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Phytoremediation of *Oryza sativa* with Media Substrates for The Sewage Effluent Treatment in Constructed Wetland

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Abstract: This study investigates the ability of the plant, *Oryza sativa*, to purify effluent from a sewage treatment plant through a constructed wetland system. A laboratory-scale constructed wetland using different media substrates was used to treat effluent from the National Water Research Institute of Malaysia sewage treatment plant, with the aim of enhancing water quality. The objectives were to design a subsurface flow constructed wetland based on hydraulic retention time and available area, evaluate the pollutant removal efficiency of various media substrates, and determine the phytoremediation capacity of *Oryza sativa* for effluent treatment. The findings suggest that *Oryza sativa*, when combined with different media substrates, significantly improved several water quality indicators, such as biological oxygen demand, ammoniacal nitrogen, total nitrogen, aluminum, nitrite, nitrate, total coliform, E-coli, and fecal coliform. The most significant reduction was in fecal coliform, which decreased by 99.7% when *Oryza sativa* was combined with K1 media. Other improvements included a 66.7% decrease in biological oxygen demand with *Oryza sativa* and gravel, and lava rock, a 53.3% reduction in aluminum with *Oryza sativa* and gravel, a 23.1% reduction in E.coli with *Oryza sativa* and gravel, a 21.7% reduction in total coliform with *Oryza sativa* alone, a 17.7% decrease in nitrate with *Oryza sativa* and K1 media, a 12.5% decrease in nitrite with *Oryza sativa* and gravel, a 10.5% decrease in ammoniacal nitrogen with Oryza sativa and gravel, and a 6.7% reduction in total nitrogen with *Oryza sativa* alone. These findings suggest that *Oryza sativa*, when combined with various media substrates, has substantial potential for purifying sewage effluent through phytoremediation.

Keywords: phytoremediation, *Oryza sativa*, wastewater, sewage effluent, constructed wetland

1. Introduction

Vegetation plays a crucial role in all phytoremediation applications, whether in soil or wetlands. Plants deemed safe for phytoremediation and related activities must possess specific qualities or characteristics. It is essential to utilize plants that can tolerate high concentrations of toxic pollutants (Sharma, 2018). For the treatment of sewage treatment plant effluent, the *Oryza sativa* (rice plant) has been identified as a suitable option due to its ability to thrive in wetland environments, high nutrient uptake capacity, an extensive root system, and tolerance to a range of contaminants, including heavy metals (Yuliasni et al., 2023). *Oryza sativa* has been widely used in constructed wetlands for the treatment of several type of wastewaters, including domestic sewage, industrial effluents, and agricultural runoff (Ali et al., 2020). *Oryza sativa* has been identified as a promising candidate for use in constructed wetlands due to its ability to effectively remove a range of contaminants, including heavy metals, organic compounds, and nutrients (Bhatia and Goyal, 2013). Studies have shown that rice plants can accumulate and remove significant quantities of pollutants from wastewater through various mechanisms, including uptake, adsorption, and microbial degradation facilitated by the plant's root zone (Omondi and Navalia, 2021). In addition to their pollutant removal capabilities, rice plants are also known for their ability to thrive in a wide range of environmental conditions, making them a suitable choice for constructed wetland systems. The integration of rice plants into constructed wetland systems has been the focus of several studies, which have demonstrated the potential of this approach to effectively treat sewerage treatment plant effluent. These studies have highlighted the ability of rice plants to remove a wide range of pollutants, including organic matter, nutrients, and heavy metals, from the wastewater.

2. Materials and Methods

2.1 Site Selection

The experiment was conducted at the National Hydraulic Research Institute of Malaysia facility in Seri Kembangan,

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Selangor, within a controlled greenhouse environment. This location was chosen due to the presence of an existing sewage treatment plant and the availability of sufficient land area to accommodate the study.

