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Unexpected heroes: invasive macrophytes boost performance of faltering waste stabilization ponds

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ABSTRACT

Waste stabilization ponds (WSPs) are a widely used technology for removing organic matter and pathogens from wastewater. However, their effectiveness is often hindered by hydraulic overload, sludge accumulation, and the proliferation of invasive macrophytes–the impacts of these challenges are not fully understood. In this study, standard methods were used to assess WSPs under these operational challenges for the removal efficiency of physical, nutrient, and organic pollutants, as well as faecal indicator bacteria (FIB); the extent of macrophyte occupancy; and the level of FIB colonizing invasive macrophyte roots. Results indicated high removal efficiencies (73.38–99.67%) for turbidity, total nitrogen, NO_3^-, NO_2^-, NH_3, PO_4^{3-}, BOD_5, total coliforms, *Escherichia coli*, enterococci, and *Clostridium perfringens*, whereas total dissolved solids, electrical conductivity, and total phosphorus showed considerably lower removal (33.5–45.2%). Significant correlations ($r = 0.47$–0.96, $P < 0.05$) were found between the removal efficiencies of physical, nutrient, and organic pollutants, as well as FIB, and macrophyte occupancy. Furthermore, high densities of FIB colonizing the macrophyte roots suggest that these roots may have positively contributed to the performance of the WSPs. The final effluent complied with East African standards for all pollutants except turbidity, BOD_5, and FIB. This study reveals that ignoring WSP maintenance significantly compromises treatment performance, with potential implications for public health, and underscores the role of macrophytes in enhancing the performance of faltering WSPs.

Key words: effluent quality, invasive macrophytes, removal efficiency, waste stabilization ponds, wastewater treatment, WSPs maintenance

HIGHLIGHTS

- Waste stabilization ponds (WSPs) characterized by increased hydraulic overloading and invasive macrophytes were studied.
- Physicochemical and faecal indicator bacteria (FIB) levels showed greater variation between treatment stages than between sampling intervals.
- Physicochemical and FIB variables reflected the impact of inadequate WSP maintenance.
- Invasive macrophytes played a significant role in removing pollutants from wastewater.
- The maintenance status of WSPs directly and significantly influences treatment performance and final effluent quality.

INTRODUCTION

The rapid expansion of urban areas, particularly in developing regions, is generating unprecedented volumes of wastewater (Fito & Van Hulle 2021; Rentschler *et al.* 2023; Xian *et al.* 2023), which frequently exceed the capacity of existing treatment infrastructure (Preisner 2020; Wear *et al.* 2021; Rangel-Buitrago *et al.* 2024). The subsequent discharge of inadequately treated effluent degrades aquatic ecosystems through mechanisms like eutrophication and toxin bioaccumulation (Jan *et al.* 2022) and poses direct public health risks via water-borne pathogens and chemical contaminants (Okoh *et al.* 2010). Consequently, developing and implementing robust, context-appropriate sanitation technologies are critical for safeguarding both environmental and public health (Andersson *et al.* 2016).

Globally, waste stabilization ponds (WSPs) represent a scalable and common solution for the removal of pathogens and organic matter from wastewater (Gruchlik *et al.* 2018; Coggins *et al.* 2019; Edokpayi *et al.* 2021; Kadri *et al.* 2021). These treatments occur in shallow, constructed basins that utilize a combination of natural biological (e.g., photosynthesis by algae, activity of microbes) and physical (e.g., settlement of solids and sunlight irradiation) processes (Polprasert & Kittipongvises 2011; Gruchlik *et al.* 2018; Mwamlima *et al.* 2025). The operational simplicity, low cost, and minimal energy demands of WSPs make them a favored alternative to energy-intensive mechanical systems (Edokpayi *et al.* 2021), leading to their widespread adoption across the developing world (Izdori *et al.* 2019; Achag *et al.* 2021; Edokpayi *et al.* 2021; Kadri *et al.* 2021). A primary operational issue in these contexts is hydraulic overload, where influent volumes from expanding populations exceed the original design capacity of the ponds (Sinn & Lackner 2020; Sinn *et al.* 2023). For example, Mwamlima *et al.* (2025) reported that the Kaliti wastewater treatment plant in Addis Ababa, Ethiopia, although originally designed to handle 7,500 m^3/day, was by 2021 receiving 47,500 m^3/day. Although the principle that overloading impairs treatment is theoretically acknowledged (Hoko & Toto 2020), there is a distinct lack of field-based research that precisely measures the resulting performance loss in actual, full-scale WSPs, especially concerning the removal of organic matter and pathogens. Filling this knowledge void is essential to formulate improved operational guidelines, meet discharge regulations, advance the targets of SDG 6, and facilitate the safe reuse of water in regions facing scarcity.

For decades, Tanzania, like many African nations, has relied extensively on WSPs for domestic and industrial wastewater treatment (Yhdego 1989; Kaseva *et al.* 2008; Zacharia *et al.* 2019). While these systems are operational nationwide, comprehensive performance data – particularly regarding the removal of organic matter and pathogens – is lacking for the vast majority. This gap is critical, as WSP effluent is commonly discharged into freshwater bodies (Machibya & Mwanuzi 2006) and widely used for crop irrigation (Kihila *et al.* 2014; Nyomora 2015; Zacharia *et al.* 2019) and aquaculture (Mhongole *et al.* 2017). Although this reuse supports food security (Kihila *et al.* 2014), it raises significant public health concerns. Studies in similar contexts consistently detect enteric pathogens and FIB in crops and fish from systems using WSP effluent (Hounmanou *et al.* 2016; Verbyla *et al.* 2016), prompting calls for further treatment. However, WSP performance is highly variable and site-specific, influenced by operational conditions like loading rates and maintenance, as well as environmental factors like climate. Consequently, data from one system are not directly transferable to another. This variability underscores an urgent necessity: independent, site-specific performance assessments are essential to manage WSPs effectively, optimize their treatment efficiency, and develop safe, context-appropriate wastewater reuse strategies.

