

Physicochemical and microbial analysis of sugarcane press mud reveals ligninolytic and plant growth-promoting bacteria with soil amendment potential

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Abstract

Press mud is an acidic by-product of sugarcane processing that is commonly discarded, despite containing components with potential agricultural value. This study assessed sugarcane mill press mud through physicochemical, microbiological and enzymatic analyses to evaluate its environmental implications and suitability as a soil amendment. The material was slightly acidic (pH 6.4) and rich in essential nutrients, including potassium (1061.06 ppm), magnesium (624.96 ppm), calcium (461.06 ppm) and phosphorus (513.11 ppm). However, elevated metals such as aluminium (2083.22 ppm), iron (2342.57 ppm), manganese (85.90 ppm), zinc (60.84 ppm), copper (17.70 ppm), lead (3.10 ppm) and chromium (4.18 ppm), together with the detection of the pollutant 2,4-di-tert-butylphenol (2,4-DTBP), suggest the need for proper regulated application to mitigate environmental risks. Microbial profiling across acidic (6.4), neutral (7.0) and alkaline (9.5) conditions revealed diverse bacterial taxa. Acidic conditions yielded *Escherichia coli* (PQ001953), *Bacillus licheniformis* (PQ001957) and *Moraxella catarrhalis* (PQ047632); neutral conditions favored *Herbaspirillum seropedicae* (PQ008927), *Enterococcus faecium* (PQ012564) and *Micrococcus luteus* (PQ012569); while alkaline conditions supported *Bacillus subtilis* (PQ012572), *Listeria ivanovii* (PQ060442) and *Paracoccus pantotrophus* (PQ012574). Notably, *E. coli*, *H. seropedicae* and *M. luteus* exhibited pronounced ligninolytic enzyme activity, indicating a capacity to degrade complex organic substrates. Plant growth trials using mustard (*Brassica campestris*) demonstrated that a 5:1

soil-to-press-mud ratio significantly enhanced plant growth relative to untreated soil. Collectively, these findings indicate that when applied in controlled quantities, press mud, represents a promising bioresource with valuable ligninolytic and plant growth-promoting microbial communities, while warranting careful oversight due to its contaminant load.

Keywords: Press mud · Biofertilizer · Distillery waste · PGPB · Organic pollutants · Metal contaminants

Introduction

Industrial waste recycling plays a crucial role in sustainability by reducing the effect on environment, lowering greenhouse gas emissions, conserving natural resources, and maintaining the soil ecology. This strategy not only enhances environmental stewardship but also encourages economic efficiency, aligning industrial practices with global sustainability goals for various industries (Krishna et al. [2020](#)). Among various waste management strategies, the direct use of industrial waste containing organic compounds in specific ratio with diverse bacterial communities, either as a soil amendment or through composting, represents one of the most feasible and environmentally friendly approaches for recycling agro-industrial waste into valuable by-products (Bano et al. [2025](#)). However, sustainable recycling of industrial wastes remains challenging due to the presence of unknown pollutants and the complexity of selecting suitable carrier materials that support biofertilizer producing bacterial communities (Desai et al. [2023](#); Gavrilescu et al. [2015](#)). In this context, sugarcane press mud represents a promising agro-industrial residue that can be effectively repurposed for sustainable applications.

Sugarcane press mud is an important solid waste byproduct during sugar production. With over 704 operational sugar mills in India, the industry produces approximately 8 million tons of press mud annually, representing a substantial resource for potential reuse in agriculture and environmental applications. Press mud is a compressed waste product, generated in the sulfitation (3%) and the carbonation process (7%) during cane juice filtration in sugar industries (James and Pandian [2016](#)). After discharge, sugar mill press mud retains a spongy, amorphous texture, exhibits a dark brown color and consists of sugar fiber, cane wax,

coagulated colloids, albuminoids, flavonoids, soil particles, organic salts, and various mineral elements (Sathish and Harsha [2020](#)). When incorporated into soil, press mud helps prevent erosion, crust formation, and cracking by modifying soil pH and hydrogen ion concentration. It also improves soil porosity and promotes microbial growth (Ansari and Gaikar [2014](#)).

Bioconversion of press mud is gaining momentum as a feasible green strategy in waste management, allowing the conversion of agricultural and industrial solid waste to high-value products that support environmental sustainability (Poria et al. [2022](#)). In addition, co-composted press mud with distillery spent wash resulted in a bio-compost that significantly improved finger millet yield and enhanced overall soil health (Byakodi and Babu [2022](#); Prabhavathi et al. [2019](#)). Press mud has several other applications, including biogas production, combustion, and use as a raw material for wax substitutes and ink (Arulazhagan et al. [2024](#)). The chemical properties of sugarcane press mud have been analysed to support safe application in agriculture and aquaculture practices (Saleh-e-In et al. [2012](#)). Despite the diverse potential applications of press mud, its effective use as a soil amendment for sustainable agriculture and its impact on the native soil microbial community remains poorly understood. This gap in knowledge limits its broader adoption in agro ecological practices. Currently, distilleries are increasingly adopting zero liquid discharge (ZLD) technologies, where spent wash is incinerated following multi-stage evaporation, further highlighting the need for integrated waste valorization strategies (Yogarajachari et al. [2023](#)).

Previous literature has shown the potential of sugar industry press mud as an alternative source of organic fertilizer. For instance, Khan et al. ([2012](#)) reported that applying around 40 tons of press mud per hectare resulted in maximum wheat yield. Additionally, press mud has been used for soil reclamation (Sheoran et al. [2020](#)), and composted press mud has shown benefits in enhancing crop productivity and soil health. However, the microbial dynamics involved in press mud composting and the characterization of organic waste streams from the sugar industry remain insufficiently explored. Research is needed to understand microbial interactions, optimize the composting process, and evaluate the long-term impacts on soil ecosystems to support its safe and effective reuse in sustainable agriculture (Lima and Beacorn [2022](#)).

Composting is a biologically driven process which is accomplished by the conversion of organic materials are decomposed and stabilized by microbial communities (SARKER et al.,

2013). Some studies have highlighted the role of specific bacteria, such as *Cellulomonas* (HSMP-2), in the bioconversion of press mud. Other studies have reported that sugar cane press mud-adapted bacteria enhanced the soil macro- and micronutrient levels, indicating their potential as biofertilizer (Patil et al. 2013). Recent investigations using 16 s rDNA sequencing showed a variable carbon-nitrogen ratio and predominance of Firmicutes in fresh press mud samples. Environmental exposure and organic enrichment were found to promote microbial diversity, including the emergence of Acidobacteria, Chloroflexi, and Planctomyces during metabolite production and consumption in a model microbial system (Uchimiya et al. 2022). Despite these insights, comprehensive characterization of the inorganic and organic chemical composition of press mud and the identification of the dominant bacterial taxa responsible for its conversion into biofertilizer, remains limited.

Ligninolytic enzymes such as laccase and manganese peroxidase (MnP) are key determinants of microbial versatility in organic- and metal-rich environments. Laccase catalyses the oxidation of phenolic compounds and contributes to the breakdown of lignin and other recalcitrant organic matter, thereby reducing the toxicity associated with phenolic byproducts (Athanasίου et al. 2024). MnP complements this process by oxidizing Mn^{2+} to Mn^{3+} , which acts as a diffusible oxidant capable of degrading lignin-derived substrates and humic substances (Wang et al. 2025). Their combined action has been widely implicated for detoxification of agroindustrial wastes and the enhancement of nutrient cycling, improving soil fertility and microbial colonization potential (Morsi et al. 2020; Wang et al. 2018). Screening PGPB isolates for laccase and MnP activities in sugarcane press mud, therefore, provides crucial insight into their ability to degrade complex organics, tolerate stressors, and contribute to sustainable soil amendment.

The study adopts an integrated approach to evaluate the potential of sugarcane press mud as a biofertilizer for enhancing soil health in agricultural practices. Using Fourier transform infrared spectroscopy (FTIR), Inductively coupled plasma mass spectrometry (ICP-MS), and Gas chromatography-mass spectrometry (GC-MS), the organic and inorganic constituents were characterized, including trace metals and potential organic pollutants. Environmental toxicity was assessed through seed germination assays conducted before and after bioconversion. The dominant indigenous bacterial strains were identified and evaluated for plant growthpromoting attributes. The findings offer new insights into the safe and

sustainable reuse of sugarcane press mud, bridging microbiological and environmental perspectives to support circular bioeconomy practices in agriculture.

Materials and Methods

Sample collection

The sugar mill press mud samples were collected from the disposed site of M/S K.M. Sugar Mill Pvt. Ltd. Ayodhya UP, India, located at (26.70483 N, 82.13186 E). Ten 5.0 kg polythene bags that had been washed with sterile distilled water and sanitized were used to collect the fresh press mud samples. The sample was dehydrated at room temperature and crushed for preparation and passed through a 4 mm sieve for physicochemical analysis and biological characterization.

