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Enhancing Water Quality and Sustainable Organic-Phosphate Fertilizer Production through Phytoremediation of Cattle Farm Effluent using Artificial Wetlands.

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Abstract

Eutrophication, which results from the excessive introduction of phosphorus into water bodies, poses a critical ecological challenge, although it is not the exclusive factor driving this phenomenon. The indispensability of phosphate fertilizers for global food production, coupled with the gradual depletion of natural phosphate reserves, underscores the urgency of sustainable phosphorus management. This research investigated the efficacy of two aquatic plant species, *Salvinia* and *Pistia*, for phosphate removal from cattle farm effluent while also exploring their potential use as organic fertilizers. These two species have been extensively employed in phytoremediation studies, underscoring their established roles in environmental remediation processes. Additionally, the study measured alterations in water quality parameters within an artificial wetland (AW) designed for wastewater treatment. Cattle farm effluent was collected during the cleaning and washing of the animal farm. Macrophytes were collected within a 500 m diameter area of the pond using the systematic random sampling technique. The selected macrophytes were grown in identical plastic tanks filled with cattle farm effluent. Following a 21-day growth period, the harvested plants were subjected to drying at 70°C and were then ground into smaller particles to be transformed into fertilizer. The collected water samples were analyzed for residual phosphate concentrations and other water quality indicators, including pH, temperature, conductivity (EC), total dissolved solids (TDS) and dissolved oxygen percentage (DO%). Measurements of water quality indicators were replicated three times to report the average data and standard deviations. In this experimental setup, different water samples (AW I, AW II, AW III and AW IV) served as the experimental units, with corresponding dilution factors of 1:3 for AW I and AW III, and 1:2 for AW II and AW IV. The plant species involved in the study were *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. Statistical analysis employing Minitab 17 software enabled quantitative comparisons. Results demonstrated a consistent reduction in phosphate concentrations within the AWs over time, affirming their phosphate-removing potential. The phosphate removal efficiency of *Salvinia* was 37.87±6.50% and 35.69±1.32% for AW I and AW II, respectively. Similarly, for AW III and AW IV, *Pistia* demonstrated remarkable removal rates of 84.32±4.26% and 47.51±3.98%, respectively. Phosphate content in the fertilizers derived from *Salvinia* was 35.71±1.48 mg/kg and 29.44±0.91 mg/kg for AW I and AW II, respectively, while *Pistia* fertilizers from AW III and AW IV contained 38.00±2.29 mg/kg and 31.56±1.23 mg/kg, respectively. Furthermore, the investigation underscored the effectiveness of AWs as a potent phytoremediation technology for wastewater treatment, as evidenced by the water quality parameter analysis. This study not only highlights the promising potential of aquatic plants for sustainable phosphate management but also underscores the vital role of AWs in addressing phytoremediation potential in aquatic ecosystems.

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1. Introduction

Phosphorus (P) is an essential nutrient for all life forms, and it plays a key role in fundamental biochemical reactions involving genetic material and energy transfer and in structural support of organisms (Ruttenberg, 2003). P is found primarily in the form of phosphates, also known as orthophosphates (Kumar et al., 2019). P is a non-substitutable plant nutrient crucial for agriculture as it plays a significant role in plant growth and development.

Phosphate is typically provided to plants through various sources and methods including natural soil P, phosphate fertilizers and decomposition of organic matter, mycorrhizal associations, and P amendments (Zhu *et al.*, 2018). Among these, phosphate fertilizers derived from phosphate rock extraction and processing are widely utilized for their concentrated and accessible P content.

In Sri Lanka, phosphate deposits at Eppawala and Ridigama, particularly the Eppawala Phosphate Deposit (EPD), have been mined since the mid-1970s to produce rock phosphate fertilizers (Dushyantha *et al.*, 2017). However, global P reserves are depleting rapidly due to intensive mining, with projections suggesting potential depletion within 50–100 years. Given that approximately 90% of extracted P is used in agriculture, this scarcity poses challenges for plant growth, leading to stunted development and overall reduced plant health (Cordell, 2010; Cooper *et al.*, 2011).

In response to the depletion of phosphate reserves, there is a growing emphasis on sustainable phosphorus management. Cattle farm effluents, rich in phosphate from manure and urine, hold significant fertilizer value. When applied to agricultural fields, they contribute to plant growth and productivity, aligning with the broader goals of sustainable nutrient management in cattle farming.

Commonly, natural water bodies contain phosphate in very low concentrations. However, when high phosphate containing water reaches bodies of water, it leads to eutrophication. Effective and environmentally friendly removal of phosphate from water bodies is vital to prevent these adverse effects on human activities and biodiversity.

Phytoremediation is a cost-effective, emerging green technology to remediate polluted soil, water, and air using plants. In particular, artificial wetlands (AWs) that employ aquatic plants to remove pollutants through various processes have proven effective for wastewater treatment. AWs mimic natural wetland ecosystems that remove pollutants from wastewater through physical, chemical, and biological processes (Greenway, 2007).

The correct selection of plant species is the most significant aspect of successful phytoremediation. Aquatic plants, specifically those that thrive in aquatic environments like wetlands, ponds and lakes, are used in the phytoremediation of water bodies that are contaminated with various pollutants and have long-lasting applicability (Ali *et al.*, 2020). In this study, phosphate sorption by *Salvinia molesta* and *Pistia stratiotes* was investigated. These two plant species are invasive plants in Sri Lanka. They were selected due to their ability to absorb and accumulate high amounts of phosphate from contaminated water bodies (Mustafa & Hayder, 2021).

S. molesta, a floating aquatic plant, has been utilized in phytoremediation. Their ability to thrive in nutrient-rich water bodies and tolerance to high levels of pollutants are preferred desirable characteristics (Mendes *et al.*, 2021). *P. stratoites* (water lettuce) is an aquatic plant that grows rapidly and is a high biomass crop that has been widely used in phytoremediation projects. Water lettuce has been employed to remove excess nutrients, particularly phosphate, from wastewater and polluted water bodies (Lu *et al.*, 2010). *S. molesta* and *P. stratoites* plants have been employed for the remediation of cattle farm effluent. They adsorb phosphate from water and accumulate it in their tissues. This capacity makes them potentially useful to produce phosphate organic fertilizers.

Therefore, this research aimed to investigate the effectiveness of phytoremediation by aquatic plant species *Salvinia molesta* and *Pistia stratiotes* in removing phosphate from wastewater, particularly cattle farm effluent. The study also explored the potential use of these plants as organic fertilizers rich in phosphate content. Additionally, the study sought to assess the impact of phytoremediation on water quality parameters in an artificial wetland designed for wastewater treatment.

2. Materials and Methods

2.1 Plant selection and nutrient source

S. molesta and *P. stratiotes* aquatic macrophytes were used to adsorb the phosphate from cattle farm effluent**.** Cattle farm effluent was used as a nutrient source for aquatic macrophytes.

2.2 Chemical reagents and preparation methods

Selenium powder, lithium sulphate, 30% hydrogen peroxide and Conc. H_2S0_4 were used to prepare the digestion mixture. Ammonium molybdate, sulfuric acid

(2.5 M), potassium antimony tartrate and ascorbic acid were used in the modified single solution method for phosphate determination. Potassium dihydrogen phosphate was used to prepare the phosphate stock solution.

