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Microbial community structure in constructed wetland receiving salinity and polluted water

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ABSTRACT

Constructed wetlands are considered sustainable systems to treat various contaminants in wastewater depending on the structure and bacterial functions. This research investigated the identity of bacterial species that exist in constructed wetlands and their ability to remove saline and pollutants. A horizontal wetland system was constructed, receiving an artificially salinity-polluted water. Evaluation of salinized and polluted water was done physically and chemically. Bacterial species were isolated and identified by morphological and chemical analysis, and confirmed by 16SrDNA test, and a phylogenetic tree was constructed to reveal the relationship among the bacterial species. Results showed that pH amounted to 6.4 and temperature 18 °C, whereas the values of EC, TDS decreased (from 5.5 to 1.18 µS/cm), (from 1036 to 750 mg/L), respectively, in 12 hours. Concentrations of other pollutants were also reduced for PO_4 to 1.2 mg/L and for NO3 to 2.2 mg/L. Efficiency removal of heavy metals were 100% for Cd and Ni, and ranged between (38–66%) for Pb and Zn. Analysis of 16SrDNA revealed various bacterial genera such as Enterobacter, Stutzerimonas, Stenotrophomonas, Areomonas and Pseudomonas that showed strong positive statistical correlation with removal of pollutants and salinity, such as TDS, heavy metals, and nutrients like PO_4 and NO_3. These results declared that each genus has a special functional role to remove or eliminate contaminants in a constructed wetland. Thus, it can be concluded that wetlands are mainly directed by a bacterial community that has special connections and an interactive network. Therefore, further investigations are required to understand the dynamic and metabolic pathway for that community.

Keywords: bacterial genera, bioremediation, heavy metals, salinity water, wetland.

INTRODUCTION

Wetland ecosystems have been known for their role to improve the quality of water, biochemical cycles, carbon sequestration and diversity of biological communities on earth. One of these is bacterial community that play a crucial part in wetland via supporting multiple bacterial species that utilises pollutants for preserving clean water supplies as bioremediation capacity or promoting biodiversity. Moreover, complex and varied ecosystems are demonstrated by the way of microbes to support the endurance, productivity, and biological efficiency of wetlands via controlling greenhouse gases, balancing nutrient flows, and assisting in minimising pollution, which in general is the core of wetland function (Vymazal, 2010, Mohammed, 2017).

The participation of microorganisms is natural in the biogeochemical cycles of a wetland (Sánchez-Carrillo et al., 2013, Reddy et al., 2022, Mellado and Vera, 2021). For instance, the nitrification and de-nitrification processes in nitrogen cycle occur via releasing nitrogen gas into the atmosphere after forming nitrates from ammonium. Vice versa, the transformation of nitrogen from the atmosphere into bioavailable form occurs in a wetland by nitrogen-fixing bacteria to assist the growth of plants. Also, their contribution in decreasing eutrophication phenomena by obstructing extreme bloom of algae that could terminate the aquatic system zone via eliminating nitrogen gas (Ramond et al., 2022). Likewise, in the carbon cycle, fixing carbon dioxide into organic matter or transforming it into methane gas or carbon dioxide under

anaerobic conditions significantly affects climate change (Segers, 1998). Similarly, in the phosphorus cycle, it is preserving the productivity of plants through phosphorus availability as an essential factor in plant metabolism (Dai et al., 2020).

However, bacteria play a vital role in reducing salinity in wetlands through several biological and chemical processes. Halophilic and halotolerant bacteria, such as those from the *Proteobacteria, Cyanobacteria*, and *Firmicutes phyla*, contribute to salt removal by bioaccumulation, biosorption, and ion exchange mechanisms (Ma et al., 2024). Additionally, specific organisms reduce toxic contaminants as an eco-friendly tool to enhance the quality of water and soil in the environment by eliminating pollutants like heavy metals, insecticides, and other pollutants (Al-Jwaid et al., 2018, Camargo et al., 2016, El-Liethy et al., 2022, Mohammed et al., 2025). However, their existence in these environments ranged widely because of dependence on salinity, nutritional availability, and hydrology (Vymazal, 2010, Shahid et al., 2020). This research aimed to explore the role and impact of bacterial communities in mitigating salinity and reducing pollutants. The anticipated results aim to deepen the understanding of bacterial functions in wetlands, thereby informing future restoration and management strategies for effectively controlling salinity and pollutants in aquatic environments.

METHODOLOGY

Structure and design of constructed wetland

The experimental design of the wetland consists of two basins work as a subsurface horizontal wetland system located in engineering technical college. These two basins were connected in series, each one was filled with 22±3 mm round gravel (10 cm as top and bottom layers) and 40 cm sand with 25% w/w of reed powder as main substance, giving an average porosity value of 0.4. The basin measurements were [(60 width × 30 length × 60 depth) cm] with a longitudinal slope of 1% was planted with common reed, as shown in Figure 1.