Physicochemical characterization of the collected samples and garden soil

For the determination of pH and electrical conductivity (EC) (Systronics, conductivity TDS meter 308); 20 gm of the sample was dissolved in 50 ml distilled water and measured using a digital pH meter (Hanna, LMPH10H). Potassium, phosphorus, and total carbon, were quantified following standard procedures (Singh et al. 2018). The total nitrogen content was measured using the micro-Kjeldahl distillation method (Okeke et al. 2022). Using the Agilent 8900 ICP-MS Triple Quad (US), heavy metals (including Al, Cr, Mn, Fe, and Ni) were detected in acid-digested samples using Inductively coupled plasma mass spectrometry (ICP-MS). Sodium concentrations were measured with a flame photometer, while Ca^{2+} and Mg^{2+} levels in the soil extract were calculated using the Versenate method (Zhao et al. 2023). The F^- and Cl^- concentration was measured using the selective ion electrode (Agilent, 1260 Infinity II), while NH_4^+ , SO_4^{2-} , NO_3^- and PO_4^{3-} were analyzed as per the method mentioned in (APHA, (American 2012).

Garden soil (loamy) used as a control in the experiment was collected from the premises of Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow. The soil was autoclaved prior to use to eliminate microbial contamination (physico-chemical characteristics shown in Table 1).

To ensure data reliability, quality assurance & quality control (QA/QC) protocols were followed throughout the analyses. Instrument calibration was carried out using multi-element standard solutions (Merck, Germany) with calibration curves ($R^2 \geq 0.999$). For ICP-MS, certified

reference material (CRM, NIST SRM 2711a Montana Soil) was analyzed in parallel with the samples, and the recovery rates for the metals ranged between 92 and 106%. Procedural blanks and replicate analyses (10% of total samples) were included, with relative standard deviations (RSDs) below 5%. For ion analysis, standard addition methods were applied periodically to check for matrix interference. These steps ensured the robustness, precision, and accuracy of the reported results.

Scanning electron microscopy (SEM energy dispersive X-ray spectroscopy (EDX), and elemental analysis of sugarcane press mud

The powdered distillery press mud was analyzed for surface morphology using SEM and elemental composition using EDX. Initially, the press mud sample was dried by placing it on aluminum foil and heating them in an oven for 3 to 4 h at approximately 60 °C. After drying, they were stored in a desiccator for 2 h to prevent moisture absorption. To prepare the samples for analysis, a thin conductive coating of gold and palladium was applied to their surface. The coated samples were then mounted on stubs for SEM imaging. A field emission SEM (JOEL JSM-6330 F, JOEL Ltd., USA) coupled with a WVEX-EDS detection unit was used to perform SEM and EDX analyses in order to examine both the microstructure and elemental composition of the press mud (Asberry et al. [2014](#)).

FTIR analysis of collected press mud

FTIR spectroscopy (Nexus-890, Thermo Electron Co., Yokohama, Japan) was employed to identify the functional groups of the organic compounds present in the distillery press mud. For the analysis, oven-dried press mud samples were combined with high-purity anhydrous KBr powder at a ratio of 1:30 (w/w). Then, using a hydraulic press, a pressure of 8–10 tons cm^{-2} was applied for 10 min to generate a thin KBr pellet. The samples were kept at a constant temperature of 25 °C while their infrared (IR) spectra were acquired in the wavelength range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} . After collection, the infrared data were processed for subsequent analysis.

Extraction and identification of organic compounds from sugarcane press mud in acidic and alkaline pH medium

Soli-liquid extraction

Ethyl acetate was used to perform a solid-liquid extraction process to separate organic components of the press mud. In a 250 mL separating funnel, 20 g of powdered press mud was dissolved with 20 mL of 99% pure ethyl acetate from Molychem, India. The entire mixture was given a vigorous shake at intervals to facilitate the extraction and solubilization of organic compounds in acidic conditions ($\text{pH} < 2.0$), following the protocol outlined by Chandra and Kumar (2017a). This extraction process was performed thrice to ensure complete recovery of the organic compounds. The organic phase was then separated, dehydrated using anhydrous sodium sulfate (Na_2SO_4), and dried under a stream of nitrogen gas. For further GC-MS analysis, the resultant dry residue was passed via a 0.22 μm syringe filter (Millipore Ltd., Bedford, MA, United States) after being dissolved in 1.0 ml of ethyl acetate.

Gas chromatography-mass spectrophotometry (GC-MS) analysis

The extracted organic compounds from all the press mud samples were derivatized with trimethylsilyl (TMS), following the procedure demonstrated by Fabbri et al. 2002. In this process, 50 μL of pyridine along with 80 μL of TMS BSTFA & TMCS were added to 300 μL of the sample. To ensure that the leftovers were dissolved, the mixture was heated to 70 $^{\circ}\text{C}$ for 30 min while being shaken occasionally. The GC-MS system (Thermo Scientific Trace GC Ultra Gas Chromatograph), connected to a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific, Miami, FL, United States) had a TriPlus auto-sampler, was filled with a 2.0 μL aliquot of the silylated sample. Separation was performed using a DB-5MS capillary column [30 m $\times$ 0.25 μm I.D. $\times$ 0.25 mm film thickness, 5% phenyl, 95% methylpolysiloxane (v/v)]. Helium (He) was used as the carrier gas, with a flow rate of 1.1 mL min^{-1} . Initial oven temperature was set at 65 $^{\circ}\text{C}$ (held for 2 min), followed by increases to 230 $^{\circ}\text{C}$ (6 $^{\circ}\text{C min}^{-1}$), and then to 290 $^{\circ}\text{C}$ (20 min) at 10 $^{\circ}\text{C min}^{-1}$ (Chandra and Kumar 2017a). The temperatures of the ion source and transfer line were kept at 220 $^{\circ}\text{C}$ and 290 $^{\circ}\text{C}$, respectively. With an electron energy of 70 eV and a solvent delay of 7.0 min, the mass spectrometer ran in positive electron ionization (+ EI) mode. Full scan mode (mass range 45–800 amu) was used initially to confirm

the organic compound's derivatization. The compounds were identified by comparing their mass spectra (m/z) with the National Institute of Standards and Technology (NIST) library and by matching retention times with standard compounds.

Isolation and screening of bacteria from press mud

The serial dilution method was employed for bacterial isolation on nutrient agar (NA) plates, as detailed by Pandey et al. (2019). After that, the plates were placed in the incubator for 24 h at 30 ± 2 °C till bacterial colonies were observable. Individual colonies were subsequently re-streaked onto fresh agar plates at different pH: acidic (pH 6.4), neutral (pH 7.0), and alkaline (pH 9.0) purified culture was evaluated for survival under variable pH conditions. From each pH condition, three colonies exhibiting distinct morphology and vigorous growth were selected for further morphological & biochemical analysis, following the protocol outlined by Abbasi et al. (2011).

Identification of isolated bacteria via 16s rDNA sequencing and phylogenetic analysis

The Bacterial Genomic DNA Isolation Kit from HiMedia was used to recover genomic DNA from the bacterial strains. Every strain was grown in Nutrient Broth (NB) (HiMedia) on a shaker running at 220 rpm for 24 h at a regulated temperature of 30 °C. After centrifuging the cultures for 10 min at 24 °C at 4000 rpm, the supernatant was discarded.

Using particular primers, P027F and 1378R, which were intended to target a region of roughly 1500 bp from the isolated genomic DNA, the 16 S ribosomal DNA genes were amplified (Klindworth et al. 2013). One unit of HighFidelity KOD Taq DNA Polymerase, 200 μ M of each dNTP,

10X buffer, 2 mM $MgSO_4$, 0.2 μ L of each primer (UNI-16GT-F: AGAGTTTGATCCTGGCTCAG and UNI-16-GT-R: GGTTACCTTGTTACGACTT), and 1 μ L of template DNA were used to prepare each polymerase chain reaction (PCR), bringing the total reaction volume to 25 μ L.

The PCR amplification protocol involved an initial denaturation at 94 °C for 4 min, followed by 30 cycles consisting of 30 s of denaturation at 94 °C, 1 min of annealing at 63 °C, and 1 min of extension at 68 °C, culminating in a final extension step at 68 °C for 7 min. The PCR products were then purified using a PCR Purification Kit (NorgenBiotek, Canada), and sequencing was

performed by Biokart India Pvt. Ltd. To identify the bacterial sequences, the BLAST tool from the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/BLAST) was used for comparison with existing 16 S rDNA sequences. CLUSTAL W was used for further sequence alignment, and MEGA 11 software's maximum likelihood (ML) approach was utilized for making a phylogenetic tree in accordance with the earlier procedure (Chandra and Kumar 2017b).

Bioassays for plant growth promoting (PGP) traits and lignolytic enzyme activity

Assay for indole-3-acetic acid (IAA) production

Nine press mud isolated bacterial strains (three from each pH) (see Sect. 2.6) were cultured in yeast malt dextrose (YMD) broth (HiMedia, India) for 72 h at 28.2 ± 2 °C. The cultures went through a centrifuge for 30 min at 3000 rpm following incubation. The supernatant was carefully collected into a fresh test tube, and 2–3 drops of orthophosphoric acid were added, followed by 4 mL of Salkowski reagent, which consisted of 50 mL of 35% perchloric acid (HClO_4) and 1 mL of 0.5 M ferric chloride (FeCl_3) solution. The development of a pink color suggested the presence of IAA production (Linu et al. 2019).

Assay for N_2 fixation

To test the bacterial isolates' nitrogen-fixing capability, a glucose nitrogen-free mineral medium (GNFMM); composition: glucose (10 g L^{-1}), dipotassium hydrogen phosphate (1.0 g L^{-1}), magnesium sulfate (0.2 g L^{-1}), calcium carbonate (1.0 g L^{-1}), sodium chloride (0.2 g L^{-1}), ferrous sulfate (0.1 g L^{-1}), sodium molybdate (0.005 g L^{-1}), final pH 7.0 ± 0.2 was prepared. Bromothymol blue (BTB) was added to the medium, giving it its green color. The bacterial strains were injected into the GNFMM + BTB medium using a fixed amount of 50 μL of bacterial suspension with a concentration of around 10^5 CFU mL^{-1} . The cultures were maintained at 28 ± 2 °C for 48–72 h to measure growth and nitrogen fixation (Singh et al. 2023).