The WSPs situated at Solomon Mahlangu Campus (SMC), Sokoine University of Agriculture (SUA), Morogoro, Tanzania, have been in operation for more than 30 years. The WSPs treat wastewater from the SMC hospital, SMC staff residences, SMC undergraduate's student hostels and Chief Albert Luthuli Primary School. Initially designed for a population of 300 individuals, the WSPs now cater to a population of >8,000 due to increased student enrollment and staff recruitment, exceeding their design capacity and leading to hydraulic overloading. The system is also affected by invasive macrophytes, with emergent species (*Typha latifolia* and *Cyperus esculentus*) growing in shallow areas due to sludge accumulation, and surface floating species (*Pistia stratiotes* and *Ludwigia adscendens*) dominating deeper zones of the WSPs. These invasive macrophytes hinder UV radiation penetration, crucial for pathogen inactivation, and limit algal growth necessary for oxygen production to oxidize organic matter (Yeh *et al.* 2011). Despite the challenges posed by invasive macrophytes in poorly maintained WSPs, studies in constructed and natural wetlands have shown that these plants can effectively remove contaminants from wastewater through filtration, adsorption, absorption, and microbial activity in the rhizosphere (de Vasconcelos *et al.* 2021; Kalengo *et al.* 2021). However, information on the occurrence of various invasive macrophyte species and their role in pollutant removal within poorly maintained WSPs is currently unavailable – even though WSPs are the most widely used wastewater treatment technology. For the first time, this study evaluated WSPs characterized by hydraulic overloading and a variety of invasive macrophytes, assessing their removal efficiency, the extent of invasive macrophyte occupancy and its relation to removal efficiency, and the role of invasive macrophyte roots in contaminant removal from wastewater. The hypothesis was that the studied WSPs can effectively eliminate most of the physicochemical pollutants and FIB from raw wastewater with the help of invasive macrophytes. This research provides a critical case study on the operational challenges of aging WSP infrastructure, with a specific focus on the underreported impact of invasive macrophytes in such an overloaded and poorly maintained system. The results of this study not only assist facility managers in enhancing the removal efficiency of the studied WSPs but also inform environmental engineers about the importance of designing WSPs that can utilize suitable aquatic macrophytes to improve treatment system performance and enable the use of effluent water.

MATERIALS AND METHODS

Description of the study area

The investigated WSP system is located at the SMC within Morogoro municipality, Tanzania. The municipality lies at the foothills of the Uluguru Mountains, approximately between latitudes 6°50′ and 7°00′ S and longitudes 37°45′ and 37°65′ E (Figure 1), at an altitude of 457 m above sea level.

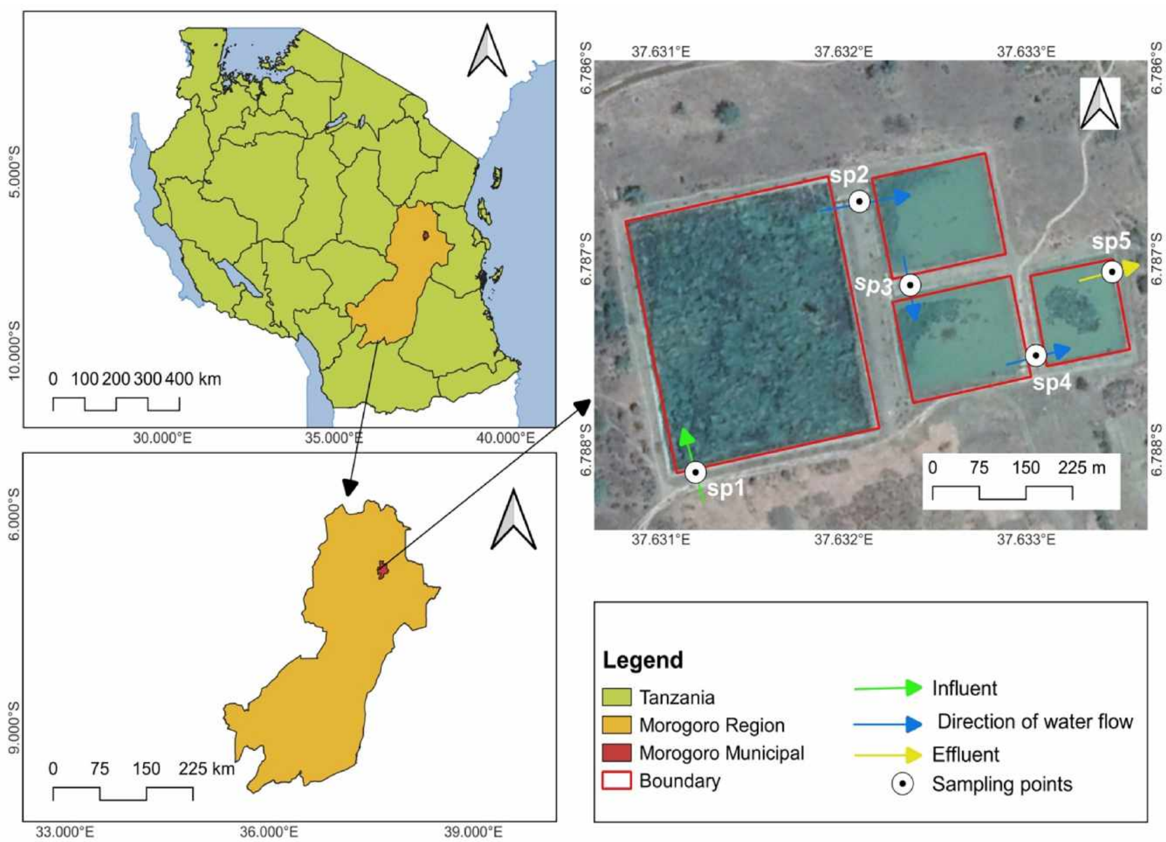

Figure 1 | Layout of the studied waste stabilization ponds and water quality sampling points.

The region experiences a tropical subhumid climate, with an annual average temperature ranging from 18 to 30 °C and an average annual rainfall ranging from 600 to 1,800 mm (Mkonda 2015). Rainfall is bimodal, featuring long rains from March to May and short rains from October to December. The WSP system comprises an anaerobic pond, a facultative pond, and two maturation ponds arranged in series (Figure 1); their specific surface areas and depths are shown in Table 1. No flow meter is installed to measure the quantity of the influent entering the WSP system, and there is no routine testing of effluent to ensure compliance with guidelines. Additionally, the WSP system is neither fenced nor tagged with warning signs, leading to the cattle, goat, sheep and other animals accessing and watering directly from the ponds. The ponds are heavily colonized by invasive macrophytes, including the emergent species *T. latifolia* and *C. esculentus*, and the free-floating species *P. stratiotes* and *L. adscendens* (Figure 2). Given that these four species of macrophytes were present in each pond of the investigated WSPs, the percentage of surface area of each pond occupied by each invasive macrophyte species was estimated as described by Ten Caten *et al.* (2022). Across the ponds, the occupancy was highest for *P. stratiotes* (20–75%) and *T. latifolia* (4–73%), and lowest for *L. adscendens* (5–13%) and *C. esculentus* (2–14%). *T. latifolia* and *C. esculentus* were predominantly established in shallow areas, due to sludge accumulation, while

Table 1 | Dimensions of the studied wastewater stabilization ponds

Pond	Surface area (m^2)	Depth (m)	Volume (m^3)
Anaerobic	8,285	4.0	25,908
Facultative	1,315	2.4	2,984
Maturation♯1	1,435	1.5	2,141
Maturation♯2	775	1.5	1,579

