Microbial Analysis and Compliance Levels of Wastewater Treatment Using a Nature-Based Solution of Phytoremediation System at National Police College, Rwamagana District, Rwanda

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Abstract

This research evaluated the microbial status and compliance level of wastewater treated through a nature-based phytoremediation system in Rwanda. Physicochemical parameters such as pH, temperature, total suspended solids (TSSs), total dissolved solids (TDSs), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) were measured and analyzed. The microbial contents, such as total heterotrophic bacteria count, fecal coliform bacteria, total coliform bacteria, *E. coli*, and helminth eggs, were also investigated. Morphological identification and biochemical tests were conducted via standard techniques. After water treatment in different chambers, the physicochemical parameters were analyzed, and the mean values of the microbial indicators were all within the compliant limits of the Rwanda established water quality standards. The results revealed a decrease in the pH, TSS, BOD_5, COD, TP, HE and microbial counts as the wastewater passed through the initial and middle stages. However, there was a statistically significant decrease in the physicochemical parameters and microbial indicators as the water passed through the final stages of water treatment. These relative and logarithmic decreases indicate polishing and pathogen removal efficiency at the end of the treatment. Therefore, the National Police College

phytoremediation system is effective at removing both organic pollutants and pathogenic microorganisms.

Keywords: *Compliance, Microbial, Microorganisms, Nature-based, Phytoremediation, Rwanda, Wastewater*

Introduction

Rwanda has made significant strides in terms of environmental protection and sustainable development, with wastewater (ww) management being a critical component of the national environmental agenda. However, ww generation and discharge into various surfaces and groundwater bodies present severe threats of water pollution at the local, national and global levels. In many countries, the ww treatment processes required to recover these waters to acceptable quality standards are either nonexistent or inadequate. Water is key to economic growth and development; hence, strict laws in alignment with global protocols for sewage treatment before dumping into water bodies or open environments to ensure the safety of life, the sustainability of the environment, improved water management and enhanced overall ecosystem health have been passed [1].

Sustainable development goal 6 declares the importance of achieving clean water and sanitation for all, and to achieve this goal, the world must reduce freshwater pollution by eliminating dumping and minimizing the release of hazardous chemicals into water bodies [2]. According to the UNICEF report 2023, Rwanda strives to achieve this goal, and only 57% of the population has access to safe drinking water [3]. The growing challenge of water resource management is attributed to the growing population and robust quest for socioeconomic development. Tremendous efforts have been made to launch a Rwandan National Policy (RNP) for the management of the water and sanitation sector, which consists of strategies and programs for the development and rehabilitation of human resources and social and economic infrastructures [4]. Consequently, ww treatment through nature-based solutions (NBSs) or biological processes is a key part of robust policy.

Relentless global effort is evolving through extensive research in search of the best ww treatment techniques, which focus on key issues involving ww purification, reuse, availability and improvement of the quality for human consumption via sustainable NBS. Apart from the global standards of the WHO, the Rwanda Standards Board (RSB) and the Rwanda Environment Management Authority (REMA) have established water quality standards [5] that specify maximum permissible limits for various physicochemical and microbiological parameters in treated ww before discharge into the environment.

Nature-based solution (NBS) systems, particularly phytoremediation (phytoextraction, rhizofiltration, phytodegradation, rhizodegradation and phytostabilization) systems, have gained prominence as eco-

friendly alternatives to conventional ww treatment methods. These systems utilize plants and associated microorganisms to remove, degrade, or sequester contaminants from wastewater while providing additional ecosystem services. Sustainable NBS, as an evolved method of ww treatment, employs innovative technologies such as biological treatment, membrane technologies, resource recovery, circular economic approaches (integrated systems that recover water, energy, and nutrients), and smart water management systems essential for conserving water resources and reducing environmental pollution [6] without causing harm to the environment. It is therefore important that feasible techniques for tackling water pollution and protecting the ecosystem from the harmful impacts of pollution in the present-day environment are intelligently developed.

The National Police College (NPC) in Rwamagana District represents an institutional facility requiring effective ww treatment solutions that are both economically viable and environmentally sustainable. Only a few studies have evaluated the microbial community during ww treatment; therefore, this study comprehensively investigated the exact roles that microbial communities play in full-scale phytoremediation systems, thereby highlighting a pertinent gap addressed by this study. The capacity to optimize and expand this NBS for wider use in Rwanda and similar settings is limited by the scarcity of available data; therefore, by assessing the microbial processes involved in site treatment through each treatment chamber, this research seeks to bridge the wide knowledge gap.

Materials and methods

The Study Area

This study was carried out at a domestic facility in Rwamagana District located in the Eastern Province of Rwanda, within the coordinates of 1° 54' 12. 471" S and 30° 25.796 " E. (latitude -1.953513, longitude 30.438644.) at the elevation of 1,476.45 Masl. (Fig 1). This study site is located approximately 50 kilometers east of Kigali, the country's capital city [7]. The region is characterized by a tropical subhumid climate with two distinct seasons: wet and dry. The monthly temperature ranges from 22 °C - 23 °C, and the annual rainfall ranges from 1100 mm to approximately 2000 mm during the wet and dry seasons.

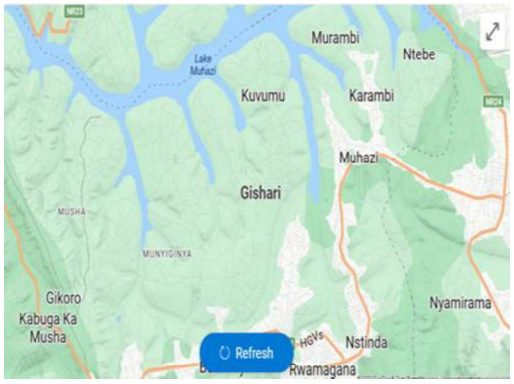

Fig. 1: Map of Rwanda showing the study site area (NPC, Rwamagana)

Ethics approval and consent to participate

Not applicable

Design of the Wastewater (ww) Treatment System

The ww treatment system consists of seven (7) chambers/units, which include the influent chamber (Inlet), anaerobic baffled reactor/bioreactor (ABR), first blackstone flotation (FBF), shallow aeration basin (SAB), second blackstone flotation (SBF), phytoremediation bed (PB), and outlet/shallow maturation basin (SMB). The influent chamber receives the ww directly, and the ABR is built with reinforced concrete and designed to receive ww and accompanying solid waste in volume (Fig. 2). The chamber consists of a series of interconnected compartments within a single reactor designed to regulate the flow of incoming ww and retain sludge efficiently, while the digestate is released in ww. This configuration enhances the separation of bacterial trophic groups and provides high tolerance to both hydraulic and organic shock loads. Physical retention and anaerobic microbial biodegradation produce the digestate within the membrane of the bioreactors. The liquid digestate is then discharged after accumulating to a certain volume through an outlet into the FBF (Fig 3). The FBF belt in the system is designed to retain flocs and optimize the digestate flow rate while allowing the injection of atmospheric air. It is bedded with black stones of various sizes and shapes in convoluted unit-like structures that increase the ww retention time to enable the absorption of contaminants through the large surface area of black stones. It also enables ww to interface with the multidimensional rough surfaces of black stones, which are suitable habitats for microbes, thereby increasing the ww retention time. The black stones traditionally purify water by absorbing impurities and contaminants in soluble substances within a solution on a proper surface through easing the area available for adsorption.

Fig 2: Anaerobic Baffled Reactor/Bioreactor (ABR) chamber

Fig 3: First Blackstone Flotation (FBF)

These SABs are units with dimensions of (4 × 3 × 1.5) m and are built with a concrete cement block with built-in cells (compartmentalized to facilitate the integration of atmospheric air into the ww) within the structure, which allows for mechanical agitation of the ww while it circulates from one cell to another (Fig. 4). This process promotes flocculation and supports aerobic microbial activity, which is essential for organic matter breakdown. This system allows for oxygen transfer, mixing, biological decomposition and nitrification. Pollution reduction and odor control are achieved in this chamber through a reduction in biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in ww. Additionally, anaerobic conditions that would otherwise produce hydrogen sulfide and other odorous compounds should be prevented. The SBF in the system is designed to retain flocs and optimize the digestate flow rate while allowing the injection of atmospheric air. It is bedded with black stones of various sizes and shapes in convoluted unit-like structures that increase the ww retention time to enable the absorption of contaminants through the large surface area of black stones. It also enables ww to interface with the multidimensional rough surfaces of black stones that are suitable habitats for microbes (Fig. 5), thereby increasing the ww retention time over a large surface area ratio.

Fig 4: Shallow Aeration Basin (SAB)

Fig 5: Second Blackstone Flotation (SBF)

The phytoremediation bed (PB) is the core treatment unit responsible for both nitrification and denitrification processes. The bed features compartments with moderate flow rates that hold ww beneath specially selected plant species, i.e., vetiver (*Chrysopogon zizanioides*) Source: French & Maynard, 2022).

These grow on a simulated substrate composed of four distinct, juxtaposed layers. Wastewater flows through the treatment bed via vertical or horizontal subsurface pathways or occasionally via surface flow. A combination of surface and subsurface flow systems is referred to as amphibian flow. As shown in Fig. 6, the PB is built with concrete cement blocks of dimensions (8 × 12 × 1.5) m with cells within which plant species grow. This green approach detects, degrades, and removes various types of pollutants from the environment. The plant species take up pollutants from ww and detoxify their toxic effects.

SMBs are shallow, open basins that facilitate natural disinfection through prolonged solar exposure, serving as a form of tertiary treatment. The effluent from the aeration basin may either be discharged into the natural environment or redirected for reuse, such as in landscape irrigation. This is where the treated ww exits the entire system of purification after an extended retention time at the phytoremediation and aeration chambers. It enters through the influent and circulates in the bioreactor while mechanical agitation takes place; then, it discharges into the flocculation.

Fig 6: Phytoremediation Bed (PB)

Fig 7: Outlet/Shallow Maturation Basin (SMB)

Sampling Techniques

The ww samples (influent to effluent) were collected for microbial diagnostic evaluation and compliance studies. The grab technique was used to collect ww samples systematically from seven chambers/units beginning from the exit chamber in a backward direction (Fig. 8) to the influent unit via standard procedures by directly filling the sample container [8]. This technique was appropriate, adequate and practical because ww samples were collected from a point where they were well mixed near the center of the flow channels within each chamber at approximately 40 to 60% of the water depth where possible [8]. Five (5) samples each were collected from seven (7) chambers/units in addition to the composite sample, resulting in a total of forty (40) samples. Samples of ww were collected in two (2) sterilized water samples (100 cm^3 for microbiological analysis and 1000 cm^3 bottles for physicochemical analysis). Another approximately 200 cm^3 portion of ww was collected in a 250 cm^3 beaker for in situ testing at the site. A HACH HQ40d multimeter was used to measure the temperature, pH and total dissolved solids (TDS) by dipping the probes into the ww samples, and the readings were recorded accordingly.

Sample collection activities proceeded progressively from the least suspected contaminated area to the most suspected contaminated area. It commenced from the effluent chamber (SMB) to the influent unit (Inlet), and ww samples were collected into well-labeled sample collection bottles, placed in an ice pack and transported to the laboratory. Samples of ww were collected in equal volumes and mixed as composite samples.