2.3 Instrumentation

Phosphate concentrations were quantified employing the UV Spectrophotometer (Analytic Jena Specord 210 plus, Germany) through the Ascorbic Acid Blue method. The digestion unit BLOC-DIGEST (P-SELECTA, TuV NORD 4000631) was used for the digestion of fertilizers in ascorbic acid phosphate determination. The multiparameter instrument (EUTECH PCD650, Singapore) was used to measure conductivity (EC), temperature, ion-specific concentrations, dissolved oxygen percentage (DO%) and total dissolved solids (TDS) directly. The pH of the solutions was measured using the HACH Hd 30Q portable pH meter.

2.4 Experimental design:

Sample collection and source

Cattle farm effluent was collected from the animal farm, Faculty of Agriculture, University of Peradeniya. Four bottles (5.0 L) of effluent samples were collected at every sampling period. Effluent samples were collected at the end of the wastewater treatment systems of the cattle farms before being discharged into the sewers and/or receiving water bodies.

S. molesta and *P. stratiotes* were collected from the Polgolla reservoir, Kandy, based on their high abundance. Macrophytes were systematically sampled within a 500 m diameter area of a pond using random sampling techniques. Plant species were identified at the herbarium of the Royal Botanical Gardens in Peradeniya, Sri Lanka.

Experimental Setup

The experiment was conducted in identical plastic tanks filled with cattle farm effluent. Selected macrophytes were grown in these tanks, creating artificial wetlands (AWs). The macrophytes were allowed to grow within the AWs for a standardized 21-day period. After the growth period, harvested plants were subjected to post-growth processing. The collected samples underwent a thorough analysis for water quality parameters, including residual phosphate concentrations, pH, temperature, conductivity (EC), total dissolved solids (TDS), and dissolved oxygen percentage (DO%).

Replication and Statistical Analysis

Water quality parameter measurements were replicated three times, and the average data and standard deviations are reported. The MINITAB software package (ver. 2017) and Excel (ver. 2016) were used for data analysis. Time series graphs were used to visualize and analyze data points that were collected over a continuous period of time. These graphs were used to identify trends, patterns, and fluctuations in the residual phosphate concentrations and water quality parameter variations in the artificial wetlands over time. The one-way ANOVA test was conducted to assess potential differences in means of phosphate removal efficiency among the four AW systems.

2.5 Analytical methods

Digestion method

Digestion mixture: Selenium powder (0.42 g) and lithium sulphate (14.00 g) were added to 350 mL of 30% hydrogen peroxide and mixed well. Conc. H_2S0_4 (420 mL) was slowly added with care to the mixture while cooling the latter in an ice bath.

Soil (0.200 ± 0.001 g), following grinding and sieving to contain particle sizes > 0.15 mm, and plant material (0.200 ± 0.001 g) were weighed into numbered digestion tubes (75 mL), and the digestion mixture (4.4 mL) was added to each tube. A blank digestion mixture (40 mL) was digested for standard compensation. Digestion was carried out at 360°C for 2.0 h until the solution became colorless and any remaining solids turned white. If a color was observed, it was further heated for 1.0 h more. Digestion was continued for 2.0 h to ensure that all the H_2O_2 was boiled off. Samples were allowed to cool. To each tube, distilled water (about 50 mL) was added and mixed well until no more sediment was present. Solutions were transferred to volumetric flasks after cooling. The volume was made up to 100 mL with deionized water and was mixed well. Samples were allowed to settle so that a clear solution could be taken for analysis. The P content was determined as phosphate in the digests using a UV-Vis spectrophotometer at a wavelength of 882 nm. The working standards were made by using 2.5 mL of the digested blank after dilution, as described previously.

Modified single solution method for phosphate determination

Ammonium molybdate (20.000 g) was dissolved in 500 mL of deionized water in a volumetric flask. The solution of 2.5 M sulfuric acid was prepared by diluting 70 mL of concentrated sulfuric acid in 500 mL of distilled water. Potassium antimony tartrate (0.274 g) was dissolved in a 100 mL volumetric flask and diluted to the mark. Ascorbic acid (1.320 g) was dissolved in 75 mL of deionized water in a volumetric flask.

The reagent was prepared by mixing together 125 mL of 2.5 M sulfuric acid, 37.5 mL of ammonium molybdate, 75.0 mL of ascorbic acid solution and 12.5 mL of potassium antimony tartrate solution. The sample (40.0 mL) was pipetted into a 50 mL calibrated flask, and 8 ml of the mixed reagent was added, followed by dilution to volume with distilled water. The solution was mixed well. After not less than 10 min the optical density of the solution was measured at 882 nm using a UV-vis spectrophotometer, and the phosphate concentration was determined from the standard curve.

2.6 Experimental setup for phytoremediation of cattle farm effluent

This experiment used the collected cattle farm effluent. It was initially filtered using a muslin cloth to remove debris. Due to the phytotoxicity of the concentrated cattle farm effluent, the samples were diluted before the experiment to reduce the concentration of potentially toxic substances. Thereafter, the nutrient composition and chemical properties (pH and EC) were determined.

S. molesta and *P. stratiotes* plants were collected from open tanks and cleaned with running tap water to remove surface dirt and senescent plant parts. Young plants at the same stage of growth were selected and blotted with tissue paper, weighed, and then introduced to the cattle farm effluent. The same number of plants with similar weights were added to every AW. As a control, known weights of *S. molesta* and *P. stratiotes* were introduced to a tank filled with tap water.

The experimental setup for the AW study was performed in three identical plastic tanks, with dimensions of 72 cm in diameter and 33 cm in depth. The study was conducted for 21 days. Since these plants effectively reach their maximum capacity for pollutant uptake and remediation, research findings indicate that the saturation point of phytoremediation using *Salvinia* and *Pistia* was achieved in less than 21 days (Ng et al., 2017; Nivetha et al., 2016).

In the design and implementation of this experiment, several methodological choices were carefully considered to optimize the efficacy and reliability of the AW study. The initial concentration of the effluent raised concerns about potential phytotoxicity. To address this, a strategic decision was made to dilute the samples, thereby minimizing the concentration of potentially harmful substances. The tanks were filled with cattle farm effluent in a dilution ratio (effluent: water) of 1:3 and 1:2 and were planted with *Salvinia*, and *Pistia* separately. The selection of these dilution ratios was dependent on a combination of factors, including the specific nutrients present in the effluent, the pollutant removal performance of the chosen plant species, and the treatment goals.

The water samples were checked for residual phosphate concentration, pH, temperature, EC, TDS, and DO%. Water samples were monitored at three-day intervals using the multi-parameter instrument. Four AW experimental units were monitored (Table 1).

Table 1: Artificial wetland monitoring units

No.	Experimental unit	Dilution factor	Plant species
1	AW I	1:3	*Salvinia*
2	AW II	1:2	*Salvinia*
3	AW III	1:3	*Pistia*
4	AW IV	1:2	*Pistia*

A known weight of saturated *S. molesta* and *P. stratiotes* was oven dried at (70ºC) to determine the phosphate content. Phosphate concentrations were determined by the ascorbic acid blue method (Murphy and Riley 1962).