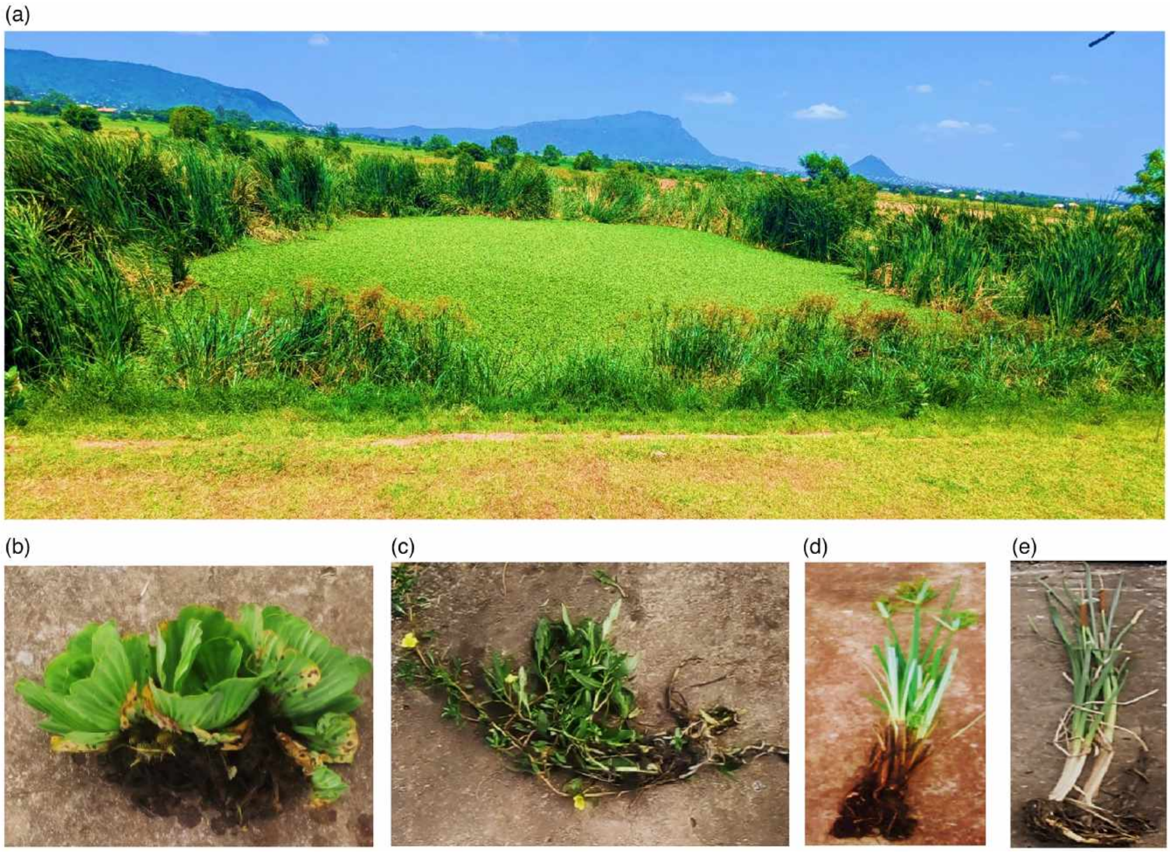

Figure 2 | Image a shows a waste stabilization pond (maturation♯1) with invasive macrophytes, including the floating species *Pistia stratiotes* (b), *Ludwigia adscendens* (c), and the emergent species *Cyperus esculentus* (d) and *Typha latifolia* (e).

P. stratiotes and *L. adscendens* covered the deeper zones. This extensive occupancy by invasive macrophytes is known to obstruct light penetration and suppress algal growth (Yeh *et al.* 2011), which can compromise pond performance. The treatment efficacy of this WSP system and its associated ecosystem has not been formally evaluated. Furthermore, there is no established monitoring program to assess compliance of effluent quality with national or international effluent standards or to guide systematic maintenance. The final effluent is discharged without further treatment. The effluent enters the Ngerengere River, which is used for watering cattle, sheep, and goats, irrigating varieties of vegetables, presenting a potential pathway for environmental contamination and public health risks (Lewerin *et al.* 2019).

Water sampling and measurement of water quality variables

To capture temporal dynamics and ensure statistical robustness, eight biweekly sampling events were conducted from November 2024 to February 2025. This period corresponded to the dry season, with no rainfall recorded within 72 hours prior to each sampling event, minimizing dilution effects on influent wastewater characteristics. Water samples were collected aseptically from five locations: the influent (raw wastewater) and the effluent of the anaerobic pond, facultative pond, maturation pond #1, and maturation pond #2 of the WSP system

(Figure 1). These locations were selected because effluent from each pond provides an integrated sample representative of treatment performance within that pond. Overall, 40 water samples were aseptically collected using sterilized 500 mL glass bottles. On-site measurements of physical parameters were performed at each site using a calibrated turbidimeter (TB200, Orbeco Hellige) for turbidity and a multiparametric meter (PCE-PHD 1, PCE Instruments, Meschede, Germany) for electrical conductivity (EC), pH, total dissolved solids (TDSs), and temperature; eight measurements were taken per parameter per site. All water samples were promptly placed in a dark, cool box maintained at approximately 4 °C and transported to the SUA laboratory for analysis. Nutrients (NO_2^-, NO_3^-, PO_4^{3-}, NH_3, total nitrogen (TN), and total phosphorus (TP)), organics (BOD_5), and FIB (total coliforms (TC), *Escherichia coli*, enterococci (ENT), and *Clostridium perfringens* (CP)) were analyzed according to standard methods (APHA 2022).

In the laboratory, BOD_5 was determined using a calibrated dissolved oxygen probe following the 5-day incubation method at room temperature (Method 5210). TN was analyzed by alkaline persulfate digestion, which oxidizes nitrogenous compounds to NO_3^-, followed by spectrophotometric quantification of NO_3^- (Method 4500-NO_3^- C). NH_3 was determined by the phenate method (method 4500-NH_3 G). TP was measured by acid persulfate digestion to convert phosphorus to orthophosphate, followed by the ascorbic acid method and spectrophotometric measurement (Method 4500-P E). NO_2^- and NO_3^- were analyzed spectrophotometrically using the diazotization method (Method 4500-NO_2^- B) and the cadmium reduction method (Method 4500-NO_3^- E), respectively.

The enumeration of CP involved heat shock treatment, where water samples were heated at 75 °C for 15 minutes to eliminate vegetative background bacteria and select for CP spores (Mushi 2018). In contrast, TC, *E. coli*, and ENT were analyzed from nonheated aliquots. For all FIB, appropriate decimal dilutions (10^{-4} to 10^{-1} mL) of the water samples were filtered through 0.45-μm pore-size, 47-mm diameter cellulose nitrate membrane filters (Whatman, Sartorius, Vienna, Austria) using the membrane filtration technique. The filters were aseptically transferred onto selective agar media. TC and *E. coli* were cultured on chromocult coliform agar (CCA; Merck, Darmstadt, Germany), ENT on membrane enterococcus agar (MEA; Merck), and CP on tryptose sulfite cycloserine agar (TSCA; VWR Prolabo Chemicals, Darmstadt, Germany) supplemented with D-cycloserine. All incubations were performed for 24 hours. CCA and MEA plates were incubated at 37 °C. TSCA plates for CP were incubated at 44 °C under anaerobic conditions generated using an anaerobic jar and gas packs. Following incubation, colonies were identified and enumerated based on characteristic phenotypes: on CCA, pink colonies were considered TC and dark blue to violet colonies as *E. coli*; on MEA, pink to maroon colonies were considered ENT; and on TSC, black colonies that fluoresced under UV light were presumptively identified as CP. Representative colonies from each agar type were subjected to confirmatory tests as described by Byamukama *et al.* (2005). Results for all bacteria were calculated and reported as colony-forming units per 100 mL (cfu/100 mL).

