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Distillery wastewater detoxification and management through phytoremediation employing *Ricinus communis* L.

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Abstract

This study aimed to assess the phytoremediation potential of *Ricinus communis* L. for heavy metals remediation via rhizospheric bacterial activities for distillery wastewater detoxification and management. Results revealed that distillery wastewater contained high levels of metals and other physico-chemical pollution parameters that could cause environmental pollution and aquatic toxicity. The identified bacterium produced several plant growth-promoting compounds including siderophores, ligninolytic enzymes, and indole acetic acid that resulted in nutrient enhancement and improved mineralization of metals in the plants during stress conditions. The bioconcentration factor (BCF) of all the metals examined were > 1, which showed that these metals are accumulating in the root, shoot, and leaves of *Ricinus communis* L. Most of the metals are stabilised in the roots but Pb, Cd and Zn were translocated more to the shoots (TC>1). The ability of *Ricinus communis* L. to grow in metals-containing distillery wastewater and reduce heavy metals and organic contaminants suggests that it can be used to provide an effective treatment of distillery wastewater. The use of *Ricinus communis* L. is an eco-friendly tool for the reduction of organometallic contamination and protecting agricultural land.

Keywords: Distillery wastewater, Phytoextraction, Scanning electron microscopy, Translocation factor, Remediation strategy *Ricinus communis* L.

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1. Introduction

The development of the industry is a major reason for high levels of environmental pollution (Esmaeili and Beni, 2015; Sharma and Rath, 2020). Substantial amount of metals are released and discharged directly into receiving water and soil as a result of anthropogenic activities, causing problems in human health and the environment (Jiang et al., 2019; Sharma et al., 2021a). There are nearly 320 distilleries currently in operation in India, discharging approximately 1500 tons of wastewater and sludge into the aquatic environment daily. Distilleries wastewater (DWw) contains very high organic and suspended solids levels, and due to its dark color, reduces the photosynthetic activity and dissolved oxygen content in water. Melanoidins are the most recalcitrant color pollutants in DWw and known to cause environmental and health concerns (Arimi et al., 2015; Chowdhary et al., 2017). The presence of harmful carcinogenic and androgenic pollutants such as endocrine- disrupting chemicals, recalcitrant toxic compounds, phenolics, melanoidins, organic acids, heavy metals and other persistent toxic compounds made sugarcane molasses-based distilleries a major source of environmental pollution (Tripathi et al., 2021a). Reuse of distillery wastewater for crop irrigation could result in some of these metals being taken up via contaminated food and stored in the lungs and other organs (Benson et al., 2018; Huang et al., 2018; Ngo et al., 2020). Many bacterial species, such as *Micrococcus* sp. *Flavobacterium, Pseudomonas, Bacillus* and *Enterobacter have been investigated for heavy metals bioremediation.* Bacteria have excellent biosorbent ability due to the high surface ratio and large number of active chemisorption sites in their cell walls (Delil et al., 2020). Bacterial communities like *Bacillus, Rhodococcus, Paenibacillus, Acidovorax, Alcaligenes, Mycobacterium,* and *Pseudomonas* are also able to enhance the phytoremediation process. For example, they can increase metal availability via the production of polysaccharides and polymers (Zhang et al., 2020). In addition to physico-chemical factors such as soil quality temperature, moisture, and light characteristics that influence plant root growth and development, these rhizobacteria also affect the efficiency of phytoremediation (Liu et al., 2017). By adopting various strategies, including the production of antioxidant enzymes, subcellular localization of metals, and organic acid exudation, these plant-growth-promoting bacteria, such as *Pseudomonas* and *Bacillus,* alleviate abiotic stresses in plants (e.g., heavy metal toxicity), enhance plant growth (Wang et al., 2020) and promote plant life (Sharma et al., 2020a).

The toxicity of metals can affect the reproductive system of plants as well as marine organisms, resulting in delayed egg hatching, physical deformity in fish, and larval death (Fatima et al., 2014). Numerous fish species are strongly affected by pollution, such as endocrine hormone deficiencies, genetic anomalies, and oxidative stress (Javed et al., 2016). *Caenorhabditis elegans* has been used as a model for the toxicity assessment of industrialization and urbanization wastewater (Jiang et al., 2016; Cadkova et al., 2020). According to the Environmental Protection Agency (EPA), heavy metals such as Cu, Cd, Pb, As, Cr, Ni, and Hg are the most common environmental pollutants.

Even low concentrations of heavy metals can cause a range of toxicity to humans and plants (Sharma et al., 2020f, 2021b). Cadmium, arsenic, and lead are amongst the two most toxic contaminants worldwide (Oconnor et al., 2019). Any plant species, with high tolerance, can thrive and reproduce in metallic soils i.e., hyper-resistance to usually toxic amounts of heavy metals in growing substrates. For example, the phytoremediation ability of species of *Salicaceae* was recently reported (Simiele et al., 2020). Phytoremediation using native plant species, in particular, is an attractive and environmentally friendly strategy to remediate hazardous metals from wastewater (Ekperusi et al., 2019). Some of the most important measures of phytoremediation efficiency are biological concentration factors such as the bioconcentration and translocation factors. Hence, metal-accumulating plants like *Cannabis sativa, Zea mays, Thelypteris palustris, Chenopodiaceae Typha latifolia, Nicotiana tabacum*, and *Helianthus annuus* L. are highly efficient in metal absorption due to their large biomass. As a green metal decontamination technique that is economically viable, environmentally friendly, and socially acceptable (Singh et al., 2017; Sharma et al., 2020c), phytoremediation can be applied to improve distillery wastewater management. *Ricinus communis* L., is a species of perennial flowering plant in the spurge family, *Euphorbiaceae.* It can tolerate high amounts of metals (Yeboah et al., 2020) and has been shown to phytoextract heavy metals in distillery sludge (Kumar et al., 2021). However, the role of rhizobacteria has not been fully explored. This research investigates the relationship between metals and other co-pollutants with *Ricinus communis* L., the role of the plant growth promoting rhizobacteria and their joint phytoremediation potential.