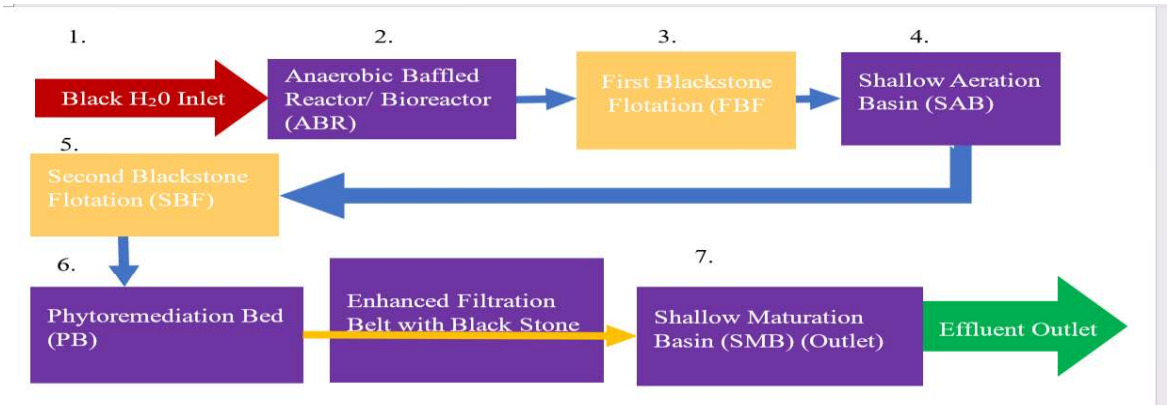

Fig. 8: Flow process of black water treatment at the Gishari police training school

Wastewater analysis

Standard methods were used for data collection and analysis in this research. Physicochemical parameters such as pH, temperature, total suspended solids (TSSs), total dissolved solids (TDSs), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) were measured and analyzed. The microbial contents, such as total heterotrophic bacteria (THB) count, fecal coliform (FC) count, total coliform (TC) count, *E. coli* count, and Helminth egg (HE) count, were also investigated. Morphological identification and biochemical tests involving catalase, coagulase, motility, indole and oxidase tests were conducted via standard techniques [9].

Results

Physicochemical parameters and compliance limits at various treatment stages (chambers)

As shown in Table 1 and Fig. 9, the eight (8) physicochemical parameters investigated were pH, temperature, TSS, TDS, BOD, COD, TN and TP.

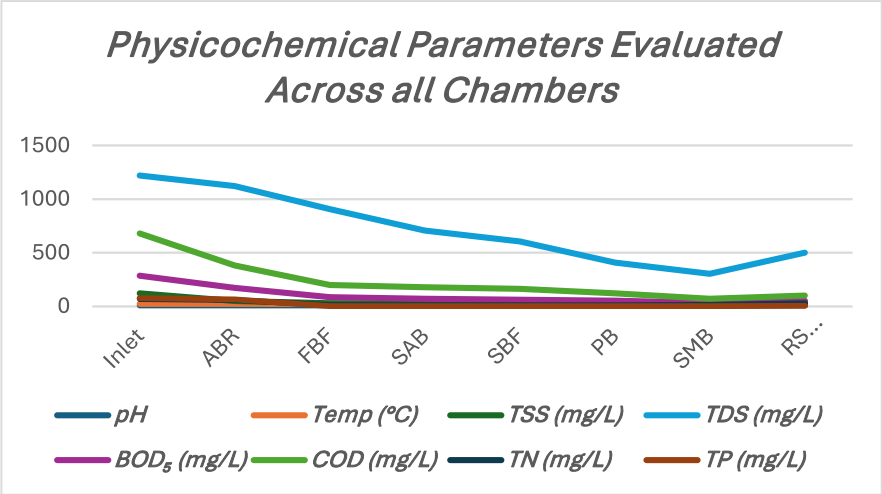

Fig 9: Physicochemical parameters and compliance limits at various treatment stages (chambers)

pH values of the ww in the various treatment chambers

The mean pH values of the ww in the various treatment chambers are shown in Table 1 and Fig. 10. The results indicated that the highest value of pH (7.8±0.4) was recorded at the inlet, followed by

7.6±0.3 and 7.4±0.2 at the ABR and FBF, respectively. However, the lowest mean value of 7.1±0.2 was recorded at the SMB, followed by 7.1±0.4, 7.2±0.1 and 7.2±0.4 for chambers PB, SBF and SAB, respectively.

The temperature of the ww in the various treatment chambers

As shown in Table 1 and Fig. 10, the mean values of Temp (°C) were recorded. The results revealed that the highest value of 23.2±2.1 was recorded at Inlet, followed by 22.9±1.9 and 22.7±1.5 at the ABR and FBF, respectively. However, the lowest mean value of 21.3±1.4 was recorded at the SMB, followed by 21.3±1.9, 22.3±0.4 and 22.3±1.5 for chambers PB, SBF and SAB, respectively.

The TSS content in the ww in the various treatment chambers

As shown in Table 1 and Fig. 10, the mean TSS (mg/L) values were recorded. The results revealed that the highest value of 120±45 was recorded at Inlet, followed by 50±28 and 28±12 at the ABR and FBF, respectively. However, the lowest mean value of 22±8 was recorded at the SMB, followed by 25±28, 26±10 and 27±9 for chambers PB, SBF and SAB, respectively.

TDS in the ww in the various treatment chambers

The mean values of TDS (mg/L) were recorded as shown in Table 1 and Fig. 10. The results indicated that the highest value of 1220±28 was recorded at Inlet, followed by 1120±58 and 908±7 at the ABR and FBF, respectively. However, the lowest mean value of 304±35 was recorded at the SMB, followed by 409±28, 606±15 and 708±7 for chambers PB, SBF and SAB, respectively.

The BOD in the ww in the various treatment chambers

As shown in Table 1 and Fig. 10, the mean values of BOD_5 (mg/L) were recorded, and the results revealed that the highest value of 285±52 was recorded at Inlet, followed by 173±35 and 85±15 at the ABR and FBF, respectively. However, the lowest mean value of 32±9 was recorded at the SMB, followed by 52.5±15, 60.5±42 and 72±35 for chambers PB, SBF and SAB, respectively.

The COD in the **ww in the various treatment chambers**

As shown in Table 1 and Fig. 10, the mean values of COD (mg/L) were recorded, and the results revealed that the highest value of 680±78 was recorded at Inlet, followed by 380±58 and 199±28 at the ABR and FBF, respectively. However, the lowest mean value of 72±28 was recorded at the SMB, followed by 120±58, 162±68 and 179±58 for chambers PB, SBF and SAB, respectively.

TNs in the ww in the various treatment chambers

For TN (mg/L), as shown in Table 1 and Fig. 10, the mean values were recorded, and the results revealed that the highest value of 69.5±12 was recorded at Inlet, followed by 57.4±10 and 12.25±7 at the ABR and FBF, respectively. However, the lowest mean value of 12.01±5 was recorded at the SMB, followed by 12.04±7, 12.14±7 and 12.18±7 for chambers PB, SBF and SAB, respectively.

The TPs in the ww in the various treatment chambers

As shown in Table 1 and Fig. 10, the mean values of TP (mg/L) were recorded, and the results revealed that the highest value of 4.96±3.2 was recorded at Inlet, followed by 3.16±2.1 and 2.33±0.8 at the ABR

and FBF, respectively. However, the lowest mean value of 1.95±0.5 was recorded at the SMB, followed by 2.08±0.7, 2.16±2.1 and 1.64±3.2 for chambers PB, SBF and SAB, respectively.

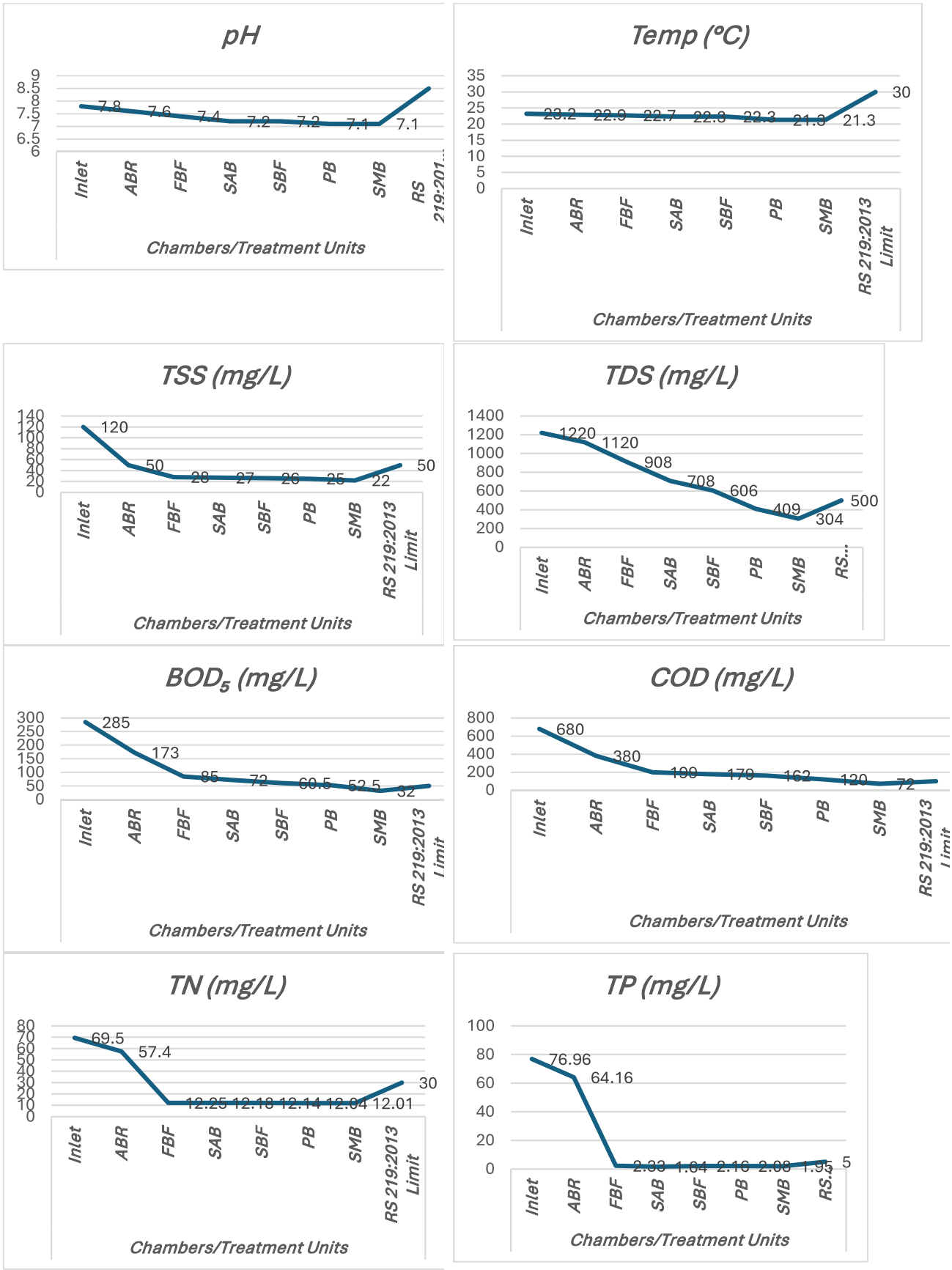

Fig 10: Physicochemical parameters and compliance limits at various treatment stages (chambers)

