Water Air Soil Pollut (2023) 234:717
https://doi.org/10.1007/s11270-023-06738-2

Eco-friendly Degradation of Tannery Sludge with Coir Pith and Nava Rasa Karaisal

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Received: 12 May 2023 / Accepted: 1 November 2023 / Published online: 11 November 2023

Abstract The disposal of tannery sludge (TNS) without treatment causes severe toxic metal contamination in the ecosystem and food chain. Hence, eco-friendly solid waste management is essentially needed for the tanneries. The aim of this study was to degrade the raw TNS into composted sludge using coir pith (CP) as an adsorbent for organic compounds and other pollutants and Nava Rasa Karaisal (NRK) as a consortium of microorganisms. The TNS treated with CP and NRK showed decolorization and a reduction in pH. After the treatment, the electrical conductivity decreased from 24.7 to 6.31 µS/cm; total dissolved solids (28.4 ppm), salinity (21.2 ppm) and dissolved oxygen (7.5 mg/L) in the untreated TNS were reduced to 10.9 ppm, 4.9 ppm, and 4.6 mg/L in the TNS+CP+NRK treatment, respectively. SEM analysis showed small, smooth-walled structures on the surface of composted TNS compared to rough textures on the untreated TNS. Energy-dispersive spectroscopy analysis showed a higher number of elements in NRK (16) than in untreated sludge (8). Four different bacteria and six fungi were dominant in the treated tannery waste. The effect of different dilutions (12.5%, 25%, 50%, 75%, and 100%) of treated TNS on seed germination of *Vigna radiata* L. was carried out to check the feasibility of using the treated TNS for plant irrigation and growth. The results showed maximum growth at lower concentrations (12.5% and 25%). The results indicated that TNS treatment using CP and NRK is a potential eco-friendly approach to remediating the sludge waste.

Keywords Tannery sludge · Nava Rasa Karaisal · Coir pith · *Vigna radiata* L

Abbreviations

TNS	Tannery sludge
CP	Coir pith
NRK	Nava Rasa Karaisal
EC	Electrical conductivity
DO	Dissolved oxygen
TDS	Total dissolved solids
SEM	Scanning electron microscopy
EDS	Energy-dispersive spectroscopy
h	Hours

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DNA Deoxyribonucleic acid
PCR Polymerase chain reaction

1 Introduction

The tannery is one of the oldest industries in the world and this is a place where hides are tanned to leather. It is typically characterized as a pollutant-generating industry that produces a wide variety of highly toxic chemicals. Tannery waste is known to pose a serious environmental risk because of its high chemical content, which includes salinity, organic load, inorganic matter, dissolved solids, suspended solids, ammonia, and nitrogen. Specific pollutants are also present in the tannery waste, like sulfide, chromium, chloride, sodium, and other salt residues and heavy metals (Apte et al., 2005; Leghouchi et al., 2009; Yang et al., 2022). Tannery sludge (TNS) is a solid waste produced as a result of various physico-chemical treatments in tanning treatment plants. In particular, the tannery sludge contains solid wastes like hides, skins, fats, buffing dust, several organic and inorganic compounds, and heavy metals from the effluent (Rigueto et al., 2020). The dark color of TNS is due to the existence of residual components, which are resistant to environmental factors including light, pH, and microbial attack. The TNS is characterized by a strong offensive odor and turbidity, which may vary depending on the usage of chemicals in the tannery industry (Prabhakaran et al., 2022). The sludge severely reduces photosynthetic activity, making it microtoxic to aquatic life (Chandra et al., 2011). The hazardous pollutants in untreated tannery effluents are released into the land and deposited as sludge. It causes environmental pollution and increases health risks to humans due to the presence of heavy metals, toxic chemicals, chloride, dissolved salts, and other pollutants (Zhao & Chen, 2019).

Coir industries in India constitute about 70% of the coconut husk and 7.5 million tons of coir pith (CP) are produced per year in Tamil Nadu. More than 98% of coir wastes are used for economic purposes such as landfilling, hydroponics, and manuring (Kumar & Ganesh, 2012). The physical, chemical, and biological qualities of the soil are improved when the CP is added as a soil amendment in a variety of soil situations. (Hossain et al., 2012). It is also one of the most important wastes because of its high lignin (31%), cellulose (27%), and potash contents (Anandhraj et al., 2012). It is enormous, accumulated in the coir industry, and difficult to dispose of in the environment (Tharani et al., 2019). CP has a water-holding capacity that is between 5 and 6 times its weight, and it gently releases water into the soil. As great organic manure and a sustainable alternative to peat moss, composted CP is utilized (Ningshen & Daniel, 2013). Burning has historically been used to dispose of CP wastes, which has led to a number of environmental issues, including the release of carbon dioxide and global warming. However, this undesirable substance is considered useful biomass and can be converted into manure using biological sources, which can be effectively used for increasing the yield of crops (Sundaram & Malliga, 2013). Agricultural wastes are gaining importance in preparing eco-friendly and low-cost biosorbents for the removal of dyes from wastewater (Abrouki et al., 2020). Biosorption plays an important role in microbial remediation as the extracellular materials immobilize heavy metals by attaching to the anionic functional groups of the cell surface (Kapahi & Sachdeva, 2019). CP acts as a low-cost, non-toxic, and biocompatible biosorbent for removing various types of dyes from effluent water. CP, as a biosorbent, offers an outstanding adsorption capacity for many pollutants. Moreover, it improves water infiltration and nutrient availability to crops and reduces the amount of phenolic compounds entering the water resource (Kalaibharathi et al., 2019). According to Chowdhury & Fatema, (2016), partially composted CP can be used as biofertilizers while also keeping TNS moist. The addition of CP significantly reduces the physicochemical parameters of tannery wastewater as well as the chromium levels (Hashem et al., 2021).

Bioremediation is an eco-friendly approach to breaking down hazardous substances in tannery waste into less toxic substances (Saimoon Rahman et al., 2018). Different types of sludge were treated with different methods to get various products. For example, oily biological sludge generated from wastewater treatment plants in petroleum refineries was co-digested with sugarcane bagasse anaerobically to produce methane (Ghaleb et al., 2020). Basically, biological treatments of organic contaminants are based on the degradation ability of microorganisms (Kalaibharathi et al., 2019). These microbes are crucial in the remediation of contaminated soil because they have the mechanism to endure metal toxicity. The mechanisms include extracellular and intracellular sequestration, production of metal chelators, precipitation, enzymatic detoxification, and volatilization (Ojuederie & Babalola, 2017; Yin et al., 2019). Nava Rasa Karaisal (NRK) is a mixture of five cow-based products, such as cow dung, urine, curd, ghee, and four other ingredients: bananas, besan flour, jaggery, and a handful of soil. It contains microorganisms such as bacteria and fungi that improve soil fertility and provide all of the nutrients needed for plant growth. These microorganisms act as decomposers in the ecosystem and have been used to reduce the heavy metals and contaminants in tannery waste. Both indigenous and exogenous bacteria have the capacity to decontaminate tannery waste (Kalaibharathi et al., 2019). In general, microorganisms play a vital role in bioremediation by breaking down harmful substances due to their diverse metabolic activities (Araujo et al., 2021). NRK can be applied as a biofertilizer, vermicompost, and biopesticide to increase soil fertility and produce food grains without the health risks associated with the use of conventional fertilizers and pesticides (Shrikant & Sekhar, 2016). Microorganisms in NRK are essential for degrading the lignin in CP and reducing the heavy metal pollutants in tannery waste due to their ability to produce extracellular enzymes (Reghuvaran & Das Ravindranath, 2010). The combined application of microorganisms containing NRK and CP would be an excellent absorbent and adsorbent during the tannery waste treatment process (Josepine et al., 2020).