Detection of faecal indicator bacteria on macrophyte root surfaces

The invasive macrophytes were aseptically harvested by firmly grasping the leaves to lift the plant from the pond. Loosely adhered sediment was removed from the roots by gentle shaking, following the method of King *et al.* (2021). The roots were then aseptically excised. Triplicate root samples, each weighing 20 g, were collected from the invasive macrophytes *T. latifolia*, *C. esculentus*, *P. stratiotes*, and *L. adscendens* in each pond. Each sample was placed in a separate sterile Whirl-Pak bag, stored on ice, and transported to the laboratory for analysis of root-colonizing bacteria: TC, *E. coli*, ENT, and CP. In the laboratory, bacteria were detached from the roots by adding 200 mL of sterile wastewater and vigorously shaking on an orbital shaker at 250 rpm for 6 minutes. The samples were left to stand for 5 minutes to allow root debris to settle. One milliliter of the supernatant was serially diluted and analyzed for TC, *E. coli*, ENT, and CP using the membrane filtration technique with the appropriate cultivation media and incubation conditions, as previously described. After incubation, the concentrations of all FIB were quantified from each agar plate and expressed as colony-forming units per gram of root wet weight (cfu/g).

Data analysis

Descriptive statistics, including the median, minimum, and maximum for the measured physicochemical parameters, were calculated using Microsoft Excel 2010. Given that the data did not meet the assumptions of normality and homogeneity of variance, as assessed by the Shapiro–Wilk and Levene's tests respectively, nonparametric tests – the Kruskal–Wallis test and Spearman's rank correlation analysis – which account for outliers, were employed for the respective analyses. A non-parametric-based coefficient of variation was calculated according to the method of Byamukama *et al.* (2005) as (75th percentile − 25th percentile)/median. The removal efficiency (RE) for each pollutant was calculated using the standard formula: RE (%) = [(C_influent − C_effluent)/C_influent] × 100, where C_influent and C_effluent are the concentrations in the influent and effluent, respectively. Physicochemical and microbiological quality of WSP effluent was assessed for compliance with the East African Standard for discharging wastewater to the environment (EAS 1172: 2023). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 16.0). Differences in physico-chemical and microbiological variables among sampling points were assessed using the Kruskal–Wallis test, followed by pairwise Mann–Whitney U tests for post-hoc analysis. The relative contribution of each FIB group to the roots of a given macrophyte species was calculated as the proportion of the absolute counts of that group on that macrophyte species to the sum of the absolute counts of the same group on all macrophyte species sampled in the corresponding pond of the waste stabilization pond. Counts were normalized per gram of fresh root mass prior to calculation. Relationships between the percentage of pond area occupied by specific invasive macrophyte species (occupancy) and the removal efficiencies of various parameters were determined using Spearman's Rank correlation algorithm. To control the family-wise error rate arising from multiple comparisons in Spearman's correlation analysis, the Bonferroni correction was applied, as it provides strong control of false-positives across all comparisons. For any parameter where the concentration was below the detection limit (DL), a value of DL/2 was substituted for statistical analysis. The significance level for all tests was set at $p < 0.05$.

RESULTS AND DISCUSSION

This study evaluates the performance of a full-scale WSP system characterized by sludge accumulation, hydraulic overload, and prolific aquatic macrophyte growth. To our knowledge, it is the first study to assess a WSP system simultaneously burdened by these combined operational drawbacks. Data retrieved from analyzed wastewater samples revealed that all measured physico-chemical and microbiological parameters were consistently detected above their respective DLs, with the exception of nitrite. Nitrite was below the DL of 0.005 mg/L in 25% of samples (10 out of 40). This is likely attributed to the reduction via bacterial denitrification, a process favored by anoxic conditions, such as those resulting from sludge accumulation and high organic loading (Ahmed *et al.* 2023; Tracey *et al.* 2023).

Table 2 shows the levels of physicochemical variables across the treatment stages of the investigated WSP system. For all physicochemical variables except temperature, pH, and TP, the within-site CV (0.01–5.73) was significantly lower than the between-site CV (7.37–13.25). This suggests that the different treatment stages (anaerobic, facultative, and maturation) were the primary driver of the observed physicochemical variability, rather than temporal variations within each stage. The lack of significant change in pH, temperature, and TP across the treatment stages is consistent with previous reports (Zacharia *et al.* 2019; Shaheen *et al.* 2024) and suggests that physical and biological activities within the treatment stages had no impact on these parameters. This is likely because pH and temperature are more strongly influenced by climatic conditions, whereas TP removal typically requires specific chemical or advanced biological processes that do not occur reliably in the simple, passive environment of a WSP system. In contrast, a consistent decrease in EC and TDS was observed across the treatment stages. Although this trend contrasts with the findings of Shaheen *et al.* (2024), the divergence may be due to the uptake of metal and nutrient ions by invasive macrophytes present in the studied system (Moreno Castro *et al.* 2025) – macrophytes that were absent in the WSPs studied by Shaheen *et al.* (2024). Turbidity was substantially reduced, primarily in the anaerobic pond, with subsequent stages showing no further removal. de Vasconcelos *et al.* (2021) studying the efficiency of using aquatic macrophytes for the treatment of aquaculture effluents, concluded that systems with aquatic macrophytes were effective at reducing turbidity. This reduction can be attributed mainly to the extensive rhizome system and dense, fibrous root network of the dominant invasive macrophyte species, *Typha latifolia*, which facilitated removal in the anaerobic pond by promoting sedimentation, filtration, and entrapment of suspended solids, as well as uptake of dissolved ions. The anaerobic pond was also the most effective stage at reducing NH_3 and TN. While all stages contributed to the removal of NO_3^- and PO_4^{3-}, substantial reductions in NO_3^- occurred in the anaerobic pond, whereas PO_4^{3-} removal was most pronounced in the maturation pond. Similarly, the maturation pond was the most effective at removing NO_2^-. We attribute the overall nutrient removal to a

Table 2 | Physico-chemical parameters of raw wastewater and effluents of various treatment stages of the investigated waste stabilization ponds

