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Chemical and Structural Characterisation of Bio-Earth as a Sustainable Substrate for Wastewater Treatment in Constructed Wetlands

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Constructed wetlands (CWs) are growing as sustainable, nature-based solutions for wastewater treatment, esteemed for their cost-effectiveness, ecological benefits and nutrient removal capabilities. This study introduces bio-earth, a newly engineered substrate recovered from biomineralized legacy waste, for its first application as a sustainable medium in CWs. Using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDAX), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and hydraulic and microbial analyses, the bio-earth was found to possess optimal properties for wetland applications including high porosity (40%), good permeability (3.19×10^{-3} cm/s) and a mineral composition (e.g. quartz, calcite, hematite) that enhances structural integrity, pH buffering capacity and contaminant adsorption. Moreover, bio-earth effectively immobilised heavy metals and supported diverse microbial populations (e.g. *Bacillus* and *Pseudomonas*), which are critical for pollutant degradation. Domestic wastewater treatment using vertical-flow CWs with the bio-earth-based system achieved significant removal efficiencies for BOD₅ (68%), COD (72%), nitrate (60%), TSS (78%) and a 2.1-log reduction in coliforms, showing better results than the unplanted control during the 24-week study. According to these results, bio-earth is a promising, eco-friendly substrate for circular waste management. Further field-scale studies are recommended to evaluate its long-term performance in full-scale wetland systems.

Keywords: Constructed wetland, Media, Waste management, Sustainable development, Bio-earth, Wastewater treatment.

INTRODUCTION

Water scarcity has become a major global challenge due to rapid population growth, expanding urbanisation, growing industrial demands, widespread pollution and increasing climate change impacts in the 21st century [1]. In response to these stresses, freshwater quality and quantity are depleting rapidly, resulting in severe consequences for drinking water supplies, agricultural irrigation and industrial processing. More than 2 billion people, according to a United Nations report, live in water-stressed countries and this number is expected to rise significantly in the next few decades [2]. Thus, there is a great need for sustainable management of water resources, particularly in wastewater treatment and reuse. Meanwhile, conventional process treatments such as activated sludge, chemical coagulation and membrane filtration generally have significant drawbacks, including high costs, energy consumption and the generation of secondary pollutants that require further treatment and disposal [3]. These disadvantages would be felt

predominantly by resource-constrained countries, where uptake of such advanced technologies has been limited due to financial, technical and infrastructural constraints. This leaves an urgent need for wastewater treatment systems that are cost-effective, energy-efficient and environmentally sustainable, especially in diverse settings including low-income and water stressed ones [4].

In recent years, interest in nature-based, low-impact treatment technologies that use natural processes, renewable materials and waste-derived resources has been growing. For instance, constructed wetlands (CWs) mimic natural wetland ecosystems and leverage plant-soil-microbial activity to filter and degrade pollutants [5]. The use of CWs can be appealing since they are economical, energy-efficient solutions capable of treating a broad range of contaminants, including organic matter, nutrients and heavy metals [6]. Selecting appropriate media to support plant growth and to serve as the habitat of the microbial communities responsible for pollutant degradation is an important factor in CW design and management

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operations [7]. The efficiency of constructed wetlands is moderately influenced by substrate properties, with media selection playing a pivotal role in overall system performance [8]. Usually, suitable media should be characterised by good porosity with maximal water flow, support for plant roots and nutrient storage for plants and microorganisms. The right media can further enhance the ability of the system to remove nutrients, nitrogen and phosphorus from organic and inorganic contaminants [9].

Generally, used media such as sand, gravel, soil and rock are inexpensive and easily available [10]. However, they tend to exhibit limitations, including clogging, low surface area for microbial action and poor nutrient adsorption capacity that restrict their performance [11]. Some of the newer media, such as biochar, zeolite and industrial byproducts (*e.g.* slag, fly ash) have been investigated to overcome such limitations. Biochar, with its high surface area and porosity, enhances nutrient adsorption and microbial activity, whereas zeolite excels at ammonium removal *via* ion exchange [12]. Steel slag, an industrial byproduct, is effective at removing phosphorus and heavy metals and offers a green waste-recycling alternative [13]. Composite media, integrating materials such as biochar and zeolite, have been found to exhibit synergistic behaviour, with high rates of pollutant removal (*e.g.* 95% nitrogen and 85% phosphorus removal) and enhanced hydraulic performance [14]. Innovative approaches to media selection, such as bio-earth derived from legacy waste and recycled materials, can significantly improve the efficiency and sustainability of constructed wetlands.

Legacy waste is generated over time by improper disposal of solid waste, especially in poorly managed landfills or open dumps. Various factors contribute to this issue including rapid population growth, urbanisation and a lack of appropriate waste management systems. With increasing population and urbanisation, the amount of solid waste generated exceeds the handling capacity of waste management systems of cities, resulting in persistent waste issues [15]. The environmental

and health hazards arising from legacy waste include contamination of soil and water, greenhouse gas emissions and the spread of diseases. Minimising environmental and health damage requires creative solutions that integrate efforts in waste management, resource recovery and environmental protection to address the legacy waste problem [16]. One feasible approach to managing legacy waste is biomining, which helps recover value from such waste and mitigates the negative impacts of abandoned waste. This process begins with sorting the waste into different types through mechanical and manual methods, followed by the removal of recyclables, hazardous materials and large debris. The remaining waste undergoes microbial and chemical treatment to speed up the breakdown of organic matter [16]. Microorganisms play a crucial role in degrading organic materials, thereby reducing the total waste volume and transforming it into bio-earth, a type of bioengineered organic soil [17]. Bio-earth, which can achieve physical, chemical and biological properties at minimal cost, has thus been developed as the advancement substrate for CW applications [18].

The objective of this study is to characterise bio-earth and evaluate its suitability as a sustainable substrate for CWs. This work aims to study the physical, chemical, mineralogical and hydraulic attributes of bio-earth and determine how these properties support pollutant adsorption and plant growth. Furthermore, the study assesses the treatment performance of bio-earth-based vertical flow CWs, with and without *Canna indica*, for the removal of organic matter and nutrients from domestic wastewater.