2.7 Calculation

The efficiency of phosphate removal from cattle farm effluent by the plants was calculated using the equation mentioned by Kamiyango *et al.* (2011).

$$\text{Phosphate removal efficiency} = \frac{(C_1 - C_2)}{C_1} * 100$$

C_1= Initial phosphate concentration (mg/kg)

C_2 = Final phosphate concentration (mg/kg)

2.8 Preparation of macrophyte phosphate organic fertilizer

After plants were saturated with phosphate by absorbing phosphate from the cattle farm effluent, *S. molesta* and *P. stratiotes* plants were taken out and washed. Then they were blotted with tissue papers to drain water, and were first air dried and then oven dried at 70ºC for 2 days. Dried plants were ground and converted to powder form using a blender. The final total amount of phosphate in plant tissues was measured by wet digestion using a digestion mixture of H_2O_2, H_2SO_4, Se, and Li_2O_4 at 360°C for 2.5 h (Anderson and Ingram, 1993).

3. Results

3.1 Selection of macrophyte species for phytoremediation

The correct selection of plant species for phytoremediation plays a significant role in the development of remediation methods (Fischerova *et al*., 2005). Based on the literature survey (Nizam *et al*., 2020; Tripathi & Shukla 1991; Wickramasinghe & Jayawardana, 2018) and their local availability, *S. molesta* and *P. stratiotes* were selected for detailed studies of phytoremediation of cattle farm effluent.

3.2 Characteristics of cattle farm effluent used in this study

The cattle farm effluent was filtered, and the nutrient composition and other chemical parameters determined are given in Table 2, which shows the average values of experiments conducted in five trials.

Table 2: Nutrient composition and other chemical properties of cattle farm effluent from Mawelawaththa farm (mean±SD) used in the experiments

Parameter	**Quantity/values**
pH (1:5)	7.68±0.1
Electrical conductivity (1:5) (µS mm^{-1})	30.32±0.07
Available phosphate concentration (1:2) (mg/kg)	67.51±2.68
Total nitrogen concentration (mg/kg)	293.67±0.25
Available potassium concentration (mg/kg)	416.59±2.05
Total Carbon %	0.04±0.00

Note. SD = Standard deviation

The data reveal that cattle farm effluent is a good source of the nutrients, N, P and K.

3.3 Phosphate concentration variation of AW over time

In this experimental setup, different water samples served as the experimental units, with corresponding dilution factors of 1:3 for AW I and AW III and 1:2 for AW II and AW IV. The plant species involved in the study are *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. The phosphate concentration variations were recorded and plotted for each wetland (Figure 1). The plot illustrates the changes in phosphate concentration in effluent in plastic tanks (artificial wetland) over the duration of the observation period.

In AW I, the starting phosphate concentration of 41.02 mg/kg showed a general trend to decrease with time. It reached its lowest point at 25.49 mg/kg on day 21. AW II had a similar pattern, with the phosphate concentration gradually decreasing from 61.54 mg/kg to 39.57 mg/kg on day 21.

AW III showed the most significant variation in phosphate concentration. It experienced a sharp decrease from 39.85 mg/kg on day 0 to 6.24 mg/kg on day 21. AW IV also demonstrated a decreasing trend in phosphate concentration. It gradually decreased from 58.83 mg/kg to 30.84 mg/kg on day 21.

On the other hand, *Salvinia* and *Pistia* control unit phosphate concentrations remained lower and constant over the observed period.

Overall, the phosphate concentration trends in the AW indicate a general decrease in phosphate levels over time. AW III showed the most significant reduction, while AW I, AW II, and AW IV displayed relatively consistent declines with some fluctuations along the way.

3.4 Phosphate removal efficiency of selected macrophytes from cattle farm effluent treated AWs.

Table 3 gives the phosphate removal efficiency of *S. molesta* and *P. stratiotes,* 21 days after growing them in cattle farm effluent treated AWs.

The one-way ANOVA test was conducted to assess potential differences in means among the four AW systems (AWI, AW II, AWIII, and AWIV). The null hypothesis, stating that all means are equal, was rejected based on a significant F-value of 113.49 and a p value of 0.000 ($p < 0.05$). This indicates that there is at least one significant difference among the means of AWs.

Tukey pairwise comparisons were then performed to identify specific group differences. Grouping information revealed that AW III (mean = 84.32) is significantly different from AW IV (mean = 47.51), and both of these groups are distinct from AW I (mean = 37.87) and AW II (mean = 35.69). However, there was no significant difference between AW I and AW II.

In summary, the analysis demonstrates that there are significant differences in means among the AWs. Specifically, AW III exhibits the highest mean, significantly differing from AW IV, which in turn is distinct from AW I and AW II.

Table 3: Phosphate removal efficiency (mean±SD) of *S. molesta* and *P. stratiotes* in cattle farm effluent treated AWs.

Experimental unit	**Dilution factor**	**Plant species**	**Phosphate removal efficiency (%)**	**Grouping**
AW I	1:3	*S. molesta*	37.87±6.50	B C
AW II	1:2	*S. molesta*	35.69±1.32	C
AW III	1:3	*P. stratiotes*	84.32±4.26	A
AW IV	1:2	*P. stratiotes*	47.51±3.98	B

Note. SD = Standard deviation

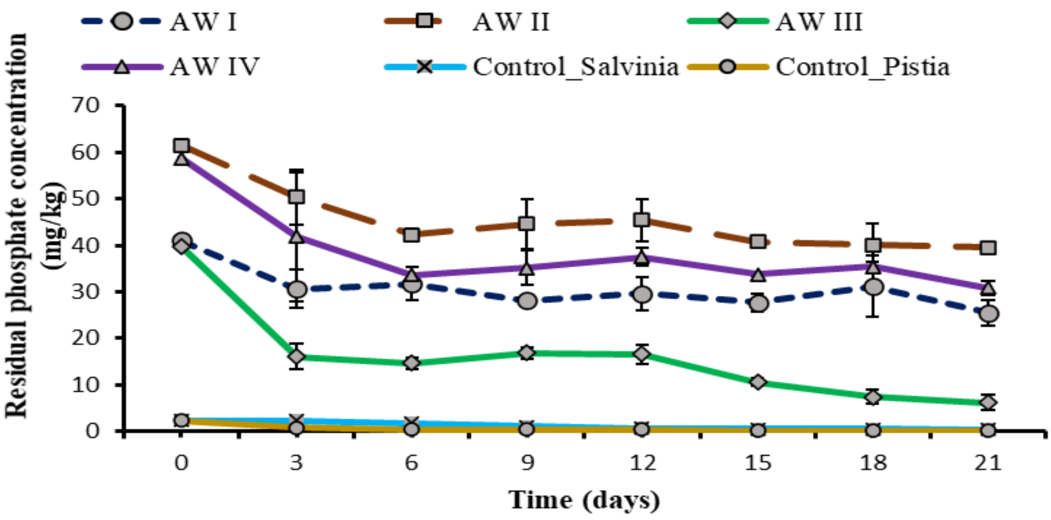

Figure 1: Temporal trends in phosphate concentration in the four AWs over a period of 21 days

Overall, *P. stratiotes* had significantly ($p < 0.05$) higher phosphate removal efficiency and phosphate uptake compared to *S. molesta* in the two tested concentrations.