Therefore, developing an efficient treatment technique to protect the environment becomes vital. Many strategies have been tried for waste degradation, displaying different levels of efficiency (Jagaba et al., 2021). The literature survey showed that not many studies were carried out on utilizing CP and NRK together for treating TNS. Hence, the present study aims to investigate the decomposition of TNS with the help of microorganisms present in NRK along with CP and the characterization of the treated TNS. The physicochemical analysis and the degradability of contaminants in tannery waste by the use of CP and NRK were studied. The bacteria and fungi in the composted TNS were isolated and identified. Further to this, the effect of application of composted TNS on the seed germination of *Vigna radiata* L. (Mung bean) was studied. *V. radiata* was used for this study because it is an easily available edible legume crop, and the seed germination capacity is fast so that the experimental results can be obtained quickly.

2 Materials and Methods

2.1 Collection of Samples

TNS were collected from tannery industries located in Sembattu, Tiruchirappalli, India. The collected samples were then transferred aseptically into dry containers and stored at 4 °C for further studies. CP was collected from coir industries near Srirangam, Tiruchirappalli, India, and stored in clean, dry containers at room temperature for further studies. Raw materials for NRK (cow dung, cow urine, milk, curd, ghee, banana, besan flour, jaggery, and a handful of fertile soil) were purchased from a local market in Tiruchirappalli, India. NRK was prepared freshly whenever required. Seeds of *V. radiata* were purchased from a local seed center and stored at 4 °C for further studies.

2.2 NRK Preparation and TNS Treatment

NRK (10 L) was made by combining five cow-based products, such as cow dung (200 g), urine (200 mL), curd (200 mL), ghee (10 mL), and four other ingredients: bananas (two pieces), besan flour (200 g), jaggery (200 g), and a handful of soil (Kalaibharathi et al., 2019). For 10 days, the preparation was kept in a shaded area and stirred well twice daily (in the morning and evening) to allow fermentation to occur. Wire mesh was used to cover the barrel to protect it from contamination from outside sources.

To treat the TNS, both CP and NRK consortiums containing effective microbes were added to the TNS in a sterile plastic tray. The TNS, NRK, and CP were mixed evenly in a ratio of 1:1:1 to get an appropriate consistency and the prepared homogeneous mixture was incubated for 30 days to obtain composted TNS. Fig. 1 represents the outline of the methodologies used for this study.

2.3 Physicochemical Parameters

Treated TNS (after 30 days of incubation) was dissolved in distilled water (1:1 ratio) and stirred to

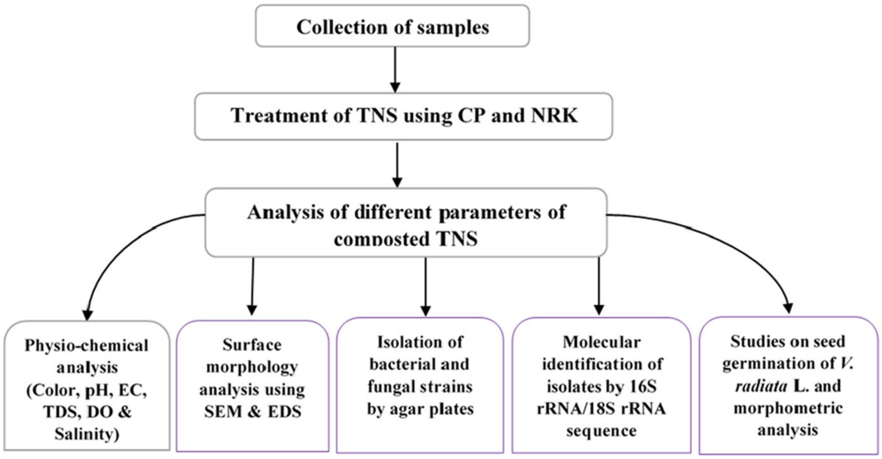

Fig. 1 Flowchart showing an outline of the methodologies used for this study

create a homogeneous solution, which was then filtered through the Whatman filter paper. Important parameters such as color reduction, pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), and salinity were monitored to demonstrate the reduction of toxicity and contamination in the composted TNS using standard analytical methods (AOAC, 2000; APHA, 1998).

2.4 Surface Morphology

The surface morphology of the dried sample was observed using a scanning electron microscope (SEM) with an energy-dispersive spectroscopic (EDS) attachment. After air drying, the samples (0.71 to 1.0 mm) were covered with a thin layer of gold (10 min) using a sputter coater. The coated samples were examined with 20 Kilo Electron Volts (KEV) in a JEOL JSM-5400 SEM/EDS unit (Ajayan et al., 2015).

2.5 Isolation and Identification of Bacteria and Fungi from Treated Tannery Waste

An aliquot of 0.1 mL of the appropriate dilution of serially diluted samples of treated tannery waste was spread onto nutrient agar plates for bacterial isolates and incubated at 37 °C for 24 h. Likewise, the Rose Bengal Agar plates for fungal isolates were incubated at 27 °C for 48 h, and the number of developed colonies was counted and stored for identification purposes. The phenol-chloroform method was used to extract total genomic DNA from the culture samples. The quality of the genomic DNA was determined using a 0.7% agarose gel and a 1 kb DNA ladder as a size standard, and the quantity was determined using a UV-Vis spectrometer (Labman, India).

Amplification of the 16S rRNA gene was carried out for all the samples using universal 27 forward (5′AGAGTTTGATCCTGGCTCAG 3′) and 1492 reverse (5′ GGTTACCTTGTTACGACTT 3′) primers. All of the samples showed the predicted band being amplified, and the amplicon synthesized by the PCR was confirmed and purified with the GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) to get rid of the primer dimer and other carryover contaminants. Using a 100-bp DNA ladder and a 2% agarose gel, the product's quality was evaluated. The 18S rDNA amplification was carried out using universal fungal primer pairs ITS4 (5′-TCC TCCGCTTATTGATATGC-3′) ITS5 (3′-GGAAGT AAAAGTCGTAACAAGG-5′). DNA amplification was achieved using a PCR reaction kit (GCC Biotech DNA Isolation Kit) (Gontia et al., 2014).

Using the BigDye® Terminator 3.1 sequence kit, amplified PCR products were purified and processed for cycle sequencing (Applied Biosystems, Foster City, CA, USA). Following cycle sequencing, the unincorporated dNTPs, ddNTPs, and primer dimers were removed from the products using the ethanol-EDTA purification process. The samples were denatured at 95 °C for 5 min after the purified cycle sequencing products were dissolved in 12 µL of Hi-Di formamide. Using the Genetic Analyzer 3500 (Life Technologies Corporation, Applied Biosystems®, California 94404, USA), denatured products were sequenced both forward and backward. Mega software version 11 was used to create the phylogenetic tree (Tamura et al., 2021).

2.6 Seed germination

The healthy seeds of *V. radiata* L. were purchased from a local seed center and were used for this study. The effects of various dilutions (12.5, 25, 50, 75, and 100%) of composted tannery waste and untreated tannery waste on the seed germination of *V. radiata* L. were examined. The study was carried out in sterilized Petri dishes using a single layer of Whatman No. 1 filter paper (125-mm diameter) as the bed for the seeds. Ten healthy and undamaged seeds of similar size were uniformly distributed in each sterilized Petri dish, which contained the test solution, and incubated at 28 °C in a dark environment for 96 h. The germination percentage was observed in each test solution at 24-h intervals, and the growth parameters such as hypocotyls and radical length of *V radiata* L. were observed and recorded on the fifth day.

The germination percentage was calculated using the formula:

$$\text{Germination percentage} = \frac{\text{No.of seeds germinated}}{\text{Total no.of seeds}} \times 100$$

All experiments were conducted in triplicate and the mean values were represented with ± standard deviation.

3 Results and Discussion

The discharged wastes from tannery industries may accumulate and pollute the surrounding area. In the present study, an attempt was made to treat the TNS using a low-cost method using CP and NRK and convert the composted TNS into fertilizer. For individual treatments, a 1:1:1 ratio of the TNS, CP, and NRK was mixed and incubated for 30 days. After the incubation period, the color reduction and physicochemical parameters were analyzed.

