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CONTENT

Lesson Learned from Yangon to Mandalay on Wastewater Treatment Systems

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ABSTRACT

This paper presents a comparative analysis of wastewater management practices in Myanmar's major cities, Yangon and Mandalay, with a focus on drawing valuable lessons from Yangon's experiences and proposing recommendations for the improvement of Mandalay's domestic wastewater management, drawing insights from a SWOT analysis. Both cities are facing challenges due to rapid urbanization, leading to untreated discharge into the environment. The study identifies common challenges in both Yangon and Mandalay, such as limited treatment capacity, environmental concerns, and funding gaps. The decentralized-centralized strategy is a successful approach for Yangon even though the capacity is not high. Results showed that 17.5% of Decentralized Wastewater Treatment Systems (DEWATS) users were highly satisfied and 45% were somewhat satisfied. Yangon's experience with centralized systems showed that it took several years to cover the entire city for treatment, resulting in issues to cover revenue expenditures. If Mandalay adopts a similar, it will likely encounter the same issues. A recommended approach would be to implement an integrated system with DEWATS, which offers a better solution. The recommendations for sustainable wastewater management in Mandalay include active stakeholders' involvement in decision-making, promoting community participation, and providing training. Transparency and shared responsibility are crucial for success. Addressing membrane fouling, sludge disposal, and implementing monthly fees are essential for sustainable implementation. An integrated approach along with environmental and social impact assessments are necessary to develop a cost-effective and efficient wastewater treatment system while safeguarding public health and the environment. These insights offer broader implications, guiding developing countries towards more effective and environmentally responsible wastewater management practices.

1. INTRODUCTION

Poor sanitation in developing countries makes urban wastewater management (WWM) difficult. Rapid urbanization and population growth have led to harmful impacts on the environment, public health, and economy, with almost half of developing countries lacking proper sanitary disposal (Laugesen et al., 2010; WHO, 2022). Wastewater treatment systems (WWTS) aim to mitigate these issues, with centralized systems suitable for densely populated areas (Fisher, 1995; USEPA, 2005a; World Bank, 2012; ADB, 2020) and decentralized systems more appropriate for small rural or peri-urban communities, offering potential for reuse (West, 2001; Parkinson and Tayler, 2003; Seidenstat et al., 2003). Trained personnel and good operation and maintenance (O&M) can boost performance and lower costs (Tokich, 2006; Massoud et al., 2009). Population growth, urbanization, and outdated systems complicate for Myanmar (IGES, 2019). Myanmar has 77-84% urban sanitation coverage, so water- and sanitation-related diseases kill 18% of that under-5 (Kamp, 2017). Recent literature has emphasized the urgent need for modernized, cost-effective, technical support, and better environmental protection laws (WHO, 2006; YCDC, 2018; Thin, 2018; Naing et al.,

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2020). For developing countries, integrated approach between onsite treatment and centralized system was popular (Ho, 2005; Chen et al., 2011; Eales et al., 2013; Pham and Kuyama, 2013; Narayanamoorthy et al., 2022). This encourages a dynamic and multispectral approach to plans sustainable water and wastewater management based on community needs (Molinos et al., 2011; Eales et al., 2013; Capodaglio et al., 2016; Padilla et al., 2019; Mehariya et al., 2021). Sustainable integrated WWM is necessary for urban (Nagara et al., 2014; UN, 2015; Naing et al., 2020) and the proposed of integrated Decentralized Wastewater Treatment Systems (DEWATS) into the WWM chain is shown in Figure 1. Several research gaps were found in terms of comparative studies between different urban areas. Capodaglio et al. (2016) and Ho (2005) noted the absence of such comparative studies in their review of literature. This research's originality is enhanced by the incorporation of significant contributions by Laugesen et al. (2010), Nagara et al. (2014), and Gürel (2017) in the literature review. Unlike previous studies that focused solely on individual aspects of wastewater treatment, this research uniquely combines a comparative approach with SWOT analysis. The approach allows to identify the strengths, weaknesses, opportunities, and threats, providing valuable insights into the factors that influence their performance and also can help decision-making on WWM. This approach simplifies on the complex interactions between factors, providing valuable insights for sustainable WWM strategies. The analysis also can help to identify areas for improvement in sustainable construction and operations for decision-making on WWM, which can lead to reduced energy, capital, and O&M costs (Nowak et al., 2015; Akhobadze, 2018; Riaz, 2022).

The study focuses on the cities of Yangon and Mandalay to address global WWM issues in rapidly urbanizations. The objectives of this study are to assess the current situation of WWTS in Yangon and Mandalay, and to propose recommendations for implementation in Mandalay, drawing from the lessons learned in Yangon. These recommendations will not only enhance the WWM in these cities but will also serve as valuable lessons for other developing countries facing similar challenges in WWM. By analyzing WWTS, the study adds new insights to these specific challenges and opportunities. These findings are consistent with the observations made by JICA (2014), Khin and Myint (2018), and IGES (2019) in their respective studies. Using SWOT analysis brings a fresh perspective to the research, enhancing comprehension of various factors at a more profound level. This multidimensional approach is a contribution that enhances the understanding of complex urban WWM as suggested by Than (2010) and Narayanamoorthy et al. (2022) in their research on integrated approaches to urban WWM.

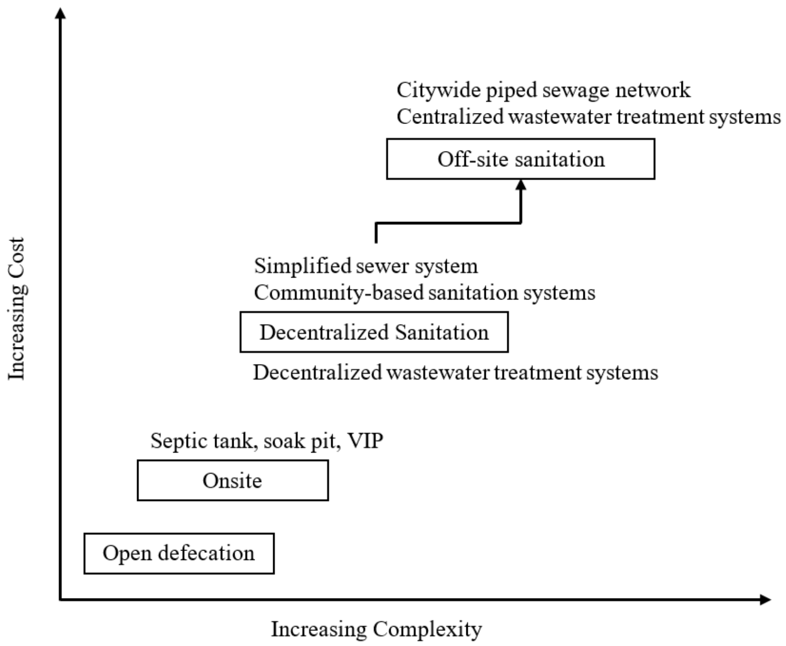

Figure 1. Proposed integrated DEWATS between onsite and offsite system (Eales et al., 2013; Pham and Kuyama, 2013)

2. METHODOLOGY

2.1 Area of studies and data collection process

Mandalay, Myanmar's third capital is the second largest city on the east bank of the Irrawaddy River. Kandawgyi Lake and the Irrawaddy River to the west. Mandalay's population was 1,360,138 (GAD, 2019; City Population, 2022). Mandalay City Development Committee (MCDC) has responsibility for 84.92% of the MCDC's 236,815 households which have upgraded sanitation facilities (Zin and Soe, 2010; Thin, 2018; Daeweaung et al., 2022). MCDC handles urban development, city planning, land administration, and taxation. The City Hall has 14 departments, including Water and Sanitation Department. The master plan for a CWTS in Amarapura township (Than, 2010; Naing et al., 2020). Field questionnaires assessed waste management, decision-making, and user opinions.

Yangon, on the east bank of the Yangon River, is a large city, 776 km south of Mandalay (Fan et al., 2022; Kohno et al., 2022). According to the 2019 GAD report, Yangon has 5.16 million people, four districts and 44 townships, with the Yangon City Development Committee (YCDC) managing 33 of them (GAD, 2019). YCDC's has 23 departments, manage waste and sanitation (Lwin et al., 2017; YCDC, 2018; MWEP, 2019). Pollution Control and Cleansing Departments (PCCD) manage domestics wastewater, 14 CWTS are planned for Greater Yangon 2040.

2.2 Factors influencing the choice of wastewater treatment systems

Selecting WWM is a challenging but necessary decision for Mandalay. Learning from Yangon's experiences and weakness, Mandalay should focus on identifying and improving major influencing factors. The key factors were examined using a review of relevant literature and selected factors in key management categories (Massoud et al., 2009; Sujaritpong and Nitivattananon, 2009; Schweitzer et al., 2014; Capodaglio et al., 2016). The factors considered are sustainability, social acceptability, public health protection, regulations, and planning. The selection process should take into account investment cost, population density, technology efficiency, and operation and maintenance. Innovative technologies and alternative financing models should be explored, especially in densely populated areas with limited land availability and skilled labor (UNESCAP, 2017; Padilla et al., 2019; Orak et al., 2021). The study has selected environmental, socioeconomic, technical, and institutional factors, along with 14 subfactors, to guide the decision-making process for WWM as shown in Table 1.

Table 1. Influencing factors on decision-makings on wastewater management

Environmental	Socioeconomics	Technical	Institutional
• Effluent quality • Resource recovery • Environmental protection	• Costs • Social acceptability • Willingness to pay • User's satisfaction • Revenue expenditure	• O&M • Skillful workers availability • Monitoring program	• Coordination • Policy and regulations • Land availability

2.3 Survey questionnaires

To evaluate the current situation of study areas, the primary sources of data were household surveys, official documents, and interviews with municipal officers were conducted. The household surveys were comprised of two parts: one with CWTS users in seven townships in downtown areas, and the other with DEWATS users in selected housing estates as illustrated in Figure 2. The Yamane formula (Yamane, 1967) was used to conduct 400 surveys, evenly distributed between DEWATS users and CWTS users. These surveys aimed to gather demographic information, assess the current state of wastewater systems in residents' homes, and examine their satisfaction levels and factors perspectives. However, one study found that the equations assumed that the population is homogenous, which may not be the case in practice which can lead to biased results if the sample does not represent the population accurately (Cochran, 1977). The systematic random sampling method was used to select 28 households in each township for centralized users and 28 households in each housing section for decentralized users, who were geographically dispersed in the Yangon study area. Demographic information, household size, types of homes, and the significance of choosing factors were gathered through the surveys. The respondents were asked about their satisfaction with the current preferences and willingness to pay system.

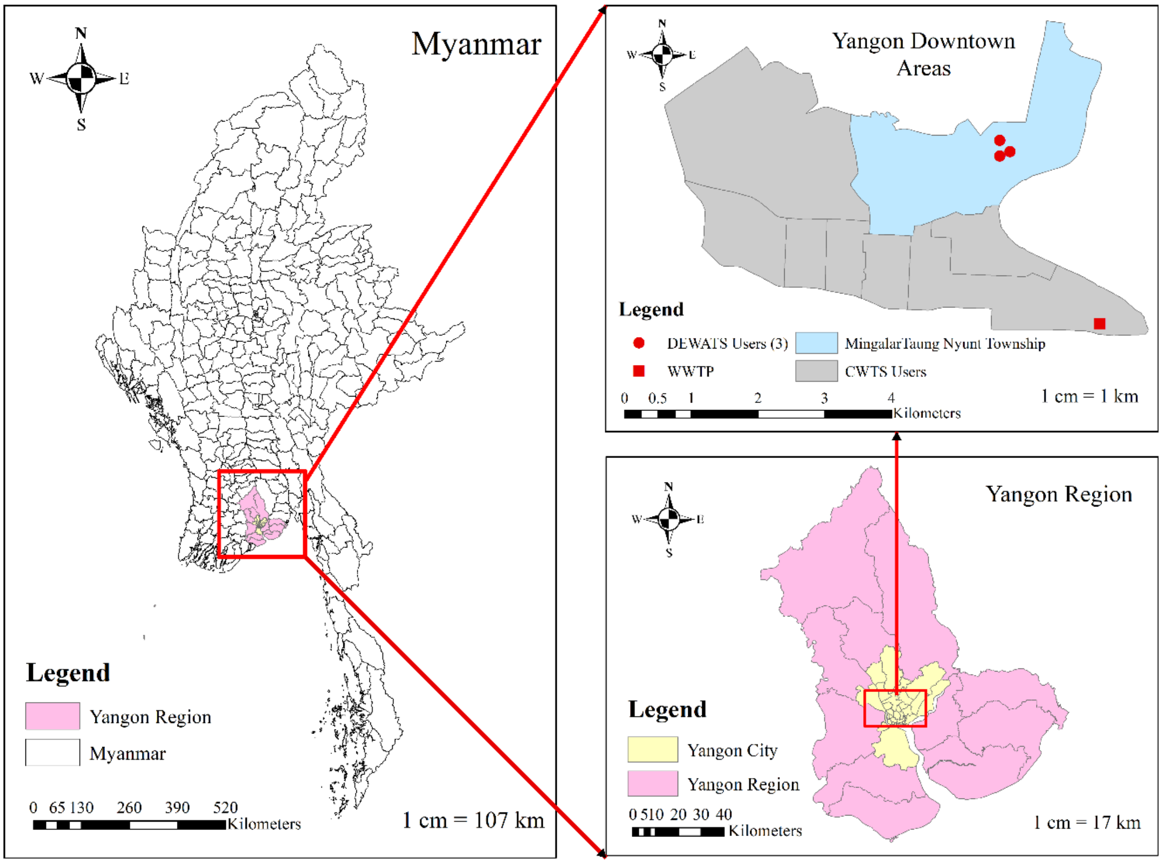

Figure 2. Map of data collection areas and points in Yangon

Data collection in Mandalay was also conducted through questionnaires to residents, official documents, and online interviews with municipal officers. Mandalay is located in the central dry zone, adjacent to the Irrawaddy River, and Kandawgyi Lake and Taung Tha Man Lake (Sanchez et al., 2019). The survey in Mandalay included 400 samples, with half of the respondents are DEWATS users and the other half utilizing onsite systems, given the absence of centralized systems in the city. The population was sampled using the systematic random sampling method, with 28 households selected in each township for onsite users and 28 households selected in each housing block for decentralized users, who were geographically across Mandalay. The survey collection points in Mandalay are illustrated in Figure 3.

2.4 SWOT analysis

The study used SWOT analysis to assess WWM lessons for Mandalay, identifying strengths, weaknesses, opportunities, and threats based on 14 selected subfactors. Data was collected through questionnaire surveys, interviews with municipal officials, and field visits, enabling factors affecting both cities' WWM. SWOT analysis proved valuable in identifying current and future factors influencing these solutions, complementing monitoring, decision-making, and management processes (Nagara et al., 2014; Akhobadze, 2018; Riaz, 2022). The study's insights can be used to inform wastewater management planning and decision-making, facilitating comparisons with practices in other cities. By using SWOT analysis, the study highlights key areas for improvement and strategic actions to enhance the effectiveness of WWM for Mandalay.

3. RESULTS AND DISCUSSION

3.1 Wastewater management situations in Yangon

In Yangon, 80% of households use onsite systems, mostly septic tanks. Using vacuum trucks, the YCDC transports sludge to CWTS and use as fertilizer (YCDC, 2018). In Botahtaung, Latha, Pabedan, Pazundaung, Kyauktada, Dagon, and Lanmadaw, a CWTS processes sewage at a rate of 14,775 m^3/day. The plant separates the sewage into 13,829 m^3/day of greywater flow and 946 m^3/day of black water. However, this capacity only serves 7% of the city's population, indicating insufficient coverage for the domestic wastewater needs of the entire city (Premakumara, 2017; Khin and Myint, 2018).

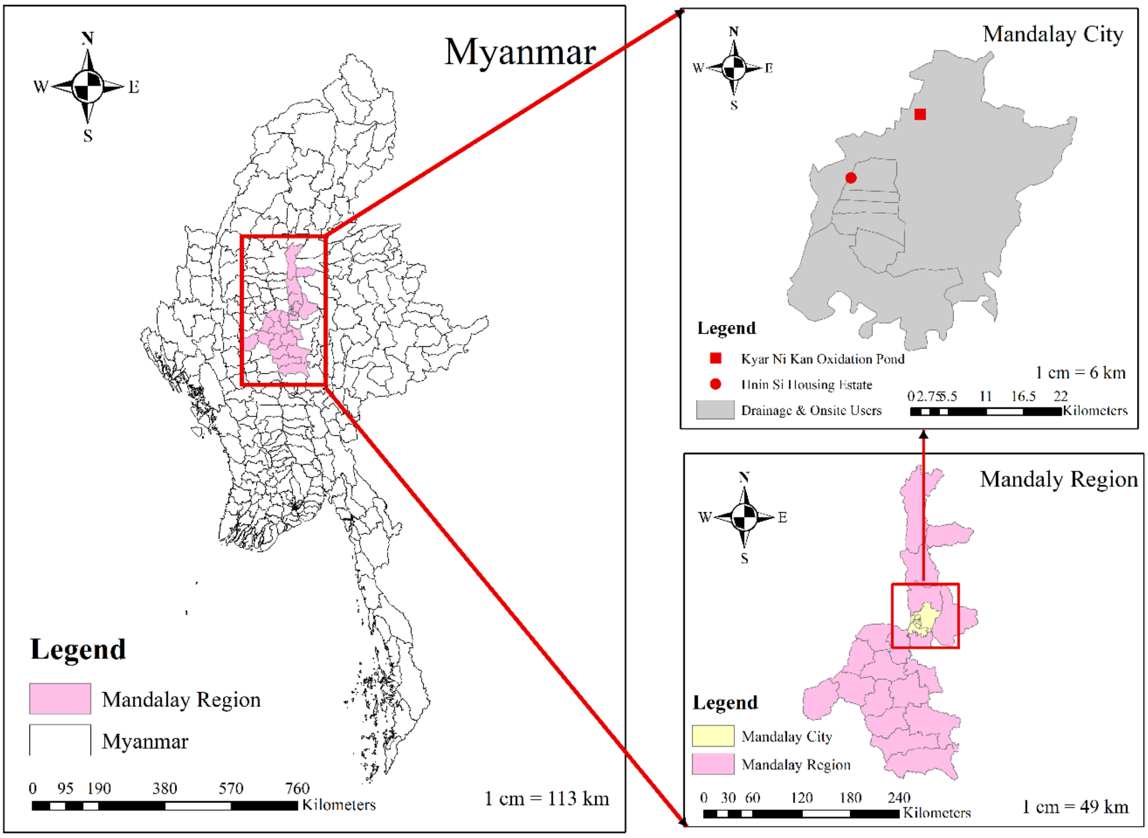

Figure 3. Map of data collection areas and points in Mandalay

There are plans to increase the percentage of the population receiving treatment to 49% by 2040 (Min, 2018). The WWTP treats 300,000 people and monitors effluent water daily to meet National Environmental Quality Emissions Guidelines (NEQEG) (JICA, 2014; ECD, 2015). Due to technological inconsistencies, some drainage directly enters the rivers. 10% of city households use membrane bio-reactor (MBR) DEWATS, before flowing drainage, wastewater is treated. Only 3% of residents use an unimproved pit latrine (Lwin et al., 2017; YCDC, 2018), as shown in Figure 4 and the survey sampling distribution details listed in Table 2. In Yangon, approximately 17.5% of DEWATS users expressed a high level of satisfaction with their system, while 45% reported being somewhat satisfied. Among centralized users, 60% stated that they felt neither satisfied nor dissatisfied, and 17.5% indicated a level of satisfaction. When asked about important factors in choosing WWTS, 36% considered all factors important, with 32% prioritizing socio-economics, 12% environmental, 11% technical, and 9% institutional considerations.

Table 2. Distribution of surveyed results in Yangon

Characteristics	DEWATS Users	CWTS Users
Number of respondents	200	200
Gender	70% female, 30% male	66% female, 34% male
Age	28% under 30 years old, 59% 31-50 years old, 13% 51 years old and above.	31% under 30 years old, 54% 31-50 years old, 15% 51 years old and above.
Education level	6% Basic Education, 8% University Level, 86% Graduates	4% Basic Education, 4% University Level, 92% Graduates
Household Size	6% below 3, 78% 4 to 6, 16% above 6 members	6% below 3, 27% 4 to 6, 67% above 6 members
Household Income	30% less than 300,000 MMK, 30% 300,001 MMK to 600,000 MMK, 40% more than 600,001 MMK	10% less than 300,000 MMK, 20% 300,001 MMK to 600,000 MMK, 70% more than 600,001 MMK

Table 2. Distribution of surveyed results in Yangon (cont.)

Characteristics	DEWATS Users	CWTS Users
Type of sanitation facility	68% Flush toilet, 32% Pour flush toilet,	45.5% Flush toilet, 50% Pour flush toilet, 4.5% Pit latrine
Willing to pay O&M (per month)	55% less than 50,000 MMK, 30% between 50,000 MMK to 100,000 MMK, 15% more than 100,000 MMK	15% less than 50,000 MMK, 36% between 50,000 MMK to 100,000 MMK, 49% more than 100,00 MMK
Users' satisfaction on current system	17.5% very satisfied, 45% Somewhat satisfied, 15% Neither satisfied nor dissatisfied, 12.5% Somewhat dissatisfied, 10% Very dissatisfied	5% very satisfied, 17.5% Somewhat satisfied, 60% Neither satisfied nor unsatisfied, 12.5% Somewhat unsatisfied, 5% Very unsatisfied

(MMK=Myanmar Kyats, 1 USD=2,095/- MMK (as of July 2023))

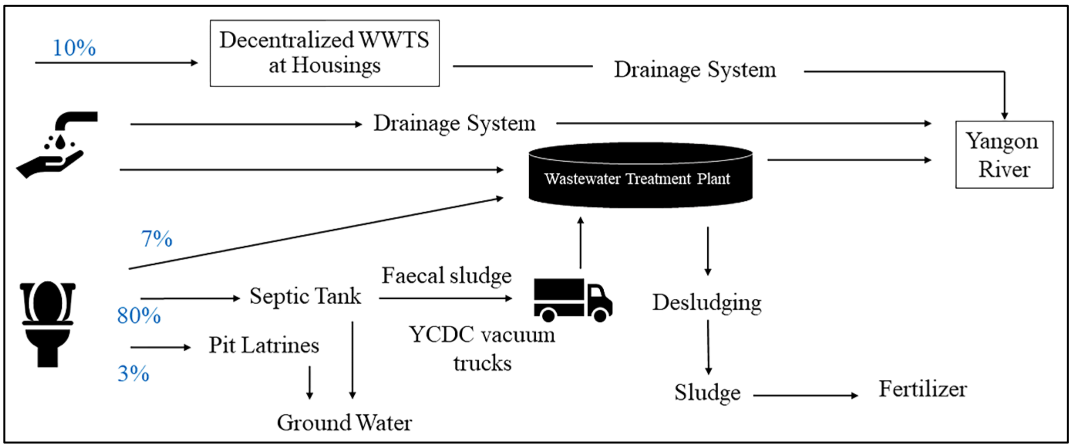

Figure 4. Sanitation flow chart of Yangon

3.2 Wastewater management situations in Mandalay

Water and Sanitation Department of Mandalay manages and administers the water supply and sanitation services for 155,880 households in the downtown area (DOP, 2015). The monthly fees for water supply service are below 1 USD, and there are no charges for domestic wastewater treatment. The city has well-planned public drainage systems, and conduct regular maintenance. Sludge is collected using vacuum trucks, and effluent monitoring is carried out monthly to comply with NEQEG (Zin and Soe, 2010; Grzybowski et al., 2019). Approximately 94% of households use onsite system, with septic tank and pit latrines. (Thin, 2018, Naing et al., 2020) Sludges are dried and used as fertilizer, and treated water is discharged near the drainage. Around 6% of households still practice open defecation, leading to contamination of underground water. Mandalay has only one DEWATS system, located in the Hnin Si Housing Estate in the Aungmyaythazan Township and others are onsite. The sanitation flowchart in Mandalay is presented in Figure 5 and the survey sampling distribution details are presented in Table 3.

Based on interviews with municipal officers, Mandalay has limited domestic wastewater treatment capacity, with only 17% of the total generated being treated and the total treatment capacity of all the WWTS facilities were approximately 20,000 m^3/day as of 2017. About 39% of DEWATS users mentioned being somewhat satisfied with their current system, and 28.5% reported feeling neither satisfied nor dissatisfied. Among the onsite users in Mandalay, 31% expressed a level of satisfaction, while 30% expressed a high level of satisfaction. Regarding the selection of factors, 39% of respondents highlighted the importance of all factors, with 29% emphasizing socioeconomics as most crucial. Technical and institutional factors were considered significant by 11% of the participants, while 10% placed importance on environmental aspects.

3.3 SWOT analysis

The findings of the analysis are summarized in Tables 4 and 5 with strengths and weaknesses categorized as internal factors, while opportunities and threats were classified as external factors.

Table 3. Distribution of surveyed results in Mandalay

Characteristics	DEWATS Users	Onsite Users
Number of respondents	200	200
Gender	45% female, 55% male	41% female, 59% male
Age	23% under 30 years old, 63% 31-50 years old, 14% 51 years old and above.	22% under 30 years old, 67% 31-50 years old, 11% 51 years old and above.
Education level	25% Basic education, 11% University level, 64% Graduates	13% Basic education, 13.5% University level, 73.5% Graduates
Household size	12% below 3, 69% 4 to 6, 19% above 6 members	8% below 3, 71% 4 to 6, 21% above 6 members
Household income	27% less than 300,000 MMK, 42% 300,001 MMK to 600,000 MMK, 31% more than 600,001 MMK	15% less than 300,000 MMK, 35% 300,001 MMK to 600,000 MMK, 50% more than 600,001 MMK
Type of sanitation facility	71% Flush toilet, 29% Pour flush toilet,	63% Flush toilet, 29% Pour flush toilet, 8% Pit latrine
Willing to pay O&M (per month)	44% less than 50,000 MMK, 45.25% between 50,000 MMK to 100,000 MMK, 10.75% more than 100,000 MMK	54% less than 50,000 MMK, 32% between 50,000 MMK to 100,000 MMK, 14% more than 100,00 MMK
Users' satisfaction on current system	22.5% very satisfied, 39% Somewhat satisfied, 28.5% Neither satisfied nor dissatisfied, 6% Somewhat dissatisfied, 4% Very dissatisfied	30% very satisfied, 31% Somewhat satisfied, 20.5% Neither satisfied nor dissatisfied, 12% Somewhat dissatisfied, 6.5% Very dissatisfied

(MMK=Myanmar Kyats, 1 USD=2,095/- MMK (as of July 2023))

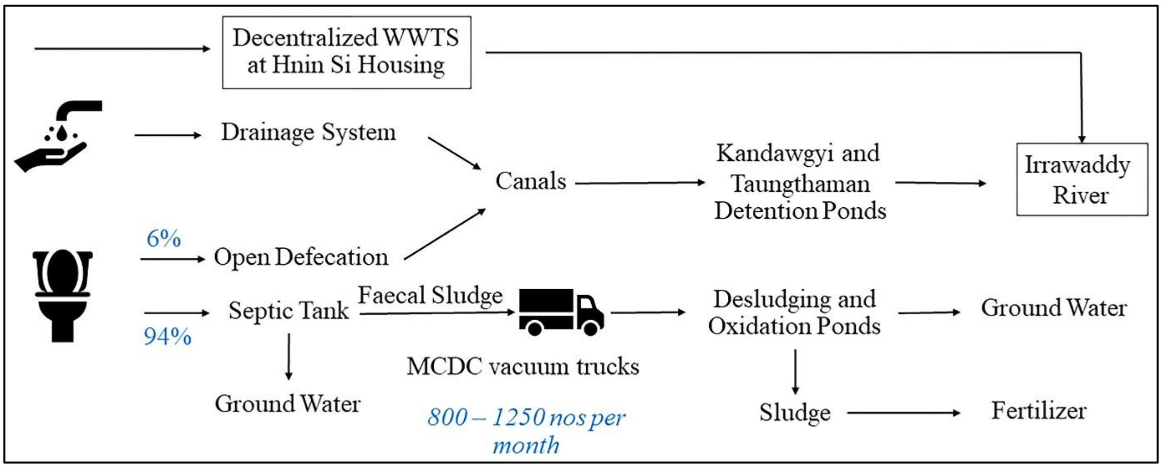

Figure 5. Sanitation flowchart of Mandalay

Table 4. SWOT analysis of CWTS in Yangon

Strengths	Weaknesses
• Improves water quality and protects the environment • Cost-effective and efficient • Ensures compliance with regulations and standards for public health • Promotes resource recovery and circular economy practices • Provides accountability and transparency	• Inconsistent effluent quality • Limited resource recovery • Construction and operation may have environmental impacts • High energy requirements • High capital and O&M expenses • Affordability and willingness to pay create financial challenges • Lack of trained personnel • Land availability major challenges • Incomplete coverage of the city and limited domestic wastewater
Opportunities	**Threats**
• Modernizing and optimizing treatment systems • Using environmentally friendly technology to improve performance	• Insufficient treatment • Limiting reuse potential • Costs and funding gaps due to development and operations

Table 4. SWOT analysis of CWTS in Yangon (cont.)

Opportunities	Threats
• Reusing wastewater to save costs • Investigating financing strategies or grants to obtain funds • Regular effluent quality monitoring • Investing in training and capacity building for operators and maintenance employees • Best practices in O&M • Fostering collaboration among local and international private sectors	• No users fee collection • Lack of experienced skillful workers and technicians • Land high price and limit availability • Residents' inability or unwillingness to pay appropriate fees • Insufficient monitoring and weak regulatory resulting in compliance issues and environmental concerns

The CWTS in Yangon has several strengths, including its positive impact on water quality, cost-effectiveness, and compliance with regulations. It also promotes resource recovery and transparency. Problems include insufficient treatment, limiting reuse potential, costs and funding gaps due to development and operations, no user fee collection, lack of experienced skilled personnel, high land prices and limited, residents' unwillingness to pay fees, insufficient monitoring, and weak regulatory compliance causing environmental concerns (MWEP, 2019; Ortega et al., 2022). Opportunities for improvement in systems, adopting environmentally friendly technology, and reusing to save costs. The system must address threats such as insufficient treatment, funding gaps, and weak regulatory monitoring to ensure effective operation and mitigate environmental concerns (Aung et al., 2020).

Table 5. SWOT analysis for DEWATS for Yangon

Strengths	Weaknesses
• High-quality effluent • Low energy consumption • Low-sludge production • Flexibility and Affordability • Odor control • Minimal capital and O&M costs • Tailored to meet local community and environmental needs • Land-Saving • Creates employment opportunities for local communities • Less O&M	• Limited capacity for large volumes • Susceptibility to fouling and membrane damage • Regularly require maintenance and replacement of membranes • Challenges in disposing of sludge • Requires skilled technicians and operators • Requires adequate land • O&M and monitoring costs • Initial investment can be a challenge, with limited funding • Revenue generated may not cover all O&M costs, leading to financial unsustainability in some cases
Opportunities	**Threats**
• Affordable for most commercial wastewater treatment needs • Complements other sustainable infrastructure projects • Ensure compliance with regulations • Job creation • O&M monitoring ensures efficient system functioning • Community payment ensures long-term sustainability • Fostering collaboration among local and international private sectors	• Lack of community acceptance due to unfamiliarity with benefits • Regulatory obstacles in acquiring authorizations and approvals • Weak or ineffective regulations and enforcement hindering adoption • Lack of skilled workers for O&M • Climate change and natural disasters • Incorrect O&M leading to negative impacts

The DEWATS in Yangon is an affordable solution to produce reusable treated water which provides flexibility, affordability, and best solutions to meet local needs while creating employment opportunities. However, it faces challenges in limited capacity for large volumes, membrane fouling and damage, and proper sludge disposal. Skilled technicians and adequate land are essential for successful operation. Weak regulations and enforcement, lack of skilled workers, and poor O&M monitoring can cause a negative impact to the system and the environment. A comprehensive plan must prioritize community engagement to address these challenges. They are sustainable solutions which aims to ensure access to clean water and sanitation for all (Nguyen et al., 2005; UN, 2021).

3.4 Lessons learned and recommendations for Mandalay

Based on the experiences and challenges faced in Yangon's CWTS and DEWATS systems by using SWOT analysis, Mandalay can draw valuable lessons to improve its wastewater treatment approach. To ensure successful implementation and sustainability, Mandalay should prioritize resource recovery and effluent quality while addressing funding gaps and enforcing strong regulatory frameworks. Mandalay can learn from Yangon's CWTS by addressing weaknesses such as insufficient treatment, funding gaps, and weak regulatory monitoring. The key points to prioritize in Mandalay include resource recovery, effluent quality improvement, modernizing systems, adopting environmentally friendly technology, and establishing effective regulatory frameworks to enforce compliance. The implementation of DEWATS in Mandalay should take into account the limited land, technical expertise and funding, as well as potential challenges such as membrane fouling and damage, sludge disposal, and O&M costs that may cause financial instability (Tchobanoglous et al., 2004; USEPA, 2005b). The development plan for Mandalay should therefore prioritize the implementation and sustainability considering their potential benefits. Prioritizing community engagement, in decision-making and implementing user fee collection, regulatory frameworks, and monitoring can improve the wastewater treatment system's performance and prevent environmental impacts (ISO, 2006; Sanchez et al., 2019). Collaboration among community is necessary for implementing and sustaining WWTS in Mandalay. Training and stronger regulations and enforcement can address the shortage of skilled trained personnel. Learning from Yangon's experience in adopting integrated approaches, Mandalay can customize and create job opportunities while producing reusable treated water. The scarcity of water in Mandalay during hot summers and frequent droughts can also be addressed by utilizing greywater reuse (Mainali et al., 2011). Table 6 compares the key aspects of each system, highlighting their strengths and weaknesses from Yangon to Mandalay.

Table 6. Comparative analysis of wastewater treatment systems in Yangon and Mandalay

Factors	Sub factors	Lessons learned from Yangon	Proposed strategies for Mandalay
Environmental	Effluent quality	Membrane fouling	Invest in advanced treatment tech for high-quality effluent
	Resource recovery	Successful resource recovery but no reuse	Implement greywater reuse to conserve water during droughts
	Environmental protection	Environmental and Social impact assessments were doing.	Need impact assessments and mitigation plans
Socioeconomics	Costs	Ongoing O&M cost challenges	Explore sustainable financing
	Social acceptability	Inadequate community engagement	Prioritize community engagement
	Willingness to pay	Inadequate user fee collection	Implement user fee collection
	User satisfaction	DEWATS received higher satisfaction levels with most users being "very satisfied" or "somewhat satisfied," while centralized had a higher proportion of users in the "neither satisfied nor dissatisfied"	DEWATS received higher satisfaction levels, with most users very satisfied or somewhat satisfied, while for onsite, satisfaction was more
	Revenue expenditure	Weak revenue generation	Improve revenue generation
Technical	O&M	Sludge disposal challenges	Develop comprehensive sludge disposal plan
	Skillful workers availability	Enough trained personnel	Invest in skilled training
	Monitoring program	Inadequate monitoring	Implement robust monitoring
Institutional	Coordination	Collaboration between stakeholders necessary	Foster collaboration for successful implementation
	Policy and regulations	Weak regulatory enforcement	Establish and enforce strong regulatory frameworks
	Land availability	Limited	Plan for adequate land for wastewater treatment plants

The recommended approach for Mandalay's wastewater treatment includes approaching an integrated strategy, promoting water conservation and reuse, considering the suitability of different systems, and ensuring transparency and stakeholder involvement. Technical training, renewable energy use, and environmental assessments are essential for sustainability. Collaboration among various stakeholders is crucial for a comprehensive and efficient wastewater management system, leading to continuous improvement and better environmental outcomes. A comparative table has been created to provide a clear overview of each recommendation's focus and potential impact as listed in Table 7.

Table 7. Recommendations for improving domestic wastewater management in Mandalay

Recommendation	Focus	Potential impact
Adopting an integrated strategy	Strategy	Improved system efficiently and resilience
Implement water conservation and reuse	Water management	Addressing water scarcity
Promoting shared responsibility and transparency	Governance	Effective and inclusive decision-making
Promoting environmental education and public health benefits	Public awareness	Increased public acceptance and support
Training and capacity building	Skill development	Efficient O&M
Reducing Environmental pollution with renewable energy	Sustainability	Lower environmental impact
Environmental and Social Impact Assessments (ESIA)	Environmental and social impact	Responsible and acceptable practices
Life cycle assessment (LCA)	Environmental assessment	Informed decision-making and planning
Involving community in decision-making	Stakeholder Engagement	Align strategies with local needs
Development user fee collection systems and enhance regulatory frameworks	Funding and compliance	Sustainable financing and adherence
Collaboration among private sectors	Stakeholder collaboration	Comprehensive and inclusive approaches
Continuous improvement based on lessons from Yangon	Learning and adaptation	Enhanced domestic wastewater management

4. CONCLUSION

Based on the obtained results, Yangon relies mainly on onsite systems. Only 7% of the city's population is served by the CWTS, creating insufficient coverage and some technological inconsistencies lead to direct drainage into rivers. In Yangon, 17.5% of DEWATS users were highly satisfied, while 45% were somewhat satisfied; among centralized users, 17.5% were satisfied and 60% expressed neither satisfied nor dissatisfied. The study revealed those factors in choosing WWTS included prioritizing socioeconomics (32%), environmental concerns (12%), technical aspects (11%), and institutional considerations (9%). Similar to Yangon, most households in Mandalay (94%) use onsite systems, primarily septic tanks and pit latrines. Only 17% of the total wastewater generated is treated due to limited capacity, leading to contamination of underground water from open defecation by 6% of households. In SWOT analysis, Yangon showed an affordable solution, but it faced challenges with limited capacity, technology, sludge disposal, and lack of skilled personnel. Mandalay can learn from Yangon's experiences to improve its approach by prioritizing water conservation, high effluent quality, reuse and customization on addressing funding gaps and regulatory enforcement. Yangon's centralized wastewater treatment system took years to treat the entire city, requiring revenue expenditure. Mandalay may face the same issue if it adopts a similar. Thus, implementing a DEWATS-integrated system is the better option. Then, in Mandalay, it is important to carefully consider implementing of monthly fee. In addition, prioritizing community engagement, training, and monitoring should be included in the intervention plan.

The study provides valuable insights for policymakers through its analysis of 14 subfactors under environmental, socioeconomic, technical, and institutional aspects of WWM, despite limitations in self-reported survey data and information gathering challenges. Nevertheless, the study offers valuable lessons from Yangon's WWTS, adaptable and implementable in Mandalay for sustainable WWM. The study's findings and recommendations contribute to a better understanding of wastewater treatment and offer valuable insights for sustainable strategies in developing countries. These may assist in developing sustainable WWM strategies for the country and other developing countries.

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REFERENCES

Akhobadze GN. SWOT analysis of industrial wastewater. IOP Conference Series: Materials Science and Engineering 2018;451(1):Article No. 012212.

Asian Development Bank (ADB). Asian Water Development Outlook 2020: Advancing Water Security across Asia and the Pacific [Internet]. 2020 Available from: http://dx.doi.org/10.22617/SGP200412-2.

Aung HH, Myint TY, Khaing NN. Assessment of surface water quality along Pazundaung Creek, Yangon City. IOP Conference Series: Earth and Environmental Science 2020;496(1):Article No. 012013.

Capodaglio AG, Cecconet D, Molognoni D. Small communities decentralized wastewater treatment: Assessment of Technological Sustainability. Proceedings in the 13th IWA Specialized Conference on Small Water and Wastewater Systems; 2016 Sep 14-16; Athens, Greece; 2016.

Chen DC, Maksimovic C, Voulvoulis N. Institutional capacity and policy options for integrated urban water management: A Singapore case study. Water Policy 2011;13(1):53-68.

City Population. MYANMAR: Administrative Division [Internet]. 2022 [cited 2022 Mar 23]. Available from: https://www.citypopulation.de/en/myanmar/admin/.

Cochran WG. Sampling Techniques. 3rd ed. New York: John Wiley and Sons; 1977.

Daeweaung T, Zaw T, Aung E, Zaw M, War K, Shwe T. Climate change vulnerability assessment for Pyu and Paleik Lakes, Mandalay Region, Myanmar. Myanmar; Biodiversity and Nature Conservation Association (BANCA); 2022.

Department of Population (DOP). The 2014 Myanmar population and housing census Yangon Region Report [Internet]. 2015 [cited 2023 Apr 21]. Available from: http://www.dop.gov.mm/en/.

Eales K, Blackett I, Siregar R, Febriani E. Review of Community-Managed Decentralized Wastewater Treatment Systems in Indonesia. Water and Sanitation Program Technical Paper; WSP. Washington, DC: World Bank; 2013.

Environmental Conservation Department (ECD). National Environmental Quality (Emission) Guidelines. Naypyitaw, Myanmar: ECD; 2015. p. 1-72.

Fan P, Chen J, Fung C, Naing Z, Ouyang Z, Nyunt KM, et al. Urbanization, economic development, and environmental changes in transitional economies in the global south: A case of Yangon. Ecological Processes 2022;11(1):Article No. 65.

Fisher M. The economics of water dispute resolution, project evaluation and management: An application to the Middle East. International Journal of Water Resources Development 1995;11:377-90.

Grzybowski M, Lenczewski ME, Oo YY. Water quality and physical hydrogeology of the Amarapura Township, Mandalay, Myanmar. Hydrogeology Journal 2019;27:1497-513.

Gürel E. SWOT analysis: A theoretical review. Journal of International Social Research 2017;10:994-1006.

Ho G. Technology for sustainability: The role of onsite, small and community scale technology. Water Science and Technology 2005;51(10):15-20.

Institute for Global Environmental Strategies (IGES). Quick Study on Waste Management in Myanmar: Current Practices and Potential for Sustainable Waste Management. Japan: Institute for Global Environmental Strategies; 2019.

International Standard Organization (ISO). ISO 14040:2006 Environmental Management Life Cycle Assessment Principles and Framework. Switzerland: International Standard Organization; 2006.

Japan International Cooperation Agency (JICA). The project for capacity development on urban environmental management in Yangon City in the Republic of the Union of Myanmar final report. Tokyo: Japan International Cooperation Agency; 2014.

Kamp KJ. Diarrhea among children under five in Myanmar: A systematic review. Journal of Health Research 2017; 31(1):77-84.

Khin A, Myint A. Existing Yangon City Development Committee Wastewater Treatment Plan and Future Plan. Yangon, Myanmar: Yangon City Development Committee; 2018.

Kohno H, Takahashi M, Niina D, Tokiwa S. Construction of Container Terminal in the Yangon River. In Smart Rivers. Singapore: Springer; 2022.

Laugesen CH, Fryd O, Koottatep T, Brix H. Sustainable Wastewater Management in Developing Countries: New Paradigms and Case Studies from the Field. Reston: American Society of Chemical Engineers Press; 2010.

Lwin M, Maung N, Murakami M, Hashimoto S. Scenarios of phosphorus flow from agriculture and domestic wastewater in Myanmar (2010-2100). Sustainability 2017;9(8):Article No. 1377.

Molinos M, Hernández F, Sala R, Garrido M. Economic feasibility study for phosphorus recovery processes. Ambio 2011; 40:408-16.

Mainali B, Ngo HH, Guo WS, Pham TTN, Wang XC, Johnston A. SWOT analysis to assist identification of the critical factors for the successful implementation of water reuse schemes. Desalination and Water Treatment 2011;32(1-3):297-306.

Massoud MA, Tarhini A, Nasr J. Decentralized approaches to wastewater treatment and management: Applicability in developing countries. Journal of Environmental Management 2009;90(1):652-9.

Mehariya S, Goswami RK, Verma P, Lavecchia R, Zuorro A. Integrated approach for wastewater treatment and biofuel production in microalgae biorefineries. Energies 2021;14(8): Article No. 2282.

Min T. Current Activities on Domestic Wastewater Treatment in Yangon City, Myanmar [Internet]. 2018 [cited 2023 Jun 16].

Available from: https://www.jeces.or.jp/relays/download/257/1391/404/2205/?file=/files/libs/2206/202304181110415669.pdf&file_name=Speaker%20-%206.

Myanmar Water Engineering and Products (MWEP). Water Supply and Wastewater Management in Myanmar. Yangon, Myanmar: Myanmar Water Engineering and Products; 2019.

Nagara G, Lam WH, Lee NCH, Othman F, Shaaban MG. Comparative SWOT analysis for water solutions in Asia and Africa. Water Resources Management 2014;29(1):125-38.

Naing W, Harada H, Fujii S, Hmwe C. Informal emptying business in Mandalay: Its reasons and financial impacts. Environmental Management 2020;65(1):122-30.

Narayanamoorthy S, Brainy JV, Sulaiman R, Ferrara M, Ahmadian A, Kang D. An integrated decision-making approach for selecting a sustainable waste water treatment technology. Chemosphere 2022;301:Article No. 134568.

Nguyen VA, Nga P, Hieu T, Morel A. Potential decentralized wastewater management for sustainable development from Vietnamese experience. Proceedings of the Water Environment Federation 2005;2005:917-46.

Nowak O, Enderle P, Varbanov P. Ways to optimize the energy balance of municipal wastewater systems: Lessons learned from Austrian applications. Journal of Cleaner Production 2015;88:125-31.

Orak S, Ercan O, Karahasan B, Yilmaz G. Public-private partnerships in wastewater treatment in Turkey. Water 2021;13(5):Article No. 603.

Ortega L, Alcalá J, Poyatos M, Martín J. Wastewater reuse for irrigation agriculture in Morocco: Influence of regulation on feasible implementation. Land 2022;11(12):Article No. 2312.

Padilla A, Morgan J, Güereca L. Sustainability assessment of wastewater systems: An environmental and economic approach. Journal of Environmental Protection 2019;10:241-59.

Parkinson J, Tayler K. Decentralized wastewater management in peri-urban areas in low-income countries. Environment and Urbanization 2003;15(1):75-90.

