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THE EFFECTIVENESS OF BATCH CULTURE PHYTOREMEDIATION SYSTEMS USING HYDROMACROPHYTES TO INCREASE WATER QUALITY ON LEAD-POLLUTED IRRIGATION WATER

MIMATUN NASIHAH, TRI ARDYATI, DIAN SISWANTO AND CATUR RETNANINGDYAH*

Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, 65145 Malang, East Java, Indonesia.

**Corresponding author: catur@ub.ac.id*
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Abstract: Lead (Pb) pollution harms aquatic ecosystems, especially when Pb pollutes irrigation water. Hence, it needs to be addressed, one of which is by phytoremediation. This research aims to evaluate the success of the phytoremediation of Pb-contaminated irrigation water using local hydromacrophytes in a polyculture through a batch culture system. The results of the research showed that local hydromacrophytes in polyculture were able to improve the quality of irrigation water as indicated by a decrease in Total Suspended Solids (TSS), conductivity, Biochemical Oxygen Demand (BOD), and nitrate levels on the 7th day and Total Dissolve Solid (TDS), orthophosphate, Total Organic Matter (TOM), and Pb on the 14th day. Meanwhile, Chemical Oxygen Demand (COD) decreased on the 22nd day while Dissolved Oxygen (DO) increased since the 7th day of incubation. The results of the one-way ANOVA on bacterial abundance showed a significant increase on day 28 after incubation, which notes that bacteria play an active role in the phytoremediation process while the environment supports bacterial activity. The results of Next-Generation Sequencing (NGS) analysis after 28 days of incubation showed that irrigation water was dominated by more than 50% of the *Actinobacteria* genus. This research provides new knowledge about the role of hydromacrophytes and bacteria in the phytoremediation process of Pb-polluted irrigation water.

Keywords: Hydromacrophytes, bacteria, bioremediation, heavy metals, irrigation water.

Introduction

Increased industrial activity leads to the release of heavy metal waste into irrigation water sources, negatively impacting physical, chemical, and biological qualities. One of the heavy metals that are toxic to the environment is Lead (Pb), which reduces the concentration of micronutrients in plants (Yildirim *et al*., 2019). Other than that, Pb also causes respiratory, digestive, cardiovascular, and neurological disorders in humans and animals (Boskabady *et al*., 2018), especially in children and has long-term effects on adults (Le *et al*., 2019). Therefore, efforts must be made to overcome this problem through environmentally friendly and sustainable methods.

One method to reduce Pb concentrations and improve irrigation water quality is phytoremediation processes (Sabreena *et al*., 2022). Phytoremediation is an environmentally friendly and cost-effective technique that utilises plants to clean polluted soil and water (Bamagoos *et al*., 2022). Moreover, several studies have shown the effectiveness of phytoremediation in reducing heavy metal concentrations in water (Pang *et al*., 2023) phytoremediation has gained increasing attention from worldwide researchers and scientists due to its cost-effectiveness and environmental friendliness. Hence, this review first discussed soil and water remediations. Phytoremediation can be divided into five techniques to remove heavy metals from the polluted environment, namely, phytostabilisation (phytosequestration) such as using local hydromacrophytes with batch culture systems (Retnaningdyah, 2017). The use of phytoremediation can reduce heavy metal concentrations that negatively impact human health and aquatic ecosystems (Aghili & Golzary, 2023), improve irrigation water quality, prevent eutrophication, are more accessible, and cost-effective than other remediation techniques (Retnaningdyah, 2017).

Phytoremediation involves bacterial species in complex interactions between plants and bacteria. Hydrophytes release organic compounds such as organic acids, carbohydrates, and phenolic compounds through their roots, which serve as substrates for bacteria (Oriebe *et al*., 2023). Additionally, bacteria contribute nutrients such as nitrogen and phosphorus, which are essential for plant growth (Kumari, 2017). The interaction between hydromacrophytes and bacterial communities is interesting. Therefore, bacterial abundance and diversity must also be observed.

Our study specifically addresses the issue of irrigation water contaminated with heavy metal Pb, a significant environmental concern in agricultural areas (Orosun *et al*., 2023). Nonetheless, there is a scarcity of phytoremediation studies aimed at addressing this problem. Previous research often neglects the role of bacteria in facilitating the phytoremediation process and the comprehensive effects of phytoremediation on changes in the bacterial community structure. Therefore, this research provides new insights that could lead to developing more effective and sustainable phytoremediation strategies, emphasising the role of bacteria in mitigating heavy metal pollution in irrigation water.