EXPERIMENTAL

Study area: Thiruthangal (9.48° N, 77.81° E) is a rapidly growing town in the Virudhunagar district of India. It is famous for its cultural heritage, industrialisation and urbanisation. Fig. 1 shows the geographical locations of the study area. The town consists of about 19,446 households, with a

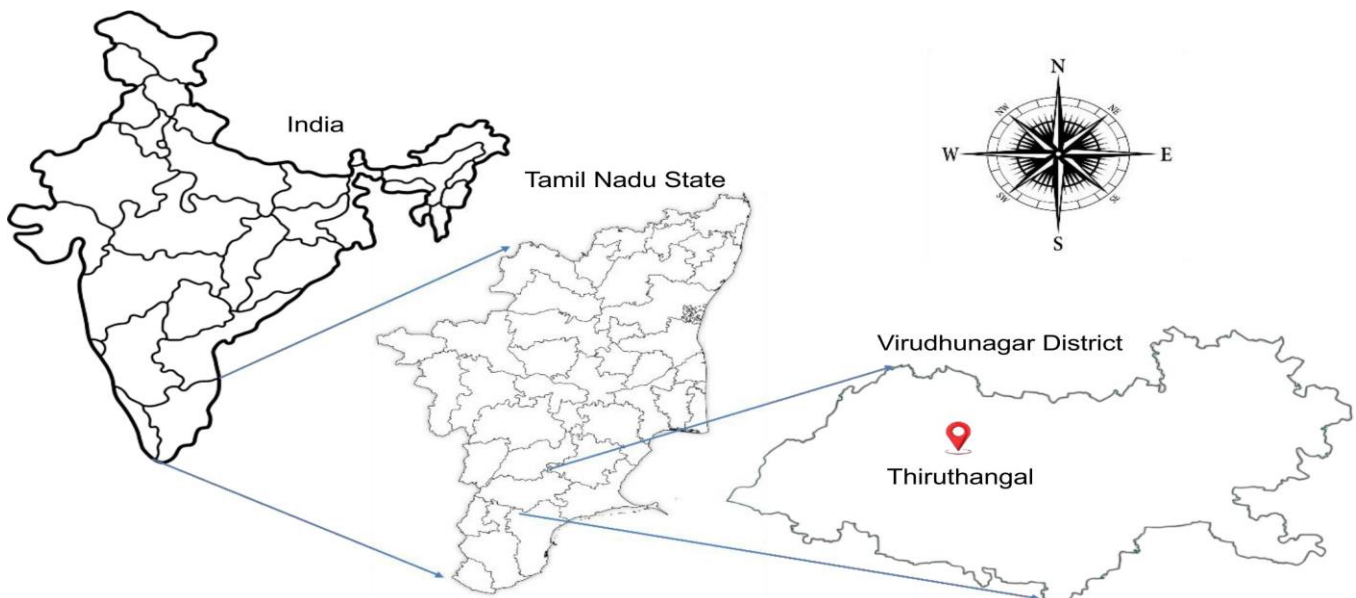


Fig. 1. Geographical locations of the study area

large percentage involved in small-scale industries, commerce and agriculture. High population growth, economic activities and urbanisation have increased the generation of municipal solid waste over time, posing grave challenges for waste management. About 16 tons of solid waste are generated daily in the town and collected from residential, commercial and public areas. The waste is transported and dumped in a separate dumping area and over time, legacy waste accumulates. Legacy waste comprises organic and inorganic materials, mainly plastics, construction waste, recyclable materials and degraded organic waste. Legacy waste has the potential to yield severe environmental pollution, groundwater contamination and health issues if not managed appropriately [19].

Biomining is one such eco-friendly approach to waste management that relies on the scientific excavation, sorting and processing of deposited waste. This approach guarantees not only a reduction in the size of inherited waste but also the retrieval of high-value materials like bio-earth that can be reused for multiple purposes including agriculture, land development and building purposes. Thiruthangal's project is a benchmark for other urban centres with similar waste management challenges. By incorporating state-of-the-art waste-processing technologies, the town will reduce environmental degradation, advance circular-economy principles and raise the overall living standards of its inhabitants.

Biomining process: The biomining procedure for legacy waste management entails a systematic, technology-based method for excavating, treating and separating accumulated landfill waste [20]. This procedure makes it possible to recover resources and reduce reliance on landfills by developing by-products from waste, such as bio-earth. The most important steps in the biomining process include excavation, stabilisation, screening and segregation to maximise waste utilisation and environmental sustainability [21]. The first step in the biomining process is the excavation of legacy waste from landfill sites. Over time, landfill waste becomes highly compacted, making excavation a critical process that requires heavy machinery such as excavators, loaders and conveyor belts. This phase involves the careful retrieval of waste from different layers of the landfill to ensure efficient processing in subsequent stages. The excavated waste is a heterogeneous mixture of organic refuse, plastics, textiles, metals, glass, construction debris and other miscellaneous items. The waste, after excavation, is stabilised by bio-culture treatment to minimise organic content, pathogens and odour.

Bio-culture is the process of applying microbial solutions and enzymes to accelerate the breakdown of biodegradable waste. This phase is important for eliminating foul smells, minimising pathogen loads and enhancing the quality of segregated waste for subsequent processing. The biological treatment also improves moisture balance, facilitating easy screening and segregation of waste by size and composition. After stabilisation, the excavated waste is screened to separate materials into particle-size fractions. screening machines and mechanical sieves to effectively segregate coarse inert, recyclables and fine organic fractions [22]. Fig. 2 presents the sequential screening and segregation process in biomining. The debris particles of sizes greater than 150 mm consist mainly of inert materials, bricks, coconut shells and shoes.

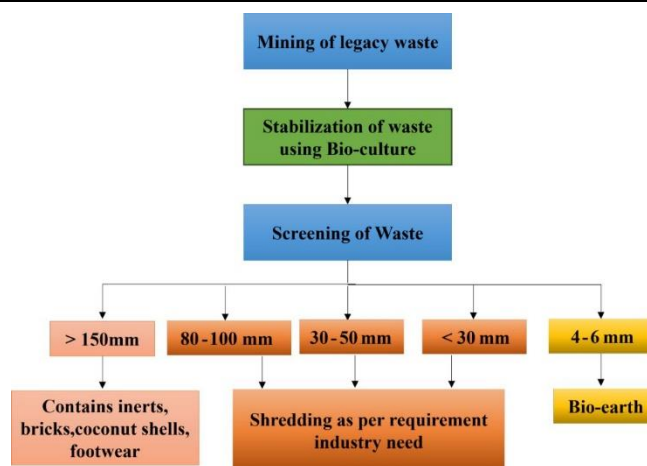


Fig. 2. Process flowchart of biomining legacy waste for bio-earth production

These materials have low recyclability and are therefore mostly disposed of or used for backfilling and construction. Intermediate fractions (30-50 mm and 80-100 mm) are made up of industrial waste, textiles and plastics.

These products are shredded according to industrial needs and recycled as refuse-derived fuel for power use. Particles less than 30 mm are also screened to recover fine organic material. Fine particles of size 4-6 mm are processed into nutrient-enhanced bio-earth, which can be used for soil amelioration, land reclamation and other environmental applications. Segregation and screening in a systematic manner ensure that different fractions of wastes are gainfully utilised with a reduced environmental footprint and maximum conversion of waste to resource.