Variables statistics		Quality of water from: Influent	Anaerobic pond	Facultative pond	Maturation pond ♯1	Maturation pond ♯2	*P*-value
Physical parameters:							
Temperature (°C)	Median	26.8	26.5	25.7	25.6	27.0	0.781
	Min.	26.6	24.5	24.3	24.0	26.6	
	Max.	27.1	27.0	26.0	27.0	27.1	
	CV	0.02	0.06	0.07	0.08	0.02	
	EAS					20–30	
pH	Median	7.55	7.65	8.32	7.17	7.07	0.567
	Min.	7.01	7.48	7.89	7.06	6.86	
	Max.	7.81	8.1	8.76	7.27	7.11	
	CV	0.08	0.07	0.10	0.03	0.02	
	EAS					5–9	
EC (mg/L)	Median	1,014	899	679	574	673	0.004
	Min.	978	893	669	567	624	
	Max.	1,145	916	682	607	805	
	CV	0.14	0.02	0.01	0.05	0.23	
	EAS					2,500	
TDS (mg/L)	Median	507.5	450	340	285	337	0.004
	Min.	489	447	335	284	313	
	Max.	573	458	342	304	403	
	CV	0.14	0.02	0.02	0.05	0.22	
	EAS					1,500	
Turbidity (NTU)	Median	244.5	29.5	45	92.2	87.7	0.002
	Min.	2.88	19.9	37.9	45.2	220	
	Max.	362	88.1	50.6	365	601	
	CV	1.15	1.80	0.26	2.61	1.02	
	EAS					30.0	
Nutrients:							
NH_3 (mg/L)	Median	6.08	2.17	1.28	0.0168	0.00248	0.001
	Min.	5.31	0.36	0.19	0.034	0.0019	
	Max.	7.28	2.79	4.78	0.0813	0.00428	
	CV	0.29	0.92	2.70	3.70	1.38	
	EAS					6.08	
NO_2^- (mg/L)	Median	0.16	0.29	0.01	0.01	0.03	0.043
	Min.	0.005	0.02	0.003	0.01	0.005	
	Max.	0.25	0.45	0.01	0.01	0.10	
	CV	1.47	1.18	0.79	0.49	3.36	
	EAS					1.0	
NO_3^- (mg/L)	Median	6.17	0.06	0.05	0.023	0.14	0.001
	Min.	4.81	0.04	0.02	0.02	0.01	
	Max.	9.46	0.20	0.07	0.026	1.01	
	CV	0.57	2.09	0.86	2.40	5.73	
	EAS					10	
TN (mg/L)	Median	13.36	8.2	3.75	0.08	0.0434	< 0.001
	Min.	10.8	6.0	2.4	0.03	0.0097	
	Max.	15.74	9.0	6.8	0.12	1.5	
	CV	0.357	0.27	1.08	0.68	2.76	
	EAS					20	
PO_4^{3-} (mg/L)	Median	1.78	1.60	1.45	0.80	0.35	0.028
	Min.	0.76	1.31	0.3	0.25	0.11	
	Max.	2.19	1.81	1.8	1.06	0.65	
	CV	0.71	0.15	0.73	0.93	1.35	
	EAS					15.33	
TP (mg/L)	Median	7.72	4.18	4.31	4.74	4.23	0.052

(*Continued.*)

Table 2 | Continued

Variables statistics		Quality of water from: Influent	Anaerobic pond	Facultative pond	Maturation pond ♯1	Maturation pond ♯2	*P*-value
	Min.	6.75	2.98	4.12	3.42	4.29	
	Max.	8.02	4.46	4.81	6.79	6.04	
	CV	0.14	0.28	0.12	0.58	0.26	
	EAS					5.0	
Organics:							
BOD_5 (mg/L)	Median	233	133	103	91	63	0.001
	Min.	226	125	89	69	61	
	Max.	255	155	122	98	69	
	CV	0.11	0.19	0.22	0.31	0.11	
	EAS					50	

n = 5 sites × 8 individual samples per site; CV, coefficient of variation; EC, electrical conductivity; TDS, total dissolved solids; TN, total nitrogen; TP, total phosphorus; BOD_5, five-day biological oxygen demand; EAS, East African standards for wastewater discharged on land and into water bodies.

combination of microbial denitrification (Ahmed *et al.* 2023), sedimentation of organic matter and adsorbed nutrients (Gomes *et al.* 2024), and direct nutrient uptake by invasive macrophytes (Ali *et al.* 2024; Blandford *et al.* 2024). A consistent and substantial decrease in BOD_5 was observed across all stages. This reduction was attributed to sedimentation of organic particles (Gomes *et al.* 2024) coupled with heterotrophic bacterial decomposition. Given the noted absence of algal communities, the oxygen required for this decomposition was likely supplied by the root systems of the invasive macrophytes (Rehman *et al.* 2023).

The influent wastewater was characterized by high median concentrations of TC (9.1×10^7 cfu/100 mL), *E. coli* (7.6×10^7 cfu/100 mL), and ENT (4.4×10^7 cfu/100 mL). The concentration of CP was approximately an order of magnitude lower (2.6×10^6 cfu/100 mL). A significant reduction in all microbiological parameters was observed between the influent and the final effluent ($P < 0.05$, $n = 40$; Figure 3). The final effluent concentrations were 1.7×10^6 cfu/100 mL for TC, 3.8×10^5 cfu/100 mL for *E. coli*, 7.8×10^5 cfu/100 mL for CP, and 6.9×10^4 cfu/100 mL for ENT. Analysis of the treatment stages revealed distinct removal patterns for FIB: the anaerobic and facultative ponds were primarily responsible for reducing TC, while *E. coli* and ENT were consistently reduced across all stages. CP was substantially removed in the facultative and maturation ponds. The extent of reduction for TC, *E. coli*, and ENT was significantly higher than for CP ($P < 0.05$; Figure 3). This disparity can be explained by CP's spore-forming capability, which confers resistance to environmental stressors and treatment processes (Hu *et al.* 2021). Furthermore, its reversible adsorption to solids may facilitate transport through the system and subsequent release, impeding complete elimination (Medeiros *et al.* 2019). Despite the operational challenges facing the investigated WSP system, the treatment stages achieved substantial removal of FIB. However, when compared with well-maintained, optimally operated WSP systems reported in the literature (Shaheen *et al.* 2024), the FIB reduction by the investigated system was much lower, underscoring the importance of consistent maintenance and proper operation.

The removal efficiency of various pollutants by the investigated WSP system is shown in Figure 4. Overall, the median removal efficiency for all pollutants was 70 ± 6.9%, demonstrating strong performance given the maintenance drawbacks. However, the removal efficiency varied significantly among pollutants. TP, EC, and TDS had lower removal efficiencies (33.59 to 45.2%) compared with other pollutants – namely NH_3, NO_3^-, NO_2^-, TN, PO_4^{3-}, BOD_5, turbidity, TC, *E. coli*, CP, and ENT – which exhibited higher removal efficiencies (73–99.67%). This discrepancy is common in conventional WSP systems, which are optimized for organic matter degradation and pathogenic reduction (Coggins *et al.* 2019; Edokpayi *et al.* 2021; Kadri *et al.* 2021) but lack effective mechanisms for stripping dissolved ions or facilitating the assimilation, sedimentation, and precipitation processes required for extensive TP removal. The disparity between higher PO_4^{3-} removal compared with TP has been also reported in previous studies (Kalengo *et al.* 2021). While PO_4^{3-} can be rapidly removed from the water column – primarily through abiotic mechanisms such as adsorption onto metal oxides (Fe, Al, and Mn) and precipitation with calcium or iron, as well as biotic mechanisms such as assimilation by macrophytes and microbes – release of adsorbed PO_4^{3-} back into the water column due to changing redox conditions was unlikely in this study, as PO_4^{3-} decreased consistently across treatment stages throughout the sampling period (Table 2). In contrast, TP, a significant portion of which is bound in particulate matter, is