Pham NB, Kuyama T. Decentralized Domestic Wastewater Management in Asia - Challenges and Opportunities. Policy Brief - Series 1, Water Environment Partnership in Asia (WEPA). Institute for Global Environmental Strategies (IGES); 2013.

Premakumara DG. Waste management in Myanmar: Current status, key challenges and recommendations for national and city waste management strategies [Internet]. 2017 [cited 2023 Oct 10]. Available from: https://www.unep.org/ietc/resources/report/waste-management-myanmar-current-status-key-challanges-and-recomendations-national.

Riaz S. SWOT Analysis of sustainable use of wastewater in Pakistan. International Journal of Legal and Social Sciences. 2022;1(1):59-80.

Sanchez J, Myat SS, Kyaw PP. SFD Report: Mandalay. Myanmar: University of Lausanne; 2019.

Schweitzer R, Grayson C, Lockwood H. Mapping of water, sanitation and hygiene sustainability tools. Triple-S Working Papers 2014;10:Article No. 43.

Seidenstat P, Haarmeyer D, Hakim S. Reinventing Water and Wastewater Systems: Global Lessons for Improving Water Management. New York: John Wiley and Sons, Inc.; 2003.

Sujaritpong S, Nitivattananon V. Factors influencing wastewater management performance: Case study of housing estates in suburban Bangkok, Thailand. Journal of Environmental Management 2009;90(1):455-65.

Tchobanoglous G, Ruppe L, Leverenz H, Darby J. Decentralized wastewater management: Challenges and opportunities for the twenty-first century. Water Science and Technology: Water Supply 2004;4(1):95-102.

Than M. Waste Management in Myanmar: Current Situation, Key Issues, and Challenges. Naypyitaw, Myanmar: Naypyitaw City Development Committee; 2010.

The General Administration Department (GAD). Township Profiles of Yangon Region. Naypyitaw, Myanmar: Ministry of Home Affairs; 2019.

Thin KM. Water supply and Wastewater Management in Mandalay City. Mandalay, Myanmar: United Nations Industrial Development Organization; 2018.

Tokich SH. Wastewater Management Strategy: Centralized v. Decentralized Technologies for Small Communities. Enschede, Netherlands: Center for Clean Technology and Environmental Policy; 2006. p. 27.

United Nations (UN). Water and sanitation [Internet]. 2015 [cited 2023 Jul 13]. Available from: https://sustainabledevelopment.un.org/topics/waterandsanitation.

United Nations (UN). Goal 6: Ensure access to water and sanitation for all [Internet]. 2021 [cited 2023 May 4]. Available from: https://www.un.org/sustainabledevelopment/water-and-sanitation/.

United Nations Economic and Social Commission for Asia and the Pacific (UNESCAP). Policy Guidance Manual on Wastewater Management. Bangkok, Thailand: United Nations Economic and Social Commission for Asia and the Pacific; 2017.

United State Environmental Protection Agency (USEPA). Decentralized Wastewater Treatment Systems; A Program Strategy; EPA 832-R-05-002; Office of Water, United States Environmental Protection Agency. Washington, DC, USA: USEPA; 2005a.

United State Environmental Protection Agency (USEPA). Handbook for Managing Onsite and Clustered (Decentralized) Wastewater Treatment Systems; EPA/832-B-05-001; Office of Water, United States Environmental Protection Agency. Washington, DC, USA: USEPA; 2005b.

West S. Centralised management: The key to successful on-site sewerage service. Proceedings of the On-site’01 Conference, Armidale; 2001 Sep 22-27; University of New England; 2001.

World Bank. Environmental Management Framework for Kyrgyz Health and Social Protection Project. Kyrgyzstan: World Bank; 2012.

World Health Organization (WHO). Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquaculture. WHO; 2006.

World Health Organization (WHO). Sanitation [Internet]. 2022 [cited 2023 Mar 28]. Available from: https://www.who.int/news-room/fact-sheets/detail/sanitation.

Yamane T. Statistics: An Introductory Analysis. 2nd ed. New York: Harper and Row; 1967.

Yangon City Development Committee (YCDC). Existing Yangon City Development Committee Wastewater Treatment Plan and Future Plan. Yangon City Development Committee; 2018.

Zin M, Soe S. Reduction of wastewater pollutants of Mandalay City slaughterhouse. Proceedings of the Second International Conference on Science and Engineering; 2010 Dec 2-3; Yangon: Myanmar; 2010.

Evaluation of Water and Sediment Quality by Bacteriological Diversity Studies on Certain Locations of the Diyala River, Baghdad

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ABSTRACT

With a focus on the impact of the Rustumiya sewage treatment facility, the physio-chemical and indicator bacterial contamination pollution of the Diyala River (Iraq) was examined. The Diyala River in Baghdad was sampled on a monthly basis from March 2022 to February 2023 for surface water and sediment. The findings demonstrated seasonal and geographical variation in physio-chemical parameters (pH, EC, salinity, and TDS) and bacteriological markers (TC, FC, *E. coli*, and FS). The research procedures and methods showed that some physicochemical parameters (pH, EC, TDS) in sediment exceeded their standard limits concerning aquatic life protection in spring and summer. The level of bacteriological pollution in the water and sediments, evaluated by tracking of faecal indicator bacteria (*E. coli* and Enterococci), during the autumn and winter seasons had the largest concentration of bacterial contaminants. In this study, the main finding was the majority of the studied stations had water classified as not recommended or unacceptable and sediment classified as acceptable or conservatively accepted. The source of contamination, determined by the ratio value of faecal coliform bacteria to faecal Streptococcus, was from human origin in the majority of studied stations, and from mixed origin during the spring and summer. Bacterial indicators in this research exhibited both quantitative and qualitative fluctuation, suggesting their potential utility as a bio-indicator for contamination of water and sediment in the Diyala River.

1. INTRODUCTION

The majority of cases where the faecal contamination factor (FC:FS ratio) was greater than 4 showed that the area was predominately affected by human-borne faecal contamination. The release of untreated wastewater into rivers or rivers that have received inadequate treatment from water treatment facilities is a major contributor to the detriment of the aquatic ecosystem. This is due to the fact that heavy water is discharged into rivers without treatment. Since man began storing the earth, water systems have been the most heavily used natural resource (Yehia and Sabae, 2011; Khaled, 2016; Hawraa and Mrooj, 2018; Humudat et al., 2020). Human and animal waste, as well as temperature, oxides, salt, and pesticides are examples of biological pollutants that have deleterious effects on microbial populations in water and sediments (Filimon et al., 2010; Diwan et al., 2018; Abdus et al., 2019).

Due to their quick adaptation to new conditions, bacteria provide excellent sensors for monitoring microbiological contamination of surface water (Gunda and Mitra, 2016; Kirschner et al., 2017; Chen et al., 2019). Bacteria may be used as indicators of water quality from two perspectives: either they indicate fecal pollution of the water supply or they pose a health concern (Baghed et al., 2005).

Although there is no consensus on how best to measure microbial contamination in water systems, total coliform bacteria (TCB) and fecal coliform bacteria (FCB) have historically been used as microbial indicators of water (APHA, 2005). While FCB are not directly harmful to humans or animals, their presence in water systems is a sign that waste from humans and other warm-blooded species has made its way there (Yehia and Sabae, 2011; Sudip et al., 2021).

Commonly used indicators include coliform bacteria, which may have been reduced from total

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coliform to fecal coliform and faecal Streptococci (Kistemann et al., 2002). Commonly used indicators include coliforms, which may have been reduced from total coliforms to faecal coliforms and faecal Streptococci (Messner et al., 2017; Ramos-Ramírez et al., 2020).

The removal and degradation of pollutants and their conversion into lower risk compounds through sedimentation, aeration, filtration, chlorination and other processes at treatment plants such as the Al-Rustamiya Wastewater Treatment Plant are important steps in the process of reducing pollutant levels. Water Spill Hazard (RSTP). Pollution of most of the rivers and streams in Baghdad governorate is increasing exponentially due to a combination of factors including the carelessness of citizens and the irregularities of many authorities dumping waste directly into the rivers. The Diyala River is considered to be one of the largest rivers in the province, stretching for more than 150 km. Early roads in Iran, considered one of the most important tributaries of the Tigris. Several writers have examined the Diyala River, focusing on water quality (Al-Lami et al., 1996; Ayad, 2017; Abd Alkhder et al., 2019).

Bacterial pollution in the water body is one of the major issues concerning the sanitary quality of drinking and recreational water. The pollution of water samples by bacteria might become the cause of a severe epidemic of enteric diseases. So, the purpose of this research was to study the prevalence of the bacterial indicator in the Diyala River in Iraq, both in its water and in its sediments.

2. METHODOLOGY

2.1 Area of study

The objective of this research was to investigate the degree to which the bacterial indicator was prevalent in the Diyala River in Iraq, both in the water and the sediment. At Sanandaj in Iran's Zagros Mountains, the Diyala River begins its journey. This river serves as the border between Iran and Iraq for more than 30 km. The river drains an area that is 32,600 km^2, and its length is 574 km (UN-ESCWA and BGR, 2013). The objective of this research was to investigate the degree to which the bacterial indicator was prevalent in the Diyala River in Iraq, both in the water and the sediment.

The three main tributaries of the river are Don Lo, Sarawang and Vander. It crosses the Hemreen Mountains before branching into many other streams including Diyala, Al-Khalis, Rose, Haronia, Shahraban, Mahrute and Khraisan. Three dams were built on the river; their numbers are as follows: Darbandekhan (three times 109 m^3), Diyala (a controlled dam), and Hemreen (four times 109 m^3).

One of the most important projects is the Al-Rustumiya treatment facility, which provides services to the Rusafa neighborhood in the eastern part of Baghdad. The community's sewage is discharged into the Diyala River, which eventually flows into the Tigris River. The former Al-Rustmiya Sewage Treatment Plant consists of two stations, the first being the Al-Rustmiya South Station. This station has been in operation since 1963 and is comprised of the integrated projects three zeros (F0) and expansion I (F1), respectively. Since 1984, the Extension II (F2), also known as the Al-Rustumiya north station, has been in service. The Diyala River will ultimately become the site of the plant's discharge (Al-Sakini, 2016). Four sites along the Diyala River were chosen. Site (1) was about 800 meters north of the Rustamiya Wastewater Treatment Plant. Site (2) was located at the new Diyala Bridge after the outflow of the north Rustamiya into the Diyala River, which was approximately 1.8 km away from the first site. The site (3) was located on the army channel after the outlet south of Rustamiya in the Diyala River, which was approximately 3 km away from the second site. The fourth site is located near the AL-Rasool Bridge, which is about 3 km away from the third site (Figure 1).

2.2 Sample collection

Water and sediment samples will be collected monthly at four different locations from March 2022 to February 2023. The water was sampled from mid-stream, from the surface layer (25-35 cm deep) directly into sterile flasks. These samples are analysed at the environmental laboratory as soon as practicable after collection. Sediment samples were collected using a van veen scoop sampler; from which the samples were aseptically transferred into sterile glass containers. The samples were then placed in thermo insulated bags, and transported to the environmental laboratory for analyses.

2.3 Physicochemical analysis

Use a pH meter as a sample, and measure the pH value of the river water after calibration with a standard solution (buffer solution). EC conductivity was analyzed using the HANNA-type electrical conductivity meter and the results were expressed in units of microsiemens/cm (µs/cm). Total dissolved solids (TDS) were measured using a multimeter (manufactured by Hanna) and expressed in mg/L. The salinity was measured according to (APHA, 2017) based on the conductivity measurement and the following equation were uses to calculate the salinity.

$$\text{Salinity\%} = \text{EC} - 14.78/1589.08 \quad (1)$$

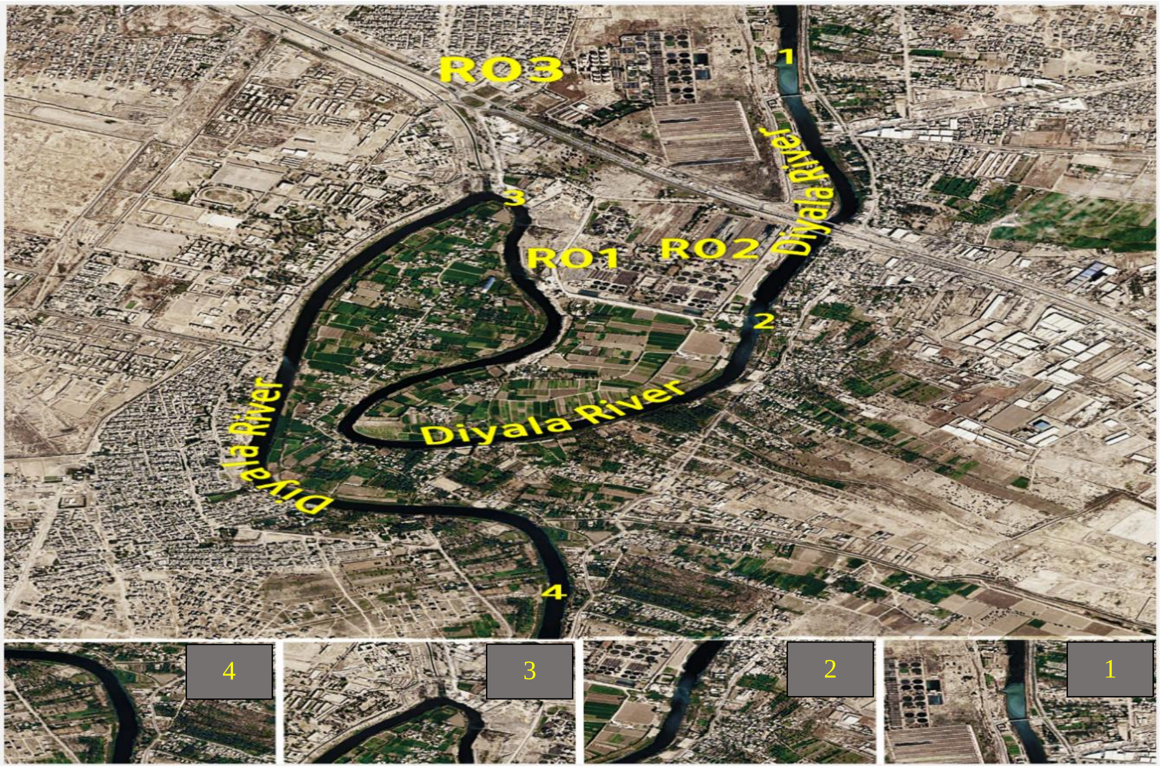

Figure 1. Study area with sampling stations

2.4 Bacterial identification

Total bacterial count (TCB) and fecal coliform bacteria (FCB) were determined using the most probable number (MPN) method. Incubate for 48 h at 37°C for total coliforms and 24 h for fecal coliforms at 44°C (in a water bath), and a positive result is indicated by an increase due to gas evolution in the tubes, and the identification was finalized by microscopic inspection and biochemical tests. Using the MPN technique, positive tubes showed severe turbidity and purple color after incubation in azide-dextrose broth at 37°C for 48 h (APHA, 2005; Filimon et al., 2010). There were three tubes used for each of the three decimal dilutions of each sample. Use of MacConkey broth for inferential TC and FC testing next, a gram stain is used to validate the results of the TC test using vivid green bile. The EC medium was tested at 44.5±0.5°C to confirm cases of FC (by water bath). The MPN technique was employed to identify faecal Streptococci, with azide-dextrose broth serving as the presumptive test and ethylvioletazide broth serving as the confirmatory test.

3. RESULTS AND DISCUSSION

3.1 Grain size analysis

Soil physicochemical characteristics at the locations of the different sediments of the Diyala River are listed in Table 1. According to Table 1, this kind of soil is called sandy loam. A grain size study showed that sand ranged from 612 to 712 mg/kg of soil, silt from 140 to 260 mg/kg of soil, and clay from 108 to 148 mg/kg of soil.

3.2 Sediment composition analysis

The Diyala River deposition site was characterized using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) of sediment grain and pore structure. The sediment from site 1, site 2, site 3 and site 4 was magnified at a magnification scale of 20 μm according to (Wang et al., 2017).

Table1. Grain size analysis of different sediment sites in the Diyala River at Baghdad city

Locations	Grain size analysis (g kg/soil)			
	Sand	Clay	Silt	Tissue class
Site 1	632	108	260	Sandy loam
Site 2	612	128	260	Sandy loam
Site 3	692	108	200	Sandy loam
Site 4	712	148	140	Sandy loam

Figure 2 (a1-a4) shows the energy dispersive X-ray spectroscopy (EDS) curve for sediments, showing that (SE, C, Si, Ca, Al, Mg, K, Fe) for site 1, (SE, C, Si, Ca, Al, Mg, K, Fe, O, Na) for site 2, (SE, C, Si, Ca, Al, Mg, K, Fe, O, na) for site 3 and (SE, Si, C, Ca, Al, Fe, In, O, Mg, Ba, Co, P, S) for site 4 are present in relatively high amounts.

Figure 2 (b1-b4) shows that the scanning electron microscope (SEM) has been curved upwards to describe the comparisons showing the surface shape, particle distribution, contaminant distribution and the morphological properties according to (Rashid and Faisal, 2018; Safia et al., 2021).

3.3 Water and sediment analysis

The distribution and seasonal changes of the physical and chemical properties of the Diyala River water and sediments and bacterial pollution indicators are shown in Figures 3 and 4.

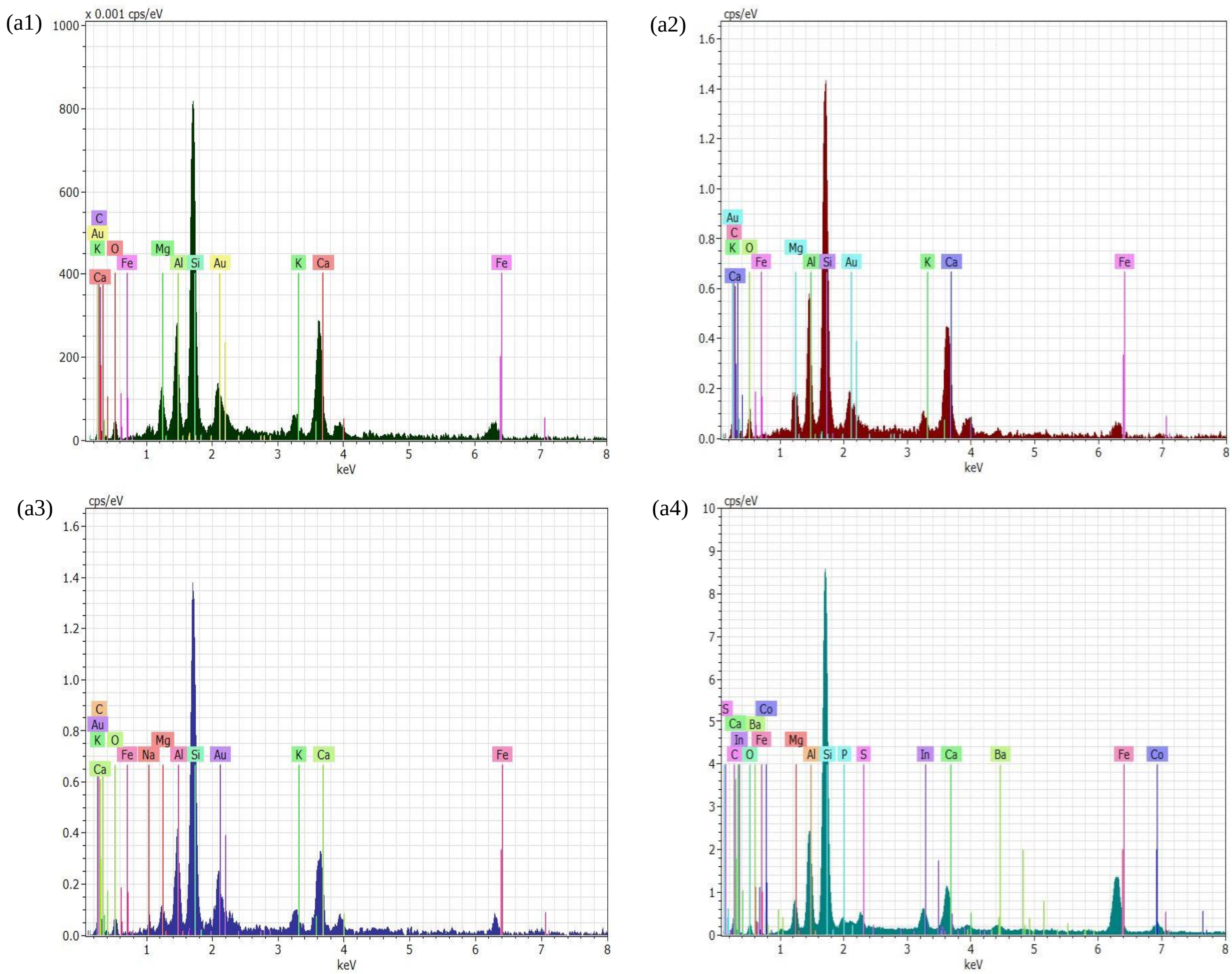

Figure 2. EDS for the composition and SEM images for the sediment samples: (a) EDS, (b) SEM

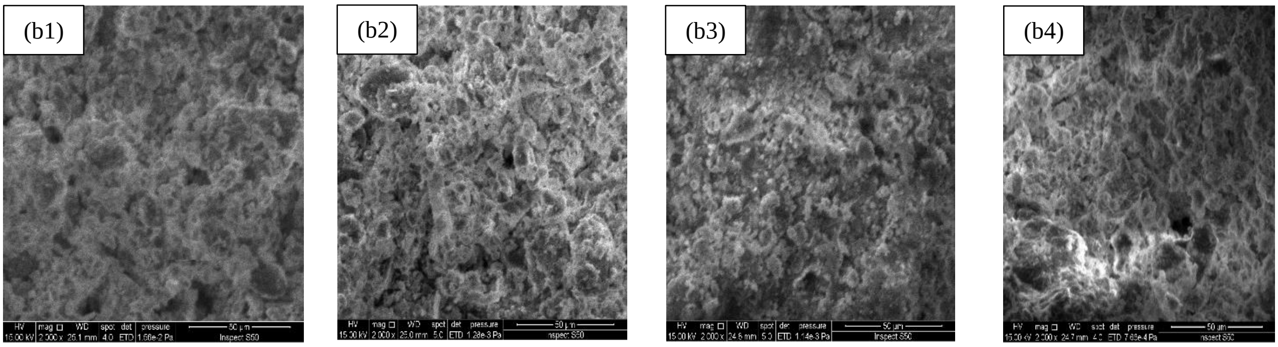

Figure 2. EDS for the composition and SEM images for the sediment samples: (a) EDS, (b) SEM (cont.)

pH values were consistent throughout the duration of the study period in all the studied stations, which is common in waters and sediment (Hashim and Rabee, 2014; Muhanned et al., 2020). High pH values were recorded in the summer at water site 4 and sediment site 1, while lower values were recorded in the winter at water site 1 and in the autumn at sediment site 4. This may be due to the decomposition of organic matter, which can be felt in large quantities at these stations. This value was within the Iraqi Water Standards (1967), as shown in Figure 3 (a-b).

Within a period, the study recorded the highest values of the EC and salinity (2,900 S/cm, 1.86%) in water, respectively, at sites 1, 2, and 3 during the summer and (2,600 S/cm, 1.66%) in sediment, respectively, at site 2 in the spring. while the lowest values were 1,900 S/cm and 1.22% in water, respectively, at site 4 during autumn and 1,180 S/cm and 0.76% in sediment, respectively, at site 4 in winter, as shown in Figure 3 (c-f). Higher value readings of conductivity and salinity may be due to the rainfall, which will cause the salts to drift from the area surrounding the river and the discharge of the Rustumiya sewage treatment plant (RSTP), increasing salt concentrations as well as many other pollutants (Ghayda et al., 2022). These values are close to those measured in other studies of this river (AL-Khaledy, 2003; AL-Sarraf, 2006).

The total dissolved solids (TDS) values showed a high value (1,856, 3,260) mg/L at water site 2 during spring and sediment site 2 in summer, The lower values (902, 770) mg/L were measured in water sample point 3 in autumn and sediment sample point 4 in winter. A high value may be due to the rainy season and a period of high discharge. These values in sediment exceeded the Iraqi Water Standards (1967), as shown in Figure 3 (g-h).

In this research, the total coliform bacteria (TCB) concentrations in the water samples varied between 3.6 and 2,300 MPN/100 mL from site 4 to site 2, and the TCB concentrations in the sediment samples varied between 3.6 and 1,100 MPN/100 mL from site 4 to site 2. According to these findings, the highest values were seen throughout the winter and autumn seasons. Increased bacterial activity led to a higher concentration of organic matter (as seen in Figure 4 (a-b)) (Othman et al., 2012). High concentrations of TCB were found across all research locations and time periods. High levels of suspended solids and nutrients in runoff water may lead to a decline in aquatic microflora and increased incidence of TCB in winter and autumn (Ankit et al., 2019).

Figure 4 (c-d) show that the total number of faecal *E. coli* found in water and sediment varied widely across all study sites, ranging from 3 to 1,150 MPN/100 mL in water and from 2 to 150 MPN/100 mL in sediment. (Kirschner et al., 2017) note that fecal coliforms are the greatest markers for gauging recent fecal contamination, which comes mostly from raw and processed sewage as well as dispersed influences from agricultural land and pasture.

In this study, the prevalence of faecal indicator bacteria was determined in samples collected from a large river in a rural area. According to studies (Quattare et al., 2011), this river was more polluted than others since it flowed through fields. In water, the MPNs for *E. coli* and *S. faecalis* range from 0.5 to 35 MPN/100 mL and 3 to 75 MPN/100 mL, respectively, and in sediment 0.5 to 20 MPN/100 mL, 0.5 and 6 per 100 mL, respectively the number of cells. Faecal Streptococci are appraised to have certain benefits over the coliform bacteria as indicators as they are more resistant to environmental pressure and chlorination than coliforms (Gerba et al., 2019).

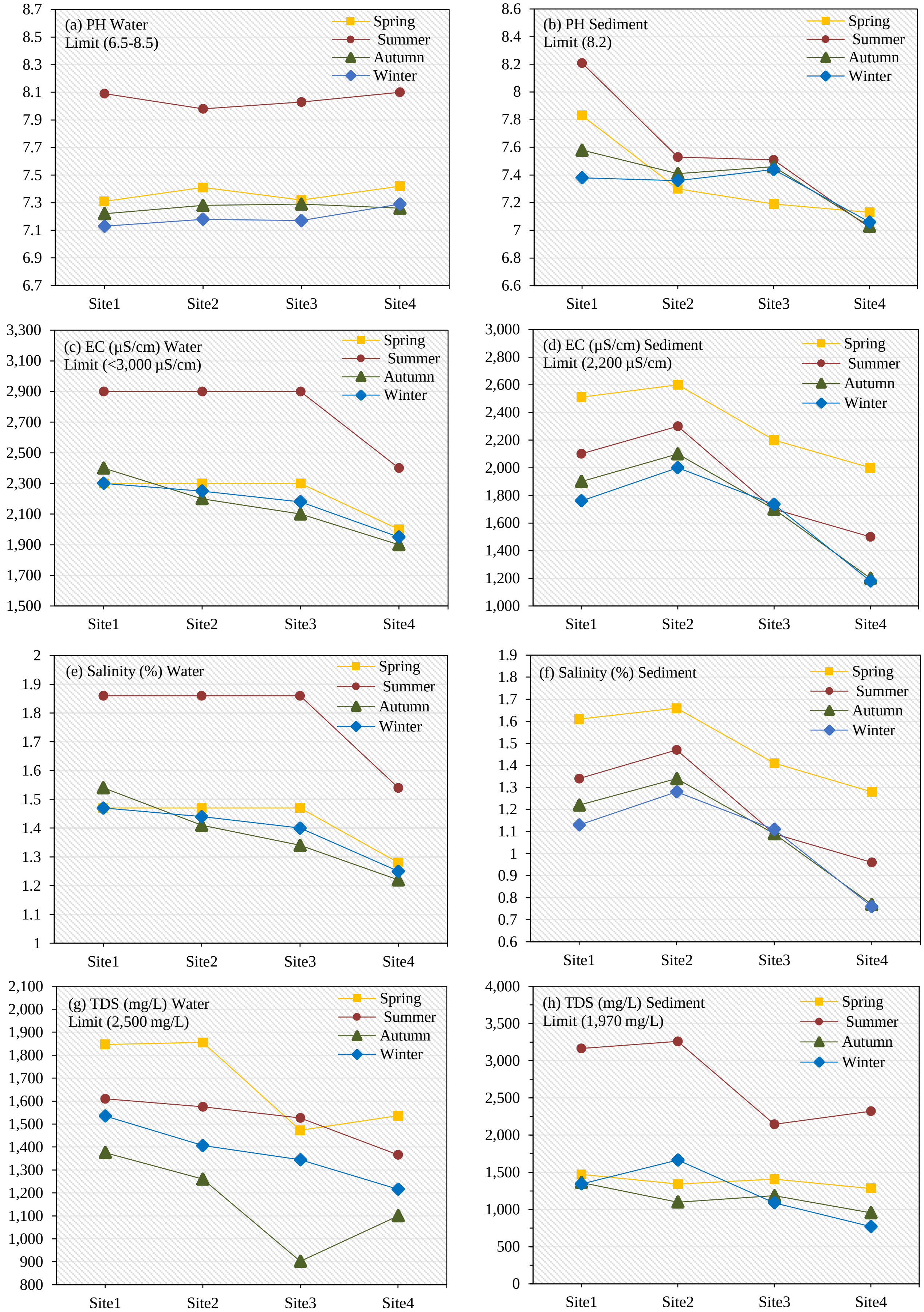

Figure 3. Seasonal variation of some physicochemical parameters (pH, EC, Salinity, TDS)

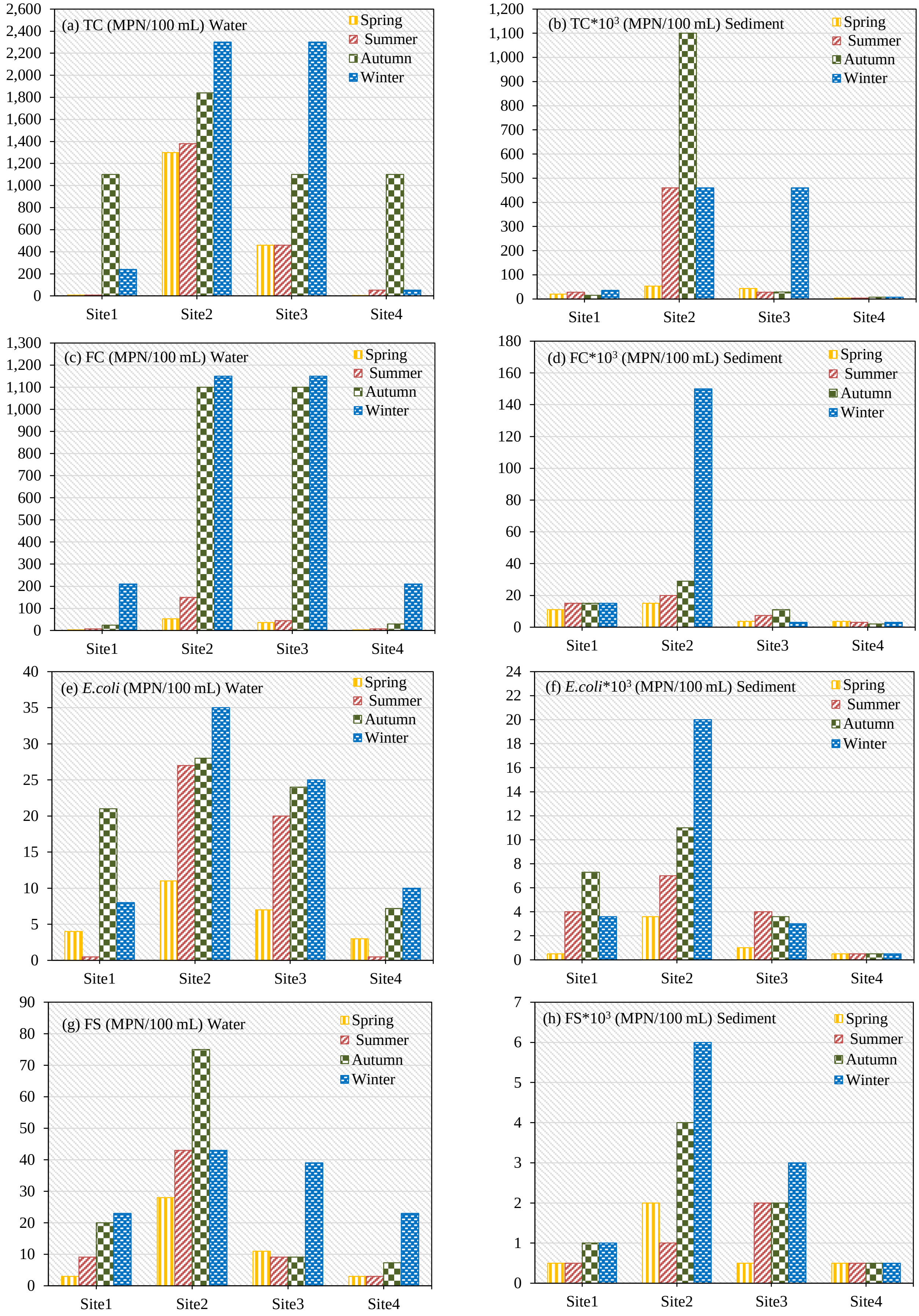

Figure 4. Seasonal variations of bacteria indicator (MPN/100 mL) in water and sediment

As illustrated in Figure 4 (e-f), the river's land and RSTP sediments affect these microorganisms. The results showed that insolation, turbidity, temperature, salinity, dissolved oxygen, and organic matter may all have effects on the variation of bacterial index values and counts in different seasons and locations in the Diyala River (Yehia and Sabae, 2011). High levels of sun radiation and high temperatures may hasten the demise of the indicator bacteria, which may explain why summer months have the lowest numbers (El-Shenawy, 2005).

As predicted from heavy, untreated water, the investigation found a rise in pollution factors in the water entering the station. Discharging this water to the Diyala River without treatment will worsen the river's low level, lack of flow, and dry conditions.

3.4 FC:FS ratio in water and sediments

Surface and groundwater samples were analyzed for the presence of bacteria by comparing the levels of faecal *E. coli* and faecal Streptococci (Baudisova, 2009). A ratio of 4 or more indicates pollution from humans, a ratio of 0.7 to 4 indicates contamination from a combination of sources, and a ratio of less than 0.7 indicates pollution from wild animals. The seasonal variation of this percentage indicates that the source of pollution at most stations is anthropogenic pollution in spring and summer. While it was of mixed origin during the winter, as shown in Table 2. These results can be attributed to human activities and sewage waste in most seasons of the year. While the source of mixed pollution can be attributed to the waste of animal farms in addition to precipitation during this period. These results agree with other studies (Donderski and Wilkk, 2002; Shawky and Saleh, 2007; Raji et al., 2015).

3.5 Water and sediment quality evaluation

Due to the high level of bacterial contamination of the river's water and sediments (WHO, 1989), it is questionable whether the use of Diyala River water for irrigation would increase the risk of infection transmission (Obasohan et al., 2010). WHO proposes an average value of 1,000 MPN/100 mL for fecal coliforms in vegetable irrigation wastewater.

Table 3 provides practical guidance for planning and implementing a freshwater quality research and monitoring program (UNEP and WHO, 1996). Therefore, the water quality of the Diyala River was assessed according to the approved test limits for bacterial indicators (TC, FC and FS) in water and sediment (UNEP and WHO, 1996). By applying the World Health Organization's criteria for assessing water quality based on bacterial indicators, it was observed that most of the surveyed sites were rated as not recommended or unacceptable in water, and acceptable or conservatively acceptable in sediment, as shown Table 4 and Table 5.

Table 2. Indicate the source of bacterial contamination as a ratio (FC/FS)

Locations	(FC/FS) Water				(FC/FS) Sediment			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Spring	Mixed	Mixed	Mixed	Mixed	Human	Human	Human	Human
Summer	Mixed	Mixed	Human	Mixed	Human	Human	Mixed	Human
Autumn	Mixed	Human	Human	Mixed	Human	Human	Human	Mixed
Winter	Human	Human	Human	Human	Human	Human	Mixed	Human

Table 3. Evaluation of water and sediment quality in Diyala River (UNEP and WHO, 1996)

Bacterial indicator	Number of cells/100 mL (Water)	Number of cells/100 mL (Sediment)	Water quality	Symbol
(TC)	50>	50,000>	Acceptable	A
	51-500	51,000-500,000	Conservatively accepted	CA
	501-1,000	501,000-1,000,000	Not recommended	NR
	1,000-10,000	1,000,000-10,000,000	Unacceptable	UA
	10,000<	10,000,000>	Contaminated	C
(FC) or (FS)	10>	10,000>	Acceptable	A
	11-100	11000-100000	Conservatively accepted	CA
	101-1,000	101000-1000000	Not recommended	NR
	1000-10000	1000000-10000000	Unacceptable	UA
	10,000<	10,000,000<	Contaminated	C

Table 4. Water quality evaluation according to the approved limits for testing water (TC, FC, FS) MPN/100 mL

Locations	(TC)				(FC)				(FS)			
	Site1	Site2	Site3	Site4	Site1	Site2	Site3	Site4	Site1	Site2	Site3	Site4
Spring	A	UA	CA	A	A	CA	CA	A	A	CA	CA	A
Summer	A	UA	CA	CA	A	NR	CA	A	A	CA	A	A
Autumn	UA	UA	UA	UA	CA	UA	UA	CA	CA	CA	A	A
Winter	CA	UA	UA	CA	NR	UA	UA	NR	CA	CA	CA	CA

Table 5. Sediment quality evaluation according to the approved limits for testing sediment (TC, FC, FS) $*10^3$ MPN/100 mL

Locations	(TC)				(FC)				(FS)			
	Site1	Site2	Site3	Site4	Site1	Site2	Site3	Site4	Site1	Site2	Site3	Site4
Spring	A	CA	A	A	CA	CA	A	A	A	A	A	A
Summer	A	CA	A	A	CA	CA	A	A	A	A	A	A
Autumn	A	UA	A	A	CA	CA	CA	A	A	A	A	A
Winter	A	CA	CA	A	CA	NR	A	A	A	A	A	A

4. CONCLUSION

- The physico-chemical parameters (pH, EC, TDS) in sediment were exceeded their standard limits concerning of aquatic life protection at spring and summer.
- Bacterial contamination levels in Diyala River water and sediments were assessed by tracing faecal indicator bacteria (*Escherichia coli* and Enterococci). The concentrations of *E. coli* and fecal bacteria in the river water were highest in autumn and w inter.
- Fecal coliform, faecal Streptococcus and *Escherichia coli* were detected in all water samples, indicating the seriousness of the pollution of Diyala River to human health.
- The index factor FC:FS values of most samples were greater than 4, indicating the dominance of human fecal contamination.
- The water quality of the Diyala River in the study area was deteriorated by the Rustumia sewage treatment plant.

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REFERENCES

Abd Alkhder AM, Luay MA, Raaed SA. The effect of DiyalaI River drain on the environment and the two types of Iraqi Fish in the Tigris River. Plant Archives 2019;19(1):949-58.

Abdus S, Ashra F, Fazle R, Nusrat HN, Naim U, Masud R. Study of sanitary conditions and quantitative estimation of bacterial flora in Tank Goby (*Glossogobius giuris*) Fish and pond. International Journal of Fisheries and Aquatic Research 2019;4(4):10-4.

AL-Khaledy SH. Ecological and Bacteriological Study in The South Part of Diyala River [dissertation]. College of Science for Women, University of Baghdad; 2003.

Al-Lami AA, Sabri AW, Kassim TI, Rasheed KA. The ecological effect if Diyala River on Tigris River,1-Limnology. Journal College Education for Women, University of Baghdad 1996;7(1):84-92.

Al-Sakini AY. Rustumiya Sanitation Project and its effects on polluting Diyala River. Journal of Basra Arts 2016;76:243-68.

AL-Sarraf MA. Ecological and Taxanomical Study for Phytoplankton in AL-Adaim and Diyala Tributaries and Their Effects on Tigris River [dissertation]. College of Science for Women, University of Baghdad; 2006.

Ankit D, Chandra A, Shubhakriti U, Aarti N, Singh KP. Isolation and characterization of coliform bacteria and bacteriophages from Ganga River in Northern Himalayan Regions. International Journal of Current Microbiology and Applied Sciences 2019;8(11):1582-92.

American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington, USA: APHA; 2005.

American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. 23rd ed. Washington, USA: APHA; 2017.

Ayad GH. The physico-chemical properties of southern part of Diyala River water. Iraqi Journal of Science 2017;58(C4): 2322-31.

Baghed VS, Gopal K, Dwivedi S, Tripathi RD. Bacterial indicators of faecal contamination of the Gangetic River system right at its source. Ecological Indicators 2005;5:49-56.

Baudisova D. Microbial pollution of water from agriculture. Plant, Soil and Environment 2009;55:429-35.

Chen L, Li F, Zhi XS, Zhang P, Dai Y, Xiao YC, et al. Pollution characteristics and health risk assessment of microorganism pollutions in the Beiyun River. Huan Jing Ke Xue 2019; 40:633-9.

Diwan V, Hanna N, Purohit M, Chandran S, Riggi E, Parashar V, et al. Seasonal variations in water-quality, antibiotic residues, resistant bacteria and antibiotic resistance genes of *Escherichia coli* isolates from water and sediments of the Kshipra River in Central India. International Journal of Environmental Research and Public Health 2018;15(6):Article No. 1281.

Donderski W, Wilkk I. Bacteriological studies of water and bottom sediments of the Vistula River between Wyszogrod and Torun. Polish Journal of Environmental Studies 2002;11(1):33-40.

El-Shenawy MA. *Staphylococcus aureus* and fecal indicators in Egyptian coastal waters of Aqaba Gulf and Red Sea. Egyptian Journal of Aquatic Research 2005;31(2):113-24.

Filimon MN, Borozan A, Sinitean A, Popescu R, Oance RT, Verdes D. Bacteriological studies on Timis and Bega Rivers with a role in appreciating pollution. Annals of the Romanian Society for Cell Biology 2010;15(2):217-20.

Gerba CP, Pepper IL, Brusseau ML. Environmental and Pollution Science. Academic Press; 2019: p. 191-217.

Ghayda YA, Sara AH, Husam AA, Sedik AK. Environment impact assessment of Al-Rustumiya wastewater treatment plant in Baghdad-IRAQ. Innovative Infrastructure Solutions 2022;7(2):1-15.

Gunda NS, Mitra SK. Rapid water quality monitoring for microbial contamination. The Electrochemical Society Interface 2016;25(4):73-8.

Hashim AG, Rabee AM. Impact of Diyala Tributary on the quality of Tigris River Water. Journal of International Environmental Application and Science 2014;9(4):493-504.

Hawraa MR, Mrooj SA. Evaluating the water of the Hilla River through the study of physical and chemical properties and microbial content. Journal of University of Babylon for Pure and Applied Sciences 2018;26(7):18-31.

Humudat YR, Al-Naseri SK, Al-Fatlawy YF. Assessment of microbial contamination levels of water in hemodialysis centers in Baghdad, Iraq. Water Environment Research 2020;92:1325-33.

Iraqi Water Standards. Regulation No. 25 on the Preservation of Rivers and Public Waters from Pollution. Iraqi: Official Gazette; 1967. p. 108.

Khaled SB. Microbial contamination of vegetable crop and soil profile in arid regions under controlled application of domestic wastewater. Saudi Journal of Biological Sciences 2016; 23(1):83-92.

Kirschner AK, Reischer GH, Jakwerth S, Savio D, Lxenmaier S, Toth E, et al. Multiparametric monitoring of microbial faecal pollution reveals the dominance of human contamination along the whole Danube River. Water Research 2017; 124(1):543-55.

Kistemann AT, Claben T, Koch C, Dangendorf F, Fischeder R. Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. Applied and Environmental Microbiology 2002;68:2188-97.

Muhanned RN, Fatema SM, Enaam KA, Eman HA. The effect of Diyala tributary on ecological factors of Tigris River. Journal of Physics: Conference Series 2020;1664:Aticle No. 012134.

Messner MJ, Berger P, Javier J. Total coliform and *E. coli* in public water systems using undisinfected ground water in the United States. International Journal of Hygiene and Environmental Health 2017;220(4):736-43.

Obasohan EE, Agbonlahor DE, Obano EE. Water pollution: A review of microbial quality and health concerns of water, sediment and fish in the aquatic ecosystem. African Journal of Biotechnology 2010;9(4):423-7.

Othman AA, Rabeh SA, Fayez M, Monib M, Hegazi NA. El-Salam canal is a potential project reusing the Nile Delta drainage water for Sinai desert agriculture: Microbial and chemical water quality. Journal of Advanced Research 2012;3(2):99-108.

Quattare NK, Passerat J, Servais P. Fecal contamination of water and sediments in the Scheldt drainage network. Environmental Monitoring and Assessment 2011;183(1-4):243-57.

Raji MI, Ibrahim YK, Tytler BA, Ehinmidu JO. Faecal coliforms (FC) and faecal streptococci (FS) ratio as a tool for assessment of water contamination: A case study of river Sokoto, northwestern Nigeria. Handbook on the Emerging Trends in Scientific Research 2015;3:8-11.

Ramos-Ramírez L, Romero-Bañuelos CA, Jiménez-Ruíz EI, Palomino-Hermosillo YA, Saldaña-Ahuactzi Z, Martínez-Laguna Y, et al. Coliform bacteria in San Pedro Lake, western Mexico. Water Environment Research 2020;93(4):1-9.

Rashid HM, Faisal AH. Removal of dissolved cadmium ions from contaminated wastewater using raw scrap zero-valent iron and zero valent aluminum as locally available and inexpensive sorbent wastes. Iraqi Journal of Chemical and Petroleum Engineering 2018;19(4):39-45.

Safia MK, Hassan F, Abdelazim N, Ahmed T. Measuring the engineering properties of landfill leachate-contaminated soil in Egypt. Euro-Mediterranean Journal for Environmental Integration 2021;6(23):1-12.