Sample collection: The structured collection of the bio-earth samples at the Thiruthangal biomining site made it representative and reliable in the laboratory analysis. The locations were well spread at the site to ensure that spatial differences in the composition and stages of degradation of the waste were taken into consideration. Three different depths were used, *i.e.* the surface layer (0-10 cm), which consisted of freshly deposited material; the middle layer (10-30 cm), which contained moderately decomposed material; and the bottom layer (30 or more), which comprised the older and stabilised material. Such depthwise sampling has allowed wide knowledge on physical, chemical and biological properties within the bio-earth. Collection and prevention of cross contamination of the samples was achieved through the use of stainless-steel augers and hand shovels, washed after and before use. Exactly 5-10 kg of content was collected at each location of sampling. The collected material was stored in sealed plastic containers labelled with essential details such as date, location, depth and intended analyses. Bio-earth were stored in a cool, dry place, shielded from sunlight and analysed in the laboratory within 24 h. This methodical sampling and handling process-maintained sample quality and supported accurate experimental results.

Washing pretreatment: After being collected, the bio-earth samples were pre-treated with sequential aqueous leaching (repeated washing) to get rid of soluble contaminants, fine particles and heavy metal fractions that were easy to break down. The washing process involved three washes with

deionised water (1:5 w/v) while being stirred mechanically for 30 min each. After that, the supernatant was poured off. After being washed, the bio-earth was left to air dry at room temperature for 48 h before being used in the vertical flow constructed wetland (VFCW) experiments and further studied. This pre-treatment makes sure that the bio-earth is stable in the environment and can be used in constructed wetlands.

Characterisation: FTIR analysis (IRTracer-100, Shimadzu) was performed using KBr pellets over $4000\text{--}400\text{ cm}^{-1}$ to identify functional groups. XRD (PANalytical X'Pert PRO, $\text{CuK}\alpha$, 40 kV, 30 mA) was used to determine mineral phases over $2\theta = 5^\circ\text{--}70^\circ$ and SEM-EDX (ZEISS EVO 18, Oxford Instruments) was employed to examine surface morphology and elemental composition under high vacuum at 15 kV.

Porosity: Porosity was determined using the water saturation method. Oven-dried samples were saturated and weighed to calculate pore volume relative to the total volume.

Permeability: A constant-head permeability test was conducted using a rigid-wall permeameter setup (EIE Instruments Pvt. Ltd., India), in accordance with ASTM D2434. Bio-earth media was compacted in the column and flow was measured under a fixed hydraulic gradient.

Particle size distribution: Sieve analysis was carried out according to ASTM C136 using standard sieves (0.075–4.75 mm). Coefficient of uniformity and curvature were calculated to determine grading characteristics.

Specific gravity and void ratio: Specific gravity was measured using a pycnometer (ELGI, India) as per IS:2720 (Part 3) and void ratio was calculated based on bulk and particle density values.

Microbial analysis: Total culturable bacteria were enumerated using serial dilution and plate count method on nutrient agar and cetrimide agar (HiMedia, India). Colony-forming units (CFU/g) were recorded after 24–48 h

incubation at 37°C . Identification of dominant species (*e.g.*, *Bacillus* and *Pseudomonas*) was conducted *via* Gram staining and biochemical tests.

Heavy metal analysis: Heavy metals were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES; Agilent 5110, USA). Bio-earth (1 g) were digested using a tri-acid mixture ($\text{HNO}_3\text{:HClO}_4\text{:H}_2\text{SO}_4 = 3\text{:}1\text{:}1$) in a microwave digestion system (Anton Paar Multiwave PRO, Austria). The filtrate was diluted and analysed following APHA 3120-B guidelines.

Experimental design: Two lab-scale vertical flow constructed wetland (VFCW) units were built from PVC containers ($0.6\text{ m} \times 0.4\text{ m} \times 0.3\text{ m}$) to test bio-earth as a primary substrate (Fig. 3). Each unit contained a 10 cm base layer of washed gravel (10–20 mm) for drainage, topped with a 15 cm layer of bio-earth as the main filtration medium. Headspace (5 cm) was maintained to allow for water distribution, plant growth and oxygen diffusion. One unit was planted with *C. indica*, while a second, unplanted unit served as a control. Both units received raw domestic wastewater from the campus sewage treatment plant, applied daily at a hydraulic loading rate of $0.05\text{ m}^3/\text{m}^2\text{-day}$ to balance contact time with clogging risk. The systems operated outdoors under natural conditions and underwent a two-week stabilisation period before data collection to allow plant establishment and microbial biofilm development.

Physico-chemical analysis of wastewater and performance evaluation: Monitoring was done to measure the treatment performance of the bio-earth-based VFCW systems. This entailed a thorough physico-chemical description of the influent (raw) wastewater and the post-treated effluent from two units, the planted with *C. indica* and the unplanted control. The sampling and analysis were carried out on a weekly schedule for a thorough 24-week operating time to observe

