 4

Heavy metal accumulation (mg kg^{-1} DW) in the root, shoot, and leaves of selected plant species growing contaminated site of pulp and paper industry wastewater. All values are mean ($n = 3$) ± SD and presented in milligrams per kilogram plant dry weight. Mean ±SD followed by different letters in same column are significantly different (one-way ANOVA; Tukey's test, $p \leq 0.05$)

Plants name	Plant part	Fe	Zn	Cu	Cd	Mn	Ni	Pb	Hg	Cr	As
Plant-1	Root	322.04 ± 27.51	68.33 ± 6.84	98.25 ± 0.24	5.03 ± 0.54	36.17 ± 0.03	13.01 ± 0.20	3.04 ± 0.56	5.67 ± 0.00	6.03 ± 0.05	3.26 ± 1.06
	Shoot	333.23 ± 67.34	44.21 ± 0.59	72.14 ± 5.24	2.01 ± 0.09	21.04 ± 1.04	4.79 ± 0.01	2.17 ± 0.14	7.59 ± 0.15	5.67 ± 0.00	2.69 ± 0.02
	Leaves	186.53 ± 23.60	34.56 ± 0.23	63.47 ± 1.01	3.25 ± 0.10	10.27 ± 0.27	5.07 ± 0.02	1.29 ± 0.03	2.48 ± 0.29	3.05 ± 0.36	2.13 ± 0.30
Plant-2	Root	463.25 ± 11.54	36.97 ± 1.01	13.67 ± 1.01	2.01 ± 0.02	66.25 ± 1.46	8.25 ± 0.05	6.33 ± 0.23	9.84 ± 2.01	5.69 ± 0.05	5.34 ± 0.56
	Shoot	364.86 ± 63.51	43.16 ± 3.03	16.97 ± 1.05	2.16 ± 0.11	53.67 ± 1.06	5.69 ± 0.06	8.69 ± 0.01	5.67 ± 3.07	5.49 ± 0.03	2.64 ± 0.24
	Leaves	254.33 ± 28.34	39.15 ± 3.05	12.33 ± 2.01	1.09 ± 0.23	34.11 ± 1.00	4.69 ± 0.20	7.58 ± 1.06	4.21 ± 1.69	3.24 ± 1.05	3.48 ± 0.11
Plant-3	Root	263.47 ± 0.67	64.38 ± 0.03	69.31 ± 6.33	4.31 ± 0.04	59.34 ± 1.32	7.94 ± 0.00	1.08 ± 0.04	16.49 ± 0.33	3.64 ± 0.64	88.67 ± 3.57
	Shoot	231.49 ± 64.59	46.25 ± 0.01	31.25 ± 3.71	3.66 ± 0.09	56.17 ± 2.36	3.47 ± 0.06	11.94 ± 3.67	8.67 ± 0.01	4.67 ± 3.00	31.49 ± 2.94
	Leaves	188.60 ± 0.01	51.24 ± 0.05	41.36 ± 2.36	4.03 ± 0.05	42.37 ± 1.09	5.12 ± 0.05	5.47 ± 4.97	6.34 ± 0.05	1.09 ± 0.00	49.67 ± 1.00

Table 5
Bioconcentration and translocation of different heavy metals accumulation (mg kg^{-1} DW) by plants at wastewater site.

Native plants	Fe	Zn	Cu	Cd	Mn	Ni	Pb	Hg	Cr	As
	Bioconcentration Factor (BCF)									
Plant-1	38.70	82.43	6.45	179.32	63.69	46.42	39.47	19.04	21.72	43.86
Plant-2	26.89	152.36	46.37	448.75	35.68	73.21	18.95	10.97	23.02	26.77
Plant-3	47.31	87.49	209.98	9.147	38.15	76.07	111.11	6.54	35.98	1.612
	Translocation Factor (TF)									
Plant-1	1.03	0.64	0.73	0.39	0.58	0.36	0.71	1.33	0.94	0.82
Plant-2	0.78	1.16	1.24	1.07	0.81	0.68	1.37	0.57	0.96	0.49
Plant-3	0.87	0.71	0.45	0.84	0.94	0.43	11.05	0.52	1.28	0.35