Shawky ZS, Saleh AR. Evaluation of the microbial quality of the river NILE waters at Damietta branch, Egypt. Egyptian Journal of Aquatic Research 2007;33(1):301-11.

Sudip S, Rittick M, Debasis M, Divya Jain, Devvret V, Samanwita D. Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. Energy Nexus 2021;1:Article No. 100008.

United Nations Economic and Social Commission for Western Asia (UN-ESCWA), Bundesanstalt für Geowissenschaften und Rohstoffe (BGR). Shared tributaries of the Tigris River. In: Invent tory of Shared Water Resources in Western Asia. Beirut, Lebanon: UN-ESCWA, BGR; 2013.

United Nation Environment Program (UNEP), World Health Organization (WHO). Water Quality Monitoring: A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. WHO; 1996.

Wang H, Liu T, Feng S, Zhang W. Metal removal and associated binding fraction transformation in contaminated river sediment washed by different types of agents. PLoS ONE 2017;12(3):e0174571.

World Health Organization (WHO). Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture. Geneva: WHO; 1989.

Yehia HM, Sabae SZ. Microbial pollution of water in El-Salam Canal, Egypt. American Eurasian Journal of Agricultural and Environmental Sciences 2011;11(2):305-9.

Streptomyces sp. Strain SRH22: A Potential Bioremediation Agent for Glyphosate-Contaminated Agricultural Soils

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ABSTRACT

Glyphosate, also known as N-phosphonomethylglycine, is the herbicide that is widely used across the globe. As there are concerns over its potential toxicity to non-target soil species, there is a growing interest in identifying glyphosate-degrading microorganisms in soil. Biodegradation, by actinobacteria, is a very promising approach to eliminate this pesticide from contaminated environments. The present work isolated and identified actinobacteria capable of degrading glyphosate from Saharan agriculture, as well as determined how the application of this herbicide affects the abundance of actinobacteria present in soil. It was observed that the use of glyphosate led to an increased abundance of actinobacteria in the soil compared to the untreated soil. Among this population, an actinobacterial strain was isolated from glyphosate contaminated soil by the enrichment method, and was identified to possess the greatest capability to degrade glyphosate at 50 mg/L. The identification of this strain was achieved through a combination of cultural, morphological, biochemical, and molecular techniques. This included the use of 16S rDNA sequencing, leading to its successful classification as *Streptomyces* sp. strain SRH22. This strain was assigned the accession number OQ302556 by the National Center for Biotechnology Information (NCBI). A rapid, sensitive, and straightforward spectrophotometric technique was employed for the quantification of glyphosate. Results showed that the optimal biodegradation (90.2%) was obtained under a temperature of 30 degrees, a PH of 7.2, and an inoculum volume of 4% timed over six days. This work shows that the *Streptomyces* SRH22 presents good potentiality to be used as a bioremediation agent for agricultural soils in the Algerian Sahara.

1. INTRODUCTION

The use of pesticides is an important and necessary practice in agriculture due to their ability to kill pests and reduce crop diseases, primarily for economic reasons (Sabzevari et al., 2022). Pesticides are widely used throughout the world, including in Saharan agronomy in Algeria (Supreeth et al., 2016; Arias-Estévez et al., 2008; Belhadi et al., 2016) However, the use of pesticides in agriculture can have severe environmental consequences, including air, soil, and water pollution. Exposure to these chemicals can also harm non-target organisms, leading to fatalities from accidental poisoning. Among the most dangerous pesticides, we found organophosphates

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(OPs), which are extensively used and known to persist in the environment for long periods (Jiang et al., 2019; Briceño et al., 2018; Cardozo et al., 2019).

Glyphosate, commonly known as N-phosphono-methyl-glycine, is an extensively used herbicide in the Sahara Region of Algeria. It is an organophosphate pesticide that targets weeds by inhibiting the activity of the enzyme EPSPS which affects the production of amino acids, reducing the level of these essential compounds (De Castilhos Ghisi et al., 2020; Maeda and Dudareva, 2012). Glyphosate is the active ingredient in the commercial product Fortin SL (Zhan et al., 2018), which has been registered and sold in Algeria for years and used in agriculture such as vegetable crops, citrus orchards, and palm groves in the Saharan agricultural soils of Algeria. Recently, the European Union authorized its use until December 15th, 2023.

Glyphosate continues to be used in Algeria, and its half-life in the environment ranges from 0.8 to 151 days. This is influenced by variation in environmental conditions and soil type (Bai and Ogbourne, 2016). Reports from experts suggest that the harmful residues of this herbicide can accumulate in soil and water, posing a potential environmental hazard (Firdous et al., 2020). To eliminate this pesticide, bioremediation has been identified as the most effective, and economical, mechanism which involves the use of microorganisms to restore various environmental sites that have been negatively impacted (Manogaran et al., 2017; Uqab et al., 2016; Briceño et al., 2017; Mishra et al., 2021; Rossi et al., 2021).

Actinobacteria, a type of Gram-positive bacteria with a filamentous structure, are abundant in soil and have remarkable abilities to degrade xenobiotic compounds such as pesticides (Alvarez et al., 2016). While several studies have shown that actinobacteria can degrade different chemical classes of pesticides, including organochlorines such as lindane, y-chlordane, and methoxychlor (Fuentes et al., 2017), most reports on glyphosate biodegradation have focused on microorganisms other than actinobacteria (Manogaran et al., 2017; Fuentes et al., 2017; Hadi et al., 2013; Adelowo et al., 2014; Malla et al., 2023; Yu et al., 2023).

Due to the enzymatic diversity of actinobacteria, and the lack of research on the biodegradation of glyphosate in arid soils by these bacteria, we sought to explore their metabolic potential in this area. Our main focuses are on the isolation and identification of actinobacteria that could degrade glyphosate, a commonly used herbicide in Algerian agriculture. Additionally, we aimed to assess the potential of this strain in mitigating the negative effects of glyphosate on the actinobacterial population in contaminated soil.

2. METHODOLOGY

2.1 Geographic location

The study was carried out in the city of Ouargla, situated in the northeast of the northern Sahara, which is located about 750 km to the south of Algiers.

Ouargla is bordered by the wilaya of El-Oued to the northeast; the wilaya of Djelfa to the northwest; the wilaya of Illizi to the southeast and by the wilaya of Ghardaïa to the west (Abdelhak, 2020).

2.2 Effect of glyphosate on the number of actinobacteria

To evaluate the impact of glyphosate herbicide on the actinobacteria population, in arid soil of Ouargla, two soil samples were examined: one treated with glyphosate and another untreated. The selective GLM and Bennett medium were used to count the actinobacteria, and their numbers were determined by applying a serial dilution technique. The soil was first mixed with distilled water to create a stock solution, which was then diluted several times to create a series of dilutions up to 10^{-5}. Next, from each dilution, an amount of 0.1 mL was inoculated onto the nutrient medium and incubated at a temperature of 30°C, for 21 days to allow for bacterial growth and enumeration.

2.3 Isolation and identification of actinobacteria

Isolation by enrichment: Actinobacteria were isolated from agricultural soil located in Ouargla City, Algeria, through an enrichment method using Vendermesse's minimum medium (MSM) with 50 mg/L of glyphosate as a carbon source, according to the protocol of Abraham and Gajendiran (2019).

2.4 Glyphosate tolerance of actinobacterial isolates

Following the protocol of Briceño et al. (2012), several actinobacterial isolates were used to determine their ability to degrade glyphosate pesticide at varying concentrations (1, 10, 25, and 50 mg/L). Based on the results, one of the actinobacterial isolates was identified as having a high potential for biodegrading glyphosate pesticide.

2.4.1 Morphological, biochemical and physiological characterization

The selected actinobacterial isolate was subjected to a study of its macro and micro morphological characteristics. The isolate was streaked on various nutrient media, including ISP2, ISP7, ISP9, GLM, Bennett, and YEMA, then incubated during a week at 30°C to observe macroscopic characteristics. Following this, Gram staining and spore morphology were examined using a light microscope. To evaluate the bacterial isolate's ability to use different sugars as a carbon source, such as D-fructose, galactose, glucose, lactose, sachharose, mannitol, and citrate (Shirling and Gottlieb, 1966). In addition, various amino acids (aspartic acid, laproline, arginine, threonine, histidine, asparagine, tyrosine, and methionine) were tested as a nitrogen source (Williams et al., 1983). Other tests conducted included catalase production (Li et al., 2016), gelatin hydrolysis (Minotto et al., 2014), starch hydrolysis (Tatsinkou Fossi et al., 2005), and melanoid pigment production (Lee et al., 2014). The strain's tolerance to different pH levels (2, 5, 9, 12), temperatures (4°C, 28°C, 37°C, 40°C, 50°C), and NaCl concentrations, ranging from 2% to 15%, were also tested using ISP2 medium.

2.4.2 Amplification of 16S ribosomal RNA gene and nucleotide sequencing through polymerase chain reaction (PCR)

DNA extraction was performed using the DNeasy Power Soil kit. A pair of forward (27F 5′AGAGTTTGATCCTGGCTCAG-3′) and reverse (1429R 5′-GGTTACCTTGTTACGACTT-3′) primers were used for the PCR reaction. The denaturation of target DNA was done at 95°C for 5 min, followed by amplification with 30 cycles at 94°C for 1 min, then, at 55°C for 1 min, and at 72°C for 1.50 min. The PCR mixture was then maintained at 72°C for 10 min (Boufercha et al., 2022). The amplified PCR products were purified and sequenced at Eurofins genomics (Konstanz-Germany) using universal bacterial RNA16S primers (27F) (Moreira et al., 2021). Identification was performed using the BLAST software. Phylogenetic analysis was conducted by aligning the bacterial 16S rRNA gene sequences with reference sequences available in the GenBank database. The Neighbor-Joining method was implemented using the MEGA software (version 11) to construct the phylogenetic tree.

2.5 Growth kinetics

To record the growth curve of the actinobacterial strain, the protocol of Briceño et al. (2012) was used with simple modification. Briefly, 4% of the bacterial pellet was cultivated in liquid MSM medium, with 50 mg/L of glyphosate as the sole carbon source, and placed on a shaker at 100 rpm at 30°C for 10 days.

2.6 Biodegradation of glyphosate by action-bacterial isolate

To evaluate the rate of glyphosate biodegradation, a spectrophotometric technique proposed by Bhaskara and Nagaraja (2006) was used. 30 mL of MSM medium, with glyphosate at 50 mg/L, were prepared in 50 mL erlens and inoculated with an actinobacterial isolate pellet at a concentration of 4%. Abiotic controls were also prepared, followed by incubation, during a ten day period at 30°C under 200 rpm. 1 mL of the culture was taken every day and passed to centrifugation at 6,000 g for 10 min at room temperature. It was then filtered through a PVDF polyvinylidene fluoride membrane filter of 0.22 µm. The filtered liquid was mixed with a solution of a mixture containing 0.5 mL of ninhydrin and 0.5 mL molybdate (at 5% for each solution). A standard curve was also prepared under the same experimental conditions, added by glyphosate at concentrations from 4 to 14 mg/L. The tubes were covered with aluminum foil to prevent exposure to light, and incubated at 100°C for 5 min followed by cooling at room temperature. Finally, 3 mL of distilled water was subsequently added to reach a final volume of 5 mL. The absorbance measurements were taken using a UV-visible spectrophotometer (UV-1800A, Shimadzu, Japan) at 570 nm. The experiment was carried out in triplicate, and the percentage of glyphosate biodegradation was calculated using the following formula.

$$\text{Biodegradation percentage (\%)} = (M1 - M2)/M1 \times 100\%$$

M1 and M2 represent the concentrations of glyphosate in the untreated and treated samples, respectively, using the actinobacterial isolate.

2.7 Effect culture conditions on glyphosate biodegradation

A 4% inoculum of actinobacterial isolate was introduced into 30 mL of Vendermesse's minimum medium, which contained 50 mg/L of glyphosate. The flasks with different pH levels (2, 5, 7, 9, 12), various temperatures (4°C, 10°C, 20°C, 25°C, 30°C, 37°C, and 40°C) and various concentrations of actinobacterial inoculum (2%, 4%, 5%, 7%, and 9% w/v) were incubated at 30°C with agitation at 100 rpm/min for 10 days. During the incubation period, the glyphosate concentration was measured every 24 h (Bhaskara and Nagaraja, 2006).

2.8 Statistics analysis

All experimental procedures were performed in triplicate with standard error. GraphPad Prism version 8.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to carry out statistical analyses. ANOVA analyses (one-way and two-way) of variance and Tukey's test were utilized with a significance level of $p \leq 0.05$ (95% confidence interval).

3. RESULTS

3.1 Effect of glyphosate on the number of soil actinobacteria

Actinobacteria isolated from herbicide-treated and untreated soil samples, on both GLM and Bennett media, were recognised by their characteristic aspects and then enumerated. Their numbers in the untreated soil were 220 CFU/mL in the GLM medium and 300 CFU/mL in the Bennett medium. On the other hand, in the treated soil, the number was 500 CFU/mL in the GLM medium and in the Bennett medium, it was 300 CFU/mL (Figure 1).

3.2 Morphological characteristics of action-bacterial isolate SRH22

One strain among the eight actinobacterial isolates was selected for this study based on its superior tolerance to the highest glyphosate concentration 50 mg/L. This actinobacterium SRH22 showed a circular colony morphology, with a pasty texture and firm adherence to the agar, with yellow substrate mycelium, grey aerial mycelium and Gram-positive. The shape of spore chains was Retinaculum-Apertum. When it was grown on ISP2 medium and ISP7, the isolate produced yellow pigments. The isolate showed good growth on ISP2, GLM, Bennett, and YEMA media (Figure 2 and Table 1).

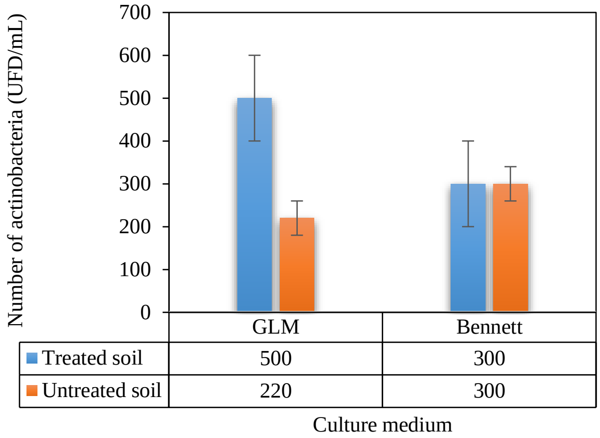

Figure 1. Determination of number of actinobacteria in treated and untreated soil with Glyphosate pesticide.

3.3 Biochemical and physiological characteristics of the actinobacterial isolate SRH22

The actinobacterial isolate SRH22 showed positive hydrolysis of starch, gelatin and casein, as well as the ability to produce catalase, coagulate and peptonize milk. In addition, negative results were observed on Nitrate reductase, RM, VP, H_2S and mobility. The isolate SRH22 used glucose, D-fructose, and galactose as a source of carbon, and lactose, saccharose, citrate, and mannitol were not utilised. All tested nitrogen sources, including aspartic acid, proline, arginine, threonine, histidine, asparagine, tyrosine, and methionine, were utilized by the isolate. SRH22 showed moderate growth on media containing 2% and 5% sodium chloride, poor growth on 9%, and no growth on 15% NaCl. It was capable of growing across the pH range tested (2, 5, 7, 9, and 12), with optimal growth at pH 7. The isolate showed strong growth at 28°C and 37°C, moderate growth at 40°C, weak growth at 4°C and no growth at 50°C (Table 2).

Table 1. Cultural characteristics of *Streptomyces* sp. isolate SRH22 after incubation during 15days at 30°C in different nutrient media.

Medium	Growth	Aerial mycelium	Substrate mycelium	Form of spores chain	Pigmentation
ISP-2	Strong	Strong, gray	Present	Retinaculum-Apertum	Yellow
ISP-7	Moderate	Weak, gray	Present		
ISP-9	Moderate	Weak, gray	Present		
GLM	Strong	Strong, gray	Present		
Bennett	Strong	Weak, gray	Present		
YEMA	Strong	Weak, gray	Present		

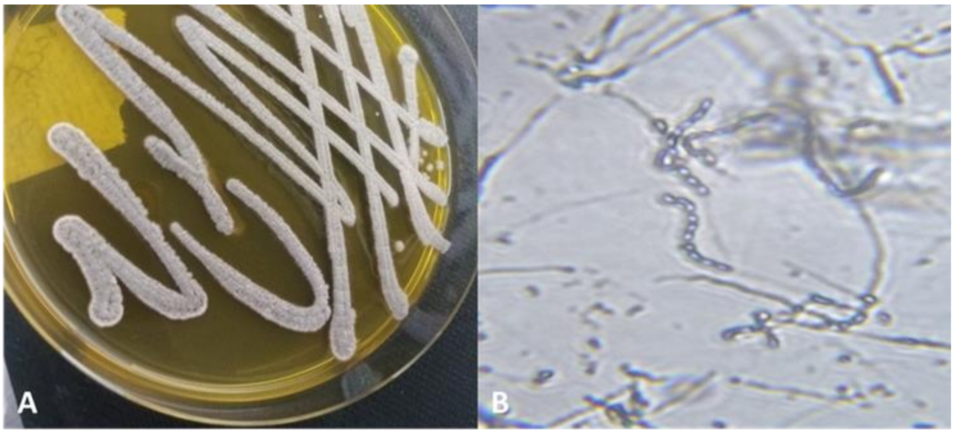

Figure 2. (A) Morphological image of the actinobacterial isolate SRH22 growth on ISP-medium after 7 days; (B) Microscopic observation of the isolate SRH22 (G.100)

Table 2. Biochemical and physiological characteristics of the actinobacterial isolate SRH22

Enzyme activity	Result
Production of H2S	-
Reaction RM	-
Reaction VP	-
Nitrate reduction	-
Catalase	+
Starch hydrolysis	+
Gelatin hydrolysis	+
Casein hydrolysis	+
Coagulation of skim milk	+
Peptonization of skim milk	+
Utilisation of nitrogen sources	Result
Aspartic acid	+
The proline	+
Arginine	+
Threonine	+
Histidine	+
Asparagine	+
Tyrosine	+
Methionine	+
Utilisation of carbon sources	Result
Glucose	+
Galactose	+
D-Fructose	+
Lactose	-
Saccharose	-
Mannitol	-
Citrate	-
Growth at NaCl %	Result
2%	++
5%	++
9%	+
15%	-
Growth at different pH	Result
2	++
5	++
7	+++
9	++
12	++
Growth at different temperatures	Result
4	+
28	+++
37	+++
40	++
50	-

3.4 Molecular identification

The phylogenetic tree was created by comparing the actinobacterial isolate's 16S rRNA gene sequence, which contained 948 nucleotide bases, with 18 *Streptomyces* 16S rRNA gene sequences from NCBI databases. The actinobacterial isolate presented 88% to 99% similarity with *Streptomyces* species such as *Streptomyces ambofaciens* strain S8-36 (accession no. MW339011), *Streptomyces humiferus* JCM 3037 (accession no. MT760387), *Streptomyces violaceoruber* strain EA128 (accession no. MW642118), *Streptomyces marrokonensis* strain 2 (accession no. MW695204), and *Streptomyces tricolor* strain CIAD-CA43 (accession no. MK96859). According to the phylogenetic tree analysis, our actinobacterial isolate is closely related (88%) to *S. coelicolor* sp. strain A3 (accession no. OP315308), as shown in Figure 3. Based on the morphological, molecular, and phylogenetic analyses, the action-bacterial isolate was identified as *Streptomyces* sp. strain SRH22 under Accession No OQ302556.

3.5 Growth kinetics of SRH22 isolate in liquid medium

The actinobacterial isolate SRH22's growth on liquid MSM medium, supplemented with 50 mg/L glyphosate as a carbon source, was determined by monitoring the amount of cell dry mass in mg/mL (Figure 4). The isolate showed good growth, without any lag phase, for the first 24 h, and the logarithmic phase persisted until the 6th day when the isolate reached its growth optimum of 0.382 mg/mL. The growth rate remained unchanged thereafter. In comparison, the actinobacterial growth exhibited significant variation (p-value=0.0022). The biotic control, which represents the culture without pesticide, was also included in the study.

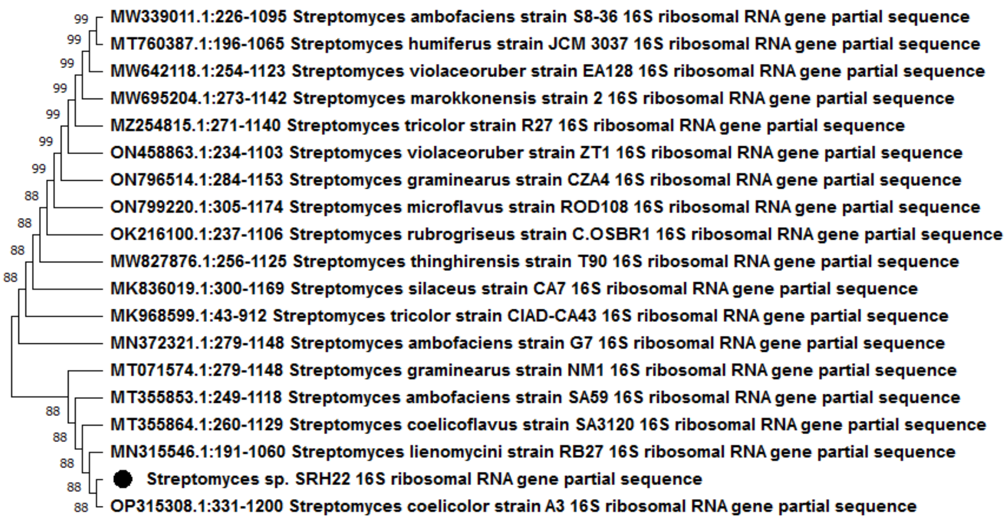

Figure 3. Phylogenetic tree analysis of *Streptomyces* sp. strain SRH22

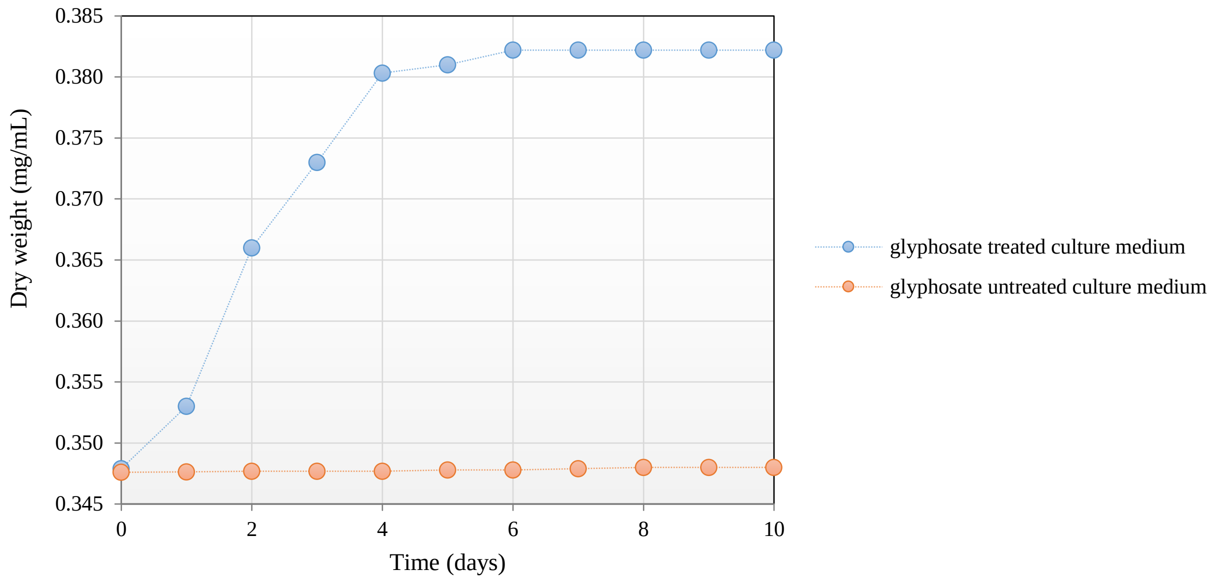

Figure 4. Growth kinetics of strain SRH22 with glyphosate at the concentration 50 mg/L

3.6 Kinetics of glyphosate biodegradation

The degradation of glyphosate, by the isolate SRH22, was demonstrated by the disappearance of the purple color that indicates the C-N bond between glyphosate and ninhydrin, while the control tube retained the purple color. Within the first 24 h, the isolate SRH22 exhibited an estimated percentage of glyphosate degradation of 74%. Over the next 6 days, degradation rates increased with time and reached a maximum degradation capacity of 90.2%, which showed significant differences (p-value<0.0001). After 6 days of incubation, the percentage of degradation became stable for the remaining days (Figure 5).

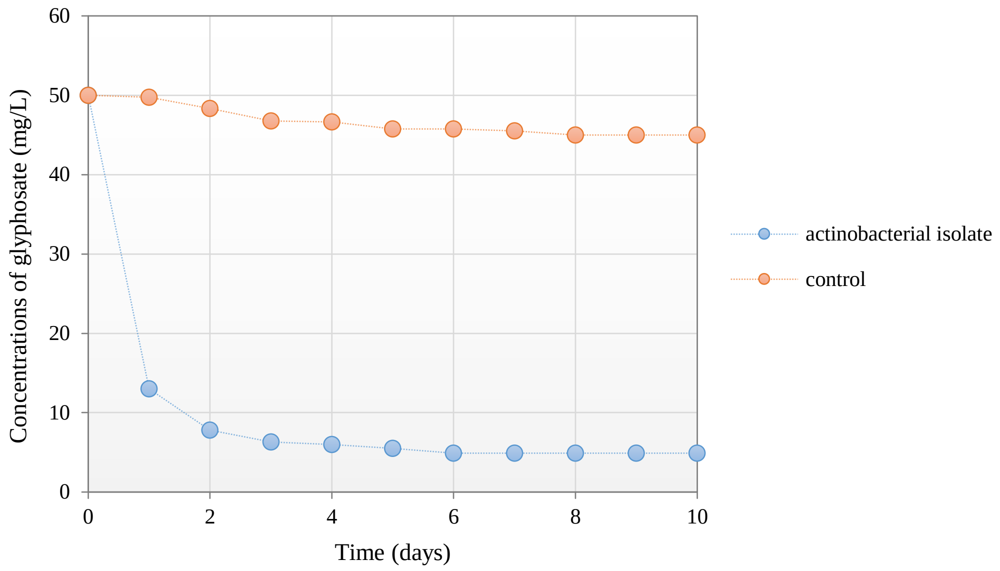

Figure 5. Determination of the quantity of the degraded glyphosate by *Streptomyces* sp. strain SRH22

3.7 Effect of culture conditions on glyphosate biodegradation

3.7.1 Effect of pH on glyphosate biodegradation

Figure 6 showed the effect of pH on glyphosate biodegradation. The highest percentage of degradation was observed at pH 7.2, after 6 days of culture, which amounted to 90.2%. The lowest biodegradation rate was marked at acidic pH 2 and 5 with the percentage of 14.59% and 18.36% respectively. At Basic pH, 9 and 12, the strain SRH22 removed 32.88% and 30.35% respectively. However, at neutral pH 6.5 and 7.5, good degradation was observed reaching 58.24% and 69.97% respectively. The independent variance pH had a significant impact on the glyphosate degradation percentage, with an increase from pH 2 to 7.2 causing the degradation rate to increase from 14.59% to 90.2%. However, there was no significant difference in glyphosate biodegradation from the 5th day when the pH was $\leq$7±0.2 and from the third day when the pH was $\geq$7±0.2 (p-value>0.05).

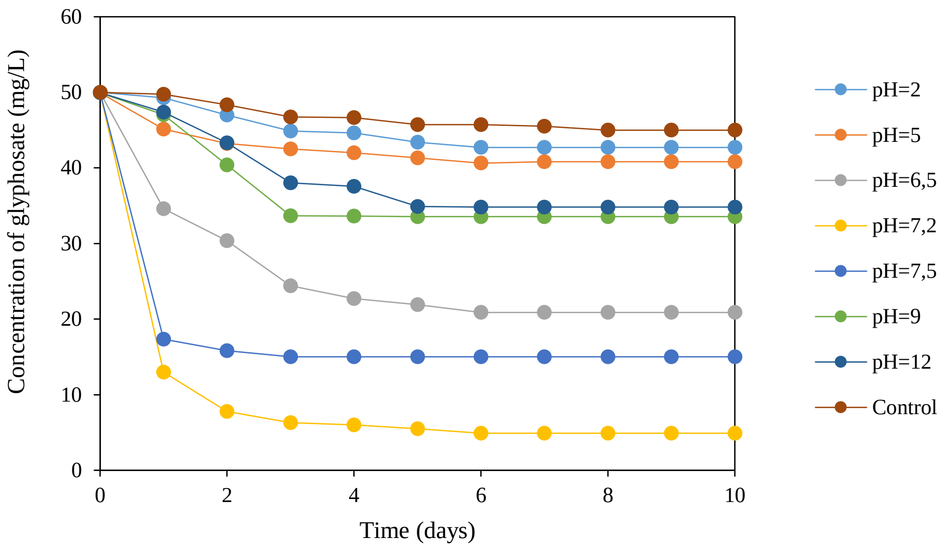

Figure 6. Effect of pH on glyphosate biodegradation by *Streptomyces* sp. strain SRH22

3.7.2 Effect of temperature on the bio-degradation of glyphosate

The results of glyophosate degradation, under different temperatures, indicate that the most efficient temperature was 30°C with 90.2% of glyphosate elimination during 6 days of incubation (Figure 7). The results showed that the percentage of degradation was proportional to the increase in temperature.

SRH22 bacteria was able to degrade 14.18%, 19.86%, 37.16%, 45.17%, and 68.87% at temperatures of 4°C, 10°C, 20°C, 25°C, and 37°C respectively. However, at temperature 40°C, a decrease in the rate of biodegradation was observed as 43.80%. These findings suggest that temperature is a crucial factor that impacts the degradation rate of glyphosate. The effect of temperature on glyphosate biodegradation was significant at temperatures ranging from 4°C to 30°C, over an interval of 3 to 6 days. Additionally, with an increase in temperature from 37°C to 40°C, the effect was significant over an interval of 1 to 2 days with a p-value of less than 0.001.

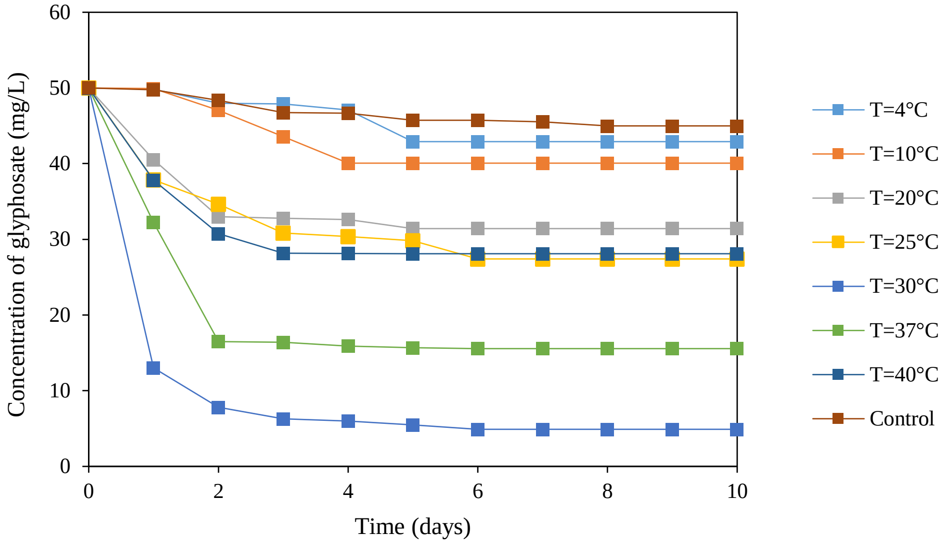

Figure 7. Effect of temperature on glyphosate biodegradation by *Streptomyces* sp. strain SRH22

3.7.3 Effect of inoculum on glyphosate biodegradation

The results of glyphosate degradation, with different inoculum size, indicate that the highest biodegradation rate of 90.2% was observed within 6 days with inoculum volume 4%. During the first six days of incubation and at inoculum volumes of 2%, 5%, 7%, and 9%, the degradation progressively decreased to 17.31%, 89.32%, 38.82%, and 32.72% respectively (Figure 8). The inoculum size has an impact on glyphosate degradation by the SRH22 strain. An inoculum size of 4% resulted in a high percentage of degradation (90.2%), while inoculum sizes smaller or larger than the optimal 4% had a negative effect on degradation. The results suggest that the impact of the inoculum size on glyphosate degradation was significant (p-value<0.001).

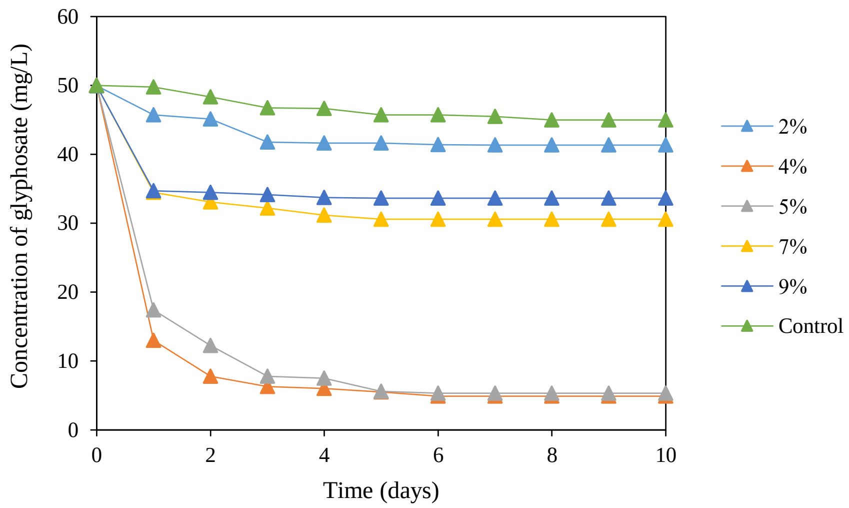

Figure 8. Effect of different inoculum volumes on glyphosate degradation by *Streptomyces* sp. strain SRH22

4. DISCUSSION

The use of herbicides in agriculture in Ouargla, a region in the Algerian Sahara, has led to the accumulation of pollutants in the soil. This poses a significant challenge for soil remediation in these arid land ecosystems (Sviridova et al., 2015; Benslama and Boulahrouf, 2013). The biological approach to soil remediation is considered more ecologically friendly and less expensive than physical-chemical techniques (Zhan et al., 2018). Glyphosate is a widely used herbicide efficiently eliminated by microorganisms (Bhatt et al., 2021a), Over time, various studies have confirmed the ability of different microorganisms to degrade glyphosate (Abosereha et al., 2022; Elarabi et al., 2020; Ermakova et al., 2017). However, studies on the biodegradation of glyphosate by the genus *Streptomyces* are rare (Singh et al., 2019; Lipok et al., 2009; Obojska et al., 1999), although this particular genus of *actinomycetales* being the most dominant in soil, accounting for more than 95% of the identified bacteria (Barka et al., 2016).

Several works have isolated different species of the genus *Streptomyces* from arid soils, which offers a promising prospect for treating these contaminated areas with this bacterial genus (Boudemagh et al., 2005; Korayem et al., 2015; Reghioua et al., 2006; Souagui et al., 2015). In this context, *Streptomyces* sp. SRH22 has been identified as a potential bioremediation agent for glyphosate-contaminated agricultural soils in the Algerian Sahara. The strain SRH22 was found to effectively use glyphosate as the sole source of carbon.

The herbicide glyphosate showed a positive impact on the actinobacterial population in treated soil, with an increase in their numbers compared to untreated soil with glyphosate. This result is consistent with findings from other researchers who have shown that when glyphosate is used in soil it becomes soluble and inactive, losing its antimicrobial effect. This encourages soil microorganisms to use it as an energy and nutrient source (Prankle et al., 1975; Kuklinsky-Sobral et al., 2005). Similarly, Araujo et al. (2003) observed a significant increase in the population of actinobacteria in soil in the presence of glyphosate. However, other studies suggest that glyphosate in soil can stimulate or inhibit soil microorganisms, depending on the pesticide type and soil characteristics (Carlisle and Trevors, 1986; Subhani et al., 2000).

In this study, among eight isolated strains, only one actinobacterium showed the highest tolerance to various tested concentrations of glyphosate. According to the cultural, macroscopic, and microscopic characteristics, and 16S rRNA identification, the isolate was named *Streptomyces* sp. SRH22. The SRH22 were found to use glyphosate at 50 mg/L as the sole source of carbon in MSM liquid medium, which is the case for the majority of microorganisms that use glyphosate as a source of nutrients for their growth (Hernandez Guijarro et al., 2018).

Glyphosate degradation was quantified using the UV-spectrophotometric method, proposed by Bhaskara and Nagaraja. (2006), which is simple, cost effective, and easy to use in developing countries (Nnamonu and Nkpa, 2012). In this reaction, glyphosate reacts with ninhydrin in the presence of molybdate to form a blue-purple complex. The intensity of the color produced is proportional to the amount of glyphosate present in the sample (Xu et al., 2018).

The *Streptomyces* sp. SRH22 showed direct growth during the first 24 h of incubation, without any adaptation period, utilising approximately 74% of glyphosate as a carbon source. This result could be attributed to the adaptation of the actinobacterium to the herbicide, resulting in an efficient degradation metabolism. Similar results have been observed in other studies involving the degradation of glyphosate by the strain *Chryseobacterium* sp. Y16C (Zhang et al., 2022) and the degradation of Lindane, γ-chlordane, and methoxychlor by the genus *Streptomyces* (Fuentes et al., 2017). After 6 days of culture, the *Streptomyces* sp. SRH22 removed 90.2% of glyphosate, and the biodegradation rate remained stable at 90.2% for 10 days. Our results are very satisfactory compared to other studies. For instance, Singh et al. (2019) reported that an actinobacterium assigned to the genus *Streptomyces* sp. used 89.77% of glyphosate after 7 days of culture. Other bacteria, such as *Bacillus subtilis* and *Rhizobium leguminosarum*, used 87.64% and 86.17% of glyphosate, respectively, after 336 h (Singh et al., 2019). Kryuchkova et al. (2013) found that the bacterium *Enterobacter cloacae* K7 was able to utilise 50% of the initial glyphosate concentration 5 mM after 5 days of incubation. The strains *Ochrobactrum* sp. B18, *Pseudomonas citronellolis* ADA-23B, *Ochrobactrum* sp. Ge-14 and *Ochrobactrum* sp. DGG-1-3 isolated by Gongora-Echeverría et al. (2020) degrade 60% of glyphosate at initial concentration 50 mg/L after 15 days of incubation. However, Zhang et al. (2022) showed a 100% degradation of glyphosate by a new isolate *Chryseobacterium* sp. Y16C in 4 days. The difference in the ability of the bacteria to degrade glyphosate is linked to the specific characteristics of the strain (Ermakova et al., 2017).

The biodegradation of glyphosate at different pH, temperatures and inoculum volume is important to show the most favorable conditions for effective degradation of glyphosate. The strain *Streptomyces* sp. SRH22 presented an effective degradation rate, at a neutral pH 7.2, however, lowest rate of biodegradation was founded at acidic pH. This result is similar with studies of Zhan et al. (2022) who reported that the strain *Chryseobacterium* sp. Y16C degrade 100% of initial glyphosate concentration at pH range of 7-9 and, however acidic pH 5 and 6, the degradation was 73.63% and 81.08% respectivelly. Manogaran et al. (2017) found that the optimium pH for glyphosate degradation by the isolate *Burkholderia vietnamiensis* strain AQ5-12 was 6 and 7 and low biodegradation rate was observed at acidic pH of 4, 4.5. These results were expected, as most studies on glyphosate biodegradation have shown that the majority of bacteria prefer neutral or alkaline pH to eliminate glyphosate (Singh and Walker, 2006).

The Biodegradation of glyphosate, by the strain SRH22, was higher at temperature 30°C, same temperature was found by many studies such as Zhan et al. (2022), Hadi et al. (2013) by the strain *Ochrobactrum* sp. GDOS. The pH and temperature are critical factors that affect the biodegradation process of pesticides, affecting the structure of enzymes and causing their denaturation, which affects the ability of the strain to degrade the pesticide.

The highest degradation percentage was observed at an inoculum concentration of 4%, and it decreased as the inoculum percentage was either increased or decreased, which is consistent with the findings of Zhang et al. (2014). However, Nourouzi et al. (2012) found that the values of the degradation rates of glyphosate by bacteria increased with an increasing of the initial inoculum size. These findings highlight the potential of *Streptomyces* sp. SRH22 as an environmentally friendly and cost-effective alternative to traditional physical-chemical methods of soil remediation in the Algerian Sahara. Further research is needed to investigate the long-term efficacy of this bioremediation strategy and the potential development of herbicide resistance within the actinobacterial population.

5. CONCLUSION

Streptomyces sp. strain SRH22 was isolated from agricultural soil in Ouargla and demonstrated a good tolerance and ability to grow in the presence of high concentrations of glyphosate (50 mg/L) as the only source of carbon. Identification was performed using both phenotypic and molecular methods. The study determined that the most favorable conditions for glyphosate degradation were at pH 7.2, a temperature of 30°C, during 6 days, and a volume of inoculum of 4%, resulting in a 90.2% glyphosate biodegradation percentage. Additionally, the research found that glyphosate had a positive impact on the number of soil actinobacteria. These findings suggest that the *Streptomyces* SRH22 strain holds promising potential as a bioremediation agent in Saharan agricultural soils.

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REFERENCES

Abosereha NA, Salima RG, El-Sayeda AF, Hammadb MA, Elsayeda GM. In-vitro biodegradation of glyphosate using genetically improved bacterial isolates from highly polluted wastewater. Egyptian Journal of Chemistry 2022; 65(132):669-81.

Abraham J, Gajendiran A. Biodegradation of fipronil and its metabolite fipronilsulfone by *Streptomyces rochei* strain AJAG7 and its use in bioremediation of contaminated soil. Pesticide Biochemistry and Physiology 2019;155(1):90-100.

Adelowo FE, Olu-Arotiowa OA, Amuda OS. Biodegradation of glyphosate by fungi species. Journal of Bioscience and Bioengineering 2014;2(1):104-18.

Alvarez A, Saez JS, Costa JSD, Colin VL, Fuentes MS, Cuozzo SA, et al. Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 2016;166:41-62.

Araujo ASF, Monteiro RTR, Abarkeli RB. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 2003;52(5):799-804.

Arias-Estévez M, Lopez-Periago E, Martinez-Carballo E, Simal-Gandara J, Mejuto JC, Garcia-Rio L. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agriculture, Ecosystems and Environment 2008;123(4):247-60.

Bai SH, Ogbourne SM. Glyphosate: Environmental contamination, toxicity and potential risks to human health via food contamination. Environmental Science and Pollution Research 2016;23(19):18988-9001.

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, et al. Taxonomy, physiology, and natural products of actinobacteria. Microbiology and Molecular Biology Reviews 2016;80(1):1-43.

Belhadi A, Mehenni M, Reguieg L, Yakhlef H. Phytosanitary practices of greenhouses farmers in three region of Ziban east and their potential impact on human health and environment. Revue Agricole 2016;1:9-16.

Abdelhak IM. A Geochemical Approach to Predicting the Salinity of Bare Soils Under Arid Conditions [dissertation]. Ouargla, University of Kasdi Merbah; 2020 (in French).

Benslama O, Boulahrouf A. Isolation and characterization of glyphosate-degrading bacteria from different soils of Algeria. African Journal of Microbiology Research 2013;7(49); 5587-95.

Bhaskara BL, Nagaraja P. Direct sensitive spectrophotometric determination of glyphosate by using ninhydrin as a chromogenic reagent in formulations and environmental water samples. Helvetica Chimica Acta 2006;89(11):2686-93.

Bhatt P, Bhandari G, Bhatt K, Maithani D, Mishra S, Gangola S, et al. Plasmid-mediated catabolism for the removal of xenobiotics from the environment. Journal of Hazardous Materials 2021a;420(10):Article No. 126618.

Boudemagh A, Kitouni A, Boughachiche F, Hamdiken H, Oulmi L, Reghioua S, et al. Isolation and molecular identification of actinomycet microflora, of some Saharian soils of south east Algeria (Biskra, EL-Oued and Ouragla) study of antifungal activity of isolated strains. Journal of Medical Mycology 2005;15(1):39-44.

Boufercha O, Moreira IS, Castro PML, Boudemagh A. Actinobacteria isolated from wastewater treatment plants located in the east-north of Algeria able to degrade pesticides. World Journal of Microbiology and Biotechnology 2022;38(6):Article No. 105.

Briceño G, Fuentes MS, Palma G, Jorquera MA, Amoroso MJ, Diez MC. Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil. International Biodeterioration and Biodegradation 2012;73:1-7.

Briceño G, Fuentes MS, Saez JM, Diez M, Benimeli CS. *Streptomyces* genus as biotechnological tool for pesticide degradation in polluted systems. Critical Reviews in Environmental Science and Technology 2018;48:773-805.

Briceño G, Vergara K, Schalchli H, Palma G, Tortella G, Fuentes MS, et al. Organophosphorus pesticide mixture removal from environmental matrices by a soil *Streptomyces* mixed culture. Environmental Science and Pollution Research 2017; 25(22):21296-307.

Cardozo M, de Almeida JSFD, de Cavalcante SFA, Salgado JRS, Gonçalves AS, França TCC, et al. Biodegradation of organophosphorus compounds predicted by enzymatic process using molecular modelling and observed in soil samples through analytical techniques and microbiological analysis: A comparison. Molecules 2019;25(58):2-21.

Carlisle SM, Trevors TJ. Effect of the herbicide glyphosate on respiration and hydrogen consumption in soil. Water, Air, and Soil Pollution 1986;27:391-401.

