Integrating phytoremediation into treatment of pulp and paper industry wastewater: Field observations of native plants for the detoxification of metals and their potential as part of a multidisciplinary strategy

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Abstract

This work aimed to explore the use of native herbs for the removal of heavy metals from pulp and paper industry wastewater, with the view of applying them as part of a multidisciplinary approach for detoxification. Results showed that after in-situ phytoremediation by the native herbs, the heavy metal, and metalloid contents in the wastewater were reduced by almost 60%. Heavy metal analysis of the plant tissues revealed that Fe accumulation was highest in all the tested plants. In general, the bioconcentration factor (BCF) was higher than one (>1) for all the metals except for Cd, suggesting most of the metals were concentrated in the plant tissues. In particular, As was concentrated significantly in *Momordica doica* and *Cannabis sativa* with elevated BCF of 269.46 and 131.20, respectively. High translocation factor (>1) was observed in *P. hysterophorus* and *Tribulus terrestris* for Cr (5.63) and Cd (7.53), respectively. Results showed most of the native plants examined in this study had hyperaccumulating tendency. Transmission electron microscope analysis of plant root tissues showed abundant metal depositions in the root cell wall, cytoplasm, and vacuole as strong evidence of the in-situ phytoremediation capability of these plants. Antioxidants activities of the plants such as superoxide dismutase, catalase, hydrogen peroxidase, peroxidase, and ascorbate peroxidase production were also noted to be higher than the control. These results support the use of native plants as a novel green process that can be integrated into the multidisciplinary treatment of hazardous industrial wastewater in the polluted sites.

Keywords: heavy metal pollution; detoxification technology; native plants; organometallic pollutants; antioxidants

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Introduction

Heavy metal and metalloid pollution are caused by indiscriminate discharge from various sources of industrial activities into the environment. The discharges are often accompanied by complex organic hazardous substances such as pesticides and solvents [1,2]. Effluents from several industries including dye, pesticides, textiles, pulp and paper, plastics, and paint are common sources of metal pollution in the aquatic environment [3], which is of serious concern to India and other developing countries [4,5]. Previous studies have reported that pulp and paper industry wastewater is a source of pollution even after secondary treatment. This, directly and indirectly, poses toxicity to phytoplankton and zooplankton by increasing the physico-chemical parameters in the receiving water, such as total suspended solids (TSS), chemical oxygen demand (COD), total dissolved solids (TDS), biological oxygen demand (BOD), metals (e.g., Fe, Cu, Ni, Cr, Pb, Cd, Hg, As, Mg, and Mn) and other organic pollutants [6,7]. Efficient and economical methodologies as well as cost-effective approaches are warranted to remove metals from the environment, especially as mixed organometallic waste is considered a distinctive category of contaminants [8,9]. Cadmium, Pb, Ni, and Hg are some of the most common pollutants worldwide [10,11]. Native plant species use phytoremediation as an attractive solution for remediating industrial wastewater pollutants [12]. A study has demonstrated the luxuriant growth of native plants thriving on the wastewater of the pulp and paper industry could act as pioneer vegetation, to be used in phytoremediation of various metal and metalloids complex organometallic waste and heavy metals from complex organo-metallic sources. Consequently, they could be applied to bioremediate complex hazardous wastewater containing a mixture of different heavy metals [6].

Recently, Simiele et al. [13], reported the phytoremediation potential of *Salicaceae* species. Uptake of heavy metals from the industrial waste polluted area by plants can reduce seed germination and hinder the development of shoots and roots, lead to chlorosis and wilting, decrease chlorophyll and protein contents as well as reduce the efficacy of photosynthetic processes and different enzyme activities responsible to induce the germination process [14,15,84]. Different molecules like metal ion transporters and complicating factors control the accumulation and translocation of heavy metals in a plant. Several advanced transporters are localized in the root cell plasma membrane, including channel proteins and H^+, coupled transmembrane protein, which is essential for the uptake of

metal ions [16]. They could transport various metals via the cell membranes and mediate the flow through roots to shoots of metal translocation [17].

To negate the harmful impact of pollutants, the plant uses a sequence of defense mechanisms to regulate and detoxify its systems; for example, through absorption, accumulation, and translocation of these hazardous metals by secreting out free ions from the cytoplasm. Phytoremediation is a cost-effective and environmentally sustainable technique that exploits these detoxification abilities in plants to reduce or eliminate harmful pollutants from the environment [18]. Plants such as *Typha latifolia*, *Helianthus annuus* L, *Thelypteris palustris, Cannabis sativa*, *Nicotiana tabacum, Zea mays, Ricinus communis,* and *Chenopodiaceae* can accumulate high levels of metal and have been used in the phytoremediation of metals [19,20,21,85]. However, the use of native plants is less well documented.

Detailed studies on the characteristics and impact of pollutants present in pulp and paper mill waste and their phytoremediation potential of native plants are still limited. Many heavy-metal phytoremediation studies were achieved in pot experiments using single metal [e.g. [21, 22]]. Since the heavy metal phytoremediation process by growing plant species in an open field at any contaminated site is regulated by several factors, more representative studies using field samples are needed to provide better insight into the plant-metal interactions. For example, a study should consider factors such as the age of plants, type of plant, type of soil and soil texture, pH of the soil, concentration of metals, characteristics of organic content, and cation exchange capacity of the soil [23,24]. In India, there are estimates of more than 850 pulp and paper units, including small paper industries [25]. The industry has a production capacity of 400 TDP (ton per day) of papers, which is not only a source of soil and aquatic pollution but also affects the food chain. To safeguard human health and the environment, cost-effective and environment-friendly remediation technology is needed. Therefore, this study focused on the evaluation of potential native plants growing on the wastewater disposal site and along the effluent discharge drain where the dissolved pollutants of effluent also directly interacting with these plants. The information indicated the effectiveness of using native plants to remove heavy metals and their potentials to be integrated into a multidisciplinary strategy to detoxify heavy metals in pulp and paper industry wastewater.

2. Materials and methods

2.1. Site description and samples collection

Samples were collected from the K.R. Pulp and Paper Limited, Shahjahanpur (27°50′31.8″N 79°51′15.7″E), Uttar Pradesh, India, which produced Kraft paper as well as writing paper, utilizing sugarcane bagasse, wood pulp, and recycled paper also as raw material for manufacturing. The K R pulp and paper industry (plant) was established in the year 1998. The industry has a wastewater treatment system but due to the inadequate treatment facility, the discharged wastewater did not meet the required pollution standard which caused adverse effects in the environment. A number of native plants grew along the disposal drain where fibrous sludge was deposited. Their root zone interacted with flowing wastewater directly. Therefore, to assess the potential of the growing plants to bioremediate the wastewater, a path of the experimental site was created and with 50 days wastewater holding time with hydrological flow rate 5000 L/minute. The samples were collected at an interval of 15 days. Repeated sampling was carried out from March-June 2018 and 2019 periodically. The wastewater samples were collected from the discharge drains in 20 L sterile plastic jerrycan (Tarsons Production Pvt. Ltd., USA). Six native plant species were collected based on their abundance and luxuriant growth. These included *Tribulus terrestris* (Zygophyllaceae), *Parthenium hysterophorus* (Asteraceae), *Momordica doica* (Cucurbitaceae), *Alternanthera sessilis* (Amaranthaceae), *Cannabis sativa* (Cannabaceae), and *Calotropis procera* (Apocynaceae). Moreover, soil samples (one kg in sterile polythene) were also collected from normal land for confirmation of metals status. The collected plants were thoroughly washed first with the water and then with a 10 mmol L^{-1} solution of calcium chloride solution to remove the dirt and adhering particles.

2.2. Physico-chemical assessment

The samples were analyzed for their physicochemical properties, including the total pollutant loads in the pulp and paper industry wastewater. The electric conductivity (EC) and pH of the wastewater samples were measured using an Orion pH meter (Model-960, Thermo Scientific, FL, USA) and Orion conductivity meter, respectively. The total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), total phenols, total nitrogen, and ions (K^+, Na^+, and Cl^-) were analyzed using the standard methods of American Public Health Association [25]. The concentration of lignin and chlorophenols were measured as per the method described previously [26]. The

concentration of metals in the wastewater was determined by atomic absorption spectrophotometry (AAS) (ZEEnit 700, Analytic Jena, Germany) as mentioned in the APHA standard method 3030H [82].

2.3. Scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis

Scanning electron microscopy (SEM) analysis of wastewater samples was completely dried at 37 °C. The samples were placed on an aluminium stub and coated with a block of platinum through a sputter coater (SC 7620 Mini Sputter Coater, Quorum Technology Ltd., UK) [3]. The samples were analyzed by a JEOL JSM 6490 LV (Tokyo, Japan) SEM at different magnifications and an accelerating voltage of 15 kV. Element composition analysis, a selected area was investigated by SEM with an energy-dispersive X-ray spectroscopy (EDAX) system JEOL JSM 6490 LV (Tokyo, Japan).

2.4. UV-visible spectroscopy and Fourier-transform infrared spectroscopy analysis

To observe the absorbance characteristics of different organic and inorganic pollutants, UV–visible (UV-Vis) spectroscopy and absorption spectra were obtained between 250 and 700 nm at 37 °C (Thermo Fisher Scientific Shanghai spectrophotometer Evolution 2001, China). Fourier-transform infrared spectroscopy (FT-IR) analyses of the wastewater samples before (WS-1) and after (WS-2) phytoremediation was recorded by a FT-IR Perkin Elmer Spectrum-II spectrometer. The sample was centrifuged at 6000 rpm and then dried for 6 h in an oven at 65 °C to remove the moisture content. To make the pellets, 100 mg of potassium bromide (KBr) and 2 mg of dried sample were mixed as per the previously mentioned method [3]. The KBr based pellets were compressed into a thin disk using a hydraulic press (CAP-15 T) at 15 tons pressure. The disks were fixed in an FT-IR spectrometer (ThermoNicolet 6700) and analyzed in the spectral region of 4000–400 cm^{-1} with potassium bromite (KBr) pallets.