In this study, the dried ground *Salvinia* and *Pistia* powders were named *Salvinia* phosphate organic fertilizer and *Pistia* phosphate organic fertilizer, respectively. The phosphate content of these fertilizers were determined and they are reported in Table 4.

Table 4: Phosphate content variations in *Salvinia* and *Pistia* derived organic fertilizers (mean±SD)

Experimental unit	**Dilution factor**	**Fertilizer source**	**Before treatment Phosphate content (mg/kg)**	**After treatment Phosphate content (mg/kg)**
AW I	1:3	*S. molesta*	1.58±0.12	35.71±1.48
AW II	1:2	*S. molesta*		29.44±0.91
AW III	1:3	*P. stratiotes*	1.86±007	38.00±2.29
AW IV	1:2	*P. stratiotes*		31.56±1.23

Note. SD = Standard deviation

For both fertilizer sources, macrophytes treated by concentrated cattle farm effluent (1:2) showed a lower phosphate content compared to the more diluted AWs (1:3). When considering the plant species in equally treated concentrations, *Pistia* phosphate organic fertilizer had a higher phosphate content than *Salvinia* phosphate organic fertilizer.

3.5 Water quality parameter variations in AW over time

3.5.1 pH variation of AW over time

In this experimental arrangement, AW I and AW III underwent a dilution factor of 1:3, while the dilution factor for AW II and AW IV was 1:2. The plant species employed in the experiment were *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. The pH values were recorded and plotted for each wetland system. Figure 2 illustrates the changes in pH levels over the duration of the observation period.

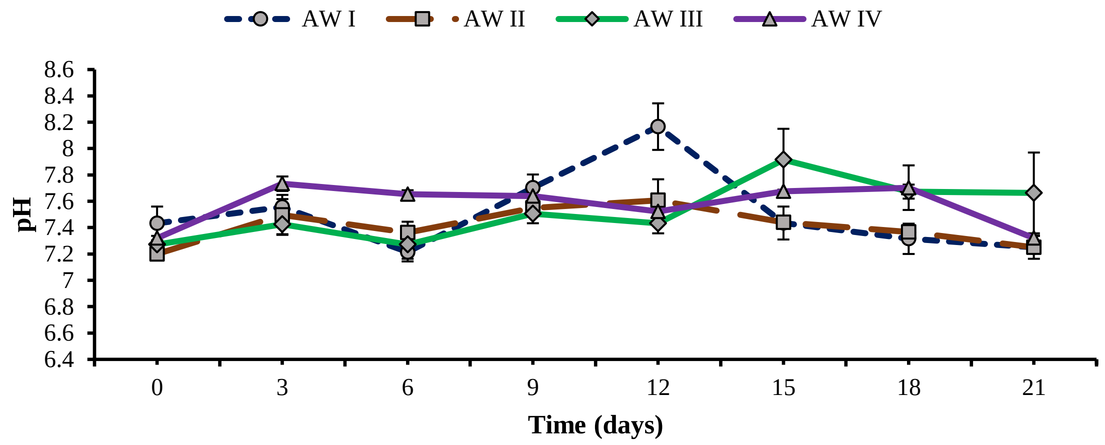

Figure 2: Temporal trends in pH variation in the four AWs over 21 days

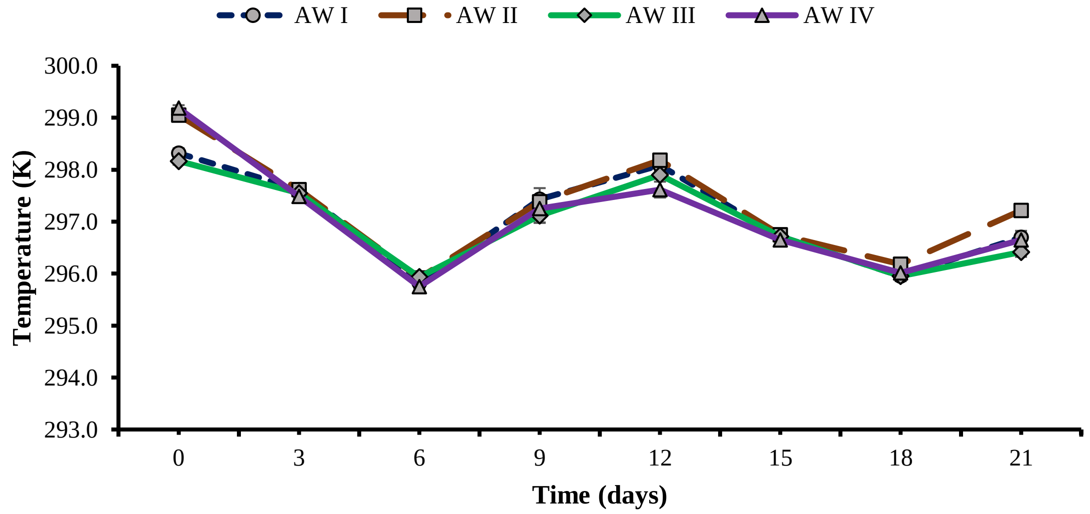

Figure 3: Temporal trends in temperature in the four AWs over 21 days

In the AW I, the pH values exhibited a range from 7.21 to 8.17. Up to 9 days, though the pH varied slightly with time, it was still within the neutral range. On day 12, the pH reached 8.17, indicating a more alkaline condition when compared to the other measurements. From days 15 to 21, the pH continuously decreased in a narrow range.

AW II: The pH values ranged from 7.20 to 7.61, indicating a near-neutral condition. The initial day measurement was 7.20; subsequently, the pH increased to 7.50, but by the 6th day, it had decreased slightly to 7.36. The pH increased again to 7.55 by day 9 and further to 7.61 by day 12. These values suggested a shift towards a more neutral or slightly alkaline condition. Afterwards, the pH continuously decreased and reached 7.25 on the final day.

AW III: On the first day, the pH was measured to be 7.27. After three days, the pH increased to 7.43, and it remained relatively stable around this pH until the 12th day. Then the pH increased noticeably to 7.92 by the 15th day. By day 21, the pH was 7.66, remaining relatively stable and maintaining a slightly alkaline pH level.

AW IV: On the initial day, the pH was 7.32. After 3 days, the pH increased to 7.73, moving towards a more alkaline pH. On the 18th day, the pH remained relatively stable at 7.70. Finally, on the 21st day, the pH returned to 7.32. Throughout the observed time period, the pH values fluctuated within a slightly acidic to slightly alkaline range.

The most significant trends include the distinct pH changes observed on specific days. For example, in AW I, the sharp increase on day 12 and in AW III, the noticeable increase on the 15th day, are noteworthy trends.

In summary, the trends observed in the four AWs varied. AW I exhibited a fluctuating pH within a relatively narrow range between slightly acidic and slightly alkaline values. AW II showed a similar trend but with a narrower pH range than AW I. AW III had a pH range from slightly acidic to slightly alkaline, with noticeable fluctuations. AW IV also showed pH fluctuations within a slightly acidic to slightly alkaline range, but with a narrower range compared to AW III.