3.1 Decolorization

The color of tannery waste differs from industry to industry and depends on the chemicals used for tanning. (Arasappan et al., 2015; Muthukaruppan & Parthiban, 2018). Therefore, the color is regarded as the first indicator of contaminants in wastewater that affect the aesthetics of water bodies (Josepine et al., 2020). The initial color of the TNS was gray. The effluent is colored due to the presence of biodegradable organic compounds and a significant amount of inorganic chemicals used in the processing, such as salt and chromium. The color of the control tannery sludge remained the same until the 30th day. However, the composted TNS (TNS + CP + NRK) showed a brownish-black color on the 30th day (Fig. 2a and b). The color change could be due to the degradation of organic matter by the microbes present in the NRK. During the degradation processes, the microbes could have consumed the tannery residue particles along with the CP as nutrients for their growth. The CP acts as an adsorbent, neutralizes the residue particles in TNS, and also maintains the moisture content. The color of tannery waste may change after treatment with microorganisms due to chemical constituent decontamination (Mythili & Karthikeyan, 2011). It was reported that the color changed because the combined action of CP and cyanobacteria converted inorganic compounds to organic ones (Bhagat & Malliga, 2015; Lakshmi & Malliga, 2014; Shanmugapriya & Malliga, 2013). The odor is produced

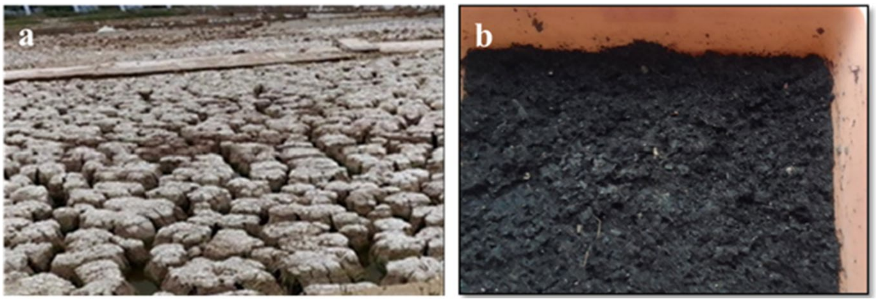

Fig. 2 **a** TNS and **b** composted TNS

by the putrefaction of the organic residues from the processed skin and hides (Zereen et al., 2013).

3.2 Effect of pH

The investigation of pH plays an important role because it acts as an indicator of the availability of metal ions present in the wastewater, thereby influencing the overall properties of the treatment sample (Amanial, 2016). The effect of pH on different treatment parameters on 30th-day analysis is shown in Fig. 3a. The obtained results of the analysis range from 4.0 to 12.6. On the 30th day, the pH of the raw sludge was 9.8 and the pH of the CP was 12.6, but in NRK, the pH was 4. Discharge of untreated tannery effluent with low or high pH will pollute the environment (Amaro de Sales et al., 2021). In this study, the pH value decreased in composted TNS and all other treatments. This could be due to the accumulation of organic acids and it also indicates the efficiency of the microbes to biodegrade the effluent (Noorjahan, 2014). The alkaline pH of the coir pith was due to its high content of calcium carbonate. The alkaline environment of the coir pith also allowed for the growth of microorganisms, which further degraded the sludge and helped reduce the amount of heavy metals in the sludge. This resulted in a decrease in the toxicity of the sludge, making it safe to be used as fertilizer. During the breakdown of pollutants, the pH plays a significant and crucial role in microbial metal uptake by affecting the metal speciation and solution chemistry as well as the surface characteristics of bacterial cells (Kumaran et al., 2011).

3.3 Effect of EC

The measure of EC shows the amount of ions dissolved in wastewater. It can also be said that it is a measure of the ability to conduct electricity. The amount of ions present is directly proportional to EC. The obtained results of EC range from a minimum of 6.3 μS/cm to a maximum of 28.0 μS/cm (Fig. 3b). The EC for TNS, CP, and NRK were 24.7 μS/cm, 11.3 μS/cm, and 28 μS/cm respectively. More chemical discharge as cations and anions in wastewater is indicated by increased conductivity in untreated tannery effluent (Ram Bharose & Singh, 2017). However, the EC values decreased with all the treatments. EC level (6.31μS/cm) significantly reduced in composted TNS. This shows that the degradation process has been carried out by microbes, and they reduce the organic and inorganic substances and salts present in TNS. Zereen et al., (2013) observed the highest EC (3.5 μS/cm) in 100% of tannery effluent and the lowest (0.75 μS/cm) in the pretreated tannery effluent. The raw tannery effluent containing a high content of EC (35.3 mS/cm) was reported by (Sharma & Malaviya, 2016). The reason for the increasing EC value is due to the presence of organic and inorganic substances and salt used during skin processing (De Sousa et al., 2017; Deepa et al., 2019).

3.4 Analysis of TDS

TDS is the measure of total inorganic substances and salts dissolved in water consequently leading to the measure of the turbidity of water. Compared to all other treatments, the highest levels of TDS were found in the control samples: TNS (28.4 ppm), CP (17.3 ppm), and NRK (20 ppm). High levels of TDS are not suitable for the application of tannery waste as a plant fertilizer due to the presence of dissolved organic and inorganic salts like chloride, sodium, nitrates, nitrites, carbonate, bicarbonates, sulfate, and phosphates (Nagarajan & Ganesh, 2015; Patel et al., 2022). Consumption of water with a high concentration of total dissolved solids may cause digestive tract issues, respiratory problems, nervous system problems, and cancer (Subba Rao et al., 1998). The TDS level was reduced in treated TNS with CP (10.9 ppm) and NRK (9.8 ppm) and the maximum level of reduction was observed in composted TNS (4.2 ppm) (Fig. 3c). This clearly indicates that the microbial population in the composted TNS degraded the dissolved organic materials in the sludge for their growth and metabolism (Oljira et al., 2018). Jahan et al., (2015) reported a high level of TDS (21.3 g/L) in the raw sample and the maximum level of reduction was observed in a treated tannery using *Eichhornia crassipes* (77.32%).

3.5 Analysis of DO

DO is one of the most important indicators of water quality. The high DO level in untreated TNS (7.5 mg/L) and low level in CP (4.3 mg/L) were noted on the 30th day. After the treatment, the level of DO was gradually reduced in all the