2.5. Extraction and characterization of pollutants

2.5.1. Solid-liquid extraction

Dichloromethane (DCM) was found to have the optimum extractability of the residual wastewater organic pollutants. The organic pollutants in the wastewater before and after phytoremediation were extracted using DCM to identify a broad range of organic compounds present [26]. A fresh wastewater sample (20 mL) was weighed and placed in an Erlenmeyer flask (500 mL), to which 25 mL of DCM was added and mixed vigorously. For sample preparation the dry residue was dissolved in 2.0 mL DCM (Millipore Ltd., Bedford,

Massachusetts, USA), a 3.0 mL sample was obtained for the gas chromatography-mass spectroscopy (GC-MS) analysis.

2.5.2. Characterization of organic pollutants through GC-MS

Extracted samples (200 µL) were transferred into GC vials and dried with nitrogen gas. The characterizations of pollutants were performed by the addition of 50 µL pyridine to the sample followed by silylation using 80 µL trimethylsilyl BSTFA and TMCS. The sample mixture was heated at 70 °C for 30 min with periodic shaking, after which it was subjected to the GC-MS analysis. The organic pollutants were identified by comparing their mass spectra (*m/z*) with recorded values at different retention times (RT) in the NIST library [3].

2.5.3. Digestion of plants for metal estimation

The estimation of concentrations of metals in the aerial parts of the potential native plants was measured. The samples were incubated at 70 °C for 7 days to dry, dried samples were then kept in a muffle furnace at 460 °C for 6 h. The samples were digested in 2% HNO_3 and filtered through a 0.45 µM glass fibre filter [27]. The accumulation of heavy metals in the plant parts was measured by atomic absorption spectrometry (AAS-ZEEnit 700, Analytic Jena, Germany).

2.5.4. Bioconcentration factor

The bioconcentration factor (BCF) was determined to measure the in-situ phytoremediation potential according to the number of native plants, as shown in the following formula [28].

$$BCF = \frac{Metal\ concentration\ in\ plant}{Metal\ concentration\ in\ wastewater}$$

2.5.5. Translocation factor

The translocation factor (TF) was used to evaluate the ratio of the metals between plant shoot and in the wastewater in which the plant root was growing [29].

$$TF = \frac{Metal\ concentration\ in\ plant\ shoot}{Metal\ concentration\ in\ plant\ root}$$

2.6. Estimation of antioxidants and microscopic observation of stomata

To estimate the antioxidants enzymes, 250 mg of fresh leaves were homogenized in 3 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). Estimation of

superoxide dismutase (SOD)and peroxidase (POD) was recorded at 560 and 420 nm, respectively [30,31]. 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 [32]. Catalase (CAT) activity was monitored by spectrophotometric analysis at 37 °C at 240 nm [33]. For the hydrogen peroxidase (H_2O_2) content, the absorbance was recorded at 350 nm [34]. A Nikon phase-contrast microscope (Nikon YTV-55, Tokyo Japan) at low power magnification was used to observe the stomata of the native plants grown in the contaminated site. To prepare the slide for stomata observation, the peel in the upper surface of the leaf was removed, and the leaves were preserved in a watch glass containing distilled water.

2.7. Histological observations transmission electron microscopy analysis

Plant roots were cut cross-section at approximately 2.0 mm from the tips and fixed in 2.5% glutaraldehyde solution for histological observation of heavy metal accumulation. For the precipitation of trace elements from the root, H_2S saturated water was used for 30 min at 37 °C [6,35]. The root was washed again with 0.1 M sodium cacodylate buffer (SCB), at a pH of 7.2. Also, the roots were washed in 1% O_sO_4 three times at 10 min intervals between each wash for post-fixation. Fixed root was dehydrated with acetone at different concentrations (30%, 50%, 60%, 70%, 80%, 90%, 95%, and 100%) and embedded in epoxy resin medium using an Araldite-DDSA kit (Ladd Research Industries, Williston). TEM section observation was performed using an FEI TecnaiTM G2 Spirit Twin (Hillsboro, USA) transmission electron microscope at an 80 kV voltage velocity.

2.8. Statistical analysis

The values before and after the in-situ phytoremediation of metals accumulation by native plants were calculated using the Student *t*-test, one-way ANOVA or Tukey-test ($p < 0.001$). Statistical analyses of the mean concentration of metals were performed by using the SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Alteration of physicochemical properties

The physicochemical analysis of the treated wastewater showed organic and inorganic pollution loads, an alkaline pH (8.4), the presence of nitrogen (183 mg L^{-1}), sulfate (2137 mg L^{-1}), and phosphorus (161 mg L_{-1}) were noted to be above the permissible limit (Table 1). The

results revealed that the presence of the native plants was able to improve the quality of the wastewater, probably due to the higher bioavailability of metals and organic compounds at an acidic pH around the root hairs, resulting in an increased uptake of heavy metals. The hydraulic retention time was 30 days, and the flow rate of wastewater was 500 lit/hr in the study area. The creation of an acidic environment might be due to the secretion of various acidic compounds by the roots, which was reported by previous research [36,37]. The TDS value (1430 mg L_{-1}) was high before the phytoremediation, but it decreased (811 mg L^{-1}) after the phytoremediation owing to the microbial metabolic and enzymatic activities in the rhizosphere. The source of TDS in wastewater might be due to the discharge of the cellulosic material dissolved during alkali pulping [38]. Microbial strains might have developed resistance mechanisms to enable them to utilize or detoxify the pollutants due to their broad range of nutritional behavior. These resistant microorganisms have the ability to release detoxifying enzymes for adaptation at low pH in the presence of lignocellulosic wastes, could therefore survive and persist in the discharged effluent. Consequently, they could ultimately utilize the organic pollutants of effluent [39]. It is worthful mentioning that wastewater-containing metals could be harmful to microplankton, which impacting the aquatic ecosystem to the food chain [40].

The ratio of BOD (8974 mg L^{-1}) and COD (24670 mg L^{-1}) was noted after phytoremediation which increased from the original ratio i.e., 1.77 of BOD (3574 mg L^{-1}) and COD (13860 mg L^{-1}) (Table 1). This indicated the discharged pulp and paper wastewater was not easily biodegradable. This was ameliorated by the growing native plants which increased the BOD/COD ratio, suggesting bioremediation of pollutants present in the pulp and paper mill wastewater. Moreover, the EC (1570 ms cm $^{-1}$) value was noted high reflecting the salt and ions contents of the wastewater. This corroborated with previous observations of pulp and paper mill waste [41]. Besides, lignin and chlorophenols compounds were derived directly from the plant material during chemical treatments, and they were dissolved in wastewater at high pH, thus further contributing to the waste toxicity.

Parameters	Sediment value (Mean)	Values (Mean±SD) before phytoremediation	Values (Mean±SD) after phytoremediation	Mean % reduction	Permissible limit (EPA, 2002)
pH	7.0	8.4±0.24	6.7±0.50[b]	20.2	5-9
Color	743	2340±105	1599±48.50[a]	31.7	Dark Brown
Total solid (TS)	-	1618±108	802±49.84[c]	50.4	-
Total dissolved solid (TDS)	-	1430±31.25	811±24.66[a]	43.2	-
Total suspended solid (TSS)	-	73±1.21	37±3.21[c]	49.3	35
Chemical oxygen demand (COD)	-	24670±254.00	13860±363.45[a]	43.8	120
Biological oxygen demand (BOD)	-	8974±172	3574±125[a]	60.2	40
Electrical conductivity (EC)	-	1570 ±84.00	577±65.43[b]	63.2	1000
Total Phenols	2.04	456±22.23	281±28.02[b]	38.4	0.50
Total nitrogen	4.2	183±4.10	78±9.84[b]	57.4	143
Sulphate	2.6	2137±09.70	1160±76[b]	45.7	250
Phosphorus	3.01	161±5.84	47±7.54[b]	70.8	200
Cl^-	1.1	4.51±0.20	4.91±1.61[NS]	-8.9	1500
Na^+	9.24	331±11.20	140±12.66[b]	57.7	200
K^+	7.01	19.8±0.80	12.83±1.4[b]	35.2	-
Lignin	0	42100±114.21	19280±1865[b]	54.2	-
Chlorophenol	0	324±10.23	80±5.01[a]	75.3	3.0
Heavy metals					
Iron (Fe)	2.01	95.35±1.89	43.16±3.54[a]	54.7	2.00 mg L^{-1}
Zinc (Zn)	1.02	48.40±0.40	37.50±4.80[a]	22.5	2.00 mg L^{-1}
Copper (Cu)	3.01	3.28±0.07	1.91±0.52[NS]	41.8	0.50 mg L^{-1}
Cadmium (Cd)	0.2	9.36±0.01	5.40±0.85[a]	42.3	0.01 mg L^{-1}
Manganese (Mn)	1.01	19.00±0.27	12.21±2.02[a]	35.7	0.20 mg L^{-1}
Nickel (Ni)	0.05	4.24±0.04	2.5±0.50[b]	41.0	0.10 mg L^{-1}
Lead (Pb)	0.02	0.64±0.01	0.35±0.08[c]	45.3	0.05 mg L^{-1}
Chromium (Cr)	0.02	0.76±0.01	0.30±0.05[b]	60.5	0.10 mg L^{-1}
Arsenic (As)	0.001	0.41±0.01	0.28±0.08[NS]	31.7	0.1 g/m^3

Table 1. Physico-chemical characteristics of discharged wastewater along with heavy metals content collected from M/s K. R. Pulp and Paper industry Ltd. *Shahjahanpur,* Uttar Pradesh, India.