De Castilhos Ghisi N, Zuanazzi NR, Fabrin TMC, Oliveira EC. Glyphosate and its toxicology: A scientometric review. Science of the Total Environment 2020;733:Article No. 139359.

Elarabi NI, Abdelhadi AA, Ahmed RH, Saleh I, Arif IA, Osman G, et al. *Bacillus aryabhattai* FACU: A promising bacterial strain capable of manipulate the glyphosate herbicide residues. Saudi Journal of Biological. Sciences 2020;27(9):2207-14.

Ermakova IT, Shushkov TV, Sviridov A, Zelenkova NF, Vinokurova NG, Baskunov BP, et al. Organophosphonates utilization by soil strains of *Ochrobactrum anthropic* and *Achromobacter* sp. Archives of Microbiology 2017;199(5): 665-75.

Firdous S, Iqbal S, Anwa S. Optimization and modeling of glyphosate biodegradation by a novel *Comamonas odontotermitis* P2 through response surface methodology. Pedosphere 2020;30(5):618-27.

Fuentes MS, Raimondo EE, Amoroso MJ, Benimeli CS. Removal of a mixture of pesticides by a *Streptomyces consortium*: Influence of different soil systems. Chemosphere 2017; 173:359-67.

Gongora-Echeverría VR, García-Escalante R, Rojas-Herrera R, Giacoman-Vallejos G, Ponce-Caballero C. Pesticide bioremediation in liquid media using a microbial consortium and bacteria-pure strains isolated from a biomixture used in agricultural areas. Ecotoxicology and Environmental Safety 2020;200(5):Article No. 110734.

Hadi F, Mousavi A, Noghabi KA, Tabar HG, Salmanian AH. New bacterial strain of the genus *Ochrobactrum* with glyphosate-degrading activity. Journal of Environmental Health 2013; 48(3):208-13.

Hernandez Guijarro K, Aparicio V, De Geronimo E, Castellote M, Figuerola EL, Costa JL, et al. Soil microbial communities and glyphosate decay in soils with different herbicide application history. Science of the Total Environment 2018;634(9):974-82.

Jiang B, Zhang N, Xing Y, Lian L, Chen Y, Zhang D, et al. Microbial degradation of organophosphorus pesticides: Novel degraders, kinetics, functional genes, and genotoxicity assessment. Environmental Science and Pollution Research 2019;26(21):21668-81.

Korayem AS, Abdelhafez AA, Zaki E, Saleh MM. Optimization of biosurfactant production by *Streptomyces* isolated from Egyptian arid soil using Plackett-Burman design. Annals of Agricultural Science 2015;60(2):209-17.

Kryuchkova YV, Burygin G, Gogoleva NE, Gogolev YV, Chernyshova MP, Makarov OE, et al. Isolation and caracterisation of a glyphosate-degrading rhizosphere strain, *Eterobacter cloacae* K7. Microbiological Research 2013; 169(1):99-105.

Kuklinsky-Sobral J, Araujo WL, Mendes R, Pizzirani-Kleiner AA, Azevedo JL. Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 2005;273(1-2):91-9.

Lee LH, Zainal N, Azman AS, Mutalib NSA, Hong K, Chan KG. *Mumia flava* gen. nov., sp. nov., an actinobacterium of the family *Nocardioidaceae*. International Journal of Systematic and Evolutionary Microbiology 2014;64(Pt-5):1461-7.

Li Q, Chen X, Jiang Y, Jiang C. Cultural, physiological, and biochemical identifcation of actinobacteria. In: Dhanasekaran D, Jiang Y, editors. Actinobacteria: Basics and Bio-technological Applications. InTech; 2016. p. 88-111.

Lipok J, Wieczorek D, Jewginski M, Kafarski P. Prospects of in vivo 31P NMR method in glyphosate degradation studies in whole cell system. Enzyme and Microbial Technology 2009; 44(1):11-6.

Maeda H, Dudareva N. The shikimate pathwayand aromatic amino acid biosynthesis in plants. Annual Review of Plant Biology 2012;63(1):73-105.

Malla MA, Dubey A, Kumar A, Yadav S, Kumari S. Modeling and optimization of chlorpyrifos and glyphosate biodegradation using RSM and ANN: Elucidating their degradation pathways by GC-MS based metabolomics. Ecotoxicology and Environmental Safety 2023;252(4)Article No. 114628.

Manogaran M, Shukor MY, Yasid NA, Johari WLW, Ahmad SA. Isolation and characterisation of glyphosate-degrading bacteria isolated from local soils in Malaysia. Rendiconti Lincei 2017;28(2):471-9.

Minotto E, Milagre LP, Oliveira MT. Enzyme characterization of endophytic actinobacteria isolated from tomato plants. Journal of Advanced Scientific Research 2014;5(2):16-23.

Mishra S, Pang S, Zhang W, Lin Z, Bhatt P, Chen S. Insights into the microbial degradation and biochemical mechanism of carbamates. Chemosphere 2021;279(2):Article No. 130500.

Moreira IS, Lebel A, Peng X, Paula MLC, Goncalves D. Sediments in the mangrove areas contribute to the removal of endocrine disrupting chemicals in coastal sediments of Macau SAR, China, and harbour microbial communities capable of degrading E2, EE2, BPA and BPS. Biodegradation 2021;32(5):511-29.

Nnamonu LA, Nkpa NN. Use of bufers in spectrophotometric determination of N-phosphonomethylglycine by the ninhydrin colour reaction. IOSR Journal of Environmental Science Toxicology and Food Technology 2012;1(1):6-10.

Nourouzi MM, Chuah TG, Choong TSY, Rabiei F. Modeling biodegradation and kinetics of glyphosate by artificial neural network. Journal of Environmental Science and Health, Part B 2012;47(5):455-65.

Obojska A, Lejczak B, Kubrak M. Degradation of phosphonates by *Streptomycete* isolates. Applied Microbiology and Biotechnol 1999;51(6):872-6.

Prankle P, Meggitt W, Penner D. Rapid inactivation of glyphosate in soil. Weed Science 1975;23(3):224-8.

Reghioua S, Boughachiche F, Zerizer H, Oulmi L, Kitouni M, Boudemagh A. Antibacterial activity of rares actinomycetes isolated from arid soil samples of the southeast of Algeria. Antibiotiques 2006;8(3):147-52.

Rossi F, Carles L, Donnadieu F, Batisson I, Artigas J. Glyphosate-degrading behavior of five bacterial strains isolated from stream biofilms. Journal of Hazardous Materials 2021; 420:Article No. 126651.

Sabzevari S, Hofman J. A worldwide review of currently used pesticides monitoring in agricultural soils. Science of the Total Environment 2022;812:Article No. 152344.

Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology 1966;16(3):313-40.

Singh BK, Walker A. Microbial degradation of organophosphorus compounds. FEMS Microbiology Reviews 2006;30(3):428-71.

Singh S, Kumar V, Sidhu GK, Singh J. Kinetic study of the biodegradation of glyphosate by indigenous soil bacterial isolates in presence of humic acid, Fe (III) and Cu (II) ions. Journal of Environmental Chemical Engineering 2019; 7(3):Article No.103098.

Souagui Y, Grosdemange-Billiard C, Tritsch D, Kecha M. Antifungal molecules produced by a new salt-tolerant and alkaliphilic *Streptomyces* sp. BS30 isolated from an arid soil. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 2015;87(2):527-35.

Subhani A, El-ghamry A, Changyong H, Jianming X. Effects of pesticides (herbicides) on soil microbial biomass: A review. Pakistan Journal of Biological Sciences 2000;3(5):705-9.

Supreeth M, Chandrashekar MA, Sachin N, Raju NS. Effect of chlorpyrifos on soil microbial diversity and its biotransformation by *Streptomyces* sp. HP-11.3. Biotech 2016;6(2):Article No.147.

Sviridova AV, Shushkova TV, Ermakova IT, Ivanova EV, Epiktetova DO, Leontievskya AA. Microbial degradation of glyphosate herbicides (review). Applied Biochemistry and Microbiology 2015;51(2):188-95.

Tatsinkou Fossi B, Tavea F, Ndjouenkeu R. Production and partial characterization of thermostable alpha amylase from ascomycete yeast strain isolated from starchy soil. African Journal of Biotechnology 2005;4(1):14-8.

Uqab B, Mudasi S, Nazir R. Review on bioremediation of pesticides. Journal of Bioremediation and Biodegradation 2016;7(3):Article No.1000343.

Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ. Numerical classification of *Streptomyces* and related genera. Journal of General Microbiology 1983;129(6):1743-813.

Xu ML, Gao Y, Li Y, Li X, Zhang H, Han XX, et al. Indirect glyphosate detection based on ninhydrine reaction and surface enhanced Raman scattering spectroscopy. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 2018;197:78-82.

Yu J, Jin B, Ji, Q, Wang H. Detoxification and metabolism of glyphosate by a *Pseudomonas* sp. via biogenic manganese oxidation. Journal of Hazardous Materials 2023;48(5):Article No. 130902.

Zhan H, Feng Y, Fan X, Chen S. Recent advances in glyphosate biodegradation. Applied Microbiology and Biotechnology 2018;102(12):5033-43.

Zhang H, Zhang Y, Hou Z, Wu X, Gao H, Sun F, et al. Biodegradation of triazine herbicide metribuzin by the strain *Bacillus* sp. N1. Journal of Environmental Science and Health, Part B 2014;49(2):79-86.

Zhang W, Li J, Zhang Y, Wu X, Zhou Z, Huang Y, et al. Characterization of a novel glyphosate-degrading bacterial species, *Chryseobacterium* sp. Y16C, and evaluation of its effects on microbial communities in glyphosate-contaminated soil. Journal of Hazardous Materials 2022;432:Article No. 128689.

Investigation of Subsurface and Geological Structures Contributing to Collapse Sinkholes in Covered Karst Terrain, Northeast Thailand

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ABSTRACT

This study focuses on covered karst terrain situated in Phu Pha Man District, Khon Kaen Province, Northeast Thailand, where records of collapse sinkholes are limited. Here, we investigate the subsurface characteristics and potential causes of sinkhole formation within this area using geophysical methods, hydrogeological techniques, and precipitation analysis. We collected field data by measuring groundwater levels, and conducting electrical resistivity tomography (ERT) surveys. We identified eight cover-collapse sinkholes of various shapes and sizes. Analysis of the groundwater flow indicated that the predominant flow direction runs from north to southeast. Examination of rainfall data showed a progressive increase in total rainfall on a yearly basis, with a significant precipitation event preceding the initial occurrence of sinkholes. The ERT results revealed the presence of highly resistive bedrock, water-saturated layers, and potential cavities. Notably, the tomograms indicated variations in resistivity values, suggesting the presence of irregular surfaces of limestone bedrock and weathered zones as characteristics of karst settings. Intense precipitation is a possible dominant trigger for the formation of the sinkholes. This study contributes to understanding sinkhole formation in karst environments and provides key information for hazard mitigation, not only in the Phu Pha Man District but also in areas with similar geological settings.

1. INTRODUCTION

Sinkholes are closed depressions with internal drainage observed on the surface, and formed by the presence of underground cavities or voids (Gutiérrez et al., 2014; Kaufmann et al., 2018). These voids gradually develop through physical-chemical weathering of underlying fractured bedrocks (Williams, 2008; Billi et al., 2016). Sinkholes are common in karst landscapes, characterized by the predominance of soluble rocks that can naturally dissolve through the circulation of groundwater within subsurface fractures (Heidari et al., 2011; USGS, 2018). When water infiltrates the soil, it combines with carbon dioxide released from organic matter, resulting in increased groundwater acidity. This process leads to the dissolution and erosion of soluble rocks in the shallow subsurface (Waltham et al., 2005; Kaufmann et al., 2011).

The formation sinkholes can be influenced by geological processes, climatic processes, and/or human activities (Kidanu et al., 2016; Youssef et al., 2016). Several factors can induce or accelerate sinkhole formation, such as intense rainfall events (Van Den Eeckhaut et al., 2007; Tufano et al., 2022), hydrological alterations (Doğan and Yilmaz, 2011; Pando et al., 2013), leakage from underground aqueducts (Richardson, 2013; di Santolo et al., 2018), and processes related to mining operations (Fidelibus et al., 2011; Ammirati et al., 2020).

Numerous studies have been conducted worldwide to investigate subsurface karst, deformation structures, and sinkhole development using various methods (e.g., Margiotta et al., 2012; Theron and Engelbrecht, 2018). Integrated geophysical surveys in karst environments have employed electrical, seismic, gravimetric, and electromagnetic methods to detect subsurface voids (Kruse et al., 2006; Kaufmann, 2014; Cueto et al., 2018). The analysis of groundwater flow paths has been shown to contribute to the creation of conceptual models for hydrogeological systems within

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karst areas (Nam et al., 2020; Al-Halbouni et al., 2021). Long-term geophysical monitoring and precipitation records have been utilized to gain insights into the interaction between subsurface conditions and dynamics of infiltration within karst systems (Watlet et al., 2018).

Sinkhole collapses in Thailand which occur in areas characterized by karst landforms such as limestone, gypsum, or salt, pose significant threats to human lives and property (DMR, 2011). Extensive research has been conducted to investigate the mechanisms underlying sinkhole development in karst regions of Thailand, employing various approaches including geomorphology and geophysics (Furukawa and Pichai, 1989; Giao et al., 2011; Yordkayhun, 2021). However, there remains a paucity of studies specifically focused on the causes of shallow collapses, particularly within rock salt strata (Satarugsa, 2011) and carbonate rocks (Arjwech et al., 2021) in northeast Thailand.

This study aims to examine the potential factors contributing to the occurrence of sinkhole hazards in a buried karst area where such events have not been previously documented. On April 19th, 2021, unexpected events unfolded when the Phu Pha Man district experienced the sudden emergence of two massive collapse sinkholes. Subsequently, six additional sinkholes were discovered, as indicated in the report by DMR (2021). Our aim is to gain a comprehensive understanding of the underlying causes and mechanisms associated with sinkhole formation in the area. To achieve our research objective, we employ the geophysical method of Electrical Resistivity Tomography (ERT) to investigate the subsurface characteristics of the sinkhole-affected region. In addition, we utilize supportive data, including measurements of groundwater levels and historical precipitation records. By combining these approaches, our findings could provide valuable insights for identifying sinkhole-prone areas, which contribute to enhancing hazard mitigation planning.

2. METHODOLOGY

2.1 Geological setting and study site

The Phu Pha Man District is located on the westernmost part of Khon Kaen Province and is geologically situated on the western edge of the Khorat Plateau (Figure 1). The district's geological setting comprises mostly sedimentary rocks deposited during the upper Paleozoic to Mesozoic periods (DMR, 2007; Booth and Sattayarak, 2011). Additionally, there are sparse occurrences of extrusive igneous rocks from the Triassic to Permian age (PTrv). The Permian rocks primarily consist of shallow-marine deposits and epeiric carbonate platforms, forming karst landforms. These rocks are represented by the Ratburi (P1) and Saraburi (Ps) groups, found in the northeastern and middle parts of the district, respectively. Lithological logs indicate that the Ratburi group consists of thin- to medium-bedded limestone intercalated with thin-bedded grey shale (Chonglakmani and Sattayarak, 1984; Chaodumrong et al., 2007). The Saraburi group is characterized by fossiliferous limestone, shale interbedded with limestone, shale, sandstone, siltstone, and tuffaceous sandstone (Ueno and Charoentitirat, 2011). The Khorat Plateau Basin was formed as a result of the collision between the Sibumasu (Shan-Thai) and Indochina continental blocks during the Permo-Triassic period (Minezaki et al., 2019). This collision led to the uplift and profound erosion of Permian carbonate platforms during the Indosinian I Event (Booth and Sattayarak, 2011). Subsequently, the Khorat Group was deposited from the Triassic to Cretaceous periods and primarily consists of terrigenous sediments. In the Phu Pha Man District, the Huai Hin Lat (TRhl), Nam Pong (Trnp), and Phu Kradung (Jpk) formations are present. The depositional environments of the extensive limestone formations overlying carbonate units in this area are similar to those in Saraburi Province, which has experienced a cluster of sinkhole collapses (DMR, 2005; Ponta et al., 2013).

The study site is situated on the eastern border of the Phu Pha Man District, ~110 km northeast of Khon Kaen City. The site encompasses an area of ~2 km^2, spanning across the Phu Pha Man and Huai Muang Sub-Districts (in Figures 1 and 2, highlighted with a yellow-black rectangle). Geological formations in the area include massive limestone boulders located in the western section and clast-supported conglomerate outcrops found in the eastern part of the site. The surficial layer is predominantly covered by unconsolidated soils consisting of Quaternary fluvial deposits, underlain by rocks from the Khorat group. The soils in the area are classified by the Land Development Department (2005) as a mixture of sandy clay loam and silty clay. The land in this area is extensively utilized for agricultural purposes, primarily for sugarcane and rice cultivation. A limestone quarry is also located nearby, within 2 km from the study site.

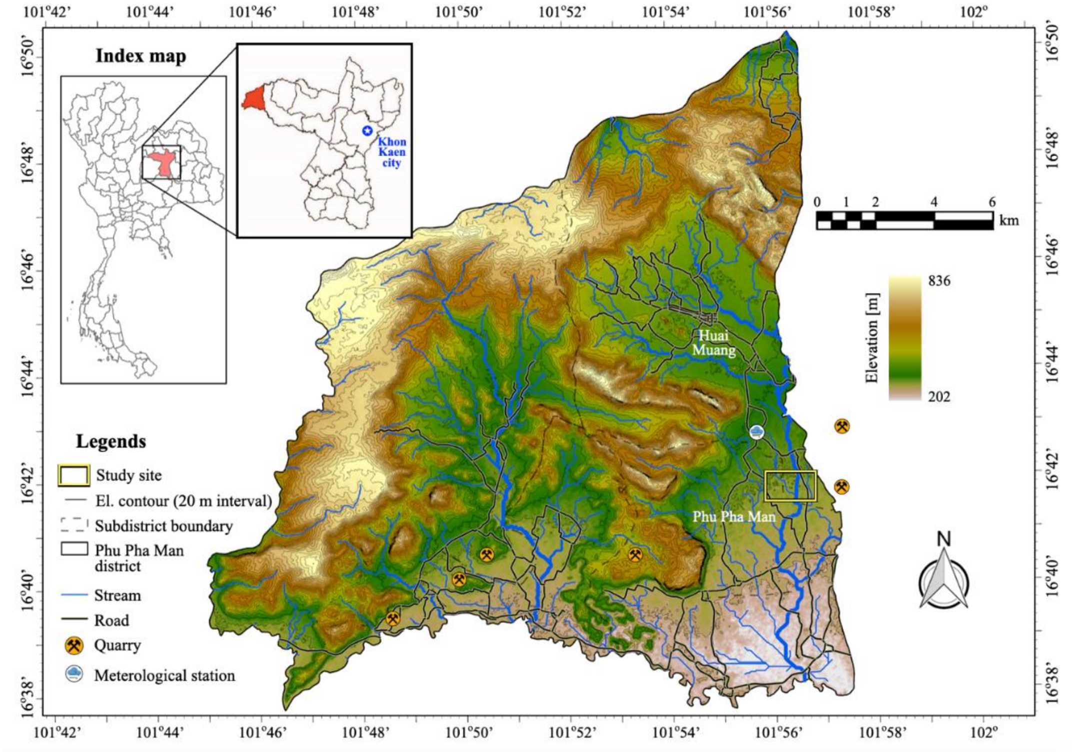

Figure 1. Topographic map of Phu Pha Man District. An index map shows the location of Khon Kaen Province in Northeastern Thailand. The study site is marked by a yellow-black rectangle.

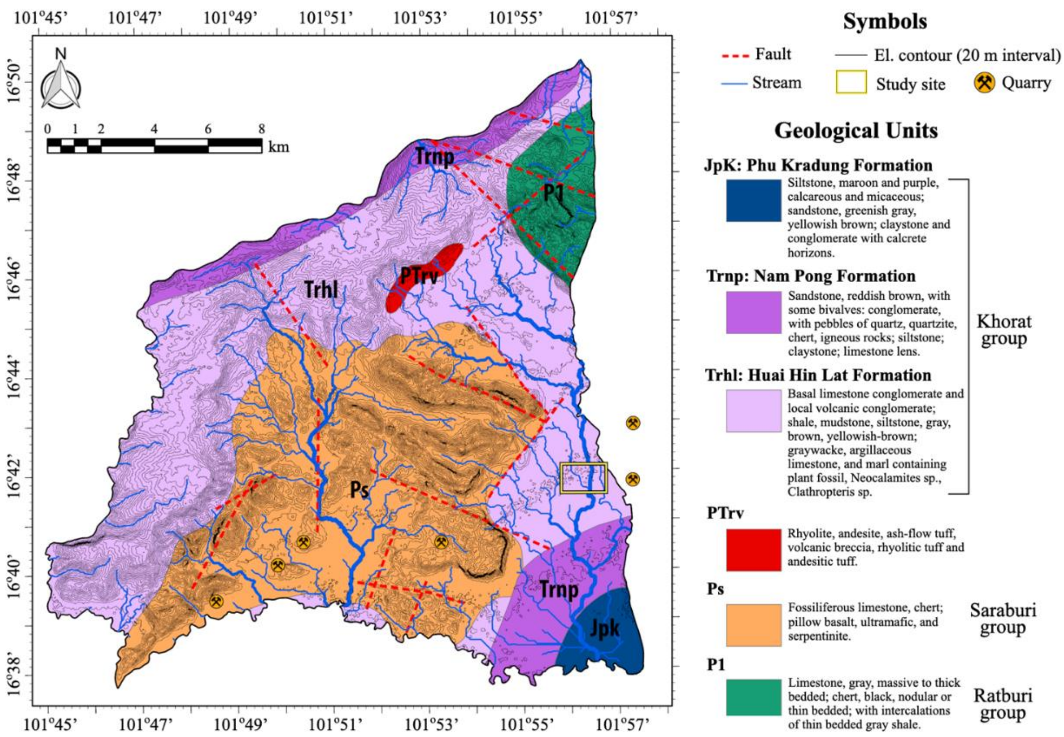

Figure 2. Geological map of Phu Pha Man District and locations of quarries (Modified from DMR (2007))

2.2 Field data acquisition

We began visiting in the middle of 2021 to verify and map subsidence features identified through preliminary aerial imagery study. Further field inspections then conducted to gather more detailed information. Based on the findings from the initial site visits, preferred locations for ERT surveys were determined. Additionally, another round of site visits took place from late 2021 to early 2022 to perform groundwater level measurements and carry out geophysical fieldwork.

Transient groundwater level measurements were conducted within the study site during the crop harvesting period (December 2021 to January 2022). A total of 11 local wells, primarily drilled for agricultural purposes, were identified and distributed across the study area. At each well location, the depth to the local groundwater table was measured using a flat tape water level indicator, and the precise coordinates were recorded using RTK GNSS positioning systems. Subsequently, the collected hydraulic head data from the field measurements were utilized to generate a groundwater flow contour map.

In addition to water table measurements, rainfall data from the years 2019 to 2021 were utilized as supplementary information for this study (Upper Northeastern Meteorological Center, 2022). The data was obtained from a meteorological station located in the Huai Muang Sub-District, ~2 km northwest of the study site (Figure 1).

ERT is a geophysical method that provides an image of the electrical resistivity structure in a vertical plane beneath a linear array of metal electrodes inserted in the ground and connected together by a multi-core cable (Everett, 2013). By measuring the voltage developed across pairs of electrodes during current injections and withdrawals, information about the electrical resistivity spatial distribution can be obtained. In this study, ERT data was collected using the IRIS SYSCAL Pro instrument from January to February 2022. A total of nine 2D ERT profiles, designated as PPM_1 to PPM_9, were conducted in the vicinity of the observed surficial collapses, covering a combined array length of 1,755 m (Figure 3). The ERT survey utilized a dipole-dipole measurement protocol because it is the most effective array for karst mapping, as discussed in Zhou et al. (2002). A typical electrode spacing of 5 m was used, except for PPM_6 to PPM_9, which had an electrode spacing of 2.5 m. The positions and elevations of the electrodes were accurately acquired using RTK GNSS positioning systems. Most ERT profiles were oriented approximately in the N-S direction, perpendicular to the trend of the observed sinkholes identified during the preliminary field investigation. There is only one profile (PPM_3) that extended from W to E. The selection of each ERT survey location attempted to capture a broad subsurface image, aiding in the identification of sinkhole-related features. The acquired ERT data were processed using topographic reconstruction methods implemented in Res2DInv software. The finite-element algorithm was selected to discretize and optimize a geoelectrical model. The L-1 norm inversion method was used to minimize the sum of absolute derivation between the measured and the calculated apparent resistivities for each iteration.

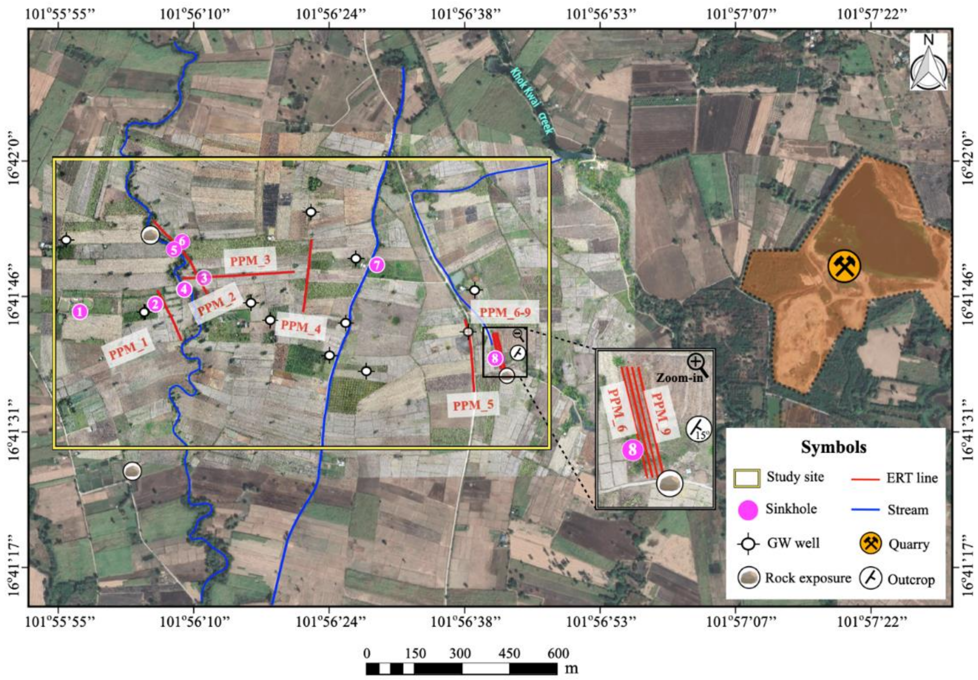

Figure 3. An overlay aerial photograph on satellite imaginary showing details of the study site

3. RESULTS AND DISCUSSION

3.1 Sinkhole formation

During site visits in 2021, field investigations and physical measurements were conducted within an area of ~2 km^2. As a result, eight cover-collapse sinkholes were identified (Figure 4(a-d)). Among these sinkholes, three exhibited partial filling with water at varying levels. The surficial sinkholes displayed a range of shapes, transitioning from nearly circular to irregular as their size increased. Detailed dimensions of all sinkholes are provided in Table 1. The sinkholes can be categorized into two groups: (1) three collapses located further inland, distanced from surface water drainages in the western parts of the site, referred to as sinkholes #1, #2, and #3; (2) five relatively larger sinkholes situated near streams or irrigation waterways, designated as sinkholes #4 to #8. In the study site, the frequency of sinkhole formation is increasing, as several additional collapses were reported by local residents after the harvesting period in early 2022. However, this study does further discuss these recent sinkholes.

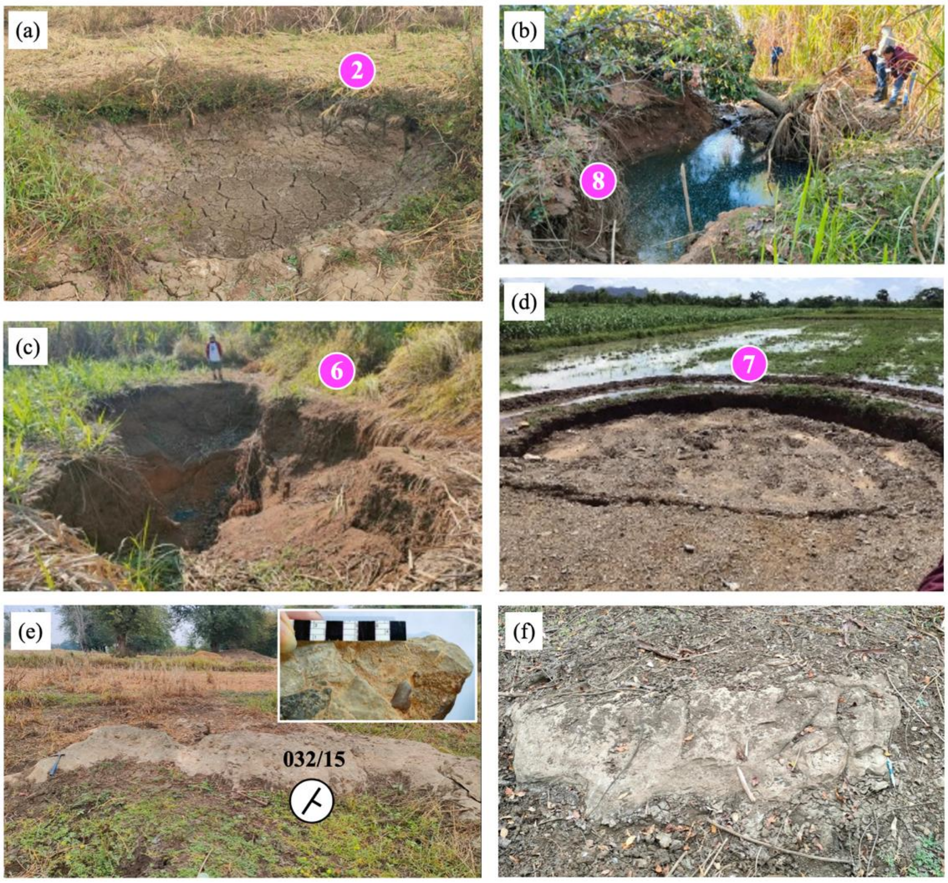

Figure 4. (a-d) Examples of existing sinkholes; (e) Conglomerate outcrop found in the east; (f) Massive limestone boulder exposed in the west

Table 1. Dimensions of collapse sinkholes

Group	Sinkhole No. #	Width (m)	Length (m)	Depth (m)	Estimated volume (m^3)
1	1	8.6	9.4	Filled* (3.2)	203.17
	2	0.7	0.7	0.4	0.15
	3	4.2	5.1	Filled* (1.2)	20.19
2	4	4.0	5.5	Flooded	N/A
	5	8.7	9.3	Partially flooded (>4)	>254.17
	6	3.1	10.0	5.2	126.61
	7	7.0	7.8	Filled	N/A
	8	5.0	6.0	Flooded	N/A

*The depth acquired from geological hazard report by DMR (2021).

3.2 Groundwater flow and rainfall

Figure 5 illustrates the distribution of groundwater wells within the study site, with a noticeable sparsity observed in the western region compared to the middle and eastern areas. The hydraulic head data indicates that higher groundwater levels are predominantly located in the north and northwest parts of the site. Consequently, groundwater generally flows from the N to SE direction in the western to middle sections, gradually transitioning to W to E flows in the eastern part. Additionally, the groundwater elevation map reveals larger gradients in the eastern part of the site.

Figure 6 presents the precipitation records obtained from the meteorological station near the study site during 2019-2021. The pattern of monthly accumulated rainfall does not appear to exhibit a clear systematic trend over the three-year period. However, the total amount of rainfall clearly increased over the study period. In particular, an exceptional heavy rainfall event or monsoon occurred in April 2021, preceding the regular rainy season (typically in mid-May). During this event, the precipitation exceeded 200 mm, a significant increase from the ~5 mm recorded in March of the same year. These sudden and intense rainfall events coincided with the initial occurrence of collapse sinkholes in the area. Additionally, a prolonged dry period with limited rainfall from November 2020 to March 2021, combined with groundwater pumping for agricultural activities, contributed to the decline of the groundwater table in the area.

3.3 ERT results

2D tomographic images were arranged into the fence diagram in order to visualize the continuity of the subsurface structures in the study area (see Figures 7 and 8). Inverted ERT sections (tomograms), generated after six iterations of the reconstruction algorithm, exhibited misfits of less than 10%, indicating a good fit to the measured data. Three principal zones can be broadly identified in the tomograms. The surficial layer of the tomograms exhibited low resistivity values (1-120 Ωm), represented by dark blue to dark green colors, which are interpreted as overburden fluvial sediments. The moderate resistivity zone (120-1,200 Ωm), appearing at a depth of ~5-10 m in all sections, likely indicates water-saturated layers and/or weathered rocks. The zone of high resistivity (>1,200 Ωm), depicted by orange to dark purple colors, corresponds to the sedimentary bedrock.

ERT profile PPM_1 was deployed near the collapse sinkhole #2 (top left panel in Figure 7). The tomogram for this profile does not show any indication of highly resistive bedrock within the investigated depth of 35 m. However, a high resistive anomaly was observed at the location of 85-100 m, marked with a '?' symbol, which is likely caused by an air-filled cavity. ERT profile 2 exhibits a relatively wide range of bulk resistivity values down to a depth of 60 m and

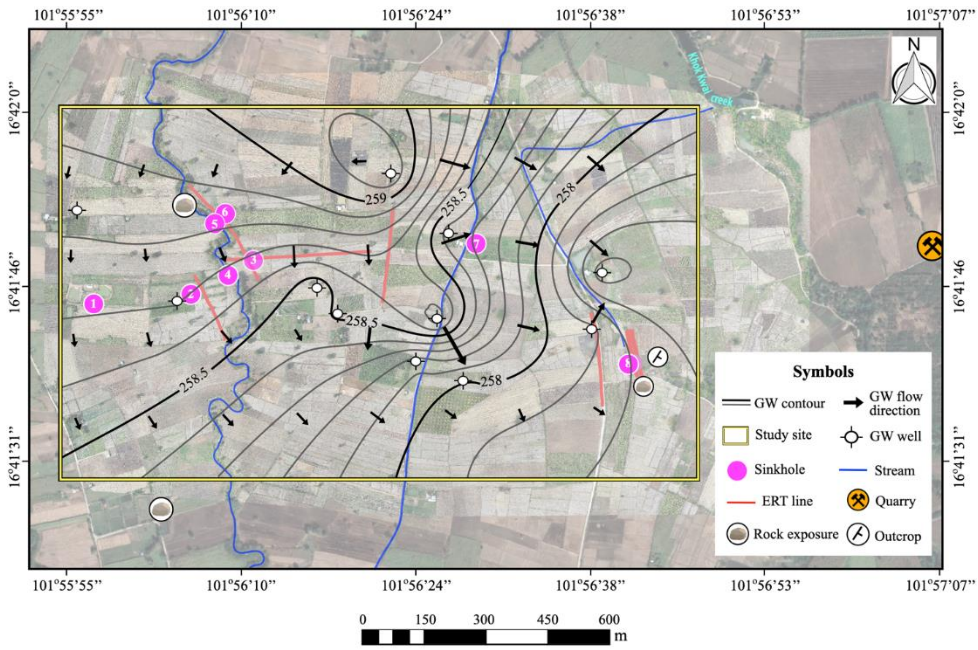

Figure 5. Groundwater (GW) elevation map of study site. Note that the length of arrow is an indicative of flow direction and proportional to hydraulic gradient at each location.

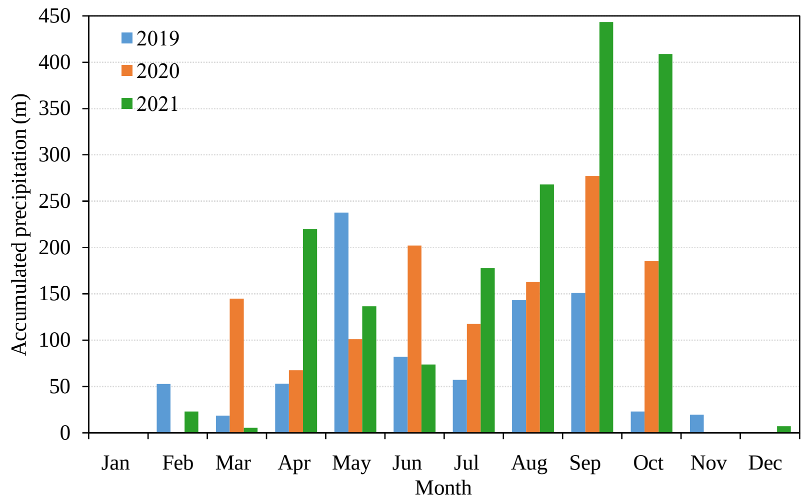

Figure 6. Monthly accumulated rainfall during 2019-2021 at the meteorological station located near the study site

shows a high spatial distribution of Earth resistivity. In the southern half of the section, there are notably high resistivity values, starting at around 10 m depth, which can be interpreted as limestone bedrock based on the presence of exposed rocks nearby. A water-saturated zone is identified in the middle of the transect, extending from depths of 10-30 m, coinciding with the partially flooded area near sinkhole #5. Additionally, a moderate resistive anomaly was observed at a location of 200 m (marked as '??' symbol), which is interpreted as a cavity filled with water-saturated soils. ERT profile PPM_3 intersects profile PPM_2 at location ~55 m on PPM_3 and ~60 m on PPM_2. This section is located adjacent to sinkhole collapse #3 at a location of 60 m and does not exhibit any indication of electrical subsurface anomalies. The top layer in the eastern section of profile PPM_3, spanning from the surface to a depth of ~5 m, corresponds to variably saturated topsoil of a paddy rice field. Along the transect, between locations of 80-260 m, a highly weathered zone is observed with a moderate range of resistivity values, extending from 15-65 m in depths. The highly resistive bedrock in the eastern half of the section, below 20 m, indicates the continuity of an underlying unit that dips eastward. ERT profile PPM_4, a distinct irregular shape was observed at depths around 10-30 m of the substratum surface, which is indicative of karst settings. The presence of uneven surfaces can be attributed to spatial variations in lithology and differential rates of weathering within the limestone bedrock.

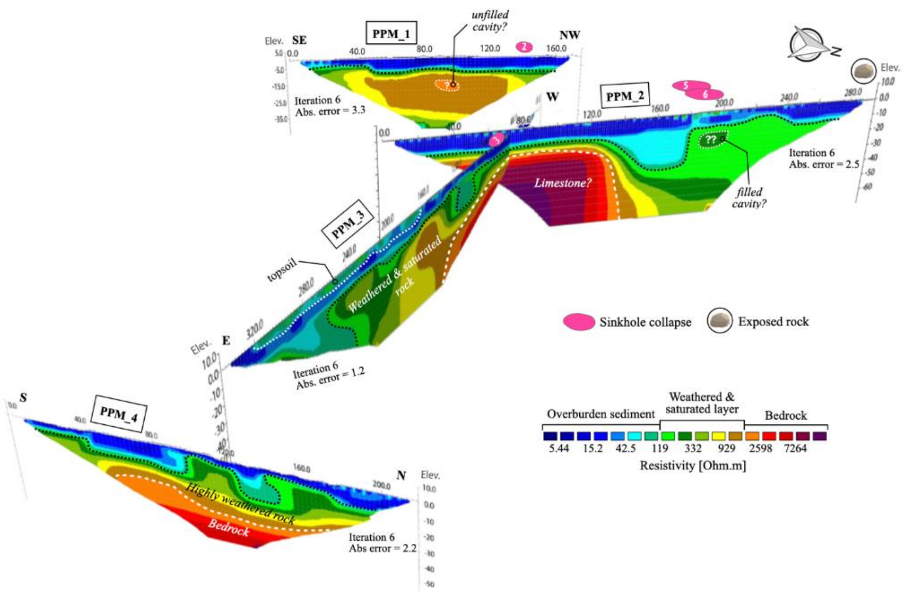

Figure 7. Geoelectrical resistivity fence diagram of 2D inversion tomograms shows subsurface features and inferred cavities in the western part of the study site.

Figure 8 presents the fence diagram illustrating the ERT inversion images of PPM_5, PPM_6, PPM_7, PPM_8, and PPM_9, which cover the eastern part of the study site. Note that these profiles extend in SE to NW direction. The interpreted bedrock in profile PPM_5 appears to be relatively shallower but more resistive when compared with the profiles in the western part of the study area (PPM_2, PPM_3, and PPM_4). In this section, the top layer shows a slightly increased thickness and higher conductivity in the NW part. Similarly, the middle layer, within the moderate resistivity range, appears to be thicker, indicating increased weathering of rocks in the NNW half of the transect. Furthermore, a zone of low resistivity, indicating a highly weathered layer, is observed in the SE part of the transect, specifically at locations 40-60 m, extending from the surface to depths of 20 m. This low resistivity zone is likely caused by loam soils with a relatively high water content. Four geoelectrical tomograms, namely PPM_6 to PPM_9, were conducted with a length of 115 m to reach investigation depth of ~25 m. The electrode separation for this measurement protocol was 2.5 m, and the survey lines were parallel to each other with a spacing of 5 m. Profile PPM_6 was positioned ~100 meters east of profile PPM_5. This transect was located within a close proximity of less than 5 m east of the flooded open collapse #8, which can be seen at the location ~35 m along the transect (second panel from the top in Figure 8). Between the locations of 40-50 m, an irrigation drainage was identified, which serves to convey surface water to the sinkhole (water ingress). The tomograms PPM_7, PPM_8 and PPM_9 exhibit characteristics similar as the one observed in PPM_6. In these transects, a zone characterized by low resistivity values, indicated by the dashed gray lines, is more pronounced in the southwestern half. Furthermore, this zone appears to become more continuous and slightly more resistive across adjacent tomograms, suggesting a potential groundwater flow path in the west to east direction. Notably, the water from the drainage directly feeds sinkhole #8 and cannot be traced from the surface. In contrast, the resistivity of the uppermost layer in the northwestern half decreases as the ERT surveys move eastward, indicating an increase in water content within the overburden soils. Additionally, the underlying bedrock in this half of the transects (PPM_6 to PPM_9) appears to be less weathered in the eastward direction. Scattered boulders were observed on the surface in the southwestern parts of profiles PPM_7 to PPM_9. These rock exposures were characterized as conglomerate, predominantly composed of limestone clasts. Additionally, a conglomerate outcrop located ~60 m east of profile PPM_5 was measured (see Figure 4(e)). The outcrop exhibited a NE-SW orientation with a dip of 15° in the SE direction (032/15).

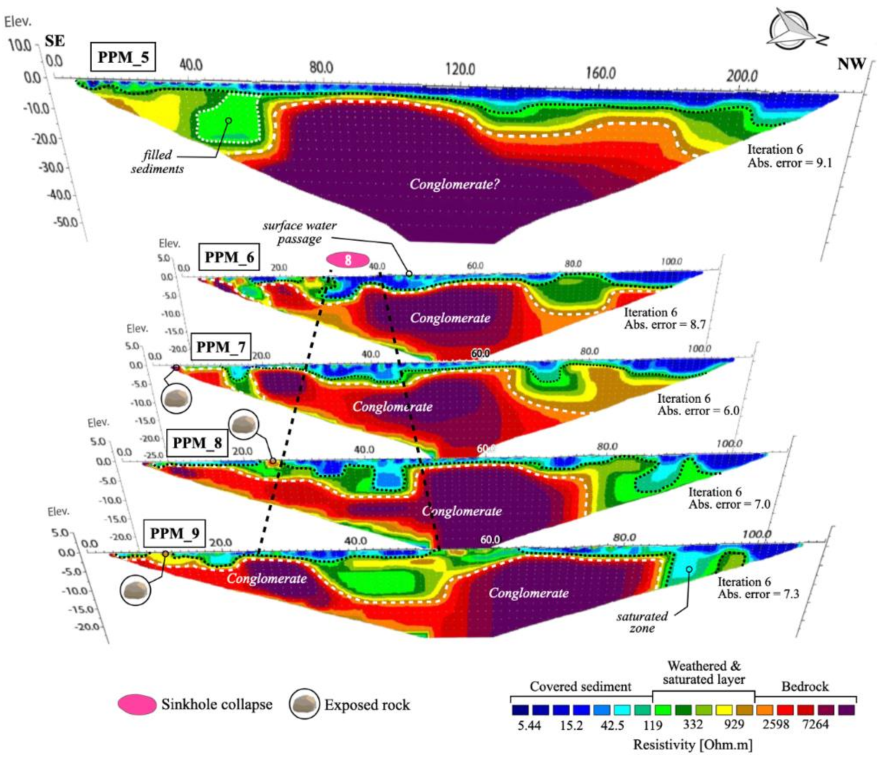

Figure 8. Fence diagram of 2D ERT inversion images shows subsurface features in the eastern part of the study site.

4. DISCUSSION

The findings of this study suggest that the presence of continuous large-scale cavities related to fractures in the bedrocks of the study area is unclear based on geophysical results. However, based on the prevailing lineation trend of existing surface collapses, it is speculated that fracture zones may extend in a west-east direction. Additionally, the results indicate that the triggering factors contributing to sinkhole collapses differ from others found within northeast Thailand. Arjwech et al. (2021) mentioned that anthropogenic factors such as quarry dewatering and blasting mainly influence the occurrence of sinkholes in Nong Bua Lamphu Province.