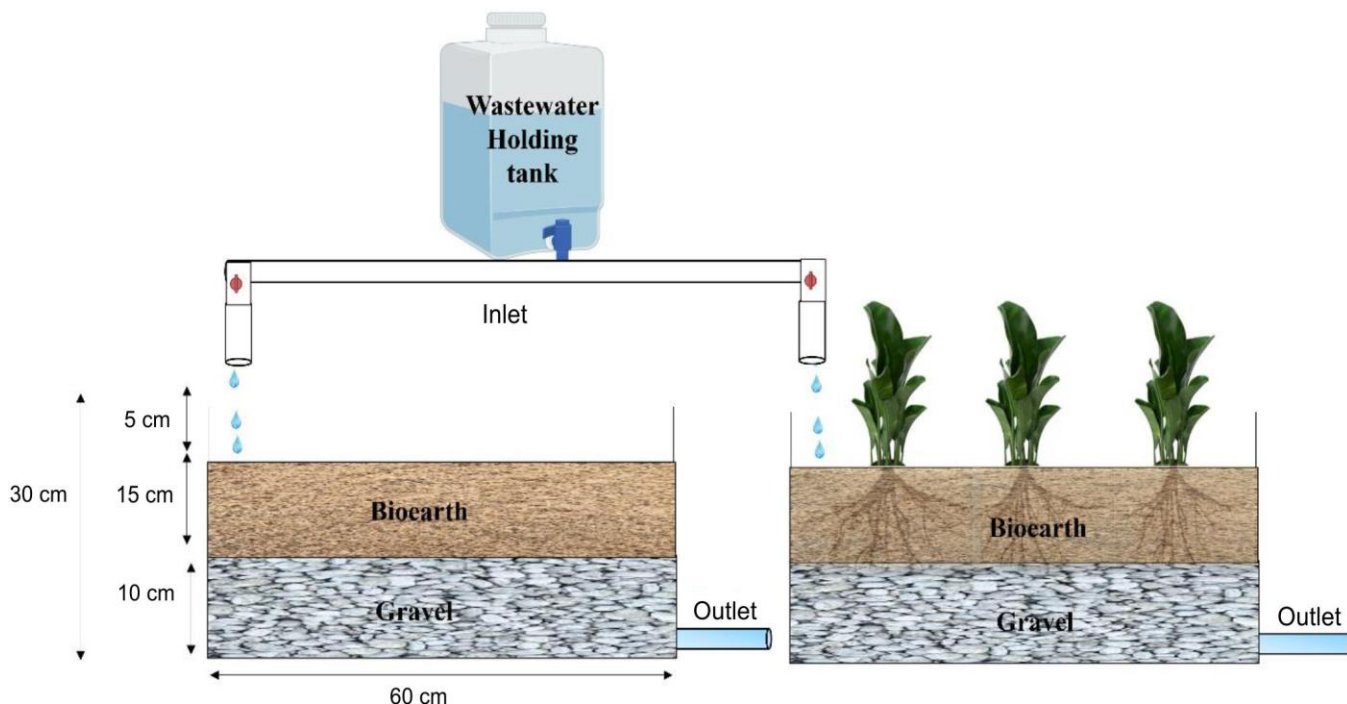


Fig. 3. Schematic diagram of the laboratory-scale vertical flow constructed wetland system

both short-term adaptation and long-term stability of treatment. All analytical steps were carried out in triplicate ($n = 3$) for statistical reliability, strictly following the standardised protocols described in the current edition of the American Public Health Association's (APHA) Standard Methods for the Examination of Water and Wastewater [23].

The selected parameters were analysed to evaluate water quality improvement. Basic properties such as pH and electrical conductivity (EC), indicating acidity/alkalinity and ionic strength, respectively, were measured using calibrated multi-parameter digital meters (Eutech Instruments pH700 and Hanna Instruments HI 98301). TDS as a direct measure of the sum of the inorganic salts present was measured directly from filtered samples through the integrated TDS function of the conductivity meter. The organic pollution load was quantitatively assessed through two key parameters: COD, which measures the oxygen equivalent of the organic matter susceptible to oxidation by a strong chemical oxidant, was determined using the closed reflux titrimetric method [24]; and the 5-day biochemical oxygen demand (BOD₅), which assesses the oxygen consumed by microorganisms while decomposing organic matter under aerobic conditions over 5 days, was analysed *via* the standard dilution method [25].

In nutrient removal, the levels of major nitrogen and phosphorus species were monitored. The ammonia-nitrogen (NH₄⁺-N), nitrate-nitrogen (NO₃⁻-N) and phosphate-phosphorus (PO₄³⁻-P) were analysed with a double-beam UV-Visible spectrophotometer (Labman Scientifics, LMSP-UV1900) through standard colorimetric procedures. The efficiency of removal was calculated using the following equation:

$$\text{Removal efficiency (\%)} = \frac{\text{Conc.}_{\text{influent}} - \text{Conc.}_{\text{effluent}}}{\text{Conc.}_{\text{influent}}} \times 100$$

This method permits a strong and quantitative comparison of the treatment efficiency between the planted and unplanted bio-earth systems for an extended period of time.

Statistical analysis: All experiments were done three times ($n = 3$ for each sampling event) and the results are shown as mean \pm standard deviation (SD). The Shapiro-Wilk test ($p > 0.05$) was used to check if the data distribution was normal and Levene's test was used to check if the variances were the same. Two-tailed Student's *t*-tests were used to compare two groups (for example, planted *vs.* unplanted systems). One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test was used to compare more than two groups (for example, bio-earth *vs.* sand *vs.* gravel). Statistical significance was set at $p < 0.05$. Pearson's correlation coefficient was used to look at the relationship between removal efficiencies and environmental parameters (temperature, pH). All statistical analyses were done using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Morphological studies: Fig. 4 displays the SEM micrograph of bio-earth with its porous nature, rough surface texture, microbial biofilm development and heterogeneous particle size distribution, making it a valuable substrate for constructed wetlands. The SEM micrograph reveals a porous and rough surface morphology of the bio-earth material, characterised by

interconnected voids and micro-pores. Such structural features facilitate efficient water flow through the medium, preventing stagnation and maintaining uniform hydraulic conductivity. This is essential for the efficient functioning of CWs, as stagnant conditions can induce anaerobic environments and reduce treatment efficiency [26]. Moreover, the interconnected pore network facilitates the simultaneous development of aerobic and anaerobic zones within the system, thereby improving treatment performance [27].

Bio-earth particle surfaces are rough and irregular and has high specific surface area, which improves the physical adsorption of impurities. This characteristic is highly useful for removing particulate matter and toxic metals from wastewater [28]. The rough surfaces are excellent substrates for the development of microbial biofilms. These biofilms consist of pollutant-degrading microorganisms, *e.g.* *Pseudomonas* and *Bacillus*, which are the key organisms responsible for the degradation of organic pollutants and the removal of nitrogen and phosphorus by nitrification, denitrification and phosphorus uptake [29]. The micro-voids and crevices in bio-earth serve as microhabitats for microbial populations. These microhabitats support the growth of varied microbial populations, *e.g.* aerobic, anaerobic and facultative bacteria, responsible for the degradation of complex organic and inorganic pollutants [30]. The occurrence of bacteria improves the biodegradation of organic material and the conversion of nutrients, *e.g.* the conversion of ammonia to nitrate and nitrate to nitrogen gas. The morphology of bio-earth allows for effective trapping of nutrients.

The porous structure and high adsorption capacity of bio-earth contribute to nutrient removal, thereby reducing the risk of eutrophication in downstream water bodies [31]. Its heterogeneous particle distribution provides structural stability, minimising compaction and maintaining effective water infiltration and microbial activity, which are essential for sustained CW performance. The material also exhibits good mechanical and chemical stability, ensuring durability under prolonged wastewater treatment conditions [32]. Derived from organic waste, bio-earth is a sustainable and cost-effective substrate, particularly advantageous in resource-limited settings due to its local availability. SEM analysis further confirms its suitability, revealing a rough, porous surface with high surface area that supports efficient water flow, microbial colonisation and nutrient retention in constructed wetland systems.