Stones of Ø100 ±20 mm were placed in the first 10 cm each basin. Each basin experienced cyclic of wet and dry periods with the artificial salinity polluted water generated by peristaltic pumps. Each cycle was completed in six hours a day (given 350 min wet period and 10 min dry period).

Letters of the location in constructed wetland descripted as following:

a) source of water that enters the first basin.
b) after staying in the first basin
c) after staying in the second basin
d) effluent from the first basin, which us used as influent for the second basin
e) effluent water from the system.

Preparation of artificial polluted water

Artificial salinity-polluted water was synthesised in the laboratory to simulate Shat Al- Arab water quality. By using chemical compounds purchased from HIMEDIA company, for Lead II $PbCl_2$, Zinc acetate dehydrate [$C_4H_6O_4Zn.2H_2O$], Cadmium sulphate octahydrate [$CdSO_4.83H_2O$] and $NiSO_4.6H_2O$ for Nickel(II) sulphate [$NiSO_4.6H_2O$] salts for Pb, Zn, Cd and Ni, respectively. While, Mono-potassium Phosphate (KH_2PO_4) was purchased from IBI scientific company and Potassium nitrate [KNO_3] from THOMAS BAKER Company. They were used the following concentrations: 5 mg/L, 5 mg/L, 0.5 mg/L, 0.5 mg/l, 5 mg/L and 12 mg/l for Pb, Zn, Cd, Ni, PO_4 and NO_3 respectively. After that they were mixed with tap water that contains various concentrations of salts that are parallel with the concentrations of the Shat Al-Arab water.

The system operated in batches from November 2018 to June 2019; however, for this study, water samples were collected during the spring months from February to May (90 days). This timeframe allowed for sufficient microbial growth. Water samples were taken from the inlet at time zero, from the outlet of the first basin after six hours of treatment, and from the outlet of the second basin after twelve hours, with collections occurring weekly.

Evaluation of the physiochemical properties of artificial water

A volume of 50 ml of water samples were collected in three different locations (A, C and E) and three periods of total cycle of 12 hours (at initial time, six hours later and finally at 12 hours). In order to monitor the salinity of water that enter the wetland, electrical conductivity (EC) was measured by using a portable conductivity meter (Hanna Instruments) and results were expressed in (µS/cm) and TDS in (mg/L). Additional parameters were also recorded, like pH and temperature degree.