All the values are means of triplicate (n = 3) ± SD. Unit of all parameters are in mg L^{-1} except pH, color (Co-Pt. Unit) and EC (μmhoscm^{-1}). 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 $p > 0.05$.

Elevated levels of lignin, chlorophenols, chloride, and other salts in the wastewater were also noted to be higher than the permissible levels (Table 1). Due to their lipophilic properties, chlorophenols tend to be absorbed in soil [42]. The increase in COD can be due to the release of various wood extracts and chemicals used in the process of pulping, leading to the presence of complex compounds in the wastewater and adversely affect the photosynthesis activity [43,44]. Similar observations regarding the discharge of recalcitrant pollutants of biologically treated effluent have also been reported in previous works, noted for the pollutants present in the black liquor of discharged effluent [45,46]. The physico-chemical analysis showed the quality of in-situ degraded wastewater was significantly enhanced ($p < 0.001$) after phytoremediation. For example, after phytoremediation, the concentration of all heavy metals (mg L_{-1}) in the wastewater was reduced: Fe (95.35–43.16), Zn (48.40–37.50), Cu (3.28–1.91), Cd (9.36–5.40), Mn (19.00–12.21), Ni (4.24–2.5), Pb (0.64–0.35), Cr (0.76–0.30), and As (0.41–0.28). Although the levels remained above the currently permissible limit prescribed by the US EPA, the improvement of water quality was observed in all parameters except for chloride concentration. More significantly, the toxic metal content in the

wastewater was reduced (between 22.5% and 60.5%). These reductions demonstrated the in-situ phytoremediation potential (Table 1). The presence of heavy metals might be explained by the alkali pulping and bleaching processes of this industry and bioaccumulation by plants that are used as raw materials. Metals have strong binding properties with lignocellulosic materials, thus forming cationic molecules. Therefore, during the waste treatment, organic pollutants might be degraded, and metals have been released as residual pollutants [26]. In water and soil, many heavy metals persist causing oxidative stress and carcinogenicity in fish and human health via the food chain [47].

Several researchers have reported the use of constructed wetland for the removal of contaminant from pulp paper wastewater and the role of phytoremediation is recognized [86,87]. However, the choice of plants used often come from a well-established but conservative list e.g., *Typha* and *Phragmites* sp. This study provides valuable information on the performance of six native plants in a field setting and their potentials to further improve the quality of the discharged wastewater. (Fig. 1).

3.2. Morphology view and element content analysis

Lignocellulosic organic polymers and heavy metals were observed during the SEM analysis of the morphological structure of two wastewater samples (Fig. 2a–b). The findings revealed complex and unusual shapes combined with enlarged rods or tubular structures (Fig. 2a–b), and an organic polymer (lignin) along with a heterogeneous compound on the surface of the wastewater. The rod shape structure might be the lignin polymers or cellulose present in wastewater. Similar observations of the granulated appearance of lignin with the complete structure have been reported in earlier studies [48,49]. This finding provided strong evidence of the presence of the metallic complex of lignocellulose. The abnormal structure often illustrated the complexation of lignin with various heavy metals and hydroxyl, carbonyl, and phenolics compounds [50]. Also, the EDAX analysis of two different samples showed different toxic elements in the wastewater (Fig. 2c–d). There was also considerable amount (%) of iron (1.99–3.86), oxygen (64.52–51.83), Mg (1.36–1.48), Al (10–9.27), and Si (17.21–28.90) in the effluent and wastewater. These findings also corroborated with the results of a previous study of wastewater from another discharged pulp paper waste site [3]. The presence of hazardous metals such as Fe, Cu, and Zn in groundwater would affect human health beyond the permissible level.

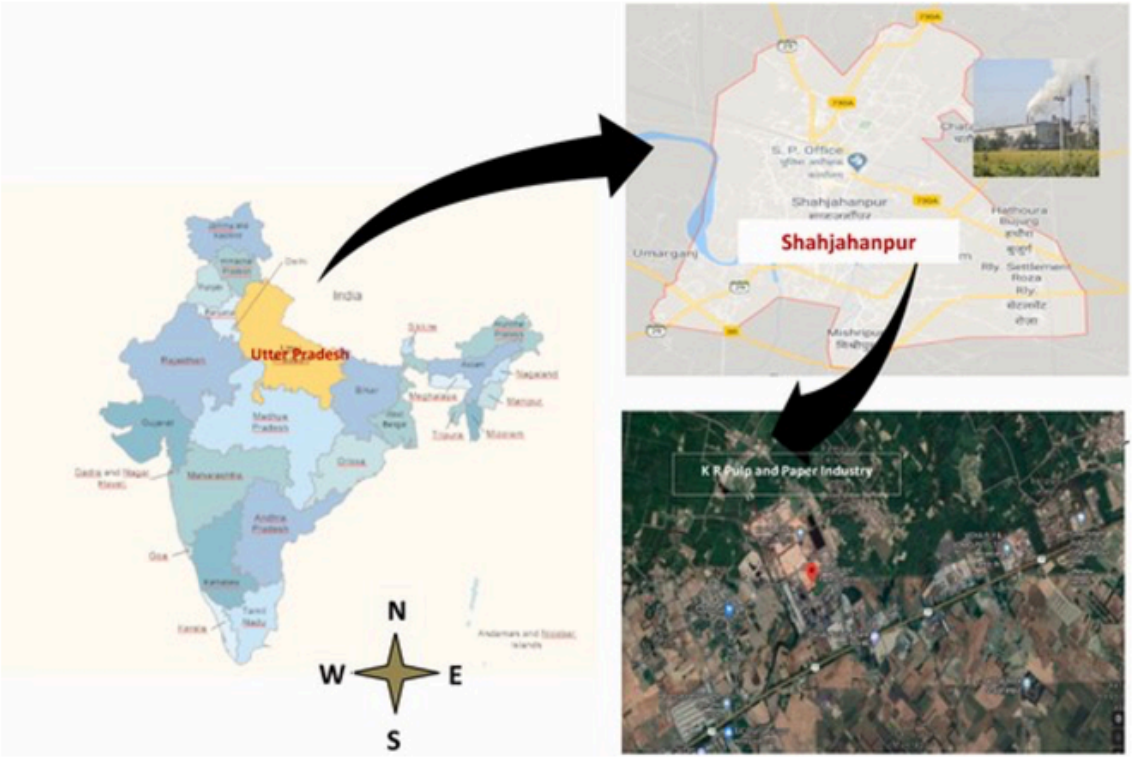

Fig. 1. Geographical location map of sampling site i.e., K.R. pulp and paper industry Ltd Shahjahanpur (Utter Pradesh), India. (Satellite view adapted from Google Map: Google Maps, 2020. *K R Pulp and Papers Ltd,* 1:500. Google Maps [online] [Accessed 1 Dec 2020]).

3.3. UV-Vis spectral and FT-IR analysis

The UV-Vis spectral analysis was performed between a wavelength range of 250–700 nm. This analysis revealed the availability of the dissolved organometallic compounds in the wastewater before and after the phytoremediation [51]. The results showed noticeable differences in the peak absorption at the UV-region, and the maximum absorbance was noted as λ_{max} 310 before the phytoremediation, as shown in Fig. 3a [102][102]. While, after the phytoremediation, some peaks disappeared, and the maximum absorbance was noted at λ_{max}270, as shown in Fig. 3b. The change of peak area and height indicated the conversion of compounds into complex metabolites [46]. This suggests the organometallic compounds have been removed by the native plants, either via physical adsorption or absorption into the plant tissues. The active uptake of organometallic compounds was further investigated; the resulting changes in the morphology of the plant tissues are discussed in Sections 3.7 and 3.8.

The FT-IR spectrum was used to analyze a wide range of functional groups with strong and weak bonds in the organic compounds and polymers of two different wastewater samples, i.e., WS-1 (wastewater before phytoremediation) and WS-2 (wastewater after

phytoremediation). This analysis identified different functional groups of various organic pollutants in the wastewater. The FT-IR analysis was performed in the range of the IR region at 4000–500 cm^{-1}, as shown in Fig. 3c. The results showed that the WS-1 broadband at 3436 cm^{-1} indicated the presence of alcohol functional groups and the strong intensity of phenol bonds with O-H due to the presence of various organic compounds. The broadband region at 2923 cm^{-1} indicated the presence of functional groups, namely alkenes C-H, alkyls C═C, and alkene. This demonstrated the presence of fatty acids, which were by-products of the pulping process during papermaking. The absorption at 1021 cm^{-1} represented the functional group of sulfur, namely sulfoxide S═O, and ether linkage =C-O-C, and their bonds are strong and medium, respectively. Ether is produced by the grafting of epichlorohydrin onto the lignin from the pulp and paper industry. The comparative analysis with WS-2, which was collected near the plant root, showed broadband at 3480,781, and 681 cm^{-1}. The peaks were decreased at 3480 cm^{-1} and were related to the functional group O-H, where the presence of alcohols and organic acids have been noted. The broadband at 687 cm^{-1} represented an aromatic compound with the Ar C-H bond, which was medium-strong, and alkynes bonds, namely =C-H stretches, that were strong and sharp. The FT-IR results indicated that the change of function groups occurred due to the exchange of metabolites in the wastewater during the in-situ phytoremediation.