2. Materials, methods and processes

2.1. Sample collection and site description

The sample was collected from M/s Unnao distillery located at Unnao, Uttar Pradesh, India (26°320″ N, 80°30′0″E). Freshly disposed distillery wastewater samples were collected in the pre-sterilized tank (20 L) near the disposal site (Tripathi et al., 2021b). Luxuriantly growing *Ricinus communis* L. (*Spurges*) plants were collected based on their abundance. To eliminate the adherent soil particles, the extracted plants were vigorously cleaned with a calcium chloride.

2.2. Estimation of heavy metals and Physico-chemical parameters

The total metal (Cr, Mn, Fe, Cu, Ni, Zn, and Pb,) contents in the DWw was measured using atomic absorption spectrophotometry (AAS) (ZEEnit 700, Analytic Jena, Germany) (APHA, 2012; Sharma et al., 2020). The physico-chemical properties of the samples from both locations (wastewater and near the rhizospheric zone of the plant), including pH (Orion pH meter-Model-960, Thermo Scientific, FL, USA), electrical conductivity (EC), chloride (Cl^-), sodium (Na^+), BOD, COD, and nitrate, were analyzed and estimated. The phenol content of DWw was measured using the American Public Health Association's standard methods (APHA, 2012; Chandra et al., 2018b).

2.3. Scanning electron microscopy examination of DWw

Detailed microscopic analyses of microbial and metals attachment as well as organic polymers formation were carried out using Scanning Electron Microscopy (SEM, QUANTA FEG 450, FEI, and the Netherlands) analysis. For SEM analysis of metals and other carbonyl compounds in DWw, one gram of the DWw sample was put in a hot air oven till dry (Evolution-201, Thermo Science, USA) (Sharma et al., 2021a).

2.4. *Organic pollutant analysis through gas chromatography-mass spectroscopy*

To identify the organic compounds in the DWw samples, analysts used gas chromatography-mass spectroscopy (GC–MS). Trimethylsilyl was used to derivatized the extracted samples (TMS) and the prepared samples were injected into the GC–MS instrument (Trace GC Ultra Gas Chromatograph; Thermo Scientific, FL, USA) (Sharma et al., 2020e).

2.5. *Isolation and plant growth-promoting activity of isolates*

2.5.1. *Indole-3-acetic acid and siderophores activity*

Siderophores formation was observed by inoculation of a 24 h culture of the isolated bacterial strain on to Chrome-azurol-S (CAS) medium. Siderophore production was confirmed by the development of orange coloration around the colonies. For the estimation of indole-3-acetic acid (IAA) activity of the isolated bacterium, the culture was inoculated into tryptone yeast extract broth containing L tryptophan (200 μg / mL) and incubated for 72 h at 28 °C in the dark. IAA production was confirmed by the development of red color upon the additional of Salkowski reagent and further incubation for 1 h at 37 °C

2.5.2. *Ligninolytic enzyme activity*

The ligninolytic enzyme activity of the bacterial isolated was investigated using various substrates (phenol red, Azur B, and guaiacol). Production of manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase was observed on the plate according to the standard procedure (Sharma et al., 2020).

2.6. *DNA isolation and sequencing of the isolated strain*

2.7. *DNA isolation and sequencing of the isolated strain*

The bacterium was isolated by serial dilution and plate streak method. For the identification of the isolated bacterium, the DNA was isolated from a fresh bacterial colony for 16S rRNA sequencing (Himedia Pvt Ltd). The bacterial strain was identified using the Cowan and Steels Manual for Bacterial Identification (Barrow and Feltham, 1993). Total DNA was extracted from an overnight bacterial culture using a genomic DNA extraction kit (Real Biotech Corporation). Universal primers 16Sf (5′ CAGCAGCCGCGGTAATAC 3′) and 16Sr (5′ TACGGCTACCTTGTTACG 3′) were used for amplification of the 16S rRNA gene. The PCR reaction mixture contained an assay buffer 5 μl, forward primer 1 μl, reverse primer 1 μl, dNTP 1 μl, template 2 μl, tag polymerase 1 μl and the final total volume was made up 50 μl with Milli Q. Polymerase chain reaction was performed in a thermocycler (Sure Cycler 8800; Agilent Technologies, Malaysia) under the following conditions, denaturation at 94 °C for 1 min, followed by annealing at 55 °C for 1 min and extension at 72 °C for 2 min, for 35 repeated cycles. Approximately 1500 bp region of the gene was amplified and the amplification product was gel purified using QIA gel extraction kit and sequenced. All query sequences and other homologous sequences available online in the NCBI (National Centre for Biotechnology Information) nucleotide database were saved in a single FASTA file format after retrieval.