Elemental composition: Fig. 5 illustrates the EDX spectrum of the bio-earth sample, revealing a predominance of oxygen (43.8%) and carbon (16.9%), indicating the coexistence of organic and mineral constituents that support nutrient cycling and microbial processes [33]. The presence of silica (10.5%) contributes to structural stability and helps in maintaining the integrity of the wetland matrix and root support [34]. Elements such as calcium (7.2%) and iron (3.5%) play key roles in adsorption and precipitation mechanisms, facilitating the removal of heavy metals and inorganic contaminants. Also, trace elements including aluminium, magnesium and potassium enhance the cation exchange capacity, thereby improving nutrient retention and purification efficiency [35]. These compositional features highlight the suitability of bio-earth as a durable and effective substrate for pollutant removal in constructed wetland systems.

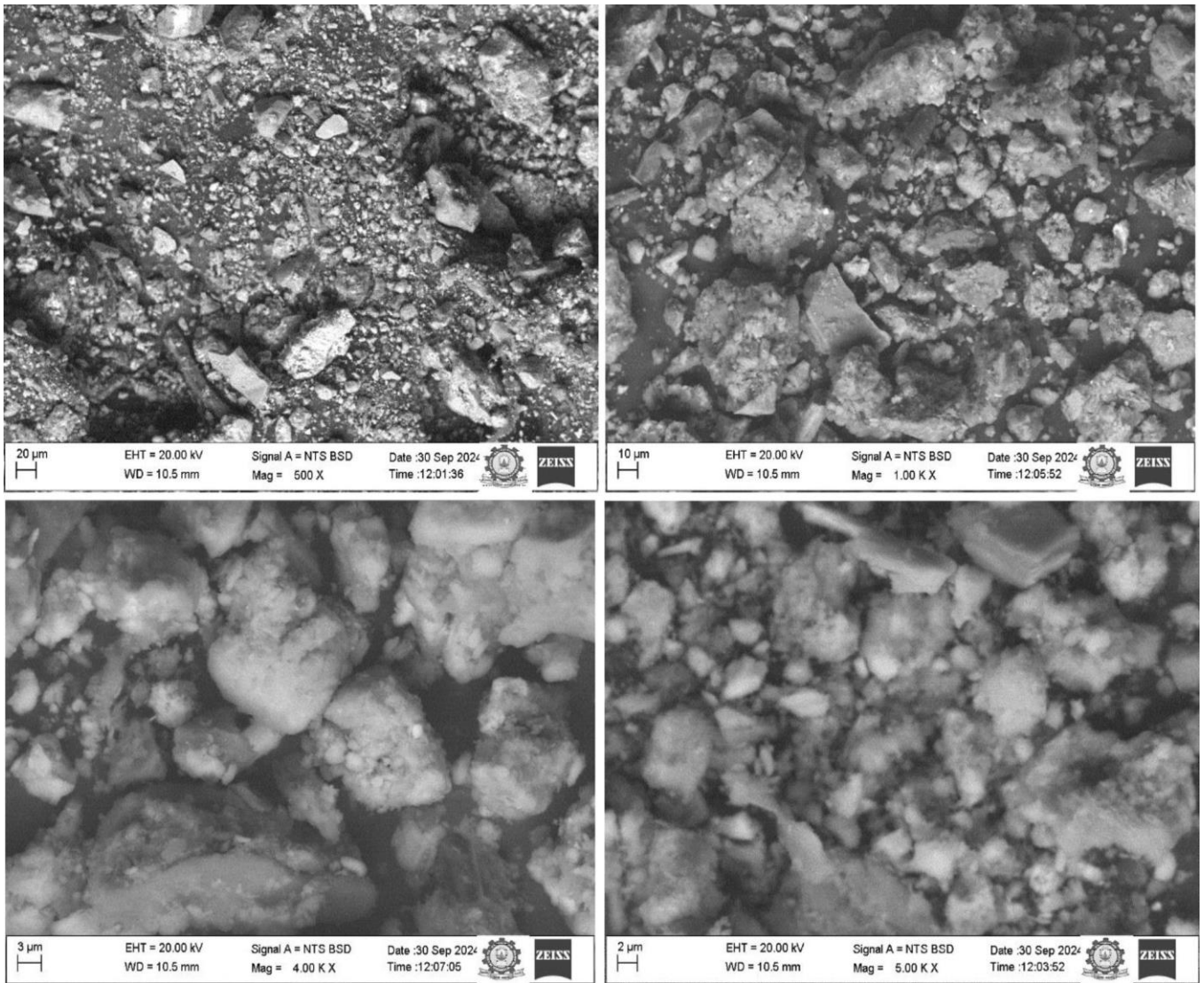


Fig. 4. SEM micrographs of the bio-earth sample

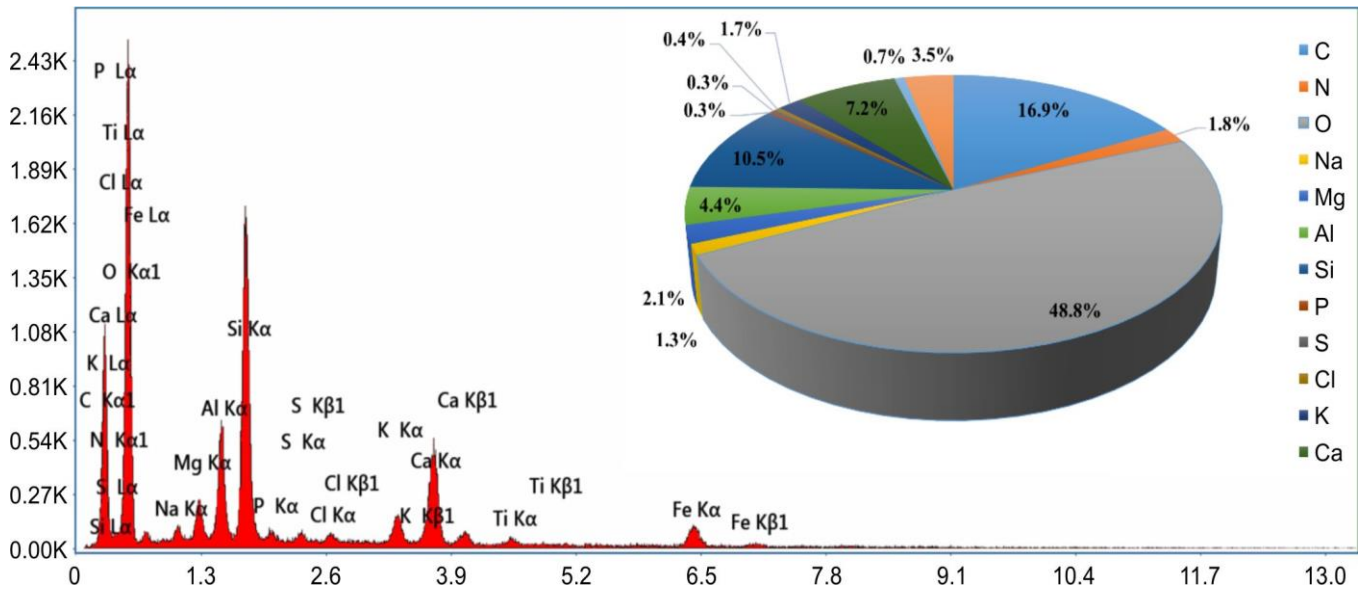


Fig. 5. EDAX spectrum and elemental composition of bio-earth

Mineralogical composition: The XRD pattern of bio-earth (Fig. 6) confirms the presence of crystalline phases such as quartz, calcite and hematite. Quartz imparts mechanical strength and maintains pore architecture, facilitating water flow and microbial colonisation [36]. Calcite contributes to pH buffering, helping to stabilise the medium within a range favourable for microbial activity [37]. Hematite enhances adsorption capacity, promoting the removal of heavy metals such as lead, arsenic and cadmium through surface interactions and ion exchange [38]. Together, these mineral phases provide structural stability and functional reactivity, enabling effective pollutant removal *via* combined physical, chemical and biological mechanisms, and supporting the suitability of bio-earth as a substrate in constructed wetlands.

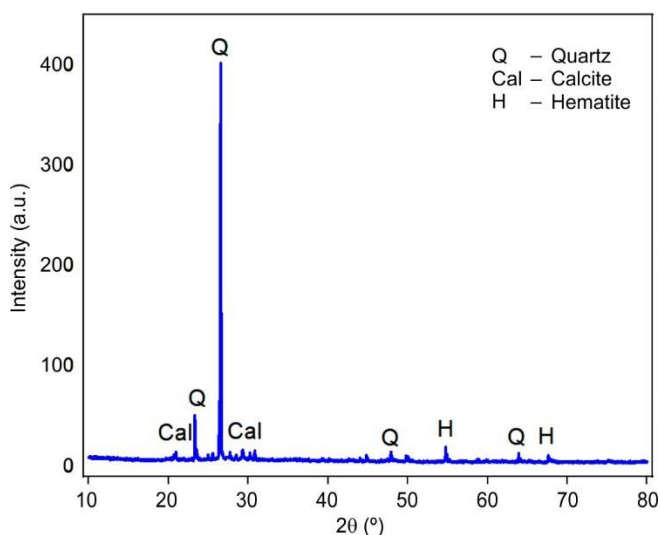


Fig. 6. XRD pattern of bio-earth

Organic composition: The FTIR spectrum (Fig. 7) confirms the presence of key functional groups in bio-earth. A broad band at 3500–3200 cm^{-1} corresponds to O–H stretching indicating moisture retention and organic constituents [39,40], while peaks near 2900 cm^{-1} are attributed to C–H stretching of organic matter. The peaks in the 1400–900 cm^{-1} region are associated with CaCO_3 , reflecting buffering capacity and signals within 1000–500 cm^{-1} correspond to Si–O and Al–O vibrations, confirming silicate and aluminate phases that enhance adsorption [41]. These features support the suitability of bio-earth as an effective substrate in constructed wetlands.