Table 1: Mean values (mv) of physicochemical parameters and compliance limits at various treatment stages (chambers)

S/No	Parameter	Chambers/Treatment Units							RS 219:2013 Limit	Compliance Status
		Inlet	ABR	FBF	SAB	SBF	PB	SMB		
1	pH	7.8±0.4	7.6±0.3	7.4±0.2	7.2±0.4	7.2±0.1	7.1±0.4	7.1±0.2	6.5-8.5	✓ Compliant
2	Temp (°C)	23.2±2.1	22.9±1.9	22.7±1.5	22.3±1.5	22.3±0.4	21.3±1.9	21.3±1.4	<30	✓ Compliant
3	TSS (mg/L)	120±45	50±28	28±12	27±9	26±10	25±28	22±8	50	✓ Compliant
4	TDS (mg/L)	1220±28	1120±58	908±7	708±7	606±15	409±28	304±35	500	✓ Compliant
5	BOD_5 (mg/L)	285±52	173±35	85±15	72±35	60.5±42	52.5±15	32±9	50	✓ Compliant
6	COD (mg/L)	680±78	380±58	199±28	179±58	162±68	120±58	72±28	100	✓ Compliant
7	TN (mg/L)	69.5±12	57.4±10	12.25±7	12.18±7	12.14±7	12.04±7	12.01±5	30	✓ Compliant
8	TP (mg/L)	76.96±3.2	64.16±2.1	2.33±0.8	1.64±3.2	2.16±2.1	2.08±0.7	1.95±0.5	5	✓ Compliant

RS = Rwandan standard

The microbial indicators at various treatment stages (chambers)

As shown in Table 2 and Fig. 11, five (5) microbial indicators, namely, TC, FC, THB, *E. coli* and HE, were documented.

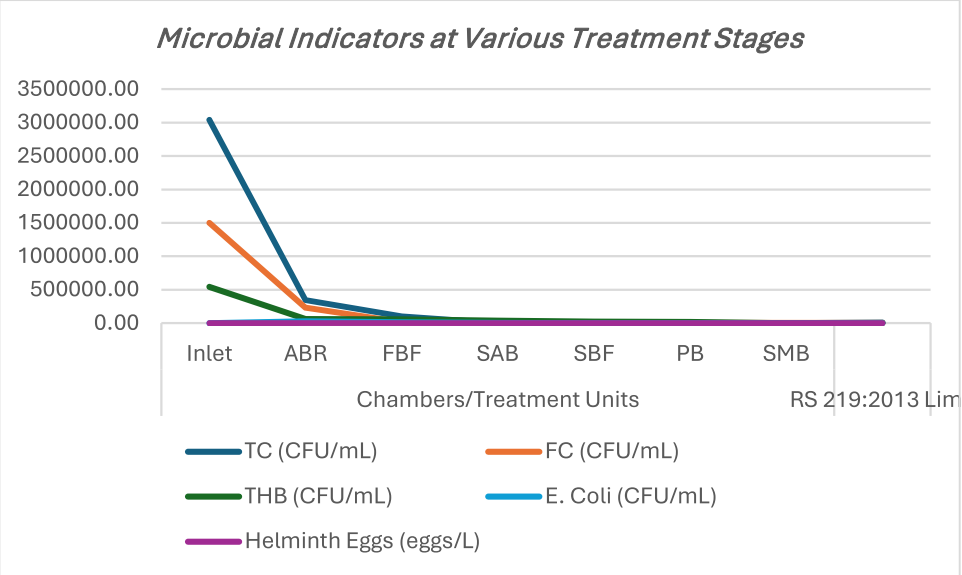

Fig. 11: Microbial indicators at the various treatment stages

The TC counts in the various chambers

The results of the TC count are shown in Table 2 and Fig. 12. The mean values (mv) were recorded, and the results revealed that the highest mean value (hmv) of TC (CFU/100 mL), 3.04x107, was recorded at Inlet, followed by $3.48x10^6$ and $1.01x10^6$ at the ABR and FBF, respectively. However, the lowest mean value (lmv) of $6.80x10^2$ was recorded at the SMB, followed by $5.20x10^3$, $7.20x10^4$ and $3.36x10^5$ for chambers PB, SBF and SAB, respectively.

FC counts in the various chambers

As shown in Table 2 and Fig. 12, the results of the FC (CFU/100 mL) counts revealed that an hmv of 1.50x107 was recorded at Inlet, followed by $2.32x10^6$ and $1.51x10^5$ at the ABR and FBF, respectively. However, an lmv of 2.2×10^3 was recorded at the SMB, followed by $5.50x10^4$, $6.50x10^4$ and $1.00x10^5$ for the PB, SBF and SAB chambers, respectively.

The THB counts in the various chambers

As shown in Table 2 and Fig. 12, the results of the THB (CFU/mL) count revealed that an hmv of 5.44x105 was recorded at the Inlet, followed by $2.32x10^6$ and $5.64x10^4$ at the ABR and FBF, respectively. However, an lmv of 1.5×10^2 was recorded at the SMB, followed by $2.x10^3$, $2.28x10^4$ and $3.91x10^4$ for the PB, SBF and SAB chambers, respectively.

E. coli isolated from the various chambers

As shown in Table 2 and Fig. 12, the results for *E. coli* (CFU/100 mL) revealed an hmv of 3.84x105 at Inlet, followed by $2.60x10^4$ and $1.34x10^4$ at the ABR and FBF, respectively. However, lmv of $0.23x10^2$ was recorded at the SMB, followed by $1.80x10^3$, 3.80x103 and $6.10x10^3$ for chambers PB, SBF and SAB, respectively.

HE isolated from the various chambers

As indicated in Table 2 and Fig. 12, the results were HE (eggs/L). An hmv of 48±8 was recorded at Inlet, followed by 22±4 and 17±4 at the ABR and FBF, respectively. However, lmv of 0<1 was recorded at the SMB, followed by 7±1, 10±4 and 15±4 for chambers PB, SBF and SAB, respectively.

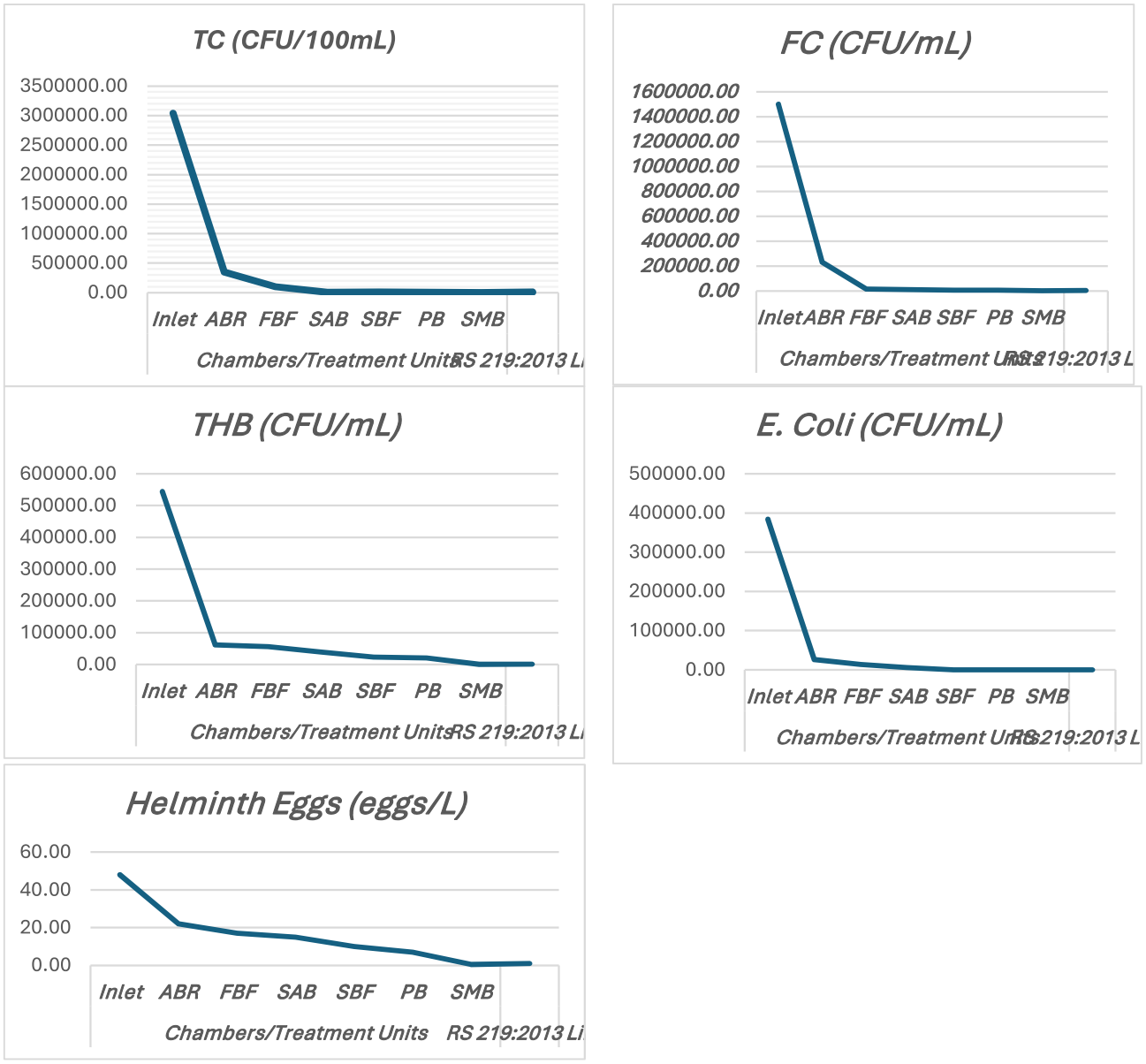

Fig. 12: Microbial indicators at various treatment stages

Table 2: Mean values of microbial indicators at various treatment stages (chambers)

S/No	Microbial indicator	Chambers/Treatment Units							RS 219:2013 Limit	Compliance Status
		Inlet	ABR	FBF	SAB	SBF	PB	SMB		
1	TC (CFU/100 mL)	$3.04x10^7$	$3.48x10^6$	$1.01x10^6$	$3.36x10^5$	$7.20x10^4$	$5.20x10^3$	$6.80x10^2$	<10,000	✓ Compliant
2	FC (CFU/100 mL)	$1.50x10^7$	$2.32x10^6$	$1.51x10^5$	$1.00x10^5$	$6.50x10^4$	$5.50x10^4$	$2.2×10^3$	<5,000	✓ Compliant
3	THB (CFU/mL)	$5.44x10^5$	$6.11x10^4$	$5.64x10^4$	$3.91x10^4$	$2.28x10^4$	$2.x10^3$	$1.5×10^2$	500	N/A
4	*E. coli* (CFU/100 mL)	$3.84x10^5$	$2.60x10^4$	$1.34x10^4$	$6.10x10^3$	$3.80x10^3$	$1.80x10^3$	$0.23x10^2$	≤126	✓ Compliant
5	HE (eggs/L)	48±8	22±4	17±4	15±4	10±4	7±1	<1	<1	✓ Compliant

Physicochemical analysis

As shown in Table 1 and Fig. 13, six (6) of the physicochemical parameters (TSS, TDS, BOD_5, COD, TN and TP) decreased (or slightly fluctuated) across the treatment units from Inlet → SMB. The key insights from the above findings could be summed into the two stages of progressive improvement (each stage reduces the pollutant load, with the most dramatic changes in COD, BOD, TN, and TDS). and critical stages where major nitrogen reduction is experienced between ABR → FBF, compliance is achieved for TDS and TC at PB. The final polishing stage achieves full compliance across all parameters at SMB, with stable compliance of pH, Temp, and TP within the limits throughout. For each of the parameters, the TSS (mg/L) sharply decreased from 120 at Inlet → 22 at SMB. The TDS (mg/L) showed a major decrease from 1220 → 304, with compliance achieved at PB onward. The BOD_5 (mg/L) gradually declined from 170 → 32 and was only compliant at SMB. The TN concentration (mg/L) sharply decreased after the ABR (69.5 → 12.01), which was consistent with the FBF, and the TP concentration (mg/L) always remained consistent; however, it decreased from 4.96 → 1.95, with slight fluctuations midstream.