DNA isolation and sequencing of the isolated strain In estimating the content of the metal deposited in the *Ricinus communis* L., plant tissue of the root, the shoots and leaves were cut into small pieces and oven-dried for 5 days at 70 °C until a consistent weight was reached. The absorptions of Zn, Cr, Cu, Mn, Ni, Fe, Pb, and Cd in the plant were determined by atomic absorption spectrometry (AAS) (Sharma et al., 2020b). Bioconcentration factors (BCFs) and translocation factors (TFs) were calculated to determine the efficiency of metal accumulation. The calculation of BCFs as the metals in the root and wastewater, while TFs is defined as the metal concentrations in the shoot and root (Yoon et al., 2006). The statical analysis of results verifies data inconsistency and validity data will be interpreted standard deviation mean. Using program Graph Pad, all data were subjected to Tukey’s Statistical Analysis Test (Ott, 1984) (Graph Pad Software, San Diego, CA).

3. Results and Discussion

3.1. Physico-chemical characteristic

The concentrations of heavy metals and other physico-chemical parameters in DWw were all significantly above the permissible limit (Table 1). The pH of the DWw ranged from 8.40 and EC measured at 1877.62 (μmhoscm^{-1}), but the samples obtained near the rhizospheric zone of *Ricinus communis* L. had neutral pH (7.01) and reduced EC (890.30 μmhoscm^{-1}). Similarly, the BOD (4234.96 mg L^{-1}), and COD (11452.22 mg L^{-1}) values near the rhizospheric zone of *Ricinus communis* L., also decreased. The combined action of hydroxides, carbonates, bicarbonates, calcium, and magnesium ions, which were used to adjust the pH of diluted molasses during the fermentation process, resulted in a higher pH value in distillery wastewater. The observation of these parameters revealed the phytoremediation potential of metals in DWw. The pH of distillery sludge ranged from 3.84 to 5.64 in our research, which was ideal for plant growth and development. The interaction of rhizospheric microorganisms with organometallic contaminants in DWw could explain the small decrease in in-situ phytoremediation wastewater pH. Furthermore, the plant–microbe interactions in the rhizosphere can increase metal bioavailability by secreting amino acids, organic acids, protons, and enzymes, which reduces wastewater pH and enables plants to thrive in polluted environments. In a contaminated environment, the low pH of wastewater could increase metal solubility and plant phytoextraction efficiency. Results showed that *Ricinus communis* L. can phytoremediate DWw. Eutrophication could be caused by the accumulation of high concentrations of ions and other co-pollutants in distillery wastewater, posing a danger to aquatic life. Different metals (in mg L^{-1}) like Mn (7.96), Cr (3.32), Zn (15.65), Cu (2.00), Fe (378.66), Pb (4.79), and Ni (7.89) were present in high concentration in the DWw. A three-fold reduction in most metal concentrations was observed in the samples collected near *Ricinus communis* L. (in mg L^{-1}) - Mn (2.45), Cr (1.76), Zn (7.19), Cu (0.30), Fe (123.77), Pb (0.86), and Ni (2.50). The high concentration of heavy metals in distillery wastewater may be attributed to the condensation, molasses fermentation, and distillation processes, which are then discharged as raw effluent after subject to anaerobic treatment once. Other factors that affect metal bioavailability and phyto-uptake in soils include pH, metal content, and water content, as well as the existence of organic acids, chlorides, sulfides, carbonates, organic compounds, and other co- pollutants in the root zone (Pinto et al., 2015).

Table 1. Physico-chemical characteristics of discharged distillery wastewater before and after plant growth, collected from M/s Unnao Distillery Pvt. Ltd. Unnano Uttar Pradesh, India.

Parameters	Distillery wastewater	After plant growth	CPCB, (2017)
pH	8.40 ± 0.26	7.01 ± 0.18[a]	7.54 ± 0.01
Biological oxygen demand	4234.96 ± 77.76	1800.23 ± 65.40[a]	47.00 ± 0.00
Chemical oxygen demand	11452.22 ± 188.32	5980.54 ± 119.08[a]	79.00 ± 0.01
Electrical conductivity	1877.62 ± 45.03	890.30 ± 28.70[a]	950
Total dissolve solid	9978.67 ± 230.32	5043.78 ± 100.87[a]	70 ± 0.00
Chloride	2578.54 ± 68.78	1076.43 ± 49.08[b]	11.82 ± 0.01
Total nitrogen	200.49 ± 7.68	123.61 ± 4.70[a]	9.90 ± 0.00
Phenol	7000 ± 63.90	233.09 ± 30.80[c]	–
Sulphate	14,543.76 ± 454.32	7654.10 ± 73.32[b]	–
Phosphate	40.03 ± 1.30	20.40 ± 0.79[b]	3.40 ± 0.01
Na^+	455.98 ± 5.89	59.34 ± 5.9[NS]	0.01
K^+	234.45 ± 09.19	129.76 ± 0.76	0.02
Heavy metals (mg L^{-1})			
Mn	7.96 ± 0.42	2.45 ± 0.34[b]	0.15
Cr	3.32 ± 0.78	1.76 ± 0.57[c]	0.01
Zn	15.65 ± 0.64	7.19 ± 0.34	1.28
Cu	2.00 ± 0.10	0.30 ± 0.05[NS]	0.19
Fe	378.66 ± 5.23	123.77 ± 6.67	1.45
Pb	4.79 ± 0.34	0.86 ± 0.32[c]	0.02
Ni	7.89 ± 0.87	2.50 ± 0.40[a]	0.04

All the values are Mean ± SE. (n = 3); Unit of all parameters is 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 $p > 0.05$, Central pollution control board (CPCB).