2.2 Experimental Setup

The study was conducted in a greenhouse using a series of lab-scale constructed wetland units with an HDPE water tank to effectively treat the discharge from the NAHRIM STP. The design was based on a literature review of the hydraulic retention time (HRT) and the surface area of the water tank, which are key parameters governing the performance and efficiency of constructed wetlands. The hydraulic loading rate (HLR) was determined by dividing the volume of the experimental water tank by the specified HRT of the wetland system. The HRT was set at 12 hours. The effluent volume, based on manual measurements, ranged from 0.22 l/s to 2.15 l/s, likely due to fluctuations in the overall wastewater treatment capacity of the plant. The subsurface flow (SSF) constructed wetland was constructed using a water tank with a square area of 0.8 m² and a depth of 0.35 m, with inlet and outlet were designed to control water flow and prevent short-circuiting, as also depicted in Fig. 1.

Fig. 1: Inlet and outlet structure

Three different media substrates were tested (Fig. 2): gravel, lava rock, and K1 filter media, each with a depth of 0.3 m. The choice of appropriate media is crucial for SSF constructed wetlands, as it supports plant roots and microbial activity, provides a substrate for plant establishment, and facilitates the growth of microorganisms responsible for various treatment processes. Gravel with a larger particle size, such as the 25 mm used in this study, offers advantages over smaller substrates. Larger gravel has higher porosity, allowing for greater water infiltration and contact with the wetland's microbial communities. Additionally, the coarser substrate is less prone to clogging, which can be an issue in constructed wetlands and impede the system's functionality. The porosity of the lava rock and K1 bio media are also key parameters that influence the overall treatment performance of the constructed wetland. Evaluating the porosity characteristics can help optimize the design and operation of the wetland system to maximize treatment efficiency and minimize operational challenges.

Fig. 2: Substrates (a) gravel, (b) lava rock, and (c) K1

The wetland plant used was *Oryza sativa*, also known as rice or paddy, and was approximately 1 month old at the time of the study. *Oryza sativa* is well-documented for its effectiveness in constructed wetland systems for wastewater treatment. Each experimental tank was planted with *Oryza sativa* at a density between 90 and 100 plants. The plant roots were thoroughly washed to remove soil, then positioned with supporting structures within perforated PVC pots. Additionally, a high-density foam sheet with 6-inch apertures was installed to accommodate fifteen 6-inch PVC pots per tank. The high-density foam floated on the water surface, allowing the plant roots to be fully submerged. The experimental tank setup has been illustrated in Fig. 3 and its cross-section presented in Fig. 4.

Fig. 3: Experimental setup

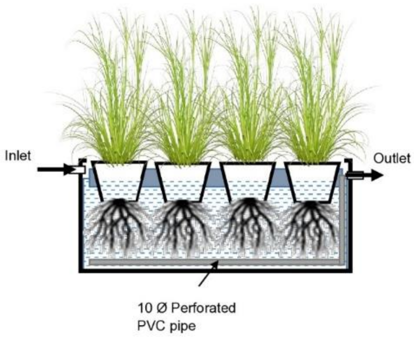

Fig. 4: Section of SSF CW

The SSF constructed wetland in the experimental tanks will be fed with effluent from the storage tank and have a total

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volume of 250 litres. The SSF design allows greater exposure of the wastewater to the natural processes within the wetland, facilitating more effective treatment. The HLR will be 23 l/hr, controlled by a flow meter in each experimental tank. The system will operate continuously with a 12 HRT, providing sufficient contact time between the wastewater and the wetland for effective treatment. One experimental tank with the same setup but no media substrate will serve as a control.

2.3 Water Quality Data Collection

There are two methods of data collection, in-situ and ex-situ:

a) In-situ measurements involve directly measuring water quality parameters at the inlet and outlet of the constructed wetland system using an Aqua Troll multiparameter water quality probe (Fig. 5).
b) Ex-situ measurements involve collecting water quality parameters from the inlet and outlet of the constructed wetland system for further analysis at the NAHRIM Water Quality Lab (Fig. 6).