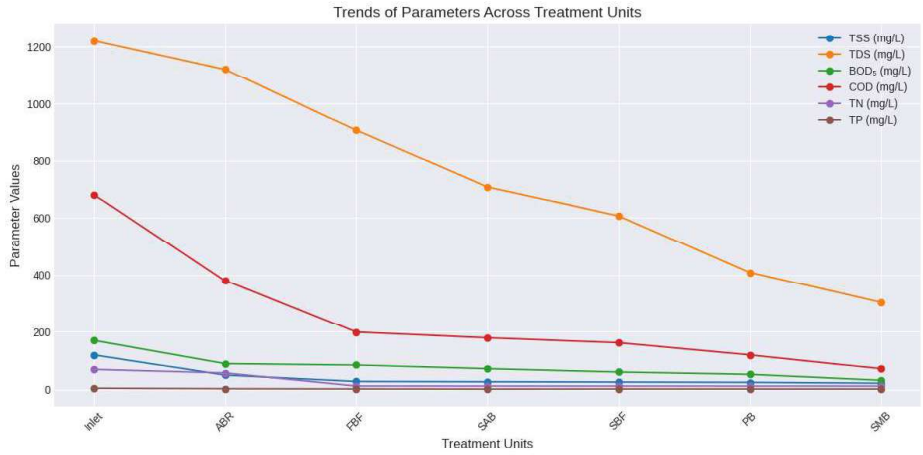

Fig. 13: Trends of some physicochemical parameters in the ww treatment

Pollutant removal

As shown in Table 3, across each of the ww treatment stages in the various units/chambers, six (6) parameters, including the TSS, TDS, BOD, COD, and TN TP, were observed to decrease from the highest pollution status to below the compliance level (WHO, RSB). With respect to each of the parameters, the TSS experienced a major decrease at Inlet → ABR (58.3%: 120 → 50 and $\chi^2 = 0.31$), followed by gradual polishing.

Table 3: Responses of the physicochemical parameters to phytoremediation at various treatment stages (units/chambers)

S/No	Parameter	Chamber/Unit	± error Values	Variation between chambers	(%) Reduction	(X^2) value	Key insights in the treatment unit
1	pH	Inlet	7.8 ± 0.4	Inlet → ABR Δ = −0.2	**2.56** (7.8 → 7.6)	**0.16**	Major drop at **ABR→ FBF**, then gradual polishing
		ABR	7.6 ± 0.3	ABR → FBF Δ =−0.2	**2.63** (7.6 → 7.4)	**0.31**	
		FBF	7.4 ± 0.2	FBF → SAB Δ =−0.2	**2.70** (7.4 → 7.2)	**0.20**	
		SAB	7.2 ± 0.4	SAB → SBF Δ = 0.0	**0** (7.2 → 7.2)	**0.00**	
		SBF	7.2 ± 0.1	SBF → PB Δ =−0.1	**1.38** (7.2 → 7.1)	**0.06**	
		PB	7.1 ± 0.4	PB → SMB Δ =−0.1	**1.40** (7.1→ 7.1)	**0.00**	
		SMB	7.1 ± 0.2	**End**	**Decrease**		
2	Temp (°C)	Inlet	23.2 ± 2.1	Inlet → ABR Δ = −0.3	**1.29** (23.2 → 22.9)	**0.011**	Major drop at **SBF→ PB**, then gradual polishing
		ABR	22.9 ± 1.9	ABR → FBF Δ =−0.2	**0.87** (22.9 → 22.7)	**0.007**	
		FBF	22.7 ± 1.5	FBF → SAB Δ =−0.4	**1.76** (22.7 → 22.3)	**0.036**	
		SAB	22.3 ± 1.5	SAB → SBF Δ = 0.0	**0** (22.3 → 22.3)	**0.00**	
		SBF	22.3 ± 0.4	SBF → PB Δ =−1.0	**4.48** (22.3 → 21.3)	**0.265**	
		PB	21.3 ± 1.9	PB → SMB Δ = 0.0	**0** (21.3 → 21.3)	**0.00**	
		SMB	21.3 ± 1.4	**End**	**Fluctuate**		
3	TSS (mg/L)	Inlet	120 ± 45	Inlet → ABR Δ = −70	**58.3** (120 → 50)	**1.75**	Major drop at **Inlet → ABR**, then gradual polishing
		ABR	50 ± 28	ABR → FBF Δ = −22	**44.0** (50 → 28)	**0.52**	
		FBF	28 ± 12	FBF → SAB Δ = −1	**3.60** (28 → 27)	**0.004**	
		SAB	27 ± 9	SAB → SBF Δ = −1	**3.70** (27 → 26)	**0.006**	
		SBF	26 ± 10	SBF → PB Δ =−1	**3.80** (26 → 25)	**0.001**	
		PB	25 ± 28	PB → SMB Δ = −1	**12.0** (25 → 22)	**0.011**	
		SMB	22 ± 8	**End**	**ORE = 81.6%**		
4	TDS (mg/L)	Inlet	1220 ± 28	Inlet → ABR Δ = −100	**8.2** (1220 → 1120)	**2.41**	Largest improvement at **SBF → PB**
		ABR	1120 ± 58	ABR → FBF Δ = −212	**18.9** (1120 → 908)	**13.17**	
		FBF	908 ± 7	FBF → SAB Δ = −200	**22.0** (908 → 708)	**408.16**	
		SAB	708 ± 7	SAB → SBF Δ = −102	**14.4** (708 → 606)	**37.99**	
		SBF	606 ± 15	SBF → PB Δ = −197	**32.5** (606 → 409)	**38.47**	
		PB	409 ± 28	PB → SMB Δ = −105	**25.7** (409 → 304)	**5.49**	
		SMB	304 ± 35	**End**	**ORE = 75.0%**		
5	BOD_5, (mg/L)	Inlet	285±52	Inlet → ABR Δ =−112	**39.3** (285 → 173)	**3.19**	Major drop at **Inlet → ABR** and final polishing at **PB → SMB**
		ABR	173 ± 35	ABR → FBF Δ = −88	**50.9** (173 → 85)	**5.34**	
		FBF	85 ± 15	FBF → SAB Δ = −13	**15.3** (85 → 72)	**0.12**	
		SAB	72 ± 35	SAB → SBF Δ = −11.5	**16.0** (72 → 60.5)	**0.044**	
		SBF	60.5 ± 42	SBF → PB Δ = −8	**13.2** (60.5 → 52.5)	**0.032**	
		PB	52.5 ± 15	PB → SMB Δ = −20.5	**39.0** (52.5 → 32)	**1.37**	
		SMB	32 ± 9	**End**	**ORE = 88.8%**		
6	COD (mg/L)	Inlet	680 ± 78	Inlet → ABR Δ = −300	**44.1** (680 → 380)	**9.52**	

		ABR	380 ± 58	ABR → FBF Δ = −181	**47.6** (380 → 199)	**7.90**	Strong reductions at **Inlet → ABR** and **ABR → FBF**, then final polishing at **PB → SMB**.
		FBF	199 ± 28	FBF → SAB Δ = −20	**10.1**(199 → 179)	**0.096**	
		SAB	179 ± 58	SAB → SBF Δ = −17	**9.5** (179 → 162)	**0.036**	
		SBF	162 ± 68	SBF → PB Δ = −42	**25.9** (162 → 120)	**0.22**	
		PB	120 ± 58	PB → SMB Δ = −48	**40.0** (120 → 72)	**0.56**	
		SMB	72 ± 28	**End**	**ORE = 89.4%**		
7	TN(mg/L)	Inlet	69.5 ± 12	Inlet → ABR Δ = −12.1	**17.4** (69.5 → 57.4)	**0.60**	**ABR → FBF** is the critical stage for nitrogen removal
		ABR	57.4 ± 10	ABR → FBF Δ = −45.15	**78.6** (57.4 → 12.25)	**13.68**	
		FBF	12.25 ± 7	FBF → SAB Δ = −0.07	**0.6** (12.25 → 12.18)	**0.00005**	
		SAB	12.18 ± 7	SAB → SBF Δ = −0.04	**0.3** (12.18 → 12.14)	**0.00002**	
		SBF	12.14 ± 7	SBF → PB Δ = −0.10	**0.8** (12.14 → 12.04)	**0.0001**	
		PB	12.04 ± 7	PB → SMB Δ = −0.03	**0.2** (12.04 → 12.01)	**0.00001**	
		SMB	12.01 ± 5	**End**	**ORE = 82.7%**		
8	TP (mg/L)	Inlet	4.96 ± 3.2	Inlet → ABR Δ = −1.80	**36.3** (4.96 → 3.16)	**0.22**	Overall downward trend, but **SAB → SBF shows a slight increase**
		ABR	3.16 ± 2.1	ABR → FBF Δ = −0.83	**26.3** (3.16 → 2.33)	**0.14**	
		FBF	2.33 ± 0.8	FBF → SAB Δ = −0.69	**29.6** (2.33 → 1.64)	**0.044**	
		SAB	1.64 ± 3.2	SAB → SBF Δ = **+0.52**	**31.7**(1.64 → 2.16)	**0.018**	
		SBF	2.16 ± 2.1	SBF → PB Δ = −0.08	**3.7** (2.16 → 2.08)	**0.0013**	
		PB	2.08 ± 0.7	PB → SMB Δ = −0.13	**6.3** (2.08 → 1.95)	**0.023**	
		SMB	1.95 ± 0.5	**End**	**ORE = 60.7%**		

Responses of Physicochemical Parameters to Phytoremediation at Various Treatment Stages (Units/Chambers)

1. **pH**

As shown in Table 3 and Fig. 10, the largest decrease in pH was −0.2, which was consistent with the first three transitions. This steady average rate of decrease (−0.2 pH units per step) was shown in early chambers (Inlet → ABR → FBF → SAB). Later, the chambers (SAB → SBF → PB → SMB) stabilized (0 or −0.1). The position with the greatest decrease (statistically significant) with the largest normalized χ^2 was observed at ABR → FBF ($\chi^2 \approx 0.31$). This means that the most significant (most statistically significant) decrease relative to uncertainty occurs between ABR and FBF.