Hydraulic properties-porosity and permeability: Bio-earth exhibits a porosity of 40% and a permeability of 3.19×10^{-3} indicating favourable characteristics for application in constructed wetlands (CWs). The high porosity enhances water retention and supports the development of both aerobic and anaerobic zones, which are crucial for microbial-driven pollutant removal processes [42]. Its permeability facilitates efficient water flow through the medium while preventing excessive waterlogging [43]. These properties support its suitability as a sustainable and effective substrate for CW systems.

Physical properties: The sieve analysis indicates a moderately well-graded medium (coefficient of uniformity = 6.2 and a coefficient of curvature = 0.6126), which promotes a balanced interaction between permeability and filtration

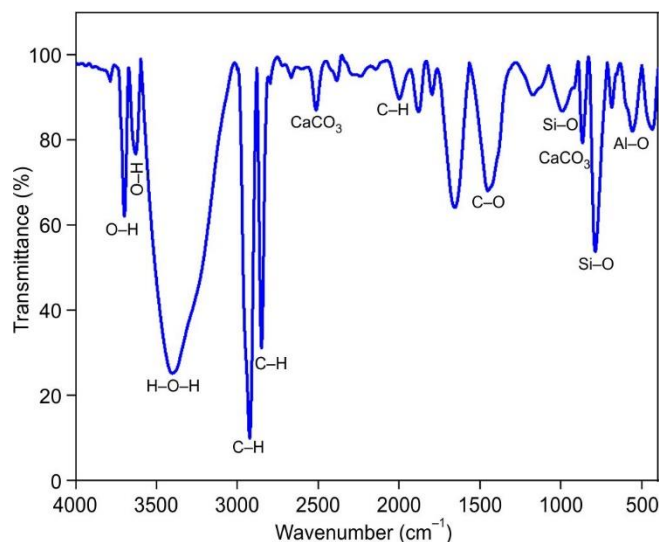


Fig. 7. FTIR spectrum of bio-earth

efficiency [44]. This gradation influences particle packing, which in turn is reflected in the void ratio (0.66), providing adequate pore space for water flow while supporting microbial attachment and contaminant retention [23]. The relatively low specific gravity (1.78) suggests a lightweight structure that may enhance porosity and hydraulic behaviour. These properties are interdependent: gradation controls pore distribution, the void ratio governs flow and aeration, and specific gravity affects media stability. Collectively, this combination supports effective filtration, sufficient permeability and favourable conditions for microbial growth including oxygen transfer that enhances aerobic degradation in constructed wetlands.

Environmental suitability: The compositional analysis of bio-earth indicates low concentrations of potentially toxic metals, supporting its suitability as a filter medium in constructed wetlands [45]. Lead (1.69 ± 0.02 mg/kg), cadmium (0.01 ± 0.003 mg/kg) and nickel (1.07 ± 0.05 mg/kg) are present within safe limits, suggesting minimal risk to plant growth, microbial activity and overall wetland stability. In contrast, the presence of essential micronutrients such as copper (94 ± 5 mg/kg) and zinc (135 ± 11 mg/kg) can promote plant development and enhance microbial processes involved in pollutant degradation and nutrient cycling, although their accumulation requires monitoring to avoid long-term toxicity [45]. To address this, the incorporation of biochar as an amendment can effectively reduce heavy metal mobility through adsorption, ion exchange and complexation mechanisms. This modification improves the safety and performance of bio-earth by enhancing its adsorption capacity, supporting microbial activity and strengthening pollutant removal efficiency. Consequently, biochar stabilised bio-earth emerges as a more reliable and sustainable substrate for wastewater treatment in constructed wetland systems.