Fig. 5: In-situ measurement device

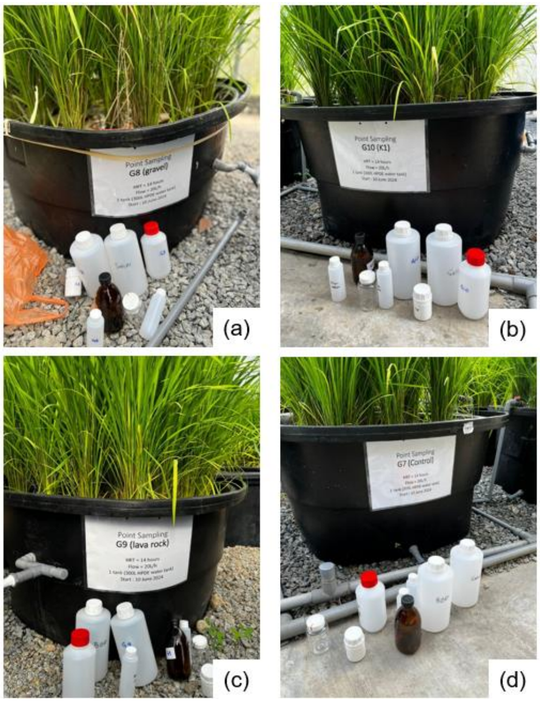

Fig. 6: Sampling for lab analysis (a) *Oryza sativa* with gravel, (b) *Oryza sativa* with K1, (c) *Oryza sativa* with lava rock and (d) *Oryza sativa* without any media substrate

2.4 Pollutants Removal Efficiencies

Removal efficiencies of pollutants from the wetland systems were calculated using the following equation.

Removal rate (%) = $(Ci - Co) \times 100/Ci$

where,
Ci = Inlet Concentration
Co = Outlet Concentration

3. Results and Discussion

Table 1 presents the significant chemical, physical, and biological water quality parameters for the inlet and outlet.

Table 1: Chemical, physical and biological water quality characteristics of inlet and outlet

	Inlet	Outlet			
		Control	Gravel	L. rock	K1
pH	7.3	7.25	6.18	6.07	6.12
Temp.	30	29.5	29.6	30	28.5
DO	7.7	3.4	2.67	2.63	2.06
BOD	6	4	2	2	3
NH_3	1.9	3.3	1.7	1.8	1.8
NH_3-N	4	7.8	4.3	4.7	4.8
TN	4.6	6.7			
Al	0.15	0.16	0.07	0.08	0.1
NO_2^-	0.24	0.32	0.21	0.22	0.25
NO_3^-	7.13	6.54	6.65	8.07	5.87
SO_4^+	17.05	15.14	16.58	17.74	16.93
PO_4^{3-}	0.24	0.25	0.28	0.26	0.26
NH_4^+	2.3	3.51	2.18	2.26	2.33
TC	>35,000	>27,400	>36,300	>33,800	>32,900
E. coli	2,600	3,800	2,000	2,100	2,100
FC	>50,200	4,100	1,700	4,700	164

The use of *Oryza sativa* plants in phytoremediation successfully reduced pollutant levels, as shown by improvements in water quality measurements like biological oxygen demand (BOD), ammoniacal nitrogen, aluminum, nitrate, total coliform (TC), E. coli, and fecal coliform (FC). In contrast, DO and pH levels exhibited the opposite trend. However, two parameters, total nitrogen and nitrite, remained high compared to standards.

The average pH in the effluent of the sewage treatment plant was 7.3, with reductions observed in the control, gravel, lava rock, and K1 tanks. The average DO concentration in the effluent was 7.7 mg/L, but declined to 3.4 mg/L, 2.67 mg/L, 2.63 mg/L, and 2.06 mg/L in the respective experimental tanks.

The initial E. coli concentration in the wastewater was 2,600 cfu/100 ml. The system with plants and gravel achieved the greatest reduction, reaching 2,000 cfu/100 ml. The systems with plants and lava rock or K1 media had similar reductions, both reaching 2,100 cfu/100 ml. In contrast, the control treatment had a higher E. coli concentration of 3,800 cfu/100 ml.