Assay for siderophore production

The bacterial isolates were streaked onto chrome azurol S (CAS) agar plates and incubated for 5 days at 28 ± 2 °C. The appearance of a color change in the medium from blue to yellow in the inoculated regions indicated the production of siderophores by the bacterial strains (Nithyapriya et al. 2024).

Assay for phosphate, potassium and zinc solubilization

The phosphate solubilization ability of the isolated bacterial strains was evaluated by spot inoculating 10 μL of bacterial suspension (prepared at a concentration of 10^4 CFU mL^{-1}) on Pikovskaya's agar plates using a sterile metal loop. The plates were incubated for 72 h at 30 °C. Phosphate solubilization activity was demonstrated by the development of clear zones surrounding the bacterial colonies (Patel et al. 2021). Similarly, potassium solubilization potential was evaluated on Aleksandrov agar plates under the same conditions, with clear zones indicating potassium solubilization (Dhaked et al. 2017). The zinc solubilization capability of the isolated bacterial strains was assessed by spot inoculating them onto zinc solubilizing agar plates using a sterile metal loop, followed by incubation for 48 h at 30 °C. Zinc solubilization activity was demonstrated by the development of clear zones surrounding the bacterial colonies (Dhaked et al. 2017). For calculating the solubilization index of strains for phosphate, potassium, and zinc solubilization, the following formula was used:

Solubilization index (SI) Total diameter of the clear zone and colony/Diameter of the colony alone.

Screening of laccase producing bacterial strains

For the screening of laccases producing bacterial strains, 1.0 g press mud sample was added to 100 mL minimal salt media (MSM), which contains (g L^{-1}) D-glucose 10.0; peptone 5.0; CaCl_2 0.01; Na_2HPO_4 2.4; K_2HPO_4 2.0; NH_4NO_3 0.1; MgSO_4 0.01, trace element (1 mL^{-1}) and at pH 7.5 ± 0.1 . The flasks were incubated for seven days at 120 rpm at 37 ± 1 °C in an incubator shaker (Orbitek, Scigenic India). After serially diluting a 1 mL sample (10^{-1} – 10^{-8}) with distilled water, 100 μL of the diluted sample was spread onto MSM agar plates and incubated at 30 °C. Laccase activity was then assessed following the method of Kumar et al. (2020), using guaiacol as the substrate. The appearance of reddish-brown halos around colonies indicated the presence of a laccase-producing bacterium.

Screening of lignin peroxidase producing bacterial strains

Guaiacol and veratryl alcohols, two lignin model compounds, were used to assess the bacterial ligninolytic potential following the methodology outlined by Taylor et al. (2012). The medium contained KH_2PO_4 (0.53 g L^{-1}), NH_4NO_3 (5 g L^{-1}), MgSO_4 (0.5 g L^{-1}), K_2HPO_4 (4.55 g L^{-1}), 0.1 g L^{-1} of yeast extract, either guaiacol or veratryl alcohol (0.1% v/v), and agar (15 g L^{-1}).

¹). Each plate was inoculated with 5 μ L of the isolated bacterial suspension. To identify the deterioration zones, the plates were flooded with Gram's iodine solution after being incubated for 16–18 h at 30 °C. Following the method described by Bandounas et al. (2011), isolates exhibiting ligninolytic activity were assessed for their capacity to decolorize methylene blue (MB) and azure B (AZ). Dye agar plates contained KH_2PO_4 (0.53 g L⁻¹), NH_4NO_3 (5 g L⁻¹), MgSO_4 (0.5 g L⁻¹), K_2HPO_4 (4.55 g L⁻¹), yeast extract (0.1 g L⁻¹), glycerol (40 mM), dye (100 mg L⁻¹ MB or AZ), and agar (15 g L⁻¹). Each plate was aseptically inoculated with 5 μ L of an 18-hour bacterial culture. Following a 24- to 48-hour incubation period at 30 °C, the plates were routinely checked for growth and the development of decolorization zones (Dube et al. 2023).

Screening of manganese peroxidase (MnP) producing bacterial strains

For isolation of potential MnP producing bacteria, 1 g of collected press mud sample was added to a prepared glucose peptone dipotassium phosphate magnesium sulphate (GPM) broth (g L⁻¹) containing glucose (1%), peptone (0.1%), K_2HPO_4 (0.1%), MgSO_4 0.7% (0.05%), which was then maintained at 120 rpm in incubator shaker at 37 °C for seven days. Following incubation, 1 mL of the enriched sample and 9 mL of sterile water were transferred to a sterile flask, where they were further diluted to achieve a 10⁻⁶ dilution. Each GPM plate received 1 mL of the previously diluted bacterial culture. The inoculated plates underwent incubation at 37 $\pm$ 1 °C for 48 h to allow the bacterial colonies to develop (Kumar and Chandra 2018). To obtain pure cultures, individual colonies were streaked onto fresh plates for 48 h at 37 °C. MnP production by the bacterial isolates was then assessed using phenol red as an indicator, which underwent oxidization in the presence of MnP activity, indicated by the change of the red color to yellow around the bacterial colonies, and the zone of color change was measured (Kheti et al. 2023).

Effect of press mud on plant growth (Brassica campestris)

A pot study was carried out by mixing autoclaved garden soil with press mud in the ratio of 5:1 to assess the effect of press mud-amended soil on mustard plant growth (*Brassica campestris*). The press mud amended soil was filled in a 50 $\times$ 30 cm earthen pot containing 4% moisture and 10–12 mustard seeds were sown in each pot. To prevent fungal contamination, the seeds were surface sterilized in 0.1% HgCl_2 for two minutes. They were

then rinsed three times with double-distilled water (Bharagava and Chandra 2010). Three separate outdoor runs of the experiment were carried out, each with an additional pair of controls. After 7 days, each pot was moistened with a water sprinkler to maintain the moisture in the soil. Seedlings were visible after 15 days. Further, the plant growth was monitored to observe the effect on stem and leaf growth. After 40 days, the plant showed flowering, and pod formation was observed after 50 days. Every pot was maintained in its natural state, with a temperature of 20–30 °C and a moisture content of 50–55%. The light intensity was 1700 lx, and the photoperiod lasted 10 h. A lux meter was used to measure the light intensity (Lux meter-1131, Korea).

Statistical analysis

Each experiment was performed in triplicate. Standard deviation (SD) was calculated to assess in variability and ensure reliability of the results for physico-chemical parameters and their impact on seed germination as mentioned previously (Bharagava and Chandra 2010). Data analysis, t-test and ANOVA analyses and were carried out using Microsoft Excel 2010 (Microsoft, Redmond, WA). Error bars indicate mean $\pm$ SD with sample size $N = 3$. In every parameter analyzed through ANOVA i.e. root length, shoot length, Leaf number, total dry weight, the interaction effect between treatment and day is highly statistically significant (p -value < 0.05). This indicates that the performance difference between treatments is not constant but changes significantly depending on the Day of measurement.

Results

Physicochemical analysis of sugarcane press mud

The physicochemical analysis of sugarcane press mud revealed high elemental concentrations, indicating potential environmental concerns. The measured pH was 6.4 ± 0.1 , indicating a slightly acidic environment. Sodium concentration was 70.31 ± 0.01 ppm and potassium concentration was 1061.06 ± 0.04 ppm, both significantly exceeding the USEPA permissible discharge limit of 5–9 ppm for sodium and 10 ppm for potassium. Magnesium and calcium were present in substantial amounts (624.96 ± 0.03 ppm and 461.06 ± 0.01 ppm, respectively), while phosphorus reached 513.11 ± 0.02 ppm, approaching the USEPA limit of 750 ppm. Chloride, sulphate, and nitrate levels were considerably higher than permissible limits,

suggesting potential risks of soil and water contamination. Trace elements such as aluminum (2083.22 ± 0.03 ppm) and iron (2342.57 ± 0.03 ppm) were detected at elevated levels, and heavy metals such as titanium (85.82 ± 0.01 ppm), manganese, zinc, and lead (3.10 ± 0.001 ppm) were also present above recommended standards (Table 1).