2. **The temperature (°C)**

As shown in Table 3 and Fig. 10, the largest raw temperature (°C) decrease was −1.0 °C between SBF → PB. The rate of decrease in early chambers shows a small decrease (−0.2 to −0.4 °C), whereas the largest raw decrease of −1.0 °C occurred between SBF → PB. The position with the greatest decrease (statistically significant) was the largest normalized χ^2, which was observed at SBF → PB ($\chi^2 \approx 0.265$). This means that the most significant decrease relative to uncertainty occurs between SBF and PB. This means that the average rate of decrease is small (−0.2 to −0.4 °C) in the early chambers, whereas the highest statistically significant decrease occurs between SBF and PB, with a decrease of −1.0 °C.

3. **The TSS (mg/L)**

As shown in Table 3 and Fig. 10, the largest decrease in raw material content was −70 mg/L between Inlet → ABR. The rate of decrease showed the greatest decrease at the beginning, at −70 mg/L (Inlet → ABR), and afterwards, smaller decreases were recorded (−22, then −1 to −3 mg/L). The position of the greatest decrease (statistically significant) was the largest normalized χ^2, which was at Inlet → ABR ($\chi^2 \approx 1.75$). This means that the most significant decrease relative to uncertainty occurs between Inlet and ABR. The average rate of decrease is steep initially (−70 mg/L), then moderate (−22 mg/L), and finally stabilizes with small drops (−1 to −3 mg/L), with the greatest statistically significant decrease occurring between Inlet and ABR. The overall removal efficiency (ORE) was 81.6%.

4. **TDS (mg/L)**

As shown in Table 3 and Fig. 10, the largest decrease in raw material content was −212 mg/L (ABR → FBF). The rate of decrease steadily decreased across all the chambers, with decreases ranging from −100 to −212 mg/L. The position of highest decrease (statistically significant), with the largest normalized χ^2, is at FBF → SAB ($\chi^2 \approx 408.16$). This means that the most significant decrease relative to uncertainty occurs between FBF and SAB. This means that the average rate of decrease was between 100–212 mg/L

per step, indicating strong removal efficiency across units. The greatest statistically significant decrease occurred between FBF and SAB, even though the largest raw drop occurred between ABR and FBF, with an ORE of 75.0%.

5. The BOD_5 (mg/L)

As shown in Table 3 and Fig. 10, the largest normalized chi-square occurs at ABR → FBF ($\chi^2 \approx 5.34$), meaning that this stage shows the most statistically significant reduction relative to uncertainty. The second strongest was Inlet → ABR ($\chi^2 \approx 3.19$), confirming that the early treatment step was also highly impactful. The final polishing step (PB → SMB) contributes moderately ($\chi^2 \approx 1.37$). The middle stages (FBF → SAB, SAB → SBF, SBF → PB) have very small χ^2 values (<0.12), indicating that the reduction in χ^2 values is minor compared with the measurement error. Chi-square normalization revealed that the most significant BOD_5 reductions occurred at ABR → FBF ($\chi^2 \approx 5.34$) and Inlet → ABR ($\chi^2 \approx 3.19$), with a secondary contribution at PB → SMB ($\chi^2 \approx 1.37$) and an ORE of 88.8%.

6. The COD (mg/L)

As shown in Table 3 and Figure 10, the largest decrease in raw COD was −300 mg/L (Inlet → ABR). The rate of decrease in the COD decreases sharply at the beginning: −300 mg/L (Inlet → ABR) and −181 mg/L (ABR → FBF), and the latter decreases are smaller (−20 to −48 mg/L). The position of the greatest decrease (statistically significant) showed that the largest normalized χ^2 was at Inlet → ABR ($\chi^2 \approx 9.52$). This means that the most significant decrease relative to uncertainty occurred between Inlet and ABR, and a second significant decrease occurred at ABR → FBF ($\chi^2 \approx 7.90$). This implies that the average rate of decrease is steep initially (−300 mg/L, then −181 mg/L), followed by smaller drops downstream, and the highest statistically significant decrease occurs between Inlet and ABR, with a secondary strong decrease between ABR and FBF, with an ORE of 89.4%.

7. TN (mg/L)

As shown in Table 3 and Fig. 10, the largest decrease in raw material content was −45.15 mg/L (ABR → FBF). The rate of decrease corresponding to the largest decrease was −45.15 mg/L (ABR → FBF). After FBF, the TN values stabilized at ~12 mg/L, with negligible changes. The position with the greatest decrease (statistically significant) was the largest normalized χ^2 at ABR → FBF ($\chi^2 \approx 13.68$). This means that the most significant decrease relative to uncertainty occurs between the ABR and FBF. Therefore, the average rate of decrease was steep initially (−12.1 mg/L, then −45.15 mg/L), followed by stabilization, and the highest statistically significant decrease occurred between the ABR and FBF, with an ORE of 82.7%.

8. **The TP (mg/L)**

As shown in Table 3 and Figure 10, the largest decrease in raw material content was −1.80 mg/L (Inlet → ABR). The rate of decrease associated with the greatest decrease was −1.80 mg/L (Inlet → ABR). This trend followed a moderate decrease (−0.83, −0.69), after which the values stabilized with small changes. There was a slight increase (+0.52 mg/L) between SAB and SBF, likely due to variability or release. This means that the position of highest decrease (statistically significant) and the largest normalized χ^2 was at the Inlet → ABR ($\chi^2 \approx 0.22$). This means that the most significant decrease relative to uncertainty occurs between Inlet and ABR. The average rate of decrease is steep initially (−1.80 mg/L), moderate in the middle (−0.83, −0.69), and then stable. The most statistically significant decrease occurred between Inlet and ABR, with a small rebound (increase) observed between SAB and SBF, which may indicate local variability in phosphorus release/uptake, with an ORE of 60.7%.

Microbial load response to phytoremediation at various treatment stages (units/chambers)

As shown in Table 4 and Figs. 12 & 13, the responses of the microbial load (TC counts, FC counts, THB counts, *E. coli* counts and HE counts) to phytoremediation at various treatment stages (units/chambers) were documented.

1. **The TC count (cfu/ml)**

As shown in Table 4 and Fig. 13, the largest log reduction occurred between SBF → PB (≈ 1.14 log units), and the rate of decrease revealed that the TC decreased dramatically from 3.04×10^7 → 3.48×10^6 in the first step (Inlet → ABR), and a steady reduction continued across units, with the most pronounced microbial removal occurring between SBF → PB. The position of the greatest decrease (raw decrease) was between Inlet → ABR (-2.69×10^7 CFU/100 mL), and the relative/log decrease was at SBF → PB (≈ 1.14 log reduction). It can be inferred that the largest raw decrease in TC occurred between Inlet and ABR, and the highest relative/logarithmic decrease occurred between SBF and PB, with the highest microbial removal efficiency at that stage and an ORE of 99.9%.

2. **FC count (cfu/ml)**

As shown in Table 4 and Fig. 13, the largest raw decrease was Inlet → ABR (-1.27×10^7 CFU/100 mL), and the largest log reduction was PB → SMB (≈ 1.40 log units). The rate of decrease showed a very large initial decrease from Inlet → ABR (-1.27×10^7 CFU/100 mL), followed by steady reductions, with smaller steps in the middle, and the final stage (PB → SMB) showed the strongest relative removal. The position of the greatest decrease (raw decrease) was between the Inlet → ABR, and the relative/logarithmic decrease was between PB → SMB. It can therefore be inferred that the largest decrease in the number of fecal coliforms occurred between Inlet and ABR. The greatest relative/logarithmic decrease occurred

between PB and SMB, which presented the strongest microbial removal efficiency at the final stage, with an ORE of 99.8%.

3. **The THB count (cfu/ml)**

As shown in Table 3, the largest log reduction was PB → SMB (≈ 1.12 log units), closely followed by SBF → PB (≈ 1.06 log units). The rate of decrease showed a large initial decrease between Inlet and ABR (-4.83×10^5 CFU/mL), with smaller decreases in the middle stages, and strong microbial removal occurred again in the final stages (SBF → PB → SMB). The highest decrease (raw decrease) was between Inlet → ABR, and the relative/logarithmic decrease was PB → SMB (≈ 1.12 log units).

It can therefore be inferred that the largest raw decrease in THB occurred between Inlet and ABR, whereas the highest relative/logarithmic decrease occurred between PB and SMB, with the strongest microbial removal efficiency at the final stage and an ORE of 99.7%.

4. ***E. coli*** **(cfu/ml)**

As shown in Table 4 and Fig. 13, the largest raw decrease was Inlet → ABR (-3.58×10^5 CFU/100 mL), and the largest log reduction was PB → SMB (≈ 1.89 log units). The rate of decrease showed a large initial decrease between Inlet → ABR (-3.58×10^5 CFU/100 mL) and moderate decreases in the middle stages (-1.26×10^4 to -2.0×10^3), and the final stage (PB → SMB) presented the strongest relative removal. The position of highest decrease (raw decrease) was between the Inlet → ABR, whereas the relative/logarithmic decrease was between PB → SMB. It can therefore be inferred that the largest decrease in *E. coli* raw matter occurred between Inlet and ABR, whereas the highest relative/logarithmic decrease occurred between PB and SMB, with the strongest microbial removal efficiency at the final stage and an ORE of 99.9%.

5. **The HE (eggs/L)**

As shown in Table 4 and Fig. 12, the greatest decrease in raw material occurred at Inlet → ABR (−26 eggs/L), and the initial decrease from Inlet → ABR (−26 eggs/L) was strong; moreover, moderate decreases in raw material content occurred at the middle stages (−2 to −5 eggs/L), and the final stage (PB → SMB) presented another strong decrease (~−6 eggs/L). The position of the greatest decrease (statistically significant) in the raw data was between Inlet → ABR. The chi-square normalized decrease was between PB → SMB ($\chi^2 \approx 18.0$), indicating the most statistically significant removal relative to uncertainty. It can therefore be inferred that the largest decrease in Helminth Eggs occurred between Inlet and ABR, whereas the greatest statistically significant decrease occurred between PB and SMB, reflecting the highest removal efficiency at the final stage and an ORE of 97.9%.