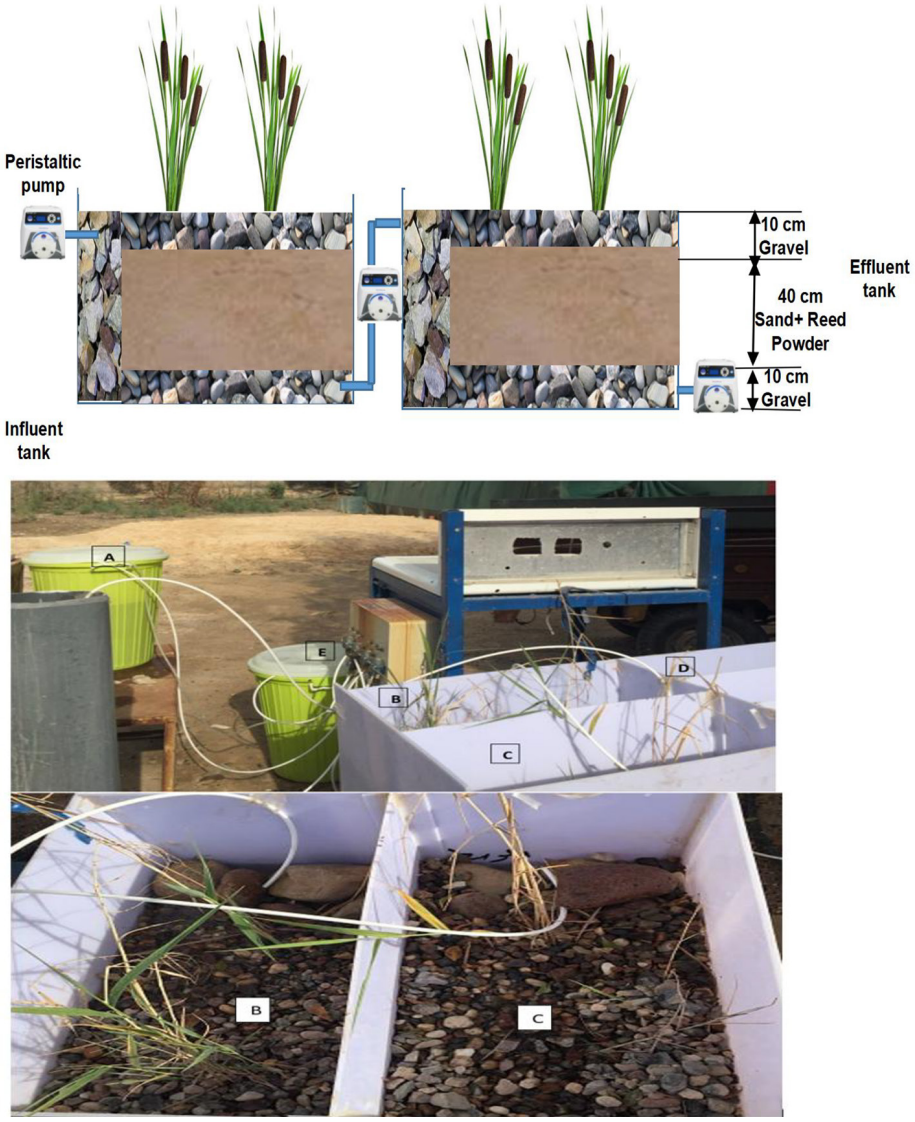

Figure 1. Artificial constructed wetland (structure and real image)

Nutrients, such as phosphate PO_4 and nitrate NO_3 were analysed using a Hach DR/3900 spectrophotometer following standard operating procedures (Agency, 1978, Miranda et al., 2001), while heavy metal concentrations were detected by using an ICP–OES instrument.

Collection of water samples for microbial analysis

Water samples for microbial identification were taken by choosing three points (A, C, E) in the designed constructed wetland as illustrated in Figure 1. Sterilised needles and syringes sized 10 ml were used to transport water into 50 ml polyethylene bottle samples. Then, 0.1 ml of each sample was spread on the nutrient agar in Petri dishes after labelling them according to site and date. After twenty four hours of incubation at 25 °C, several colonies appeared on the plates with different sizes, shapes and colours. Purification and isolation of bacterial colonies were done according to (Al-Jwaid, 2018). In brief, a colony from each shape was inoculated and sub-cultured several times for purification and identification through the morphological characteristics, Gram stain methods, biochemical traits according to Bergey's Manual of Systematic Bacteriology (Boone, 2001) and finally confirmed by DNA analysis. Some samples were stored in a freezer at -20 °C for later use.

Isolate DNA and 16SrDNA gene fragments

One ml of each purified bacterial sample was incubated in 25 ml of nutrient broth overnight, prior to transferring this broth into a 1.5

ml Eppendrof tube. Then, all bacterial samples were sent to Macrogen Inc.[1007,254 Beotkkot-ro, Geumcheon-gu, Seoul 153-781, Rep. of Korea (Tel:+82-2-2113-7000)], for 16SrDNA gene fragments analysis.

The universal primers forward (27F), length (bp)20, with sequences (5-AGAGTTTGATCCTGGCTCAG-3) and backward Primers (1492R), length (bp)19, with sequences (5-GGTTACCTTGTTACGACTT-3) for 16SrDNA were used (Srivastava et al., 2008). Later on, all sequence data of isolated bacteria were compared with the data in Gene-bank database using the blast online programme in NCBI web site (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification (Al-Jwaid, 2018).

Constructing a phylogenetic tree

The phylogenetic tree was done by aligning the molecular sequence data, then saving them in FASTA format. Then, they were imported into MEGA12 (Molecular Evolutionary Genetics Analysis, version 12) software. The data were accurately verified throughout the whole process to guarantee appropriate alignment and remove all confusing marks. Phylogenetic analysis was carried out using the maximum likelihood (ML) technique, which is considered as one of the most reliable approaches for inferring evolutionary connections. The substitution model was selected using MEGA12 with using a bootstrap evaluation 1.000 repeats for evaluating the reliability of constructed tree. At the end of this process, phylogenetic tree was created and visualised and saved as an image form for interpretation and publication (Tamura et al., 2021).

Generation of a heat map

Visualisation of the relationship between bacterial concentrations and efficiency to remove contaminants was established via heat map, through Microsoft Excel Programme. The data organised into a two-dimensional matrix, where X-axis columns corresponded to the studied pollutants (PO_4, NO_3, Pb, Cd, Zn, and Ni) and Y axis rows represented bacterial abundance and the values of removal efficiency were created and expressed as percentages under these conditions. Then, in Excel by utilising "Conditional Formatting" tool heat map was produced, specifically, through applying a colour scale gradient to show differences in eliminating efficiency. Dark colour displayed lesser, meanwhile, bright colour presented greater values which allow a clear visual variation. The colour bar a side was used to reveal the percentage scale (Bessler, 2023).

Analysis of the data by canonical correlation analysis (CCA)

Canonical correlation analysis is a multivariate statistical model that assists in the study of linear interrelationships between two sets of variables. The first set of variables represents the independent variable and the second set considers dependent variables (Mohammed et al., 2020). In this study, CCA was applied to quantify the correlations between independent variables represented by microbial genus and the dependent variables represented by pollutant removal. CCA was completed using the IBM SPSS 24 software package.