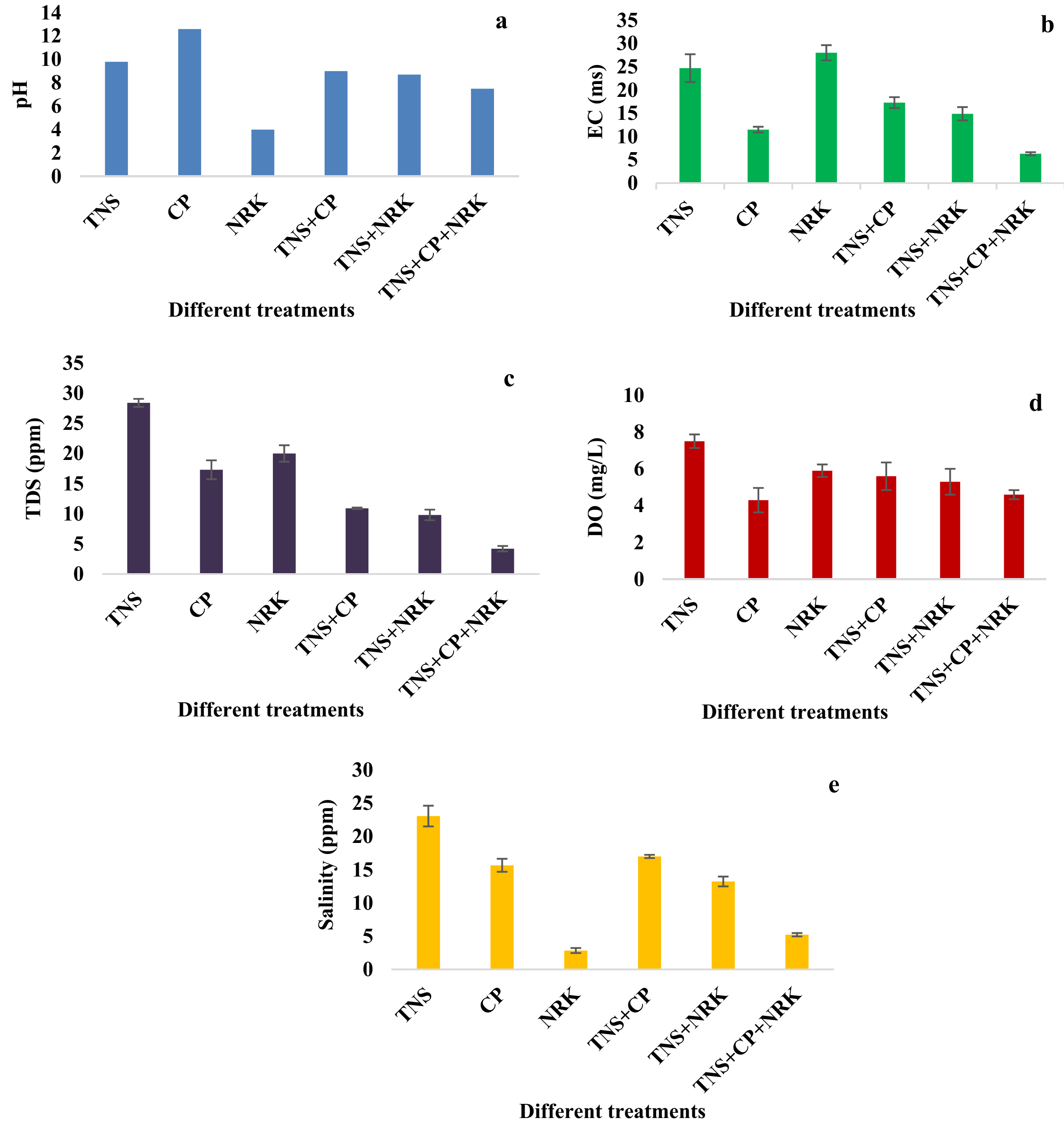

Fig. 3 Physio-chemical analysis of untreated and treated TNS with CP and NRK: **a** pH, **b** EC, **c** TDS, **d** DO, and **e** salinity (TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal)

treatments when compared to untreated TNS. The composted TNS contains 4.6 mg/L of DO which indicates the degradation of organic matter by the microbes present in the composted TNS (Fig. 3d). When the number of aerobic organisms increases, the DO level decreases (Vijayanand & Hemapriya, 2014). Balasubramanian & Dhevagi, (2016) reported that a similarly low level of DO (3.4 mg/L) was found in the treated tannery wastewater using domestic wastewater.

3.6 Analysis of Salinity

Salinity plays a significant role in the treatment of tannery wastes because high salinity content has a negative impact on the performance of various physicochemical properties. Moreover, the high salinity in tannery sludge and wastewater hampers the growth of microbial biomass, changes the soil pH thus affecting the flora and fauna of the environment. The presence of dissolved impurities such as Na+, Cl−, and NO− can raise the salinity of TNS, rendering it unfit for irrigation or consumption (Nosheen et al., 2000). The salts are unaffected by the pretreatment method of the effluent and remain a burden on the environment (Sampathkumar, 2001). Chemicals used in tanning, such as sodium carbonate, sodium bicarbonate, sodium chloride, and calcium chloride, cause the soil to become more alkaline, which raises the pH of the soil (Mondal et al., 2005). The results revealed that NRK has a low salinity value (2.37 ppm) when compared to other treatments, but control TNS showed a high salinity content (21.2 ppm). Analysis of the composted TNS showed a high reduction (4.9 ppm) in salinity level when compared to all other treatments and untreated TNS (Fig. 3e). The reduction of salinity in composted sludge may be due to the presence of microorganisms present in NRK that have the ability to degrade the chemicals dissolved in water. A report suggests that diverse microorganisms are capable of remediation, such as *Nostoc linckia*, *B. megaterium*, *Rhizopus stolonifer*, *B. subtilis, Lecythophora* sp., and *Saccharomyces cerevisiae* (Raklami et al., 2022).

3.7 Analysis of the Morphological Changes of Treated and Untreated TNS

SEM analysis was performed to study the morphological changes of 30 days of treated TNS using CP and NRK and untreated TNS. SEM studies revealed that the accumulation of residual particles with rough textures in the control TNS could be due to the presence of salts and chemicals in the raw sludge (Fig. 4a). This could be due to the presence of salts and chemicals in the untreated sludge. Similar research reported that hardened and thick particles were observed in the surface area of the tannery sludge due to the presence of heavy metals (Malaiskiene et al., 2019). Similarly, the SEM images of the coir pith showed some thick and dense structure of fiber on the surface (Fig. 4b) while NRK showed some tubular structure where some nutrients were attached to the surface of the tubular structure (Fig. 4c). Chen & Yang, (2005) reported that after tannery wastewater treatment, *Scenedesmus* sp. appeared as a rough surface compared to the normal cell surface in SEM analysis. Crystallized salt deposition on the biomass surface might be the result of the surface protuberance on the biomass after treating tannery wastewater. The SEM studies reveal that before heavy metal absorption, the cells appeared to be plump, having smooth surfaces in a loosely bound form. After interaction with heavy metals, precipitates in the form of round globules and amorphous substances aggregate all over the cell surface of *Pseudomonas aeruginosa* (Chatterjee et al., 2011).

After the 30th day, the TNS + CP + NRK results revealed that the bacterial cells were either lysed or in the decline phase due to the lack of nutrients. Composted TNS showed small, smooth particles that indicate the degradation of residual particles by bacteria and fungi present in the NRK (Fig. 4d). The morphological features and surface properties of adsorbent materials are frequently studied using SEM analysis (Joothi, 2015). It has been stated that the hardened and thick particles were observed in the surface area of the TNS due to the presence of heavy metals (Malaiskiene et al., 2019). Raw coir fiber had a rougher surface in scanning electron micrographs than alkali-treated fiber, which was determined to have a clean, smooth surface (Pani & Mishra, 2019). Due to their high surface area to volume ratio and the availability of electronegative charges on the surface of their cell walls, microorganisms make suitable nucleation sites for the creation of grained minerals (Baldi et al., 1990).

3.8 Analysis by Energy-Dispersive Spectroscopy

Energy-dispersive spectroscopy (EDS) analysis was used to determine the presence of elements in the tannery, CP, NRK, and the combined treatments. After 30 days of incubation, more types of elements were found in NRK (16) and CP (13) and treated TNS using CP and NRK (13) compared to TNS (8). Carbon (C), oxygen (O), sodium (Na), molybdenum (Mo), aluminum (Al), magnesium (Mg), phosphorus (P), and silicon (Si) levels were changed from TNS to composted TNS with CP and NRK.