3.4. Identification of organic compounds

The GC-MS analyses of the organic pollutants were extracted from the discharged wastewater after the secondary treatment before and after phytoremediation, this showed the presence of the different metabolic products of the lignocellulosic complex (Fig. 4a–b). The compounds, their relative abundance, and similarity percentages were identified at different RT using the NIST library (Table 2). The detailed nature of the identified compound indicated the presence of various metabolic products of lignin from wood generated during pulping and bleaching processes, which transformed various chlorinated toxic organic wastes. The GC-MS chromatogram of the main hazardous compounds before phytoremediation in the wastewater at RT-9.06, RT10.90, RT-13.73, RT-20.55, and RT-27.96 were identified, as listed in Table 2. Out of these, some were of endocrine-disrupting chemicals (EDCs) nature, identified as 2-methyl-4-keto-2-pentane-2-of TMS (RT9.06), octadecanoic acid (13.73), trimethylsilyl ester, hexadecanoic acid, trimethylsilyl ester (RT-10.90), and tetradecanoic methyl ester (RT-27.64), which have been reported as mutagenic and EDCs by the earlier

report (USEPA, 2012) [52]. Moreover, octadecanoic acid was detected in *Eucalyptus camaldulensis*, and it is reported as an extract of ethanol or benzene. The tetradecanoic and hexadecanoic acids were essentially plant-based fatty acids in humic substances, which have also been reported [53]. Moreover, eicosanoic (CAS) (RT-37.45) detected in the wastewater before the phytoremediation was also reported as EDCs by different regulatory agencies [52]. The eicosane was detected in the wastewater as an alkane group compound generated during the pulping and bleaching processes, and after secondary treatment.

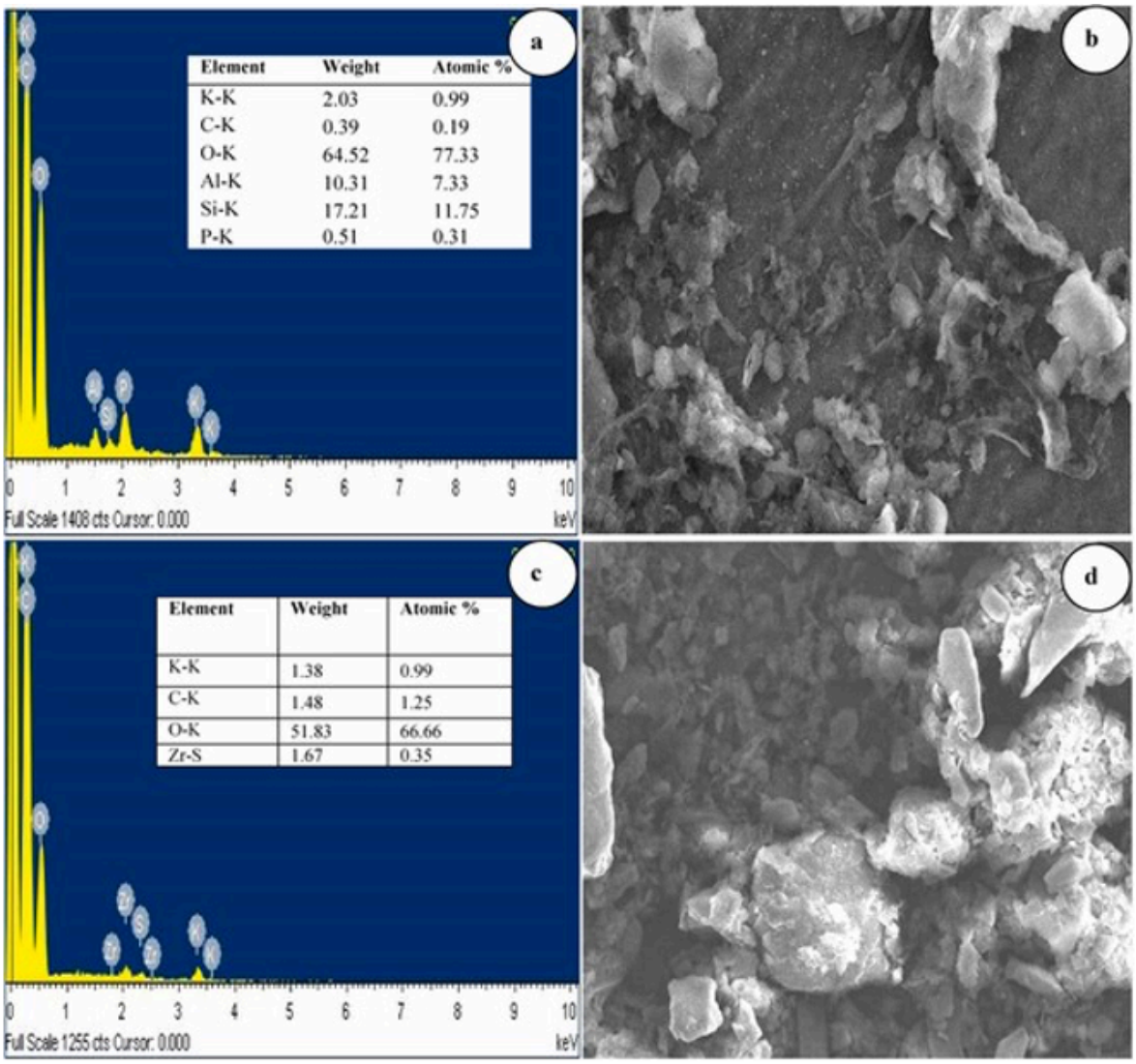

Fig. 2. Scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis of pulp and paper industry wastewater (a and b) before phytoremediation, (c and d) after phytoremediation.

Also, β-sitosterol (RT-48.77) was identified in the wastewater sample despite of the biological treatment of the industry. β-sitosterol is a plant sterol with a chemical composition like cholesterol and could have been generated during the wood waxing process of chemical

treatment; these EDCs can lead to DNA damage and carcinogenic effects. The chromatogram of organic pollutants in the wastewater sample after phytoremediation is shown in Fig. 4b. The major peaks are noted at RT25.54 (1-monopalmitin-ditms), RT-28.81 (1, 2, diphenyl-s (t-butyl) acephenanthrylene), RT-33.32 (2′ 6′-dihydroxyacetophenone), and RT 42.27 (Cinnamic acid-α-phenyl-trimethylsilyl ester). The compounds of the major peak are noted at RT-9.06, 13.17, and 20.55 also disappeared after phytoremediation. After the phytoremediation, the detected compounds at RT-19.56 (lactic acid, trimethylsilyl ether, trimethylsilyl) were observed in alkaline conditions. Lactic acid might be produced due to the fermentation and hydrolysis of cellulosic wood components during the pulping process. Cinnamic acid (RT-42.27), a by-product of lignin and hemicelluloses, was detected after the in-situ phytoremediation. During the chemical treatment of the alkaline pulping process, the ester linkages break down. Cinnamic acids are produced by the cleavage of ester linkages in guaiacol, which also produces esters by the reaction of their carboxyl and phenolics groups. Furthermore, at RT-47.17 ethanedioic acid, bis (trimethylsilyl) ester, or oxalic acid was identified. An unknown organic compound related to the carboxylic group was detected after phytoremediation. The results demonstrated that the native plants could breakdown the major toxic organic pollutants present in the wastewater into less harmful organic compounds and acids.

3.5. Heavy metals accumulation analysis

Plants collected from the wastewater bed were able to accumulate heavy metal throughout their parts, i.e., roots, shoots, and leaves (Table 3). Results revealed that the concentration of Fe was highest in *P. Hysterophorus* (in total 1109.5 mg kg^{-1}) leaves and shoots (518.80–348.93 mg kg^{-1}), followed by the root of *T. Terrestris* (504.20 mg kg^{-1}). Iron is an essential micronutrient, the synthesis of many enzymes and certain plant pigments is necessary and it also helps reduce sulfate and nitrate for energy production inside plants. A similar accumulation pattern of Zn in the plant was observed in different native plants. The highest concentration of total Zn (87.69 mg kg^{-1}) was observed in *T. Terrestris*, while the highest concentrations of Zn in roots, leaves, and shoots were observed in *M. dioica* (39.92 mg kg^{-1}), *P. hysterophorus* (47.83 mg kg^{-1}), and *A. Sessilis* (44.39 mg kg^{-1}), respectively. Zn is a valuable free ion micronutrient that is also a protein molecule and acts as a functional, structural, or regulatory cofactor for many enzymes [54].