Table 1 Physicochemical analysis of sugarcane press mud $\pm$ SD with their permissible discharge limit as per USEPA

Parameters	Press mud	Garden Soil	Max permissible discharge limit (USEPA 2002)
Color	Brown	Dark Brown	
pH	6.4 ± 0.1	7.3 ± 0.03	-
Sodium	70.31 ± 0.01	28.2 ± 0.02	5–9
Ammonium	35.70 ± 0.02	6.61 ± 0.04	60.00
Potassium	1061.06 ± 0.04	86.03 ± 0.04	10
Magnesium	624.96 ± 0.03	598.84 ± 0.06	-
Calcium	461.06 ± 0.01	321.32 ± 0.45	-
Fluoride	0.45 ± 0.01	0.97 ± 0.57	-
Chloride	32.49 ± 0.02	26.01 ± 0.04	15.00
Phosphorus	513.11 ± 0.02	321.01 ± 0.02	750.00
Phosphate	54.63 ± 0.02	31.22 ± 0.02	-
Sulphate	72.26 ± 0.01	37.02 ± 0.02	2.00
Nitrate	421.95 ± 0.01	28.61 ± 0.04	750.00
Total Nitrogen (%)	3.19	0.5 ± 0.03	-
Total sodium (ppm)	3063.00	29.31 ± 0.06	-
TOC (%)	18.60 ± 0.03	03.56 ± 0.01	10.0
Metals/trace elements			
B	1.10 ± 0.03	11.02 ± 0.02	-
Al	2083.22 ± 0.03	1103 ± 0.01	-
Ti	85.82 ± 0.01	56.01 ± 0.65	1.0
V	6.17 ± 0.01	2.35 ± 0.14	-
Cr	4.18 ± 0.03	1.03 ± 0.02	-
Mn	85.90 ± 0.01	43.06 ± 0.32	0.20
Fe	2342.57 ± 0.03	2154.95 ± 0.64	2.00
Co	1.01 ± 0.01	1.54 ± 0.54	7.00
Ni	3.30 ± 0.02	1.91 ± 0.95	0.10
Cu	17.70 ± 0.03	6.75 ± 0.85	0.05
Zn	60.84 ± 0.03	46.45 ± 0.75	2.00
As	1.71 ± 0.02	0.98 ± 0.34	10.00
Se	24.59 ± 0.01	17.64 ± 0.85	0.50
Sr	17.76 ± 0.02	11.65 ± 0.14	-
Mo	0.12 ± 0.02	0.09 ± 0.84	-
Cd	0.15 ± 0.02	0.04 ± 0.65	0.01
Ba	32.79 ± 0.03	56.84 ± 0.01	-
Pb	3.10 ± 0.001	1.09 ± 0.65	0.05

All the values are in ppm except pH, total nitrogen, and total organic carbon (TOC)

Scanning electron microscopy and energy dispersive X-ray spectroscopy

SEM-EDX analysis revealed that sugarcane press mud contained significant amounts of oxygen (55.38%), magnesium (4.44%), aluminium (1.68%), silicon (12.03%), phosphorus (trace amounts), sulfur (2.58%), potassium (1.54%), calcium (12.62%), and iron (4.47%) (Fig. 1a, b). These results highlighted the mineral-rich and heterogeneous composition of the press mud, reflecting both soil-derived minerals and processing residues.

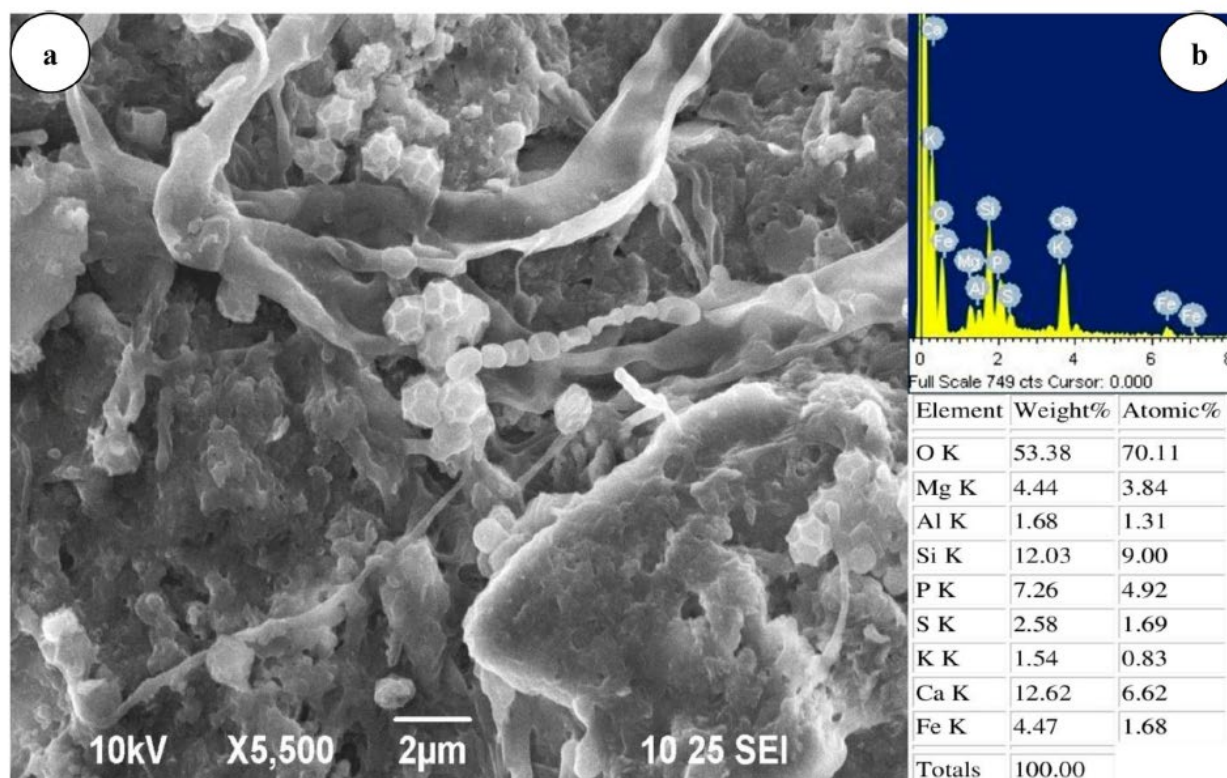


Fig. 1 (a) SEM image showing the presence of bacterial community in press mud (b) Elemental analysis of press mud

FTIR analysis of press mud

The FTIR analysis of press mud indicated the presence of a wide variety of functional groups. A strong peak at 3403.0 cm^{-1} corresponded to N-H stretching of primary amines, while peaks at 2968.9 cm^{-1} and 2921.1 cm^{-1} suggested amine salts. The band at 2854.3 cm^{-1} indicated the presence of alkanes, whereas the peak at 1647.4 cm^{-1} was attributed to C = N stretching of imine or oxime groups. Phenolic O-H bending was recorded at 1423.3 cm^{-1} and 1386.0 cm^{-1} , while C-O stretching of alcohols appeared at 1085.3 cm^{-1} . Halogen compounds were represented by C-Br and C-I stretching at 602.8 cm^{-1} and 554.6 cm^{-1} , respectively.

Additionally, C-C stretching in cycloalkanes was identified at 468.0 cm^{-1} , indicating the presence of lignin and other aromatic structures (Fig. 2; Table 2).

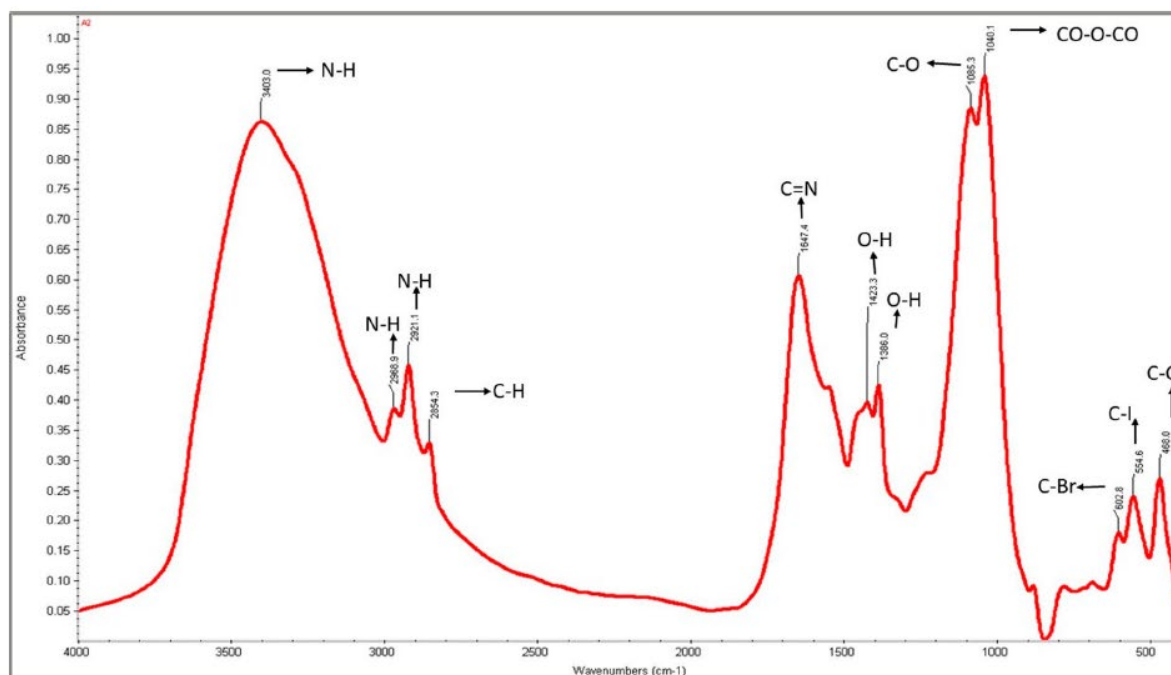


Fig. 2 Fourier transform infrared (FTIR) spectra of the press mud

Table 2 Functional group detected by FTIR analysis of press mud from distillery

Bond range(cm^{-1})	Wavenumbers (cm^{-1})	Bond	Functional group
3500–3400	3403.0	N-H stretching	Primary amine
3000–2800	2968.9	N-H stretching	Amine salt
3000–2800	2921.1	N-H stretching	Amine salt
3000–2840	2854.3	C-H stretching	Alkane
1690–1640	1647.4	C=N stretching	Imine/oxime
1440–1395	1423.3	O-H bending	Phenol
1390–1310	1386.0	O-H bending	Phenol
1085–1050	1085.3	C-O stretching	Primary alcohol
1050–1040	1040.1	CO-O-CO stretching	Anhydride
690–515	602.8	C-Br stretching	Halogen compound
600–500	554.6	C-I stretching	Halogen compound
430–480	468.0	C-C stretching	Cycloalkane

Organic compounds analysis by GC-MS from ethyl acetate extracted sample of press mud under acidic (pH6.4) and alkaline (pH 8.0) conditions

The GC-MS analysis of organic compounds extracted from press mud under acidic (pH 6.4) and alkaline (pH 8.0) conditions revealed the presence of several toxic compounds. Under acidic conditions, compounds such as 10-undecenoic acid octyl ester, carbetamide, thymol, 2,4-dimethyl-2,3-heptadien-5-yne, and methyl-(1-phenylethyl)-hydroxylamine were detected. In alkaline conditions, the compounds identified included 4-octadecenal, hexadecane, dodecyl acrylate, 1-hexadecanol 2-methyl-, and 9-hexadecenoic acid 9-octadecenyl ester (Fig. 3a & b and Table S1).