The upward migration of voids in the cohesive covering soil, characterized by its high clay content, occurs above pre-existing dissolution fissures located in the limestone bedrock through roof breakdown or erosion (Ayalew et al., 2004; Sauro et al., 2019). The dry season from November 2020 to March 2021 led to a decrease in groundwater levels within the study site, resulting in a loss of buoyant support in the cavity's roof. During the period before the onset of the regular rainy season, particularly in April 2021, the abrupt change in precipitation at the site suggests a potential increase in overburden weight due to rainfall infiltration and recharge from nearby surface drainages (Van Den Eeckhaut et al., 2007; Theron and Engelbrecht, 2018). The combination of increased water input to the ground and a decline in the water table may accelerate the processes of arch cavity growth and upward migration (Youssef et al., 2020). As the cavities progressively grow, they eventually breach the ground surface (Tufano et al., 2022), observed as depressions resulting from the first surficial collapses in April 2021. This drastic increase in water percolation, a result of high-intensity rainfalls continuing from May to October in the same year, is believed to be a cause of subsequent sinkholes in the study site.

The underground mining activities in the nearby limestone quarry could also potentially contribute to the formation of sinkholes (Richardson, 2013; Ammirati et al., 2020). Historical aerial imagery reveals that the quarry was operational from 2018 until the end of 2021, with the initial pit being filled with water. The excessive dewatering process associated with quarry operations may have led to a decline in regional groundwater levels within the aquifer (Gutiérrez et al., 2014). This, in turn, could have influenced the occurrence of sinkholes in the study area. The hydraulic head mapping conducted in this study indicates that the groundwater generally flows in the region is towards the western area, suggesting that the quarry serves as a potential recharge area.

Although the acquired geophysical data did not provide a clear indication of the presence of cavernous karst systems or connected fractures in this specific location, the sinkhole-prone area, where significant surface collapses are likely to occur, appears to be in close proximity to surface water drainages, such as natural creeks and irrigation canals.

5. CONCLUSION

This study demonstrates the effectiveness of using the geophysical ERT method, along with hydrogeological mapping and precipitation records, to investigate areas affected by sinkholes. In this study, we focused on imaging the subsurface structures within the Huai Hin Lat carbonate formation, which is covered by Quaternary fluvial deposits in the eastern part of the Phu Pha Man District, Northeastern Thailand. Groundwater flow analysis indicated flow directions from north to southeast in the western to middle sections of the site, transitioning to west to east flows in the eastern part. Rainfall data showed an increasing trend in precipitation over the study years, and a heavy rainfall event in April 2021 coincided with the initial occurrence of sinkholes. The results of the ERT inversion revealed valuable information about the depths, ranging from 25-60 m, and coverage area of ~2 km^2. These results also unveiled distinct spatial variations in geoelectrical resistivity, providing insights into the different degrees of weathering found in karst terrains. We also identified zones of cover materials that host cavities and have the potential to lead to collapses. The occurrence of collapses in this area can be attributed to a combination of factors, including the presence of local-scale cavities in heterogeneous cover layers above the Triassic limestone, unusually high precipitation events, the infiltration of surface water, and potentially the dewatering activities associated with the nearby limestone mine. With the limited data we have acquired, we suggest that intense rainfall could be the dominant triggering factor that has the most influence on sinkhole development. This is due to the unclear connectedness of subsurface cavities within the karst system, a lack of assessable dewatering impact, and an uncertainty regarding the spatial concentration of collapses coinciding with surface drainages. To mitigate potential future collapses, it may be necessary to implement regulatory measures limiting further intensification of land use and groundwater withdrawal.

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REFERENCES

Al-Halbouni D, Watson RA, Holohan EP, Meyer R, Polom U, Dos Santos FM, et al. Dynamics of hydrological and geomorphological processes in evaporite karst at the eastern Dead Sea: A multidisciplinary study. Hydrology and Earth System Sciences 2021;25(6):3351-95.

Ammirati L, Mondillo N, Rodas RA, Sellers C, Di Martire D. Monitoring land surface deformation associated with gold artisanal mining in the Zaruma City (Ecuador). Remote Sensing 2020;12(13):Article No. 2135.

Arjwech R, Ruansorn T, Schulmeister M, Everett ME, Thitimakorn T, Pondthai P, et al. Protection of electricity transmission infrastructure from sinkhole hazard based on electrical resistivity tomography. Engineering Geology 2021;293:Article No. 106318.

Ayalew L, Yamagishi H, Reik G. Ground cracks in Ethiopian Rift Valley: Facts and uncertainties. Engineering Geology 2004;75(3-4):309-24.

Billi A, De Filippis L, Poncia PP, Sella P, Faccenna C. Hidden sinkholes and karst cavities in the travertine plateau of a highly-populated geothermal seismic territory (Tivoli, central Italy). Geomorphology 2016;255:63-80.

Booth J, Sattayarak N. Subsurface carboniferous - Cretaceous geology of NE Thailand. In: Ridd MF, Barber AJ, Crow MJ, editors. The Geology of Thailand. United Kingdom: Geological Society of London; 2011. p. 185-222.

Chaodumrong P, W XD, Shen SZ. Permian lithostratigraphy of the Shan-Thai Terrane in Thailand: Revision of the Kaeng Krachan and Ratburi groups. Proceedings of International Conference on Geology of Thailand: Towards Sustainable Development and Sufficiency Economy; 2007 Nov 21-22; Department of Mineral Resources, Bangkok: Thailand; 2007.

Chonglakmani C, Sattayarak N. Geological Map of Phetchabun Province, Scale 1:250,000. Bangkok, Thailand: Department of Mineral Resources; 1984.

Cueto M, Olona J, Fernández-Viejo G, Pando L, López-Fernández C. Karst-induced sinkhole detection using an integrated geophysical survey: A case study along the Riyadh Metro Line 3 (Saudi Arabia). Near Surface Geophysics 2018;16(3):270-81.

di Santolo AS, Forte G, Santo A. Analysis of sinkhole triggering mechanisms in the hinterland of Naples (Southern Italy). Engineering Geology 2018;237:42-52.

Doğan U, Yılmaz M. Natural and induced sinkholes of the obruk plateau and karapınar-hotamış plain, Turkey. Journal of Asian Earth Sciences 2011;40(2):496-508.

Department of Mineral Resources (DMR). Risk Map of Subsidence Sinkhole in Khon Kaen Province. Bangkok, Thailand: Department of Mineral Resources, Ministry of Natural Resources and Environment; 2005.

Department of Mineral Resources (DMR). Geologic map of Khon Kaen Province, Scale 1:1,000,000. Bangkok, Thailand: Department of Mineral Resources, Ministry of Natural Resources and Environment; 2007.

Department of Mineral Resources (DMR). Handbook of Reducing the Impact Disaster. Bangkok, Thailand: Department of Mineral Resources, Ministry of Natural Resources and Environment; 2011 (in Thai).

Department of Mineral Resources (DMR). Report on sinkhole inverstigation in Phu Pha Man District, Khon Kaen Province [Internet]. 2021 [cited 2021 May 20]. Available from: https://phupaman.go.th/public/list/data/detail/id/2930/menu/1554/ (in Thai).

Everett ME. Near-Surface Applied Geophysics. New York, USA: Cambridge University Press; 2013.

Fidelibus MD, Gutiérrez F, Spilotro G. Human-induced hydrogeological changes and sinkholes in the coastal gypsum karst of Lesina Marina area (Foggia Province, Italy). Engineering Geology 2011;118(1-2):1-9.

Furukawa H, Pichai W. Salt and sinkhole: Corrosion as a principal factor governing topography and mass movement in Northeast Thailand. Japanese Journal of Southeast Asian Studies 1989;27(1):3-34.

Giao PH, Prechavit N, Manop R. Electric modeling and imaging of sinkholes developed after the 26 December 2004 Tsunami in the Karstic Limestone in the Southern Thailand. Proceedings of First EAGE South-East Asia Regional Geology Workshop-Workshop on Palaeozoic Limestones of South-East Asia and South China; 2011 Dec 5-8; Ipoh: Malaysia; 2011.

Gutiérrez F, Parise M, De Waele J, Jourde H. A review on natural and human-induced geohazards and impacts in karst. Earth-Science Reviews 2014;138:61-88.

Heidari M, Khanlari GR, Beydokhti AT, Momeni AA. The formation of cover collapse sinkholes in North of Hamedan, Iran. Geomorphology 2011;132(3-4):76-86.

Kaufmann G, Romanov D, Nielbock R. Cave detection using multiple geophysical methods: Unicorn Cave, Harz Mountains, Germany. Geophysics 2011;76(3):71-7.

Kaufmann G. Geophysical mapping of solution and collapse sinkholes. Journal of Applied Geophysics 2014;111:271-88.

Kaufmann G, Romanov D, Tippelt T, Vienken T, Werban U, Dietrich P, et al. Mapping and modelling of collapse sinkholes in soluble rock: The Münsterdorf site, northern Germany. Journal of Applied Geophysics 2018;154:64-80.

Kidanu ST, Torgashov ET, Varnavina AV, Anderson NL. ERT-based Investigation of a Sinkhole in Greene County, Missouri. AIMS Geosciences 2016;2(2):99-115.

Kruse S, Grasmueck M, Weiss M, Viggiano D. Sinkhole structure imaging in covered karst terrain. Geophysical Research Letters 2006;33(16):Article No. L16405.

Land Development Department. Soil types in Phu Pha Man District [Internet]. 2005 [cited 2023 Feb 20]. Available from: http://oss101.ldd.go.th/web_thaisoilinf/62_soilgroup/62sg_desc/desc_33gm.html/ (in Thai).

Margiotta S, Negri S, Parise M, Valloni R. Mapping the susceptibility to sinkholes in coastal areas, based on stratigraphy, geomorphology and geophysics. Natural Hazards 2012;62:657-76.

Minezaki T, Hisada KI, Hara H, Kamata Y. Tectono-stratigraphy of late carboniferous to Triassic successions of the Khorat Plateau Basin, Indochina Block, Northeastern Thailand: Initiation of the Indosinian Orogeny by collision of the Indochina and South China blocks. Journal of Asian Earth Sciences 2019;170:208-24.

Nam BH, Kim YJ, Youn H. Identification and quantitative analysis of sinkhole contributing factors in Florida's Karst. Engineering Geology 2020;271:Article No. 105610.

Pando L, Pulgar JA, Gutiérrez-Claverol M. A case of man-induced ground subsidence and building settlement related to karstified gypsum (Oviedo, NW Spain). Environmental Earth Sciences 2013;68:507-19.

Ponta G, Memon B, LaMoreaux J, Julawong J, Wongsawat S. Karst Landforms in the Saraburi Group Limestones, Thailand. Proceedings of the Thirteenth Multidisciplinary Conference on Sinkholes and the Engineering and Environmental Impacts of Karst; 2013 May 6-10; National Cave and Karst Research Institute, Carlsbad, New Mexico: USA; 2013.

Richardson S. Sinkhole and Subsidence Record in the Chuniespoort Group Dolomite, Gauteng, South Africa [dissertation]. Pretoria, University of Pretoria; 2013.

Satarugsa P. The Lessons learnt from geophysical investigation of sinkholes in rock salt in Thailand. Proceedings of International Conference on Geology, Geotechnology and Mineral Resources of Indochina; 2011 Dec 1-3; Khon Kaen: Thailand; 2011.

Sauro F, Mecchia M, Piccini L, De Waele J, Carbone C, Columbu A, et al. Genesis of giant sinkholes and caves in the quartz sandstone of Sarisariñama tepui, Venezuela. Geomorphology 2019;342:223-38.

Theron A, Engelbrecht J. The role of earth observation, with a focus on SAR Interferometry, for sinkhole hazard assessment. Remote Sensing 2018;10(10):Article No. 1506.

Tufano R, Guerriero L, Annibali Corona M, Bausilio G, Di Martire D, Nisio S, et al. Anthropogenic sinkholes of the city of Naples, Italy: An update. Natural Hazards 2022;112(3):2577-608.

Ueno K, Charoentitirat T. Carboniferous and Permian. In: Ridd MF, Barber AJ, Crow MJ, editors. The Geology of Thailand. United Kingdom: Geological Society of London; 2011. p. 71-136.

Upper Northeastern Meteorological Center. Monthly rainfall of Khon Kaen Province by District [Internet]. 2022 [cited 2023 Jan 11]. Available from: http://www.khonkaen.tmd.go.th/rainmet_kk.php/ (in Thai).

United States Geological Survey (USGS). Sinkholes | U.S. Geological Survey [Internet]. 2018 [cited 2023 Feb 15]. Available from: https://www.usgs.gov/special-topics/water-science-school/science/sinkholes/.

Van Den Eeckhaut M, Poesen J, Dusar M, Martens V, Duchateau P. Sinkhole formation above underground limestone quarries: A case study in South Limburg (Belgium). Geomorphology 2007;91(1-2):19-37.

Waltham T, Bell FG, Culshaw MG, Knez M, Slabe T. Sinkholes and subsidence: Karst and cavernous rocks in engineering and construction. Chichester, United Kingdom: Springer-Praxis; 2005.

Watlet A, Kaufmann O, Triantafyllou A, Poulain A, Chambers JE, Meldrum PI, et al. Imaging groundwater infiltration dynamics in the karst vadose zone with long-term ERT monitoring. Hydrology and Earth System Sciences 2018;22(2):1563-92.

Williams P. The role of the epikarst in karst and cave hydrogeology: A review. International Journal of Speleogy 2008;37(1):1-10.

Yordkayhun S. Geophysical characterization of a Sinkhole Region: A study toward understanding geohazards in the Karst Geosites. Sains Malaysiana 2021;50(7):1871-84.

Youssef AM, Al-Harbi HM, Gutiérrez F, Zabramwi YA, Bulkhi AB, Zahrani SA, et al. Natural and human-induced sinkhole hazards in Saudi Arabia: Distribution, investigation, causes and impacts. Hydrogeology Journal 2016;24(3):625-44.

Youssef AM, Zabramwi YA, Gutiérrez F, Bahamil AM, Otaibi ZA, Zahrani AJ. Sinkholes induced by uncontrolled groundwater withdrawal for agriculture in arid Saudi Arabia. Integration of remote-sensing and geophysical (ERT) techniques. Journal of Arid Environments 2020;177:Article No. 104132.

Zhou W, Beck BF, Adams AL. Effective electrode array in mapping karst hazards in electrical resistivity tomography. Environmental Geology 2002;42:922-8.

Assessment of Long-Term Surface Water Quality in Mekong River Estuaries Using A Comprehensive Water Pollution Index

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ABSTRACT

Surface water quality (SWQ) has been degraded in the Mekong River Basin under increasing pressures of population growth, economic development, and global climate change. This study employed the comprehensive water pollution index (CWPI) to assess the spatio-temporal variation of SWQ in the downstream Mekong River estuaries. Eight water quality parameters were measured between 2005 and 2021 at 21 sampling sites downstream of the Mekong River. These parameters included total suspended solids (TSS), biological oxygen demand (BOD_5), chemical oxygen demand (COD), ammonia ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), phosphate ($P\text{-}PO_4^{3-}$), iron (Fe), and total coliform. Most of the monitoring locations in the estuaries of Ham Luong, Cua Dai, Ba Lai, and Co Chien exhibited slightly to moderately polluted conditions, as indicated by the CWPI values ranging from 0.67-2.91, 0.41-2.20, 0.27-3.02, and 0.37-2.95, respectively. TSS and Fe concentrations consistently exceeded the allowable limits, while the majority of values for $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$, and coliform remained within acceptable thresholds. Additionally, parameters indicative of organic pollution, namely BOD_5 and COD, displayed a noticeable upward trend between 2005 and 2021. SWQ exhibited significant spatial and temporal variations with TSS, organic matter, nutrients, and iron being the main areas of concern. These findings can provide guidance to policymakers involved in the assessment and enhancement of water quality in the presence of pollutant compounds that lead to a decline in water quality.

1. INTRODUCTION

Water resources are essential for living organisms, and their availability plays a crucial role in economic and economic growth activities (Le et al., 2023). In fact, water is a vital life-supporting factor, making up 70-90% of all living cells (Khan and Ansari, 2005). In addition, various economic sectors such as agriculture, industry, domestic usage, hydropower generation, fisheries, and other creative endeavors significantly depend on water resources (Effendi, 2016).

Numerous sources of pollution, including population growth, economic development, global climate change, and anthropogenic activities, have contributed to the degradation of surface water quality (SWQ) (Bojarczuk et al., 2018; Okello et al., 2015; Soares et al., 2020). Surface water pollution risks associated with socioeconomic development, such as nutrients, heavy metals, plastics, antibiotics, pesticides, and seawater intrusion, often exceed the environmental self-purification (Kroeze et al., 2016; Le et al., 2023; Liang and Yang, 2019). In turn, the deterioration of SWQ due to these pollution sources can cause adverse human health effects and diseases in humans (Kazi et al., 2009).

Regular monitoring and conservation programs are crucial on a global scale to prevent and control water pollution. Rapid techniques for assessing water quality

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can provide prompt information on the severity of water bodies pollution reliant on their physicochemical and biological parameters (Hossain and Patra, 2020). The Water Quality Index (WQI) is a popular approach used to describe SWQ due to its simple structure and user-friendly interface (Uddin et al., 2021). WQI, ranging from 0 to 100, is obtained by combining physical, chemical, and biological factors, which involves four processes: selection of parameters, transformation of raw data into a common scale, weighting, and aggregation of sub-index values (Chidiac et al., 2023). However, weight-based indices can suffer from imbalanced sensitivity if certain parameters are strongly or weakly weighted. Additionally, any changes in weight assignments can significantly impact the overall interpretation of water quality. For example, the lower concentration parameters can be influenced by higher assigned weights, which can result in false interpretations of the water quality (Juwana et al., 2012). In looking for solutions to the problem, the comprehensive water pollution index (CWPI) is proposed, which have integrated various water quality parameters into a single index (Mishra et al., 2016; Hossain and Patra, 2020). CWPI also utilizes unequal weights of environmental parameters to evaluate water quality. Furthermore, CWPI can be employed to evaluate the physical, chemical, and biological characteristics of water sources based on the available water quality standards for a given designated use (Hossain and Patra, 2020).

The Vietnam Mekong Delta (VMD) is a vulnerable region enduring a range of environmental challenges, such as rising sea levels, modifications in water flow, and nutrients from the upstream catchment. Several methodologies and techniques have been employed to gain a comprehensive understanding of this matter. Simulations from the Integrated Catchment Model predicted increased mean and flood flows, an earlier onset of monsoonal flows, and more dry spells until 2050, with nutrient fluxes potentially rising by 5% over the Greater Mekong Basin (Whitehead et al., 2019). The Self-Organizing Map classified 117 monitoring locations and pollution hot zones in the Lower Mekong Basin (LMB) based on water quality indicators monitored from 1985 to 2010, identifying eutrophication, salinity, and human interference as factors affecting water quality, particularly in the VMD (Chea et al., 2016). Water quality in the LMB was assessed using biotic and abiotic evaluation factors from 2000 to 2017, with the findings indicating degradation in the 2010s due to several factors such as flow modification, erosion, sediment accumulation, and wastewater, resulting from the rapid development of hydropower, extensive deforestation, intensive farming practices, plastic pollution, and the expansion of urban areas (Sor et al., 2021).

The risk of pollution is heightened by the extensive use of surface water in the VMD for domestic services, irrigation, and drinking water, which poses a potential threat to human, animal, and ecosystem health (Wilbers et al., 2014). Current research on water quality in the area has revealed the presence of organic pollutants, microorganisms, salts, total suspended solids, and metals. For example, the SWQ in An Giang Province, the region upstream of VMD, was assessed using water quality indicators and multivariate statistical techniques (Hong and Giao, 2022). In the Bassac river, canal pollution levels and seasonal variations in SWQ were examined using principal component analysis, water quality attributes (Wilbers et al., 2014), and the entropy-weighted water quality index (EWQI), as well as multivariate methods (Nguyen et al., 2022). In the Mekong River, the variation in the SWQ was analyzed in Ben Tre (Nguyen et al., 2018) and Tien Giang (Hong et al., 2022) Provinces using WQI and multivariate statistics, respectively. In general, existing studies mostly employ multivariate statistical approaches and water quality measures to examine short-term variations in the SWQ and identify their causes and drivers.

The objective of this study is to analyze the spatial and temporal distribution of SWQ downstream of the Mekong River in Vietnam from 2005 to 2021. Water quality data were collected at 21 locations along Cua Dai, Ba Lai, Ham Luong, and Co Chien estuaries and analyzed for eight parameters (i.e., total suspended solids (TSS), biological oxygen demand (BOD_5), chemical oxygen demand (COD), ammonia ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), phosphate ($P\text{-}PO_4^{3-}$), iron (Fe), and total coliform). Subsequently, box and whisker plots were employed to illustrate the changes in water quality parameters over time and across different locations. Finally, CWPI was calculated to assess the overall SWQ. The study outcomes are expected to provide pivotal information, primarily regarding the quality of surface water systems, and to assist in managing the coastal regions of the VMD.

2. METHODOLOGY

2.1 Study site

The Mekong Delta region is identified as the commencing at the intersection of the Mekong River and Tonle Sap River at Phnom Penh, Cambodia, and further divides into six principal channels of the Mekong River and three channels of the Bassac River before flowing into the East Sea. The Mekong River stretches for 150 km with a width ranging from 450 to 2,250 meters and a maximum depth of 10 m. The region experiences a monsoonal humid and tropical climate with an average temperature ranging between 27-30°C. Approximately 80% of the annual rainfall occurs during the rainy season, which lasts from May to October. Consequently, the Mekong River experiences a minimum discharge of around 200 m^3/s in April-May and a maximum discharge of approximately 7,000 m^3/s in September-October (Strady et al., 2017).

2.2 Data collection and processing

A total of 21 sampling sites were chosen to represent the SWQ downstream of the Mekong River from 2005 to 2021. Among these sites, three were located on the Cua Dai estuary (CD1-CD3), eight on the Ba Lai estuary (BL1-BL8), five on the Ham Luong estuary (HL1-HL5), and five on the Co Chien estuary (CC1-CC5) (Figure 1). The collection of surface water samples was carried out biannually between 2005 and 2016, in May (at the onset of the rainy season) and November (at the onset of the dry season). However, the sampling frequency was increased to four times a year between 2017 and 2021, with samples being taken in February (between dry seasons), May (at the onset of the rainy season), August (between rainy seasons), and November (at the onset of the dry season). The water samples were collected from a depth of 0.3-0.5 m below the water's surface. To assess water quality, eight water quality parameters, namely TSS, BOD_5, COD, N-NH_4^+, N-NO_3^-, P-PO_4^{3-}, Fe, and total coliform, were measured. These water quality parameters were preserved and analyzed at the laboratory of the Center for Natural Resources and Environment Monitoring of Ben Tre Province using standard methods as described in Table 1.

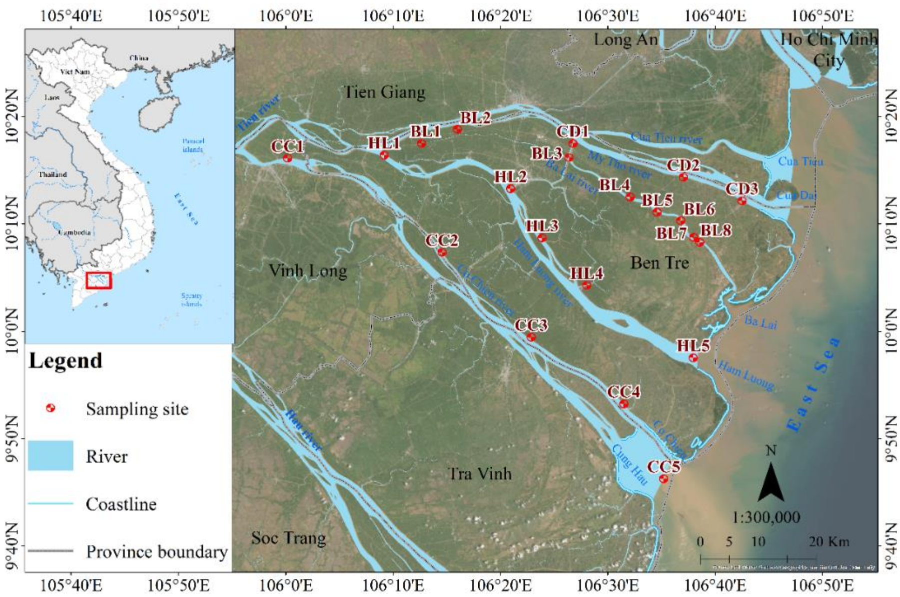

Figure 1. Map of the study area and sampling sites in the downstream of Mekong River

Table 1. Analysis methods and allowable limits of the parameters

Parameters	Units	Measurement methods	VN-Standards A2*
TSS	mg/L	SMEWW 2540D:2017	30
BOD_5	mg/L	SMEWW 5210B:2017	6
COD	mg/L	SMEWW 5220C:2017	15
N-NH_4^+	mg/L	SMEWW 4500-NH_3^+.B&F:2017	0.3
N-NO_3^-	mg/L	SMEWW 4500-NO_3^-.E:2017	5
P-PO_4^{3-}	mg/L	SMEWW 4500-PO_4^{3-}.E:2017	0.2
Fe	mg/L	SMEWW 3111B:2017	1
Coliform	MPN/100 mL	TCVN 6187-2:1996	5,000

2.3 Comprehensive water pollution index

Box and whisker plots were employed to illustrate temporal and spatial variations in water quality parameters. To assess the overall water quality, the comprehensive water pollution index (CWPI) was calculated for each sampling site using the formula (Mishra et al., 2016):

$$CWPI = \frac{1}{n}\sum_{i=1}^{n}\frac{C_i}{C_{oi}}$$

Where; n is the number of water quality parameters, C_i is the measured concentration of water quality parameter i and C_{oi} is the standard permissible concentration of water quality parameter i.

In this study, the permissible concentrations of each parameter were determined according to Vietnamese water quality standards, QCVN 08-MT:2015/BTNMT, where the A2 standard was selected as a reference value for residential use with appropriate treatment, preservation of aquatic plants, or other purposes (Ministry of Natural Resources and Environment, 2015). The surface water quality was classified using the CWPI, where a value of ≤0.20 was considered clean, 0.21-0.40 was categorized as sub-clean, 0.41-1.00 was considered slightly polluted, 1.01-2.00 was considered moderately polluted, and ≥2.01 was classified as severely polluted (Mishra et al., 2016).

3. RESULTS AND DISCUSSION

3.1 Physical and biochemical water quality parameters

Values of physical and biochemical parameters of SWQ downstream of the Mekong River are depicted in Figure 2. The average concentration of TSS in the sampling sites was 85.68±55.84 mg/L, exceeding the allowable limit by 2.86 times and higher than that of recent findings in the VMD (Table 2). TSS consists of particles suspended in water, originating from both natural and anthropogenic activities such as construction, agricultural production, the release of artificial and organic chemicals from industrial sewage, and municipal and domestic wastewater (Nguyen et al., 2020). Aquaculture practices, particularly those related to *Pangasius* catfish farms, also contribute to the contamination of surface water with high levels of TSS due to the presence of food remnants, fish excrement, and metabolic waste (Dauda et al., 2019). The substantial levels of TSS in the downstream Mekong River indicate a significant degree of riverbank erosion, given its coastal location heavily impacted by estuaries and mudflats (Giao, 2020). Therefore, specific sources of TSS have yet to be identified and may require further investigation. A high TSS concentration can lead to a drop in dissolved oxygen levels, causing hypoxic stress and adversely affecting fish species' survival, diversity, and abundance (Mueller et al., 2017). The presence of TSS in water can also result in the accumulation of heavy metals and nutrients, causing elevated pollutant levels. When TSS settles at the bottom, it can disrupt the benthic environment and harm organisms (Mueller et al., 2017). Consequently, the presence of a high TSS concentration increases the likelihood of people being exposed to environmental pollutants and water treatment costs.

BOD_5 and COD are widely used parameters for assessing the organic pollution level in water. In the study area, the BOD_5 concentration ranged from 3 to 19.3 mg/L, with a mean of 6.23±3.01 mg/L, exceeding the permissible limit by 1.03 times. In contrast, COD concentration was relatively low, ranging from 3 to 30.93 mg/L, with a mean of 11.15±5.44 mg/L, which fell within the limits of Standard A2. BOD_5 can originate from various sources such as farming waste, livestock waste, landfill waste, and untreated waste directly discharged into the environment from domestic activities and services (Giao and Nhien, 2020). To prevent saltwater intrusion, many sluices and dams have been built along the Mekong River. However, keeping these structures closed for extended periods can increase environmental pollution, particularly organic pollution (Nguyen et al., 2022; Ngo et al., 2022; Tran et al., 2022).

Nitrogen and phosphorus compounds are primary pollutants with the potential to form secondary pollutants and cause eutrophication, which adversely affects aquatic organisms (Abdel-Raouf et al., 2012). Study results also found that the average concentrations of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and $P\text{-}PO_4^{3-}$ were within the limits of Standard A2, but many values exceeded national technical regulations for surface water. The levels of $N\text{-}NO_3^-$ were suitable for daily activities but toxic to aquatic life, especially in alkaline environments (Martin et al., 2008). The presence of nutrient compounds in surface water may result from the discharge of domestic wastewater containing detergents, industrial waste, and runoff from fertilizers. Excess nutrients discharged from *Pangasius* catfish feed could also contribute to elevated nutrient levels. Additionally, the reduction in sediment flow into agricultural areas has led to soil degradation (Le et al., 2023), resulting in the overuse of fertilizers and pesticides (Chapman et al., 2016), which can lead to a high level of nutrient compounds. Therefore, it can be inferred that water quality in the water bodies downstream of the Mekong River has been contaminated with nutrients, particularly N-NH_4^+ and P-PO_4^{3-}, suggesting the potential for eutrophication to occur in the water bodies within the study area.

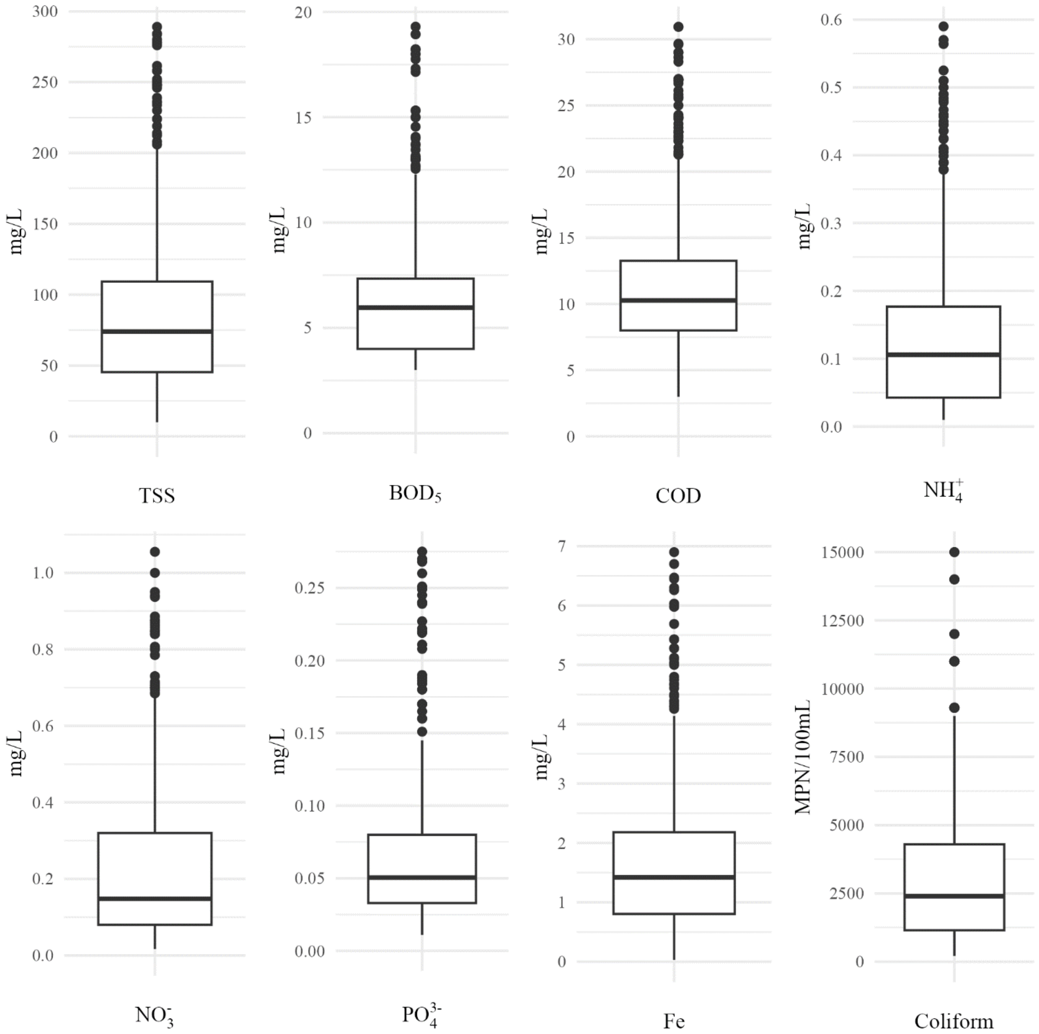

Figure 2. Surface water quality parameters downstream of Mekong River

The average Fe concentration in surface water ranged from 0.03 to 6.9 mg/L, with a mean value of 1.66±1.20 mg/L, which exceeded the limit by 1.66 times. The Mekong River was found to have a higher average iron concentration than the Bassac River. The presence of iron in surface water can be attributed to both natural factors, such as the presence of acid sulfate soil properties, and anthropogenic activities, such as washing acidic soil and intensive agricultural production (Giao, 2020). To assess water quality in the surface water monitoring program, various heavy metals such as iron (Fe), aluminum (Al), manganese (Mn), chromium (Cr), and cadmium (Cd) are measured. The selection of parameters to monitor and the monitoring locations for SWQ depends on the characteristics of pollution sources and available budget. The Center for Natural Resources and Environment Monitoring in Ben Tre Province currently uses iron to evaluate the SWQ in the downstream of the Mekong River through its monitoring program. However, there may be a need for additional updates because many studies have warned that arsenic poisoning poses the most significant health risk for both human and aquatic organisms in VMD (Strady et al., 2017).

Microbial contamination has become a significant concern in various water bodies located in the VMD, including Hau Giang, An Giang, Dong Thap, and Tra Vinh Provinces (Table 2). In contrast, the Mekong River exhibited coliform levels within the allowable limits, with a mean of 3,190±2,767 MPN/100 mL. The high presence of coliform, exceeding the national technical regulations for surface water, can be attributed to the discharge of significant amounts of organic waste into the river. The primary sources of this waste are aquaculture farms, rice fields, fish processing industries, and other industrial activities. The consumption of water with high coliform levels can lead to several health issues, such as gastrointestinal illness, fever, diarrhea, and dehydration (Divya and Solomon, 2016).

Overall, surface water in different water bodies downstream of Mekong River, VMD was contaminated with TSS, organic matter, and iron. On the contrary, microbiological pollution may not be the primary issue of water pollution in the Mekong River.

Table 2. Information regarding various surface water quality parameters in different regions of the VMD

	TSS (mg/L)	BOD_5 (mg/L)	COD (mg/L)	$N\text{-}NH_4^+$ (mg/L)	$N\text{-}NO_3^-$ (mg/L)	$P\text{-}PO_4^{3-}$ (mg/L)	Fe (mg/L)	Coliform (MPN/100 mL)
MKR	85.68 (10-289)	6.23 (3-19.3)	11.15 (3-30.93)	0.13 (0.01-0.59)	0.23 (0.01-1.05)	0.07 (0.01-0.28)	1.66 (0.03-6.9)	3,190 (210-15,000)
BR	34.8-50.8	7.3-8.3	12-12.8	0-0.1	0.34-0.38	0.1-0.23	0.3-0.47	1,156-1,657
HGP	32.8-101	6.3-14	14-25	0-0.92	0.23-0.54	0.1-0.36	0.5-2.26	3,225-15,275
AGP	53.33-59.59	13.65-24.19	21.14-37.22	0.36-2.19	-	-	-	11,067-31,363
DTP	34.60	15.03	22.34	0.29-0.45	0.99-2.70	0.1-0.59	-	1,708-25,300
TVP	57.39	7.03	24.81	-	-	0.09	-	153,229
CTC	24.7-57.9	3.3-4.7	-	-	0.2-0.3	0.2-0.3	-	19,140-28,600
TGP	78.9-121.8	8.0-8.9	14.4-17.3	0.3-0.5	0.1-0.4	0.1	-	972.9-2,261.2

MKR (Mekong River, this study), BR (Bassac River (Giao, 2020)), HGP (Hau Giang Province (Giao, 2020)), AGP (An Giang Province (Hong and Giao, 2022)), DTP (Dong Thap Province (Giao et al., 2021)), TVP (Tra Vinh Province (Le et al., 2023)), CTC (Can Tho City (Mutea et al., 2021)), TGP (Tien Giang Province (Giao et al., 2021))

3.2 Temporal changes in surface water quality

Figure 3 presents the variation in surface water quality downstream of the Mekong River over a 17-year period (2005-2021). Results from the Kruskal-Wallis test showed significant statistical differences ($p<0.001$) in all physical and biochemical parameters. TSS and Fe concentrations were mostly higher than the permissible limit. In contrast, most values of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$, and coliform remained within the allowable limit. Parameters such as BOD_5 and COD, which serve as indicators of organic pollution, tended to increase from 2005 to 2021. However, BOD_5 and COD concentrations started to exceed the permissible standard in 2016 and 2019, respectively. The increasing tendency of BOD_5 and COD could be explained by seawater intrusion. The primary reason for the rise in BOD_5 and COD levels during the period from 2016 to 2020 might be seawater intrusion in many coastal areas in VMD, where seawater intruded up to 45-50 km from the estuary. The intrusion of seawater has been recognized as one of the most significant sources of pollution responsible for declining surface water quality (Le et al., 2023).

3.3 Spatial variation in water quality

The results of the CWPI analysis at the Mekong estuaries are shown in Figure 4. The CWPI values for the Co Chien, Ham Luong, Ba Lai, and Cua Dai estuaries ranged from 0.67-2.91, 0.41-2.20, 0.27-3.02, and 0.37-2.95, respectively. Overall, most of the observation stations indicated slightly to moderately polluted conditions. In particular, the observed values at HL1 in 2005 were categorized as being in clean or sub-clean conditions with a CWPI value of 0.27.

The SWQ at the river mouth location (CD3) in the Cua Dai estuary was severely polluted in 2010, 2015, 2016, and 2019, with CWPI values of 2.20, 2.91, 2.03, and 2.73, respectively. A similar trend was observed in the Ba Lai estuary, where the SWQ at the river mouth location (BL8) was severely polluted in 2015 and 2019, with CWPI scores of 2.14 and 2.08, respectively. Furthermore, the SWQ at BL4 was severely polluted in 2010, with a CWPI of 2.20. In the Ham Luong estuary, the SWQ at the river mouth (HL5) was severely polluted in 2010, 2015, and 2019, with CWPI scores of 3.02, 2.55, and 2.99, respectively. At the Co Chien estuary, the CC5 station had severely polluted water conditions in 2009, 2010, 2015, and 2019, with CWPI scores of 2.25, 2.00, 2.96, and 2.16, respectively. Additionally, the CC4 location had severely polluted water quality in 2010, with a CWPI of 2.12.

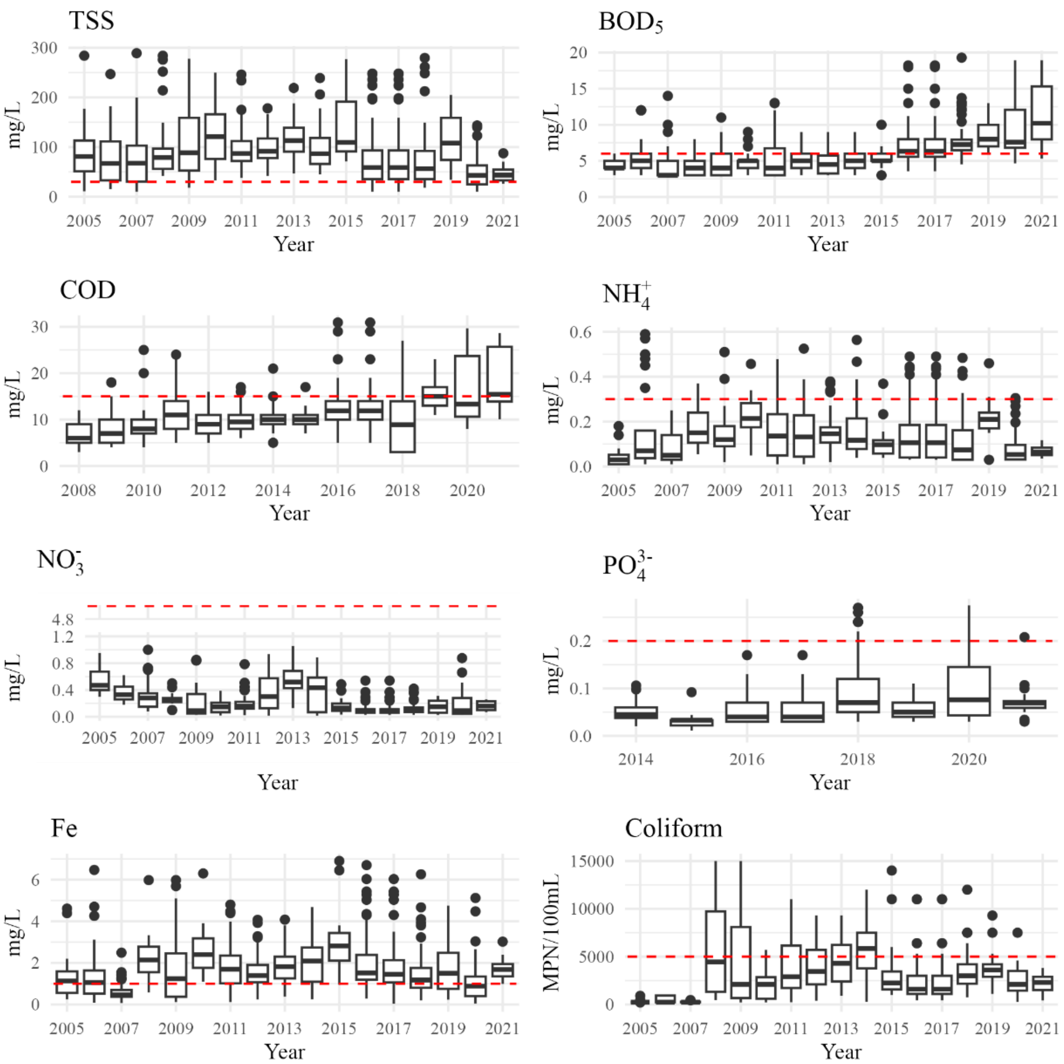

Figure 3. Box plots of surface water quality parameters downstream of Mekong River from 2005 to 2021. Dashed lines denote the Vietnamese technical regulation on surface water quality (Standard A2).

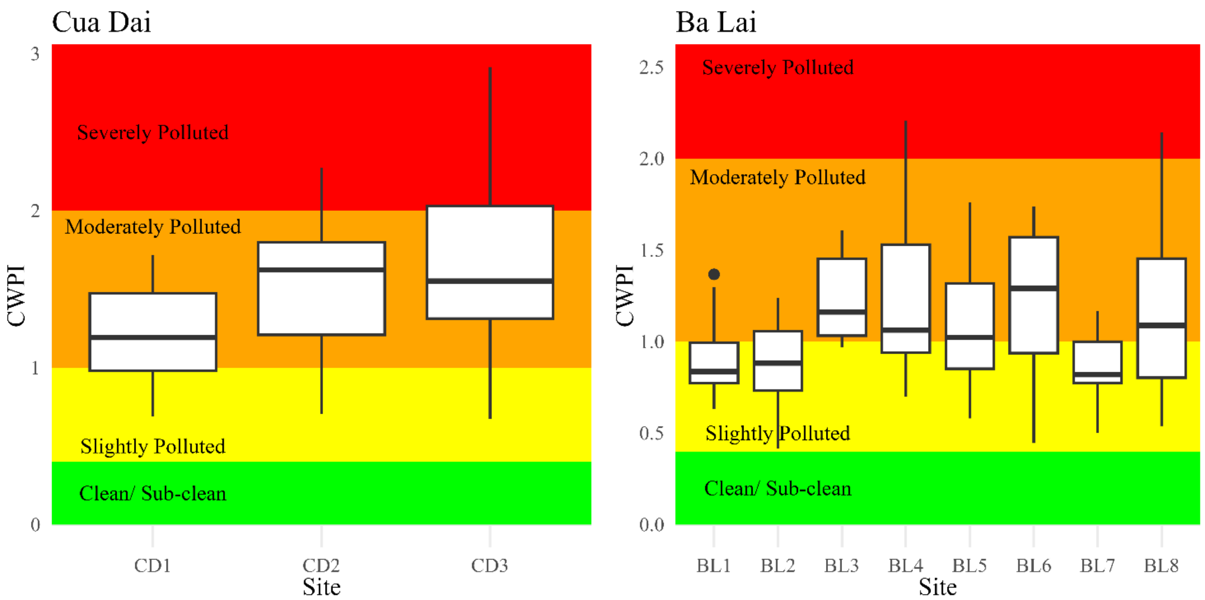

Figure 4. CWPI and the surface water quality in Cua Dai, Ba Lai, Ham Luong, Co Chien Estuaries

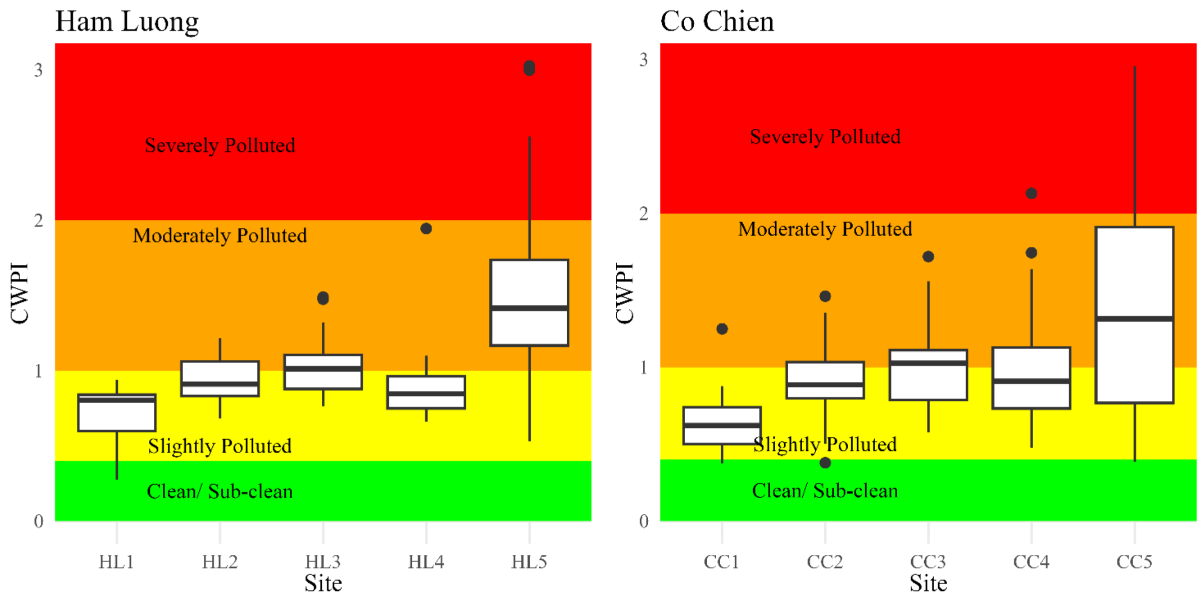

Figure 4. CWPI and the surface water quality in Cua Dai, Ba Lai, Ham Luong, Co Chien Estuaries (cont.)