Microbial analysis: Bio-earth supports beneficial microbial populations, with *Bacillus* and *Pseudomonas* detected at 2×10^2 and 2×10^1 CFU/g, respectively. These microorganisms contribute to nutrient cycling and the degradation of organic pollutants, thereby enhancing the treatment efficiency of constructed wetlands [47]. The ability of bio-earth to sustain diverse microbial communities strengthens its role as

an effective biological medium for pollutant removal and nutrient transformation. The combined physical, chemical and biological characteristics of bio-earth support its application as a constructed wetland substrate by improving adsorption capacity maintaining hydraulic stability and promoting microbial activity. Further field-scale studies are required to assess its long-term performance under operational conditions.

The bio-earth had some important microbial populations, where *Bacillus* and *Pseudomonas* occurred at 2×10^2 CFU/g and 2×10^1 CFU/g, respectively. These bacteria play a role in nutrient cycling and degradation of organic pollutants to improve the efficiency of CW [47]. The ability of bio-earth to support a wide range of microorganisms makes it an efficient biological medium to support CWs, both in the degradation of pollutants and nutrient turnover. On the whole, these results can indicate that bio-earth has gained a balance of physical, chemical and biological features. All of these aspects contribute to the use of bio-earth as a CW substrate, which has improved adsorption of pollutants, hydraulic stability and microbial survivability. Its long-term performance in operational CWs should be evaluated by conducting further field studies.

Performance evaluation of bio-earth in constructed wetlands: All data are expressed as mean \pm standard deviation (SD) based on three replicates ($n = 3$) collected over 24 weeks of operation. Data normality was verified using the Shapiro-Wilk test ($p > 0.05$) and homogeneity of variance was confirmed by Levene's test. Statistical comparisons between planted and unplanted systems were performed using two-tailed Student's t-test, while differences among bio-earth, sand and gravel were evaluated using one-way ANOVA followed by Tukey's HSD post-hoc test. Statistical significance was considered at $p < 0.05$, ensuring that the observed differences are supported by robust analysis.

Characteristics of raw wastewater: This study employed domestic wastewater representative of medium-strength sewage, providing a realistic basis for assessing the treatment performance of bio-earth-based constructed wetlands. The initial physico-chemical characteristics are presented in Table-1.

Parameters	Wastewater
pH	8.8 \pm 0.2
NH ₄	8.9 \pm 2
COD (mg/L)	440 \pm 30
BOD (mg/L)	236 \pm 36
TDS (mg/L)	1444 \pm 230
NO ₃ (mg/L)	21 \pm 3

Contaminant removal efficiency: The influent wastewater exhibited high organic load (COD: 440 mg/L; BOD: 236 mg/L) and was mildly alkaline (pH 8.8). Treatment performance was monitored over 24 weeks, during which the planted system demonstrated significantly superior efficiency compared to the unplanted control. After an initial stabilisation period of 10-12 weeks, the planted system achieved BOD and COD removal efficiencies of 68% and 72%, respectively, whereas

the unplanted system showed limited removal (20-25% for BOD and 22-28% for COD). A similar trend was observed for nutrient removal. The planted system achieved approximately 60% nitrate removal and 65-70% ammonium removal, compared to only ~20% and 25-30%, respectively, in the unplanted system. This enhanced performance is attributed to the synergistic interaction between plant roots and bio-earth. The root-mediated oxygen release promotes nitrification, while anoxic microzones within the porous bio-earth facilitate denitrification, converting nitrate into nitrogen gas. Total dissolved solids (TDS) were reduced by 30-35% in the planted system, compared to 20-25% in the control, indicating consistent ion adsorption and plant uptake without media clogging (Fig. 8). The improved treatment efficiency in vegetated systems highlights the combined role of bio-earth porosity in supporting microbial communities and plant roots in regulating micro-environments, enabling both aerobic and anaerobic processes for effective wastewater treatment.

Comparative assessment and cost analysis: Table-2 summarises the key physico-chemical and operational characteristics of conventional media (sand, gravel) and advanced materials (biochar, zeolite, slag) in comparison with the bio-earth developed in this study. The comparison includes parameters such as material source, porosity, permeability, microbial support, heavy metal retention, pH buffering capacity, circular economy benefits and reported BOD/COD removal efficiencies. Under identical VFCW conditions over 12 weeks, bio-earth demonstrated improved treatment performance, achieving 18-22% higher BOD and COD removal than sand (46% and 52%) and gravel (38% and 44%). Its hydraulic conductivity remained stable over 24 weeks (3.19×10^{-3} to 2.98×10^{-3} cm/s), whereas sand exhibited a significant decline (~40%) due to clogging after 16 weeks. From an economic perspective, bio-earth (US\$ 12-15/ton) is comparable to gravel and substantially more affordable than commercial biochar (US\$ 300-600/ton), while also contributing to waste valorisation. These findings indicate that bio-earth is a cost-effective and sustainable alternative for constructed wetlands, particularly in resource constrained settings.

Additional water quality parameters: To comprehensively assess the treatment efficacy of bio-earth based VFCWs, additional water quality parameters *viz.* total suspended solids (TSS), phosphate (PO₄³⁻-P), total coliforms and *E. coli* were analysed, as summarised in Table-3.

The TSS removal efficiency of 78% in the planted system is comparable to values reported for conventional vertical flow wetlands treating domestic wastewater [52]. Phosphate removal (54%) was moderate, consistent with observations that sand-based media typically exhibit limited phosphorus adsorption capacity due to low calcium and iron content [53]. The reduction of total coliforms and *E. coli* by 2.1 and 1.8 log cycles, respectively, indicates effective pathogen attenuation, likely due to a combination of physical filtration, natural die-off and predation by protozoa within the bio-earth matrix [54].