Cross-correlation of ICP-MS, SEM-EDX, FTIR and GC-MS findings

A comparative evaluation of the ICP-MS and SEM-EDX data shows strong consistency in the elemental composition of the sugarcane press mud. ICP-MS analysis (Sect. 3.1) reported significantly high concentrations of major and trace elements such as Fe (2342.57 ± 0.03 ppm), Al (2083.22 ± 0.03 ppm), Ca (461.06 ± 0.01 ppm), Mg (624.96 ± 0.03 ppm), and K (1061.06 ± 0.04 ppm). These findings are well-supported by SEM-EDX results (Sect. 3.2), which detected Fe (4.47%), Al (1.68%), Ca (12.62%), Mg (4.44%), and K (1.54%) as major mineral constituents of the press mud. While ICP-MS provides quantitative concentrations in parts per million (ppm) and is more sensitive to trace and heavy metals, SEM-EDX provides the relative percentage distribution at the surface level. Thus, the presence of Fe, Al, Ca, Mg and K in both analyses confirms that sugarcane press mud is mineral-rich and contains elevated levels of environmentally significant elements, reinforcing concerns regarding its uncontrolled disposal. Together, ICP-MS and SEM-EDX offer complementary confirmation of elemental contamination and heterogeneous mineral composition.

Similarly, a meaningful correlation can also be drawn between FTIR and GC-MS findings. FTIR analysis (Sect. 3.3) indicated the presence of functional groups such as hydroxyl (–OH), carbonyl (C = O), alkyl (C–H), aromatic (C = C) and ester (C–O) groups. These functional groups correspond closely with the organic compounds identified by GC-MS in Sect. 3.4. For instance, the detection of phenolic –OH bands in FTIR are consistent with the identification

of phenolic compounds such as thymol and substituted phenolics like 2,4-di-tert-butylphenol (2,4-DTBP) in the GC-MS profile. Likewise, strong C = O and C–O stretching vibrations observed in FTIR support the presence of esters and fatty acid derivatives such as 10-undecenoic acid octyl ester, dodecyl acrylate, and 9-hexadecenoic acid 9-octadecenyl ester detected in GC-MS. The alkyl C–H stretching bands also correlate with long-chain hydrocarbons like hexadecane and 4-octadecenal identified under alkaline conditions.

Therefore, FTIR served as a functional group screening tool, while GC–MS provided molecular-level identification of specific pollutants. When interpreted together, these analytical techniques confirm that sugarcane press mud contains a complex mixture of mineral elements and potentially toxic organic pollutants, including phenolic compounds, hydrocarbons, fatty acid esters, and amide derivatives. This multi-technique validation strengthens the overall conclusion that press mud is chemically heterogeneous and may pose environmental risks if improperly managed.

Bacterial identification from press mud at different pH levels

Nine dominant bacterial strains were isolated and purified, each exhibiting distinct morphological characteristics across pH levels of 6.4, 7.0, and 9.5). Bacterial identification revealed diverse microbial populations adapted to varying pH conditions. At acidic pH (6.4), *Escherichia coli* strain PBN1, *Bacillus licheniformis* strain PBN2, and *Moraxella catarrhalis* strain PBN3 were isolated. At neutral pH (7.0), the isolates included *Herbaspirillum seropedicae* strain PBN4, *Enterococcus faecium* strain PBN5, and *Micrococcus luteus* strain PBN6. At alkaline pH (9.0), the identified strains were *Bacillus subtilis* strain PBN7, *Listeria ivanovii* strain PBN8, and *Paracoccus pantotrophus* strain PBN9 (Fig. 4). The phylogeny analysis of all identified bacteria is shown in Fig. 4.

PGP and ligninolytic traits of the isolates

All nine isolates expressed a range of plant growth–promoting (PGP) traits, though the extent and combination of these functions differed noticeably among them. When the traits are considered collectively rather than individually, *M. catarrhalis* PBN3, *E. faecium* PBN5, and *M. luteus* PBN6 emerge as the most functionally competitive strains. *M. catarrhalis* PBN3 displayed strong performance in both IAA synthesis and phosphate solubilization, whereas *E. faecium* PBN5 paired high IAA production with efficient zinc and potassium solubilization as

well as the ability to fix nitrogen. *M. luteus* PBN6 also showed elevated IAA levels and nitrogen fixation, together with moderate contributions to other solubilization traits. Although traits such as siderophore release were most prominent in *B. licheniformis* PBN2 and *M. luteus* PBN6, these advantages did not develop into consistently strong performance across other PGP categories. Overall, the combined interpretation of the data suggests that *M. catarrhalis* PBN3, *E. faecium* PBN5, and *M. luteus* PBN6 possess the most balanced and agronomically relevant PGP profiles (Table 3).

A parallel evaluation of ligninolytic activities shows that only a fraction of the isolates demonstrated consistent strength across multiple enzyme systems. Despite the universal presence of MnP activity, *E. coli* strain PBN1, *H. seropedicae* strain PBN4, and *M. luteus* strain PBN6 were among the highest performers, exhibiting marked laccase activity along with enhanced lignin peroxidase and manganese peroxidase production. While several other isolates expressed one or two ligninolytic enzymes, they did not match the integrated enzymatic strength of these strains. Consequently, although ligninolytic capacity is broadly distributed among the press-mud–derived isolates, these three strains represent the most capable degraders due to their sustained, high-level activity across multiple enzyme systems (Table 3).

Table 3 PGPR attributes and lignolytic enzyme activity of various bacterial strains isolated from distillery press mud at different pH levels

S.N	Strain	Different pH of press mud	IAA	Phosphate solubilization	Zinc solubilization	Potassium solubilization	Siderophore production	Nitrogen fixation test	Lignin peroxidase	Manganese peroxidase	laccase
1	<i>E. coli</i>	pH 6	+	++	++	++	++	+	++	++	+++
2	<i>B. licheniformis</i>	pH 6	+	++	++	+	+++		+++	+	
3	<i>M. catarrhalis</i>	pH 6	+++	+++	+	++		+	+++	++	
4	<i>H. seropedicae</i>	pH 7	+++	+	+	+	++		+	+++	++
5	<i>E. faecium</i>	pH 7	+++		+++	+++		+	-	+++	+
6	<i>M. luteus</i>	pH 7	+++	+	++	++	+++	+	+	+++	+++
7	<i>B. subtilis</i>	pH 9	+	-	+++	+++	+		+++	++	+
8	<i>L. ivanovii</i>	pH 9	++		+++		+		-	+	
9	<i>P. pantotrophus</i>	pH 9	+		+++		+	+	+++	+	