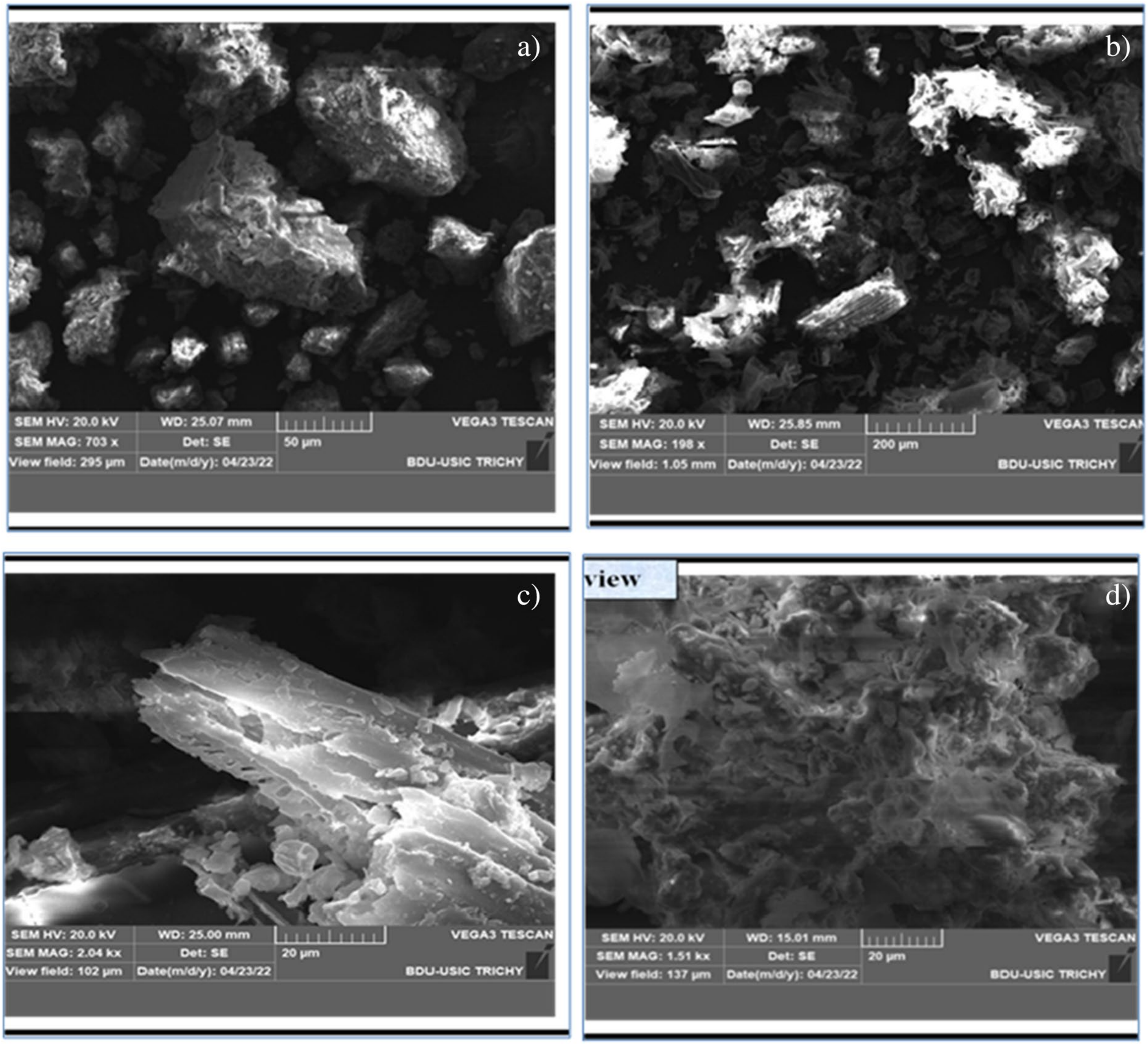

Fig. 4 SEM analysis of untreated and treated TNS along with CP and NRK. **a** Control TNS, **b** control CP, **c** control NRK, **d** TNS + CP + NRK (TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal)

Untreated TNS (TN) has the elements in the following order: Ca> O> C> Na> Mo> Mg> Si> P. CP contains a large amount of carbon (C), oxygen (O), and niobium (Nb) compared to NRK and TNS. The results revealed that the minimum particle size of composted TNS using CP with NRK (TNS + CP + NRK) exhibited significant variations of elements in the following order: O> C> Ca> Na> Mo> Cl> Fe> Mg> P> Al> Si> S> K (Fig. 5a–d). High carbon content was observed in CP, NRK, and composted TNS compared to the TNS. This carbon acts as a source for promoting microbial development, which speeds up the breakdown of contaminants in wastewater (Hamilton, 2016).

TNS contains a significant amount of calcium (Ca) along with iron (Fe) and sulfur (S), which are used in agriculture as an ameliorant as well as a nutrient source. At the same time, this sludge is highly rich in toxic constituents such as chromium (Cr), and their transformation must be understood in order to avoid potential health risks. When such wastes are applied to alkaline soils, they exchange sodium (Na) from the soil clay simplex and restore the agricultural productivity potential of

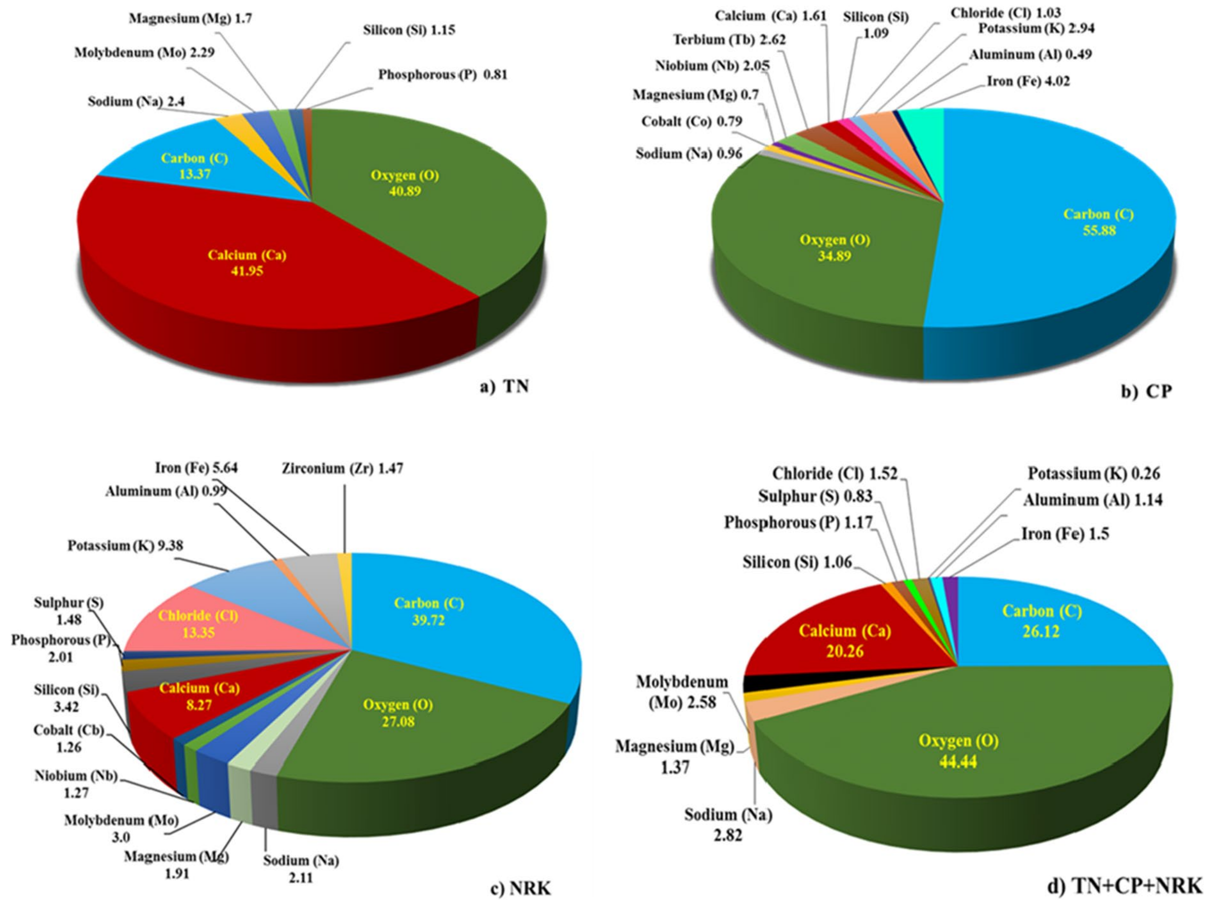

Fig. 5 EDS analysis of untreated and treated TNS using CP and NRK. **a** Control TNS, **b** control CP, **c** control NRK, **d** TNS + CP + NRK (TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal)

the soil (El Mouhri et al., 2020). The use of TNS as an alternative fertilizer for coffee seedlings is potentially feasible because this industrial waste contains nutrients such as sulfur, magnesium, calcium, nitrogen, phosphorus, potassium, and organic matter, as observed by Amaro de Sales et al., (2021). The CP treated with cyanobacteria *Oscillatoria annae* used to the reduction of heavy metals and increase the amount of NPK content (Sivakumar & Perumal, 2018). Surprisingly, the absence of chromium peaks was exhibited in treated tannery wastewater by phytoremediation process using *Scenedesmus* sp. algae compared with control (Ajayan et al., 2015).