This study aimed to determine the response to changes in the physicochemical quality of water and bacterial abundance in the phytoremediation process using local hydromacrophyte polyculture with a batch culture system against irrigation water contaminated with Pb.

Materials and Methods

Research Design

The experimental research used a complete randomised design (Anderson & McLean, 2018), which was conducted in the greenhouse of the Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang. The independent variables in this study were polyculture hydro macrophyta as phytoremediation agents (*Scirpus grossus*, *Typha latifolia*, *Vetiveria zizainoides*, *Equisetum*, *Ipomoea Aquatica*, and *Hydrilla verticilata*) and the presence or absence of additional Pb in irrigation water media used for research. The dependent variables in this study were physicochemical parameters and bacterial abundance.

The irrigation water without hydromacrophytes was used as a control. Each treatment was conducted five times in the morning, around 09:00 am to 10:00 am. Pb-Ethylenediaminetetraacetic Acid (EDTA), a complex of Pb ions (II), and EDTA in a 1:1 ratio was used. This complex was prepared by dissolving 2.684 g of EDTA in distilled water to make 1 L of solution. Subsequently, the EDTA solution was mixed with 3 mg of Pb (II) acetate dissolved in 1 L of deionised water (aquades). The mixture was stirred slowly until homogeneous, producing the Pb-EDTA complex (Shah, 2022). The detailed research conditions were:

(1) Irrigation water as control (Ir).

(2) Irrigation water with polyculture of hydro acrophytes (Ir + P).

(3) Irrigation water with the addition of Pb without polyculture of hydro acrophytes (Ir + Pb).

(4) Irrigation water with the addition of Pb and polyculture of hydro acrophytes (Ir + Pb + P).

Hydromacrophytes were planted in buckets containing a substrate of 5 cm of sand and 10 cm of gravel, followed by adding 30 L of groundwater for the acclimatisation process before treatment. This process aims to ensure that plants can survive and grow optimally in a new environment different from their place of origin (Nasution *et al*., 2020). Acclimatisation continues until plant growth stabilises, typically around 20 to 25 days (Retnaningdyah & Arisoesilaningsih, 2019).

Consequently, hydromacrophytes were grown polyculturally at each treatment, covering about 25% of the surface area. Acclimatisation of hydromacrophytes is carried out until growth is stable. Correspondingly, groundwater is replaced with treatment water of as much as 30 L. The Pb-contaminated water samples used for treatment were obtained from irrigation canals around the Wastewater Treatment Plant (WWTP) of the metal plating plant in Plosowahyu Lamongan village, East Java (Figure 1).

Water Sampling

Each water sample from 0, 7, 14, 21, and 28 incubation times was collected and placed into a 500 ml High-Density Polyethylene (HDPE) bottle. Subsequently, the bottles were placed in a cool box to maintain temperature stability during transportation. Subsequently, the samples were analysed for physicochemical parameters. For the analysis of bacterial abundance before and after treatment, water samples of each treatment were taken from the composite of each repeat for Total Plate Count (TPC) testing. Bacterial DNA extraction of water samples was performed at the Microbiology Laboratory of FMIPA Universitas Brawijaya.

Monitoring the Effectiveness of the Success of the Phytoremediation Process with Local Hydromacrophytes

Physicochemical properties of water samples were evaluated to determine the effectiveness of the phytoremediation process. The physicochemical parameters of water monitored were Total Dissolve Solid (TDS), Total Suspended Solids (TSS), conductivity, pH, temperature, DO, Biochemical Oxygen Demand (BOD), nitrate, orthophosphate, Total Organic Matter (TOM), Chemical Oxygen Demand (COD), and Pb. Each parameter is presented in Table 1.

Additionally, the abundance of bacteria and its diversity were examined as responses to improved water quality. The TPC method calculates the bacterial abundance of irrigation water in various treatments by spreading samples over agar media in Petri dishes. The agar media used is Nutrient Agar (NA), which incubates the dish for a certain period so that bacteria can grow. Consequently, the number of colonies is counted. The results are expressed as Colony-Forming Units per millilitre (CFU/ml) or per gram (CFU/g) original sample (Kasprijo & Manarni, 2020).