Table 4: Microbial responses to phytoremediation at various treatment stages (units/chambers)

S/No	Parameter	Chambers/units	± error Values	Differences between units	(%) Reduction of pollutants	(X^2) value	Key insights in the treatment unit
1	TC (cfu/ml	Inlet	3.04×10^7	Inlet → ABR Δ = -2.69×10^7	**88.6** ($3.04\times10^7 \rightarrow 3.48\times10^6$)	**0.94**	Steady decline, with the biggest drop at SBF → PB
		ABR	3.48×10^6	ABR → FBF Δ = -2.47×10^6	**71.0**($3.48\times10^6 \rightarrow 1.01\times10^6$)	**0.54**	
		FBF	1.01×10^6	FBF → SAB Δ = -6.74×10^5	**66.7** ($1.01\times10^6 \rightarrow 3.36\times10^5$)	**0.48**	
		SAB	3.36×10^5	SAB → SBF Δ = -2.64×10^5	**78.6**($3.36\times10^5 \rightarrow 7.20\times10^4$)	**0.67**	
		SBF	7.20×10^4	SBF → PB Δ = -6.68×10^4	**92.8** ($6.50\times10^4 \rightarrow 5.50\times10^4$)	**1.14**	
		PB	5.20×10^3	PB → SMB Δ = -4.52×10^3	**87.0**($5.20\times10^3 \rightarrow 6.80\times10^2$)	**0.88**	
		SMB	6.80×10^2	**End**	**ORE = 99.9%**		
2	FC (cfu/ml	Inlet	1.50×10^7	Inlet → ABR Δ = -1.27×10^7	**84.5** ($1.50\times10^7 \rightarrow 2.32\times10^6$)	**0.81**	Final SMB stage is decisive for compliance (<5,000)
		ABR	2.32×10^6	ABR → FBF Δ = -2.17×10^6	**93.5** ($2.32\times10^6 \rightarrow 1.51\times10^5$)	**1.19**	
		FBF	1.51×10^5	FBF → SAB Δ = -5.1×10^4	**33.8** ($1.51\times10^5 \rightarrow 1.00\times10^5$)	**0.18**	
		SAB	1.00×10^5	SAB → SBF Δ = -3.5×10^4	**35.0** ($1.00\times10^5 \rightarrow 6.50\times10^4$)	**0.19**	
		SBF	6.50×10^4	SBF → PB Δ = -1.0×10^4	**15.4 (**$6.50\times10^4 \rightarrow 5.50\times10^4$)	**0.07**	
		PB	5.50×10^4	PB → SMB Δ = -5.28×10^4	**96.0** ($5.50\times10^4 \rightarrow 2.2\times10^3$)	**1.40**	
		SMB	2.20×10^3	**End**	**ORE = 99.8%**		
3	THB (cfu/ml)	Inlet	5.44×10^5	Inlet → ABR Δ = -4.83×10^5	**88.23**($5.44 \times 10^5 \rightarrow 6.11 \times 10^4$)	**0.95**	Final SMB stage is decisive for compliance at 500
		ABR	6.11×10^4	ABR → FBF Δ = -4.7×10^3	**76.92**($6.11 \times 10^4 \rightarrow 5.64 \times 10^4$)	**0.04**	
		FBF	5.64×10^4	FBF → SAB Δ = -1.73×10^4	**30.67**($5.64 \times 10^4 \rightarrow 3.91 \times 10^4$)	**0.16**	
		SAB	3.91×10^4	SAB → SBF Δ = -1.63×10^4	**41.68**($3.91 \times 10^4 \rightarrow 2.28 \times 10^4$)	**0.23**	
		SBF	2.28×10^4	SBF → PB Δ = -2.08×10^4	**91.22** ($2.28 \times 10^4 \rightarrow \sim 2.00 \times 10^3$)	**1.06**	
		PB	$\sim 2.00 \times 10^3$	PB → SMB Δ = -1.85×10^3	**92.5**($\sim 2.00 \times 10^3 \rightarrow 1.50 \times 10^2$)	**1.12**	
		SMB	1.50×10^2	**End**	**ORE = 99.7%**		
4	*E. coli* (cfu/ml)	Inlet	3.84×10^5	Inlet → ABR Δ = -3.58×10^5	**93.2** ($3.84\times10^5 \rightarrow 2.60\times10^4$)	**1.17**	SMB ensures compliance (≤126)
		ABR	2.60×10^4	ABR → FBF Δ = -1.26×10^4	**48.5** ($2.60\times10^4 \rightarrow 1.34\times10^4$)	**0.29**	
		FBF	1.34×10^4	FBF → SAB Δ = -7.3×10^3	**54.5** ($1.34\times10^4 \rightarrow 6.10\times10^3$)	**0.34**	
		SAB	6.10×10^3	SAB → SBF Δ = -2.3×10^3	**37.7** ($6.10\times10^3 \rightarrow 3.80\times10^3$)	**0.21**	
		SBF	3.80×10^3	SBF → PB Δ = -2.0×10^3	**52.6** ($3.80\times10^3 \rightarrow 1.80\times10^3$)	**0.32**	
		PB	1.80×10^3	PB → SMB Δ = -1.78×10^3	**98.7** ($1.80\times10^3 \rightarrow 23$)	**1.89**	
		SMB	2.30×10^1	**End**	**ORE = 99.9%**		
5	HE (eggs/L)	Inlet	48 ± 8	Inlet → ABR Δ = −26	**54.2** (48 → 22)	**8.45**	Gradual decline, with SMB achieving full compliance (<1 egg/L)
		ABR	22 ± 4	ABR → FBF Δ = −5	**22.7** (22 → 17)	**0.78**	
		FBF	17 ± 4	FBF → SAB Δ = −2	**11.8** (17 → 15)	**0.13**	
		SAB	15 ± 4	SAB → SBF Δ = −5	**33.3** (15 → 10)	**0.78**	
		SBF	10 ± 4	SBF → PB Δ = −3	**30.0** (10 → 7)	**0.53**	
		PB	7 ± 1	PB → SMB Δ = −6	**>85** (7 → <1)	**18.0**	
		SMB	<1	**End**	**ORE = 97.9%**		

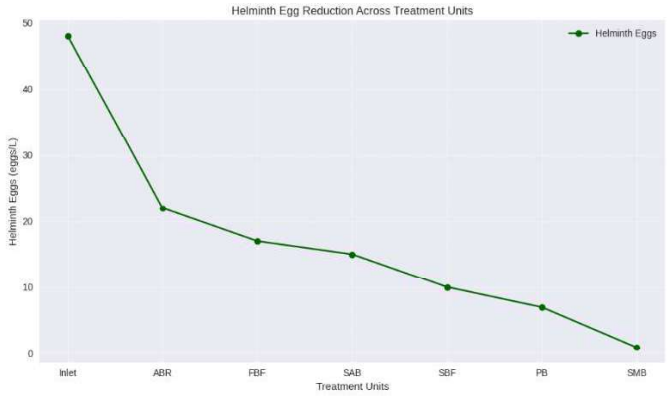

Fig 12: Linear chart of HE shows steady decline, with the final SMB stage ensuring full compliance (<1 egg/L).

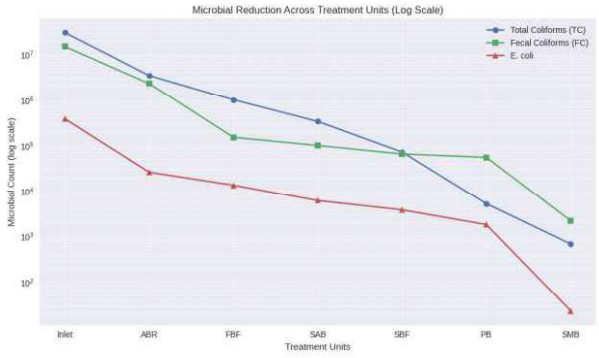

Fig 13: Log-scale chart showing TC, FC, and *E. coli* dropped by several orders of magnitude, with the most dramatic reductions at PB → SMB.

Table 5: Comparative overview of treatment performance

Parameter	Largest raw decrease (units)	Value of decrease	Highest statistically significant/logarithmic decrease (units)
pH	Inlet → ABR/ABR → FBF/FBF → SAB	−0.2 each step	ABR → FBF
Tempe (°C)	SBF → PB	−1.0 °C	SBF → PB
TSS (mg/L)	Inlet → ABR	−70 mg/L	Inlet → ABR
TDS (mg/L)	ABR → FBF	−212 mg/L	FBF → SAB
BOD_5 (mg/L)	Inlet → ABR	−80 mg/L	Inlet → ABR
COD (mg/L)	Inlet → ABR	−300 mg/L	Inlet → ABR
TN (mg/L)	ABR → FBF	−45.15 mg/L	ABR → FBF
TP (mg/L)	Inlet → ABR	−1.80 mg/L	Inlet → ABR
TC (cfu/ml)	Inlet → ABR	-2.69×10^7	SBF → PB
FC (cfu/ml)	Inlet → ABR	-1.27×10^7	PB → SMB
THB (cfu/ml)	Inlet → ABR	-4.83×10^5	PB → SMB
E. coli (cfu/ml)	Inlet → ABR	-3.58×10^5	PB → SMB
HE (eggs/L)	Inlet → ABR	−26 eggs/L	PB → SMB

Comparative overview of phytoremediation treatment performance

As shown in Table 5, at the initial stage (Inlet → ABR), most parameters (pH, TSS, BOD_5, COD, TP, microbial counts, HE) exhibited the greatest decrease in raw material. This highlights ABR as the primary treatment unit for bulk removal. During the middle stages (ABR → FBF → SAB → SBF) and when TN is also critically removed between ABR → FBF, TDS also shows a major statistically significant decrease between FBF → SAB. These stages are crucial for nutrient and dissolved solids reduction. In the final stages (SBF → PB → SMB), the microbial parameters (TC, FC, THB, *E. coli,* and HE) presented the greatest relative/logarithmic decreases. This indicates polishing and pathogen removal efficiency at the end of the treatment.

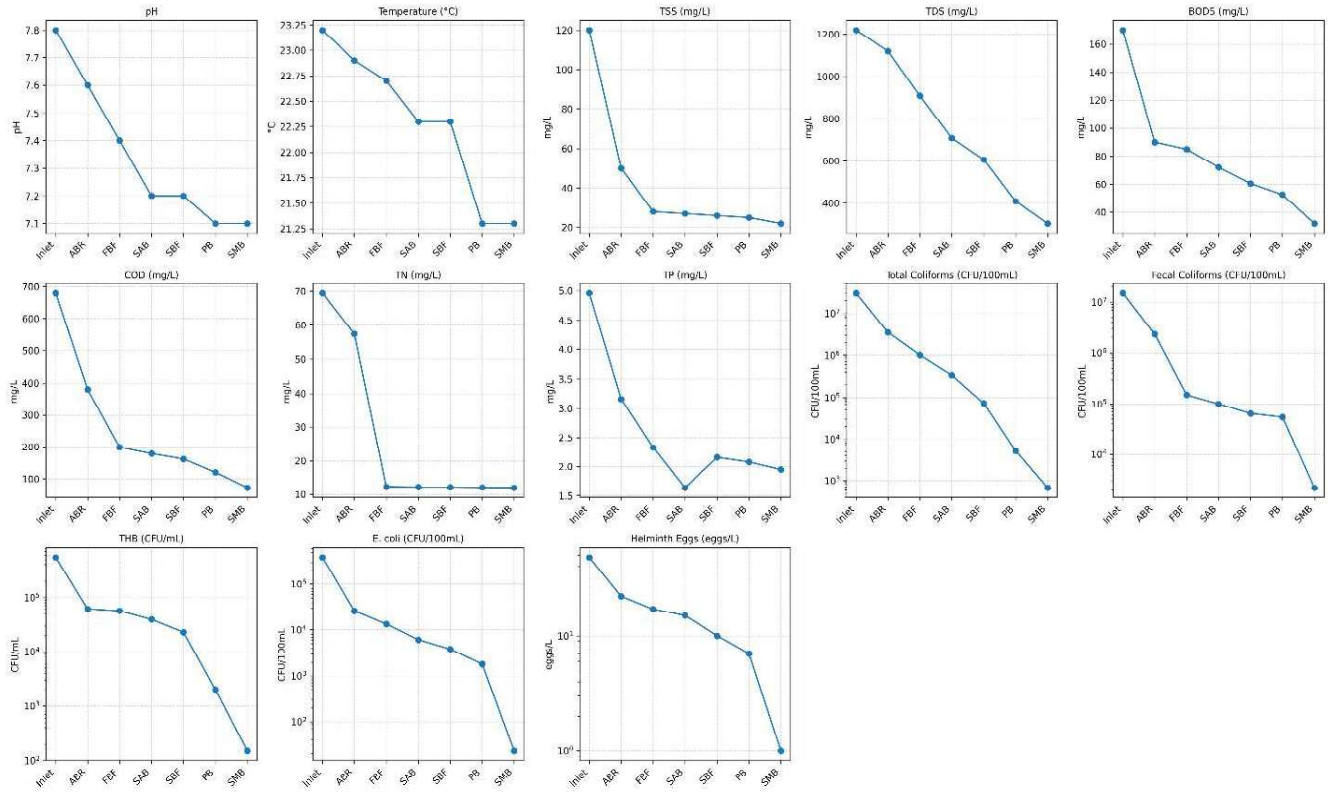

Fig 13: Physicochemical and microbial indicators of the top and bottom panels

The key insight is that the ABR was the powerhouse for the bulk removal of the COD, BOD_5, TSS, TP, and HE, which presented the largest raw decreases at this unit. FBF → SAB was critical for nutrient and dissolved solids reduction, and the TN and TDS decreased sharply at this stage. PB → SMB provides the strongest microbial polishing, i.e., *E. coli,* fecal coliforms, THB, and helminth eggs presented the greatest log reductions at this stage, ensuring pathogen safety.