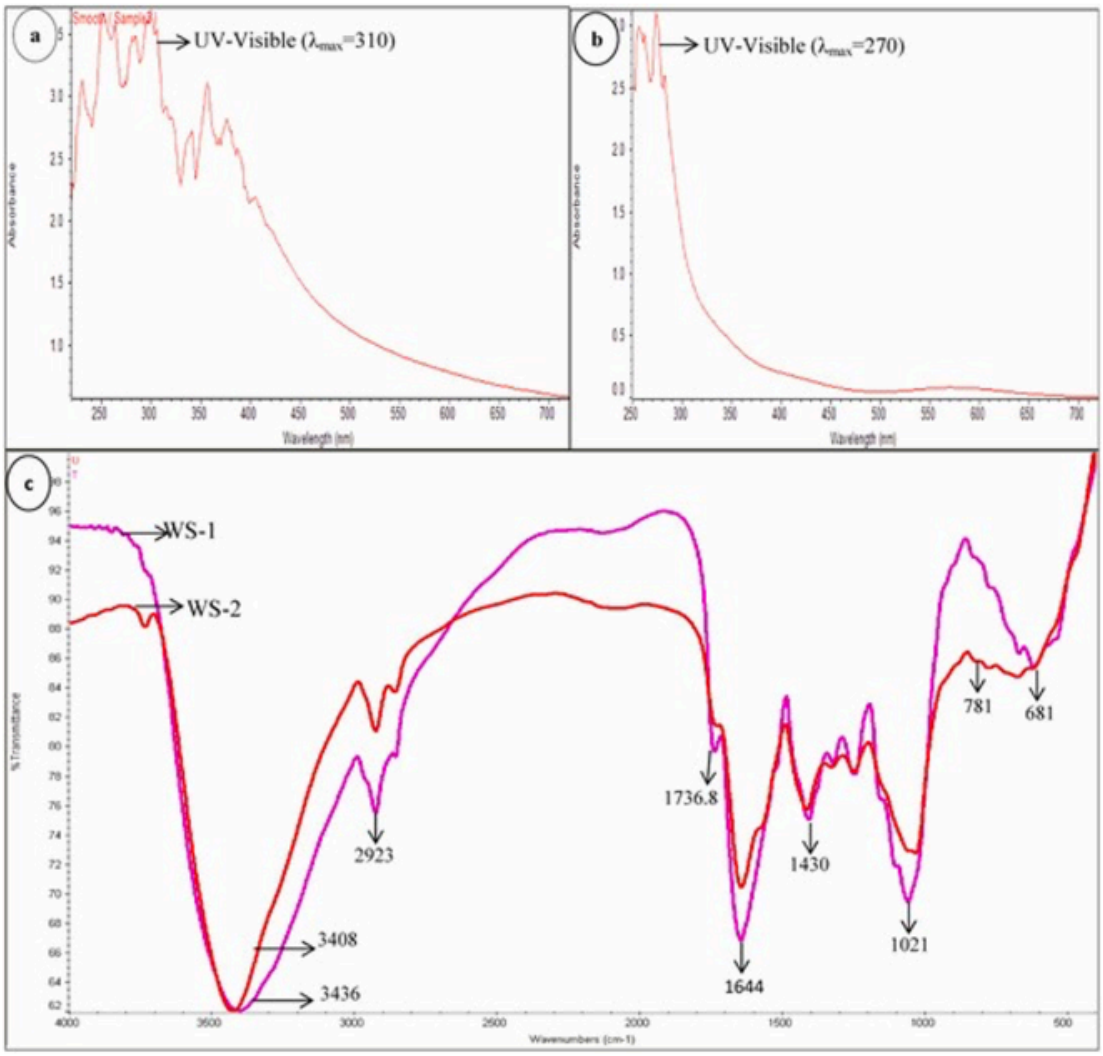

Fig. 3. Assessment of chemical constituent of pulp paper industry wastewater through UV–visible scanning spectrophotometric analysis (between 200 and 700 nm) and FTIR. (a) Spectrum analysis of UV–visible scanning spectrophotometer analysis of pulp and paper industry wastewater before phytoremediation (WS-1), (b). after phytoremediation (WS-2), (c). Comparative FTIR data analysis sample WS-1 and WS-2 before and after phytoremediation.

Besides, *Sedum alfredii* was also considered as a new species of Zn hyperaccumulation plant [55]. Also, Zn is important for RNA and DNA structure stability, DNA activity stabilization for enzymes produced, and RNA activity regulation for enzyme degradation and control of gene expression [83]. The regulation of iron uptake has been met and there is no observable toxicity detected in the plants. Excess iron can react with hydrogen peroxide and trigger the formation of harmful hydroxyl radicals, the production of antioxidants such as catalases is an indication that the native plants were able to cope with the high level of Fe present (Section 3.7). The increased lignification in the outer layers of the cortex and the vascular bundle (Fig. 5) may alter iron permeability, radial diffusion, and root-to-shoot

translocation of Fe, ultimately leading to a higher tolerance to high external iron levels [88]. Cu contributes to numerous biochemical and cellular functions like the transfer of photosynthetic electrons, and for many metalloproteinases, it is an important cofactor. However, a concentration of more than 20 mg kg^{-1} may become phytotoxic [89]. Here, we observed the native plants able to accumulate a higher concentration of Cu. The highest total Cu concentration was observed in *M. dioica* (92.04 mg kg^{-1}), while the highest concentrations of Cu in roots, shoots, and leaves were observed in *M. dioica* (57.80 mg kg^{-1}), *P. hysterophorus* (34.08 mg kg^{-1}), and *A. sessilis* (22.28 mg kg^{-1}), respectively. A previous study also presented similar observations. Cd concentrations range from 0.01 and 5 mg kg^{-1} in non-contaminated soils. Cd can be mobilized in the food chain, thereby affecting primary producers and consumers [56,57]. The investigated wastewater presented a high amount of Cd (9.36 mg kg^{-1}). *Thlaspi caerulescens* and *Arabidopsis halleri* have been identified as Cd hyperaccumulators in previous studies [58,59]. Several plants such as *Brassica campestris, Eclipta alba, Solanumnigrum, Rumex dentatus, Ranunculus sceleratus, Alternanthera philoxeroides, Phragmites communis, Typhaangustifolia*, *Cyperusesculentus,* and *Ricinus* have been previously reported for phytoremediation for several heavy metals and chlorolignin containing wastewater [47].

The concentration of total Cr was the highest in *C. procera* (29.30 mg kg^{-1}), and the highest concentrations in the root (6.98 mg kg^{-1}) and shoot (20.35 mg kg^{-1}) were observed in *C. sativa* and *M. dioica*, respectively, which showed high accumulation capacities. However, Cr enters plants through root exudates and complexation of organic acids, which increase Cr mobility through the xylem of the root [60]. The phytoremediation ability of native weeds to remove Cr in contaminated effluent from the South Kaliapani chromite mine area, Odisha, India, has been reported previously [61]. The accumulation of Pb was highest in *C. sativa* (21.67 mg kg-1), leaves of *A. sessilis* (17.65 mg kg^{-1}), and roots of *C. sativa* (9.83 mg kg^{-1}). Pb is pushed in on root surfaces by carboxylic groups of uronic mucilage acids through an active transport system [62,63]. The findings showed that selected plants compared well with reported studies and have metal tolerance ability to grow on the organometallic containing wastewater. There is wide variation intolerance, which depends on the stress duration, strength, and plant developmental stage, thus, necessitates further study to elucidate the mechanisms involved.

3.6. Bioconcentration factor and translocation factor

The ratio between heavy metal concentrations in plant roots and wastewater was investigated using the bioconcentration/bioaccumulation factor. *P. hysterophorus* showed maximum accumulation for Fe (11.63 mg kg^{-1}) followed by *A. sessilis*. However, the highest BCF was observed in *M. dioica* for As (269.46) followed by *C. sativa* (131.20), suggesting As was more readily absorbed than most metals, probably as a result of the formation of glutathione and phytochelatins (GSH/PC) complex. It was observed that most of the metals present in the wastewater were bioconcentrated in the native plants (BCF>1.0) except for Cd (Table 4). It could be attributed to the different mechanisms involved in metal resistance and also due to the fact that Cd does not have any known biological function. The deposition of metals from wastewater to plants depends on the chemical composition of the component, pH and other co-pollutants, can inhibit the mobility of metals, thus impeding their absorption and translocation in plants [28,29,64]. The highest TF was observed in *T. terrestris* for Cd (7.53), followed by *P. hysterophorus* for Cr (5.63). As a process of detoxification, ions could be sequestered in vacuole by binding with ligands, i.e., protein, organic acid, and peptides, leading to a high level of metalicious conditions [65]. The translocation value has been used to evaluate the phytostabilization of metals by the native plants growing at the disposal site of the wastewater [66]. Our data showed that except for Cd, all native plants in this study were able to bioconcentrate metals and Zn, Cr, Cu and As were mostly translocated to the shoots. BCF, and TF values that were less than one (<1.0), are deemed unacceptable for phytoremediation [67]. Our data should that except for Cd, all the native plants in this study were suitable candidates for phytoremediation. For Pb, although all the native plants were able to bioconcentrate, none of the plants produce a TF factor higher than one. Our results suggested while the other metals were translocated to the shoot, the predominant resistant mechanism of Pb in these native plants is via phytoextraction into roots, possibly binds to lignocellulose materials in the root biomass [90].

3.7. Effect of antioxidants and stomata

The native plants presented higher antioxidants enzyme activity compared to control plants growing at organometallic containing wastewater of the pulp paper industry. The activity of SOD was the highest in *C. sativa* (198.53 U/mL) and *C. procera* (196.65 U/mL) (Fig. 6). SOD is usually present in plants and algae of appropriate biochemical, enzymatic, and non-enzymatic antioxidant scavenging tools such as CAT, APX, and H_2O_2, that monitor the

concentration of reactive oxygen species (ROS) to regulate toxicity during environmental stress to sustain ionic homeostasis [69,70]. SOD and CAT catalyze superoxide decomposition and detoxification, including transforming anions of hydrogen peroxide into radical oxygen, and then converting them to O_2 and H_2O at ground level. However, that usually depends on hazardous elements as cofactors [71]. The concentration of POD was the highest in *C. procera* (191.31 U/mL) and *P. hysterophorus* (175.16 U/mL). Besides, the POD enzyme is linked to many processes, including cell formation, catabolism of auxins, lignification, as well as responses to abiotic and biotic stress [72,73]. In soybean and rice plants exposed to Cd in hydroponic experiments reported by hydroponic experiments, the enhancing of POD in conditions of toxic metals was observed as shown in Fig. 6 [74,75].

The concentrations of APX and CAT were the highest in *C. sativa* (56.30 U/mL) and *T. terrestris* (48.3 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. The APX using ascorbate to scavenge the peroxide molecule for photosynthetic machinery maintenance and another injury. Additionally, H_2O_2 content was highest in *C. sativa* (96.31 U/mL), the concentration was enhanced by the interference of toxic heavy metals, that would eliminate ROS and suppress lipid peroxidation. High level of H_2O_2 was observed, and the affected plants exhibited toxic effects in the form of plasmolysis, electrolytic leakage, and membrane damage. Metal toxicity in plants generated due to ROS causes oxidative damage, leakage of electrolytes, harm to cells, DNA inhibition, and mitochondrial toxicity [76]. Antioxidant enzymes that prevent the toxicity caused by ROS, such as SOD, APX, and CAT, were identified. They can protect plants from multiple adverse conditions [77,78]. Some antioxidants also act as substrates for phytochelatins synthesis and are essential for the detoxification of hazardous heavy metals such as Ni and Cd. In studies of plant protection mechanisms against the oxidative stress caused by heavy metals, antioxidants activity enhancement was observed in the leaves and stems of native plants. The pollutants in wastewater can cause damage to cuticular waxes, by entering the leaves through stomata and directly affecting plant morphology, physiology, and anatomical properties, and reducing transpiration rates during stress conditions. However, these native plants showed a high tolerance capacity for organometallic containing wastewater (Fig. 5a–d) [78]. The plant function for cytokinins and auxins has also been shown to regulate stomata behavior that controls open and close mechanisms in organometallic wastewater of the pulp and paper

industry [79]. The results indicated a significant level of native plant tolerance and a rapid increase in the transpiration rate.