3.5. Wastewater degradation rate (%) through phytoremediation

Phytoremediation is a cost-effective and environmentally friendly way to replant heavy metal-polluted soil. To improve phytoremediation efficiency, a better understanding of the processes underlying heavy metal elevation and tolerance in plants is needed. The remediation effectiveness (%) of heavy metals that are uptake, remediation, and translocated in plants is specified in this research. To enhance phytoremediation effectiveness, we focus on metal accumulation patterns in wild species of different parameters like antioxidant enzyme activity estimation and photosynthetic pigment analysis (Yan et al., 2020). Our finding revealed that the wastewater parameters of the pulp and paper industry were significantly reduced (>70%) in the presence of plant-1, plant-2, and plant-3 (Tables 1–3). Before discharging the wastewater into the surrounding aquatic environment, the treatment facilities are ineffective at removing contaminants, especially organometallic pollutants, and other hazardous metals. The presence of these chemicals, most of which are naturally occurring endocrine disruptors, endangers both health and the environment. Advanced oxidation techniques, like membrane filtration, reverse osmosis, or membrane bioreactors, are costly and/or require a large amount of energy input. To address this problem in developing countries, a more strategic management approach that is both environmentally and economically appealing is needed. Phytoremediation is a successful and deep technique that can be used to treat wastewater better. Native plant phytoremediation can help in both site remediation and ecological balance. Native plants have the enzymatic capacity to absorb, stabilize, biodegrade, or volatilize various inorganic and organic toxins, making their rhizosphere an ideal habitat for the microbial community. The results of this study showed that the *Ranunculus sceleratus*, *Rumex dentatus*, and *Cammelina benghalensis* plants contained in a pulp and paper wastewater-polluted site have the potential to be used in an effective care system to increase the removal of toxic organometallic pollutants.

3.6. Biological and translocation factor

The BCF and TF factor of each metal can be used to assess the ability of plants to accumulate metals from waste equations and translocate extracted metals from underground to aerial parts (Dradrach et al., 2020). Bioconcentration factors of selected plants of heavy metals in *Ranunculus sceleratus*, *Rumex dentatus,* and *Cammelina benghalensis* were calculated (Table 5). The bioconcentration/bioaccumulation factor was used to investigate the relationship between heavy metal concentrations in plant roots and wastewater (Eid et al., 2020b). The study revealed that *Ranunculus sceleratus*, *Rumex dentatus* and *Cammelina benghalensis* plant displayed lowest BCF (<1) values for Fe (38.70; 26.89; 47.31 mg kg^{-1}), Zn (82.43; 152.36; 87.49 mg kg^{-1}), Cu (6.45; 46.37; 209.98 mg kg^{-1}), Cd (179.32; 448.75; 9.147 mg kg^{-1}), Mn (63.69; 35.68; 38.15 mg kg^{-1}), Ni (46.42; 73.21; 76.07 mg kg^{-1}), Pb (39.47; 18.95; 111.11 mg kg^{-1}), Hg (19.04; 10.97; 6.54 mg kg^{-1}), Cr (21.72; 23.02; 35.98 mg kg^{-1}), and As (0.82; 0.49; 0.35 mg kg^{-1}). Plant-3 had a difficult time mobilizing these toxic compounds in the subsurface, as indicated by the low BCF values. Furthermore, the plants' low BCF values indicated that they were better suited to phytostabilization rather than phytoextraction for cleaning up polluted sites (Li et al., 2007). Phytoextraction plant species must be rapid, resistant to a wide variety of metals, and effective at moving metals from roots to shoots. The maximum TF (>1) was found in all tested metals i.e., Fe (1.03 mg kg^{-1}), Zn (1.16 mg kg^{-1}), Cu (1.24 mg kg^{-1}), Cd (1.07 mg kg^{-1}), Pb (1.37 mg kg^{-1}), Hg (1.33 mg kg^{-1}), and Cr (1.28 mg kg^{-1}). The high metals TF values accumulated in selected plants suggested that these plants are resistant to these toxic metals and can be used in the phytoremediation of these metals from contaminated sites, as previously reported (Tripathi et al., 2021a,b). The high TF value indicates higher transpirational pullout and biomass due to the rapid plant growth. Some microbial communities are living near the rhizospheric zone of plants which is very helpful for the mineralization of elements (Sharma, 2021; Sharma and Singh, 2021; Sharma et al., 2021f). The results indicate that the luxuriant growth of selected plants can adapt to the organometallic contaminated environment and accumulate multi-metals in their tissues while thriving on pulp and paper industry wastewater with no noticeable signs of morphological toxicity. This is because wastewater contains nutrients that promote the growth of the plants. As a result, this plant may be used to restore the environment at a polluted site with organometallic waste.

4. Conclusion

From the results, it was concluded that *Ranunculus sceleratus*, *Rumex dentatus,* and *Cammelina benghalensis* accumulated a significant amount of metals in their roots, shoots, and leaves when cultivated on the discharged wastewater from the pulp and paper industry containing heavy metals and other complex co-pollutants after secondary treatment. In the stressed environment, these plants released antioxidant enzymes and photosynthetic pigments. It could also be concluded that these plants would be effective to restore an industrial wastewater polluted site. However, further study should be carried out to fully comprehend the pathway for phytoremediation of different metals from such hazardous waste sites employing the phytoremediation process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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