Keys: (+)=average; (++)=more, (+++)=most

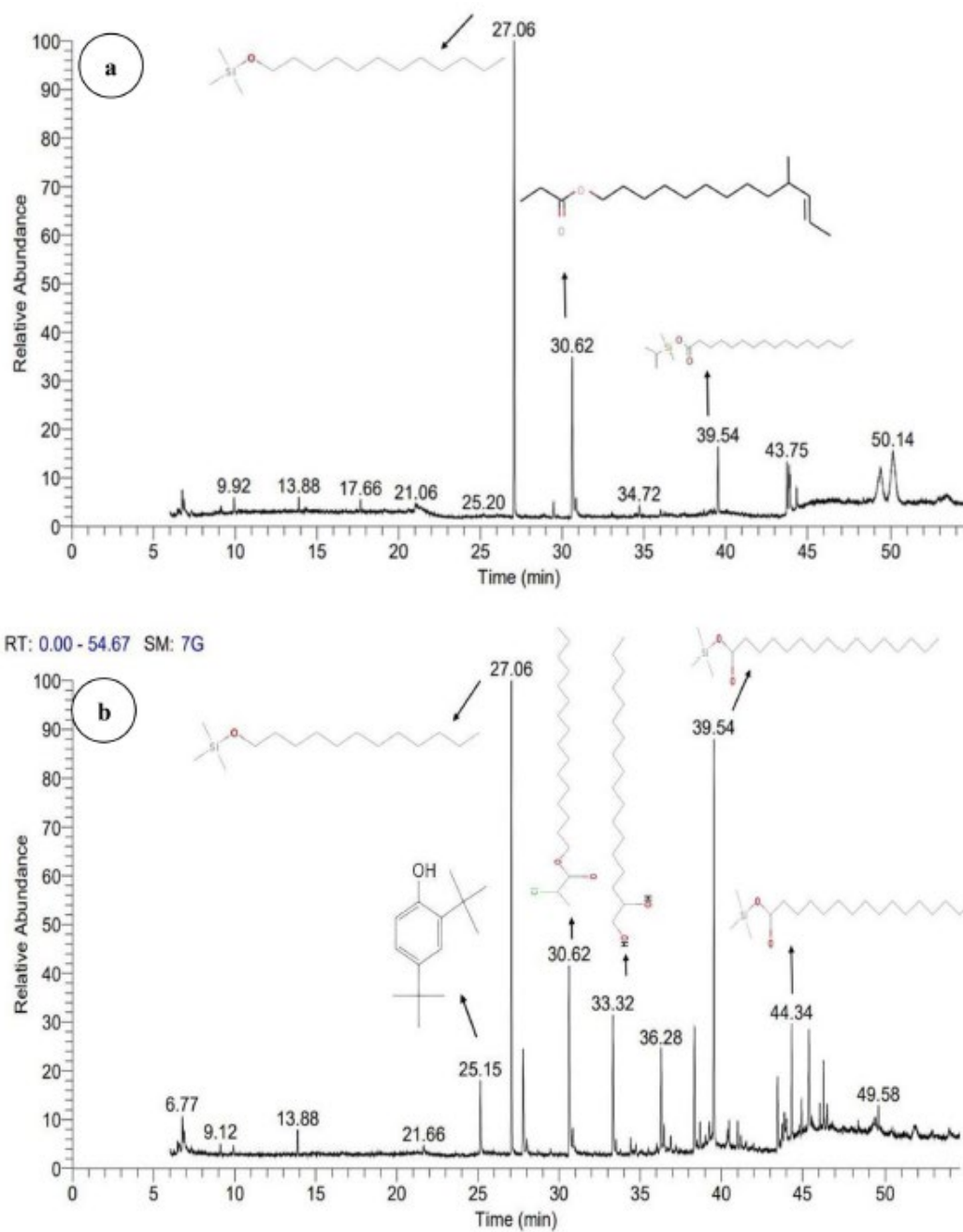


Fig. 3 GC-MS chromatogram of organic compounds extracted with ethyl acetate from distillery press mud at different pH: (a) at pH 6.4 and (b) at pH 8.0

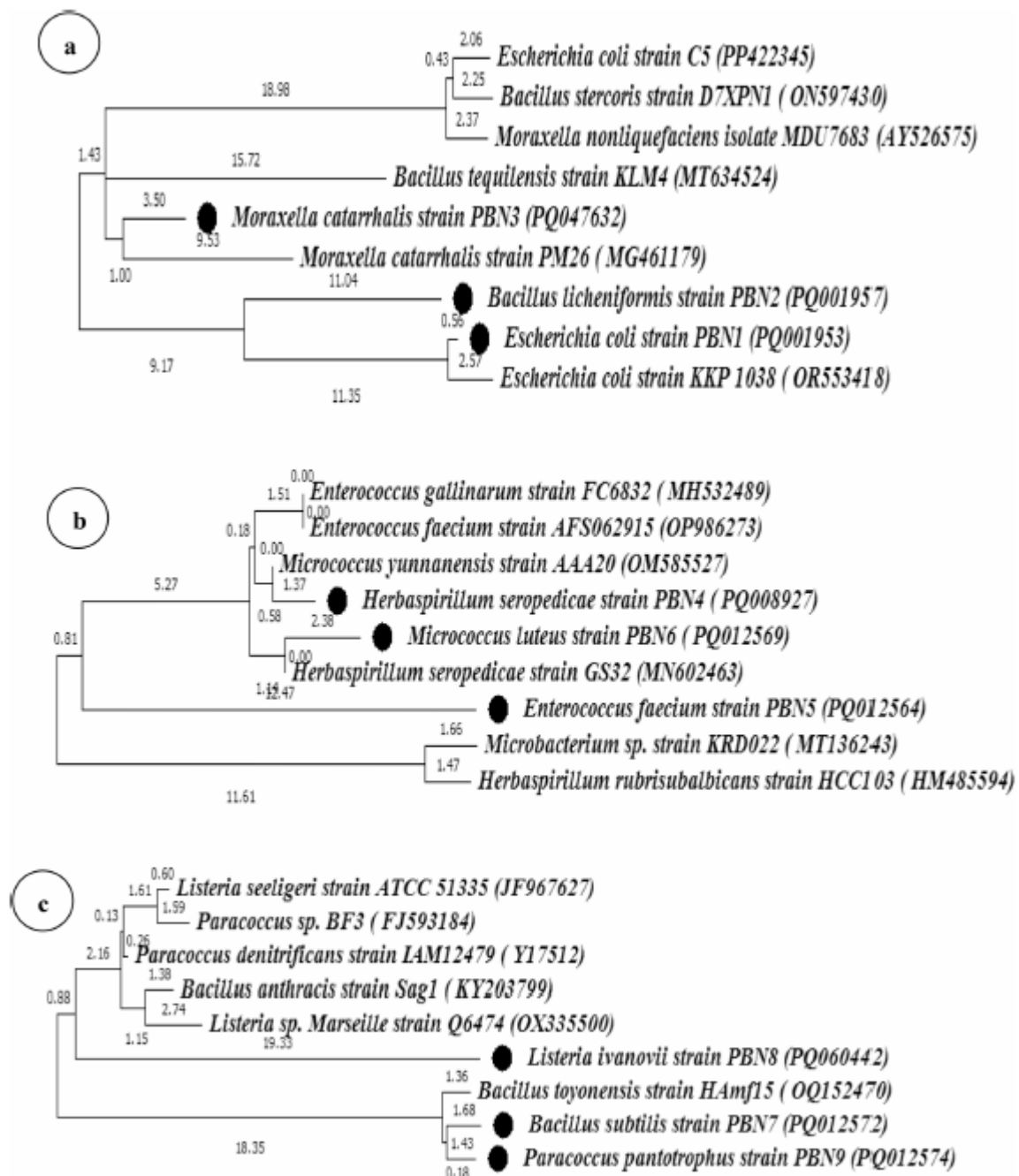


Fig. 4 Phylogenetic tree of different bacterial strains obtained at different pH levels: (a) acidic, (b) neutral, and (c) alkaline

Effect of press mud amended soil on mustard plant (*Brassica campestris*) growth

The pot experiment with mustard (*Brassica campestris*) demonstrated significant improvements in plant growth in soil amended with press mud. The obtained results were statistically analysed with one-way ANOVA to ensure the credibility of the findings. The incorporation of press-mud time points. At 25 days, root length rose from 4.63 ± 0.06 cm into

the soil significantly improved plant growth relative to in the control (a) to 6.63 ± 0.15 cm in the press-mud treated the control. Root and shoot lengths increased notably at all treatment (b), reaching 20.87 ± 0.86 cm at 75 days. Shoot length exhibited a similar trend, increasing from 11.77 ± 0.25 cm to 50.90 ± 0.52 cm over the same period. Leaf production per plant and total dry weight were also significantly enhanced, with leaves increasing from 5.67 ± 0.58 to 13.67 ± 0.58 and dry weight from 4.60 ± 0.17 g to 39.23 ± 0.06 g by 75 days. The assigned statistical letters (a/b) confirm that these differences were significant ($p < 0.05$), indicating that press mud consistently promoted vegetative growth and biomass accumulation. Root length increased by 1.29–1.39.29.39fold, shoot length increased by 2.22-fold, the number of leaves increased by 1.62–2.00.62.00-fold, and total dry weight of plants increased by 2.20–2.29.20.29-fold compared to control plants (Table 4; Fig. 5). These findings confirmed the potential of press mud to enhance plant growth when applied in low concentrations (soil-to-press mud ratio of 5:1).

Table 4 Effect of sugarcane mill press mud amended soil (5:1) on mustard plant growth

Root length (cm)		
Days	Control (mean±SD)	Press mud (mean±SD)
25	4.63 ± 0.06 f	6.63 ± 0.15 e
50	9.55 ± 0.05 d	17.42 ± 0.03 c
75	15.58 ± 0.03 b	20.87 ± 0.86 a
Shoot length (cm)		
Days	Control (mean±SD)	Press mud (mean±SD)
25	11.77 ± 0.25 f	15.53 ± 0.21 e
50	15.27 ± 0.29 d	32.70 ± 0.36 c
75	22.87 ± 0.23 b	50.90 ± 0.52 a
Leaf number/plant		
Days	Control (mean±SD)	Press mud (mean±SD)
25	5.67 ± 0.58 f	7.33 ± 0.58 e
50	10.33 ± 0.58 d	15.67 ± 0.58 c
75	7.67 ± 0.58 b	13.67 ± 0.58 a
Total dry weight (g)		
Days	Control (mean±SD)	Press mud (mean±SD)
25	4.60 ± 0.17 f	6.33 ± 0.06 e
50	10.23 ± 0.06 d	13.60 ± 0.20 c
75	17.58 ± 0.38 b	39.23 ± 0.06 a

Values are mean±SD ($n=3$). Data were analyzed using two-way ANOVA (treatment × day) followed by Tukey's post-hoc test. Different superscript letters indicate significant differences among treatments and sampling days ($p < 0.05$). Values sharing the same letter are not significantly different