3.5.2 Temperature variation of AW over time

In this experimental setup, different water samples served as the experimental units, with corresponding dilution factors of 1:3 for AW I and AW III and 1:2 for AW II and AW IV. The plant species involved in the study were *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. The temperature variations were recorded and plotted for each wetland to illustrate the changes in temperature over the duration of the observation period (Figure 3).

AW I: On the initial day, the temperature was 298.3 K. By the 6th day, the temperature had dropped to 295.8 K. On the 9th day, again, the temperature rose to 297.4 K. Throughout the observation period, the temperature showed fluctuations, but with no clear overall trend. AW II: The temperature was 299.1 K on the initial day. By the 6th day, the temperature had decreased to 295.9 K. From the 9th to the 18th day, the temperature fluctuated and reached 297.2 K on the final day.

AW III: On the initial day, the temperature was recorded to be 298.2 K. From the 3rd to the 9th day, the temperature ranged from 297.6 K to 297.1 K, with a significant drop. On the final day, it reached 296.4 K. AW IV: The initial temperature was 299.2 K. By the 3rd day, the temperature had dropped to 295.8 K. From the 6th to the 9th day, the temperature fluctuated from 295.8 to 297.3 K. Similar minor variations were observed in the temperature from the 12th to the 15th day, and it reached 296.7 K on day 21.

AW II and AW IV showed the highest initial temperatures among the four AWs. In summary, AW II and IV showed noticeably higher initial temperatures. The temperature trends in the four AWs showed variations but lacked a clear overall pattern.

3.5.3 Conductivity variation of AW over time

The dilution factor for the experimental arrangements AW I and AW III was 1:3, while it was 1:2 for AW II and AW IV. The plant species employed in the experiment were *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. The conductivity variations were recorded and plotted for each wetland (Figure 4).

AW I: Conductivity trend showed a gradual decrease from day 0 to day 21, beginning at 390.17 µS mm^{-1} and reaching 359.32 µS mm^{-1}. AW II: Exhibited a decreasing trend in conductivity from day 0 to day 9. However, from day 9 to day 21, there was a noticeable increase, with the final conductivity measuring 584.40 µS mm^{-1}.

AW III: Initially, the conductivity was 344.20 µS mm^{-1} and it decreased to 306.37 µS mm^{-1} by day 6. It then increased to 378.70 µS mm^{-1} by day 18 and then showed a decrease to 336.71 µS mm^{-1} by day 21. AW IV:

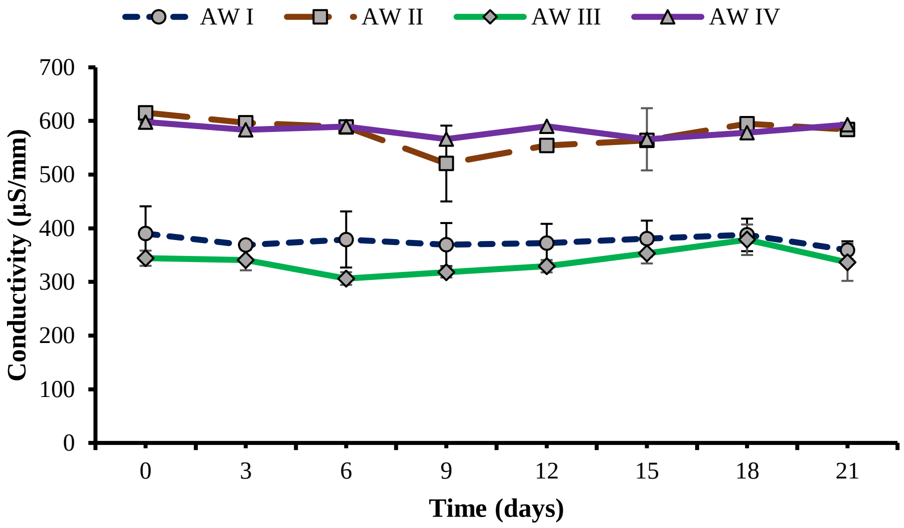

Figure 4: Temporal trends in conductivity in the four AWs over 21 days

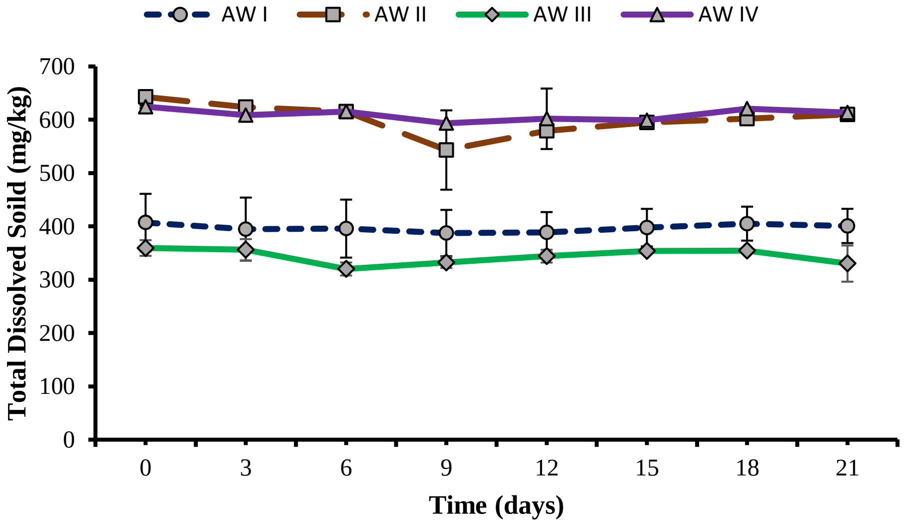

Figure 5: Temporal trends in TDS in the four AWs over 21 days

The conductivity was 598.17 µS mm^{-1} on day 0, it slightly decreased to 583.43 µS mm^{-1} on day 3, and remained relatively stable until day 21.

AW II exhibited a noticeable increase in conductivity from day 9 to day 21, distinguishing it from the overall decreasing trend observed in AW I, AW III, and AW IV.

In summary, all four AWs generally showed decreasing conductivity trends followed by fluctuations. When comparing the four trends, AW II and AW IV generally had higher conductivity levels compared to AW I and AW III.

3.5.4 Total Dissolved Solids (TDS) variation of AW over time

In this experimental setup, different water samples served as the experimental units, with corresponding dilution factors of 1:3 for AW I and AW III and 1:2 for AW II and AW IV. The plant species involved in the study were *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. The TDS variations were recorded and plotted for each wetland to illustrate the changes in TDS over the duration of the observation period (Figure 5).

In AW I, the TDS was 407.18 mg/kg initially and reached 400.85 mg/kg on day 21. In AW II, the TDS was 642.67 mg/kg on day 0, and it decreased to 543.27 mg/kg on day 9. However, from day 9 to 21, there was a gradual increase up to 610.13 mg/kg.

AW III displayed a comparatively similar trend to AW I, with a gradual decrease in TDS. It changed from 359.53 mg/kg at day 0 to reach 330.27 mg/kg on day 21. The decrease was relatively consistent, with some fluctuations observed throughout. AW IV showed a fluctuating trend, with the TDS decreasing from 624.60 mg/kg to 613.43 mg/kg from day 0 to 21, respectively.