Various chemical parameters are measured after plant growth (in mg L^{-1}): phenol (7000–233.09), sulfate (14543.76–7654.10), potassium (234.45–129.76), phosphate (40.03–20.40), and sodium (455.98–59.34), these concentrations were significantly reduced compared to the raw DWw. These results gave a very good indication of the ability of *Ricinus communis* L. to phytoremediate heavy metals and its propensity to act as a phytoremediator. The frequent use of wastewater containing metals in irrigation has also demonstrated that hazardous metals accumulate in the human diet of plant species. Heavy metal concentrations are frequently monitored using plants as biomonitors. For example, the elevated DWw metals content along with other parameters were harmful to the germination of *Phaseolus mungo* L. seeds. The organic contaminants showed strong affinity of metallic ions which reduced the metal bioavailability to plants. Plants' ability to absorb metals may be attributed to enhanced bioavailability of metal as a result of the plant–microbe interaction (Sevik et al., 2020).

3.2. Morphological view of DWw and rhizospheric wastewater

DWw SEM image revealed fine oval needle-shaped crystals, indicating the presence of metals and polymer (Fig. S1a-b). Organic polymers and metallic crystals have been documented to have needle-like crystal properties (Liu et al., 2013). The SEM images revealed that the DWw had irregular components with wide surfaces for adsorption of different contaminants along with heavy metals and organic polymers. The crystal forms revealed the existence of various heavy metals. SEM analysis study showed that most of the pollutants had intimate contact with the *Ricinus communis* L. root zone.

3.3. Identification of organic pollutants

GC–MS chromatograms and the compound identifications were presented in Figures S2a-b and Table 2. The major dominant peaks in the DWw samples were observed at RT 7.64, 13.21, s19.36, 21.86, 26.84, 36.67, 43.20, and 49.32, respectively. This compound showed > 90% correlations with compounds described in the NIST library included with the program. The characterized of compounds 2-METHYL-4-KETO- PENTAN-2-OL 1TMS (RT-7.64), Octadecanoic acid (RT-13.21), Heptadecane, 2-methyl- (CAS) (RT-19.36), Heptadecane (CAS) (RT-21.36), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) (RT-26.84), Docosane (CAS) (RT-36.67), 2-Acetamido-3-(3-hydroxyphenyl) Propanoic acid (RT-43.20), and 3-t-Butyl-5-methyl-4-hexen-2-ol (RT-49.32), respectively. The identified compounds are known to be highly toxic, they are mutagenic, androgenic, and carcinogenic to the aquatic systems and humans. Table 2 summarises the toxicity of the pollutants identified. The identification and characterization of these organic pollutants shed new information on DWw as environmental pollutants; it indicated further analysis and assessment of environmental and health risk should be carried out before the safe discharge of the DWw into the receiving water. Similarly, plant fatty acids Heptadecane, 2-methyl- (CAS) (RT-19.36), and Heptadecane (CAS) (RT-21.36) are toxic to aquatic organisms.

Other minor peaks were also observed at RT values of 19.36, 21.72, 25.44, and 33.67, but the compounds were unknown or data not reported. The formation of these new compounds may be resulting from the microbial metabolic reactions or mineralization of the organic compounds. Almost every compound discovered was under the EDCs nature of fatty acids and corbonile compounds (Chandra et al., 2018a). Sugarcane molasses extract can be the main source of phenolic and carbon compounds after fermentation in DWw. Different types of detectable aromatic compounds can be the result of the carbonyl and amine group reaction at high temperatures in the sugar industry, which exists as a complex in cane molasses known as melanoidin. The involvement of several fatty acids in the DWw would slow down the metal accumulation and bioremediation processes in plants. These organic compounds can have harmful effects on plants and aquatic organisms as they enter the aquatic environment. Other organic acids, such as dodecanoic acid, butanedioic acid, and octadecanoic acid, are also classified as EDCs compounds. The occurrence of EDCs-derived compounds in DWw has provided ample evidence of the sediment's complex composition, which contains many harmful chemicals. All these compounds were generated during the anaerobic digestion of DWw or the fermentation process. Compared to the raw DWw, the number of detectable organic compounds and peaks in samples collected near *Ricinus communis* L. growth were significantly reduced (Figure S2b). The results demonstrate that some recalcitrant organic pollutants present in DWw were removed via phytoremediation and/or microbial metabolic reactions or the mineralization of compounds into new metabolites or value-added products (Table 2). This indicated that the *Ricinus communis* L and its associate rhizobacterium able to bioremediate the organic pollutants in DWw as well as aggregate metals present in the complex organometallic waste.