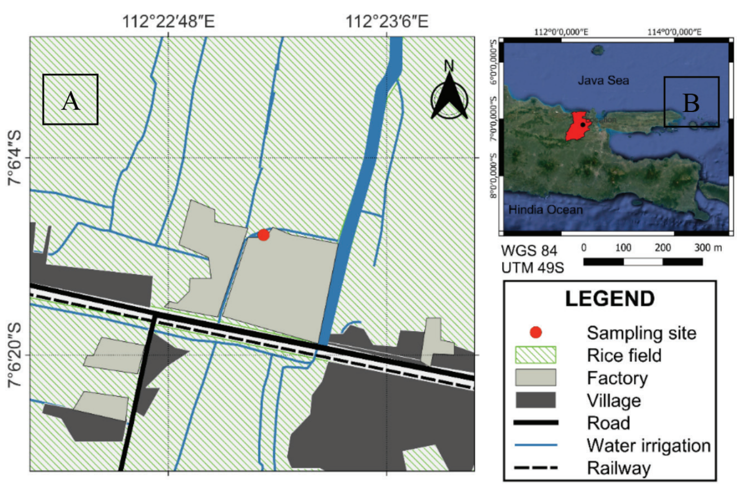


Figure 1: Map of irrigation water sampling locations around the metal plating plant in Plosowahyu Lamongan village (A). Image of sampling points around the metal plating plant WWTP (B). Map of Plosowahyu Village, Lamongan District, Lamongan Regency

Table 1: Irrigation water quality parameters and measurement methods

No.	Parameter	Measurable Quality	Measurement Method/Equipment
1	Physics	TSS (mg/L)	TSS metre
		TDS (mg/L)	TDS metre
		Conductivity (mS/cm)	Conductivity metre
		Temperature (°C)	Digital thermometer
2	Chemistry	pH	pH metre
		DO (mg/L)	DO metre
		BOD (mg/L)	Potentiometric
		COD (mg/L)	Redox titration
		Nitrate (mg/L)	Brucine methods
		Orthophosphate (mg/L)	Spectrophotometry
		Total organic matter (mg/L)	Titrimetric
		Lead (mg/L)	Atomic absorption spectrophotometry
3	Biology	Microbial abundance	Total Plate Count (TPC)
		Microbial diversity	Next-Generation Sequencing (NGS)

Using NGS methods, bacterial diversity was known using 16S rDNA analysis. The NGS test was conducted at Genetics Science Indonesia Jakarta. The stages of NGS include (1) sampling; (2) DNA extraction; (3) amplification of 16S rDNA gene using Polymerase Chain Reaction (PCR); (4) NGS sequencing uses the NGS platform to generate millions of short DNA sequences; (5) bioinformatic analysis to identify Operational Taxonomic Units (OTUs) and determine the diversity and composition of bacterial communities; (6) Alpha (in-sample) and beta (inter-sample) diversity analyses are calculated to assess the richness and evenness of bacterial communities within and between samples; and (7) the identity of genera or species present in the sample is determined based on sequenced data (Sánchez-Sanhueza *et al*., 2018).

Data Analysis

Data from measuring physicochemical parameters and Pb in each treatment were compiled using MS Excel and then, the average value was calculated. Note that improvement in irrigation water quality could be known by comparing the average value of water quality obtained with class III Water Quality Standards based on PP No. 22, 2021, concerning Water Quality Management and Water Pollution Control Annex IV.

The difference in water quality parameters of each treatment was known by conducting a one-way Analysis of Variance (ANOVA). Followed by the Tukey Honestly Significant Difference (HSD) test (if the variant was homogeneous) or Games Howell (if the variant was not homogeneous) at $\alpha < 0.05$ using SPSS for Windows release 16.0. Correspondingly, habitat similarity between treatments was determined using the Bray-Curtis similarity index. The correlation between the treatment and water's physicochemical and biological properties was conducted by biplot analysis using PAST 16.0 software.

Results and Discussion

Changes in Physicochemical Parameters of Irrigation Water During the Phytoremediation Process

Water's physicochemical properties become one indicator of irrigation water quality.

Phytoremediation using several types of hydromacrophytes, including *S. grossus*, *T. latifolia*, *V. zizainoides*, *Equisetum* sp., *I. aquatica*, and *H. verticilata*, which are grown using a batch culture polyculture system can improve the quality of irrigation water contaminated with Pb which was added at the beginning of the treatment in this study. The results showed changes in the physicochemical properties of water, especially in the parameters TDS, TSS, conductivity, pH, temperature, DO, BOD, COD, nitrate, orthophosphate, TOM, and Pb (Figures 2 and 3).