RESULTS AND DISCUSSION

Physiochemical analysis for water samples of constructed wetland

Physical and chemical properties were measured for artificial salinity-polluted water collected from three locations in the constructed wetland. Firstly, after preparation of the water and before entering the wetland location (A), then from the deep second basin (C) in the middle, and lastly from the effluent location (E). Results of physical properties, such as temperature, pH, total dissolved solid (TDS), and Electrical conductivity (EC) were established in Table 1. Total temperature of water samples in all three locations were around (18.2 °C), the pH values were approximately adjusted around (6.4 ±0.2); pH deviation and fluctuation may effect severely on stability and maintains of microbial community through altering the balance of biogeochemical processes (Rousk et al., 2010, Kumari et al., 2025). Meanwhile, results of EC showed lower values (2.65333 µS/cm)) at location (C) after 6 hours, after 12 hours, the final discharge of water out of the constructed wetland was dropped to reach (1.18 µS/cm). Whereas, TDS values showed a gradual decrease in concentrations with incubation time in wetland; at location A was (1036.7 mg/L), then six hours later reached to (936.7

mg/L), and finally after 12 hours concentrations of TDS reached (750 mg/L) with approximately 28% percentage of removal. The reduction in TDS was probably because of the existence of bacteria that have capability to adapt with salts or convert them into ions to react with other elements or metal ions to remove them (Liu et al., 2022). In turn, Bourhane et al. (2023) stated that microbial communities could impact the nitrogen and carbon cycles in wetland sediments and indirectly regulate salinity by altering osmotic conditions. Furthermore, improving desalination efficiency done by plant-associated wetlands with bacteria through assisting halophytes in absorbing sodium and chloride ions, (Zhang et al., 2022). Chiapponi et al. (2024) revealed that sulphate-reducing bacteria in saline wetlands facilitate salt removal by converting sulphate (SO_4^{2-}) into hydrogen sulphide (H_2S), which reacts with metal ions to form insoluble salts. Vymazal (2010) stated that constructed wetlands in all kinds are effectively eliminated organic materials and suspended solids.

Pollutants analysis for water samples of constructed wetland

Compounds like phosphates PO_4 (mg/L) and nitrites NO_3 (mg/L) were measured due to their influence on plant growth and microbial community in the constructed wetland. The results showed a gradual decrease in the concentrations of PO_4 and NO_3, for instance, initial concentrations of phosphate compound were (5.5 mg/L), then dropped to half the concentrations after 6 hours and ended with (1.2 mg/L) after 12 hours. Likewise, initial concentrations of nitrite compound were (14.4, 7.8, and 2.2 mg/L), respectively, as a final discharge out of the wetland, as shown in Figure 2.

Gradual elimination of these concentrations could be related to the existence of microbial communities that have special bacterial genus like: *Stutzerimonas, pseudomonas,* which play a remarkable role in nitric and phosphate compounds that are important in plant growth, as confirmed by other researchers (Lamers et al., 2012, Shahid et al., 2020). According to Vymazal (2010) nitrogen removal is much lower, unless combing different types of constructed wetlands. Likewise, phosphorus deletion would be increased by using special sorption with huge capacity (Van Nguyen, 2026). Other researchers, such as Mellado and Vera (2021) highlighted that (phylm) *Proteobacteria* in natural and artificial constructed wetlands is mainly and significantly participate in nitrogen (N), phosphorus (P), and sulphur (S) cycles, as well as organic matter degradation. Additionally, Gao et al. (2024) stated that inoculation with *Stutzerimonas stutzeri* strains eliminated emission of N_2O from two soils with contrasting textures, perhaps due to the altered the composition of microbial community and gene abundance related into in nitrification and de-nitrification of compounds in soil.

Assessment results of heavy metal concentrations added into artificial salinity-polluted water that enter the wetland, including Pb, Cd, Zn, and Ni were revealed as (5.7, 5.2, 0.54 and 0.57 (mg/L)), respectively. Six hours later, the concentrations reduced to reach 2.6, 2.1, 0.34 and 0.2 mg/L and after 12 hours in wetland, the final assessment of heavy metals concentrations were recorded for Pb and Zn at concentration (0.54 and 1.2 mg/L), respectively and not detected for Cd and Ni, as illustrated in Figure 3.