3.9 Molecular Identification and Phylogenetic Analysis of Bacterial Strains

Four different types of bacteria were identified from treated TNS using 16S rRNA sequencing. They are *Kocuria flava*, *Enterobacter hormaechei*, and two strains of *Bacillus subtilis* (GenBank Accession numbers: OM899805-OM899808). Fig. 6 depicts the phylogenetic relationship between bacterial isolates and their closest neighboring sequences in the GenBank database. These bacteria are good at decomposing organic waste in tannery waste and are utilized as a nutritional supplement. According to Fathima et al., (2012), Cr (III) resistant *Bacillus* sp. was isolated

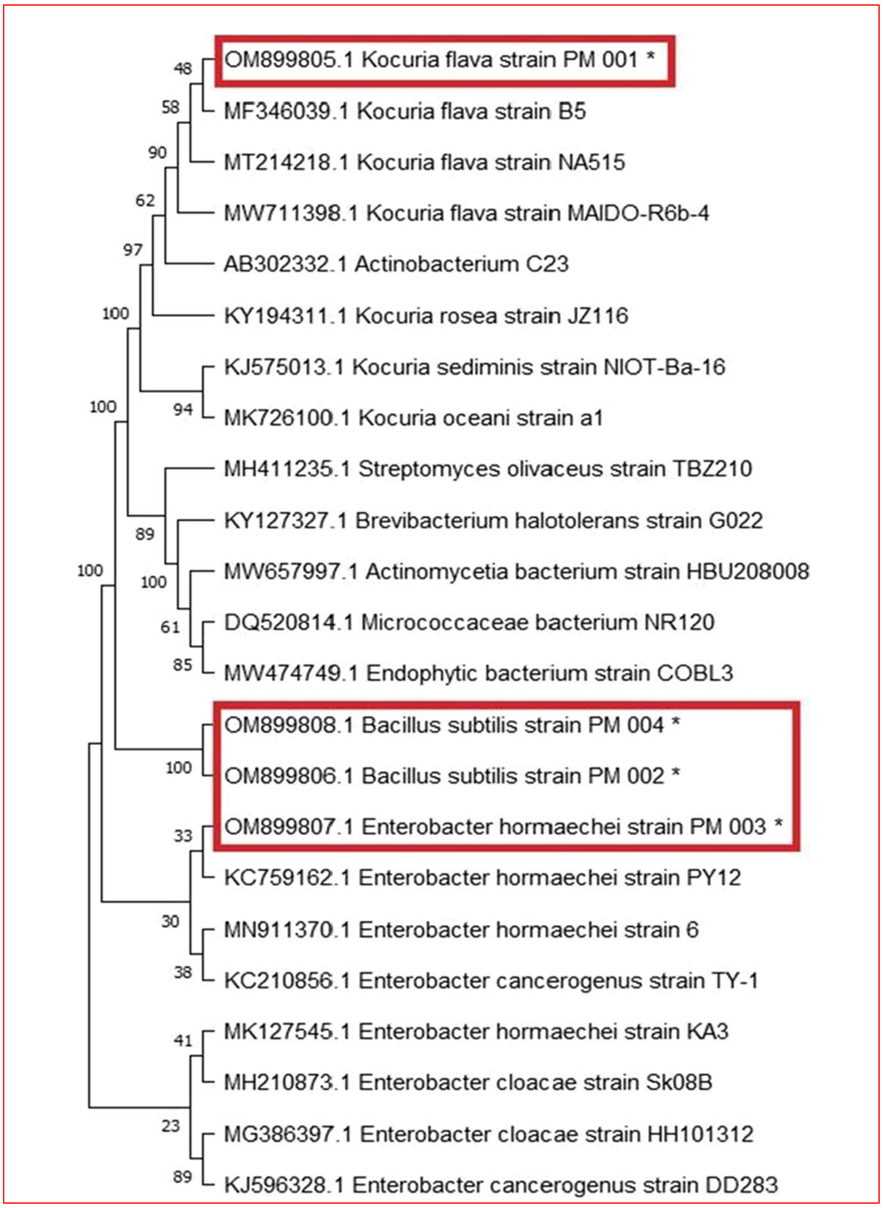

Fig. 6 Phylogenetic analysis of four bacterial strains isolated from treated TNS

from TNS and identified by 16S rRNA sequencing. The proteolytic *B. subtilis* was identified from Panchakavya and from tannery waste of the leather processing sector using the 16S rRNA sequence (Adamu et al., 2015; Saimoon Rahman et al., 2018). Vijayaraghavan et al., (2018) reported that *Raoultella ornithinolytica*, *Citrobacter freundii*, and *Shewanella xiamenensis* were identified from TNS by 16S rRNA gene sequencing. Wang et al., (1989) studied the isolation and characterization of hexavalent chromium-reducing bacteria from TNS. According to their results, *Enterobacter* sp. is resistant to chromate under both aerobic and anaerobic conditions. *Enterobacter hormaechei* has a more effective metabolism for reducing the heavy metal copper, which is present in the effluent released from industries (Irawati et al., 2022). Numerous bacteria and fungi, such as *Cellulosimicrobium* sp., *Stenotrophomonas* sp., *Bacillus* sp., *Brachymonas denitrificans*, *Thiobacillus* sp., and *Vibrios* sp., have been researched for bioremediation of tannery effluent (Vijayaraj et al., 2020). The most effective bacteria studied by Ashraf et al., (2018) for heavy metal tolerance in tannery effluent were *Enterobacter* sp., *Microbacterium arborescens*, and *Pantoea stewartii*.

3.10 Molecular Identification of Fungi

The six fungal isolates were identified as *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus terreus*, *Sarocladium implicatum*, *Sarocladium* sp., and *Aspergillus quadrilineatus* based on the nucleotide by 18S rDNA sequence (GenBank Accession number ON261387-ON261392) and the phylogenic tree constructed by neighborhood joining method (Fig. 7). These fungi identified in the composted TNS are known for their various metabolic activities and play an important role as decomposers in tannery waste