The pH value of this study meets water quality standards according to Government Regulation No. 22 of 2021 Annex IV concerning Water Quality Management and Water Pollution Control class III (7-9). The pH value in all treatments fluctuated during the 28th day incubation period. There was an increase in pH value on day 7 while on day 28, there was a decrease to 7.37-7.68 (Figure 2). This is reinforced by studies conducted by Chen *et al*. (2017), showing no difference in the pH of irrigation water in several treatments with different plant types. During photosynthesis, plants absorb CO_2 and release O_2, which can increase the pH of water. Conversely, plants release CO_2 during respiration, which can lower water pH (Wildan *et al*., 2022).

A decrease in pH value followed by a decrease in conductivity indicates a reduction in the number of inorganic ions in irrigation water. Conductivity refers to the salt content of irrigation water. The higher the conductivity value, the more the negative impact on plant growth will be. Note that the conductivity value of irrigation water is relatively low at 0.73-0.77 dS/m on the 28th day of the incubation period (Figure 2) and has complied with Food and Agriculture Organisation (FAO) standards of 0 to 3 dS/m. The decrease in conductivity in this study indicates the success of the phytoremediation process. Furthermore, Retnaningdyah *et al*. (2023) explained that the conductivity value is less than 0.7 dS/m, meaning there is no salinity problem in irrigation water. Hence, it can be used for agricultural activities.

The decrease in conductivity levels is also caused by biological processes by microbes absorbing organic and inorganic matter in waters (Sarda & Sadgir, 2015). The conductivity of water generally decreases with decreasing temperature. Cold water tends to have lower conductivity (Li *et al*., 2020). The water temperature in all treatments in this study was relatively the same because the temperature inside the greenhouse was relatively stable. The average water temperature is 25.61°C. The same relative temperature is due to the same sampling time, around 09:00 am to 10:00 am. Except for day 7, sampling was conducted at noon, so the water temperature was increased (Figure 2). Water temperature reveals changes in various water quality factors, which are the basis for controlling and improving water quality (Yang *et al*., 2018).

TDS indicates water's high and low dissolved material. This study showed a significant decrease in TDS levels from 426-435.8 mg/L to 366.8-381.6 mg/L on day 28 (Figure 2). This decrease in TDS levels is in line with research Sa'adah *et al*. (2023), which states that phytoremediation significantly reduces the organic matter content of water, which is reflected in a decrease in TDS levels. TSS are organic, inorganic, bacterial, and algal solids suspended in water. TSS levels decreased from 3.35-8.83 mg/L to 2.64-2.71 mg/L on day 28 of the incubation period (Figure 2). The TSS value in this phytoremediation process has met class III quality standards with a maximum level of about 100 mg/L. High TSS levels can reduce water quality (Leigh *et al*., 2019).

Dissolved Oxygen (DO) is a water quality parameter that shows water's ecological status and productivity (Sarda & Sadgir, 2015). The phytoremediation process of irrigation water has increased the DO value from 2.93-3.08 mg/L to 3.40-3.76 mg/L on day 28 incubation (Figure 2). The DO value in this study is at the quality standard of 3 mg/L. However, the average DO value in this study is still relatively low. A low DO value indicates that the oxygen produced in photosynthesis is used mainly for the respiration of organisms (Retnaningdyah *et al*., 2023).