The observed degradation in water quality in the years 2010, 2015, and 2019 could be attributed to significant saltwater intrusion experienced by the VMD during these years. The SWQ in the Ba Lai estuary exhibited a progressive deterioration from the upper (BL1) to the An Hoa area (BL3) due to the complex hydrological regime at this location, resulting from the confluence of three rivers, namely Ben Tre, Ba Lai, and An Hoa. The An Hoa area is frequently affected by erosion, leading to significant environmental disturbances (Tran et al., 2021). However, the SWQ from An Hoa to the Ba Lai dam (BL7) indicated an upward trend.

This study provides additional evidence that SWQ exhibits greater turbulence in the vicinity of river mouths, compared to those located further upstream. Additionally, the SWQ tends to degrade gradually as it approaches the estuary, consistent with previous research (Le et al., 2023). On the other hand, the Mekong River estuaries are being impacted by significant sediment accumulation, which has resulted in a decline in overall environmental quality (Tran et al., 2018).

The Mekong Delta region hosts relatively few large industrial zones; hence, organic pollution in its rivers predominantly stems from domestic activities and agriculture (Wehrheim et al., 2023). Effective water quality management in the Mekong River estuaries necessitates the control of external contaminants. Nonetheless, when selecting appropriate technologies, it is imperative to consider the following pivotal attributes of the Mekong Delta area: (i) limited cost recovery capacity, contingent upon personal income and prevailing economic conditions; (ii) absence of centralized wastewater treatment facilities; (iii) a predominantly flat topography characterized by intricate and interconnected river and channel systems, governed by geological and hydrological factors; and (iv) the unique culture and lifestyle of the local populace in the Mekong Delta (Huyen and Lai, 2019). To treat domestic wastewater in rural areas, septic tank coverage in the Mekong Delta region is nearly universal, except for homes located along rivers. Nevertheless, septic tanks achieve only a 45% and 25% removal of TSS and BOD, respectively (Huyen and Lai, 2019). To enhance processing efficiency, low-energy technologies such as an advanced-treatment septic system combined with constructed wetlands can be employed. In general, it is essential to establish and execute viable strategies and regulations for managing, controlling, and treating the primary sources of pollution to ensure sustainable development and a healthier environment for the Mekong River.

4. CONCLUSION

In the present study, the Comprehensive Water Pollution Index (CWPI) was assessed for 21 sampling locations downstream of the Mekong River using water quality parameters from 2005 to 2021. The CWPI clearly indicates that the majority of observation stations exhibited slight to moderate pollution conditions, with the quality of surface water deteriorating as it approaches the estuary. The accumulation of sediment has contributed to a decline in the overall environmental quality of the Mekong River estuaries. Surface water quality in the downstream Mekong River exhibited notable spatial and temporal variations, with TSS, organic matter, and iron emerging as primary areas of concern. This study clearly underscores the utility of CWPI as a valuable tool for assessing pollutants in human-impacted water bodies and for classifying river water quality. Furthermore, it is recommended that conservation plans be implemented proactively to maintain acceptable levels of TSS, organic matter, and iron in the Mekong River estuaries.

ACKNOWLEDGEMENTS

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REFERENCES

Abdel-Raouf N, Al-Homaidan AA, Ibraheem I. Microalgae and wastewater treatment. Saudi Journal of Biological Sciences 2012;19(3):257-75.

Bojarczuk A, Jelonkiewicz Ł, Lenart-Boroń A. The effect of anthropogenic and natural factors on the prevalence of physicochemical parameters of water and bacterial water quality indicators along the river Białka, Southern Poland. Environmental Science and Pollution Research 2018; 25(10):10102-14.

Chapman AD, Darby SE, Hong HM, Tompkins EL, Van TPD. Adaptation and development trade-offs: Fluvial sediment deposition and the sustainability of rice-cropping in An Giang Province, Mekong Delta. Climatic Change 2016;137(3-4):593-608.

Chea R, Grenouillet G, Lek S. Evidence of water quality degradation in Lower Mekong basin revealed by self-organizing map. PLoS ONE 2016;11(1):1-19.

Chidiac S, El Najjar P, Ouaini N, El Rayess Y, El Azzi D. A comprehensive review of water quality indices (WQIs): History, models, attempts and perspectives. Reviews in Environmental Science and Biotechnology 2023;22:349-95.

Dauda AB, Ajadi A, Tola-Fabunmi AS, Akinwole AO. Waste production in aquaculture: Sources, components and managements in different culture systems. Aquaculture and Fisheries 2019;4(3):81-8.

Divya AH, Solomon PA. Effects of some water quality parameters especially total coliform and fecal coliform in surface water of Chalakudy River. Procedia Technology 2016;24:631-8.

Effendi H. River water quality preliminary rapid assessment using pollution index. Procedia Environmental Sciences 2016; 33:562-7.

Giao NT, Cong NV, Nhien HTH. Using remote sensing and multivariate statistics in analyzing the relationship between land use pattern and water quality in Tien Giang Province, Vietnam. Water 2021;13(8):1093-109.

Giao NT, Nhien HTH. Phytoplankton: Water quality relationship in water bodies in the Mekong Delta, Vietnam. Applied Environmental Research 2020;42(2):1-12.

Giao TN. Spatial variations of surface water quality in Hau Giang Province, Vietnam using multivariate statistical techniques. Environment and Natural Resources Journal 2020;18(4): 400-10.

Hong T, Viet L, Giao N. Analysis of Spatial-Temporal variations of surface water quality in the Southern province of Vietnamese Mekong Delta using multivariate statistical analysis. Journal of Ecological Engineering 2022;23(7):1-9.

Hong TTK, Giao NT. Analysis of surface water quality in upstream province of Vietnamese Mekong Delta using multivariate statistics. Water 2022;14(12):1975-88.

Hossain M, Patra PK. Water pollution index: A new integrated approach to rank water quality. Ecological Indicators 2020;117:Article No. 106668.

Huyen DTT, Lai TD. Assessment of wastewater management in Mekong River delta region. Journal of Science and Technology in Civil Engineering 2019;13(2):82-91.

Juwana I, Muttil N, Perera BJC. Indicator-based water sustainability assessment: A review. Science of the Total Environment 2012;438:357-71.

Kazi TG, Arain MB, Jamali MK, Jalbani N, Afridi HI, Sarfraz RA, et al. Assessment of water quality of polluted lake using multivariate statistical techniques: A case study. Ecotoxicology and Environmental Safety 2009;72(2):301-9.

Khan FA, Ansari AA. Eutrophication: An ecological vision. The Botanical Review 2005;71(4):449-82.

Kroeze C, Gabbert S, Hofstra N, Koelmans AA, Li A, Löhr A, et al. Global modelling of surface water quality: A multi-pollutant approach. Current Opinion in Environmental Sustainability 2016;23:35-45.

Le TV, Nguyen DTP, Nguyen BT. Spatial and temporal analysis and quantification of pollution sources of the surface water quality in a coastal province in Vietnam. Environmental Monitoring and Assessment 2023;195(3):408-22.

Liang W, Yang M. Urbanization, economic growth and environmental pollution: Evidence from China. Sustainable Computing: Informatics and Systems 2019;21:1-9.

Martin GD, Vijay JG, Laluraj CM, Madhu NV, Joseph T, Nair M, et al. Fresh water influence on nutrient stoichiometry in a tropical estuary, Southwest coast of India. Applied Ecology and Environmental Research 2008;6(1):57-64.

Ministry of Natural Resources and Environment. National Technical Regulation on Surface Water Quality: QCVN 08-MT:2015/BTNMT. Vietnam: Ministry of Natural Resources and Environment; 2015.

Mishra S, Kumar A, Shukla P. Study of water quality in Hindon River using pollution index and environmetrics, India. Desalination and Water Treatment 2016;57(41):19121-30.

Mueller JS, Grabowski TB, Brewer SK, Worthington TA. Effects of temperature, total dissolved solids, and total suspended solids on survival and development rate of larval Arkansas River Shiner. Journal of Fish and Wildlife Management 2017;8(1):79-88.

Mutea FG, Nelson HK, Au HV, Huynh TG, Vu UN. Assessment of water quality for aquaculture in Hau River, Mekong Delta, Vietnam using multivariate statistical analysis. Water 2021;13(22):3307-24.

Ngo XQ, Yen NTM, Thai TT, Yen NTH, Van Dong N, Hoai PN, et al. Impact of a dam construction on the intertidal environment and free-living nematodes in the Ba Lai, Mekong Estuaries, Vietnam. Environmental Monitoring and Assessment 2022;194(S2):770-93.

Nguyen NTM, Vanreusel A, Lins L, Thai TT, Nara Bezerra T, Quang NX. The effect of a dam construction on subtidal nematode communities in the Ba Lai Estuary, Vietnam. Diversity 2020;12(4):137-55.

Nguyen TG, Phan KA, Huynh THN. Major concerns of surface water quality in South-West coastal regions of Vietnamese Mekong Delta. Sustainable Environment Research 2022; 32(1):46-60.

Nguyen TLC, Phan LKN, Pham AD. Water quality assessment of main rivers and canals in Ben Tre Province, Mekong Delta Vietnam. IOP Conference Series: Earth and Environmental Science 2018;191(1):Article No. 012031.

Okello C, Tomasello B, Greggio N, Wambiji N, Antonellini M. Impact of population growth and climate change on the freshwater resources of Lamu Island, Kenya. Water 2015;7(12):1264-90.

Soares ALC, Pinto CC, Oliveira SC. Impacts of anthropogenic activities and calculation of the relative risk of violating surface water quality standards established by environmental legislation: A case study from the Piracicaba and Paraopeba River Basins, Brazil. Environmental Science and Pollution Research 2020;27(12):14085-99.

Sor R, Ngor PB, Soum S, Chandra S, Hogan ZS, Null SE. Water quality degradation in the Lower Mekong Basin. Water 2021;13(11):1555-73.

Strady E, Dinh QT, Némery J, Nguyen TN, Guédron S, Nguyen NS, et al. Spatial variation and risk assessment of trace metals in water and sediment of the Mekong Delta. Chemosphere 2017;179:367-78.

Tran TT, Lam NLQ, Yen NTM, Quang NX, Vanreusel A. Biodiversity and distribution patterns of free-living nematode communities in Ba Lai River, Ben Tre Province. Vietnam Journal of Science and Technology 2018;56(2):224-35.

Tran TT, Nguyen MY, Quang NX, Hoai PN, Veettil BK. Ecological impact assessment of irrigation dam in the Mekong Delta using intertidal nematode communities as bioindicators. Environmental Science and Pollution Research 2022; 29(60):90752-67.

Tran TT, Nguyen TMY, Tran HG, Pham NH, Tran T, Ngo TT, et al. Effects of dam construction on the main-stream river, a lesson from Ba Lai Dam, Ben Tre Province. Science and Technology Development Journal-Natural Sciences 2021; 5(2):1040-54.

Uddin MG, Nash S, Olbert AI. A review of water quality index models and their use for assessing surface water quality. Ecological Indicators 2021;122:Article No. 107218.

Wehrheim C, Lübken M, Stolpe H, Wichern M. Identifying key influences on surface water quality in freshwater areas of the Vietnamese Mekong Delta from 2018 to 2020. Water 2023;15(7):1295-310.

Whitehead PG, Jin L, Bussi G, Voepel HE, Darby SE, Vasilopoulos G, et al. Water quality modelling of the Mekong River Basin: Climate change and socioeconomics drive flow and nutrient flux changes to the Mekong Delta. Science of the Total Environment 2019;673:218-29.

Wilbers GJ, Becker M, Nga LT, Sebesvari Z, Renaud FG. Spatial and temporal variability of surface water pollution in the Mekong Delta, Vietnam. Science of the Total Environment 2014;485-486(1):653-65.

Removal of BOD_5 and COD from Domestic Wastewater by Using a Multi-Media-Layering (MML) System

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ABSTRACT

This study investigated the ability of the multi-media-layering (MML) to reduce Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) levels in domestic wastewater. MML used in this study is comprised of two MMLs (MML-1 and MML-2) with a total volume for each MML of 0.056 m^3. Every MML was filled with gravel media, zeolite, activated carbon, and silica sand. The differences between MML-1 and MML-2 were only found at the height of the media, especially the height of gravel and zeolite media. This study showed that MML-1 had the highest efficiency in reducing BOD_5 (95.47%) and COD (93.10%) compared with MML-2 (BOD_5 of 85.39% and COD of 89.65%). Overall, MML showed promising results in removing pollutants from domestic wastewater. The study also suggested that the height of the gravel media and pH greatly influenced the removal of BOD_5 and COD levels in domestic wastewater.

1. INTRODUCTION

Discharging untreated wastewater into the environment can lead to a deterioration in water quality and environmental pollution (Sikiru et al., 2022). Pollutants in water bodies extend beyond industrial effluent, agriculture, hospital wastewater, or municipal activities. Domestic wastewater from household activities also significantly contributes to contaminants (Liang et al., 2018; Liu et al., 2019; Nonfodji et al., 2020; Wang et al., 2023). Greywater and blackwater are the two primary components of domestic wastewater. However, there has been a noticeable rise in the expenses associated with procuring chemicals and utilities for physicochemical domestic wastewater treatment and water body purification (Al-Ajalin et al., 2020). To mitigate this issue, an energy-saving and low-cost alternative is urgently needed. Many methods have been proposed, and of particular interest to the researchers is employing multi-layering techniques utilizing natural substances (Freitas et al., 2018).

The muti-media-layering system (MML) is regarded as a highly innovative and technologically advanced system for household wastewater treatment and environmental protection, particularly in rural areas (Lamzouri et al., 2017; Latrach et al., 2018; Hong et al., 2019). Nevertheless, its potential extends to rapidly expanding urban areas where it can effectively treat domestic sewage. MML has proven to be highly efficient in removing contaminants from various sources, including domestic wastewater and river water, textile wastewater, and industrial effluents (Supriyadi et al., 2016; Latrach et al., 2018). The MML treatment system distinguishes itself from competing treatment systems due to its lower cost, excellent treatment performance, and capacity to accommodate elevated hydraulic loading rates. Additionally, the system exhibits high adaptability to various environmental conditions, rarely clogs, is easy to maintain, and boasts a long service life of over 20 years (An et al., 2016; Lamzouri et al., 2016).

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The MML typically consists of a media layer composed of various natural materials, including soil, zeolite, activated carbon, gravel, and more, making it an attractive option for researchers seeking sustainable wastewater treatment solutions. As other sewage treatment systems utilizing porous supports, the MML employs various mechanisms, including adsorption, filtration, nitrification, denitrification, predation, and microbial degradation, to effectively eliminate contaminants from domestic wastewater (Latrach et al., 2018).

The MML has received considerable global attention in recent times owing to its demonstrated success in several countries, including China (Guo et al., 2019; Tang et al., 2020), Marocco (Sbahi et al., 2020), and Thailand (An et al., 2016; Koottatep et al., 2021). However, the MML technology in Indonesia is scarcely used for domestic wastewater treatment. The MML utilizes physical and biochemical mechanisms to remove contaminants from domestic wastewater. Alternating aerobic and anaerobic phases in a permeable layer (PL) and media-mixture layer are the primary keys to removing nitrogen through nitrification and denitrification processes (An et al., 2016; Zhou et al., 2021). Both physical and chemical adsorption techniques have demonstrated successful removal of organic materials. Meanwhile, continuous aeration processes are required for organic matter removal through decomposition and heterotrophic aerobic metabolism (Zhou et al., 2021). Furthermore, Xiao et al. (2020) have discovered a synergistic adsorption and co-precipitation mechanism for efficiently removing total phosphorus (TP), which has shown excellent results. The composition and metabolism intensity of the microbial community in the MML are also critical factors in effectively removing pollutants from domestic wastewater (Zhou et al., 2021).

Domestic wastewater has a diverse array of contaminants, encompassing biochemical oxygen demand (BOD), chemical oxygen demand (COD), Suspended solids (SS), nitrogen (N), phosphorus (P), pathogenic organisms, microplastics, and more. BOD and COD are crucial parameters used to measure the concentration of organic matter in domestic wastewater and are widely utilized due to their relative ease of measurement. Elevated BOD and COD concentrations indicate correspondingly increased SS, N, and P levels. The presence of N and P in domestic wastewater can lead to eutrophication, posing a threat to aquatic organisms through the depletion of dissolved oxygen (DO) concentrations in the water. This study aims to examine the MML's ability to remove pollutants BOD and COD from domestic wastewater and identify the best MML based on the height of the filter media (gravel, zeolite, activated carbon, and silica sand) for removing household contaminants. By manipulating the height of the filter material in the MML, it is possible to determine the most optimal MML configuration for effectively eliminating household pollutants. The height of the filter media, especially the gravel media in the MML, greatly affects the removal of BOD_5 and COD. While the other media also have diverse functions in removing pollutants from domestic wastewater.

2. METHODOLOGY

2.1 Materials

The MML material used the study was acrylic, purchased from Jaya Raya Acrylic. The filter media consisted of zeolite, activated carbon, and silica sand supplied by Surabaya Filter Air. Gravel was collected from the nearby study site. pH was determined using the Benchtop pH meter PH-B200E, and temperature was measured using TP3001, both purchased from CV. Sumber Ilmiah Persada.

2.2 Study site description

The experiment was conducted in Siwalankerto, a rural area located in the Wonocolo Sub-District of Surabaya City, which has 40 neighborhood units with over 1,200 houses and 180 rented houses. This area is characterized by a relatively high population density and a substandard drainage system, as depicted in Figure 1. The domestic wastewater generated by residents is directly discharged into the drainage channel without prior treatment, leading to presence of contaminated water with black sediment.

2.3 Explication of MML system

The study was carried out throughout the COVID-19 pandemic, characterized by a significant shift in human behavior toward predominantly domestic settings at home. To address the issue of domestic wastewater generated by the inhabitants of Siwalankerto Village amidst the COVID-19 pandemic, a laboratory-scale MML system was developed. The MML reactor comprised two identical units, as depicted in Figure 2(a). The domestic wastewater treatment MML was made of 4 mm thick acrylic material. This study was conducted by continuously flowing wastewater into the MML reactor employing a predetermined flow rate of 20 L/day. Wastewater drainage to the storage tank is facilitated by a pump. Meanwhile, the influx of wastewater is facilitated by gravitational forces, with the assistance of valves that regulate the intake of each MML. The MML was filled with four media types (gravel, zeolite, activated carbon, and silica sand) with different diameters. The diameters of each media are gravel (±2-3 cm), zeolite (±1 cm), activated carbon (±0,45 cm), and silica sand (±0.25 cm). In this study, the height difference of the media (gravel and zeolite) was utilized as a variable variation for both MMLs, while activated carbon and silica sand served as PL. The objective behind the implementation of varying heights for the media was to evaluate the distinct capacities of each medium in the removal of BOD_5 and COD.

(a)

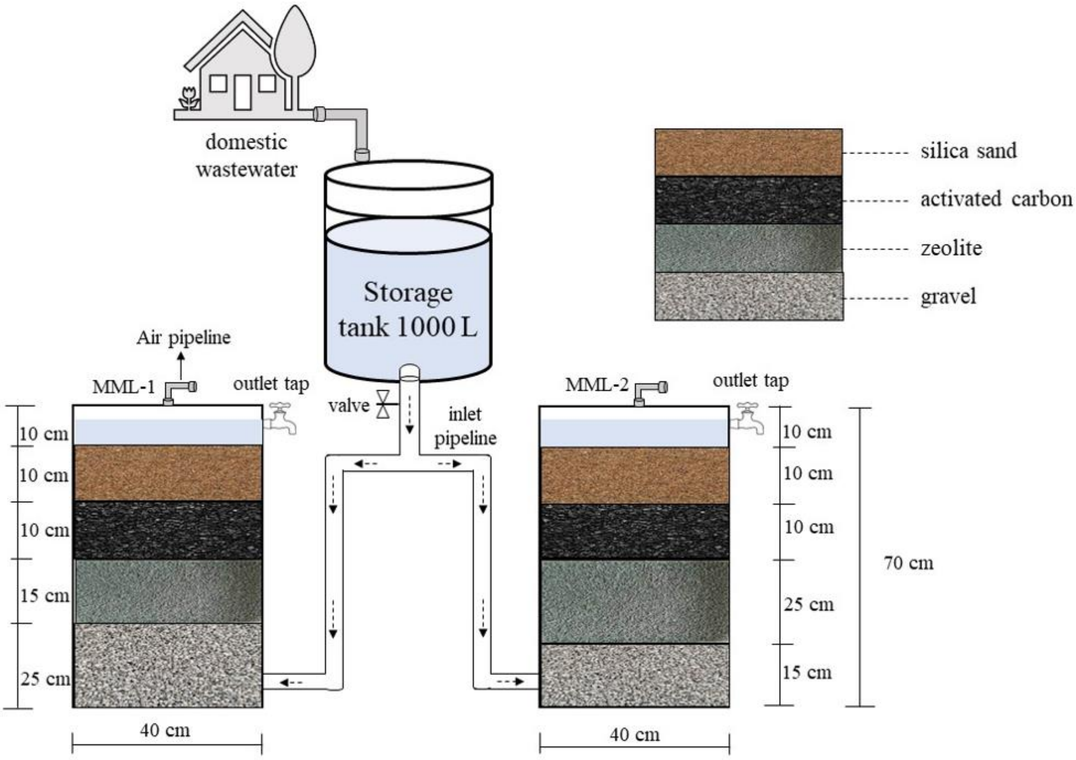
(b)

Figure 1. (a) The condition of domestic wastewater used as a sampling site; (b) Illustration of Google Earth for rural Siwalankerto, which has a high population density.

In MML-1, the gravel and zeolite media had 25 cm and 15 cm heights, respectively. Conversely, in MML-2, the gravel and zeolite media heights were reversed, with 15 cm and 25 cm, respectively. The activated carbon and silica sand heights remained consistent at 10 cm in both MML configurations. The selection of these filter media is based on their specific advantages. Gravel functions as an effective filter and substrate, fostering the growth of decomposing bacteria that facilitate the breakdown of organic matter in wastewater. In contrast, zeolite and activated carbon exhibit exceptional absorption capacities, enabling them to remove contaminants from the wastewater efficiently. In addition, silica sand serves as a filter medium and can be utilized as a substitute for the soil media often employed in previous MML systems. Replacement of filter media in MML is often carried out when there is a noticeable decline in removal efficiency and hydraulic conductivity.

The MML was designed to treat domestic wastewater with dimensions of 40 cm × 20 cm × 70 cm, with a total volume of every MML of 0.056 m^3. The domestic wastewater sampling used for this study only concentrated on five neighborhood units that generate numerous domestic wastewater. Based on the field data, it can be observed that the five neighborhood units under study consist of around 1,065 individuals residing in around 183 dwellings, together with an additional six rental houses. This population is responsible for generating domestic wastewater. All domestic wastewater from the five neighborhood units flows to a single point in the primary drainage channel, which serves as the sampling point (Figure 2(b)), before joining the leading drainage network.

(a)



(b)

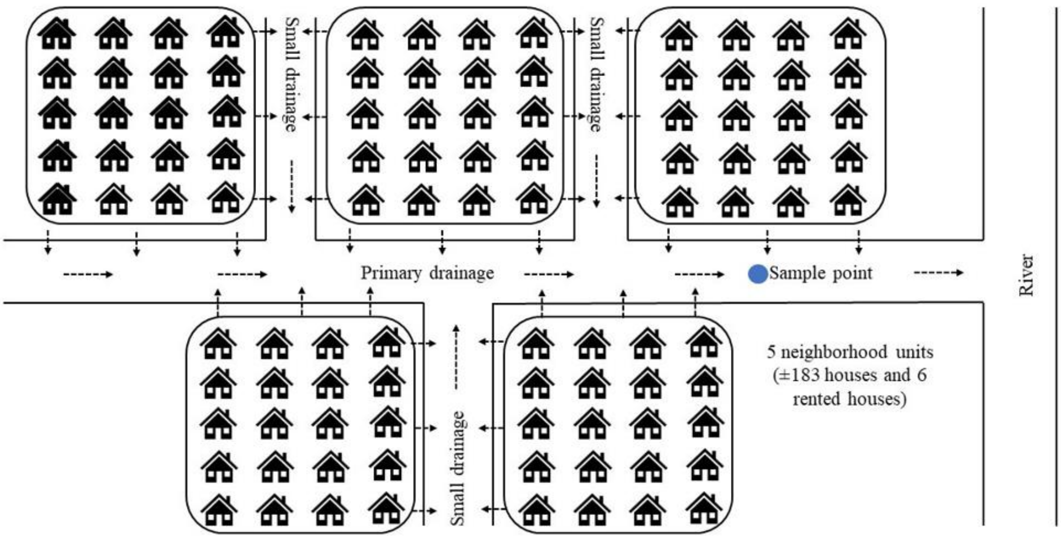


Figure 2. (a) Illustration of MML reactors in this study; (b) Illustrated distribution of domestic sewage and sampling point

2.4 Monitoring system and sampling period during the study

A total of 64 samples were collected for the two types of MML, with an average of 32 samples obtained for each system. The research data were collected during the early stages of the COVID-19 pandemic. Nevertheless, the study was primarily constrained by time limits for sample collection at the sampling location and subsequent laboratory analysis. Consequently, the research was limited to the collection of solely BOD_5 and COD data. The BOD_5 parameter was measured by determining the dissolved oxygen concentration in the sample with a 5-day incubation period. The COD parameter was analyzed using the open dichromate reflux method. Data analysis in this study involved calculating the mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Seeding and acclimatization

The BOD and COD reduction process relies on the action of microorganisms, which oxidize organic pollutants in the water. These microbes employ molecular oxygen to facilitate the decomposition of organic materials, producing carbon dioxide and water. The degradation process transpires via growth, death, decay, and cannibalism cycles. Various microorganisms participate in the breakdown of BOD and COD, including *Trichosporon cutaneum*, *Bacillus cereus*, *Klebsiella oxytoca*, *Pseudomonas* sp., as well as yeast strains such as *T. cutaneum*, etc (Meegoda et al., 2018).

This study's seeding and acclimatization were carried out for seven days, as shown in Table 1. Daily monitoring were conducted through COD testing and visual inspection to determine the growth of microorganisms within the MML. The adaptation process of microorganisms typically takes several days to reach a steady-state condition. Various techniques could be conducted to expedite the steady-state process, including adding decomposing microorganisms into the wastewater treatment MML. These microorganisms can be sourced from wastewater treatment plants (WWTP) or through effective microorganisms (EMs) liquid rich in microorganisms.

Table 1. COD analysis results during seeding and acclimatization

Day	Initial concentration (mg/L)	Effluent MML-1 (mg/L)	Effluent MML-2 (mg/L)
1	461.7	352.7	348.8
2	457.2	346.3	345.2
3	454.1	341.4	343.9
4	448.2	325.0	312.1
5	434.0	270.8	306.7
6	441.7	284.3	252.1
7	422.8	252.2	284.3

The acclimatization stage aims to obtain a stable microorganism culture capable of adapting to the specific characteristics of the liquid waste being evaluated. To accelerate the establishment of steady-state conditions for microorganisms, we introduced 1 liter of EM liquid into each MML on the first day of the study. The resulting COD number analysis directly reflects the growth of waste-decomposing microorganisms. In practice, samples from the MML were extracted and analyzed for COD to determine steady-state conditions. Steady-state conditions can be established when the COD removal efficiency exhibits less than 10% fluctuations (Hu and Grasso, 2004; Abu Shmeis, 2018). According to the COD number analysis, steady-state conditions were observed on the seventh day. This result indicates that the waste-decomposing microorganisms had stabilized and successfully adapted to their environment, enabling the progression to the running stage.

3.2 Domestic wastewater characteristics

Understanding wastewater's early properties is paramount in the context of a wastewater study. Furthermore, it can also determine the most suitable treatment technology for effectively treating wastewater. This study specifically focuses on observing the key parameters of the wastewater, such as BOD_5, COD, and pH, as showcased in Table 2, to ascertain its initial characteristics.

Table 2. Initial characteristic test results for domestic wastewater

Parameter	Quality standards*	Value
BOD_5 (mg/L)	30	230
COD (mg/L)	50	455
pH	6.0-9.0	6.7

*(East Java Governor, 2014)

Generally, the test results for domestic wastewater indicate significant deviations from the established standard quality limits, particularly regarding the BOD_5 and COD parameters. The recorded BOD_5 pollutant concentrations of 230 mg/L and COD of 455 mg/L suggest the presence of contamination in the residential wastewater of the area. The main contributors to this pollution are the escalating use of clean water and the lack of an adequate household wastewater management infrastructure. The elevated BOD_5 and COD values in domestic wastewater can be attributed to various factors, including the substantial presence of organic matter in wastewater, utilization of certain chemical products in households, improper disposal of industrial waste, population growth, and insufficient environmental awareness. Given these circumstances, selecting the MML for treating domestic wastewater is an ideal choice. This choice is supported by substantial research and empirical evidence, demonstrating its efficacy in efficiently eliminating BOD_5 and COD.

3.3 Potential of hydrogen (pH) value

The pH and temperature values were measured ex-situ using a portable multiparameter analyzer. Daily monitoring of domestic wastewater collected from the sampling site obtained the pH values in the two MMLs, ranging from 6.0-6.5, averaging 6.22. On the other hand, the average temperature value of domestic wastewater ranged from 30.2-30.4°C. Figure 3 shows the pH values for the two MMLs during the study period.

Lower pH values were observed for the two MMLs from day 4 to day 5, reaching a pH value of 6.0. Meanwhile, the temperature of domestic wastewater in the MML remained stable throughout the study period, ranging from 30-31°C. The decrease in pH value can be attributed to the fermentation process of organic matter and the presence of turbidity. Nevertheless, it is essential to note that a pH value of 6.0 falls within the acceptable range and complies with the established regulatory guidelines for household wastewater quality. The fermentation process can be optimized by monitoring the temperature remains within the ideal 30-35 °C range for the microorganisms involved (Manan and Webb, 2020).

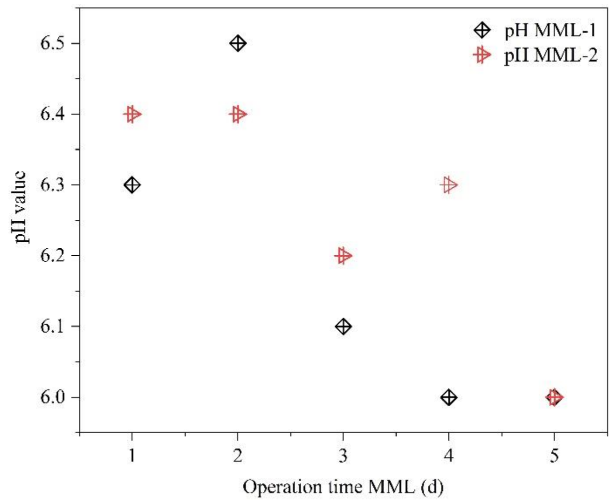


Figure 3. pH value during the studied

3.4 Effect of MML to Alleviate BOD_5 and COD

The effectiveness of MML in BOD_5 and COD removal is influenced by various factors, including the choice of filter media, hydraulic loading rate (HLR), and dimensions of the media mix layer. Previous research indicates that MSL successfully removes up to 98% of BOD_5 from domestic wastewater while achieving a COD removal rate of approximately 50% (Song et al., 2018; Hong et al., 2019). Table 3 demonstrates a significant decrease in the levels of BOD_5 and COD following treatment with MML.

Despite the study's relatively short duration of only five days, the removal efficiency for both parameters exhibited a notably high level. In MML-1, the removal rate exceeded 90%, while for MML-2, it surpassed 84%. These compelling findings demonstrate the successful application of the MML system in effectively removing contaminants from domestic wastewater. The data presented in Figures 4(a) and 4(b) provide compelling evidence of a strong association between the utilization of the MML and the reduction of contaminants in household wastewater. This reduction in pollutants is attributed to the growth and multiplication of waste-decomposing microorganisms in the MML, which is crucial in alleviating pollutant content in domestic wastewater. Furthermore, the filter media employed in both MMLs have a crucial role in reducing the levels of BOD_5 and COD pollutants through adsorption, filtration, and microbial degradation mechanisms.

Table 3. The removal of BOD_5 and COD levels after processing MMLs

Parameter	Initial concentration (mg/L)	Removal in MML-1 (mg/L)	Removal in MML-2 (mg/L)	Efficiency in MML-1 (%)	Efficiency in MML-2 (%)
BOD_5	227.34	73.55±0.22	90.45±0.36	68.02	60.67
	219.25	60.10±0.57	70.40±0.29	86.79	84.53
	224.47	48.55±0.36	68.40±0.15	78.89	70.26
	217.12	28.60±0.36	33.60±0.43	87.54	85.39
	205.05	20.60±0.43	39.50±0.15	95.47	82.83
COD	447.61	219.50±0.03	235.19±0.07	51.76	48.31
	464.66	156.76±0.06	172.56±0.12	65.55	62.07
	454.90	78.40±0.04	109.71±0.08	82.77	75.89
	439.24	31.40±0.06	47.07±0.05	93.10	89.65
	443.08	47.11±0.10	62.70±0.03	89.65	86.22

(a)

(b)

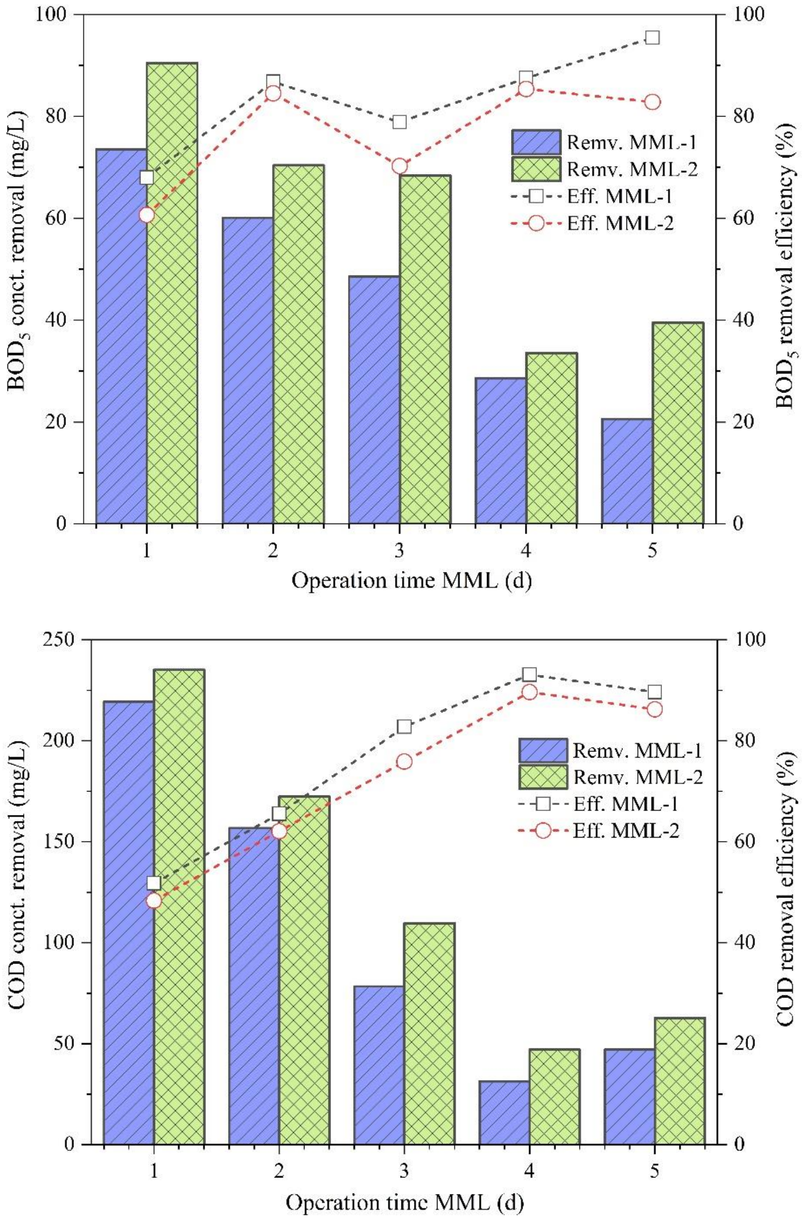


Figure 4. (a) Removal of BOD_5 and (b) removal of COD by MML

The BOD_5 and COD removal for the first day of the study was not too substantial. However, the following days showed significantly improved efficiency figures. The observation results indicate that the highest elimination rates for BOD_5 and COD levels were observed on the fourth and fifth days. MML-1 achieved the highest value for BOD_5 removal at 20.60±0.43 or 95.47% on the fifth day, whereas MML-2 reached 33.60±0.43 or 85.39% on the fourth day (Figure 4(a)). These results underscore the pronounced effectiveness of the MML in removing pollutants from domestic wastewater in a short period.

The highest COD removal rate occurred on the fourth day for both MMLs, with the highest allowance for COD in MML-1 and MML-2 being 31.40±0.06 (93.10%) and 47.07±0.05 (89.65%), respectively (Figure 4(b)). However, the removal efficiency values for BOD_5 and COD slightly decreased in both MMLs on the fifth day of the study, with a reduction of 82.83% for BOD_5 removal and 86.22% for COD removal. This decrease was due to a slight increase in temperature, which reached 32°C. The difference in the height of the gravel media between the MML-1 and MML-2 given the different results. The higher the gravel layer, the greater the removal rate for BOD_5 and COD, as the larger diameter of the gravel media allows more decomposing microorganisms to grow and multiply without causing clogging. Using filter media in MML demonstrated outstanding system performance regarding BOD_5 and COD removal. The MMLs alleviation of BOD_5 and COD is superior to that of the multi-layer artificial wetland, where the highest removal rate for BOD_5 was only 87.9% with an initial BOD_5 level of 207 mg/L and COD reaching 90.6% with an initial COD level of 381 mg/L (Lu et al., 2015).

While Hong et al. (2019) have argued that using an MML system for pollutant removal is somewhat complex, there remain unanswered questions. Other researchers have refuted this argument by demonstrating the effectiveness of MML. For instance, Sbahi et al. (2020) have reported that the use of MML for contaminant removal from domestic wastewater can significantly reduce pollutants, including more than 80% for BOD_5, ammonium (NH_4^+), nitrates (NO_3), total Kjeldahl nitrogen (TKN), and total nitrogen (TN), and up to 91% for orthophosphates (PO_4^{3-}) (TC, until 1.62 Log units).

3.5 Effect of pH on the BOD_5 and COD removal

The pH level is intricately linked to removing contaminants in domestic wastewater, specifically BOD_5 and COD, as depicted in Figure 5. A significant correlation exists between pH and the mitigation of BOD_5 and COD. Previous research has demonstrated that pH plays a fundamental role in reducing COD. Moreover, the decrease in BOD_5 and COD is influenced by various factors, including temperature and the dosage of sodium hypochlorite (NaOCl) (Danil et al., 2017).

Overall, Figure 5 demonstrates that when the pH value is at a pH of 6.0, the removal rates of BOD_5 and COD are higher. Specifically, the maximum BOD_5 removal efficiency above 85% (Figures 5(a) and 5(b)) and the maximum COD removal efficiency above 60% (Figures 5(c) and 5(d)) were observed on day 2 of the study when the pH ranged from 6.4-6.5. On the other hand, when the pH value reached 6.0, the MML-1 achieved a BOD_5 removal efficiency of 95.47%, and MML-2 reached 85.39% (Figures 5(a) and 5(b)). The COD removal efficiency was 93.10% in MML-1 and 89.65% in MML-2. This study suggests that pH 6.0 is optimal for removing BOD_5 and COD, as the pH value close to acidic conditions indicates that microorganisms' are effectively decomposing contaminants, resulting in higher pollutant removal rates. Nayl et al. (2017) suggested that the optimum pH value for COD and BOD removal percentage was 7.18. As bacteria are susceptible to pH, it is crucial to consider specific conditions when determining the optimal pH level for reducing BOD and COD.

3.6 Effect of BOD_5/COD ratio on the COD removal

The correlation between BOD and COD values might provide valuable insights in monitoring and operating urban wastewater treatment facilities. Measuring the BOD_5/COD ratio can identify the quantity of organic matter in wastewater and the microorganisms' ability to decompose waste. Moreover, the BOD_5/COD ratio indicates the organic matter output's impact on waste treatment, offering a comprehensive assessment of the success of wastewater treatment. Therefore, the BOD_5/COD ratio is a crucial factor in wastewater treatment as it provides information about the organic matter's biodegradability in wastewater. Figure 6 illustrates the effect of the BOD_5/COD ratio on COD removal.

Figure 6 provides insight into the feasibility level associated with treating domestic wastewater. In urban wastewater, the BOD_5/COD ratio typically ranges from 0.3 to 0.8 mg/L, indicating that the wastewater is easily treatable biologically. Conversely, if the BOD_5/COD ratio falls below 0.3, the waste contains several toxic components, necessitating acclimatized micro-organisms to stabilize the pollutants. Figures 6(a) and 6(b) show that the BOD_5/COD ratio ranges from 0.3 to 0.9, suggesting domestic wastewater is biologically easily treatable and requires no further processing. However, when the BOD_5/COD ratio is lower, the COD removal rate decreases, while a higher BOD_5/COD ratio leads to a higher COD removal rate, as shown on the fourth day of the study. It is important to note that the BOD_5/COD ratio's use in wastewater treatment varies depending on the level of wastewater treatment. Hence, managing biodegradable materials is paramount in evaluating the potential for short-term and long-term emissions on environmental sustainability.