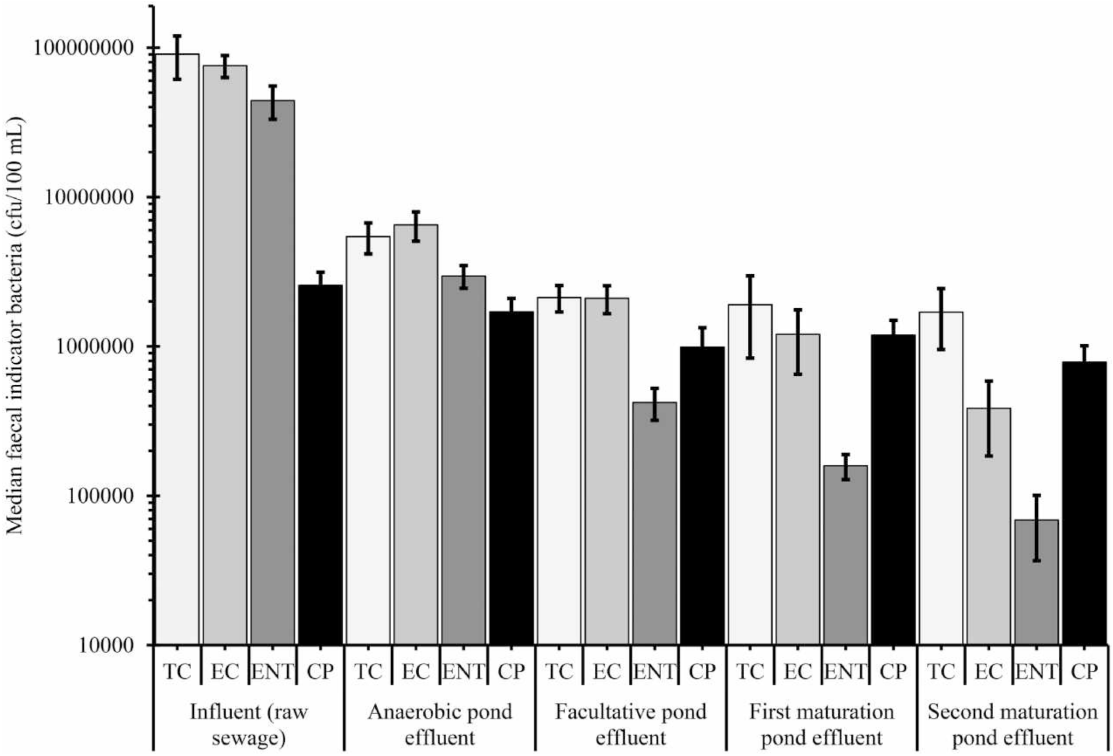

Figure 3 | Comparison of median concentrations of faecal indicator bacteria between influents and effluents of anaerobic, facultative and maturation ponds of the studied wastewater stabilization ponds. Error bars represent nonparametric coefficient of variation (CV), $n = 5$ sites × 8 individual samples per site. TC, total coliforms; EC, *Escherichia coli*; CP, *Clostridium perfringens*, ENT, enterococcus.

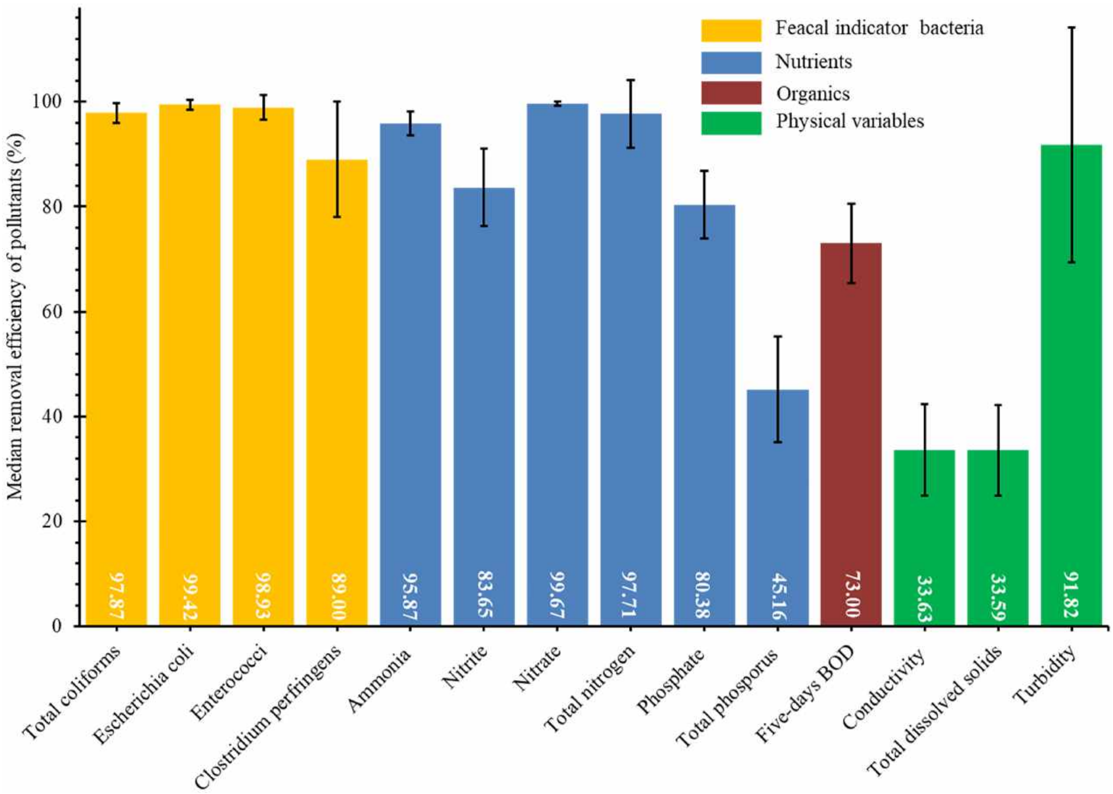

Figure 4 | Median removal efficiency (%) of faecal indicator bacteria, nutrients, organics and physical variables in wastewater treated by various treatment stages of wastewater stabilization pond system. Error bars represent nonparametric coefficient of variation.