Table 2. Detection of residual organic pollutants by GC–MS analysis from distillery wastewater before and after phytoextraction.

Retention time	Compounds name in wastewater	Toxicity
7.64	2-METHYL-4-KETO-PENTAN-2-OL 1TMS	Hypertension, Stress
8.07	Octacosane (CAS)	Irritation to mucous membranes
9.14	Ethyl 5,6-epoxyhexanoate	Acute toxicity
13.21	Octadecanoic acid	Developmental toxicity, neurotoxicity
14.03	(R,S)-1-decyn-3-ol	Carcinogenicity, reproductive
17.70	N-cetyl thiocyanate	Eye tearing, muscle weakness,
17.98	Dodecane, 4-methyl- (CAS)	nose and throat irritation
19.36	Heptadecane, 2-methyl- (CAS)	Chest pain or retrosternal burning
21.86	Heptadecane (CAS)	Nausea and vomiting
25.78	Nonacosane (CAS)	Endocrine disrupting chemicals (EDCs)
26.84	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS)	EDCs
27.66	7-Methoxy-2,3-dihydro-2-phenyl-4-quinolone	Nausea and vomiting
33.67	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS)	Acute toxicity
34.11	Tricosane (CAS)	Acute toxicity
35.84	Undecane, 2-methyl- (CAS)	Abdominal discomfort, Nausea, and vomiting
36.67	Docosane (CAS)	Bloody vomiting, liver failure
38.74	PROSTAGLANDIN F1A TMS ESTER TRI TMS ETHER	Dilate the blood vessels
38.86	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Effect on eyes
42.94	(E)-6-(*tert*-Butyldimethylsiloxy)-2-ethyl-1-(trimethylsilyl)hex-1-ene	Nausea, and vomiting
43.20	2-Acetamido-3-(3-hydroxyphenyl) Propanoic acid	Premenstrual syndrome
44.69	2,2′-di-O-methylstenosporic acid	Data not reported
45.85	6-(4′-Chlorobutyl)undecan-6-ol	Data not reported
47.78	2-Nitro-5-Bromofuran	Central nervous system damage,
47.85	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Respiratory
48.14	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Hematological
49.32	3-t-Butyl-5-methyl-4-hexen-2-ol	Gastrointestinal
Retention time	Compounds name near rhizospheric zone of *Ricinus communis* L	Toxicity
8.03	Dotriacontane (CAS)	Data not reported
13.09	1,2-O-Isopropylidene-3-O-(p-*tert*-butylbenzyl)-á-D-fructopyranose	
19.36	Trimethylsilyl n-Dodecyl Ether	Data not reported
21.72	acrylic acid decyl ester	Unknown
25.44	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS)	Data not reported
33.67	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS)	Unknown
40.06	Neopentyl 2,2-dimethylpropanoate	Data not reported
49.28	3-*tert*-Butyl-5-methyl-4-hexen-2-ol	

*RT-retention time (in minutes), (TMS) trimethylsilyl.

3.4. Plant growth-promoting activity

3.4.1. Linginolytic enzyme production

Ligninolytic enzymes are important for the breakdown and detoxification of DWw pollutants. Various microbes have been reported to be capable of degrading the complex pollutants from industrial wastewater, like bacteria, actinomycetes, cyanobacteria, and fungi (Sharma and Singh, 2021). The production of MnP and LiP suggested that the bacterial isolates could secrete extracellular enzymes to degrade the lignocellulosic materials present in the DWw during the early stages of incubation to support the plant growth-promoting activity. Manganese peroxidase is a glycosylated heme protein with a molecular mass of 40–50 kDa. The existence of all three intracellularly ligninolytic enzymes in the isolated bacterium played a significant role in the removal of a wide variety of organometallic pollutants. Laccase (EC 1.10.3.2) is a copper-containing extracellular enzyme made up of monomeric, dimeric, and tetrameric glycoproteins. Janusz et al. (2017) found this mainly in microorganisms such as actinomycetes, fungi, and bacteria. Laccase is a multi-copper oxidase that, due to the presence of guaiacol, produces a dark zone for the related radical species by single-electron oxidation of organic compounds (Ladole et al., 2020).

3.4.2. Production of indole-3-acetoc acid and siderophore

Near the rhizosphere of *Ricinus communis* L., the isolated bacterium *Enterobacter aerogenes* IITRCS-12 (KU726958.1) produced the rhizosphere signalling molecule IAA. IAA is produced by a wide range of bacteria, its role in plant growth and pathogenesis is well understood. The role of IAA is an auxin for the regulation of the plant physiological and morphological system and use L-tryptophan as a substrate. IAA carried on a medium pink color and was put to the test for IAA quantitative estimation.

Another important feature of rhizobacteria that might enhance the plant growth and development is the production of siderophore. The isolated strain exhibited the change of greenish-blue color to yellow color during the siderophore production. After incubation, the maximum yield of siderophores was observed at 36 h before the production began to decline. Furthermore, siderophores have a lower affinity for metal complexes than $FeCl_3$. Metals such as Cd, Hg, and Co inhibited the formation and growth of siderophores due to metal binding behavior and increase the production of reactive oxygen species (ROS) in bacterial cells. The production of siderophores in bacteria showed stomatal conduction and improve plant health during the accumulation of iron from DWw.