Morphological and biochemical tests

As shown in Table 6, microbial diversity was stable, with all eight (8) organisms consistently present across the seven (7) chambers (Inlet → SMB). The dominance of gram-negative rods was observed in five (5) out of 8 organisms that are gram-negative rods (*E. coli, Klebsiella, Pseudomonas, Salmonella, Bacillus* spp). Indicator organisms, e.g., *E. coli* and *Enterococcus,* are present throughout, confirming that fecal contamination indicators persist. The pathogens of concern, e.g., *Salmonella, Klebsiella, and Pseudomonas,* remain detectable even in the final chamber (SMB). Environmental spore-forming bacteria, e.g., *Bacillus* spp., are consistently present, likely because of their resilience to treatment conditions. The biochemical traits included cat/coa/Oxi/Ind/Mot with various colony morphologies (milky, yellow-gold, pink mucoid, creamy, white, pale).

Table 6: Summary of biochemical test results

S/No	Suspected organism	Gram Reaction	Shapes	Biochemical Traits (Cat/Coa/Oxi/Ind/Mot)	Colony Morphology	Presence Across Chambers (Inlet → SMB)
1	*Escherichia coli*	Gram −	Rod	Cat +, Coa −, Oxi −, Ind +, Mot +	Milky colonies	Present in all 7 chambers
2	*Staphylococcus* spp.	Gram +	Cocci	Cat +, Coa +, Oxi −, Ind −, Mot −	Yellow-gold	Present in all 7 chambers
3	*Enterococcus* spp.	Gram +	Cocci	Cat −, Coa −, Oxi −, Ind −, Mot +	Milky colonies	Present in all 7 chambers
4	*Klebsiella* spp.	Gram −	Rod	Cat +, Coa +, Oxi −, Ind −, Mot −	Pink mucoid	Present in all 7 chambers
5	*Pseudomonas* spp.	Gram −	Rod	Cat +, Coa −, Oxi +, Ind −, Mot +	Creamy colonies	Present in all 7 chambers
6	*Salmonella* spp.	Gram −	Rod	Cat +, Coa −, Oxi −, Ind −, Mot +	White colonies	Present in all 7 chambers
7	*Streptococcus* spp.	Gram +	Cocci	Cat −, Coa −, Oxi −, Ind −, Mot −	Milky colonies	Present in all 7 chambers
8	*Bacillus* spp.	Gram −	Rod	Cat +, Coa −, Oxi −, Ind +, Mot +	Pale pink	Present in all 7 chambers

Table 6 shows the presence of some suspected microbes across various chambers, including bacteria (*Escherichia coli, Staphylococcus* spp., Enterococcus spp*., Klebsiella* spp*., Pseudomonas* spp*., Salmonella* spp*., Streptococcus* spp*., and Bacillus* spp*.), and protozoans (Amoeba* spp*., Giardia* spp., *Cryptosporidium* spp*., Balantidium* spp*., Ascariasis* spp*., Euglena* spp*., Spyrogyra* spp. and *Paramecium* spp*.)* of various shapes (irregular, oval, ovular, oblong, flagellated, spiral, and slipper shaped).

Table 7: Microbial analysis summary

S/No	Unit	Suspected bacteria	Suspected protozoa/parasites	Morphology of the protozoa/parasites
1	Inlet	*Escherichia coli*, *Staphylococcus* spp., *Enterococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Streptococcus* spp., *Bacillus* spp.	*Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp	Irregular, oval, ovular, oblong
2	ABR	Same bacterial set as Inlet (*E. coli*, Staphylococcus, Enterococcus, Klebsiella, Pseudomonas, Salmonella, Streptococcus, Bacillus)	*Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp	Irregular, oval, ovular, oblong
3	FBF	Same bacterial set as above	*Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp	Irregular, oval, ovular,
4	SAB	Same bacterial set as above	*Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp, *Paramecium* spp *(appears here)*	Irregular, oval, ovular, oblong, slipper-shaped
5	SBF	Same bacterial set as above	*Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp, *Paramecium* spp	Irregular, oval, ovular, oblong, slipper-shaped
6	PB	Same bacterial set as above	*Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp,	Irregular, oval, ovular, oblong, flagellated, spiral, slipper-shaped,

7	SMB	Same bacterial set as above	*Paramecium* spp, *Ascariasis* spp, *Euglena* spp, *Spyrogyra* spp *Amoeba* spp, *Giardia* spp, *Cryptosporidium* spp, *Balantidium* spp, *Paramecium* spp	Irregular, oval, ovular, oblong, flagellated, spiral, slipper-shaped,

Morphological and biochemical tests

As shown on Fig. 14, two visualizations create a bipartite connection diagram mapping suspected microorganisms to specific treatment chambers and a multilayered circular (sunburst) multiple associations chart linking chambers, Gram-stain categories, morphology, and suspected organisms are used. Together, these charts indicate persistent microbial hubs (Bacillus, Pseudomonas, Enterococcus, and *E. coli*), biofilm- and EPS-associated colony phenotypes, and recurring algal/protozoal populations in phytoremediation and maturation basins. Key operational risks include partial pathogen persistence, biofilm-mediated carryover, and algal regrowth, which may compromise final effluent quality.

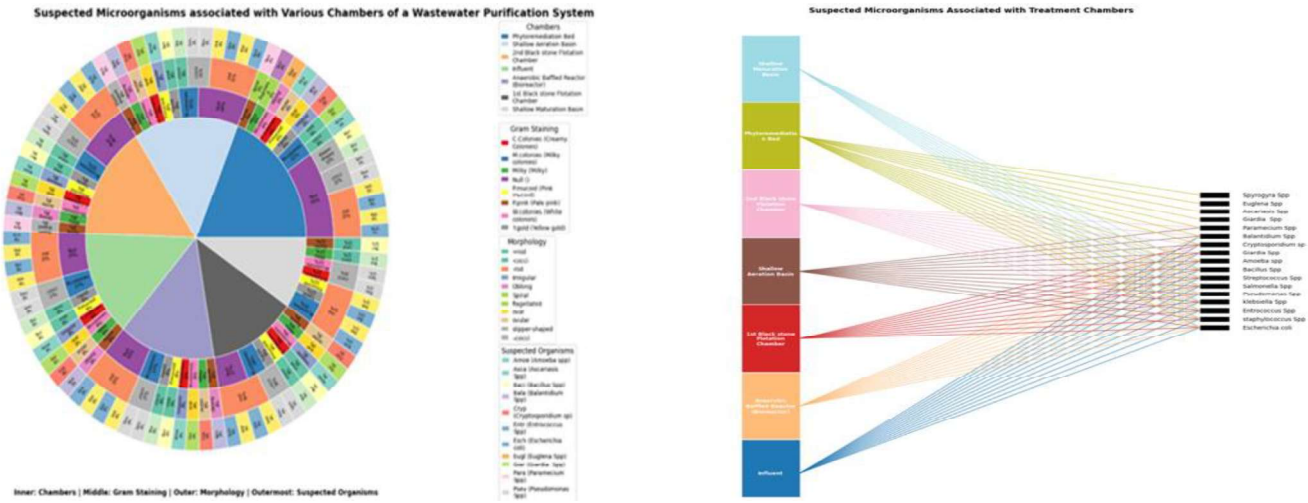

Fig 14: Guilds of microorganisms associated with various chambers

As shown in Fig. 14, the top panels (pH, Temp, TSS, TDS, BOD_5, COD, TN, and TP) show the physicochemical parameters with steep decreases (Inlet → ABR, ABR → FBF), especially for COD, BOD_5, TSS, and TN. The bottom panels (TC, Fecal Coliforms, THB, *E. coli,* and Helminth Eggs) are microbial parameters plotted on a logarithmic scale. The greatest reduction occurs in the final polishing stages (PB → SMB), where pathogen removal is most effective.

Discussion

The NPC phytoremediation system demonstrated treatment efficiencies comparable to but not exceeding those reported in similar studies across sub-Saharan Africa. The overall removal efficiency (ORE) of the

system achieves an 88.8% total BOD_5 reduction, with SMB being the only unit that meets the regulatory standard. This result closely agrees with findings from other constructed wetland systems in the region. A previous study [10] reported BOD_5 reductions of 85–92% in constructed wetlands treating domestic wastewater in Kenya, whereas [11] reported similar performance in pilot-scale wetlands in Uganda, with BOD_5 reductions ranging from 87% to 90%. These results place the NPC system's performance within the upper range of reported efficiencies for phytoremediation-based treatment in sub-Saharan Africa. Notably, the SMB (surface microbial bed) unit within the NPC system is the only component that consistently meets the regulatory standard for effluent discharge, highlighting its critical role in overall system performance. This finding is consistent with the findings of [12], who noted that subsurface flow systems can achieve greater pollutant removal due to enhanced microbial activity and reduced short-circuiting. In terms of microbial reduction, the NPC system demonstrates a fecal coliform log reduction of 3.5, meeting the World Health Organization (WHO) guidelines for restricted irrigation reuse [13], which is comparable to data from similar systems in East Africa, as seen in [14], who reported fecal coliform log reductions of 3.2-3.8 in free-water surface constructed wetlands in Tanzania, further validating the effectiveness of the NPC system in pathogen removal.

These results indicate that the NPC phytoremediation system is effective at removing both organic pollutants and pathogenic microorganisms. The system's performance in BOD_5 reduction and microbial removal contributes significantly to sustainable wastewater management in institutional settings, particularly where decentralized, nature-based treatment solutions are needed. As highlighted by [15], such systems play a vital role in enhancing wastewater reuse, reducing environmental impacts, and addressing the growing demand for sustainable water management practices in resource-limited regions. The 88.8% reduction in BOD_5 can be compared with findings from several other nature-based treatment solutions. Another study demonstrated that pilot-scale constructed wetlands in Zambia consistently achieved BOD_5 removal rates between 85% and 90%, reaffirming the robustness of phytoremediation approaches in reducing organic loads in wastewater [16].