removed primarily through sedimentation and physical filtration. The hydraulic overloading resulting from increased population growth observed in the studied WSPs may have led to insufficient hydraulic retention time for complete settling of particulate matter. Moreover, as WSPs serve as feeding grounds for waterfowl (Msaki *et al.* 2023; Damas & John 2024), their foraging and bioturbation activities disturb the sediments, leading to resuspension of particulate matter and thereby reducing net TP retention efficiency. Interestingly, the removal efficiencies for NH_3, NO_3^-, NO_2^-, TN, and PO_4^{3-} in this system were higher than those reported in previous studies in Tanzania (Zacharia *et al.* 2019) and elsewhere (Desye *et al.* 2022), suggesting the importance of invasive macrophytes in the studied WSPs. The BOD_5 removal efficiency was consistent with the findings of Zacharia *et al.* (2019) but higher than that reported in Egypt (Shaheen *et al.* 2024) and Ethiopia (Desye *et al.* 2022), though removal efficiency is inherently dependent on wastewater characteristics, WSP operational conditions, and environmental factors. Although the BOD_5 removal efficiency (73%) of the studied WSP system was high, it was substantially lower than the 95% efficiency of a system operating under optimized conditions (Achag *et al.* 2021). This indicates that the effluent still contained a substantial amount of organic matter, which can reduce the aesthetic value of the receiving water body and diminish dissolved oxygen, leading to stress and death in oxygen-sensitive aquatic organisms (Edokpayi *et al.* 2021). Conversely, the removal efficiencies for TC, *E. coli*, CP, and ENT ranged from 89 to 99.42%. However, these values were lower than the 99.999% efficiency of a WSP system under optimal conditions, confirming the discharge of partially treated wastewater. This release poses a potential threat to human health and the environment, as pathogenic microorganisms and parasites have been detected in partially treated wastewater. Similarly, there have been several reports of typhoid, cholera, and diarrheal diseases linked to human contact with partially treated wastewater and the consumption of vegetables irrigated with partially treated wastewater. Consequently, it is essential for the managers and operators of the WSP system to ensure that wastewater discharged from WSPs meets the required guidelines to prevent outbreaks of microbiological and parasite -related diseases.

The types and abundances (%) of FIB attached to the roots of the invasive macrophytes are presented in Figure 5. TC, *E. coli*, ENT, and CP were detected on the roots of *L. adscendens*, *P. stratiotes*, *C. esculentus*, and *T. latifolia*, with varying abundances. Across all species, the abundances of TC and CP were significantly higher than those of *E. coli* and ENT ($P < 0.05$; Figure 5), with bacterial abundance on the roots increasing in the order: ENT < *E. coli* < CP < TC. TC was the most abundant bacterium on the roots of *L. adscendens*, *P. stratiotes*, and *T. latifolia*, whereas CP was the most abundant on the roots of *C. esculentus*. The dominance of CP over the other bacteria on *C. esculentus* suggests a competitive advantage for CP in this specific niche. A plausible explanation is that the root environment or its exudates may be inhibitory to TC, *E. coli*, and ENT, but not to spore-forming bacteria such as CP. This is because CP forms resistant spores that confer tolerance to harsh

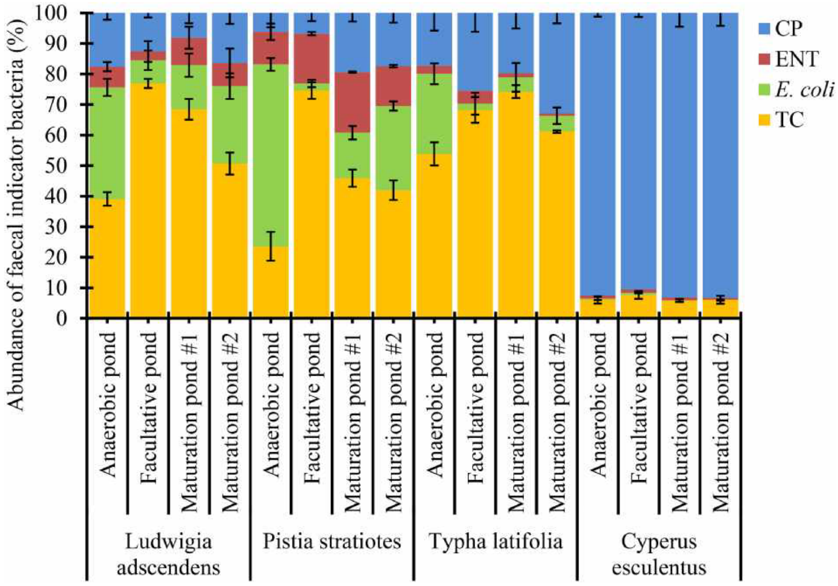

Figure 5 | Composition of faecal indicator bacteria detected on the root surface of different aquatic microphytes observed in various ponds of wastewater stabilization pond system. Error bars represent the nonparametric coefficient of variation. TC, total coliforms; CP, *Clostridium perfringens*; ENT, enterococcus.

conditions, a trait not shared by other FIB (Hu *et al.* 2021). To our knowledge, this is the first study to document the colonization of FIB on the root surfaces of macrophytes invading a WSP system. Root colonization by FIB represents a potential direct pathway for the removal of these bacteria from the wastewater column. This mechanism could contribute to the significant decline of FIB observed across the treatment stages (Figure 3) and the high removal efficiency (Figure 4). This finding is consistent with that of Bouchaala *et al.* (2025), although their work was conducted in natural wetlands with macrophyte species other than those identified in the present study. While our results provide evidence that invasive macrophytes directly remove FIB from wastewater through root colonization, these plants may also contribute to indirect removal by reducing wastewater flow velocity, thereby promoting bacterial sedimentation as previously described. Although FIB colonizing the roots of invasive macrophytes may be released into the water during disturbance or senescence events – potentially compromising effluent quality in WSP systems – macrophytes can also enhance indigenous microbial communities. This enhancement is partly attributed to thermal insulation and temperature moderation provided by macrophyte stands, which can create stable conditions that support diverse microbial assemblages, including ciliates and flagellates that exert grazing pressure on FIB (Kim *et al.* 2022). However, further research is recommended to understand the fate of root-attached FIB and to evaluate the suitability of using such invasive macrophyte species to improve the performance of WSP systems. This is particularly relevant for developing countries, where WSPs are widely used for wastewater treatment but are often characterized by poor-quality effluent.

The role of invasive macrophytes in pollutant removal was further demonstrated by statistically correlating the occupancy of each invasive macrophyte species across the WSP treatment stages with the removal efficiencies of various pollutants, using Spearman's Rank correlation metric. Significant correlations ($r = 0.40–0.96$, $P < 0.05$) were observed between the occupancy of *L. adscendens, P. stratiotes, T. latifolia*, and *C. esculentus* and the removal efficiencies of EC, TDS, turbidity, BOD_5, NO_2^-, NO_3^-, PO_4^{3-}, TN, TP, TC, *E. coli*, ENT, and CP (Table 3). An examination of the correlation coefficients revealed that no single invasive macrophyte species was consistently correlated with all pollutants. Furthermore, the patterns of correlation coefficients differed among macrophyte species, suggesting that a variety of macrophytes is necessary for the optimal removal of a broad pollutant profile. Despite multiple lines of evidence demonstrating their participation in pollutant removal, the dense growth of invasive macrophytes may obstruct light penetration. This shading can limit algal growth – which is essential for pond aeration and organic matter oxidation in typical WSPs – and may also reduce pathogen inactivation by UV light. This shift in treatment dynamics indicates that WSPs dominated by invasive macrophytes operate through fundamentally different mechanisms than conventional, algal-based WSPs. While the impact of these specific macrophyte species in WSPs is reported here for the first time, the general effectiveness of macrophytes in pollutant removal is well-established in constructed and natural wetlands (Ali *et al.* 2024; Blandford *et al.* 2024; Bouchaala *et al.* 2025). Our results align with this existing body of work. Therefore, these findings provide a basis for future research on optimizing primary pollutant removal through appropriate macrophyte(s) selection, assessing their efficacy against emerging contaminants (e.g., antibiotics, hormones, metals, and microplastics), and ultimately integrating them into design strategies to enhance treatment performance.