3.4. 16sRNA sequencing of bacteria

The isolated bacterial strain was identified using the simple local alignment search tool (BLAST) and then sent to the National Center for Biotechnology Information (NCBI) based on 16S rRNA sequencing data. Moreover, 16S rRNA sequencing revealed a close relationship between the isolated strain *Enterobacter aerogenes* IITRCS-12 (KU726958.1). The sequencing of isolating the sequence is submitted to the GenBank database. The isolated bacteria are Gammaproteobacteria, which have a wide range of adaptations in the atmosphere when anoxia and light may or may not be present, and belongs to the chemo heterotrophic community.

3.6. Total metal accumulation and bioconcentration factors

The impact of metal uptake by the *Ricinus communis* L. was further examined to evaluate its ability for phytoremediation of DWw. *Ricinus communis* L. growing at the DWw assessed the accumulated metals (Cr, Zn, Cd, Ni, Pb, Cu, Fe, and Mn). As shown in Table 3, the parameter distribution of metal uptake in different parts of the plant suggested tolerance and ability of metal accumulation shown in (Fig. 1). To withstand metal stress and toxicity, plants employ a range of defense mechanisms, including the activation of various antioxidant enzyme systems, heavy metal binding to phytochelatins/metallothioneins, metal sequestration to vacuoles, and heavy metal absorption removal (Shahid et al., 2015). A previous study reported that the metal content of DWw, combined with organic compounds, disrupted the development of various plants' roots, and stunted their seed germination (Ha et al., Analysis of metals accumulation showed that the concentration of Fe and Mn was highest in the root (78.46 ±0.50 to 60.23 ±0.68 mg kg^{-1}) of *Ricinus communis* L. Iron is an essential micronutrient for the synthesis of many enzymes and certain plant pigments and it also helps to reduce sulfate and nitrate for energy production inside the plants. The presence of Fe in plant cells demonstrated the detoxication pathway of pollutants and help in the phytoremediation process. The results showed that the high concentration of accumulation (in mg kg^{-1}) of Fe (65.29 ± 0.40), Cu (59.40 ± 0.39), and Pb (40.09 ± 0.50) in the shoot of *Ricinus communis* L. This indicated that *Ricinus communis* L had the ability to resist high concentration of metals in DWw and absorb the metals in the root, shoot, and leaves. The highest concentration of Zn (34.15 ± 0.89 mg kg^{-1}) was observed in the shoot of *Ricinus communis* L. Zn is a micronutrient that is also a protein molecule that serves as a physical, structural, or controlling co-factor for a variety of enzymes. Zn is also important for DNA and RNA structures, the stabilization of DNA function for the enzymes generated, and the regulation of RNA and gene expression regulation. *Ricinus communis* L. was also capable of accumulating higher concentrations (in mg kg^{-1}) of Cu (22.64 ± 0.78), Cd (20.59 ± 0.20), Ni (29.09 ± 0.55), and As (20.75 ± 0.09) in the root tissues.

Table 3. Heavy metal accumulation (mg kg $^{-1}$ DW) in the root, shoots, and leaves of *Ricinus communis* L plant species growing contaminated site of distillery waste site. All the values are mean of three replicates (n = 3) ± standard deviation (SD), BDL: Below detection limit, R- Root, S- Shoot, L- Leaves.

Metals (mg kg $^{-1}$)	Different parts of *Ricinus communis* L.							
	Root		Shoot				Leaves	
Cu	22.64 ± 0.78		15.34 ± 0.50				8.30 ± 0.20	
Ni	29.09 ± 0.55		20.34 ± 0.60				12.40 ± 0.50	
Pb	19.45 ± 0.40		40.09 ± 0.50				15.30 ± 0.80	
As	16.25 ± 0.70		20.75 ± 0.09				15.70 ± 0.19	
Fe	78.46 ± 0.50		65.29 ± 0.40				39.08 ± 0.14	
Mn	60.23 ± 0.68		59.40 ± 0.39				40.10 ± 0.08	
Cd	09.09 ± 0.94		20.59 ± 0.20				19.06 ± 0.30	
Zn	29.59 ± 0.40		34.15 ± 0.89				20.49 ± 0.17	
Bioconcentration factor								
	Cu	**Ni**	**Pb**	**As**	**Fe**	**Mn**	**Cd**	**Zn**
	30.65	50.98	19.09	17.05	94.90	1.78	1.00	4.45
Translocation factor								
	0.78	0.78	1.78	1.00	0.045	0.78	1.89	1.01

The microbial community near the rhizospheric zone of the plant influenced the pH and other organic compounds along with metals accumulation in the plant (Sharma et al., 2020d). After anaerobic digestion, the distillery wastewater became alkaline (pH 8.67 0.16), which could affect metal absorption in plant cells. However, translocation of metals by *Ricinus communis* L. demonstrated the potential of the plant, and the pH in the rhizospheric soil gradually decreased. It may be caused by the interaction of microorganisms and plants in the rhizosphere, which results in an excess production of acids such as citrate, succinate, gluconate, oxalate, 2-ketogluconate, acetate, and malate being released from root hairs. According to our results, DWw as waste contains several metals and organic, fatty acids, and carbon-containing compounds. The majority of DWw pollutants are anionic, with a strong cationic metal-binding propensity. The rhizospheric microbial population has improved the bioavailability of metals in plants.