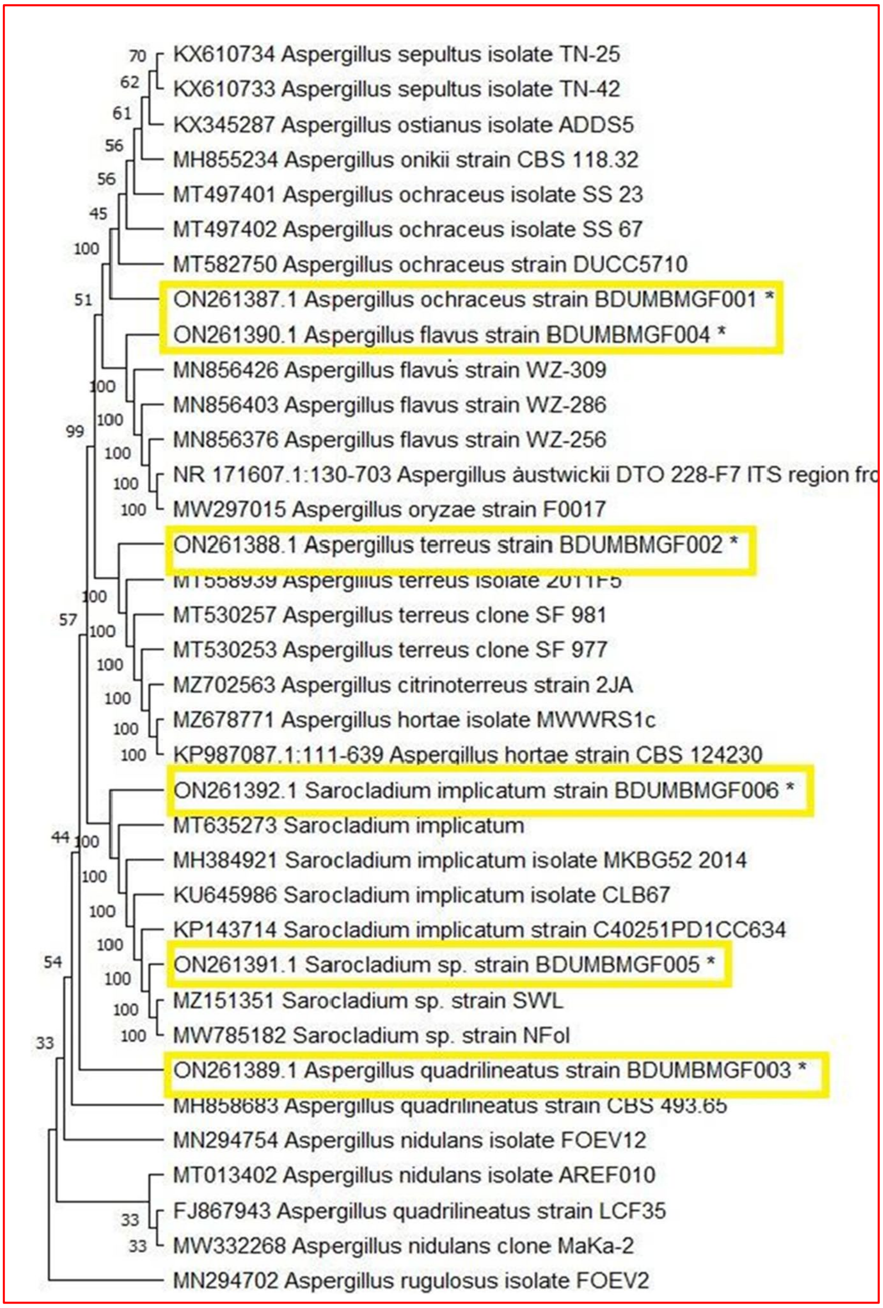

Fig. 7 Phylogenetic analysis of six fungal strains isolated from treated TNS

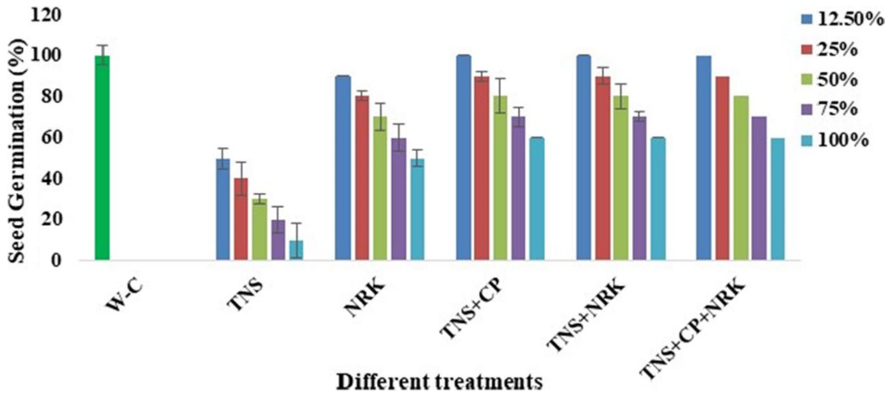

Fig. 8 Effect of different concentrations of treated TNS on seed germination of *V. radiata* L**.** W-C, water control; TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal

treatment. Various fungal species were isolated from soil polluted with waste from the tanning industry, such as *Aspergillus candidus*, *Aspergillus carneus*, *Aspergillus flavipes*, *Aspergillus flavus*, *Aspergillus unguis*, *Cephalosporium curtipes*, and *Cylindrophora hoffmannii.* Among the isolates, *Aspergillus* sp. has the ability to biosorb a high level of metal in contaminated soil (Eman & Eman, 2013). The microbial population has incredible enzymatic and metabolic ability to destroy a wide range of chemical substances in tannery waste. *A. niger*, *A. terreus*, *Paecilomyces varioti*, and *Phanerochaete chrysosporium* were isolated from tannery effluent and used to biodegrade the effluent, as reported by Deepa et al., (2019). They concluded that a mixed culture of fungal species is superior to a single fungus for tannery waste treatment. Sharma & Malaviya, (2016) investigated *A. flavus* (SPFT2), which was more effective at reducing chromium in tannery effluent and also reduced physicochemical parameters. Other investigations on

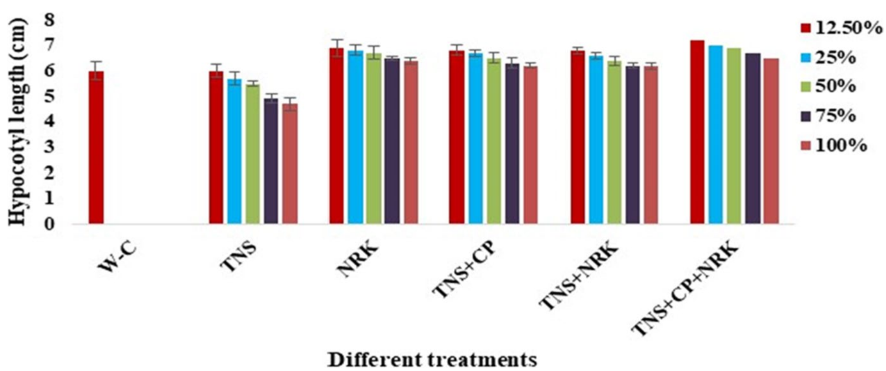

Fig. 9 Effect of different concentrations of treated TNS on hypocotyl length of *V. radiata* L. W-C, water control; TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal

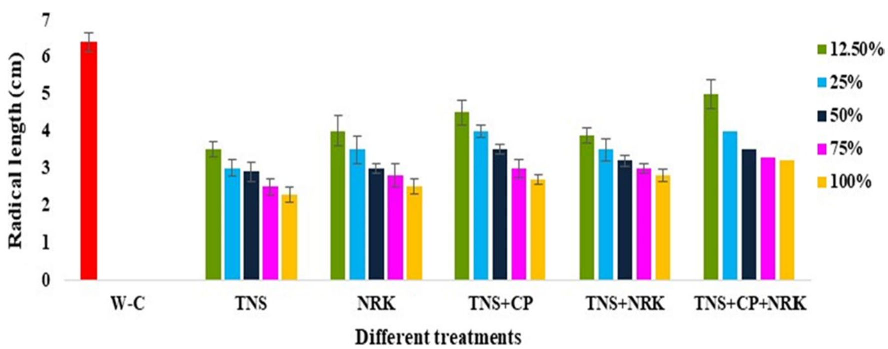

Fig. 10 Effect of different concentrations of treated TNS on radical length of *V. radiate* L. W-C, water control; TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal seeds. W-C, water control; TNS, tannery sludge; CP, coir pith; NRK, Nava Rasa Karaisal

Table 1 Tannery effluent treatment using different methods

S. No.	Source	Technique	Material used for degradation	Method	Incubation/duration	Result	Application	Reference
1	Tannery wastewater	Chemical	Graphene oxide-ferric oxide as an adsorbent (2g/L)	Nanocomposite	30 min	Removed 95% of turbidity from tannery wastewater	-	Roy et al., 2023
2	Tannery effluent	Microbial remediation	Coir pith used as an adsorbent (20g) and NRK as a microbial consortium (100ml) in 900-mL tannery	Directly added	4 days	pH, EC, Temp, TDS, salinity, Mg, Ca, and nitrate were gradually reduced in the treated tannery	Seed germination on *V. radiata* L. 12.5% of treated tanneries exhibited the highest hypocotyl and radical length	Jenifer & Malliga, 2023
3	Tannery effluent	Coagulation-Flocculation	*Moringa oleifera* as a coagulant 4000 mg/L	Directly added	24 h	Heavy metals (Cd, Cu, Fe, Mn, Pb, and Zn) were reduced	-	Jagaba et al., 2021
4	Tannery wastewater	Continuous adsorption	Beach sand as an adsorbent (3cm in inner diameter and 50 cm in height) Flow rate: 15 mL/min	Fixed sand bed column	Every 10 mins (collected the sample)	Color reduction observed (95–100%) and observed by FTIR, XRD, and SEM revealed the modifications in surface	-	El Mouhri et al., 2020
5	Tannery effluent	Biodegradation	*Enterobacter* sp. (10 mL in 100-mL tannery effluent)	Bacterial inoculum	24 h	Lowest level of Color, pH, BOD, COD, TDS, TSS, Calcium, and Magnesium were found in treated tannery effluent	Seed germination on *Oryza sativa* L.	Jenny & Malliga, 2020
6	Tannery effluent	Phytoremediation	*Moringa stenopetala* seed powder (10 g in 250 mL tannery effluent	Bio-adsorbent	12 h 25± 5 °C	BOD, COD, TDS, turbidity, conductivity, and pH were found reduced	-	Hossain et al., 2019