Fig. 5

Showing plant growth promoting attributes of bacteria isolated from sugarcane mill press mud (a) Siderophore production on chrome azurol S (CAS) agar plates; (b) Phosphate solubilization test on Pikovskaya's agar plates; (c) Potassium solubilization test on Aleksandrov agar plates; (d) Zinc solubilization test on zinc solubilizing agar plates; (e) Indole-3-acetic acid (IAA) production test after addition of orthophosphoric acid & Salkowski reagent; (f) Nitrogen production test in glucose nitrogen free mineral media; (g) Effect of press mud on *Brassica campestris* plant growth in early stage (5:1 = 5 parts soil & 1 part press mud); (h) Effect of press mud on *Brassica campestris* plant growth at flowering stage (5:1 = 5 parts soil & 1 part press mud)

Discussion

Physicochemical analysis of sugarcane press mud

The observed neutral to slightly acidic pH range suggests potential effects on nutrient availability and microbial activity when applied as a soil amendment (Neina [2019](#)). Analysis revealed that sugarcane press mud contains high levels of salts and heavy metals (Na, K, Mg, Ca, Fe, Al, Ti, Mn, Zn, Pb), often exceeding USEPA permissible limits. These elevated concentrations are likely attributed to inputs such as fertilizers, pesticides, irrigation water, inherent soil composition, and industrial processes during sugar production. The evaluated sodium concentration likely originates from the use of NaOH in the sugar extraction process, or the excessive use of sodium-based fertilizers and saline irrigation water in sugarcane fields. Naturally occurring sodium in the soil, particularly in areas with saline groundwater further contributes to this accumulation (Hailu and Mehari [2021](#)). Excessive Na and Cl can cause salinization, reduce water uptake and disrupt soil structure, while high K, Mg, and Ca may disrupt nutrient balance (Hailu and Mehari [2021](#)). Elevated phosphorus levels pose eutrophication risks if leached into water bodies, while Fe and Al toxicity can impair root development and nutrient uptake. Although potassium is essential for plant growth, concentrations exceeding the permissible limit of 10 ppm can lead to nutrient imbalance in the soil and potential toxicity (Sardans and Peñuelas [2021](#)). High K levels in press mud are largely due fertilizer application and sugarcane's natural uptake, which concentrates in the residue after juice extraction. Similarly, Mg (50–500 ppm) and Ca (700–1000 ppm) occur naturally in soils and are absorbed by sugarcane, with additional contributions from fertilizers and processing steps such as lime application and water softening. While both elements are critical for plant physiology, excessive amounts can affect soil texture and nutrient dynamics, particularly in alkaline soils (Mayland and Wilkinson [1989](#); Sanyal et al., [2009](#)). Phosphorus near the USEPA limits reflects phosphate fertilizers use and sugar clarification processes phosphorus-based chemicals (Illy et al. [2015](#)). Chloride and sulfate, often introduced through irrigation water, sulfur-based fertilizers, and refining processes, can exacerbate salinization and soil acidification, negatively impacting crop yields and microbial activity (Haj-Amor et al. [2022](#)).

Al and Fe concentrations exceeded the permissible limits, posing risks to soil structure and fertility. Excess Fe can interfere with nutrient absorption in plants (Brown 1978), while Al toxicity can inhibit root growth (Silva 2012). Elevated levels of these metals in press mud can be traced to both soil composition and industrial inputs, such as iron-based flocculants used during sugar clarification. Al is abundant in acidic soils and readily absorbed by sugarcane, while Fe, though essential for plant growth, becomes problematic at high concentration. Heavy metals including Pb and Ti represent major environmental hazards, disrupting soil microbial communities, nutrient cycling, and potentially entering the food chain through crop bioaccumulation (Naz et al. 2022). Their presence in press mud is attributed to soil mineral content, fertilizer and pesticide use, and industrial runoff. While Mn and Zn, are essential micronutrients, excessive accumulation from repeated fertilizer application can lead to toxicity. Lead contamination, often linked to industrial emissions or legacy pesticides use, poses severe risks to soil biodiversity and human health via food chain transfer.

The high concentrations of salts and heavy metals in the press mud underscore the need for effective treatment and management prior to its use in agriculture. Application of untreated press mud can degrade soil quality, disrupt microbial communities, and impair nutrient availability. Heavy metals such as Fe, Mn, and Ti and Pb, detected at levels exceeding permissible limits, pose risks to soil fertility and plant health (Naz et al. 2022). Their presence likely results from industrial effluents, fertilizers and pesticides usage, and bioaccumulation in sugarcane tissue during growth, which transfers these contaminants to press mud during juice extraction. Therefore, implementing treatment strategies to reduce metal concentrations is essential to mitigate environmental and food safety risks.

Scanning electron microscopy and energy dispersive X-ray spectroscopy

SEM-EDX and FTIR analyses confirmed that press mud is a complex mixture of mineral residues, plant-derived compounds, and agrochemical residues. The detection of amines, phenols, halogen compounds, and hydrocarbons suggests the presence of organic contaminants that could pose risks to soil biota and human health. Oxygen occurs as a component of organic matter and oxides formed during decomposition and microbial activity. Elements such as Mg and Ca, commonly associated with plant residues and fertilizers inputs, also contribute to the structural framework of organic pollutants. The presence of Al and Si suggests the inclusion of soil particles and mineral deposits during the harvesting and pressing

processes, with Si originating from soil and playing a role in strengthening plant cell walls. Trace amounts of P and S, likely derived from fertilizers and agrochemical residues, while K, a key nutrient, accumulates fertilizer use and sugarcane uptake, potentially impacting soil and water quality when present in excess. Fe contamination may result from soil minerals or mechanical processes, and at high concentrations, can form iron oxides, contributing to environmental pollution. Elevated levels of Mg, Ca, Si, and Fe indicate the potential of press mud to act as a significant pollutant source, threatening aquatic and terrestrial ecosystems if not properly managed.

FTIR analysis of press mud

IR spectra between 500 and 4000 cm^{-1} identified functional groups associated with amines, alcohols, phenols, halogen compounds and hydrocarbons. Amines likely originate from the breakdown of amino acids and proteins in sugarcane biomass during the extraction process (Valério et al. 2022). Amine salts may form through interactions of free amino acids during fermentation or decomposition (Panigrahi et al. 2021). Hydrocarbons are common byproducts of organic matter degradation, as plant materials contain significant amounts of fatty acids and aliphatic compounds (Teixeira et al. 2021). Oxime groups may result from Maillard reactions between sugars and amino compounds during processing can lead to the formation of such nitrogen-containing compounds (El Hosry et al. 2025). Phenolic compounds derive from lignin and cellulose degradation, contributing to the antioxidant properties of the by-product (Lauberte et al. 2021). Carbohydrates and their derivatives are often remnants of the incomplete juice extraction (Panigrahi et al. 2021). Halogen compounds likely result from pesticide residues or environmental exposure (Singh et al. 2020), while cycloalkane structures reflect lignin-derived cyclic components and polycyclic aromatic hydrocarbons. The diversity of organic contaminants suggests it is necessary to integrate monitoring and assessing environmental impact in the press mud management strategies (Reddy et al. 2021).

Organic compounds analysis by GC-MS from ethyl acetate extracted sample of press mud under acidic (pH6.4) and alkaline (pH8.0) conditions

GC-MS analysis detected several toxic compounds under both acidic and alkaline conditions, including known carcinogens (carbetamide), endocrine disruptors (9-hexadecenoic acid ester), and neurotoxic substances (1-hexadecanol 2-methyl-). These compounds likely

originated from complex fermentation and distillation processes involving sugarcane molasses, bagasse, and other materials used in alcohol production (Santos et al. 2020). Under acidic conditions, compounds such as 10-undecenoic acid octyl ester and carbetamide may form through the breakdown of fatty acids and pesticide residues introduced via agricultural runoff (Mitra, 2024). Thymol, derived from plant matter during fermentation, was also detected; it is toxic to microorganisms and can be associated with chronic respiratory toxicity (Marchese et al. 2018). Additional compounds, including 2,4-dimethyl-2,3-heptadien-5-yne (causes skin irritation), and methyl-(1-phenylethyl)-hydroxylamine (links to hemolytic anaemia), likely originate from the degradation of organic residues during processing.

Under alkaline conditions, GC-MS analysis identified compounds such as 4-octadecenal and hexadecane, likely byproducts of long-chain fatty acid degradation originating from sugarcane waxes or other plant oils in press mud. 4-octadecenal is associated with respiratory irritation, while hexadecane is highly toxic if ingested and aspirated (Ansari and Gaikar 2014). The detection of dodecyl acrylate suggests ester formation during the interaction of alcohols with fatty acids present in the fermentation residues; this compound is known to cause serious eye and respiratory tract irritation (Bhalerao and Vijay 2019). Neurotoxic and hepatotoxic 1-hexadecanol 2-methyl- may have originated from plant lipids during distillation. Whereas 9-hexadecenoic acid 9-octadecenyl ester, reported as an endocrine disruptor, likely results from thermal and chemical breakdown of complex plant-based fats and oils.

The detection of toxic compounds in both acidic and alkaline extracts indicates that fermentation and distillation processes in sugarcane molasses production contribute to the generation of harmful organic substances. Once present in press mud, these compounds pose a serious risk to human health and the environment, including respiratory irritation, skin and eye irritation, neurotoxicity, and even carcinogenicity. Findings in this study suggested that distillery press mud contains a diverse range of hazardous organics, necessitating composting or advanced treatment before agricultural application or environmental discharge.