The BCF and TF measure the capacity of plants to absorb heavy metals. The BCF values of metals in plants revealed that they have the potential to metal phytoremediation in the roots. The metal absorption in DWw is generally low, but *Ricinus communis* L. had exceptionally high metal accumulation in different sections, indicating that these plants have a hyperaccumulation propensity. Besides, the deposit of metals from pollutants to plant roots is mainly dependent on the properties of soil components, pH, and other complex or co-contaminants that may impede the movements of metals, thus prevents its uptake and efflux in plants. *Ricinus communis* L had the highest BCF for Ni (41.29), and Cu (9.246). As a removal process, ions can be sequestered in vacuole by binding to ligands, i.e., proteins, amino acids, and peptides, leading to an increased degree of metalicilous environments. This may be because the plant's root nodule-forming bacteria encourage metal bioavailability in DWw to the plant. The plant's high phytoremediation efficiency and stress tolerance capability rate were indicated by BCF and TF > 1. Simultaneously, these plants have shown their capacity to absorb higher metal concentrations from DWw. Metal accumulation from DWw to root is affected by the chemical nature of the element that inhibits metal mobility in plants. The translocation value has been used to assess the phytostabilization of metals by plants developing at the wastewater storage site.

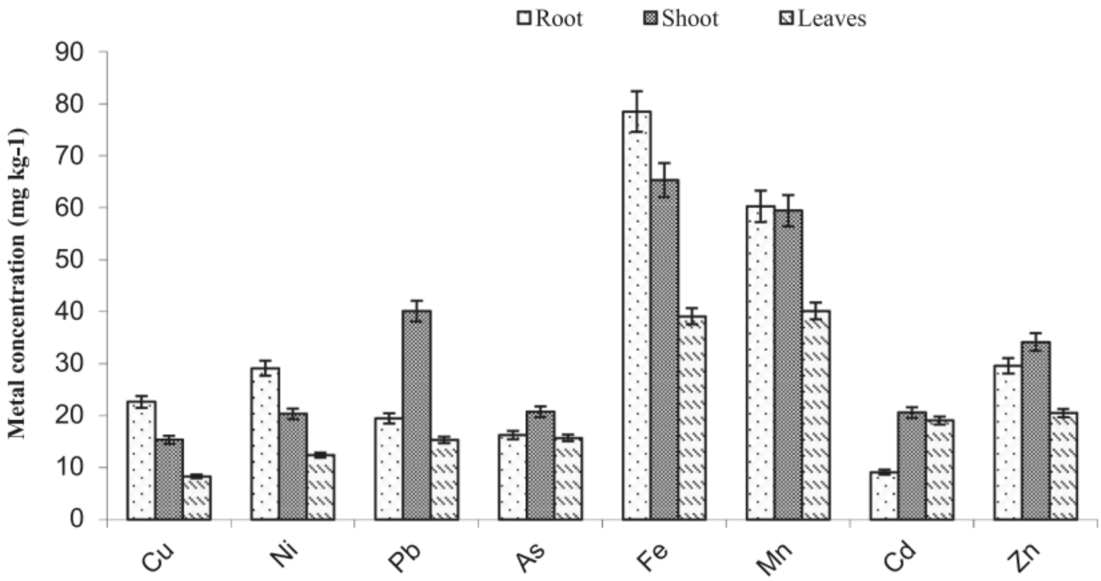

Fig. 1. Metals accumulation pattern in root, shoot and leaves of *Ricinus communis* L. growing on the distillery wastewater containing organometallic pollutants.

4. Conclusion

The results revealed that DWw containing various organic compounds and metals were generated during the fermentation process of molasses. Moreover, the detected pollutants are persistent in nature and damage the quality of soil and water. The study showed that *Ricinus communis* L. and its associated rhizobacterium had the potential to phytoremediate metals and organic pollutants present in DWw, other pollution parameters were also reduced by 50–85%. Hence, *Ricinus communis* L. may be used on a wide scale in the field for the treatment of DWw pollution. However, more research is needed to optimize their performance and integrate them with the existing wastewater treatment process.

CRediT authorship contribution statement

Sonam Tripathi & Pooja Sharma: data curation, formal analysis, investigation, methodology, visualization, writing - original draft. **Diane Purchase:** formal analysis, writing -

review and editing. **Ram Chandra:** conceptualization, methodology, funding acquisition, writing - review and editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.125192.