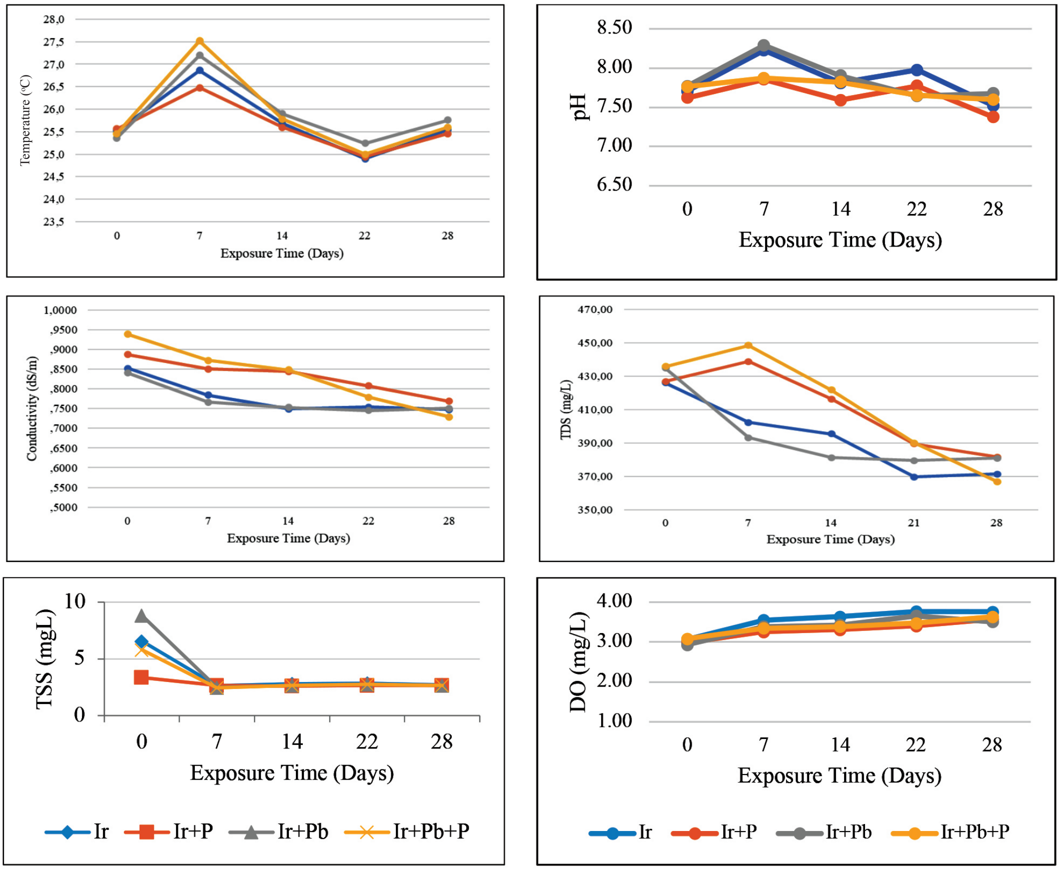


Figure 2: Results of monitoring the physicochemical quality of irrigation water during the phytoremediation model treatment process with a batch culture system of 0 to 28 days (Treatment Ir = Irrigation, Ir + P = Irrigation + Polyculture, Ir + Pb = Irrigation + Pb, Ir + Pb + P = Irrigation + Pb + Polyculture)

BOD is a parameter used to measure the amount of oxygen needed by microorganisms to decompose organic matter contained in water (Deswati *et al*., 2022). The data indicated that BOD levels on day 0 of the incubation period amounted to 2.04-2.79 mg/L and decreased to 0.74-1.28 mg/L on the 28^{th} day of the incubation period (Table 2), according to water quality standards < 3 mg/L. In treatment without hydromacrophytes, there was also a decrease because improvements in water quality could be made by bacteria through bioremediation (Oruganti *et al*., 2022). However, there is an increase in BOD on the 14^{th} day of the incubation period (Table 2). The rise in BOD decreases because old and dead hydromacrophytes are left in the water so that they rot and then increase the levels of water organic matter (Tanjung *et al*., 2019).

On the other hand, COD is a chemical parameter that measures the total amount of oxygen chemical compounds required to oxidise organic matter in water. COD concentration at the beginning of treatment ranged from 9.25-13.85 mg/L, increased on day 7 to 14.51-16.44 mg/L, and decreased to 4.92-8.27 mg/L on day 28 of the incubation period (Table 2), below the quality standard of 40 mg/L. COD concentration in irrigation + polyculture and irrigation + Pb + polyculture is higher because these two treatments are more prosperous in organic and inorganic matter derived from plant litter. The old ones then die and rot, also derived from dead microorganisms (Deswati *et al*., 2022).