Conclusion

Bio-earth demonstrated suitability as a constructed wetland (CW) substrate due to its favourable and interrelated physical and chemical properties. Its high porosity (40%) and

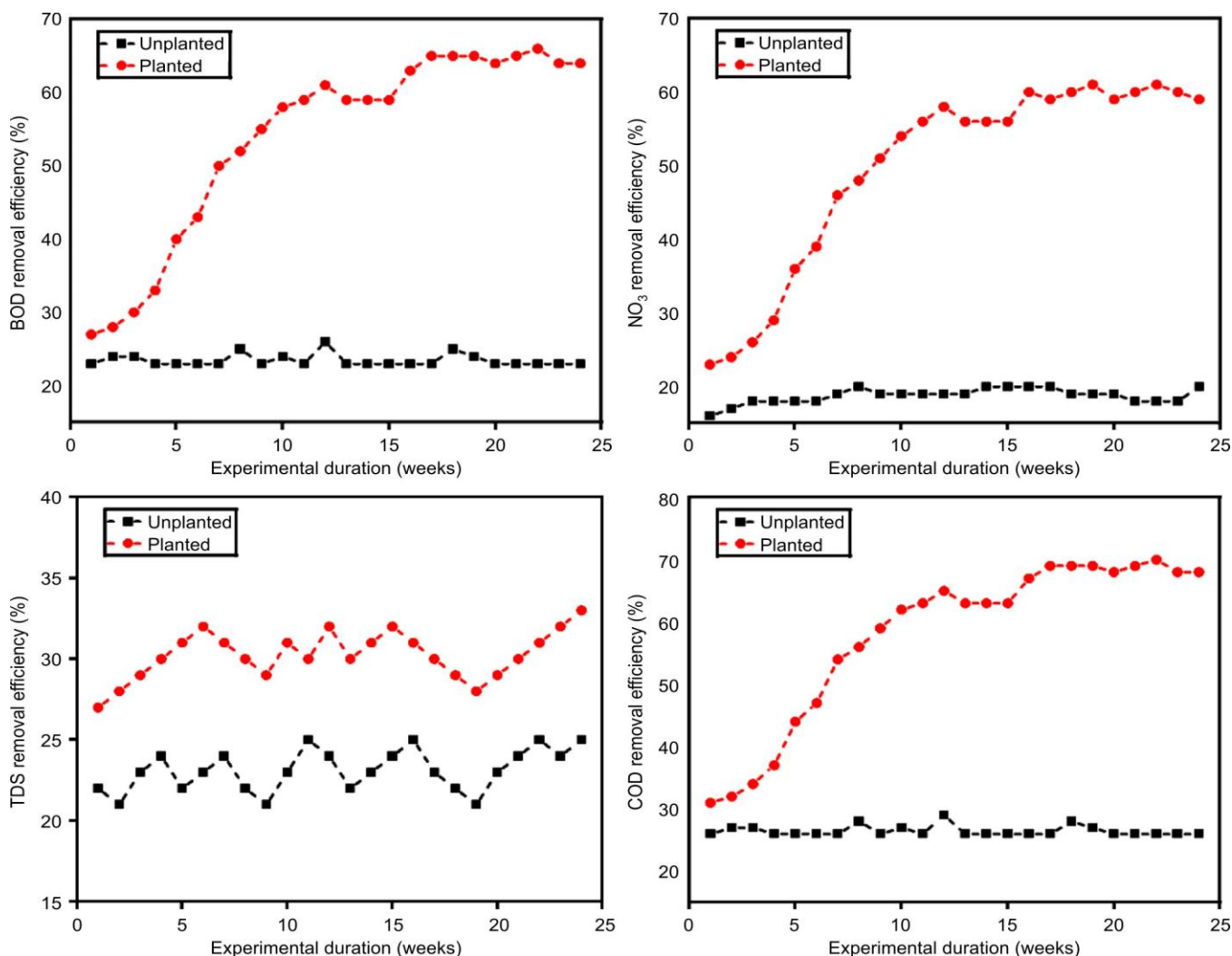


Fig. 8. Temporal variation in contaminant removal efficiency in planted and unplanted constructed wetlands over 24 weeks of operation

TABLE-2
COMPARATIVE PROPERTIES OF BIO-EARTH AND CONVENTIONAL ADVANCED CW SUBSTRATES

Substrate/parameter	Porosity (%)	Permeability (cm/s)	Microbial support	Heavy metal immobilisation	pH buffering capacity	Circular economy benefit	Reported BOD removal (%)	Reported COD removal (%)	Ref.
Sand/gravel	30–40	10 ⁻³ –10 ⁻²	Low–moderate	Low	No	Low	40–60	45–65	[48]
Biochar	40–50	10 ⁻⁴ –10 ⁻²	High	High	Variable	Moderate	70–90	75–90	[49]
Zeolite	30–50	10 ⁻³ –10 ⁻²	Low	Low–moderate	No	Low	50–70	55–75	[50]
Slag	35–45	10 ⁻⁴ –10 ⁻³	Moderate	Moderate–high	Yes (alkaline)	Moderate	55–75	60–80	[51]
Bio-earth	40	3.19 × 10 ⁻³	High	Moderate	Yes (calcite)	High (waste-derived)	68	72	This study

TABLE-3
ADDITIONAL POLLUTANT REMOVAL EFFICIENCIES IN PLANTED AND UNPLANTED BIO-EARTH VFCWS (MEAN ± SD, n = 24 WEEKS)

Parameter	Influent (mg/L)	Planted effluent	Removal (%)	Unplanted effluent	Removal (%)
TSS (mg/L)	210 ± 28	46 ± 9	78	98 ± 15	53
PO ₄ ³⁻ -P (mg/L)	12.4 ± 1.8	5.7 ± 0.9	54	8.9 ± 1.2	28
Total coliforms (log MPN/100 mL)	6.2 ± 0.3	4.1 ± 0.4	2.1 log	5.3 ± 0.3	0.9 log
<i>E. coli</i> (log CFU/100 mL)	4.5 ± 0.2	2.7 ± 0.3	1.8 log	3.9 ± 0.2	0.6 log

permeability (3.19×10^{-3} cm/s) enable efficient water flow while minimizing clogging. Mineral phases such as quartz, calcite, and hematite provide structural stability, pH buffering, and adsorption sites, while FTIR analysis confirms the presence of functional groups that support microbial activity and pollutant transformation. Pre-treatment through sequential washing effectively reduced leachable contaminants, ensuring environmental safety and long-term applicability. In vertical-flow CW experiments, bio-earth achieved significant pollutant removal in planted systems, with BOD₅ ($68 \pm 6\%$), COD ($72 \pm 5\%$), nitrate ($60 \pm 7\%$), and TDS ($35 \pm 6\%$) removal ($p < 0.01$). Performance exceeded sand and gravel, showing 18-22% higher organic removal and stable hydraulic conductivity over 24 weeks, whereas sand exhibited notable clogging. Although slightly lower than engineered media like biochar, bio-earth offers comparable efficiency to conventional substrates at a much lower cost (US\$ 12-15/ton) and supports waste reutilization. The enhanced performance in planted systems highlights the synergy between plant uptake, microbial activity and media properties. Bio-earth demonstrates potential as a cost-effective and sustainable substrate for constructed wetlands. Future studies should focus on hybrid media development (e.g. with biochar), long-term field validation, evaluation of heavy metal stability under varying conditions, and optimization for emerging contaminants and plant-media interactions to enable broader practical application.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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