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Supplementary information

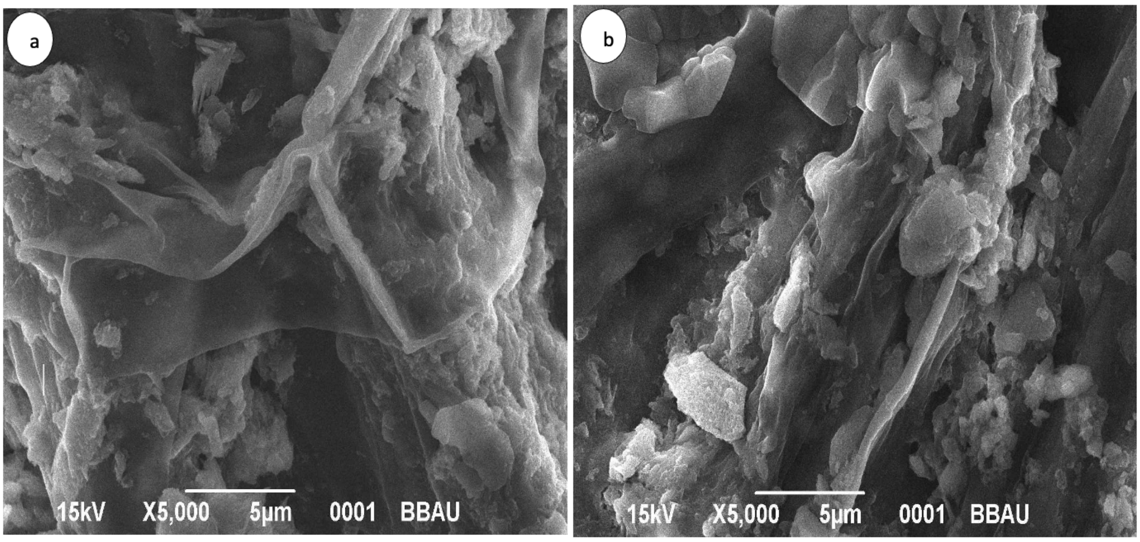

Fig S1. Scanning electron microscopy analysis of distillery wastewater sample showed different structure of pollutants (a-b)

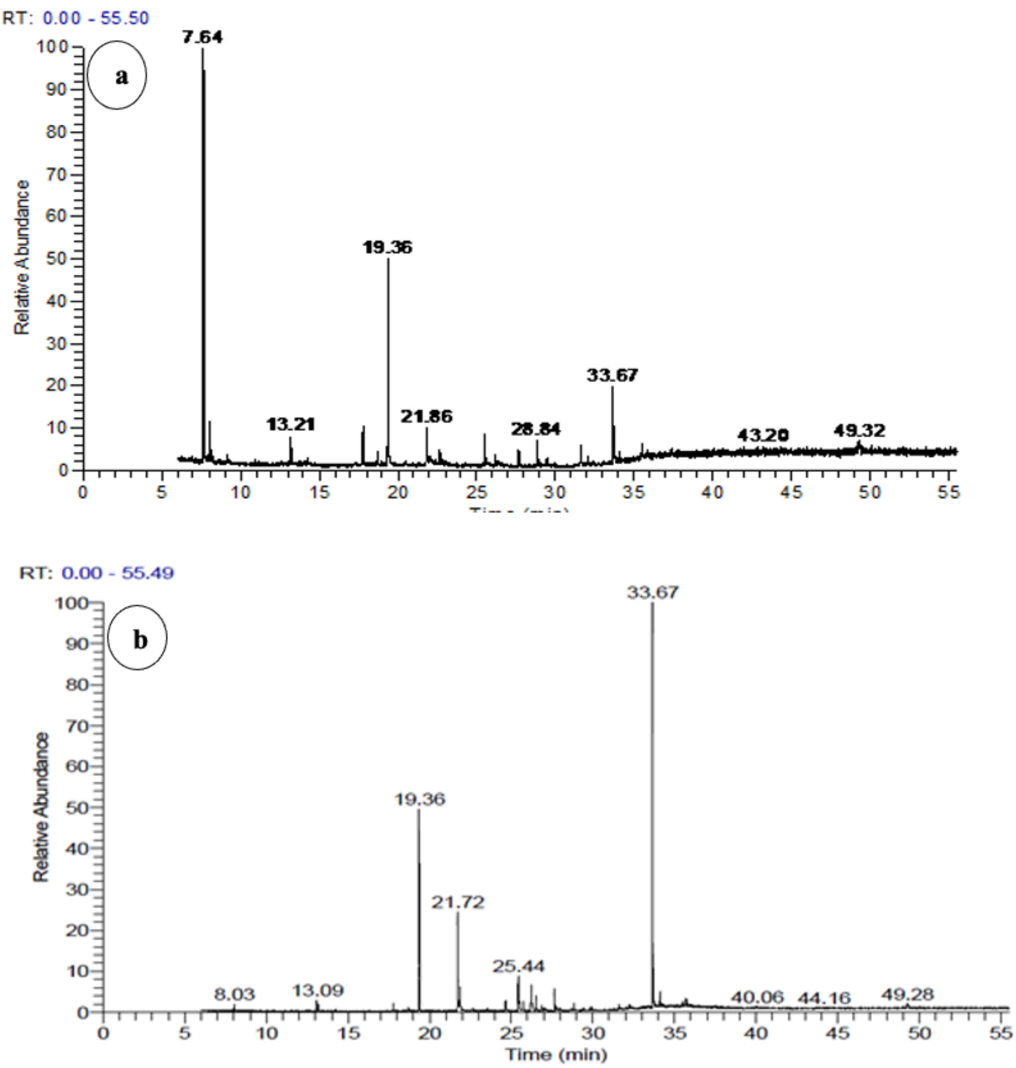

Fig. S2. Total ion chromatogram (TIC) of TMS derivatized detected organic pollutants from ethyl acetate extract of distillery waste contaminated site (a) Wastewater (b) Rhizospheric effluent