Bacterial identification from press mud at different pH levels

The microbial isolation revealed diverse bacterial taxa with plant growth-promoting (PGP) traits. Acidiphilic isolates (e.g., *B. licheniformis*) contributed to phosphate solubilization, whereas neutral isolates (e.g., *H. seropedicae* and *M. luteus*) were associated with nutrient cycling. Alkaliphilic strains (e.g., *B. subtilis* and *P. pantotrophus*) showed ligninolytic activity

and nitrogen cycling potential. Notably, *M. catarrhalis*, commonly detected in environmental samples, thrives under slightly acidic conditions, suggesting a role in organic matter decomposition (Clements et al. 2002; Hoopman et al. 2008). *B. licheniformis*, a resilient spore-forming bacterium, likely facilitates breakdown of organic compounds in press mud (Muras et al. 2021; Nunes et al. 2023). Similarly, *M. luteus* contributes to nutrient recycling in neutral environments (Muras et al. 2021), while *H. seropedicae*, a nitrogen-fixing bacterium typically associated with plant roots, may enhance nutrient availability for sugarcane plants (Canellas et al. 2013; Alves et al. 2021; Lu et al. 2022). *E. faecium*, detected at neutral pH contributes to the breakdown of complex organic compounds (Guillet et al. 2010; Mello et al. 2020). In alkaline conditions, *L. ivanovii* and *B. subtilis* exhibit adaptability and enzymatic activity, aiding organic matter decomposition (Orsi and Wiedmann 2016; Guillet et al. 2010). *P. pantotrophus*, capable of denitrification, emphasising its role in nitrogen cycling (Lu et al. 2022). These findings broaden the ecological understanding of non-traditional agricultural bacteria (e.g., *E. coli*, *L. ivanovii*, *M. catarrhalis*) while highlighting biosafety concerns regarding opportunistic pathogens.

PGP and ligninolytic traits of the isolates

E. coli exhibited the highest phosphate solubilization capacity, as indicated by the largest PSI value, followed by *B. licheniformis* and *M. catarrhalis*. Variation in PSI values suggests strain-specific efficiencies in solubilizing phosphate, likely reflecting differences in metabolic pathways and mechanisms for releasing phosphate from insoluble compounds. Strains with higher PSI values, such as *E. coli* and *B. licheniformis*, emerge as strong candidates for improving soil plant uptake (Okino et al. 2000; Kayasth et al. 2013).

All nine isolates tested positive for zinc solubilization, confirming their ability to convert insoluble zinc compounds into plant-accessible forms. Among the isolates, *E. faecium*, *B. subtilis*, *L. ivanovii*, and *P. pantotrophus* demonstrated the highest levels of solubilization (Table 3), showcasing their potential for enriching zinc-deficient soils and supporting nutrient-enriched organic fertilizer development (Sahoo et al. 2019).

K solubilization was observed in five strains: *E. coli*, *B. licheniformis*, *M. catarrhalis*, *E. faecium*, and *M. luteus* (Table 3). This highlights the potential of these strains to enhance potassium availability in the soil, a crucial nutrient for plant metabolism and growth. Among

these, *E. faecium* and *B. subtilis* exhibited the highest levels of potassium solubilization. A color change from green to yellow signified a positive result for nitrogen fixation in *M. luteus*, confirmed as positive for nitrogen fixation (Table 3). *M. luteus* is not a classical, highly efficient diazotroph, but there is evidence that some strains can fix nitrogen weakly (Dubey et al. 2021; Chang et al. 2024).

Siderophore production was detected in seven isolates, including *E. coli*, *B. licheniformis*, *E. faecium*, *M. luteus*, *B. subtilis*, *L. ivanovii*, and *P. pantotrophus*, evidenced by orange halos surrounding on chrome azurol S (CAS) agar. Notably, *B. licheniformis* and *M. luteus* demonstrated the highest efficacy in producing siderophores (Table 3).

Ligninolytic enzyme assays revealed widespread MnP activity, with *E. coli*, *E. faecium*, and *M. luteus* consistently producing multiple ligninolytic enzymes (laccase, LiP, MnP). This multi-enzyme competence positions them as promising candidates for lignin-rich substrate degradation and soil amendment. Comparable findings in agro-industrial residues confirm the role of such strains in lignin depolymerisation and organic waste valorisation (Feng et al. 2011; Ghanney et al. 2021; Ghribi et al. 2016; Kumar and Chandra 2018; Tharek et al. 2021; Singh et al. 2022).

This study provides one of the first comprehensive assessments of bacterial diversity in sugarcane press mud across pH gradients, linking community composition to PGP traits. Well-established taxa such as *B. licheniformis* and *B. subtilis* were detected, reaffirming their roles in siderophore and IAA production, phosphate solubilization, and pathogen antagonism (Nigris et al. 2018; Gohil et al. 2022; Ni et al. 2024). Importantly, additional isolates including *H. seropedicae*, *E. faecium*, and *M. luteus* have been demonstrated to possess strong PGP potential at neutral pH, consistent with previous reports in cereal and legume crops (Cordeiro et al. 2013; Patil et al. 2013; Neiverth et al. 2014; Chang et al. 2024).

A novel observation is the functional activity of bacterial taxa often overlooked in agriculture. Acidophilic isolates (*E. coli* and *M. catarrhalis*) demonstrated PGP-related traits linked to organic matter turnover and phosphorus mobilization (Tharek et al. 2021). Alkaliphilic strains, including *P. pantotrophus* and *L. ivanovii*, contributed to bioconversion processes and nutrient cycling, extending previous evidence for their role in soil fertility enhancement and expanding known functional niches. Collectively, these results highlight press mud as both a nutrient-rich substrate and a reservoir of microbial diversity with

agricultural potential. However, the recovery of opportunistic and pathogenic genera such as *Escherichia*, *Moraxella*, and *Listeria* points to the need for strain-level safety evaluation and controlled bio-composting before any biofertilizer application (Tariq et al. 2022). Future work should prioritize safe, non-pathogenic groups, particularly *Bacillus* species, that are already recognized for their reliability as biofertilizer and in soil improvement.

PGP traits such as IAA production, phosphate, Zn, and K solubilization, nitrogen fixation, and siderophore production confirm the potential of press mud-derived microbes as biofertilizer candidates. Concurrent ligninolytic enzyme activity further highlights their role in lignocellulose degradation and soil fertility enhancement, aligning with findings in *Bacillus* and *Herbaspirillum* spp. (Nigris et al. 2018; Gohil et al. 2022; Chang et al. 2024).

Effect of press mud amended soil on mustard plant (*Brassica campestris*) growth

The pot experiment assessing press mud application on mustard plant (*B. campestris*) growth showed significant enhancement across all measured plant growth parameters (Table 4). This improvement likely reflects the combined effect of macro- and micronutrients present in press mud and the activity of the PGPB which enhance soil structure and fertility, thereby promoting root development. Improved root systems support increased shoot growth, while the observed rise in leaf number suggests enhanced vegetative development, potentially facilitated by greater nutrient availability and microbial activity in the amended soil. The substantial gains in total dry weight further demonstrate that press-mud enhances overall plant productivity. Collectively, these findings highlight the potential of press-mud as a sustainable organic amendment to improve crop growth and yield, with the statistical differences (a/b) validating the reliability of these effects. All nine isolated strains exhibited strong PGP traits, including nutrient solubilization (Table 3). Notably, *B. Licheniformis* and *B. subtilis* demonstrated broad pH adaptability, suggesting their contribution to the observed plant growth response. The application of press mud at a 5:1 soil-to-mud ratio enhanced mustard growth, consistent with previous studies on wheat (Kumar et al. 2014). These benefits are attributed to both nutrients and PGP bacteria. However, the detection of toxic metals and harmful organic compounds highlighted the risk of untreated application. Controlled treatment (e.g., composting, bioremediation) is necessary to reduce contaminant loads before agricultural use. This approach would harness the benefits of nutrient enrichment and microbial diversity while minimizing potential hazards.

Conclusion

This study demonstrates that sugarcane press mud is a nutrient-rich by-product that supports bacterial strains with notable plant growth-promoting (PGP) capabilities. The physico-chemical characterization, coupled with culture-dependent isolation, enabled the identification of functionally important strains such as *B. licheniformis*, *B. subtilis*, and *H. seropedicae*, which exhibited phosphorus, zinc, and potassium solubilization, nitrogen fixation, and siderophore production. Although this work confirms the presence of PGP-positive isolates, it does not include community-level or pH-specific microbial diversity analyses. Therefore, interpretations regarding microbial assemblages across pH gradients have been reinterpreted to reflect the scope of the current findings, which are limited to strain-level observations.

Despite these benefits, the detection of potentially toxic elements (e.g., Pb, Al, Fe) and harmful organic compounds (e.g., 10-undecenoic acid, hexadecane, and endocrine-disrupting chemicals) underscores the need for caution. Direct application of untreated press mud, particularly at high doses, may pose environmental or health risks. Controlled application rates and pre-treatment strategies such as composting or bioremediation are recommended to reduce contaminant loads and enhance safety.

To conclude, sugarcane press mud shows considerable promise as a biofertilizer and soil amendment, but its sustainable use requires targeted treatment, regulated application, and further research. Future studies should include comprehensive microbial community profiling and long-term field assessments to better understand its ecological impacts, optimize its agronomic value, and ensure biosafety within sustainable agriculture and circular bioeconomy frameworks.

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