Similarly, effective BOD_5 reductions were reported by [17] at Zimbabwe, where hybrid wetland systems achieved removal efficiencies of up to 89%, comparable to the performance of the NPC system.

In terms of microbial removal, the fecal coliform log reduction of 3.5 in the NPC system was particularly significant. This finding aligns with the guidelines provided by the WHO for restricted irrigation reuse [13] and is consistent with findings from certain decentralized systems. The reports of [18] revealed that constructed wetlands in Tanzania achieved fecal coliform reductions in the range of 3.0-3.7 log units, demonstrating the capacity of phytoremediation systems to provide effective pathogen removal in line with health standards for agricultural and landscape reuses. The system's performance in terms of both organic pollutant reduction and microbial removal contributes significantly to sustainable wastewater management in institutional settings. This is particularly crucial when centralized treatment infrastructure

is unavailable, and decentralized, nature-based solutions are the most viable option. As highlighted by [15], such systems not only increase wastewater reuse but also reduce environmental impacts by lowering nutrient loads discharged into water bodies, thereby mitigating eutrophication risks. Additionally, the long-term operational benefits of phytoremediation systems such as low energy requirements and minimal chemical input contribute to their sustainability as reliable wastewater management systems. According to [19], decentralized constructed wetlands can significantly reduce operational costs over time compared with conventional wastewater treatment facilities, making them a practical solution in resource-constrained institutional settings. Moreover, the integration of these systems into institutional landscapes can provide ancillary benefits, including improved aesthetics and biodiversity enhancement [20]. Thus, the ability of the NPC phytoremediation system to remove pollutants and pathogens was effective. This position is valuable for the portfolio of sustainable wastewater management technologies, particularly in institutional contexts within sub-Saharan Africa and other resource-limited regions.

Large reductions were observed, with the COD and BOD showing large decreases at early stages and final polishing. There was dramatic removal of TN at ABR → FBF. The strongest reduction in TDS occurred between SBF and PB. Gradual polishing resulted in a continuous decrease in the TSS, COD and BOD in each step. A minor rebound was observed with the TP, which slightly increased at SAB →SBF but remained compliant. The final stage of (SMB) ensures full compliance across all the parameters. Each chamber contributes differently: ABR → FBF was critical for nitrogen, SBF → PB for salts (TDS), and PB → SMB for final polishing of COD/BOD. This pattern is consistent with findings from other multistage treatment systems. One study [21] reported that substantial COD and BOD removal often occurs in the initial anaerobic stages, with further polishing observed in subsequent aerobic or facultative stages. In the NPC system, the anaerobic baffled reactor (ABR) and freeboard buffer (FBF) stages play pivotal roles in achieving these early reductions. As shown by [22], ABR units are particularly effective in breaking down complex organic matter, leading to dramatic BOD_5 and COD reductions at this stage, often exceeding 60–70% removal.

The system also demonstrated remarkable total nitrogen (TN) removal, with the most dramatic reductions occurring between the ABR and the FBF. This aligns with findings reported by [23], who reported that anaerobic units often facilitate initial nitrogen transformations, whereas subsequent stages provide conditions conducive for nitrification and denitrification. In the NPC system, FBF appears to enhance nitrogen removal by creating an environment favorable for microbial communities responsible for denitrification, similar to a series of processes described by [24] in hybrid treatment wetlands.

Another notable result was the significant reduction in total dissolved solids (TDS), which was strongest between the subsurface biofilter (SBF) and the polishing bed (PB). This finding mirrors observations by [25] Kadlec and Wallace 2009, who reported that subsurface flow units and polishing stages are particularly effective at reducing salinity and dissolved solids, primarily through mechanisms such as

filtration, ion exchange with the substrate, and plant uptake. The stepwise reductions in TDS observed in the NPC system are consistent with the gradual polishing effects documented in similar multistage systems [26].

Gradual polishing was also evident in the total suspended solids (TSS), COD, and BOD_5 levels, with these parameters continuing to decrease step by step as the wastewater passed through each stage. Such gradual reductions are characteristic of multistage constructed wetland systems, where each chamber contributes incrementally to pollutant removal [27]. For example, the PB and final subsurface microbial bed (SMB) stages play critical roles in the final polishing of COD and BOD, ensuring that the effluent meets regulatory standards. This final stage polishing effect was described by [28], who reported that subsurface microbial beds provide a robust environment for the final breakdown of residual organics, resulting in consistently low COD and BOD levels. A minor rebound was observed in total phosphorus (TP), with a slight increase noted between the small, aerated bed (SAB) and the SBF. This phenomenon, often referred to as phosphorus release or "internal loading," is not uncommon in constructed wetlands and other nature-based systems. According to the findings of [29], phosphorus release can occur due to the remobilization of phosphorus from substrate materials under certain conditions, such as changes in redox potential or microbial activity. Nevertheless, in the NPC system, TP remained compliant with regulatory standards, indicating that the final stages maintained overall phosphorus levels within acceptable limits. Each chamber in the NPC system contributed differently to the overall treatment performance. As highlighted, the ABR → FBF stages were critical for nitrogen removal, a finding supported by [30], which identified anaerobic–aerobic transitions as essential for achieving high nitrogen removal efficiencies. Similarly, the SBF → PB stages were instrumental for salt (TDS) reduction, which is consistent with observations by [31] regarding the role of subsurface flow units in managing dissolved solids. Finally, the PB → SMB transition provided the necessary final polishing to ensure full compliance across all parameters, a result that echoes the findings of [32], who demonstrated that final-stage polishing beds in multistage systems are crucial for achieving near-complete removal of organic pollutants and suspended solids.

The NPC phytoremediation system's multistage design ensures that each unit targets specific pollutants, contributing to the overall effectiveness of the system. The system's performance across BOD, COD, TN, TDS, TSS, and TP reductions aligns with results established in other research carried out on decentralized, nature-based wastewater treatment technologies, reinforcing its value in sustainable wastewater management for institutional settings.

In the early stages of the system (Inlet → ABR), there were substantial reductions in total coliforms (TCs), fecal coliforms (FCs), and *Escherichia coli* (*E. coli*). This aligns with findings of [22], which showed that anaerobic baffled reactors (ABRs) can achieve significant early reductions in microbial loads due to prolonged retention times and enhanced sedimentation. ABRs reduce pathogens by 1–2 log units through

mechanisms such as sedimentation, anaerobic digestion, and microbial competition [33]. This explains the large initial reductions in TC, FC, and *E. coli* observed in the early stages of the NPC system.

At the midstream stages (ABR → FBF → SAB → SBF), there were moderate levels of polishing and gradual decreases in microbial loads. This pattern coincides with the findings of [30], who reported that intermediate stages in multistage wetland systems often contribute to incremental reductions in microbial loads due to the combined effects of filtration, predation by protozoa, and natural die-off. For example, freeboard buffer (FBF) and small aerated bed (SAB) units introduce aerobic conditions, which promote further microbial die-off and pathogen removal. According to [34], aerated beds can enhance microbial reduction by improving oxidative conditions and increasing contact time for pathogen inactivation. Similarly, [28] highlighted that subsurface biofilters (SBFs) contribute to additional microbial polishing by providing finer filtration and improved microbial interactions, leading to gradual decreases in microbial indicators such as TC, FC, and *E. coli*. The polishing bed (PB) stage was particularly critical for achieving major reductions in total coliform (TC) content. This observation is supported by findings from [32], who demonstrated that polishing beds serve as a final physical barrier, using dense vegetation and filtration media to capture remaining coliforms. In their study, polishing beds reduced coliform counts by up to 90%, reinforcing the importance of this stage in reducing the microbial load. However, the subsurface microbial bed (SMB) was the final decisive polishing stage that ensured full compliance for the fecal coliforms (FC), *E. coli*, and helminth eggs (HE). The role of SMB in achieving regulatory compliance is consistent with observations by [35], who reported that subsurface flow beds can achieve additional 1–2 log unit reductions in *E. coli* due to increased microbial degradation and longer hydraulic retention times. SMB units provide an environment conducive to the final removal of pathogens through microbial competition, adsorption, and natural die-off, ensuring that the effluent meets safety standards. The gradual step-by-step decrease in microbial loads across the treatment group underscores the cumulative effect of each unit. As shown in Fig. 12 and Fig. 13, while microbial loads decrease progressively across the system's units, the SMB is the essential finishing stage that guarantees compliance with regulatory standards. This is consistent with research by [36], who showed that in multistage treatment systems, early and midstream units contribute significantly to reduction, but final compliance with microbial standards is typically achieved only in the last polishing units.

The overall removal efficiency (ORE) of the physicochemical parameters in the NPC system supports its efficacy. The total reductions in BOD_5 were as follows: (88.8%) COD (89.4%), TN (82.7%), and TP (60.7%). These values correspond to results from comparable studies. For example, [21] reported BOD_5 and COD removal efficiencies of approximately 85–90% in hybrid constructed wetland systems, whereas [18] reported nitrogen removal rates between 75% and 85% in integrated wetland systems. The moderate phosphorus removal (60.7%) also aligns with findings by [29], who noted that while wetland systems are

less efficient at phosphorus removal than they are at nitrogen removal, multistage systems can still achieve 50–65% reductions through adsorption and plant uptake.

Most notably, the overall removal efficiency (ORE) for microbial load reduction was exceptionally high: TC (99.9%), FC (99.8%), total heterotrophic bacteria (THB) (99.7%), *E. coli* (99.9%), and helminth eggs (HEs) (97.9%). These values are consistent with findings from other decentralized systems. As in the study of [18]. A total coliform reduction of over 99% in multistage wetland systems was observed, whereas [36] reported that final-stage wetlands could reduce *E. coli* by 99.9% when designed with sufficient retention time and filtration layers. Helminth egg reduction, often cited as a key indicator of effluent safety in reuse scenarios, was similarly high in the NPC system. This aligns with the findings of a previous study [37], which demonstrated that helminth egg reductions of 95% or more could be achieved in systems with sufficient sedimentation and filtration stages.

Importantly, the SMB was the only unit that met the regulatory standard, and it served as the decisive polishing stage that guaranteed safe compliance with microbial standards. This final stage's importance, as echoed by [38], emphasized that to meet stringent public health guidelines such as those outlined in RS 219:2013 or the WHO 2018 guidelines, a final polishing step is often necessary for microbial safety, particularly in reuse applications where fecal coliform and helminth egg limits are strictly controlled.

Conclusion

In conclusion, the NPC phytoremediation system represents a well-coordinated, multistage approach for both physicochemical and microbial load reduction. Each stage contributes critically to incremental pollutant removal, but the SMB plays an essential role in ensuring that the final effluent meets all regulatory standards, particularly for microbial safety. The system's overall performance aligns with findings from comparable studies, reinforcing its suitability as a sustainable wastewater management solution, especially in institutional settings.

Funding Declaration

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Data availability statements

The datasets generated and/or analyzed during the current study are not publicly available at the authors may wish to use data in future as a foundation (baseline studies) for further studies/investigations and therefore would not like to release the data to the public domain but data are available from the corresponding author on reasonable request.

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