RT	Compound Name	Relative abundance	Nature of compounds	% similarity with NIST Library	Toxicity
Metabolites before phytoremediation					
9.06	2-Methyl-4-keto-2-pentan-2-ol 1TMS	41	Organic compound	67.18	Endocrine distrusting chemicals (EDCs)
10.90	Hexadecanoic acid, trimethylsilyl ester	68	Organic compound	78.01	Mutagenic and Carcinogenicity
13.46	D-Lactic acid- DITMS	80	Fatty acid	93.11	EDCs
13.73	Octadecanoic acid, trimethylsilyl ester	71	Saturated fatty acids	93.12	Ecological toxicity skin irritation
13.46	Phenol-4-ethyl-2-methoxy or 4-Ethylguaiacol	41	Organic compound	57.01	Carcinogenicity Reproductive Toxicity
19.85	(Z)-2, 2'-Dibromo-4, 4'-di-n-pentylstilbene	56	Organic compound	98.23	EDCs and fish reproduction toxicity
20.55	Dimethyl2, 2', 4, 4', 5, 5'-Hexamethoxy	36	Organic compound	90.02	Acute toxicity
27.64	Tetradecanoic methyl ester	84	Saturated fatty acids	97.13	EDCs, Animal toxicity
27.96	Pentacarbonyl {[2'-(mesitylethynyl) phenylamino]-(p-tolyl) carbene}-chromium	78	Organic compound	92.05	EDCs, comedogenic
36.09	Pentadecanoic acid, ethyl ester	84	Organic compound	74.35	EDCs,
37.45	Eicosane (CAS)	57	Acyclic, alkane	34.54	EDCs
45.10	Benzoicacid,2,6-bis [(trimethylsilyl)oxy]	81	Organic compound	95.14	Carcinogenic, Mutagenic or EDCs
48.77	β- Sitosterol trimethylsilyl		Organic compound	71.23	DNA damage, Genotoxicity
54.59	Hexadecane		Alkane hydrocarbon	41.00	EDCs, Cytotoxicity Genotoxicity
Metabolites after phytoremediation					
19.56	Lactic acid, trimethylsilyl ether, trimethylsilyl	94	Fatty acid	79.34	EDCs, hyperkeratosis
21.92	4-Mercaptobenzoic acid	93	Sulfonic benzoic	97.24	Unknown
25.54	1- Monopalmitin-DITMS	80	Fatty acid	93.11	Data not reported
28.81	1,2, diphenyl-s (t-butyl) acephenanthrylene	81	Organic compound	89.74	Carcinogenic, mutagenic
32.82	2',6'-Dihydroxyacetophenone	31	Organic compound	71.74	Induced toxicity in male rats
37.49	Phenol-4-ethyl-2-methoxy or 4-Ethylguaiacol	54	Organic compound	76.34	
40.26	9,12-octadecadienoic acid (z,z)-2,3-dihydroxypropyl ester	81	Organic compound	96.03	Hepatotoxicants and carcinogens
42.27	Cinnamic acid-α-phenyl-trimethylsilyl ester	68	Organic compound	82.02	Data not available
47.17	Ethanedioic acid,bis(trimethylsilyl)ester	42	Organic compound	24.22	Unknown

Table 2 Identified organic pollutants by GC-MS in the TMS derivatized dichloromethane extracts of pulp and paper industry wastewater secondary treatment.

3.8. Histological observations by TEM analysis

The TEM analysis of root tissue showed the presence of metals in the intracellular space, cytoplasm, vacuole, and cell wall of the plants (Fig. 7a–f). Examining the accumulation and storage of heavy metals in various plant cells is a good way to understand the plant physiological and morphological conditions after exposure to heavy metals in the contaminated site [80]. Accumulation and detoxification of metals occurred in larger amounts

in the plant cell tissues through the formation and deposition of metal granules by multi-vacuoles in the cell wall [81]. Therefore, metal deposition near the cell wall in *M. doica* is essential for the tolerance of heavy metals because it prevents free metal ions from circulating in the cytosol. This is *new* information on heavy metal detoxification by native plants. Similarly, the *A. sessilis* root tissue presented information about metal accumulation in the middle lamella, cell wall, cytoplasm, and vacuole. The anatomical observation of *C. sativa* root tissue was shown normal thickness for heavy metal accumulation in the cell wall, and the lack of cell damage or noticeable changes in the plant demonstrated its tolerance mechanism. The histological observation of metal accumulation in native plants growing on pulp paper mill contaminated sites was observed by earlier workers [83]. Based on the analysis of different parameters, native plants showed good capacity against heavy metals derived from persistent organic pollutants.

Table 3 Heavy metal accumulation (mg kg^{-1} DW) in the root, shoot, and leaves of various 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, $p \leq 0.05$), BDL= below detection limit. R- Root, S- Shoot, L- Leaves.

Plants name	Plant part	Mn	Pb	Cd	Zn	Cr	Fe	Cu	Ni	As
P. hysterophorus	**Root**	13.65±0.93^{a}	1.94±0.27^{b}	2.51±0.12^{a}	25.42±2.17^{b}	2.32±0.28^{c}	241.80±14.50^{c}	22.91±1.91^{b}	1.35± 0.50^{b}	2.36±1.01^{a}
	Shoot	13.33±1.76^{a}	Nil	Nil	13.42±1.18^{c}	13.06±0.90^{a}	348.93±9.71^{b}	34.08±2.60^{a}	0.74± 0.09^{b}	2.40± 0.42^{a}
	Leaves	11.96±2.22^{a}	4.41±0.62^{a}	1.67±0.48^{b}	47.83±4.50^{a}	4.60±0.51^{b}	518.80±40.53^{a}	14.68±1.27^{c}	3.08± 0.53^{a}	1.14± 0.36^{a}
	Total	38.94±4.91	6.35±0.89	4.18±0.60	86.37±7.85	19.98±1.69	**1109.5**±64.74	71.67±5.78	5.17± 1.12	5.90±1.79
Accumulation pattern		**R> S> L**	***L > R***	***R> L***	***L>S>R***	***S>L> R***	***L> S> L***	***S > R > L***	***L>R> S***	***S>R > L***
T. terrestris	**Root**	58.14±4.90^{a}	4.11±0.35^{a}	0.15±0.10^{c}	21.92±1.47^{b}	4.76±0.38^{b}	504.20±9.88^{a}	8.48± 1.01^{c}	2.83± 0.36^{a}	2.86± 0.60^{a}
	Shoot	Nil	2.44 ±0.09^{b}	1.13±0.12^{a}	32.12±3.31^{a}	7.01±0.42^{a}	117.26±5.35^{c}	14.53±1.47^{b}	0.95± 0.18^{c}	1.79± 0.28^{b}
	Leaves	10.24±1.99^{b}	Nil	0.35±0.40^{b}	33.65±3.25^{a}	4.88±1.10^{b}	198.01±12.94^{b}	19.15± 2.13^{a}	2.02± 0.20^{b}	1.57± 0.31^{b}
	Total	68.38±6.89	6.55±0.44	1.63±0.62	87.69±8.03	16.65±1.90	819.47±28.17	42.16±4.61	5.8±0.74	6.22±1.29
Accumulation pattern		***R>L***	***R > S***	***S> L> R***	***L > S> R***	***S> R> L***	***R> L > S***	***L ≥ S > R***	***R> L> S***	***R>S>L***
M. dioica	**Root**	48.58±1.87^{b}	0.38 ± 0.3^{a}	0.55±0.04^{b}	39.92±2.23^{a}	3.81± 0.35^{a}	377.13± 30.40^{a}	57.80±4.06^{a}	3.44± 0.23^{a}	46.25±2.60^{a}
	Shoot	43.35±1.76^{b}	Nil	1.44± 0.18^{b}	33.26±1.62^{b}	20.35± 3.92^{a}	Nil	22.18±2.99^{b}	3.36± 0.11^{a}	44.17±1.75^{a}
	Leaves	61.08±3.84^{a}	Nil	4.75± 0.66^{a}	24.50± 1.60^{c}	3.20 ± 0.29^{a}	213.06±11.19^{b}	12.06±1.29^{c}	1.70± 0.13^{b}	20.06±2.14^{b}
	Total	153.01±7.47	0.38±0.3	6.74±0.88	97.68±5.45	27.36±4.56	590.19±41.59	92.04±8.34	8.50±0.47	110.48±6.49
Accumulation pattern		***R>S>L***	***BDL***	***L>S>R***	***R>S>L***	***R>L>S***	***R>S***	***R >S >L***	***S>R>L***	***S>R>L***
A. sessilis	**Root**	15.24±1.16^{a}	5.74± 0.51^{b}	Nil	15.24±1.15^{b}	0.57± 0.07^{a}	484.40±14.41^{a}	8.62±0.85^{c}	21.15±1.04^{a}	5.92± 0.33^{b}
	Shoot	12.11±1.35^{b}	Nil	Nil	44.39±2.33^{a}	Nil	157.10±7.53^{c}	14.92±0.37^{b}	4.0± 0.20^{b}	12.51±1.79^{a}
	Leaves	8.62 ± 0.33^{c}	17.65±2.03^{a}	Nil	12.18±1.53^{b}	Nil	251.83±30.49^{b}	22.28±1.25^{a}	1.45± 0.17^{c}	8.38± 0.60^{b}
	Total	35.97±2.84	20.39±2.54	Nil	71.81±5.01	0.57±0.07	893.33±52.43	45.82±2.47	26.60±1.41	26.81±2.72
Accumulation pattern		***R>S>L***	***L>R***	***BDL***	***S>R>L***	***BDL***	***R>L>S***	***L > S> R***	***R>S>L***	***S>L>R***
C. sativa	**Root**	18.96±1.50^{a}	9.83±0.50^{a}	1.35±0.15^{a}	15.90±0.79^{b}	6.98±0.42^{c}	41.25±1.95^{a}	37.57±2.38^{a}	20.88±1.46^{a}	20.72±1.43^{a}
	Shoot	16.94±1.39^{a}	1.39±0.14^{b}	1.03±0.06^{a}	20.34±0.76^{a}	11.91±0.63^{a}	26.94±1.35^{b}	15.89±1.35^{b}	13.02±1.35^{b}	16.60±0.85^{b}
	Leaves	12.31±1.25^{b}	10.45±0.90^{a}	5.08±0.37^{b}	7.04± 0.19^{c}	9.05± 0.13^{b}	21.76±1.75^{c}	19.75± 0.89^{b}	6.82± 0.65^{c}	16.47±1.04^{b}
	Total	48.20±4.14	21.67±1.54	7.46±0.58	43.28±1.74	27.94±1.18	89.95±5.05	73.21±4.62	40.72±3.46	53.79±3.32
Accumulation pattern		***R>S>L***	***L>R>S***	***L>R>S***	***S>R>L***	***S>L>R***	***R>S>L***	***R > L>S***	***R>S>L***	***R>S>L***
C. procera	**Root**	20.25±0.90^{a}	8.68±0.38^{a}	1.30±0.17^{b}	12.15±0.40^{b}	6.74± 0.50^{c}	42.62±2.26^{a}	7.23± 0.31^{c}	18.34 ±0.74^{a}	5.41±0.60^{a}
	Shoot	13.02±0.59^{b}	2.09±0.17^{c}	1.74±0.39^{b}	23.05±1.23^{a}	13.28±0.81^{a}	28.73±1.37^{b}	17.10±0.68^{a}	17.35±0.73^{a}	4.64±0.40^{a}
	Leaves	19.86±1.70^{a}	7.96±0.26^{b}	7.32±0.30^{a}	9.74± 0.28^{c}	9.28± 0.33^{b}	20.32±2.02^{c}	9.84± 0.70^{b}	9.25± 0.42^{b}	4.14±0.79^{a}
	Total	53.13±3.19	18.73±0.81	10.36±0.86	44.90±1.91	29.30± 1.64	92.08±5.65	34.17±1.69	44.94±1.89	14.19±1.79
Accumulation pattern		***R>L>S***	***R>L>S***	***L>R>S***	***S>L>R***	***S>L>R***	***R>S>L***	***S>L>R***	***R>S>L***	***R>S>L***