Table 3 | Spearman rank correlations between occupancy of different macrophyte species occurring in the investigated WSP system and removal efficiency (%) of various physico-chemical and microbiological parameters

	Spearman rank correlation coefficients (r) for:													
	Physical variables			Nutrients						Organics	Faecal indicator bacteria			
Macrophyte	EC	TDS	Turbidity	NH_3	NO_2^-	NO_3^-	TN	PO_4^{3-}	TP	BOD_5	TC	*E. coli*	ENT	CP
L. adscendens	0.49	0.49	0.73	0.26	0.73	0.15	0.60	0.73	0.26	0.26	0.85	0.26	0.60	0.15
P. stratiotes	0.23	0.23	0.86	0.40	0.50	0.24	0.47	0.86	0.24	0.40	0.92	0.40	0.83	0.12
T. latifolia	0.40	0.40	0.83	0.50	0.47	0.49	0.50	0.83	0.33	0.50	0.96	0.50	0.86	0.24
C. esculentus	0.80	0.80	0.47	0.83	0.07	0.67	0.80	0.47	0.93	0.83	0.24	0.83	0.40	0.96

EC, electrical conductivity; TDS, total dissolved solids; TN, total nitrogen; TP, total phosphorus; BOD_5, 5 days biological oxygen demand; TC, total coliforms; ENT, enterococci; CP, *Clostridium perfringens*. The color-scaled heatmap represents the Spearman's correlation coefficients (*r*). Blue represents the least correlation coefficients ($p > 0.05$), white represents medium correlation coefficients ($p < 0.05$), and red represents strong correlation coefficients ($p < 0.002$).

The discharge from the investigated WSP system flows into the Ngerengere River and is frequently used for watering goats and cattle and for irrigating vegetables and crops. Therefore, its physicochemical and microbiological quality was assessed for compliance with the East African Standard for discharging wastewater to the environment (EAS 1172:2023). The effluent quality (Table 2; Figure 3) met the EAS standards for temperature, pH, TDS, EC, ammonia, nitrite, nitrate, TN, and TP. However, turbidity, BOD_5, and FIB – specifically TC and *E. coli* – exceeded the permissible limits of 30 NTU, 50 mg/L, 10,000 cfu/100 mL, and 400 cfu/100 mL, respectively. This noncompliance is likely attributable to a lack of periodic desludging to prevent sludge buildup. Accumulation of sludge is a primary factor influencing the hydraulics of WSPs. It reduces the effective volume and promotes short-circuiting, thereby decreasing the actual hydraulic retention time approximately in proportion to the volume occupied by sludge deposits. This reduction in hydraulic retention time negatively impacts treatment performance, ultimately compromising the quality of the effluent (Palacios-Robles *et al.* 2024). Furthermore, overgrowth of vegetation within and along the ponds may have reduced pond volume, altered wastewater flow and hydraulic retention time, and impeded the light penetration needed by algae to produce oxygen for organic matter degradation and pathogen inactivation. Additionally, the WSP system receives more wastewater than its original design capacity due to population growth. This hydraulic overloading reduces the wastewater retention time, leading to poor effluent quality. Another major issue is the lack of routine water quality and performance monitoring, which is essential for operators to identify problems early and make necessary adjustments to achieve target removal rates for pollutants, especially FIB, organic matter, and turbidity. Consequently, a comprehensive WSP management system should be established to ensure appropriate maintenance, and frequent effluent quality monitoring is performed in a timely manner. This would enhance the system's performance and, more importantly, protect public and environmental health. Furthermore, installing a flow meter is essential for accurately measuring influent flow rate. This parameter is critical for calculating theoretical hydraulic retention time and for determining the extent to which the WSP system must be expanded to meet the demands of a growing population. When combined with periodic assessments of pond volume and, where necessary, tracer studies to characterize actual residence time distribution, flow measurement provides the empirical foundation needed to optimize treatment performance, safeguard effluent quality, and ensure the long-term sustainability of pond systems. Additionally, a fence with warning signs should be installed to prevent livestock from drinking directly from the ponds and to raise community awareness about safety around the WSP system. Moreover, further investigation is warranted to explore the feasibility of integrating the observed macrophyte species into WSPs under controlled conditions to prevent escape into surrounding water bodies, alongside optimized harvesting regimes to ensure permanent removal of assimilated pollutants, appropriate hydraulic retention time to balance treatment efficiency with hydraulic loading, and management of mosquitoes (to mitigate public health risks) and waterfowl (to minimize bioturbation and faecal matter loading)—to enhance the removal of pollutants such as nutrients that are only partially removed by conventional WSP processes. Finally, the treated effluent, once safe, could be used for crop production and fish culture. The income generated could then be used to maintain the WSP system and cover the costs of ongoing effluent quality monitoring.

CONCLUSION

The studied WSP system was characterized by inadequate maintenance, hydraulic overloading due to population growth, sludge accumulation, and colonization by invasive aquatic macrophyte species. The system utilizes different treatment stages – anaerobic, facultative, and two maturation ponds – to remove pollutants from wastewater. The WSP system demonstrated high removal efficiency for turbidity, nitrite, nitrate, ammonia, TN, phosphate, BOD_5, TC, *E. coli*, ENT, and CP, but low removal efficiency for TDS, EC, and TP.

Substantial numbers of FIB were enumerated from the root surfaces of various invasive macrophyte species across the ponds. A significant positive correlation was observed between macrophyte occupancy in the WSPs and the removal efficiency of nutrients, BOD_5, turbidity, TDS, EC, and FIB. This suggests that the aquatic macrophytes played a crucial role in removing multiple pollutants from the wastewater.

The effluent discharged from the WSP system complied with standards for nitrite, nitrate, ammonia, TN, phosphate, TDS, EC, temperature, pH, and TP. However, levels of FIB, BOD_5, and turbidity exceeded permissible limits, likely due to poor maintenance. These results provide valuable insights that will guide WSP managers in developing countries to develop appropriate restoration plans for systems compromised by sludge accumulation, hydraulic overloading, and invasive macrophytes. The findings also support the potential use of suitable aquatic plants under controlled conditions to enhance pollutant removal. Further research is needed to understand the fate of root-attached FIB and the consequences of invasive macrophyte die-off for final effluent quality.

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AUTHOR CONTRIBUTION STATEMENT

L. J. M., D. M., and A. J. M. designed the study. L. J. M. conducted the experiment, performed sampling and sample analysis, and analyzed the data. L. J. M. also drafted the initial manuscript. D. M. and A. J. M. revised the manuscript. All authors reviewed and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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