Table 2: Results of monitoring the concentration of organic matter, nutrients, and Pb during the phytoremediation model treatment process with a batch culture system of 0 to 28 days

Treatment*	Day to	Water Quality Parameters				
		BOD (mg/L)	COD (mg/L)	Nitrate (mg/L)	Orthophosphate (mg/L)	TOM (mg/L)
Ir	0	2.048±0.606a**	9.252±12.668a	1.606±0.178a	0.226±0.140a	4.740±0.403a
Ir + P		2.696±0.335b	13.852±18.973b	1.443±0.218a	0.475±0.201b	5.000±1.242a
Ir + Pb		2.048±1.095a	11.112±15.222ab	1.835±0.201a	0.176±0.368a	5.880±0.649b
Ir + Pb + P		2.088±1.386a	10.864±14.876a	1.738±0.076a	0.232±0.071a	5.120±0.454a
Ir	7	1.160±0.941a	14.956±20.479a	0.188±0.035a	0.108±0.023a	4.680±0.319a
Ir + P		2.072±0.639b	16.440±22.511b	0.176±0.026a	1.277±1.168b	5.220±0.746a
Ir + Pb		1.320±0.403a	14.512±19.874a	0.256±0.114b	0.108±0.085a	5.840±0.487b
Ir + Pb + P		1.544±1.413a	16.064±22.015b	0.209±0.025b	0.485±0.212a	5.100±0.640a
Ir	14	1.568±0.665a	15.296±20.945a	0.085±0.085a	0.076±0.048a	4.500±0.187a
Ir + P		1.792±0.751a	15.958±21.851a	0.081±0.089a	0.924±0.603b	4.840±0.151ab
Ir + Pb		1.040±0.146b	14.670±20.089b	0.093±0.050a	0.046±0.021a	5.060±0.716b
Ir + Pb + P		1.296±1.122a	15.836±21.688a	0.066±0.009a	0.376±0.273ab	4.920±0.558b
Ir	21	1.520±0.585a	8.096±11.089a	0.044±0.028a	0.045±0.036a	4.180±0.109a
Ir + P		1.424±0.900a	6.240±8.545b	0.025±0.010a	0.646±0.298b	4.280±0.294a
Ir + Pb		0.952±0.590b	9.318±12.760a	0.154±0.059b	0.046±0.005a	4.880±0.661b
Ir + Pb + P		1.120±0.948a	7.726±10.587ab	0.094±0.078b	0.347±0.266b	4.800±0.565b
Ir	28	1.280±0.942a	6.870±9.409a	0.036±0.020a	0.060±0.042a	4.080±0.083a
Ir + P		1.152±0.945a	4.924±6.742b	0.023±0.009a	0.485±0.442b	4.140±0.219a
Ir + Pb		1.264±0.647a	8.268±11.323a	0.137±0.038b	0.017±0.002a	4.720±0.258b
Ir + Pb + P		0.744±0.880b	5.868±8.036ab	0.044±0.065a	0.320±0.264b	4.360±0.329ab
Quality standards		**6**	**40**	**0.008**	**0.015**	**10**

Information: *Treatment Ir = Irrigation, Ir + P = Irrigation + Polyculture, Ir + Pb = Irrigation + Pb, Ir + Pb + P = Irrigation + Pb + Polyculture.

**The same letter notation for the same parameters shows no significant difference between treatments based on the ANOVA test, followed by the HSD Tukey α = 0.05.

High levels of nitrates and orthophosphates in water can change the structure of biological communities, cause ecological disturbances and cause eutrophication. The use of hydromacrophyte plants in this study was able to significantly reduce nitrate levels from 1.44-1.83 mg/L to 0.02-0.13 mg/L and orthophosphate from 0.17-0.47 mg/L to 0.02-0.48 mg/L on the 28th day of the incubation period (Table 2). Consequently, plant removal of nitrates in water is performed through the absorption process by microorganisms attached to the roots (Kalengo *et al*., 2021). Orthophosphate levels on day 7 increased in irrigation + polyculture and irrigation + Pb + polyculture treatments due to the large amount of dead leaf litter that increased organic matter content in the water (Leigh *et al*., 2019). Plants used in accumulating pollutants in tissues can be released back into the environment when plants die or are harvested (Imron *et al*., 2019). TOM describes the entire dissolved and deposited organic matter in a body of water (Mutea *et al*., 2021). TOM values in this study ranged from 4.74 mg/L to 5.88 mg/L and decreased to 4.08 mg/L to 4.72 mg/L on the 28th day of incubation. Note that TOM levels are already under water quality standards by PP No. 20 of 2021 class III, which is 10 mg/L. Thus, high levels of TOM can indicate the presence of toxic substances in the water, posing a risk to the health of aquatic ecosystems (Carbajal-Palacios *et al*., 2014).