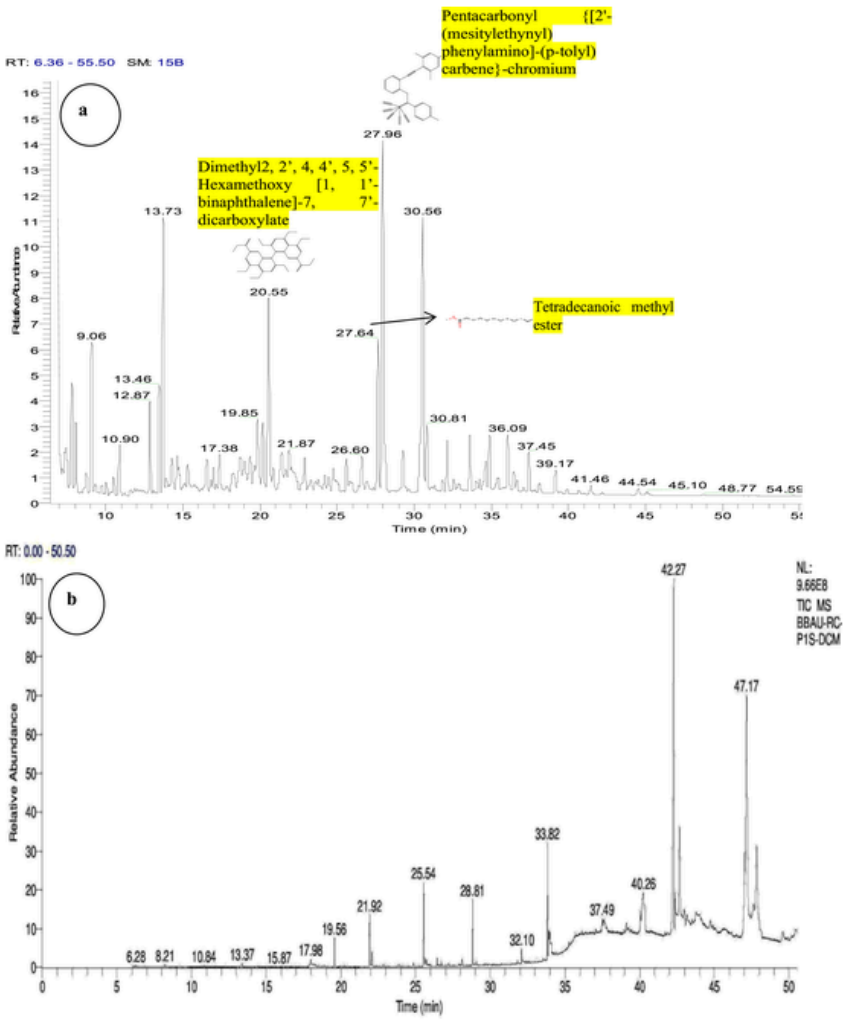

Fig. 4. Total Ion Chromatogram (TIC) of trimethylsilyl derivatized detected residual organic pollutants in the dichloromethane extract from pulp and paper industry wastewater. (a) Before the phytoremediation (b) After the phytoremediation.

Table 4. Bioconcentration factor and translocation factor of different heavy metals accumulation (mg kg^{-1} DW) by various plants of a different part in the root shoot and leave on disposal site of pulp and paper industry wastewater site.

Native plants	Bioconcentration Factor (BCF)							
	Mn	**Pb**	**Zn**	**Cr**	**Fe**	**Cu**	**Ni**	**As**
P. hysterophorus	71.84	32.98	190.40	32.75	39.43	14.31	314.7	17.34
T. terrestris	306	15.57	220.80	15.96	18.91	38.67	149.82	14.33
M. dioica	**255.6**	**168.42**	121.24	19.94	25.28	56.74	123.25	**0.88**
A. sessilis	46.76	11.14	317.58	**133.33**	19.68	38.05	20.04	6.92
C. sativa	80.21	16.51	304.40	10.88	**231.15**	**87.30**	20.30	1.97
C. procera	106.57	17.37	**398.35**	11.27	223.72	45.36	23.11	7.57
	Translocation Factor (TF)							
	Mn	**Pb**	**Zn**	**Cr**	**Fe**	**Cu**	**Ni**	**As**
P. hysterophorus	**10.40**	5.251	9.175	9.246	16.24	36.12	0.0	2.36
T. terrestris	4.025	2.312	7.123	6.249	15.24	**41.29**	0.245	5.36
M. dioica	4.022	8.015	8.258	2.561	14.27	27.56	0.558	2.94
A. sessilis	4.076	7.468	4.244	6.254	12.14	17.81	1.374	8.63
C. sativa	5.203	1.603	6.764	7.560	11.23	11.49	1.297	**11.34**
C. procera	8.24	6.124	9.182	9.266	**18.65**	19.32	0.0	6.64

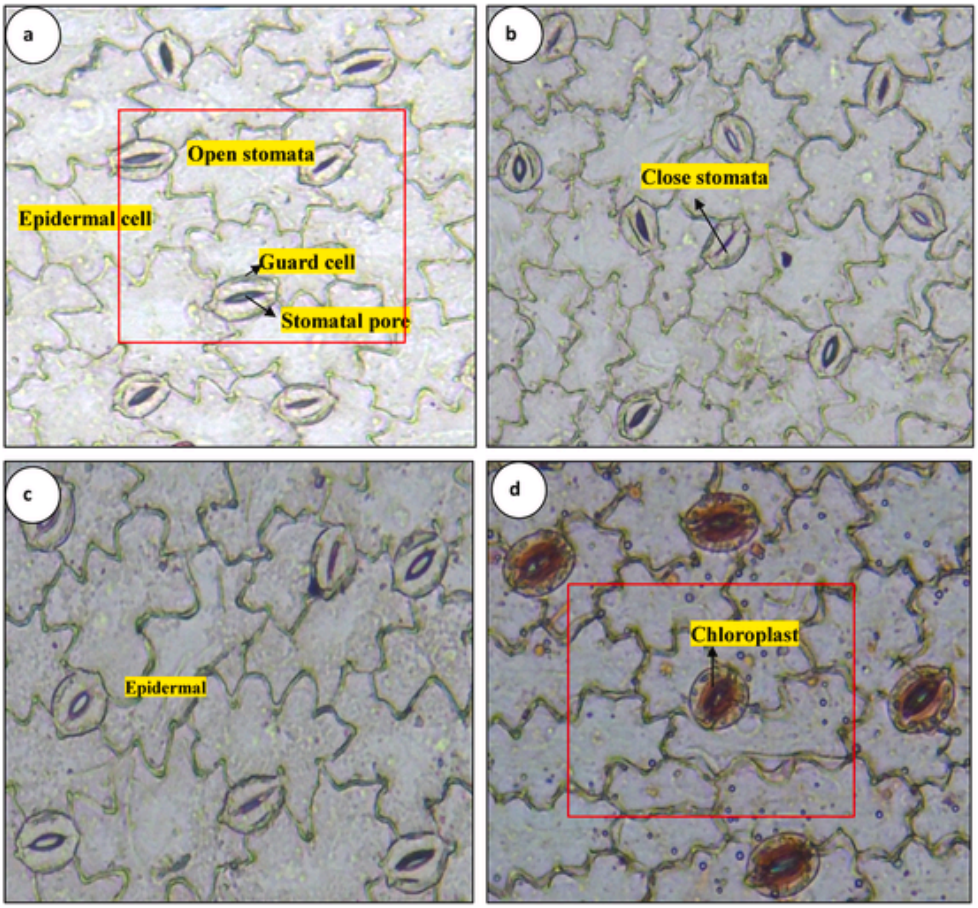

Fig. 5. Stomata observation by phase contrast microscope of in leaf portions (abaxial epidermal layer) showed the opening status of stomata apparatus of different hyperaccumulator plant grow on the contaminated site of pulp and paper industry wastewater.