These results agree with other researchers who declared removal of heavy metal concentrations as a result of the cooperation between the bacterial community and plants. This could be due to the fact that they can separate complex compounds into simple substances, and ions of toxic metals make them easily uptake by plants and improve the phytoremediation process. Additionally, thepresence of bacteria may enhance plant growth and alleviate the toxicity of metals (Shahid et al., 2020, Shylla et al., 2021, Singhaboot et al., 2026). However, other researchers highlighted a decrease in heavy metal concentrations in the wetland due to the presence of a

Table 1. Physical properties (mean ± SD) of water samples from constructed wetland at different location

Parameter	Location A	Location C	Location E
PH	6.3667 ± 0.178	6.2667± 0.2038	6.3667±0.1219
EC(µS/cm)	5.5333 ± 0.0998	2.65333±0.5163	1.18±0.27040
Temp.(°C)	18.0667 ± 1.4676	17.0667 ± 2.0842	17.0333 ± 2.4103
TDS (mg/L)	1036.667 ± 62.62469	936.66667 ± 61.6592	750 ± 52.36029

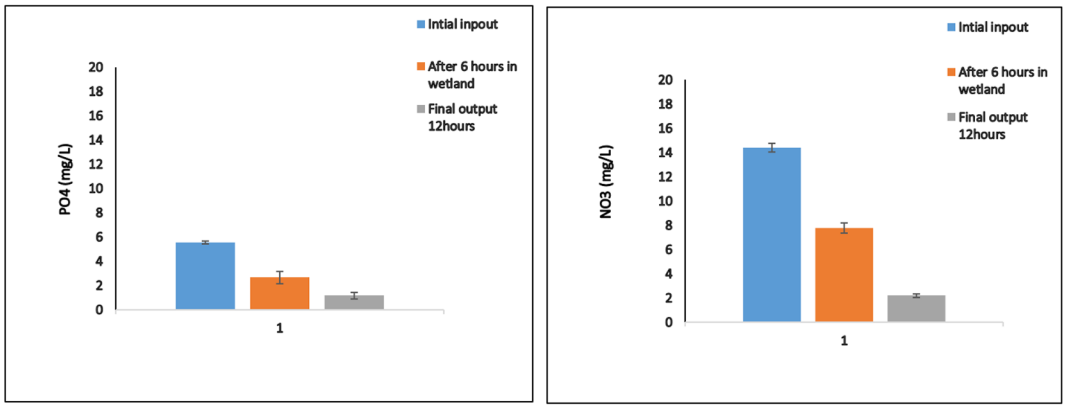

Figure 2. Concentrations of phosphates and nitrates in artificial salinity-polluted water before and after treatment in the constructed wetland

specific robust bacterial genus. For instance, Yu et al. (2020) demonstrated that genera like *Serratia* and *Pseudomonas* enhanced the removal of Cd2+ and Zn2+ from wastewater in constructed wetland with high efficiencies Cd2+ (99%), and Zn2+ (94%) through specific ability to bio-accumulate the heavy metals in the cell wall and cytomembrane. Prado et al. (2023) examined the ability of *Enterobacter chengduensis* to degrade three toxic compounds: fipronil, fipronil-sulfone and fipronil-sulfide for fourteen days, and the results of percentage removal were (96%), (92%), (79%), respectively.

Identification of bacterial species in the constructed wetland

Results of identifying bacterial species that occupied in the constructed wetland were demonstrated by using 16SrDNA gene sequence analysis

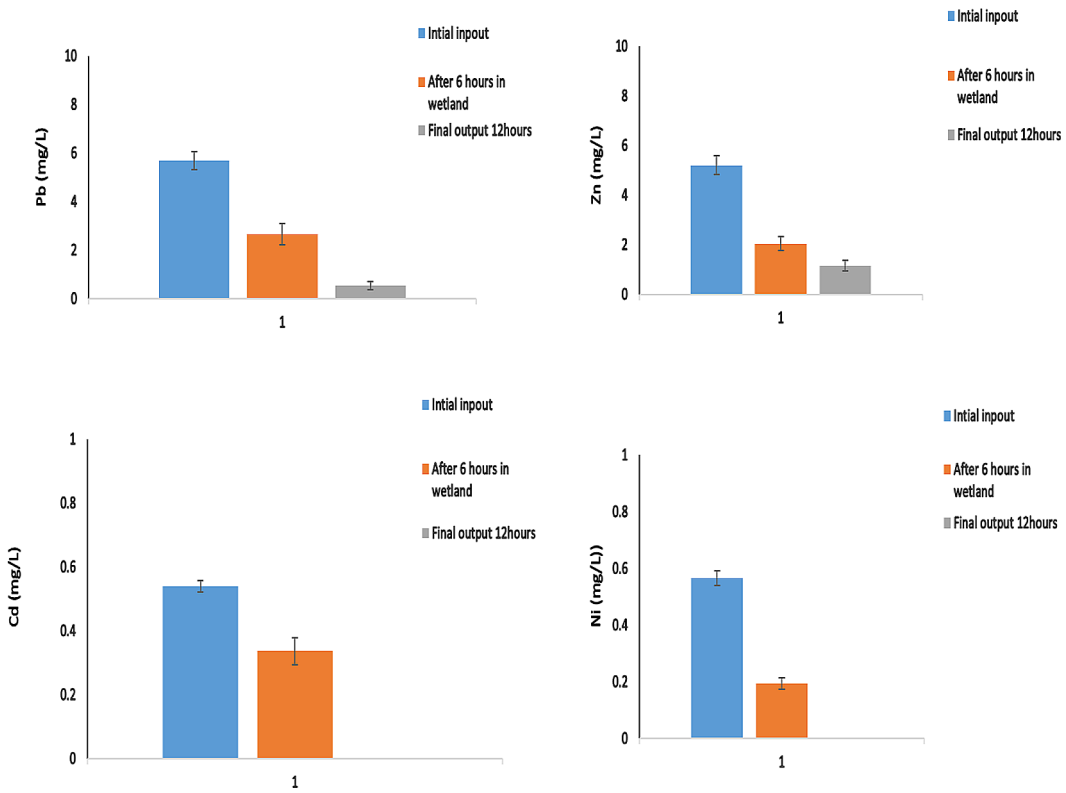

Figure 3. Concentrations of heavy metals: Pb, Zn, Cd and Ni (mg/L) in artificial salinity-polluted water before and after treatment in a constructed wetland

and matched with data in Gene-bank database with a hundred percent similarity, as shown in Table 2. It is revealed that various species belonged to one genus. Therefore, to understand the interpretation of revolutionary genes and the relationship among bacterial species, a phylogenetic tree based on 16S rDNA gene sequences applied and imaged as shown in Figure 4.

The tree displayed isolated species belonging to such genera as *Enterobacter, Stutzerimonas, Stenotrophomonas, Serratia, Cedecea,* and *Nesterenkonia*. Although, it clustered isolated species belonged to the same genus, it highly indicated their genetic similarity among species. Furthermore, tree's bootstrap values ranged from (70 to 100) which confirms the robust structure and an appropriate evolutionary relationship among the species. In addition, robust statistical support values that are equal and greater than 90 of the main branches of evolutionary tree (Moreira, 2023, Zou *et al.*, 2024).

Figure 4 shows that various species belong to the same genus like *Stenotrophomonas* were clustered closely in a phylogenetic tree due to their similarity and high capability to degrade and eliminate various pollutants from environment, as mentioned in previous research (Bhardwaj et al., 2018, Wojcieszyńska et al., 2022, Pan et al., 2025). In the same behaviour, Enterobacter genus also revealed moderate-to-high ability to reduce pollutants (Kelany et al., 2023, Prado et al., 2023). Meanwhile, isolated species belonged to *Nesterenkonia,* showing a different phylogenetic location. Because its branch is longer than other genera, this variation linked to different enzymatic systems and unique genetic background that may effect on their efficiency and metabolic activity which were proved by Amiri et al. (2016). In their study, they examined the *Nesterenkonia* sp. strain F to produce organic compound such as acetone, butanol and ethanol under aerobic conditions not for degrading of pollutants. Remarkably, the structure of phylogenetic tree exposed isolated species that exhibited similar degrading behaviours were closely clustered together. This may indicate to a possible association between genetic likeness and efficient performance (Moreira, 2023, Bianchini and Sánchez-Baracaldo, 2024, Zou et al., 2024). Therefore, constructing and analysing the phylogenetic tree is important to predict the association role among isolated bacteria from the environment.

Table 2. Identification of bacterial species in constructed wetland

No.	Species -Genus	Identity
1	*Enterobacter sp.*	85%
2	*Stutzerimonas stutzeri*	94%
3	*Enterobacter cloacae*	98%
3	*Enterobacter ormaechei*	87%
4	*Enterobacter asburiae strain MPKS02*	95 %
5	*Enterobacter cloacae*	85%
6	*Sinorhizobium sp.*	80%
7	*Stenotrophomonas muris*	87%
8	*Enterobacter bacterium*	97%
9	*Enterobacter cloacae*	98%
10	*Serratia marcescens*	83%
11	*Stenotrophomonas sp. YAU14A_MKIMI4_1 chromosome*	96%
12	*Methanotrophic alpha proteobacterium*	86%
13	*Cedecea sp. strain 256_FS2-22*	93%
14	*Aeromonas veronii*	90%
15	*Stenotrophomonas maltophilia*	86%
16	*Pseudomonas sp.*	82%
17	*Stenotrophomonas geniculata strain E119*	91%

Relationship between microbial community and pollutants removal efficiency

To assess the bacterial species that existed in the constructed wetland and pollutant removal efficiency, a correlation analysis was conducted in three points at wetland as shown in Table 3 (A, C, E). Results showed complex interactions between bacterial species such as a degradable relationship via their contributions to eliminate and remove specific pollutants with positive values (≥ 0.5), or behave neutral with positive values less than 0.5, or inhibitory connection with negative values.

The results of correlation bacterial genera, such as *Enterobacter* showed moderate positive correlation with Cd (r =0.31279), and NO_3 (0.1522) at dataset A. For PO_4 (0.1359) and TDS (0.3973) at dataset C. This indicated lower bacterial efficiency under contamination and environmental conditions, such as pH and temperature. In contrast, other researchers showed great potential to remove contaminant by that genus (Jerin et al., 2021, Ma et al., 2023, Mondal et al., 2025). Moreover, El-Shanshoury et al. (2013) demonstrated the ability and resistance of *Enterobacter* sp to accumulate heavy metals (Cd, Cu, Co, Zn and Pb) within or on external surface

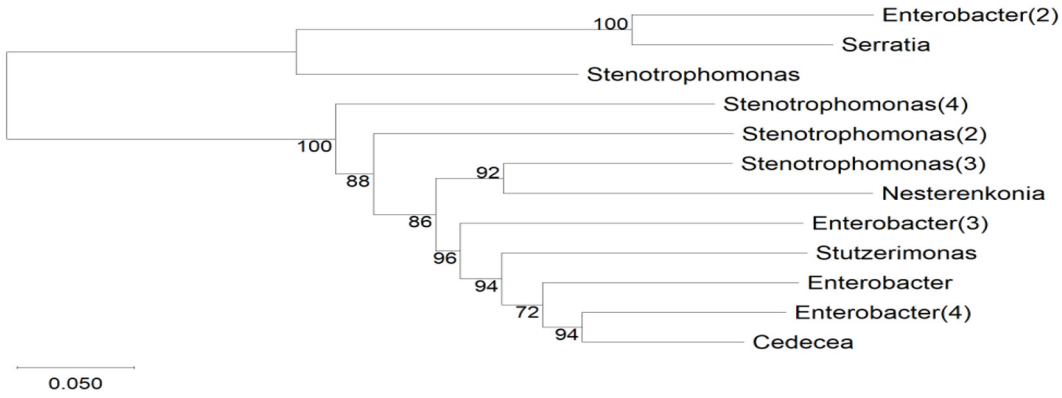

Figure 4. Phylogenetic tree for isolated bacteria in the constructed wetland

of cells with minimum inhibited concentrations under optimum conditions (pH =7 and temperature ranged from 25to -30 °C plus contact time. Anand et al. (2023) showed the performance of that genus at salinity conditions and its effect on growth of plants. Through releasing of (1-aminocyclopropane-1-carboxylic acid (ACC) deaminase) positive bacteria enhanced shoot and root length and dry weight with percentages 55%, 58% and 233%, respectively. Moreover, levels of antioxidant enzymes were increased. Additionally, Ma et al. (2024) highlighted various function capabilities of that genus such as diverse metabolic ability, improve photosynthesis and fixed nitrogen processes and may act as fermenters of three kinds of phyla: *Proteobacteria*, *Cyanobacteria*, and *Firmicutes,* respectively.

Stutzerimonas genus displayed moderate positive correlations with Zn (r = 0.59082) in dataset A and for PO_4 (r = 0.51376) in dataset E. This behaviour agreed with the research by Ali *et al.* (2024) to eliminate ciprofloxacin compounds and with Vargas-Ordóñez *et al.* (2023) to degrade and reduce Paracetamol. Another genus, *Serratia* correlated positively to remove Ni (r = 0.51446) and lower elimination with Pb, Cd and TDS in dataset C. on the other hand, *Stenotrophomonas* shows variable performance for pollutants according to (Pan et al., 2016, Pan et al., 2025). For instance, the results of correlation analysis with TDS were strongly positive (r = 0.63974) and moderate to Zn and Ni (0.373, 0.429), respectively, in dataset (C) and for PO4 (0.262) and Pb (0.475) in dataset E, which may indicate competitive inhibition properties under specific environmental conditions (Pan et al., 2025). Therefore, Wojcieszyńska *et al.* (2022) discovered that immobilising *Stenotrophomonas sp.* on a plant sponge from *Luffa cylindrica* used as a carrier was able to degrade naproxen under specific conditions (30 °C, 7.2 pH, 72 hours incubation time) with a degradation rate (3.8 µg/hour) as). Similar behaviour was shown by *Pseudomonas.* A strong positive correlation with TDS (r = 0.81011) in dataset (A) and confirmed by (Shahid et al., 2020) and moderate correlation with heavy metals at dataset E.