Pb levels on day 0 of the incubation period differed for each treatment. Pb levels in the irrigation and irrigation + polyculture treatments came from the source of irrigation water. The Pb levels were quite low (0.923 mg/L and 0.923 mg/L) because the samples were taken during the rainy season (December 2022). Meanwhile, in the irrigation + Pb and irrigation + Pb + polyculture treatments, we carried out Pb enrichment so that Pb levels were higher (1.970 mg/L and 2.114 mg/L).

Hydromacrophytes can filter pollutants from water very well. The data in Table 3 prove this and the irrigation + polyculture and irrigation + Pb + polyculture treatments significantly reduced Pb levels with a reduction percentage of 94.22% and 97.63%. Hydromacrophytes can absorb Pb ions, reducing Pb concentration in water. Furthermore, hydromacrophytes can absorb and store Pb ions in their tissues (Sa'adah *et al*., 2023). They can also break down organic compounds associated with Pb contamination such as organic matter (Annisa *et al*., 2021). Additionally, hydromacrophytes interact with bacteria in water, enhancing Pb removal through microbial processes and reducing overall Pb concentrations in wastewater. In the irrigation and Irrigation + Pb treatments, there was a decrease in Pb levels even without hydromacrophytes. This reduction is attributed to bioremediation processes facilitated by bacteria present in irrigation water (Ali *et al*., 2023). Bacteria can absorb Pb ions onto their cell surfaces and accumulate them within their cells, contributing to the overall decrease in Pb concentration in the water (Kapahi & Sachdeva, 2019). Furthermore, bacteria can interact with other microorganisms in water, further enhancing Pb removal through microbial processes (Henao & Ghneim-Herrera, 2021).

The Effect of Phytoremediation on Bacterial Diversity

Abundance of Bacteria on Day 0 and Day 28 of Incubation Time

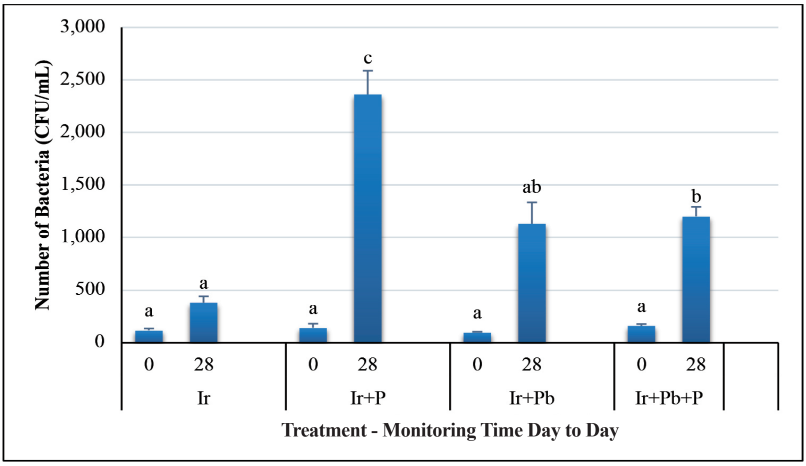
The success of phytoremediation using local hydromacrophytes through batch culture systems in various treatments is supported by bacteria associated with hydromacrophyte plant roots. It helps the process of absorption of pollutants by plant roots. The abundance of bacteria in irrigation water in the phytoremediation process with Pb enrichment can be observed in Figure 3.

Table 3: Percentage reduction in lead content

Treatment	Pb Rate (mg/L) Day to Day		Percentage Decrease (%)
	0	28	
Ir*	0.923	0.137	85.20a**
Ir + P	0.923	0.053	94.22b
I + Pb	1.970	0.108	94.52b
Ir + Pb + P	2.114	0.050	97.63b

*Treatment Ir = Irrigation, Ir + P = Irrigation + Polyculture, Ir + Pb = Irrigation + Pb, Ir + Pb + P = Irrigation + Pb + Polyculture.

**The different letter notation shows the significant difference between treatments based on the ANOVA test, followed by Tukey HSD $\alpha = 0.05$.



Description: The same notation above the bar shows no significant difference based on the Brown Forsythe test, followed by the Howell Games test α 0.05.

Figure 3: The abundance of bacteria associated with hydromacrophyte plants in some phytoremediation treatment models with 0 to 28 days through batch culture system (Ir = