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Synergistic Effect of Banana Corm-Based Microbial Organic Liquid and *Cyperus papyrus* on Lead Removal in a Subsurface Flow Constructed Wetland System

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ABSTRACT: Laboratory wastewater often contains hazardous heavy metals such as lead (Pb). A previous study found that laboratory wastewater contained Pb at a concentration of 3.52 mg/l, exceeding the Indonesian quality standard (1 mg/l; Minister of Environment Regulation No. 5/2014). In that study, laboratory wastewater was treated using a subsurface flow constructed wetland (SSF-CW) with *Cyperus papyrus*, which successfully reduced Pb concentration from 3.52 mg/l to 0.75 mg/l within 9 days. While effective, this setup required a long retention time and large space. To improve treatment efficiency, this study combined *Cyperus papyrus* with banana corm-based microbial organic liquid (MOL) containing indigenous microorganisms. A batch SSF-CW reactor (soil and gravel media) treated 1.2 l of artificial wastewater (initial Pb concentration: 3.33 mg/l) using 10% (v/v) banana corm MOL and four *Cyperus papyrus* plants. Pb concentration, pH, and plant morphology were monitored for nine days. The results showed that Pb concentration met the quality standard by day 2 (0.283 mg/l; 91.50% removal) and reached maximum removal on day 4, with Pb concentration < 0.0002 mg/l and 99.99% removal efficiency. Thus, combining *Cyperus papyrus* with banana corm MOL proved more effective in reducing Pb concentration than using *Cyperus papyrus* alone, achieving comparable results in only two days instead of nine.

KEYWORDS: Banana corm-based MOL; *Cyperus papyrus*; laboratory wastewater; lead (pb); SSF-CW

1. Introduction

The discharge of untreated laboratory wastewater presented a major environmental and public health risk due to its frequent contamination with heavy metals [1]. Heavy metals were elements with high atomic weight that were generally toxic to humans, animals, and plants [2]. When absorbed, heavy metals could bioaccumulate in living organisms due to their persistence and tendency to disrupt biological processes [3–5]. Pb was a particularly problematic heavy metal because it was non-essential, highly toxic, bioaccumulative, and persistent. Long-term and chronic exposure to Pb could cause major neurological, cardiovascular, and renal disorders

[4, 6, 7]. As a result, strict regulatory standards, such as Indonesia's wastewater limit of 1 mg/l for Pb, highlighted the need for effective treatment technologies [8]. Constructed wetlands (CWs) offered a robust, environmentally friendly, and cost-effective wastewater treatment technology that leveraged natural processes involving plants, substrates, and microbial communities [9–11]. CWs were generally classified into several types, including free water surface (FWS) systems, where water flowed above the substrate and around emergent plants, and subsurface flow (SSF) systems, where wastewater flowed through a porous medium beneath the surface [12]. SSF systems could be further divided into horizontal subsurface flow (HSSF) and vertical subsurface flow (VSSF) configurations, each offering different hydraulic and oxygen transfer characteristics [13].

Among these configurations, SSF systems were particularly advantageous because they prevented odor, minimized mosquito breeding, and provided a large surface area for microbial biofilm development [14]. Macrophytes such as *Cyperus papyrus* were particularly suitable for CWs due to their rapid growth, high biomass production, and dense root systems, which enhanced pollutant uptake and tolerance [15]. However, the primary limitation of CWs was the relatively long hydraulic retention time (HRT) required to achieve regulatory standards. A previous study reported that an SSF-CW planted with *Cyperus papyrus* required nine days to reduce Pb concentration from 3.52 mg/l to 0.75 mg/l [16]. To improve treatment efficiency and shorten HRT, bioaugmentation with specific microbial consortia was a viable strategy [17].

One potential bioaugmentation agent was local microorganisms (MOL), a fermented solution derived from local organic materials [18]. Banana corm (*Musa balbisiana*) served as an effective substrate for MOL production due to its high starch content (69.13%), which supported the growth of diverse microbial communities such as *Bacillus* sp., *Aeromonas* sp., *Aspergillus* sp., *Azospirillum* sp., and *Azotobacter* sp., organisms known for their metal resistance and biosorption potential [19–22]. The high starch content in banana corm provided an abundant and readily available carbon and energy source, thereby accelerating microbial proliferation during fermentation and enhancing overall microbial density in the MOL [23,24]. When applied as a biostimulant, this bacteria-rich MOL could strengthen the phytoremediation capacity of plants by enriching the rhizosphere microbiome, facilitating metal mobilization, and alleviating metal stress in plants [25]. This study aimed to evaluate the synergistic effect of *Cyperus papyrus* and banana corm MOL on the removal efficiency and kinetics of Pb in an SSF-CW system. The primary objective was to determine whether this integrated approach could achieve regulatory compliance for Pb in a significantly shorter time compared to a plant-only system.

2. Materials and Methods

2.1. Biostimulant (MOL) preparation and characterization.

The MOL was prepared by anaerobically fermenting 2 kg of chopped Musa balbisiana corm with 400 g of crushed palm sugar and 4 L of rice-washing water in a 10 l container fitted with a vent hose for 14 days. The resulting MOL had a pH of 4.4, a brownish-black color, and a distinct alcoholic aroma, indicating successful fermentation [26, 27].

2.2. Plant acclimatization and synthetic wastewater.

Cyperus papyrus plants, pre-selected at a height of 30–40 cm for optimal water and nutrient uptake capacity [28], were acclimatized for 7 days. This process was conducted in a plastic reactor (20 cm × 14 cm × 14 cm) filled with a 14 cm layer of sand and gravel and irrigated with clean tap water to ensure adaptation to the experimental conditions prior to phytotreatment. Following the acclimatization period, the plants remained healthy, showing no signs of wilting or mortality, thereby confirming their suitability for the subsequent phytotreatment test in a SSF-CW system. Synthetic wastewater was prepared to simulate contaminated laboratory effluent by dissolving $Pb(NO_3)_2$ in tap water to achieve a target Pb concentration of 3.33 mg/l.

2.3. Experimental setup and design.

The study was conducted using a batch-scale SSF-CW reactor (28 cm L × 20 cm W × 13 cm H) filled with a 14 cm layer of gravel and sand media. The reactor was planted with three acclimatized *Cyperus papyrus* plants (30–40 cm in height). A total of 1.2 L of synthetic wastewater was prepared at 80% of the maximum tolerable concentration determined from a previous range-finding test, resulting in a Pb concentration of 3.33 mg/l [16]. For the treatment system, the synthetic wastewater was mixed with *Musa balbisiana* MOL at a ratio of 10% (v/v) (120 mL) and then introduced into the reactor [29]. A comparative performance analysis was conducted using data from a previous study that employed an identical SSF-CW configuration without MOL addition. The reactor configurations used in this study are summarized in Table 1.

Table 1. Reactor configurations and experimental conditions.

Reactor	Plant Type	Media (Sand & Gravel)	MOL Addition	Wastewater Type
R1	*Cyperus papyrus*	Yes	Yes	Synthetic wastewater
R2	None (control)	Yes	Yes	Synthetic wastewater
R3	*Cyperus papyrus*	Yes	No	Tap water

Note: All reactors contained sand and gravel media. MOL referred to banana corm–based local microorganisms. Synthetic wastewater contained Pb at 3.33 mg/l, while tap water served as a control medium.

2.4. Sampling and analysis.

Water samples were collected from the reactor effluent at 2-day intervals over a 10-day hydraulic retention time (HRT). The samples were analyzed for Pb concentration using atomic absorption spectrophotometry (AAS) in accordance with the Indonesian standard method SNI 6989-46:2009 [30]. The pH and plant morphology were also monitored throughout the experiment. The removal efficiency was calculated as:

$$\%\ \text{Removal} = \frac{(C_0 - C_e)}{C_0} \times 100\%$$

where C_0 was the initial concentration and C_e was the concentration at time *t*.

3. Results and Discussion

3.1. Effectiveness and maximum duration for Pb removal.

The integration of MOL significantly improved the kinetics of Pb removal. As presented in Table 1 and illustrated in Figure 1, the Pb concentration decreased from an initial 3.33 mg/l to 0.283 mg/L (91.5% removal) within the first 2 days. By day 4, the removal efficiency exceeded 99.99%, and the remaining Pb concentration dropped below the AAS detection limit (<0.0002 mg/l). This low concentration was maintained until the end of the experiment (day 10).

Table 1. Pb removal efficiency in the banana corm MOL–augmented SSF-CW system.

Hydraulic Retention Time (Days)	Pb Concentration (mg/L)	Removal Efficiency (%)
0	3.33	0.00
2	0.283	91.50
4	<0.0002	>99.99
6	<0.0002	>99.99
8	<0.0002	>99.99
10	<0.0002	>99.99

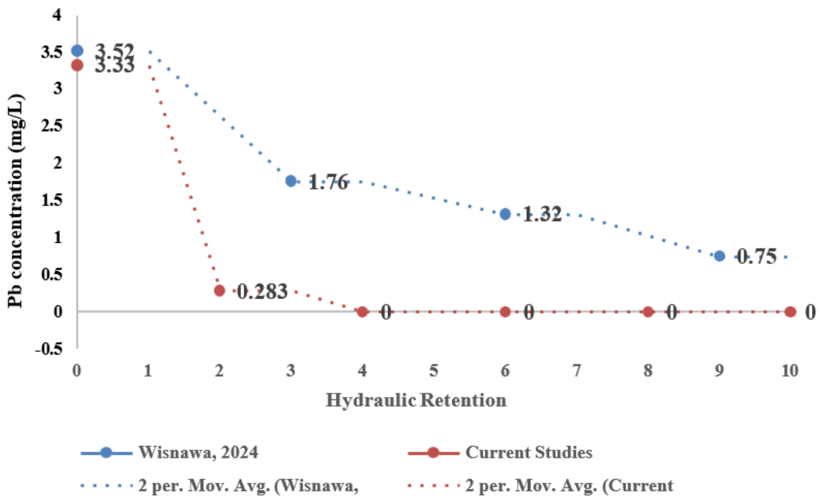

Figure 1. Comparative graph showing the rapid decline of Pb concentration in the MOL-augmented system versus the slower decline in the *Cyperus papyrus* only system.

As illustrated in Figure 1, a substantial performance gap was observed between the MOL-augmented system and the *Cyperus papyrus*-only system. The MOL-enhanced system exhibited a rapid and substantial decline in Pb concentration, achieving complete removal (<0.0002 mg/l) by day 4. In contrast, the *Cyperus papyrus* -only system showed a more gradual decline, with Pb concentration decreasing from 3.52 mg/l to 1.76 mg/l on day 3 and to 1.32 mg/l by day 6 [16]. These results indicated that bioaugmentation with banana corm–based MOL significantly enhanced microbial activity and improved the overall performance of the constructed wetland system.

The difference in removal time further highlighted the contrasting efficiencies of the two systems. The MOL-augmented system achieved the regulatory threshold (<1 mg/l) within 2 days and maintained this level throughout the 10-day period. In comparison, the C. papyrus-only system reported by Wisnawa required 9 days to reach the same threshold [16]. This finding suggested that plant-only SSF-CW systems were less efficient and highly dependent on longer hydraulic retention times, whereas the MOL-augmented system accelerated the phytotreatment process through enhanced microbial degradation and metal uptake mechanisms [31].

The accelerated Pb removal was attributed to the synergistic interaction between *Cyperus papyrus* and the microbial consortia present in the banana corm–based MOL. Initially, the MOL introduced metal-tolerant microorganisms such as Bacillus sp., Aeromonas sp., and Aspergillus niger, which acted as the first line of defense [20, 32, 33]. These microorganisms utilized heavy metals as terminal electron acceptors and detoxified them through enzymatic and non-enzymatic pathways [34]. They also exhibited strong biosorption capabilities, including extracellular adsorption onto cell walls, intracellular accumulation, and immobilization of Pb ions [33]. These processes reduced the bioavailable Pb concentration and minimized phytotoxic stress on *Cyperus papyrus* [35–37]. This initial microbial activity allowed the plants to remain healthy and functional. In turn, plant roots released exudates that stimulated microbial growth, creating an active rhizosphere. These exudates, consisting of sugars, organic acids, amino acids, and other metabolites, enhanced microbial activity and pollutant degradation [38, 39].

Furthermore, the MOL-enriched microbial community produced siderophores, organic acids, and biosurfactants that mobilized bound Pb, increasing its bioavailability for plant uptake [40, 41]. Consequently, the extensive root system of *Cyperus papyrus* efficiently absorbed the mobilized Pb while also supporting biofilm formation and phytostabilization [40, 42]. Overall, this synergistic interaction created a dynamic and self-reinforcing bioremediation system, resulting in significantly faster and more efficient Pb removal.

3.2. System pH and plant morphology.

The pH value represented the balance between hydrogen ions (H^+) and hydroxide ions (OH^-) in the system. Higher H^+ concentrations indicated acidic conditions, whereas higher OH^- concentrations indicated alkaline conditions [43]. Aquatic plants typically grew within a pH range of 7–8.5 and could not survive under highly acidic conditions (pH < 4) [26]. In this study, pH was measured every 2 days, and the results are presented in Table 2. As illustrated in Figure 2, the pH of the wastewater fluctuated between 4.9 and 6.9 during the treatment period. In the phytotreatment reactor (R1), the pH gradually increased toward neutral conditions, whereas in the control reactor (R2), the pH decreased to approximately 6.2. This trend suggested that *Cyperus papyrus* played a significant role in stabilizing the system pH. In planted systems, *Cyperus papyrus* modified water chemistry through photosynthetic CO_2 uptake, which reduced carbonic acid concentration and increased pH toward neutral conditions [44]. Plant morphological analysis was conducted to evaluate the effects of wastewater exposure on plant growth [45].

Table 2. pH variation during the phytotreatment process.

Hydraulic Retention Time (Days)	pH Value
2	6.6
4	6.6
6	6.6
8	6.6
10	6.9

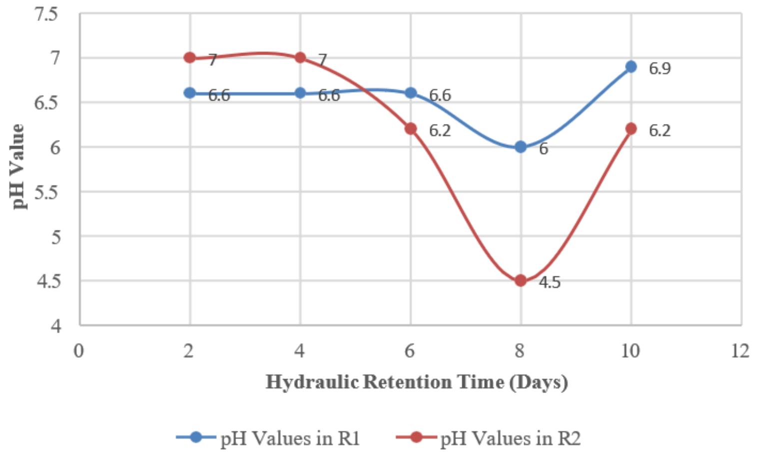

Figure 2. Comparative graph showing the comparison of pH value between R1 and R2.

Plant height was measured from day 0 to day 10, and the results are shown in Figure 3. Plant growth in the control reactor (R3) was slightly higher than in the phytotreatment reactor (R1). The average height increase was 0.12 cm in the control and 0.07 cm in the phytotreatment system. Despite this slight difference, the results indicated that wastewater exposure did not significantly inhibit plant growth. *Cyperus papyrus* demonstrated strong tolerance to Pb contamination, likely through mechanisms such as metal uptake, immobilization, and internal detoxification. This observation was consistent with previous studies showing that *Cyperus papyrus* could accumulate heavy metals such as Pb, Cd, and Ni without significant growth inhibition over extended periods [16, 46].

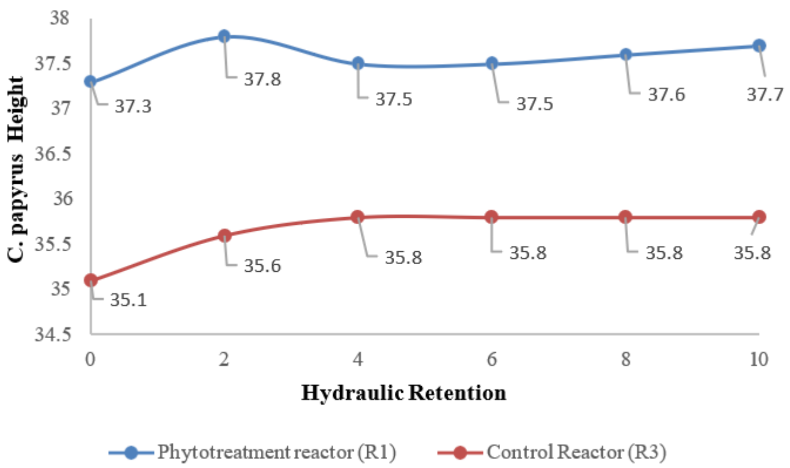

Figure 3. Comparative graph showing the *Cyperus papyrus* morphology between R1 and R3.

4. Conclusions

his study demonstrated that integrating banana corm–based MOL with *Cyperus papyrus* in a SSF-CW system created a strong synergistic effect that significantly improved Pb removal from laboratory wastewater. The combined system successfully achieved regulatory compliance (<1 mg/l) within 2 days and near-total removal (>99.99%) within 4 days, substantially outperforming the 9 days required by the plant-only system. This accelerated performance was attributed to a multifaceted synergy in which the microbial consortia in MOL rapidly immobilized and transformed Pb, thereby reducing phytotoxicity and enhancing the plant's phytoaccumulation efficiency. Overall, the bioaugmented SSF-CW system proved to be an efficient, cost-effective, and sustainable approach for treating heavy metal–contaminated wastewater. It offers a practical strategy to overcome the limitation of long hydraulic retention times typically associated with conventional constructed wetlands.

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Author Contributions

Putu Primantari Vikana Suari conceptualized the study, secured funding, designed the methodology, and supervised the experimental work. Nelson Darma Effendi conducted the laboratory experiments, collected data, and assisted in data analysis. I Gede Andy Andhika Parahita contributed to data analysis, data validation, and manuscript editing.

Competing Interests

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the work reported in this paper.

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