The effluent contained over 50,200 cfu/100 ml of FC. The unit with plants and K1 media had the highest reduction, with a concentration of 164 cfu/100 ml. This was followed by the system with plants and gravel, the control, and the system with plants and lava rock. The effluent contained over 35,200 cfu/100 ml of TC. The control system had the highest reduction, with 27,400 cfu/100 ml. This was followed by the system with plants and K1 media (32,900 cfu/100 ml), the system with plants and lava rock (33,800 cfu/100 ml), and the system with plants and gravel (36,300 cfu/100 ml).

The average BOD level in the effluent was relatively high at 6 mg/L, indicating significant organic pollution. In contrast, the BOD value was lower at 4 mg/L in the control tank, and even lower at 2 mg/L in the system with plants and gravel and plants and lava rock. The system with plants and a K1 filter had an average BOD of 3 mg/L.

The average ammoniacal nitrogen concentration in the effluent was 1.9 mg/L, exceeding the Class III limit in the National Water Quality Standards (NWQS). The control tank without plants had a slightly higher average concentration of 3.3 mg/L at the outlet. In contrast, the system with plant and gravel, lava rock, and K1 filter demonstrated lower average concentration, suggesting the plants and media were more effective in reducing ammoniacal nitrogen levels in the constructed wetland.

The effluent had a high average aluminium content of 0.15 mg/L, categorized as class III–IV based on NWQS. The concentration levels decreased in the plant with gravel, lava rock, and K1, reaching 0.07 mg/L, 0.08 mg/L, and 0.1 mg/L, respectively. Nevertheless, the aluminium concentration remained in the control system.

The effluent had a high average nitrite concentration of 0.24 mg/L. In contrast, the control had a higher nitrite level of 0.32 mg/L. The nitrite concentrations gradually decreased in the system with gravel and lava rock, reaching 0.21 mg/L and 0.22 mg/L, respectively. However, the nitrite level increased to 0.25 mg/L in the K1 media system.

The average nitrate concentration in the effluent was 7.67 mg/L. In contrast, the control showed a lower nitrate level of 5.44 mg/L. Moreover, the treated system displayed even lower average nitrate concentrations, measuring 4.95 mg/L in the plant and gravel, 0.57 mg/L in the plant and lava rock, and below 0.16 mg/L in the plant and K1. These reduced nitrate levels in the treated tanks suggest that the constructed wetland system effectively removed nitrates from the wastewater, with the presence of plants and the K1 playing a key role in this nitrate reduction.

3.1 Pollutant Removal

Table 2 compares the pollutant removal performance of SSF constructed wetlands, using *Oryza sativa* and different media substrates as gravel, lava rock, and K1, as well as a control system. This analysis evaluates the effectiveness of various substrate types in improving the water purification capabilities of these constructed wetland systems when treating effluent from a sewage treatment facility.

Table 2: Percentage removal

	Percentage removal (%)			
	Control	**Gravel**	**Lava rock**	**K1**
BOD	33	66.7	66.7	50
AN	-73.7	10.5	5.3	5.3
Al	-6.7	53.3	46.7	33.3
NO_2^-	-33.3	12.5	8.3	-4.2
NO_3^-	8.3	6.7	-13.2	17.7
TC	21.7	-3.7	3.4	6
E. coli	-46.2	23.1	19.2	19.2
FC	91.8	96.6	90.6	99.7

The system with a gravel and lava rock media achieved a BOD removal rate of around 66.7%, indicating it was more effective at improving BOD levels compared to the other treatments. In contrast, the system with K1 media achieved a 50% BOD removal, while the control showed a relatively lower BOD removal rate of 33%. These results suggest that using *Oryza sativa* and gravel and lava rock substrates in constructed wetlands can lead to significantly higher BOD removal efficiency.

The highest ammoniacal nitrogen removal was seen in the system using gravel substrate, achieving a 10.5% removal rate. The *Oryza sativa* with lava rock and K1 media had lower ammoniacal nitrogen removal rates of 5.3% each. Conversely, the control showed a negative removal rate of 73.7%, indicating ammoniacal nitrogen levels increased instead of decreasing.