Table 1 (continued)

S. No.	Source	Technique	Material used for degradation	Method	Incubation/duration	Result	Application	Reference
7	Tannery effluent	Dilution	Distilled water (25, 50, 75, and 100%)	Directly diluted with tannery effluent	-	Reduction of Color, pH, BOD, EC, TDS, TSS, and TS were observed	Seed germination and plant growth on *Zea mays*	Hailu et al., 2019
8	Tannery effluent	Bioremediation	Coir pith as an adsorbent and NRK (microbial consortium)	Directly added	30 days	12.5 % of treated effluent exhibited the good results on *Vigna radiata*	Seed germination on *V. radiata* L.	Kalaibharathi et al., 2019
9	Tannery wastewater	Bacterial remediation	*Raoultella ornithinolytica*, *Citrobacter freundii*, and, *Shewanella xiamenensis* (1-mL inoculum in 100-mL effluent)	Indigenous bacterial treatment	5 days	73% of Cr, 68% SO_2^{-4} 86% of BOD, and 80% of COD reduction observed by using *C. freundii*	-	Vijayaraghavan et al., 2018
10	Tannery sludge	Bio-fermentation	Microbial consortium including actinomycetes, lactic acid and yeast—AM 111: 1–2 kg/1 t	Warehouse	7–10 days	Raw tannery converted into organic fertilizer by using microbial consortium	Seed germination on *Osmathus fragrans* and plant growth of *Panicum virgatum*	Feng et al., 2013
11	Tannery effluent	Adsorption	Activated carbon as adsorbent: 0.5 g in 400 mL	Agitation	2 h 30 mins	Turbidity, COD, and chromium level were found decreased	-	Yasmine et al., 2012
		Electro-coagulation	Electrodes with activated carbon: 1.5 A current +0.5g	Electrolysis	2 h 30 mins			

Table 1 (continued)

S. No.	Source	Technique	Material used for degradation	Method	Incubation/duration	Result	Application	Reference
12	Tannery effluent	Bioremediation	*Bacillus, Pseudomonas* and *Micrococcus* (1% of microbial inoculum in 500 mL of effluent)	Microbial inoculum directly added to the effluent	72 h	Color, odor, pH, electrical conductivity, total solids, suspended solids, dissolved solids, BOD, COD, chromium, zinc, iron, nickel were found decreased in effluent after 72 h	Seed germination on *Vigna mungo* L. and *Helianthus annuus* L.	Mythili & Karthikeyan, 2011
13	Tannery sludge	Biological treatment	Bench scale continuous flow activated sludge reactor with mixed liquor volatile suspended solids (MLVSS): 3599 mg/L	Activated sludge process	12 h	Reduction of BOD (94%) and COD (84%) observed	-	Haydar et al., 2007

tannery-contaminated soil found that *Aspergillus* and *Penicillium* were the most prevalent fungus species among the isolates, followed by *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Trichoderma* sp. (Antunes et al., 2016).

3.11 Seed Germination

The bacteria in the composted TNS could promote plant growth. In addition, they have the potential to degrade toxic contaminants, decontaminate heavy metals by secreting substances such as siderophores (chelators) and organic acids that enhance the bioavailability of heavy metals by decreasing soil pH, improve the detoxification rates of plants, boost the enzymes for root secretion leading to the degradation of pollutants and maintain pH in soil (Chen et al., 2017; Li et al., 2020; Nedjimi, 2021; Rajkumar et al., 2012). The lower concentrations of all the treatments and the control exhibited a better seed germination percentage for *V. radiata* when compared to all the other treatments with high concentrations. However, the percentage of seed germination on the fourth day was reduced by 50% in the control TNS (Fig. 8). The seed germination percentage was inhibited by the increased concentration of untreated and treated TNS. Mythili & Karthikeyan, (2011) found that black gram and sunflower germination were inhibited by tannery effluent at higher concentrations (80 and 100%). It is caused by the stress of heavy metals and a higher level of total solids in untreated wastewater during the germination process. After 96 h, the hypocotyls length of *V radiata* was found significantly increased in composted TNS at different concentrations and water control (Fig. 9). But the radical length showed drastic reduction in all the treatments in all the concentrations when compared to water control (Fig. 10). It has been reported that the seed germination percentage was highly improved in the combined treatment of tannery effluent by CP and cyanobacterium when compared to different concentrations of individual treatment of CP, cyanobacterium (*Lyngbya* sp.), and tannery effluent. When compared to individual treatments, the findings showed that the combination treatment of *Lyngbya* sp. with CP at a dilution of 25% demonstrated the highest seed germination (Sayi et al., 2018).

Different concentrations of tannery effluent-treated seeds (*Vigna unguiculata*) showed reductions in root length, shoot length, and vigor index, whereas fresh and dry weights were increased in the untreated and treated experiments (Hussain et al., 2010). The germination of sunflower was found to be reduced as the percentage of dilution level increased in treated TNS (Silva et al., 2021). Seed germination includes many physical, physiological, biochemical, cellular, and molecular events that render the radical able to emerge from the seed (Sangeetha & Sharavanan, 2018). High concentrations of dissolved solids may prevent seed germination by enhancing the salinity and conductivity of the solute absorbed by the seed before germination (Llanes et al., 2005). The increased salinity reduces the osmotic potential to the point where it either slows or prevents the uptake of water required for germination or has toxic effects on embryo viability (Vadivudai et al., 2015). Many studies have been conducted to treat and utilize tannery sludge and tannery wastewater. Table 1 represents the different techniques, materials, and microbes used for the treatment. The treated effluents were used for the seed germination of different plants.

4 Conclusions

The use of waste byproducts as fertilizer makes a significant contribution to organic farming and may help to lessen our reliance on synthetic fertilizers. The use of CP with NRK impacted the process of degradation of heavy metals and other pollutants in the TNS into composted sludge. The four bacterial and six fungal strains identified in the composted tannery sludge act as efficient decomposers. Further to this, the composted sludge was tried as a biofertilizer for *V. radiata*. It showed promising results on seed germination, hypocotyl, and radical length. The conversion of TNS into manure or biofertilizer using microorganisms has significant ecological implications. It is possible to draw the conclusion that microbial degradation should be viewed as a crucial part of the cleanup plan for tannery waste, and microbial-assisted remediation could be considered one of the most promising, eco-friendly techniques for the restoration of tannery-polluted sites. However, further studies are warranted to ensure the suitability of this composted CP to increase the yield of *V. radiate* and other plants.

Acknowledgments The authors acknowledge Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0), MHRD, New Delhi, and Central Coir Research Institute (Coir Board), Kalavoor, Alleppey, Kerala, India for financial assistance during the period of study.

Author Contributions PM designed the research and acquired the funding. GJ and DG participated in the sample collection and conducted the experiments. GJ analyzed the data and wrote the experiment. PM, NG, and NS reviewed and edited the manuscript. SR contributed the funding. All authors read and approved the manuscript.

Funding The work was funded by Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0), MHRD, New Delhi, and Central Coir Research Institute (Coir Board), Kalavoor, Alleppey, Kerala, India for financial assistance during the period of study.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest The authors declare no competing interests.

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