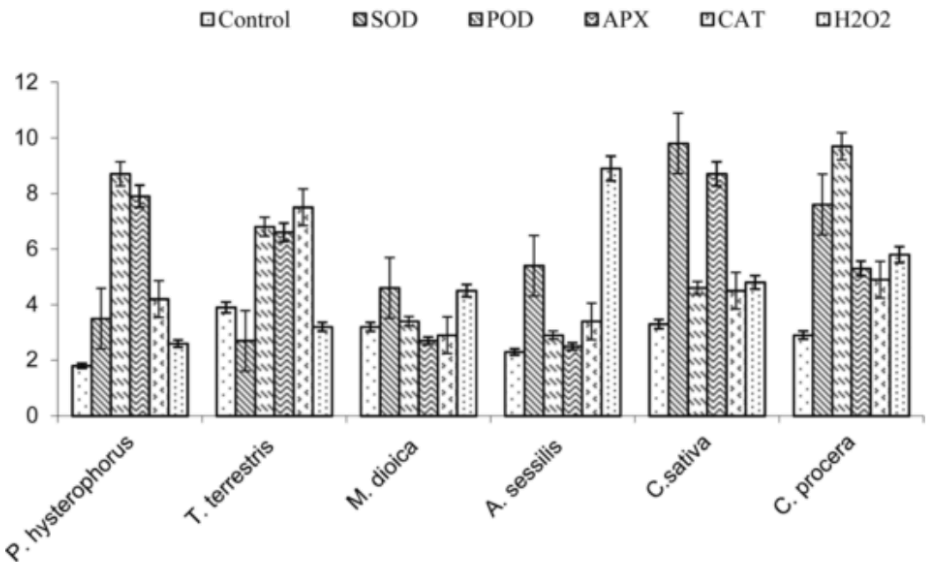

Fig. 6. Comparative analysis of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and hydrogen peroxidase (H_2O_2) activity in leaves of native plants growing at pulp and paper industry wastewater and normal agriculture land (Control).

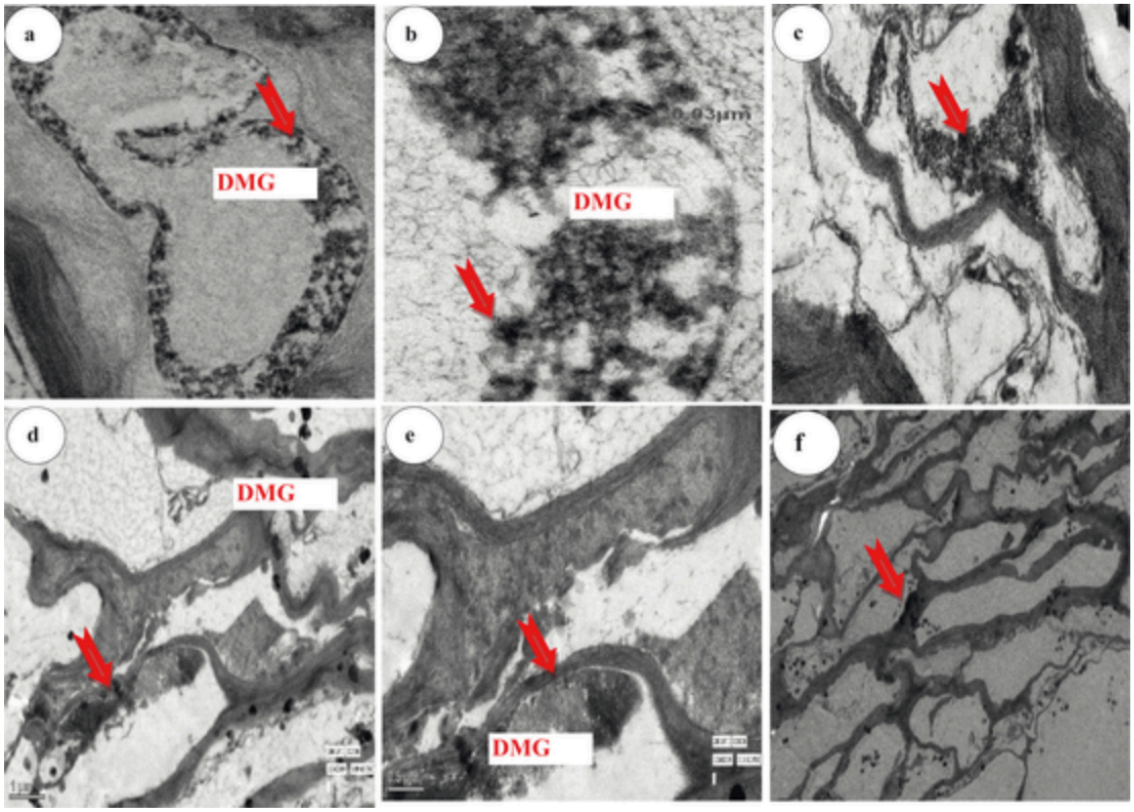

Fig. 7. Translocation electron microscopy (TEM) analysis of selected native hyperaccumulator plants root after in-situ phytoremediation of heavy metals from pulp and paper industry wastewater showing deposited metal granule (DMG) in all figures (a-f) inside the cells.

3.9. Phytoremediation as part of a multidisciplinary treatment strategy

Our data showed that the water parameters of pulp and paper industry wastewater were significantly above the permissible limit (Table 1). The treatment facilities are ineffective in removing the contaminants in the wastewater, in particular, organometallic pollutants, before discharging them into the surrounding aquatic environment. The presence of these pollutants, many are endocrine disruptive chemicalsin nature, poses danger to both human health and the environment [91–94]. This is further acerbated by the huge volume of wastewater generated by the industry- an estimated 3 bln m^3 is being produced globally [95]. As the regulations on permissible discharge tighten, the cost of wastewater treatment in the pulp and paper sector is expected to reach $1.6 billion in 2020 [96]. In developing countries such as India, the pulp and paper industry relies heavily on conventional treatment methods including traditional physicochemical (e.g., sedimentation, coagulation and precipitation, adsorption, and chemical oxidation) and biological methods (e.g., activated sludge process) [97]. More advanced and promising methods such as membrane filtration, reverse osmosis, or membrane bioreactors,

advanced oxidation techniques are expensive and/or require high energy input. A more strategic management strategy that is eco-friendly and economically attractive is therefore needed to tackle this problem in developing countries. Phytoremediation is a promising and sustainable technology that can be integrated to further treat wastewater. Phytoremediation using native plants can serve a dual purpose of site remediation and ecological restoration [98]. Native plants provide an ideal residence for the microbial community in their rhizosphere with the enzymatic ability to accumulate, stabilize, biodegrade, or volatilize various inorganic and organic contaminants [99–101]. The research carried out in this study demonstrated the capability of the six native plants obtained in a site polluted by pulp and paper wastewater, they would be ideal candidates to be used in an integrated treatment system to enhance the removal of toxic organometallic pollutant.

4. Conclusions

The study concluded that the pulp and paper industry wastewater contained several heavy metals and various detected organic compounds, including 2-Methyl-4-keto-2-pentane-2-ol 1TMS, hexadecanoic acid, trimethylsilyl ester, octadecanoic acid, trimethylsilyl ester, (Z) − 2, 2′-dibromo-4, 4′-di-n-pentylstilbene, dimethyl 2, 2′, 4, 4′, 5, 5′hexamethoxy, eicosane (CAS), β-Sitosterol trimethylsilyl, and hexadecane. Many of these organic pollutants were identified under the category of EDCs and mutagenic compounds. The studied native plants showed a major role in situ phytoremediation capability to improve the parameters of the discharged wastewater such as BOD and COD by 60% and 44%, respectively. Total phosphorous and nitrogen were found to improve by 71% and 57%, respectively; while the lignin and chlorophenol content was improved by 54% and 75%, respectively. For heavy metal removal, an improvement range between 23% and 60% was observed. Although the final concentration of the wastewater was still above the permissible limit, this field study provided evidence of metal resistance of these native plants. Further histological observations of plant tissue confirmed the accumulation of various metals in their different parts as an adaptive feature of the plants for phytoremediation of different heavy metals from the complex organometallic industrial waste in the field. The high bioconcentration and translocation factor supported the metal accumulation potential of the plant without any adverse effect, and they all tend to hyperaccumulator plants. Hence, these plant species may be used on a field scale for eco-restoration of the polluted site. However, more research is needed to optimize their performance and integrate them with the existing wastewater treatment process.

CRediT authorship contribution statement

Pooja Sharma: Writing - original draft, review & editing. **Diane Purchase:** Conceptualization, Data analysis, Writing - review & editing. **Ram Chandra:** Conceptualization, Visualization, Project administration, Funding acquisition.

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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