Overall, the TDS values in all four AWs showed a decreasing trend over time. AW II and AW IV had almost similar TDS readings on days 6 and 15, but the TSD level of AW IV surpassed that of AW II from days 6 to 21. The TDS levels of AW II and AW IV initially experienced a decrease in TDS followed by an increase, while AW I and AW III consistently exhibited decreasing TDS values with slight fluctuations throughout the observation period. When comparing the four trends, AW II and AW IV generally had higher TDS levels compared to those of AW I and AW III.

3.5.5 Dissolved oxygen percentage (DO%) variation of AW over time

In this experimental arrangement, the dilution factor for AW I and AW III was 1:3, while it was 1:2 for AW II and AW IV. The plant species employed in the experiment were *Salvinia* for AW I and AW II, and *Pistia* for AW III and AW IV. The DO variations were recorded and plotted for each wetland to illustrate the changes in DO over the duration of the observation period (Figure 6).

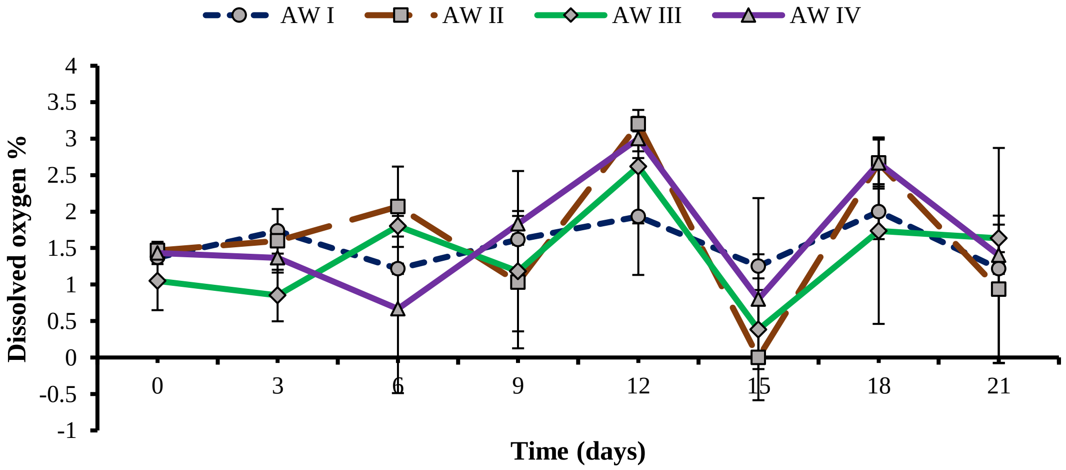

Figure 6: Temporal trends in Dissolved Oxygen % in the four Aws

In AW I, the DO% increased from 1.37% to 1.73% by day 3. It then fluctuated, reaching a peak of 2.00% on day 18 before decreasing to 1.22% on day 21. AW II exhibited a different pattern. The DO % increased significantly from 1.47% to 3.20% on day 12, indicating a substantial rise in oxygen levels. However, from day 12 to day 21, there was a notable decrease, with the DO % dropping to 0.93%.

In AW III, the DO% remained relatively stable during the initial days, ranging from 1.05% to 1.80%. However, on day 12, there was a significant increase to 2.62% before decreasing to 1.63% on day 21. AW IV showed the most varied trend. The DO% of 1.43% on day 0 reached a minimum of 0.80% on day 15, and then showed an increasing trend reaching 2.67% on day 18. On day 21, it again slightly decreased to 1.40%.

AW I experienced a peak on day 18 before decreasing on day 21. AW II, AW III, and AW IV had a distinct pattern with a significant increase in DO% on day 12.

Overall, the trends in DO% values varied among the AWs. All four AWs showed fluctuations with both increases and decreases in DO% over time, but AW I was relatively stable.

4. Discussion

Farm effluents contain high concentrations of nutrients, particularly N, P, and K, and the data revealed that cattle farm effluent is a good source of these nutrients. Cattle farm effluent is a popular choice for the recovery of phosphate because it typically contains high levels of this nutrient.

Phytoremediation is an emerging, environmentally friendly technology that can be used to clean up nutrients as a tertiary treatment process in a conventional treatment system (Jayaweera and Kasturiarachchi, 2003). Several studies have investigated the ability of farm effluent nutrients to demonstrate the potential of macrophytes to remove them. Macrophyte-based wastewater treatment systems are relatively inexpensive to construct and operate, easy to maintain, and provide effective and reliable wastewater treatments (Henry-Silva and Camargo, 2006).

The phosphate concentration trends in the AWs indicate a general decrease in phosphate levels over time. The effectiveness of AWs for removing P was tested, and it was evident from the results that these two plants can remove phosphate. This experiment showed that treatments with *Pistia* reduced the concentration of phosphate more when compared with *Salvinia* in AWs with a similar dilution ratio. In 2006, Henry-Silva and Camargo made similar observations, wherein they noted that *Eichhornia crassipes* and *Pistia stratiotes* demonstrated a notably elevated level of effectiveness in the removal of total P, recording values of 82.0% and 83.3%, respectively. Additionally, these researchers observed a similar ability in the reduction of total nitrogen, with percentages of 46.1% and 43.9% for *E. crassipes* and *P. stratiotes*, respectively. In the same experiment, *Salvinia molesta* exhibited comparatively lower removal efficiencies of 72.1% for total P and 42.7% for total nitrogen. Further, a study done by Mustafa and Hayder in 2020 found that *P. stratiotes* showed a higher rate of phosphate removal than *S. molesta* did.

Salvinia and *Pistia* are both aquatic plants commonly used in phytoremediation processes and perform differently in phosphate removal efficiency due to species-specific adaptations. Though *S. molesta* produces a thick mat (Zutshi and Vass, 1971), *P. stratiotes* can double its biomass within a month, and its long roots (capable of high rhizofiltration) are on average approximately 49 cm in length (Dean *et al*., 2022). It has recently been reported that *S. molesta* and *P. stratiotes* are widely used for the treatment of agricultural, domestic, and industrial wastewater (Akinbile & Yusoff, 2012; Mustafa & Hayder, 2021). Overall, *S. molesta* and *P. stratiotes* have both been found to be effective in removing phosphate from cattle farm effluent.

Phosphate acquired by roots is rapidly loaded into the xylem, and it moves upward in shoots, where it is unloaded into growing sinks. Also, effective nutrient uptake is assisted by other microbial processes (Akinbile & Yusoff, 2012). The right choice of plant species can increase phosphate removal. It should be added to the plant during growth stages to build up its biomass (Mustafa & Hayder, 2021). The decline in the phosphate concentration in phytoremediation system was due to the absorption by *S. molesta* and *P. stratiotes*. A substantial amount of phosphate was taken up from the cattle farm effluent throughout the 21 days due to the high growth of plants and the building up of their biomass (Ng and Chan, 2017). This mechanism of uptake significantly contributes to the transformation of cattle farm effluent into a more environmentally sustainable form.

The phosphate removal efficiency exhibited a considerable difference between AW III and AW IV when the same plant was exposed. This difference can be attributed to the concentration factor. The uptake of nutrients by plants in phytoremediation depends on the nutrient concentration in the effluent (Adler et al., 2003). Macrophytes efficiently absorb phosphate for growth and metabolic processes at lower concentrations. As concentrations increase, plants may reach a saturation level. Moreover, high concentrations of effluent can have toxic effects on plants, leading to reduced efficiency in phosphate removal.