The gravel-based constructed wetland system showed a high aluminium l removal rate of around 53.3%, followed by lava rock at 46.7% and K1 at 33.3%. In contrast, the constructed wetland without any media substrates had a negative 6.7% removal rate. These results highlight the importance of media type in determining pollutant removal efficiency within constructed wetland systems.

The gravel-based constructed wetland had the highest nitrite removal, achieving a 12.5% reduction. The lava rock substrate wetland closely matched this, reaching an 8.3% nitrite removal. In contrast, the K1 media wetland saw a 4.2% increase, while the control showed a 33.3% increase in nitrite.

The K1 media constructed wetland had the greatest nitrate removal at 17.7%. The unmediated wetland closely followed with an 8.3% removal rate. The gravel-based wetland showed a lower, yet still substantial, nitrate removal of 6.7%. In contrast, the *Oryza sativa* substrate with lava rock exhibited a negative removal of 13.2%.

The constructed wetland system incorporating *Oryza sativa* and a gravel substrate was the most effective, removing around 23.1% of E. coli. The *Oryza sativa*-based systems using lava rock and K1 media both achieved a similar E. coli removal rate of 19.2%. Conversely, the control had a negative E. coli removal rate of 46.2%.

The plant without any media substrate exhibited a 21.7% removal of total coliform. The plant with K1 media achieved a 6% reduction in total coliform. In contrast, the plant with lava rock had a 3.4% removal of total coliform. The plant in gravel media showed a negative 3.7% total coliform reduction.

These results indicate that the natural processes within the constructed wetland system also contributed to the overall water quality improvement.

The constructed wetland using K1 media displayed the highest FC removal, achieving approximately 99.7% reduction. The wetland with gravel media showed a 96.6% removal of FC. Comparatively, the lava rock-based wetland removed 90.6% of the FC. Interestingly, the control without any media substrate still managed to remove 91.8% of the FC coliform.

The highest water quality parameter improvement, in terms of percentage removal, using *Oryza sativa* with different media substrates is summarized in Table 3. The various media substrates used in this study, with *Oryza sativa*, contributed significantly to the removal of pollutants, as shown in Fig. 7.

Table 3: Highest percentage removal rate

Parameter	Media substrate	Highest removal rate (%)
BOD	Gravel and lava rock	66.7
AN	Gravel	10.5
TN	Control	6.7
Al	Gravel	53.3
NO_2^-	Gravel	12.5
NO_3^-	K1 media	17.7
TC	Control	21.7
E. coli	Gravel	23.1
FC	K1 media	99.7

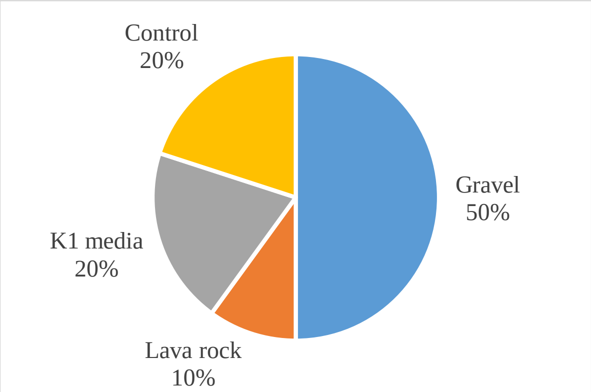

Fig. 7: Percentage removal for each substrates

4. Conclusion

This study used a lab-scale SSF constructed wetland system planted with *Oryza sativa* and different media substrates, including gravel, lava rock, and K1 filter media. The results showed significant improvements in water quality parameters in the sewage treatment plant's effluent. Key reductions were observed in BOD, AN nitrogen, nitrate, TC, E. coli, FC, and aluminum levels. These findings indicate that *Oryza sativa* is a promising plant for effective and environmentally friendly wastewater remediation through phytoremediation in constructed wetland systems.

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