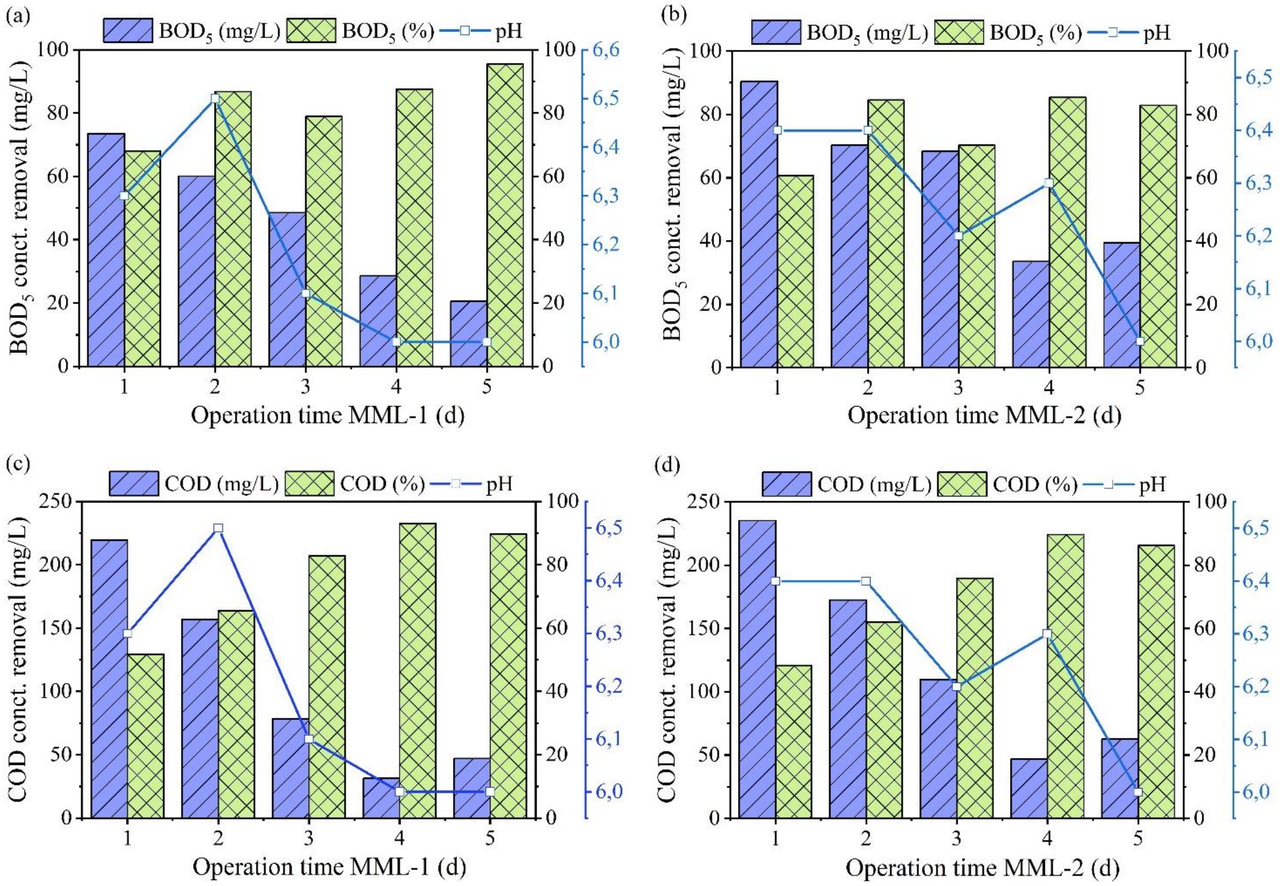


Figure 5. Effect of pH on the BOD_5 and COD removal

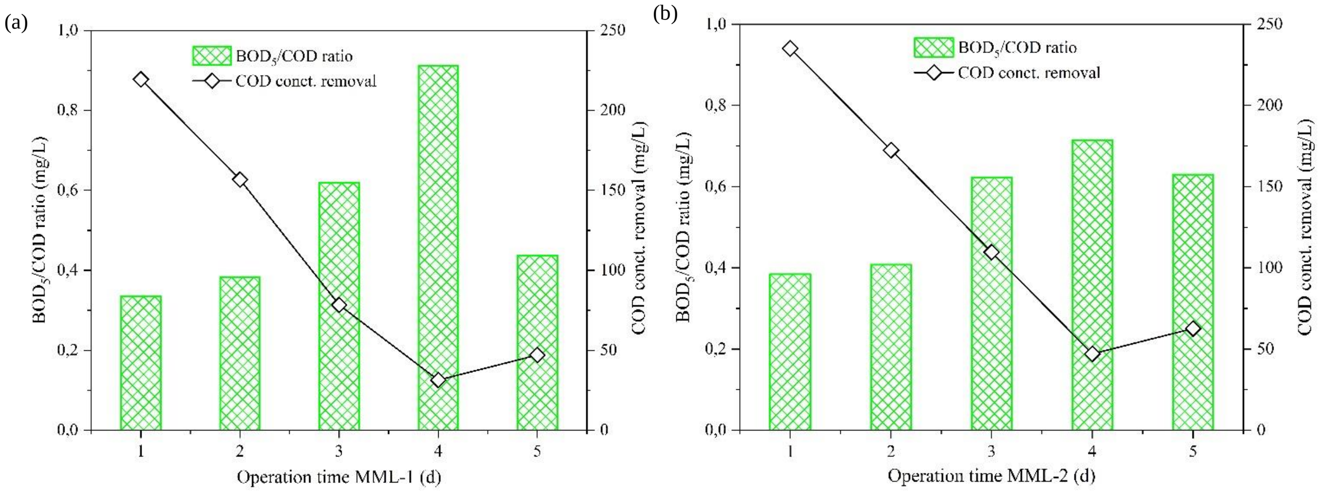


Figure 6. Effect of BOD_5/COD ratio on the COD removal

4. CONCLUSION

The use of MML for the treatment of domestic wastewater has exhibited significant efficacy in decreasing BOD_5 and COD pollutants. This is supported by the impressive removal efficiency rates, which showed MML-1 successfully removing over 93% of BOD_5 and COD, while MML-2 removed 85%. Notably, the greater depth of gravel in MML-1 resulted in more efficient removal of pollutants than in MML-2. The irregular surface of the gravel is responsible for this disparity, as it promotes the growth and multiplication of decomposing microorganisms on the media's surface. With increasing gravel media, more decomposing microorganisms thrive to break down contaminants. Hence, gravel assumes a significant role in MML in eliminating BOD_5 and COD from household wastewater.

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REFERENCES

Abu Shmeis RM. Water Chemistry and Microbiology. Volume 81: Comprehensive Analytical Chemistry. Elsevier B.V.; 2018.

Al-Ajalin FAH, Idris M, Abdullah SRS, Kurniawan SB, Imron MF. Evaluation of short-term pilot reed bed performance for real domestic wastewater treatment. Environmental Technology and Innovation 2020;20:Article No. 101110.

An CJ, McBean E, Huang GH, Yao Y, Zhang P, Chen XJ, et al. Multi-soil-layering systems for wastewater treatment in small and remote communities. Journal of Environmental Informatics 2016;27(2):131-44.

Danil R, Kirom MR, Qurtobi A. Effect of temperature and pH analysis about decreased levels of chemical oxygen demand in temperature-phased anaerobic digestion system with waste food substrates. e-Proceedings of Engineering 2017;4(2): 2174-80.

East Java Governor. Regulation of East Java Governor No. 52 of 2014 concerning amendments to East Java Governor Regulation No. 72 of 2013 concerning wastewater quality standards for industry and/or other business activities. East Java Governor; 2014.

Freitas TKFS, Almeida CA, Manholer DD, Geraldino HCL, de Souza MTF, Garcia JC. Review of utilization plant-based coagulants as alternatives to textile wastewater treatment. In: Muthu S, editor. Detox Fashion: Textile Science and Clothing Technology. Singapore: Springer; 2018.

Guo J, Jia XJ, Yang L, Zhang G, Zhang L. Effect of biosurfactant on ammonia removal from anaerobically digested swine wastewater by multi-soil-layering treatment bioreactors. Environmental Technology 2019;41(19):2510-7.

Hong Y, Huang G, An C, Song P, Xin X, Chen X, et al. Enhanced nitrogen removal in the treatment of rural domestic sewage using vertical-flow multi-soil-layering systems: Experimental and modeling insights. Journal of Environmental Management 2019;240:273-84.

Hu Z, Grasso D. Water analysis: Chemical oxygen demand. In: Worsfold P, Townshend A, Poole C, editors. Encyclopedia of Analytical Science. Elsevier; 2nd ed. 2004. p. 325-30.

Koottatep T, Pussayanavin T, Khamyai S, Polprasert C. Performance of novel constructed wetlands for treating solar septic tank effluent. Science of the Total Environment 2021;754:Article No. 142447.

Lamzouri K, Latrach L, Mahi M, Ouattar S, Bartali HE, Masunaga T, et al. Controlling biochemical oxygen demand in the multi-soil-layering using neural network tool. Journal of Materials and Environmental Sciences 2017;8(6):2013-8.

Lamzouri K, Mahi M, Ouatar S, Bartali E, Masunaga T, Latrach L, et al. Application of multi-soil-layering technique for wastewater treatment in Moroccan rural areas: Study of the operation process for an engineering design. Journal of Materials and Environmental Science 2016;7(2):579-85.

Latrach L, Ouazzani N, Hejjaj A, Mahi M, Masunaga T, Mandi L. Two-stage vertical flow multi-soil-layering (MSL) technology for efficient removal of coliforms and human pathogens from domestic wastewater in rural areas under arid climate. International Journal of Hygiene and Environmental Health 2018;221(1):64-80.

Liang J, Ning XA, Sun J, Song J, Hong Y, Cai H. An integrated permanganate and ozone process for the treatment of textile dyeing wastewater: Efficiency and mechanism. Journal of Cleaner Production 2018;204:12-9.

Liu Y, Ngo HH, Guo W, Peng L, Wang D, Ni B. The roles of free ammonia (FA) in biological wastewater treatment processes: A review. Environment International 2019;123:10-9.

Lu S, Pei L, Bai X. Study on method of domestic wastewater treatment through new-type multi-layer artificial wetland. International Journal of Hydrogen Energy 2015;40(34): 11207-14.

Manan MA, Webb C. Newly designed multi-stacked circular tray solid-state bioreactor: Analysis of a distributed parameter gas balance during solid-state fermentation with influence of variable initial moisture content arrangements. Bioresources and Bioprocessing 2020;7(1):1-18.

Meegoda JN, Li B, Patel K, Wang LB. A Review of the processes, parameters, and optimization of anaerobic digestion. International Journal of Environmental Research and Public Health 2018;15(10):Article No. 2224.

Nayl AEA, Elkhashab RA, El Malah T, Yakout SM, El-Khateeb MA, Ali MMS, et al. Adsorption studies on the removal of COD and BOD from treated sewage using activated carbon prepared from date palm waste. Environmental Science and Pollution Research 2017;24(28):22284-93.

Nonfodji OM, Fatombi JK, Ahoyo TA, Boya B, Baba-Moussa LS, Aminou T. Effects of $KMnO_4$ amounts on antibacterial properties of activated carbon for efficient treatment of northern Benin hospital wastewater in a fixed bed column system. International Journal of Hygiene and Environmental Health 2020;229:Article No. 113581.

Sbahi S, Ouazzani N, Latrach L, Hejjaj A, Mandi L. Predicting the concentration of total coliforms in treated rural domestic wastewater by multi-soil-layering (MSL) technology using artificial neural networks. Ecotoxicology and Environmental Safety 2020;204:Article No. 111118.

Sikiru S, Abiodun OJA, Sanusi YK, Sikiru YA, Soleimani H, Yekeen N, et al. A comprehensive review on nanotechnology application in wastewater treatment a case study of metal-based using green synthesis. Journal of Environmental Chemical Engineering 2022;10(4):Article No. 108065.

Song P, Huang G, An C, Shen J, Zhang P, Chen X, et al. Treatment of rural domestic wastewater using multi-soil-layering systems: Performance evaluation, factorial analysis, and numerical modeling. Science of the Total Environment 2018;644:536-46.

Supriyadi, Widijanto H, Pranoto, Dewi A. Improving quality of textile wastewater with organic materials as multi soil layering. IOP Conference Series: Materials Science and Engineering 2016;107:Article No. 012016.

Tang W, Li X, Liu H, Wu S, Zhou Q, Du C, et al. Sequential vertical flow trickling filter and horizontal flow multi-soil-layering reactor for treatment of decentralized domestic wastewater with sodium dodecyl benzene sulfonate. Bioresource Technology 2020;300:Article No. 122634.

Wang Y, Zheng K, Guo H, Tian L, He Y, Wang X, et al. Potassium permanganate-based advanced oxidation processes for wastewater decontamination and sludge treatment: A review. Chemical Engineering Journal 2023;452:Article No. 139529.

Xiao M, Hu R, Cui X, Gwenzi W, Noubactep C. Understanding the operating mode of FeO/Fe-Sulfide/H_2O systems for water treatment. Processes 2020;8(4):Article No. 409.

Zhou Q, Sun H, Jia L, Zhao L, Wu W. Enhanced pollutant removal from rural non-point source wastewater using a two-stage multi-soil-layering system with blended carbon sources: Insights into functional genes, microbial community structure, and metabolic function. Chemosphere 2021;275:Article No. 130007.

Adsorptive Removal of Chromium (VI) Ions from Aqueous Solution by Banana Pseudo Stem Adsorbent

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ABSTRACT

The presence of Cr ions in wastewater must be treated before being released into the environment due to its detrimental impact on both the environment and human health. In this study, the removal of Cr (VI) ions from an aqueous solution was investigated by adsorption using an adsorbent derived from agriculture wastes, banana pseudo stem. The adsorbent was prepared by oven-drying the banana stem waste at 105°C for 24 h. The surface structure of the adsorbent was characterized using scanning electron microscopy (SEM). Batch adsorption experiments were carried out to determine the removal efficiency of Cr (VI) ions based on four adsorption operation parameters: pH of the solution, adsorbent dosage, contact time and initial concentration of ion solution. At room temperature, the highest Cr (VI) ions removal of 88.2% was achieved using 0.5 g banana pseudo stem adsorbent, with an initial concentration of chromium solution of 500 ppm at pH 2 and after 90 min of contact time. For the equilibrium study, the experimental data were better fitted by the Langmuir isotherm model with a maximum adsorption capacity of 33.33 mg/g. Meanwhile, the kinetic isotherm was best fitted by the pseudo-second-order model. Therefore, the banana pseudo stem showed great potential as an efficient, low-cost and natural green adsorbent for Cr (VI) ions removal from an aqueous solution via adsorption.

1. INTRODUCTION

Heavy metal ions are being excessively released from industrial waste. One of the harmful metal ions that can easily be found in water sources is chromium. In the aquatic environment, this metal primarily exists in the forms of trivalent Cr (III) ions and hexavalent Cr (VI) ions. Compared to Cr (III) ion, Cr (VI) ion, requires more attention due to its poisonous, high solubility, mutagenicity, and carcinogenicity (Aharchaou et al., 2018). In humans, excessive dosage of chromium can lead to serious health issues such as ulcers, lung cancer, diarrhoea, lip and nasal irritation, asthma and kidney failure (Stambulska et al., 2018). Chromium is easily bio-accumulated at low concentrations in aquatic organisms. According to Speer et al. (2019), exposure to Cr (VI) ion in fish changed hatching times, causing DNA damage and reducing reproduction rates. Thus, removing Cr (VI) ions from water is essential for the health of all living creatures and the environment.

To date, many conventional and modern methods have been employed in the treatment of heavy metals removal from water and wastewater. Adsorption has been the most prominent due to its high removal efficiency, economical, cheaply accessible, simple operation and environmentally friendly (Sukmana et al., 2021). Recently, researchers are paying more attention to utilizing agricultural waste as the adsorbent feedstock for treating heavy metals problems in wastewater via the adsorption method. Adsorbents derived from walnut (Garg et al., 2023), soybean straw (Guo et al., 2021), corncob (Yang et al., 2018), sugarcane bagasse (Abilio et al., 2021), wheat straw (Song et al., 2021), and coconut husk (Hanafiah et al., 2020) have been applied in the study of removing Cr (VI) ions from water. Due to its renewable nature, using adsorbent made from agricultural waste can be both environmentally friendly and more cost-effective.

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Banana (*Musa* spp.) is Malaysia's second-largest cultivated fruit after durian. It is estimated about four times waste is generated for every cycle of banana production (Taib, 2019). Massive banana waste definitely creates a serious disposal issue, however, it is also a significant resource waste. The presence of abundance of lignocellulose polymers with different functional groups, (mainly hydroxyl, carboxyl and carboxylate group) in the banana biomass creates effective adsorption with heavy metal ions, dyes and other pollutants. Banana wastes like fruit peels, leaves and stems were converted into biochar and have been tested as an adsorbent for dyes and heavy metals removal (Baharim et al., 2023; Kokate et al., 2022; Liu et al., 2022).

Earlier, several studies were focusing on the Cr ions removal from aqueous solution using adsorbent and biochar or modified biochar derived from banana wastes. Selimin et al. (2022) reported in their study using banana blossom peels adsorbent with chemical treatment was able to achieve a maximum of 227.27 mg/g Cr (VI) adsorption capacity. Research by Payel et al. (2020) and Xu et al. (2018) using banana rachis biochar (carbonized at 650°C) and banana pseudostem biochar produced at 500°C for chromium removal achieved maximum adsorption capacities at 2,500 mg/g and 43.47 mg/g, correspondingly. Meanwhile, banana straw biochar loaded with magnesium chloride showed a high adsorption performance of Cr (VI) ions, with 125.0 mg/g capacity as reported by Li et al. (2020).

Even though the adsorption efficiency of the adsorbents significantly improved, the facts showed that biochar or modified biochar is non-economical since involving with high energy consumption for the carbonization procedure and utilization of hazardous chemical solution for pretreatment. Studies using natural banana waste adsorbent in removing Cr (VI) ions are still very limited. Therefore, this present study utilizes banana pseudo stem waste as biomass feedstock for producing low-cost, natural and unmodified adsorbent in removing Cr (VI) from an aqueous solution. The adsorbent was prepared by a simple and easy drying technique and without any modification or additional chemical pretreatment. The effectiveness of the banana pseudo stems adsorbent in removing Cr (VI) ions from an aqueous solution was investigated in batch mode of adsorption experiments. The adsorption operation parameters influencing Cr (VI) removal efficiency were examined, involving solution pH, adsorbent dosage, initial metal ion concentration and contact time. The experimental results were modelled using different isotherms and kinetic adsorption models.

2. METHODOLOGY

2.1 Materials

All chemicals used in this study were analytical grade (AR) reagents. 2.8287 g of $K_2Cr_2O_7$ (Systerm) was dissolved with 1,000 mL of distilled water to prepare a 1,000 ppm Cr (VI) standard stock solution. The stock solution was diluted with distilled water to the required concentration for further use in the adsorption experiment. HCl and NaOH were purchased from Sigma-Aldrich and HmbG Chemicals, respectively.

2.2 Preparation of banana pseudo stem adsorbent

The raw material, banana pseudo stems of *Musa paradisiaca* (BPS) was collected from a banana farm in Ijok, Selangor, Malaysia. The stems were washed with tap water multiple times and then cut into small pieces. After that, the stems were air-dried for five days and continued to dry at 105°C for 24 h using an oven (Venticell 55, USA). After being cooled, the produced BPS adsorbent was ground into powder, sieved at 1 mm and finally kept in a container prior to further use.

2.3 Characterization of banana pseudo stem adsorbent

The surface morphology of the BPS adsorbent was observed using scanning electron microscopy (SEM) (Zeiss supra 40, Germany) operated at 10 kV with 5.00 K magnification.

2.4 Adsorption experiments

At room temperature, the effectiveness of BPS adsorbent in removing Cr (VI) ions from aqueous solution was investigated through batch adsorption tests to determine the effects of adsorption factors, including pH, adsorbent dosage, starting concentration, and contact time. The maximum adsorption conditions were determined by changing the parameter studied while the other parameters were kept constant, as detailed in Table 1. The experiments were performed with 100 mL of Cr (VI) ions solution in a 250 mL conical flask. Prior experiment, the Cr (VI) ions solution was adjusted to a specific pH value with 0.1 M HCl or 0.1 M NaOH and was measured with a pH meter (Sartorius PB-10, China). The mixtures were agitated at 150 rpm in a temperature-controlled shaker (PROTHERM, USA) for the required time. After adsorption, the mixture was filtered and the absorbance of the Cr (VI) ions was measured with a complexing agent, 1,5-diphenyl-carbazide, at 350 nm wavelength using UV-Vis Spectrophotometer (Hitachi, Japan). Experiments were repeated three times, for the accuracy of the data analysis. The percentage removal, % R, of the Cr (VI) ions and the adsorption capacity, q_e, of the BPS were determined using the following equations.

$$\text{Percentage removal, \% R} = \left(\frac{C_0 - C_e}{C_0}\right) \times 100\% \quad (1)$$

$$\text{Adsorption capacity, } q_e = \left(\frac{C_0 - C_e}{m}\right) \times V \quad (2)$$

Where; C_0 is the initial concentration (ppm); C_e is the concentration at equilibrium (ppm); V is the volume (L); and m is the mass of the adsorbent (g).

2.5 Statistical analysis

The percentage removal of Cr (VI) ions was statistically analyzed using the IBM SPSS Statistics 27 software utilizing the Variance analysis (ANOVA) and Tukey's test for mean comparison at 95 % reliability ($p<0.05$). All data were presented as mean±standard deviation (SD).

Table 1. Conditions of batch adsorption experiments, performed at 25°C

Adsorption parameters	pH range	Adsorbent dosage range (g)	Initial concentration range (ppm)	Contact time range (min)
Effect of pH	2, 3, 4, 5, 6, 7, 8, 9, 10	0.5	100	60
Effect of adsorbent dosage	2	0.1, 0.2, 0.3, 0.4, 0.5, 0.6	100	60
Effect of initial concentration	2	0.5	100, 200, 300, 400, 500	60
Effect of contact time	2	0.5	500	10, 20, 30, 60, 90, 120

3. RESULTS AND DISCUSSION

3.1 Characteristic of banana pseudo stem adsorbent

The SEM micrographs of BPS adsorbent obtained before and after Cr (VI) ions adsorption are shown in Figure 1. Figure 1(a) demonstrates that before adsorption, the BPS particles have bundle-aligned fibres with rough lignocellulosic surface structures and uneven shapes, which can help the adsorption. Following the adsorption of metal ions, the micrograph in Figure 1(b) shows the microparticle adhesion to the fibre surface. Rigueto et al. (2021) who used banana pseudo stem in the adsorption of textile dye, also reported this same behaviour.

(a) (b)

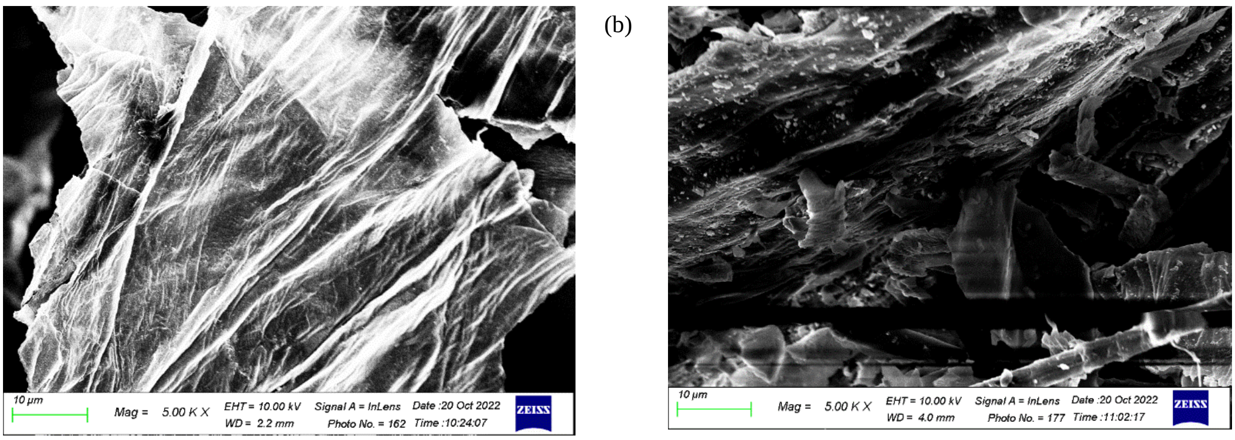

Figure 1. SEM micrograph of BPS adsorbent (a) before adsorption (b) after adsorption

3.2 Effect of adsorption experiments

The effect of four adsorption experiments is discussed in the following subsections.

3.2.1 Effect of pH

The pH of the solution affects the surface charges and the ionic state of functional groups on the adsorbent surface (Birhanu et al., 2020). Figure 2 depicts the pH solution effect from pH 2 to 10 on the removal percentage of Cr (VI) ions using the BPS adsorbent. Generally, the removal efficiency declines as the solution pH increases. The highest removal of 49.3% was achieved at pH 2, and the removal continuously decreased to 41.1% with the increase in pH to 10. Since high acidity condition creates stable cations and more formation of $HCr_2O_7^-$ which was conducive to the electron interaction between the adsorbent and chromate anions, allowing for more removal via reduction (Qasem et al., 2021). In contrast, the solubility of metal cations declines with higher pH levels, raising the probability of a precipitation occurrence. Gupta et al. (2018) reported similar trends of the pH effect on Cr (VI) removal were reported by using treated corncob biochar, with pH 2 showing the highest 93.0% removal.

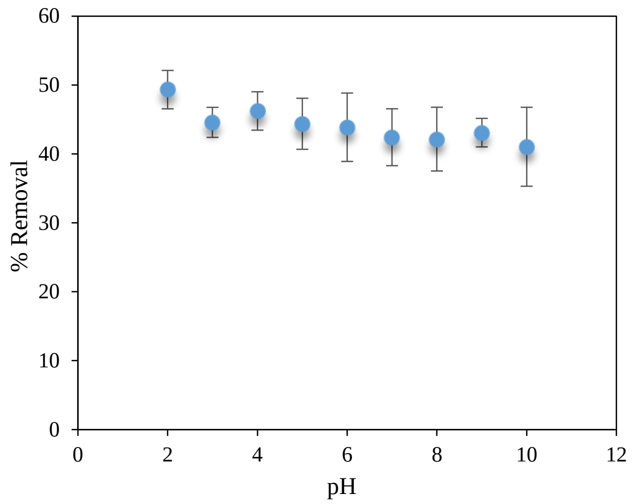

Figure 2. Effect of pH on the % removal of Cr (VI) ions

3.2.2 Effect of adsorbent dosage

The effect of adsorbent dosage on the Cr (VI) ions removal was examined by applying different dosages; 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g and the results are represented in Figure 3. The removal efficiency improved by 15.4% as the adsorbent increased from 0.1 to 0.4 g, and reached the highest removal of 46.1% with 0.5 g adsorbent dosage. There may be more surface area or active site and surface functional groups accessible for adsorption, which would explain the increase in Cr (VI) removal as adsorbent dosages increase (Garg et al., 2023). Further dosage caused no noticeable increase in the percentage removal. At this point, there were limited Cr (VI) ions in the aqueous solution which resulted in numerous unused active sites, thus the removal efficiency becoming constant or reducing. Similar adsorbent dosage effect findings on Cr (VI) ions removal have recently been published by Birhanu et al. (2020) and Bayuo et al. (2019) using groundnut shell and Ethiopian Odaracha adsorbent, respectively.

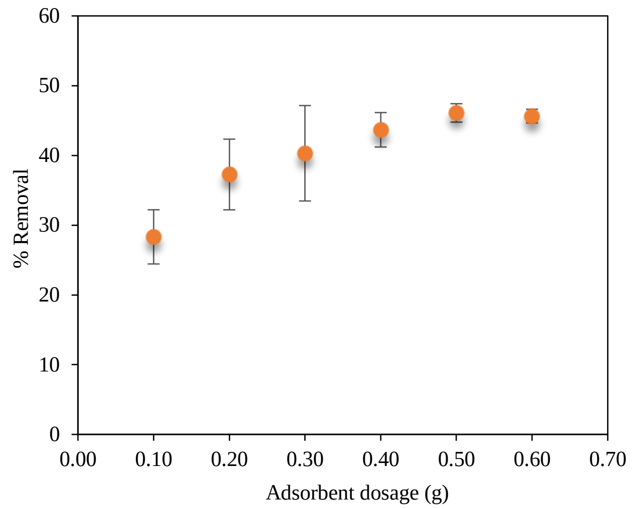

Figure 3. Effect of adsorbent dosage on the % removal of Cr (VI) ions

3.2.3 Effect of initial concentration

The initial Cr (VI) ions concentration plays an essential role as a driving force in reducing mass transfer resistance between the adsorbent's surface and the metal solution. Figure 4 shows the effect of different Cr (VI) initial concentrations on the removal efficiency. Cr (VI) ions removal was drastically increased by 35.0% with the increase in initial concentration from 100 ppm to 300 ppm. This observation might be caused by the adsorbent surface having a large number of active sites available, which would increase the Cr (VI) adsorption (Jock et al., 2021). The percentage removal continued to increase by 6.4% at 400 ppm and thereafter achieved the highest removal of 88.4% at 500 ppm. At 600 ppm, the removal efficiency of Cr (VI) decreases as insufficient availability of active sites, thus causing the saturation of the adsorbent's surface at equilibrium. Similar initial Cr (VI) concentration effects on removal trends were observed for absorbents prepared from iron-based solid waste and bentonite clay by Qi et al. (2023) and Jock et al. (2021), respectively.

3.2.4 Effect of contact time

The effect of contact time was investigated in order to determine the equilibrium time for the percentage removal of Cr (VI) ions. The experiments were run with various contact times ranging from 10 min to 120 min, and the outcomes are shown in Figure 5. The trend of Cr (VI) removal was gradually increased by 1.5% from 10 min to 60 min. This might be explained by the adsorbent's numerous available active sites, which allow for efficient adsorption. Then, the percentage removal reached the highest of 91.9% at

90 min indicating the equilibrium of the adsorption was established. Further, a longer time period will hinder any additional adsorption due to the adsorbate's difficulty to find accessible unoccupied sites. Bayuo et al. (2019) reported a similar result on the contact time effect for the Cr (VI) removal using groundnut shell adsorbent. However, with lower maximum removal (81.6%) and a longer time for achieving equilibrium (120 min) as compared to this current study.

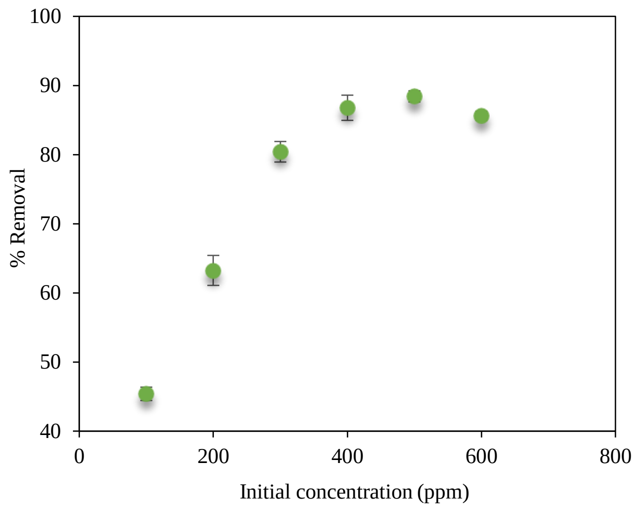

Figure 4. Effect of initial concentration on the % removal of Cr (VI) ions

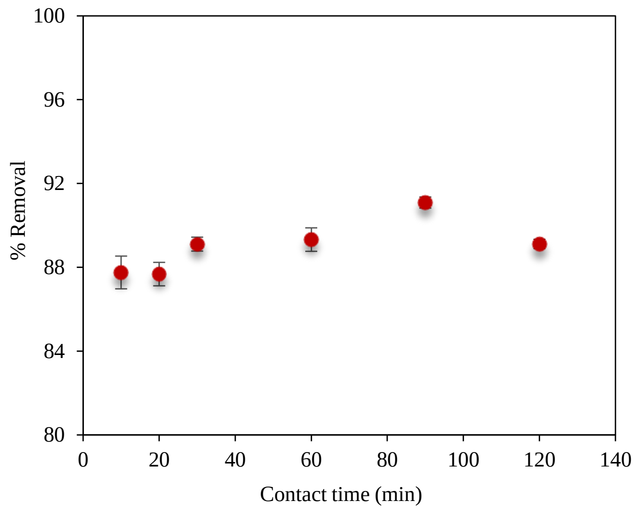

Figure 5. Effect of contact time on the % removal of Cr (VI) ions

3.3 Adsorption isotherm

Adsorption isotherm describes the adsorption distribution of Cr (VI) ions onto the surface of the BPS adsorbent solid phase of the biochar under equilibrium conditions. Two isotherm models; Langmuir and Freundlich were employed for the adsorption isotherm study. The Langmuir model assumes the adsorbate creates a monolayer on the homogenized adsorbent surface and acts as an independent entity. Meanwhile, the Freundlich model is used to simulate multilayer adsorption on heterogeneous surfaces. The isotherms are expressed by the linear equation as follows:

$$\text{Langmuir:} \quad \frac{1}{q_e} = \frac{1}{bC_e q_m} + \frac{1}{q_m} \tag{3}$$

$$\text{Freundlich:} \quad \log q_e = \frac{1}{n}\log C_e + \log K_F \tag{4}$$

Where; q_e (mg/g) is the amount of Cr (VI) ions adsorbed per unit mass of adsorbent in equilibrium, C_e (mg/L) is the Cr (VI) ions concentration at equilibrium. q_m (mg/g) and b (L/mg) are the maximum monolayer adsorption capacity and Langmuir constant, respectively. Meanwhile, K_F ((mg/g)(L/mg)1/n) is the Freundlich constant which is related to adsorption capacity and 1/n indicates the adsorption intensity. If 1/n=1, the separation within the two phases is not dependent on the concentration. If the value of 1/n<1, it shows normal adsorption. If 1/n>1, it shows cooperative adsorption.

Figure 6 shows data from equilibrium adsorption tests fitted using both isotherm equations. By comparing the linear regression correlation coefficient, R^2, the Langmuir model is better fitted to the experimental data, which concludes the monolayer adsorption of Cr (VI) ion onto the homogenous BPS adsorbent's surface. According to the Langmuir model, the maximal adsorption capacity is 33.33 mg/g. Bayuo et al. (2019) and Martín et al. (2016) reported similar adsorption isotherm results for the Cr (VI) ions removal using groundnut shells and Canary banana peels.

3.4 Adsorption kinetics

Adsorption kinetics provides crucial details regarding the reaction mechanism and the reaction's rate-limiting stage. This study applied two common models, pseudo-first-order and pseudo-second-order, to obtain the best-fitted kinetic model for the Cr (VI) ion's adsorption onto the adsorbent surface. Equations used to express the linearized pseudo-first-order and pseudo-second-order kinetic are given below:

$$\text{Pseudo-first-order:} \quad \ln(q_e - q_t) = \ln q_e - k_1 t \tag{5}$$

$$\text{Pseudo-second-order:} \quad \frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{6}$$

Where; q_e (mg/g) and q_t (mg/g) are the amount of Cr (VI) ions adsorbed in equilibrium and given time, respectively. k_1 (min^{-1}) and k_2 (g/mg·min) are the pseudo-first-order constant and pseudo-second-order constant, respectively.

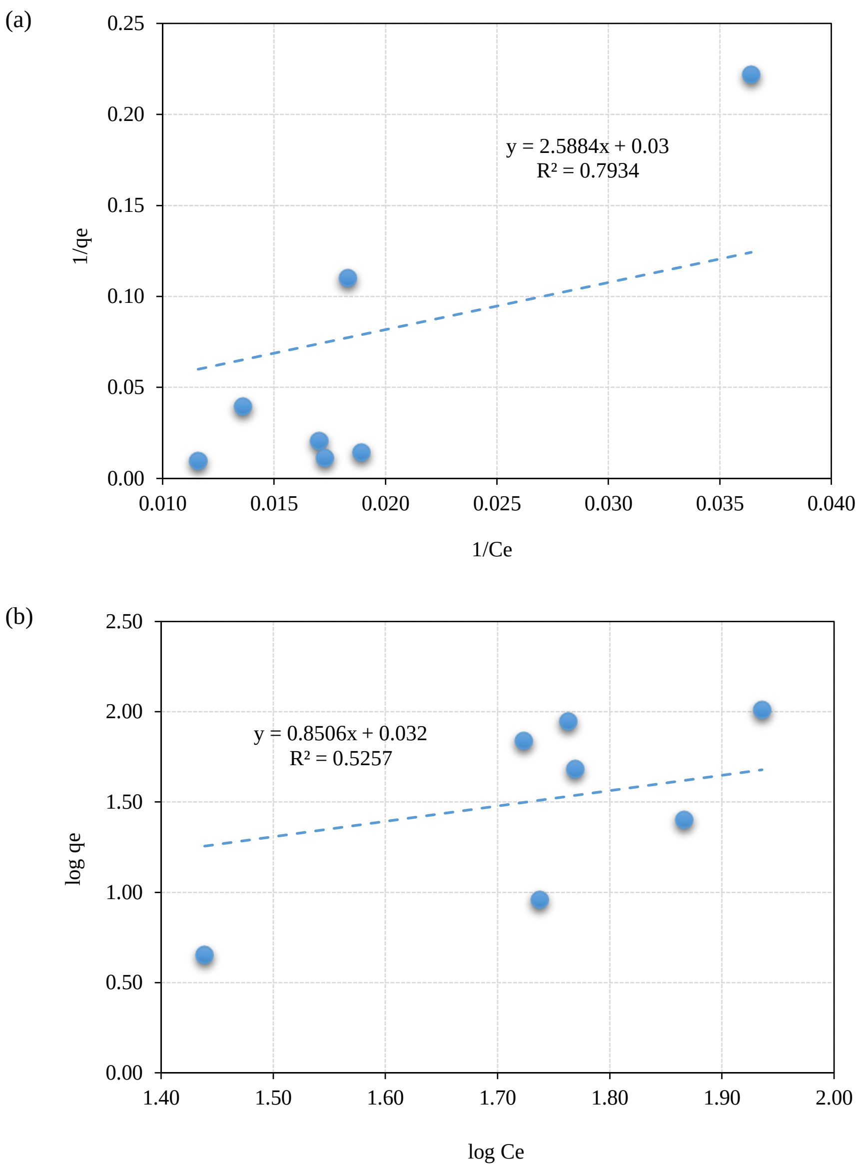

Figure 6. Linearized isotherm equation based on, (a) Langmuir; (b) Freundlich model for Cr (VI) ions adsorption

Figure 7 presents the experimental data results obtained in the kinetic study of Cr (VI) ions adsorption by BPS adsorbent. The correlation coefficient, R^2, demonstrates that the pseudo-second-order model has better fitness than the pseudo-first-order model. This implies that adsorption is influenced by the way Cr (VI) ions interact with the adsorbent surface. Additionally, the outcomes demonstrate the presence of vacant adsorption sites on the banana stem surface significantly affects the adsorption rate as opposed to the number of metal ions adsorbed. This study's findings support earlier research on Cr (VI) adsorption using various adsorbents that follow pseudo-second-order kinetics (Badessa et al., 2020; Garg et al., 2023).

3.5 Adsorption capacity comparison of various agricultural waste adsorbents

Table 2 compares the current study's adsorption capacity (q_m) for Cr (VI) ions to those recently reported by other researchers utilizing different adsorbents without chemical treatment or modifications applied. Apparently, banana pseudo stem adsorbent has a better adsorption capacity than some of these adsorbents while being comparable to others. The different adsorption capacities of these adsorbents can be attributed to a variety of factors.

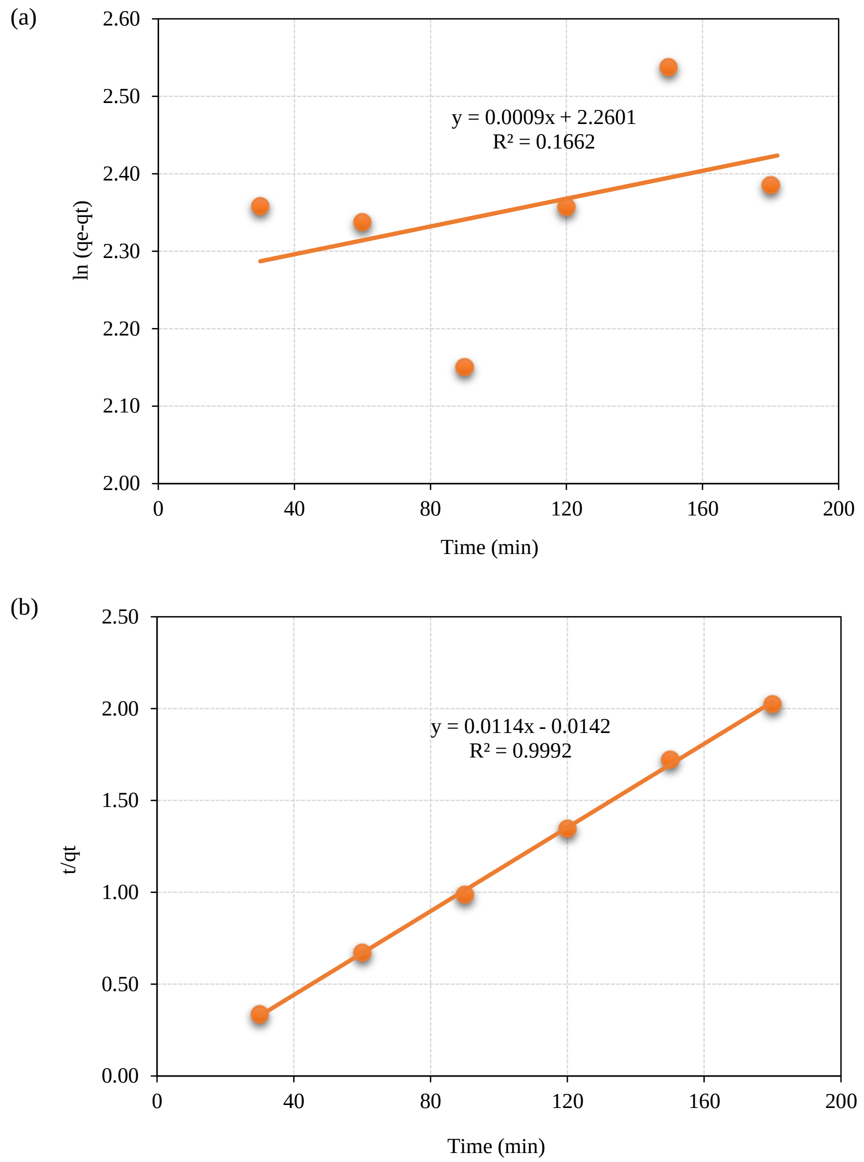

Figure 7. Kinetic of Cr (VI) ions adsorption by, (a) pseudo-first-order; (b) pseudo-second-order models

Table 2. Comparative analysis of the Cr (VI) adsorption capability of several adsorbents

Types of agriculture waste adsorbent	Adsorption capacity, q_m (mg/g)	References
Canary banana peels	10.09	Martín et al. (2016)
Palm fiber	6.00	Abubeah et al. (2018)
Groundnut shell	3.79	Bayuo et al. (2019)
Cranberry kernel shell	6.81	Parlayici and Pehlivan (2019)
Corncob	0.54	Melese et al. (2020)
Wheat straw and *E. adenophorum*	89.22	Song et al. (2021)
Sugarcane bagasse	1.49	Abilio et al. (2021)
Banana pseudo stem	33.33	Current study

4. CONCLUSION

In this study, banana pseudo stem adsorbent showed great potential for removing Cr (VI) ions from an aqueous solution. From the batch adsorption experiments, the produced adsorbent successfully removed the Cr (VI) ions up to 91.9% with the following optimal adsorption parameters, pH 2, the dosage of 0.5 g, initial Cr (VI) concentration of 500 ppm and 90 min contact time. The removal percentage increases with declining pH, rising adsorbent dosage and initial concentration, and longer contact times. The Langmuir isotherm model better fit the equilibrium experimental results, with a maximum monolayer adsorption capacity (q_m) of 33.33 mg/g. The adsorption kinetics was best described by the pseudo-second-order kinetic model, indicating the physical adsorption. In conclusion, banana pseudo stem wastes offer an attractive, eco-friendly, simple, and economically alternative adsorbent for treating and removing Cr (VI) ions from wastewater. It is recommended to convert banana pseudo stem as biochar and compare its adsorption and Cr (VI) ions removal potential to this current study. In keeping with government policy and the Sustainable Development Goals (SDG), it is expected that this study would aid the related industries to reduce the negative effects of banana plant waste and chromium contamination on the environment, public health, economy, and society.

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REFERENCES

Abilio TE, Soares BC, José JC, Milani PA, Labuto G, Carrilho ENVM. Hexavalent chromium removal from water: Adsorption properties of in natura and magnetic nanomodified sugarcane bagasse. Environmental Science and Pollution Research 2021;28:24816-29.

Abubeah R, Altaher H, Khalil TE. Removal of hexavalent chromium using two innovative adsorbents. Environmental Engineering and Management Journal 2018;17(7):1621-34.

Aharchaou I, Py JS, Cambier S, Loizeau JL, Cornelis G, Rousselle P, et al. Chromium hazard and risk assessment: New insights from a detailed speciation study in a standard test medium. Environmental Toxicology and Chemistry 2018;37(4):983-92.

Badessa TS, Wakuma E, Yimer AM. Bio-sorption for effective removal of chromium (VI) from wastewater using *Moringa stenopetala* seed powder (MSSP) and banana peel powder (BPP). BMC Chemistry 2020;14:Article No. 71.

Baharim NH, Sjahrir F, Taib RM, Idris N, Daud TAT. Methylene blue adsorption by acid post-treated low temperature biochar derived from banana (*Musa Acuminata*) pseudo stem. Sains Malaysiana 2023;52(2):547-61.

Bayuo J, Pelig-Ba KB, Abukari MA. Adsorptive removal of chromium (VI) from aqueous solution unto groundnut shell. Applied Water Science 2019;9:Article No. 107.

Birhanu Y, Leta S, Adam G. Removal of chromium from synthetic wastewater by adsorption onto Ethiopian low-cost Odaracha adsorbent. Applied Water Science 2020;10:Article No. 227.

Garg R, Garg R, Sillanpää M, Alimuddin, Khan MA, Mubarak NM, et al. Rapid adsorptive removal of chromium from wastewater using walnut-derived biosorbents. Scientific Report 2023;13:Article No. 6859.

Guo X, Hu W, Gu Z, Li J, Xie Z, Fang C, et al. Enhanced removal of aqueous chromium (VI) by KOH-activated soybean straw-based carbon. Water, Air and Soil Pollution 2021;232:Article No. 484.

Gupta GK, Ram M, Bala R, Kapur M, Mondal MK. Pyrolysis of chemically treated corncob for biochar production and its application in Cr(VI) removal. Environmental Progress and Sustainable Energy 2018;37:1606-17.

Hanafiah SFM, Salleh NFM, Ghafar NA, Shukri NM, Kamarudin NHN, Hanapi M, et al. Efficiency of coconut husk as an agricultural adsorbent in removal of chromium and nickel ions from aqueous solution. IOP Conference Series: Earth and Environmental Science 2020;596:Article No. 012048.

Jock AA, Oboh IO, Inyang UE, Ganchok LP, Adeku O. Chromium and nickel metal ions removal from contaminated water using Nigerian bentonite clay. Water Practice and Technology 2021;16(3):825-36.

Kokate S, Parasuraman K, Prakash H. Adsorptive removal of lead ion from water using banana stem scutcher generated in fiber extraction process. Results in Engineering 2022;15:Article No. 100439.

Li A, Deng H, Jiang Y, Ye C. High-efficiency removal of Cr(VI) from wastewater by Mg-loaded biochars: Adsorption process and removal mechanism. Materials (Basel) 2020;13(4):Article No. 947.

Liu X, Li G, Chen C, Zhang X, Zhou K, Long X. Banana stem and leaf biochar as an effective adsorbent for cadmium and lead in aqueous solution. Scientific Reports 2022;12:Article No. 1588.

Martín RD, Mouhaffel AG, Lisperguer RC, Rio CD. Kinetic study of absorption of chromium (VI) using canary bananas peels in contaminated water. International Journal of Innovation and Scientific Research 2016;22:139-45.

Melese T, Chala K, Ayele Y, Abdisa M. Preparation, characterization of raw corncob adsorbent for removal of heavy metal ions from aqueous solution using batch method. Africa Journal of Pure and Applied Chemistry 2020;4(4):81-90.

Parlayici Ş, Pehlivan E. Comparative study of Cr (VI) removal by bio-waste adsorbents: Equilibrium, kinetics, and thermodynamic. Journal of Analytical Science and Technology 2019;10:Article No. 15.

Payel S, Hashem MA, Sarker M, Nur-A-Tomal AS. Chromium adsorption on banana adsorbent from tannery wastewater: Optimization, isotherm, kinetics and desorption studies. Textile and Leather Review 2020;3(3):118-34.

Qasem NAA, Mohammed RH, Lawal DU. Removal of heavy metal ions from wastewater: A comprehensive and critical review. npj Clean Water 2021;4:Article No. 36.

Qi J, Li B, Zhou P, Su X, Yang D, Wu J, et al. Study on adsorption of hexavalent chromium by composite material prepared from iron-based solid wastes. Scientific Report 2023;13:Article No. 135.

Rigueto CVT, Alessandretti I, da Silva DH, Rosseto M, Loss RA, Geraldi CAQ. Agroindustrial wastes of banana pseudo-stem as adsorbent of textile dye: Characterization, kinetic and equilibrium studies. Chemistry Africa 2021;4(2):1069-78.

Selimin MA, Latif AFA, Er YC, Muhamad MS, Basri H, Lee TC. Adsorption efficiency of banana blossom peels (*Musa acuminata* Colla) adsorbent for chromium (VI) removal. Materials Today: Proceedings 2022;57(3):1262-8.

Song D, Pan K, Tariq A, Azizullah A, Sun F, Li Z, et al. Adsorptive removal of toxic chromium from waste-water using wheat straw and *Eupatorium adenophorum*. PLoS ONE 2021;11(12):e0167037.

Speer RM, Wise SS, Croom-Perez TJ, Aboueissa AEM, Martin-Bras M, Barandiaran M, et al. A comparison of particulate hexavalent chromium cytotoxicity and genotoxicity in human and leatherback sea turtle lung cells from a one environmental health perspective. Toxicology and Applied Pharmacology 2019;376:70-81.

Stambulska UY, Bayliak MM, Lushchak VI. Chromium (VI) toxicity in legume plants: Modulation effects of rhizobial symbiosis. Biomed Research International 2018;2018:Article No. 8031213.

Sukmana H, Bellahsen N, Pantoja F, Hodur C. Adsorption and coagulation in wastewater treatment: Review. Progress in Agricultural Engineering Sciences 2021;17(1):49-68.

Taib RM. Pyrolysis of Banana Pseudo-Stem and Leaf through Fast and Slow Processes [dissertation]. Penang, Universiti Sains Malaysia; 2019.

Xu S, Yu W, Liu S, Xu C, Li J, Zhang Y. Adsorption of hexavalent chromium using banana pseudostem biochar and its mechanism. Sustainability 2018;10:Article No. 4250.

Yang YM, Chen N, Feng C, Li M, Gao Y. Chromium removal using a magnetic corncob biochar/polypyrrole composite by adsorption combined with reduction: Reaction pathway and contribution degree. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2018;556:201-9.

Improving the Treatment of Saline Wastewater from Shrimp Farms Using Hybrid Constructed Wetlands Models toward Sustainable Development

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ABSTRACT

This study investigated a feasible model for treating actual shrimp farm wastewater at a pilot scale that could be applied to farms in the Mekong Delta area. The research was carried out using a hybrid constructed wetlands (HCWs) model, which included a floating constructed wetland (FCW, total area of 1,500 m^2) and a horizontal sub-surface constructed wetland (HSCW, total area of 400 m^2). The HCWs were cultivated with native plants including: *Scirpus littoralis* Schrab, *Cyperus alternifolius*, and *Paspalum vaginatum*. These plants are all adapted to the high salinity levels of shrimp farm wastewater. The system was operated for 30 days to treat shrimp farm effluent. Results indicated that the model effectively removed organic matter and nitrogen compounds from the wastewater. The treated wastewater had low concentrations of COD (10.0-15.4 mg/L), BOD_5 (7.1-12.5 mg/L), NH_4^+-N (0.04-1.11 mg/L), and TN (0.17-1.83 mg/L), which met the reliable conditions for reuse or safety requirements for discharge to aquatic systems. The findings of this study have significant implications for the sustainable management of shrimp farm wastewater in the Mekong Delta area. The HCWs model is a feasible and effective way to treat this type of wastewater, and it could be adapted to other regions facing similar challenges.

1. INTRODUCTION

Shrimp farming has become an important source of income and food security in many countries around the world, with global production reaching over 9 million tons in 2022 (FAO, 2023). However, the rapid expansion of shrimp farms has also led to significant environmental concerns, particularly with regard to the discharge of wastewater into surrounding ecosystems. Shrimp pond farming wastewater is known to contain high levels of nutrients, organic matter, and potentially harmful substances such as antibiotics and heavy metals (Boopathy et al., 2015; Li et al., 2022), which can lead to water pollution, eutrophication, and other negative impacts on aquatic ecosystems and human health (Iber and Kasan, 2021). In addition, the wastewater has high salinity concentrations, which can further exacerbate the problem of water pollution. The discharge of high-salinity wastewater from shrimp farms can result in soil and water salinization, affecting the growth and productivity of nearby crops and other vegetation (Braaten and Flaherty, 2001; Cardoso-Mohedano et al., 2018). As such, the management of shrimp farming wastewater has become an urgent priority for both environmental and economic reasons.

Various wastewater treatment solutions have been proposed and implemented to mitigate the negative impacts of shrimp farming wastewater. For instance, membrane bioreactors, advanced oxidation processes, integrated recirculating aquaculture systems (RAS), micro and biological treatment methods (Meril et al., 2022; Ng et al., 2018; Visvanathan et al., 2008). These conventional wastewater treatment methods have been found to be

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ineffective for treating shrimp farming wastewater due to their high operating cost and low effectiveness for large-scale farming. As a result, there is a need for new, innovative approaches to shrimp farming wastewater treatment.

One potential solution is the use of salt-tolerant plants for phytoremediation of shrimp farming wastewater. Salt-tolerant plants are able to tolerate the high salinity levels found in shrimp farming wastewater and can effectively remove pollutants through various mechanisms such as uptake, adsorption, and transformation (Lymbery et al., 2013; Szota et al., 2015). Pham et al. (2021) investigated the effectiveness of using the wetland system planted with *Scirpus littoralis* to treat shrimp farming wastewater. The system was operated with the high loading rate (HLR) of 1.54 m/d, the hydraulic retention time (HRT) of 1.31 h, and the salinity of 1.5%. The study found that the system achieved complete removal of nitrite and significant reductions in nitrate and COD, with reductions of 78% and 76%, respectively. In 2018, Trang and co-work studied salt tolerance between the two species: *Scirpus littoralis* and *Typha orientalis* (Trang et al., 2018). A completely randomized factorial design with three replications was used to arrange two plant species and six salinity concentrations (0, 5, 10, 15, 20, 30‰). As a result, *Scirpus littoralis* can be considered the ideal choice for a biofilter in the integration of constructed wetlands and marine shrimp production for sustainable aquaculture.

Another study found that *Juncus maritimus* had a notable horizontal flow floating treatment saltmarsh (FTS) effect on the removal of significant components of aquaculture effluent at low hydraulic retention times (Cicero-Fernandez et al., 2022). As a result, total organic carbon increased by 55%, turbidity by 53%, dissolved oxygen increased by 19%, total phosphorus increased by 86%, total suspended solids increased by 82%, and biochemical oxygen demand increased by 78%. It has been determined that certain characteristics of the native *Juncus maritimus* guarantee a 75-100% survival rate in waters with salinities as high as 38 g/L. Overall, this approach has shown promising results in laboratory and field trials and has the potential to be a cost-effective and environmentally-friendly solution for shrimp farming wastewater treatment.

In this context, the current work explores how hybrid-built wetlands (HCWs) models might be used to treat saline wastewater from shrimp farms in order to promote sustainable development. The use of local salt-tolerant plants in constructed wetlands is a promising solution for the treatment of effluents from a semi-intensive shrimp farm. This research aims to remove organic matter and nitrogen compounds from shrimp farm wastewater by using HCWs models. Out flow wastewater met reliable conditions for reuse or safety requirements when discharged into aquatic systems.

2. METHODOLOGY

2.1 Description of the research area

The experiment was carried out Bac Lieu Province - Mekong Delta which located in southern of Vietnam. The research area has a humid tropical and sub-equatorial climate condition. There are two seasons in a year which are rainy season (May to October) and hot season (November to April). The average temperature varies from 24°C to 34°C. The study was conducted during the duration of March to April, there was almost no rain during the experimental time (the average rain fall was 2 mm/month). Therefore, the salinity and water quality of shrimp farm was not much affected by rain in the experimental time.

2.2 Description of the hybrid constructed wetlands (HCWs)

The HCWs system in this study was established at a shrimp farm in Vinh Hau ward, Hoa Binh District, Bac Lieu Province, Vietnam. The schematic diagram of the system is shown in Figure 1. The HCWs consisted of three parts in sequence: an equalization pond, a floating constructed wetland (FCW), and a horizontal sub-surface constructed wetland (HSCW). The specific parameters of the HCWs were shown in Table 1. Three native plants, including *Scirpus littoralis* Schrab, *Cyperus alternifolius*, and *Paspalum vaginatum* were cultivated in the FCW and HSCW models. Before cultivating, plants were collected from brackish water natural wetland area, then acclimated with actual shrimp farm wastewater for 30 days by stepwise increase the salinity level from 5‰ to 20‰ (actual shrimp farm wastewater was mixed with fresh water). The shrimp farm effluent from the equalization pond was pumped to the FCW pond to operate the system. Afterward, the effluent from the FCW pond was then further treated by the HSCW.

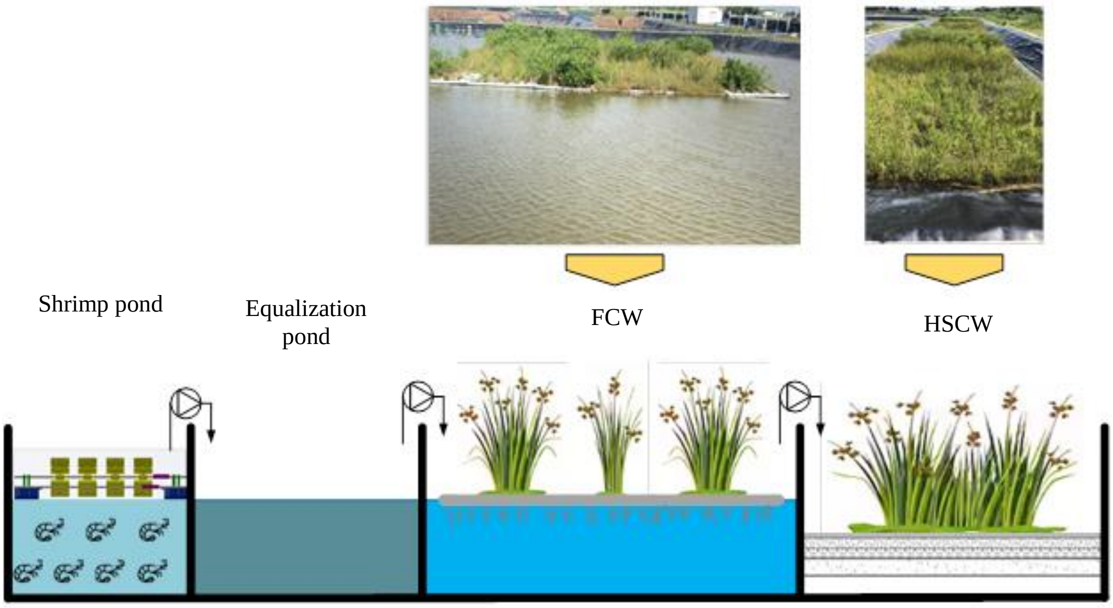

Figure 1. The schematic diagram of the HCWs

Table 1. The specific parameters of the HCWs

Units	Water/media depth (m)	Total area (m^2)	Coverage surface (%)	Substrates	Plant species
Equalization pond	1.0-1.5	1,500	-	-	-
FCW	1.0	1,500	20	-	*Scirpus littoralis* Schrab *Cyperus alternifolius* *Paspalum vaginatum*
HSCW	0.65	400	-	Gravel, sand, soil	*Scirpus littoralis* Schrab *Cyperus alternifolius* *Paspalum vaginatum*

2.3 Wastewater source

The wastewater source was taken from the super-intensive black tiger shrimp farm. On this farm area, each farming cycle lasted between 30 and 120 days, with a density of 120 shrimps/m^2. The water exchange rate varied from 10% to 20%, depending on the quality of the water. The wastewater from the shrimp ponds was collected and stored in an equalization pond and then used as the input for HCWs. The parameters of the shrimp farm wastewater, such as biological oxygen demand (BOD_5, 49.7-64.4 mg/L), chemical oxygen demand (COD, 77.8-89.5 mg/L), ammonium nitrogen (NH_4^+-N, 3.9-5.6 mg/L), total nitrogen (TN, 5.6-8.0 mg/L), and salinity (15.5-21.8‰).

2.4 Data analysis

During the operation, samples were taken from the influent and effluent of each step in the HCWs. The samples were analyzed for pH, temperature, salinity, COD, BOD_5, NH_4^+-N, and TN parameters. A mobile laboratory located at the project site was used for sample analysis. The pH, temperature, and salinity values were determined using a multiparameter water quality meter, HI98194 (Hanna, Romania). The concentrations of TN (detection method: Alkaline persulfate oxidation-UV spectrophotometry), NH_4^+-N (Nessler's reagent spectrophotometry) were determined according to the Standard Methods. The COD and BOD_5 were analyzed following the instructions outlined in Standard Methods for the Examination of Water and Wastewater, 22nd edition (APHA, 2012).

2.5 Nitrogen balance calculation

Nitrogen balance calculation can provide ideas about the pathways of nitrogen conversion in the model. The nitrogen transfer in the HSCW was similar mechanism as in the FCWs models. Because of financial limitation, the FCWs model was chosen to be an example of calculating nitrogen balance.

At the end of the experiment, nitrogen balance in the FCWs was calculated based on the methodology introduced in previous studies (Arslan et al., 2023; Zimmo et al., 2004). In the model, nitrogen from the wastewater was converted by several pathways, including denitrification/evaporation, accumulation by plants and algae, sedimentation, and nitrogen remaining in the effluent. The nitrogen mass balance calculation is illustrated in Equaltion (1).

$$N_d = N_i - (N_p + N_a + N_s + N_e) \qquad (1)$$

In Equation (1), the following variables were used:

- N_d: the mass of nitrogen converted by denitrification. This was calculated as the total nitrogen input minus the total nitrogen accumulated by plants, algae, sediment, and nitrogen contained in the wastewater.
- N_i: the amount of nitrogen injected into the model, which was considered to come only from shrimp pond wastewater.
- N_p: the amount of nitrogen accumulated in plants. This was calculated based on the nitrogen concentration and the weight of the plants.
- N_a: the amount of nitrogen accumulated in algae. This was determined based on the algae concentration and the nitrogen content contained in the algae.
- N_s: the amount of nitrogen accumulated in the bottom sediment. This was calculated based on the volume and the concentration of nitrogen in the sediment.
- N_e: the amount of nitrogen contained in the effluent wastewater.

3. RESULTS AND DISCUSSION

3.1 pH and salinity

During the 30-day experimental period, samples of influent, FCW effluent, and HSCW effluent were taken to analyze pH value and salinity (Figure 2). Results showed that the influent shrimp farm wastewater had a pH range of 7.19-8.0 (Figure 2(a)). After treatment in the FCW model, the pH value slightly increased to 7.94-8.48. This increase can be explained by the photosynthesis of algae in the FCW model, which increased OH^- ions in the water, resulting in a higher pH value. The pH of the HSCW effluent varied from 7.19-8.04. These pH values of the models indicated a suitable environment for the metabolism of aquatic organisms.

In addition, salinity concentration is a physicochemical factor strongly affecting the model's growth of microorganisms, plants, and algae. In this study, the average salinity in the influent, FCW effluent, and HSCW effluent was 19.10, 17.14, and 15.35‰, respectively (Figure 2(b)). The salinity levels were within the range suitable for the cultivated plants in the model and did not appear to inhibit the development of the plant system.

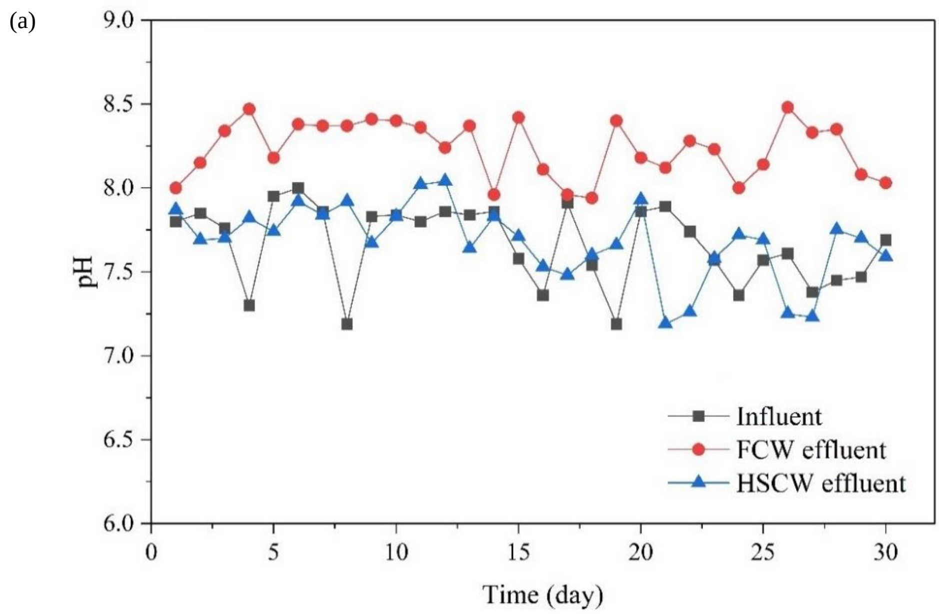

Figure 2. The pH (a) and salinity (b) values during time course

(b)

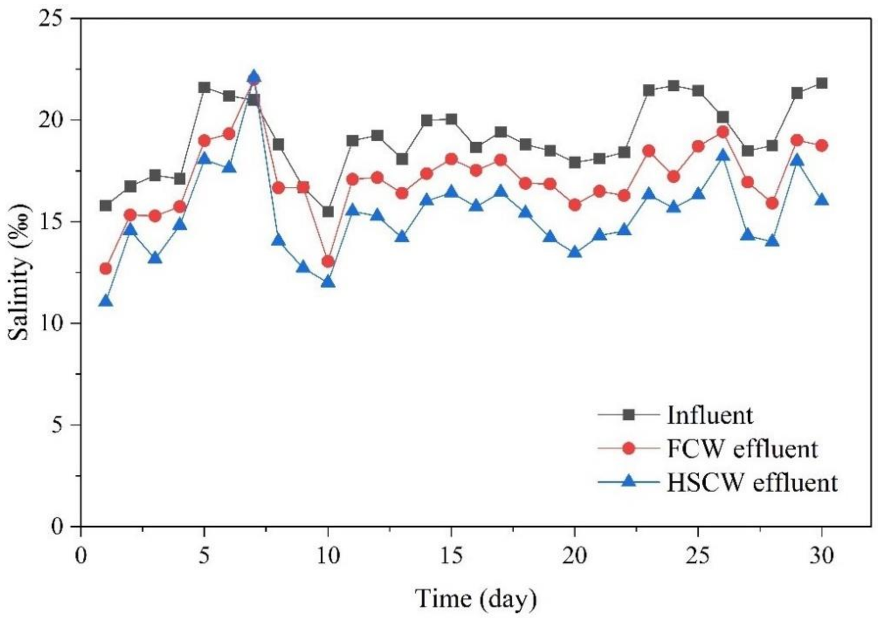

Figure 2. The pH (a) and salinity (b) values during time course (cont.)

3.2 COD and BOD_5 removal

The COD and BOD_5 concentration profile during time course is shown in Figure 3. These parameters in shrimp farm wastewater remained relatively constant over the 30-day operation period, with average influent COD and BOD_5 values of 88.1 and 57.5 mg/L, respectively. This indicates that the oxygen demand was not high. However, the wastewater may contain organic compounds from antibiotics and functional foods found in shrimp feeds, as well as feces and urine from shrimp metabolism. These components need to be treated before being released into the environment. Additionally, shrimp pond wastewater has a high salt concentration, which slows down the conversion of pollutants compared to other common types of sewage.

The results indicated that the average COD removal efficiency was 60.8% for the FCW model and 64.5% for the HSCW model. The total removal rate of the HCWs achieved 88.4% (Figure 3(a)). This result is higher than the study using *Canna indica* to improve pollutant removal efficiency and biomechanics by adding iron ions to aquaculture wastewater in the constructed wetlands of Zhimiao et al. (2019). The average BOD_5 removal rate in these models was 60.5% (FCW) and 59.5% (HSCW), and the maximum BOD_5 removal of the HCWs achieved 87.0% (Figure 3(b)). As a result, the COD and BOD_5 concentration of the effluent was stable in the range of 10.0-15.4 mg/L and 7.0-12.5 mg/L, respectively, which confirmed a safe condition for the receiver source and could be reused for the farm.

A previous study reported that 76% of the COD of White-leg shrimp farm wastewater could be removed by using horizontal subsurface flow constructed wetlands (Pham et al., 2021). In another study, the COD removal level of shrimp farm wastewater by using horizontal subsurface flow constructed wetlands reached 92.7% (Dinh, 2017). Besides, hybrid models have been applied for the treatment of different wastewater (Hu et al., 2022; Maine et al., 2022). It has been shown that the use of rotating biological contactors followed by hybrid constructed wetlands can remove 95.06% of COD in polluted rivers (Hu et al., 2022). Maine and colleagues used a hybrid system involving a free-water surface flow wetland and a horizontal subsurface flow wetland for pet-care center wastewater treatment. The system achieved the removal of 82.8% COD and 88.3% BOD (Maine et al., 2022).

3.3 Nitrogen removal

Nitrogen content is one of the important factors that need special attention for the quality control of shrimp pond water. In the pond, nitrogen compounds can be converted into different forms, of which nitrogen in the form of NH_3 is considered a toxin that affects the growth and metabolism of shrimps. Shrimp farm wastewater contains nitrogen compounds derived from dissolved feed, uneaten food, feces and shrimps' excretory products shrimp. Therefore, it is necessary to eliminate nitrogen to achieve acceptable standards before discharging it into the environment to ensure the receiving source's safety and avoid eutrophication.

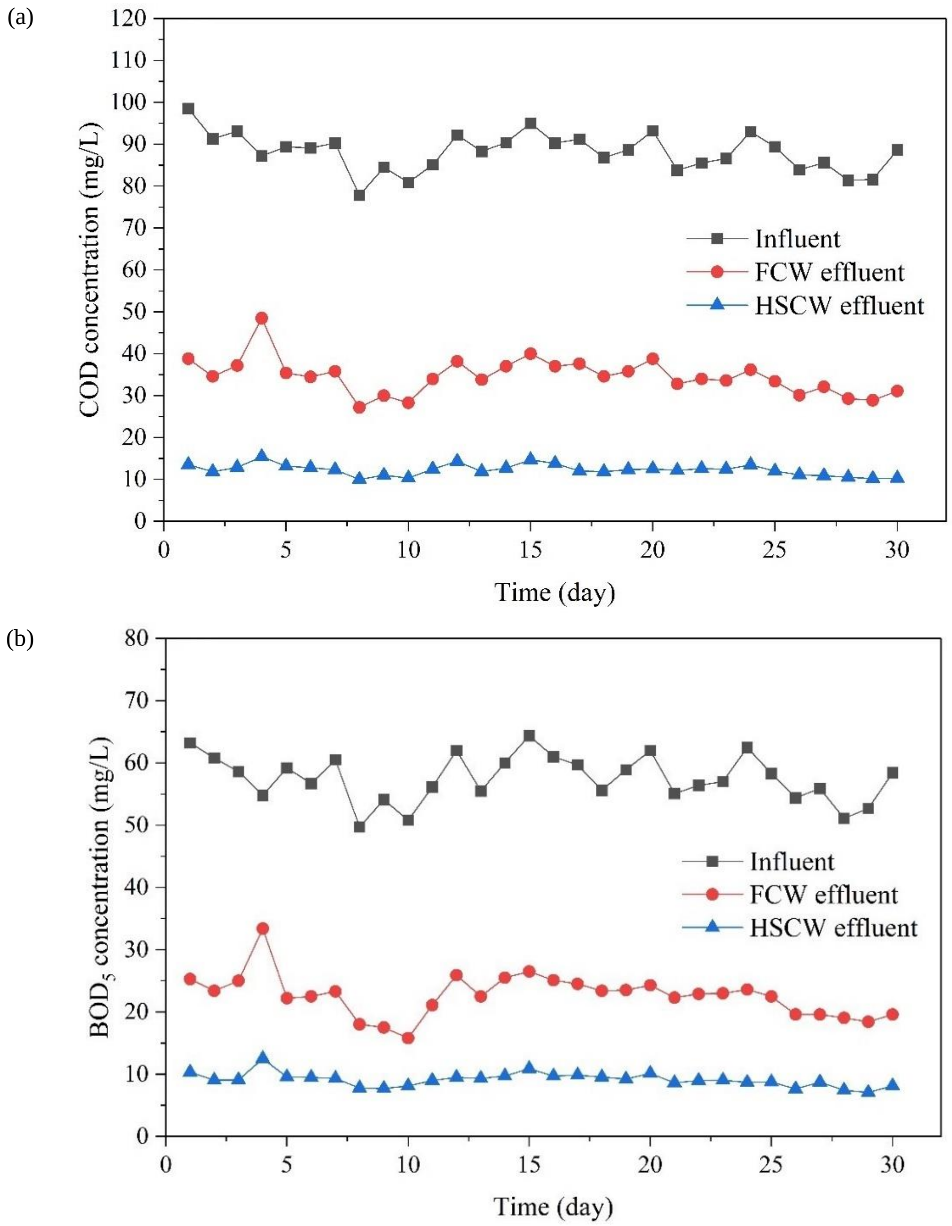

Figure 3. The change in COD (a) and BOD_5 (b) concentration over time

Figure 4 shows the change in nitrogen compounds during the operational time. In the influent wastewater, the concentration of TN varied from 5.62 to 8.02 mg/L, and NH_4^+-N fluctuated from 3.93 to 5.61 mg/L. The maximum removal of TN in the FCW and HSCW models and the total removal efficiency of the systems were 78.71, 90.32, and 97.24%, respectively. The maximum removal rate of NH_4^+-N for those steps was 92.51 and 91.67%; the ultimate removal of NH_4^+-N for all systems achieved 99.09%. According to Nasir et al. (2023), when using green microalgae to treat shrimp aquaculture wastewater, only 90.1% ammonia removal efficiency is achieved. The result illustrated that the applied system was suitable for treating nitrogen-containing shrimp farm wastewater. It was revealed that constructed wetlands and similar models performed well for nitrogen decomposition (Rampuria et al., 2020).

The main mechanisms for nitrogen degradation in constructed wetlands involve microbial conversion, chemical transformation, and physical processes (Li et al., 2021; Lu et al., 2020). Biological pathways utilize several techniques, such as nitrification, denitrification, nitrogen fixation, ammonification, and plant utilization. Nitrogen compounds are also converted through physicochemical processes such as adsorption, sedimentation, gas stripping, ion exchange, and filtration (Dinh, 2017; Dinh et al., 2021; Lee et al., 2009). Previous research has demonstrated the potential of using constructed wetland models to remove nitrogen from wastewater. Hybrid-constructed wetland systems have been shown to remove ammonium, TKN, and nitrate up to 83.0, 83.8, and 20.37%, respectively (Maine et al., 2022). Another study reported the removal of 78% nitrate matter in *Litopenaeus vannamei* farm wastewater using constructed wetlands (Pham et al., 2021). Therefore, constructed wetland models hold great promise for nitrogen treatment, especially in rural areas where land space is appropriate for modeling.

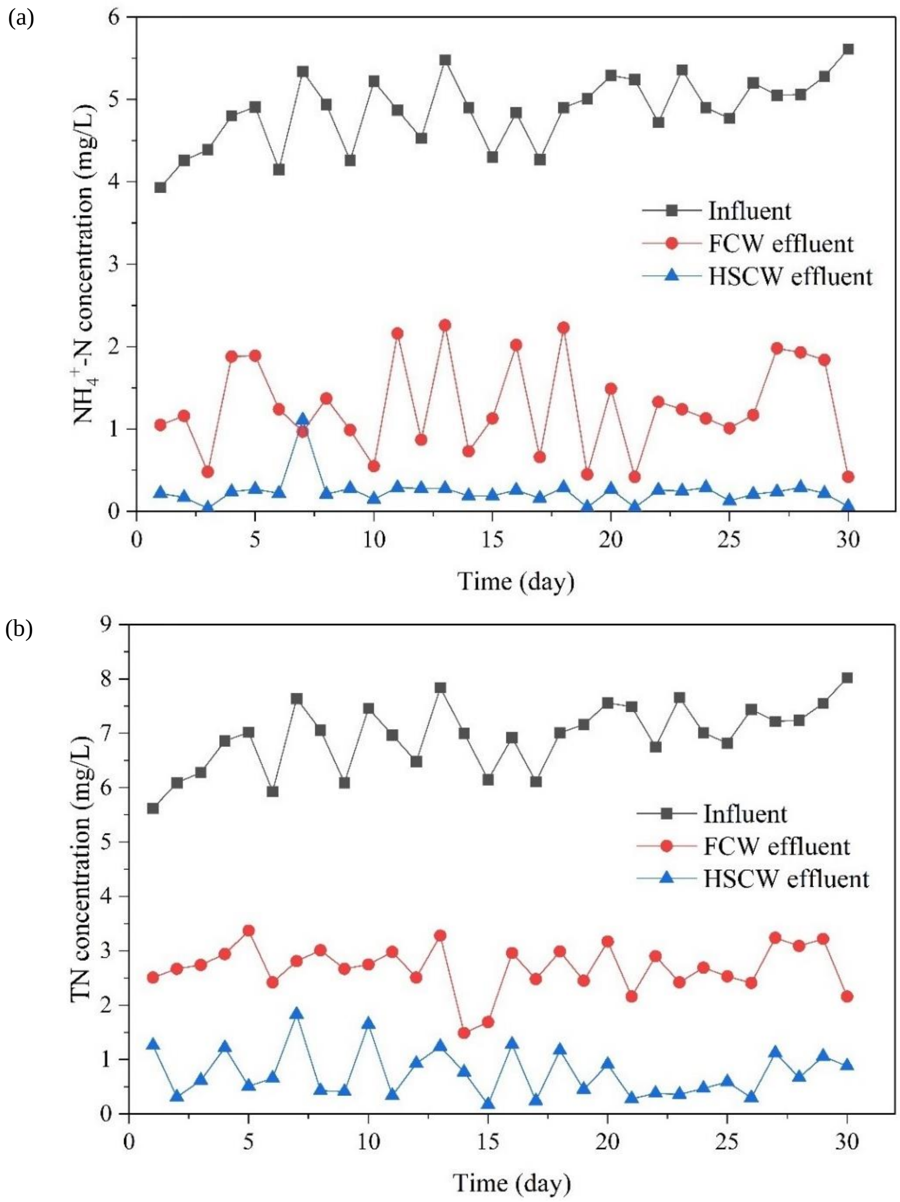

Figure 4. The change in NH_4^+-N (a) and TN (b) concentration during time course

3.4 Nitrogen balance

Nitrogen balance was calculated for the FCWs during operation to evaluate the nitrogen transition in the experimental model (Figure 5). The calculation method for the nitrogen balance considers that the nitrogen added to the model through rainfall is negligible. Towards the end of the operational period, the plants and algae in the pond were harvested, and sediment samples were taken. This work allowed for the determination of the nitrogen balance of the FCW. The findings indicated that a total of 2.272 kg of nitrogen entered the model during the operational period. Most of the nitrogen was converted through denitrification or evaporation processes (59%). In contrast, the amount of nitrogen fixed in plant cells (0.016 kg) and algae cells (0.0018 kg) was relatively small.

The previous study revealed that denitrification in the constructed wetland model was the main route of nitrogen conversion (Lee et al., 2009; Vymazal, 2007). Zimmo and colleagues indicated that the primary nitrogen conversion in the duckweed stabilization ponds was sedimentation and denitrification pathways (Zimmo et al., 2004). This result reveals that the floating constructed wetland is an excellent example of nitrogen transfer in which the dominant portion of nitrogen from the input wastewater was by denitrification and evaporation. The amount of nitrogen fixed in plants and algae cells at a particular time was insignificant because nitrogen was uptake and transferred through the metabolisms.

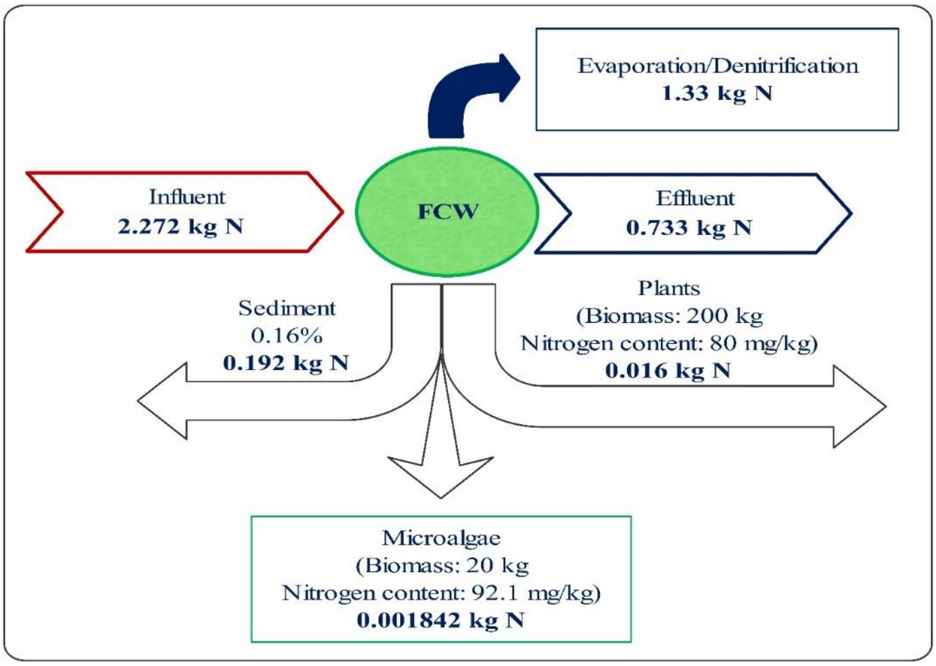

Figure 5. Nitrogen balance of the floating constructed wetland system

4. CONCLUSION

In the present study, the HCWs involve a floating constructed wetland followed by a horizontal sub-surface constructed wetland system designed at a pilot scale to treat actual shrimp farm wastewater. The findings of this study affirm the robust performance of the HCW model in wastewater treatment. Impressively, the model exhibited exceptional removal efficiencies, with values reaching 88.4% for Chemical Oxygen Demand (COD), 87.0% for Biochemical Oxygen Demand (BOD_5), 97.2% for Ammonium-Nitrogen (NH_4^+-N), and a remarkable 99.1% for Total Nitrogen (TN).

A critical aspect of this research lies in elucidating the nitrogen balance within the FCW, revealing that the principal nitrogen transformation pathways predominantly involve denitrification and evaporation processes. These outcomes underscore the potential of constructed wetland models featuring indigenous salt-tolerant flora to mitigate pollutants found in shrimp farm wastewater effectively. Moreover, the scalability of these wetland systems, utilizing pre-existing reservoirs and underutilized agricultural land, emerges as a viable prospect. Consequently, replication of this model on a larger-scale holds promise for widespread adoption, thereby facilitating the sustainable treatment of wastewater from aquaculture facilities. In doing so, it contributes to environmental preservation and bestows the added benefit of creating verdant spaces within shrimp farms in the ecologically sensitive Mekong Delta region.

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REFERENCES

American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. Washington, DC: APHA; 2012.

Arslan M, Wilkinson S, Naeth MA, El-Din MG, Khokhar Z, Walker C, et al. Performance of constructed floating wetlands in a cold climate waste stabilization pond. Science of the Total Environment 2023;880:Article No. 163115.

Boopathy R, Kern C, Corbin A. Use of Bacillus consortium in waste digestion and pathogen control in shrimp aquaculture. International Biodeterioration and Biodegradation 2015; 102:159-64.

Braaten RO, Flaherty M. Salt balances of inland shrimp ponds in Thailand: Implications for land and water salinization. Environmental Conservation 2001;28(4):357-67.

Cardoso-Mohedano J-G, Lima-Rego J, Sanchez-Cabeza J-A, Ruiz-Fernández A-C, Canales-Delgadillo J, Sánchez-Flores E-I, et al. Sub-tropical coastal lagoon salinization associated to shrimp ponds effluents. Estuarine, Coastal and Shelf Science 2018;203:72-9.

Cicero-Fernandez D, Expósito-Camargo J, Peña-Fernandez M. Efficacy of Juncus maritimus floating treatment saltmarsh as anti-contamination barrier for saltwater aquaculture pollution control. Water Science and Technology 2022;85(10):2811-26.

Dinh N. Highly efficient treatment of shrimp farm wastewater by using the horizontal subsurface flow (HSSF) constructed wetlands with phragmites Australis Plant. Asian Journal of Environment and Ecology 2017;4(3):1-9.

Dinh NT, Le AH, Phan TD. Studying on the treatment of high salinity concentration wastewater from shrimp farm by floating constructed wetlands (FCWs) models: Effect of plant cover area. Chemical Engineering Transactions 2021;89:541-6.

Food and Agriculture Organization (FAO). News and Events: Global farmed shrimp production increased in 2022 despite low demand [Internet]. 2023 [cited 2023 Feb]. Available from: https://www.fao.org/in-action/globefish/news-events/trade-and-market-news/q1-2023-jan-mar/en/.

Hu N, He J, Shi W, He J, Lv B, Liang Y, et al. Ecological restoration for the Liangtan River by Rotating biological contactors combined with hybrid constructed wetlands. Journal of Cleaner Production 2022;375:Article No. 134189.

Iber BT, Kasan NA. Recent advances in Shrimp aquaculture wastewater management. Heliyon 2021;7(11):e08283.

Lee Cg, Fletcher TD, Sun G. Nitrogen removal in constructed wetland systems. Engineering in Life Sciences 2009;9(1):11-22.

Li D, Chu Z, Zeng Z, Sima M, Huang M, Zheng B. Effects of design parameters, microbial community and nitrogen removal on the field-scale multi-pond constructed wetlands. Science of the Total Environment 2021;797:Article No. 148989.

Li Z, Li L, Sun H, Wang W, Yang Y, Qi Z, et al. Ammonia assimilation: A double-edged sword influencing denitrification of Rhodobacter azotoformans and for nitrogen removal of aquaculture wastewater. Bioresource Technology 2022;345:Article No. 126495.

Lu J, Guo Z, Kang Y, Fan J, Zhang J. Recent advances in the enhanced nitrogen removal by oxygen-increasing technology in constructed wetlands. Ecotoxicology and Environmental Safety 2020;205:Article No.111330.

Lymbery AJ, Kay GD, Doupé RG, Partridge GJ, Norman HC. The potential of a salt-tolerant plant (*Distichlis spicata* cv. NyPa Forage) to treat effluent from inland saline aquaculture and provide livestock feed on salt-affected farmland. Science of the Total Environment 2013;445:192-201.

Maine MA, Sanchez GC, Hadad HR, Caffaratti SE, del Carmen Pedro M, Di Luca GA, et al. Hybrid wetland system for a pet-care center wastewater treatment. Ecological Engineering 2022;182:Article No.106700.

Meril D, Piliyan R, Perumal S, Sundarraj DK, Binesh A. Efficacy of alginate immobilized microalgae in the bioremediation of shrimp aquaculture wastewater. Process Biochemistry 2022;122:196-202.

Nasir NM, Jusoh A, Harun R, Ibrahim NNLN, Rasit N, Ghani WAWAK, et al. Nutrient consumption of green microalgae, *Chlorella* sp. during the bioremediation of shrimp aquaculture wastewater. Algal Research 2023;72:Article No. 103110.

Ng LY, Ng CY, Mahmoudi E, Ong CB, Mohammad AW. A review of the management of inflow water, wastewater and water reuse by membrane technology for a sustainable production in shrimp farming. Journal of Water Process Engineering 2018;23:27-44.

Pham TTH, Cochevelou V, Dinh HDK, Breider F, Rossi P. Implementation of a constructed wetland for the sustainable treatment of inland shrimp farming water. Journal of Environmental Management 2021;279:Article No. 111782.

Rampuria A, Gupta AB, Brighu U. Nitrogen transformation processes and mass balance in deep constructed wetlands treating sewage, exploring the anammox contribution. Bioresource Technology 2020;314:Article No. 123737.

Szota C, Farrell C, Livesley SJ, Fletcher TD. Salt tolerant plants increase nitrogen removal from biofiltration systems affected by saline stormwater. Water Research 2015;83:195-204.

Trang NTD, Linh VC, Huu NHM, Tung NCT, Loc NX, Brix H. Screening salt-tolerant plants for phytoremediation: Effect of salinity on growth and mineral nutrient composition. Vietnam Journal of Science and Technology 2018;56(2C):9-15.

Visvanathan C, Hung N, Jegatheesan V. Hydrogenotrophic denitrification of synthetic aquaculture wastewater using membrane bioreactor. Process Biochemistry 2008;43(6): 673-82.

Vymazal J. Removal of nutrients in various types of constructed wetlands. Science of the Total Environment 2007;380(1-3): 48-65.

Zhimiao Z, Xiao Z, Zhufang W, Xinshan S, Mengqi C, Mengyu C, et al. Enhancing the pollutant removal performance and biological mechanisms by adding ferrous ions into aquaculture wastewater in constructed wetland. Bioresource Technology 2019;293:Article No. 122003.

Zimmo O, Van der Steen N, Gijzen H. Nitrogen mass balance across pilot-scale algae and duckweed-based wastewater stabilisation ponds. Water Research 2004;38(4):913-20.

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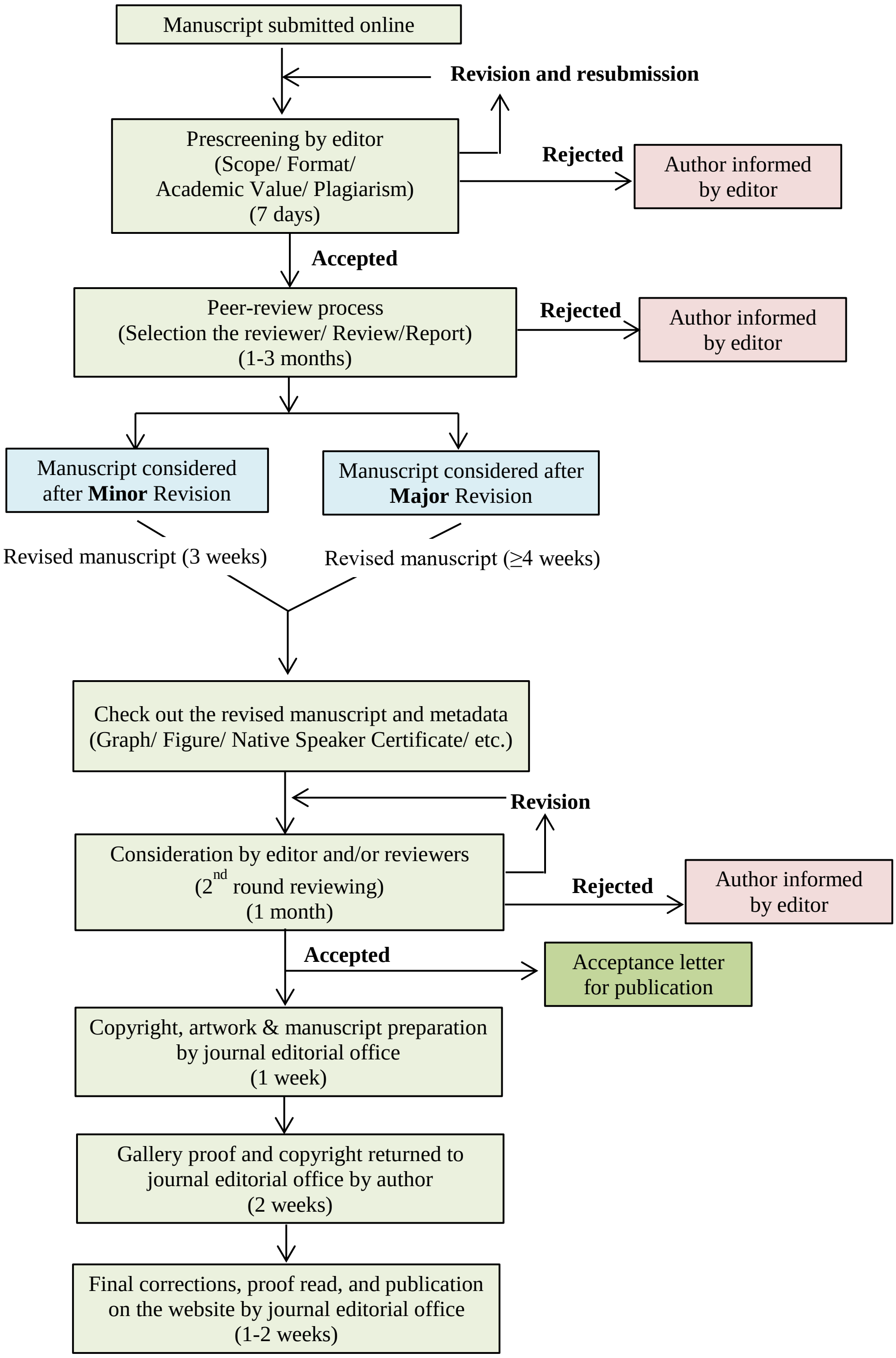

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Journal article

Muenmee S, Chiemchaisri W, Chiemchaisri C. Microbial consortium involving biological methane oxidation in relation to the biodegradation of waste plastics in a solid waste disposal open dump site. International Biodeterioration and Biodegradation 2015;102:172-81.

Published in conference proceedings

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Ph.D./Master thesis

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Website

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