On the other hand, most of the nutrients incorporated into the plant tissue can be returned to the water through decomposition if not harvested (Akinbile & Yusoff, 2012). The storage of pollutants like P in wetland sediments is a more feasible option, and they can be released back into the water after a period of time (Wong *et al*., 1999). These factors can result in a limited increase in phosphate concentration during the experiment. From similar studies conducted by several authors, it is evident that phytoremediation is an important method to remove phosphate from wastewater (Rezania *et al*., 2016; Wickramasinghe & Jayawardana, 2018; Kumar & Deswal, 2020).

There is limited research on the use of *S. molesta* and *P. stratiotes* as organic phosphate fertilizers, but some studies have explored their potential for this purpose. A study has confirmed the hypothesis that *S. molesta* has the potential to be used as an organic fertilizer (Arthur, 2007). This is due to its rapid vegetative growth, large biomass, and high concentration of mineral elements and plant hormones. The plant biomass decomposes quickly, making cytokinins and auxins readily available for uptake by crop plants (Arthur *et al*., 2007).

The biomass of *P. stratiotes* can be utilized to produce fertilizers and compost, and the utility of *P. stratiotes* as an organic phosphate fertilizer for rice cultivation has also been investigated (Polthanee *et al*., 2015). The potential of *P. stratiotes* biomass as an organic fertilizer for tomato cultivation has also been reported (Eid *et al*., 2021). Studies have shown the effectiveness of using macrophytes as organic fertilizers because they accumulate high concentrations of nutrients present in the water (Martínez-Soto *et al*., 2021).

The sediments attached to the plants and their high water content are the major drawbacks of the macrophytes in organic fertilizer production (Moeller *et al*., 2018). By carefully managing the production process and conducting detailed analysis of the nutrient content and application rates, it is possible to enhance the growth and yield of plants and thereby improve their effectiveness in organic fertilizer production.

The pH trends observed in the four AWs fluctuated within a narrow range. It is reported that the pH is increased due to the photosynthesis of plants present in water (Kumar & Deswal, 2020). The plants absorb CO_2 from water and expel oxygen; the CO_2 dissolved in water becomes carbonic acid, resulting in a low pH, but CO_2 removal increases the pH (Kumar & Deswal, 2020). The specific physiological and metabolic activities of plants may contribute to the observed pH fluctuations (Jin *et al*., 2022). AW is a complex system, and many factors, such as anaerobic circumstances in denitrification, would influence the pH. The wetland plants will increase their ability to buffer action (Songliu *et al*., 2009). This will help the pH fluctuate in a narrow range in wetlands.

The cattle farm effluent dilutions used in AWs contain various nutrients and organic matter. The uptake or release of certain ions and compounds can influence pH levels in the water. Aquatic plants absorb nutrients such as nitrogen and P from the water as they grow. This nutrient uptake affects the pH of the wetland water due to different nutrient forms and their interactions with water (Singh *et al*., 2012).

The continuous reduction in pH could be due to the absorption of nutrients and other salts by plants or the simultaneous release of H^+ ions (Dipu *et al*., 2011). However, the AW in this study does not solely adhere to this phenomenon. Instead, it underwent plant photosynthesis, resulting in increased biomass, specific physiological and metabolic plant activities, and exposure to anaerobic conditions, along with the buffering actions of wetlands. In summary, the interplay of these various processes may have created fluctuations in pH rather than a continuous decrease.

Temperature plays a crucial role in the performance of the plants in AW. A greater part of the removal of pollutants is dependent on temperature because plant uptake, nutrient cycling kinetics, and microbial activity are influenced by temperature (Kumar & Deswal, 2020). AW II and IV showed noticeably higher initial temperatures, and the temperature trends in the four AWs showed some variations but lacked a clear overall pattern. The difference in the initial temperatures among the AWs could be because the farm effluent used was high in concentration (1:2 dilution). The temperatures in AWs is also influenced by external temperature and seasonal factors. Since the whole system is exposed to a similar environment, the temperature variance in the AW showed almost a similar trend over the period, but fluctuations can occur due to changes in the environmental temperature. The plant species *Salvinia* and *Pistia* contribute to temperature regulation through activities such as evaporation, organic matter decomposition, and oxidation-reduction. It is reported that temperature is at best a secondary predictor of constructed wetland performance and that the influence of temperature may vary with plant type (Stein & Hook, 2005; Werker *et al*., 2002).

All four AWs generally showed a decreasing conductivity trend, followed by fluctuations. The conductivity levels are influenced by the dilution ratio of the farm effluent used in each AW. Both AW II and AW IV had a 1:2 dilution of farm effluent, which means that the concentration of dissolved ions and compounds in the water was relatively higher compared to the other two wetlands. This higher concentration of ions can contribute to increased conductivity levels (Bohn & Schober, 2000). Because the plant species play a role in nutrient uptake and cycling within the AWs (Stottmeister *et al*., 2003), the conductivity measurements may also change because of the differential rate of nutrient uptake (Bugbee, 2003). *Salvinia* and *Pistia* differ in their nutrient uptake rates and preferences, which can result in variations in conductivity trends among the AWs. It is reported that high conductivity inhibits the growth of *Salvinia* (Kumar & Deswal, 2020), and a conductivity of 2,683 ms cm^{-1} is toxic for *Pistia* (Sooknah & Wilkie, 2004). In this experiment, plants were exposed to concentrations below their tolerance level. It has also been observed that during phytoremediation there could be a reduction in conductivity, which is attributed to the absorption of pollutants by plants (Mahmood et al., 2005; Patel and Kanungo, 2010). Additionally, the interplay of nutrient uptake rates, species-specific responses, and adherence to tolerance levels contribute to fluctuations in conductivity measurements rather than a continuous decrease. Further, the electrical conductivity does not change rapidly (Dipu *et al*., 2010), usually making it necessary to monitor it a few times each week (Bugbee, 2003). Therefore, conductivity measurements were done every three days during the study period of 21 days, and the conductivity was shown to change only in a narrow range**.**

TDS is an important parameter in defining the water quality standards, as TDS is proportional to the degree of pollution (Jayakumar *et al*., 2009). As discussed above, both AW II and AW IV had a higher concentration of farm effluents, with the concentration of dissolved ions and compounds contributing to increased TDS levels compared to AW I and AW III. In water, TDS are composed mainly of carbonates, bicarbonates, chlorides, phosphates, nitrates, organic matter, salt, and other particles (Sarkar *et al*., 2020). A decrease in TDS reflects an improvement in the quality of wastewater due to phytoremediation (Nair and Kani, 2016). The present study showed an overall decrease in TDS, indicating an improvement in the water quality. A disadvantage of phytoremediation is the risk of contaminants collected in senescent tissues being released back into the environment (Akpor & Muchie, 2010). Therefore, the release of certain compounds back into the surrounding water may have contributed to certain increases of the TDS in this study, resulting in fluctuations in the TDS levels.

DO% is a frequently used parameter to evaluate the water quality in water bodies (Sánchez *et al*., 2007). It is a factor in purification processes and supports aerobic life forms. The trends in DO% values varied among the AWs. The application of phytoremediation would increase DO in the waterbody through the photosynthesis of aquatic plants. Photosynthesis by aquatic plants contributes to DO in water bodies during daylight hours. A decrease in water temperature due to the shade given by plants is another factor contributing to increased DO levels in water (Mika, 2022).

The oxygen levels drop to return to low anoxic conditions after a particular period of exposure. The significant explanation given for such a decline in DO is the oxidation processes. The abiotic and biotic oxidation processes consume oxygen for reactions including ferrous iron oxidation and organic mineralization, respectively (Landmeyer & Bradley, 2003; Landmeyer & Effinger, 2016). Aerobic respiration by aquatic organisms and microbial communities involved in degrading dead plants also consumes DO and contributes to the oxygen demand (Mika, 2022). Water temperature is another factor that affects the fluctuations in DO levels, not only periodically but also daily as a part of the natural ecology (Bozorg-Haddad *et al.*, 2021). Overall, among the water quality parameters, DO is a vital factor that could influence microbial activities and the efficiency required for the removal of pollutants. This research examined the fluctuations in DO levels within AWs during the experimental period. While these levels experienced variations, they consistently remained above zero percent, guaranteeing a continuous oxygen supply throughout the entire experiment.

Most plants involved in phytoremediation grow well within specific pH ranges. Fluctuations outside these ranges can affect their metabolism and nutrient uptake. Phytoremediation processes are often temperature-dependent, and different plants and microbes have specific temperature preferences. Fluctuations in temperature can influence the rate of plant growth, microbial activity, and biochemical reactions. In terms of EC and TDS, some plants are more tolerant of high conductivity and TDS levels than others. High conductivity and TDS can lead to osmotic stress in plants and can affect the solubility of contaminants. Adequate oxygen levels are crucial for the aerobic microbial processes involved in phytoremediation. Low dissolved oxygen levels can shift microbial activity toward anaerobic processes. Therefore, it is evident from this study that monitoring and maintaining an optimal range in the above water quality parameters are crucial for the success of phytoremediation.

The Central Environmental Authority (CEA) of Sri Lanka has established general standards and criteria for the discharge of industrial effluents into inland surface waters. The pH levels within the range of 6.5 to 8.5 are deemed suitable, while the temperature must not exceed 40°C. Additionally, the recommended concentration of dissolved phosphates should be kept at 5 mg/L (CEA, schedule 1, List I). It is noteworthy that, as distinct standards for farm effluents are absent, the post-treatment water quality parameters of this study align with these established limits. Remarkably, the treatment process successfully aligns with the ambient water quality standards as well. Specifically, an average pH of 6.12, a temperature of 28.44°C, and a conductivity level of 720.22 µS mm^{-1} are all within acceptable limits. This demonstrates the effective harmonization of treated water quality with broader environmental standards, affirming the responsible management of farm effluents to safeguard the integrity of inland surface waters.

The similarity between the treated water quality parameters and the CEA standards also holds broader implications for preserving biodiversity, supporting sustainable agriculture, ensuring community health, and advocating for strengthened regulations and targeted guidelines. These findings can significantly contribute to evidence-based policy development, facilitating a more comprehensive and effective approach to environmental management in Sri Lanka.

In the context of phytoremediation, fluctuations in phosphate concentrations and observed water quality parameters convey important information about the dynamic processes occurring within the AW system; for example, about processes such as phosphate removal efficiency, nutrient cycles, uptake or release of certain ions and compounds, and the photosynthesis of plants. Therefore, this study helps to understand these fluctuations, and it will be a key to evaluating the effectiveness of the phytoremediation process and its potential impact on water quality as a relatively low-cost technique to attenuate contaminants in water.

However, the real-world application of *Salvinia* and *Pistia* for phytoremediation does pose challenges. The size of the tanks or artificial wetlands required for efficient treatment must be carefully considered in relation to the amount of effluent produced per day. This consideration involves a balance between the capacity of the wetlands to accommodate the effluent volume and the need for effective remediation. The cost associated with establishing and maintaining such systems, including infrastructure, plant cultivation, and monitoring, needs to be evaluated against the potential environmental and economic benefits.

In a nutshell, this research not only successfully demonstrated the remarkable capability of *Salvinia* and *Pistia* in absorbing phosphate, but also highlighted their efficiency in reducing EC and TDS in the effluent and overall environmental protection by mitigating the impact of nutrient-rich effluent on aquatic ecosystems. These findings contribute to the main goal of the study by establishing the promising potential of artificial wetlands constructed with these plants for phytoremediation purposes. Moreover, the twofold functionality of these plants as organic phosphate fertilizers adds a practical and sustainable dimension to the research, addressing both environmental concerns and agricultural productivity. These usages highlight the many-sided significance of the study, offering valuable perceptions that bridge the gap between environmental conservation and effective agricultural resource management in the broader field of phytoremediation.

5. Conclusion

The phosphate removal efficiency of *Salvinia* was 37.87±6.50% in AW I and 35.69±1.32% in AW II. For *Pistia*, the removal efficiencies were 84.32±4.26% and 47.51±3.98% in AW III and AW IV, respectively. The phosphate contents of *Salvinia* and *Pistia* phosphate fertilizer were 35.71±1.48 mg/kg in AW I, 29.44±0.91 mg/kg in AW II, 38.00±2.29 mg/kg in AW III, and 31.56±1.23 mg/kg in AW IV. All four AWs generally showed decreasing conductivity, TDS, and phosphate concentration trends. Therefore, AWs provide an effective phytoremediation technology for wastewater treatment.

In conclusion, our research has unequivocally demonstrated the promising potential of artificial wetlands constructed with *Salvinia* and *Pistia* in the field of phytoremediation. These plants have exhibited remarkable capabilities in absorbing phosphate and effectively reducing conductivity and TDS in cattle farm effluent, affirming their pivotal role in phytoremediation. Furthermore, their dual utility as sources of organic phosphate fertilizer underscores their multifaceted significance, offering sustainable solutions for both agriculture and ecosystem management, bridging the gap between environmental conservation and agricultural productivity.

Researchers and policymakers should consider the demonstrated efficacy of *Salvinia* and *Pistia* in phytoremediation, their utility as organic fertilizers, and the practical implications regarding scale, infrastructure, and environmental impact when addressing nutrient-rich cattle farm effluent.

Future research could optimize and implement large-scale phytoremediation techniques for efficient phosphate removal from wastewater. That can be examined by performing phosphate removal from the effluent treatment tanks. Additionally, optimizing the design and scale of artificial wetlands to better match the specific characteristics of cattle farm effluent ensures practical and cost-effective implementation.

In essence, our research unequivocally establishes the prowess of *Salvinia* and *Pistia* in constructing artificial wetlands for phytoremediation, presenting not only a powerful strategy for mitigating phosphate pollution in cattle farm effluent but also offering a transformative solution that harmonizes environmental conservation with sustainable agricultural productivity. By showcasing the dual utility of these plants as both efficient pollutant absorbers and organic phosphate fertilizers, our findings illuminate a path towards a more harmonious coexistence between agriculture and the ecosystem, exemplifying the potential for innovative, nature-inspired approaches to shape a greener and more resilient future.

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7. Conflict of Interest

The authors declare that they have no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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