In contrast, *Aeromonas* and *Sinorhizobium* genera performed a significant role as the highest contributors to remove pollutants. In final dataset (E), *Aeromonas* presented robust positive correlations with PO_4 (r = 0.65413) and NO_3 (r = 0.71013) that mean great role to remove these pollutants, same behaviour to remove PO_4 and NO_3 was proved by Zainol *et al.* (2022), in the other hand Qurbani *et al.* (2022) highlight the ability of this genus to degrade heavy metals in polluted environment. In the same pathway, *Sinorhizobium* genus demonstrated strong positive associations with Zn (r = 0.57086), Ni (r = 0.68949) in dataset (A), and for Pb (r = 0.61081) in dataset (C), which showed the capability to eliminate these heavy metals, that agreed with other researchers (Oves et al., 2025, Li et al., 2024). Furthermore, Huang et al. (2014) pointed out the relationship between plants and rhizosphere microbes and their potential to remove pollutants as a sustainable agriculture tools. To sum up across all analysed data above, total results showed how bioremediation process is regulated and governed by specific interactive connection among bacterial species to resist, adapt and capable to remove contaminants under contaminated environmental conditions. Therefore, to visualise the connection between bacterial species and reduce pollutants in wetland, a heat map was generated to present this relationship, as shown in Figure 5.

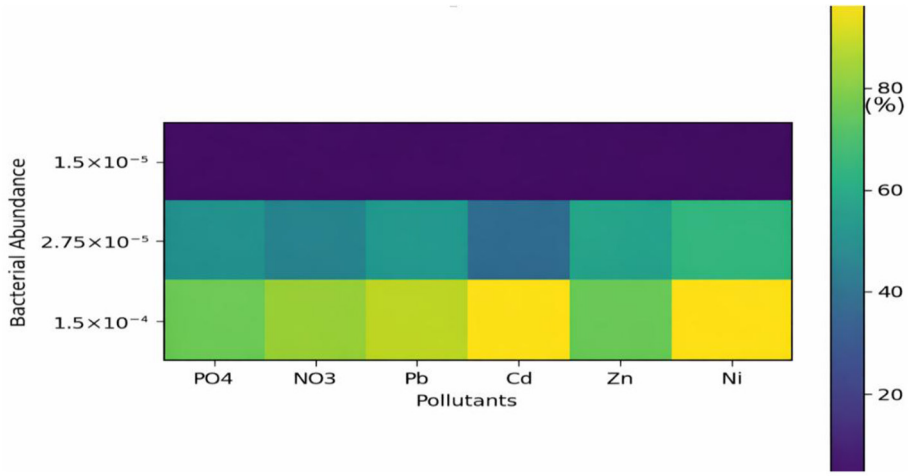

Figure 5. Heat map showing the relationship between bacterial abundance and pollutant removal efficiency in the wetland system

The heat map linked between bacterial numbers found in the wetland with removal efficiency of selected pollutants like heavy metals, including (Pb, Cd, Zn, and Ni) and nutrient compounds such as PO_4 and NO_3. The colour gradient signifies the percentages of higher efficiency removal of pollutants as long as they were in lighter shades, as shown in Cd and Ni with 100% efficiency removal and confirmed using correlation analysis test by appearing a strong positive relationship. On the other hand, it can be noticed that bacterial abundance are raised to (1.5×10^{-4}) alongside with increase the percentages of removal efficiency. This result might reflect strong bacterial activities and its influence on treatment of pollutants via bioremediation process. However, lower efficiency appeared as darker shades with percentages ranged from 38% to 66%, and reasonable bacterial abundance (2.75×10^{-5}). In turn, no elimination of pollutants was observed at smaller bacterial level (1.5×10^{-5}) with the darkest shades. Generally, the heat map in Figure 5 demonstrated the significant microbial community role to eliminate pollutants and enhance the performance of the constructed wetland.

CONCLUSIONS

Microbial community plays an effective role in constructed wetlands that are considered as sustainable and functional systems to treat wastewater from salinity and pollutants. Elimination values were significantly dropped and observed in salinity indicators EC (1.18 µS/cm) and TDS (750 mg/L). Nutrients such as PO_4 and NO_3 also decreased to reach (1.2 and 2.2 mg/L), respectively, at 12 hours. Heavy metals, such as (Pb, Zn, and particularly Cd and Ni) showed a noticeable elimination to reach (0.54 and 1.2 mg/L) for Pb and Zn, respectively, and not detected for Cd and Ni in a wetland system. Moreover, identification of bacterial genera, such as *Enterobactor, Stutzerimonas, Stenotrphomonas, Areomonas* and *Pseudomonas*, was essential to confirm that reducing contaminants due to interaction of specific bacterial inhabitants, each genus or species contributes to treat specific pollutant as long as bacterial abundance are raised to (1.5×10^{-4}) alongside with increase the percentages of removal efficiency to reach more than 98%. The results emphasised that successful constructed wetlands are dependent on microbial communities, which remain complex, and functionally effective in leading crucial processes like nutrient elimination, organic matter degradation, and heavy metal reduction. Furthermore, association with plants adds longitudinal variation within the system for better treatment performance in systems. However, research is required to explore the dynamic pathways and metabolic interactions of microbial communities to improve the design of wetland and enhance treatments under saline and contaminated conditions.

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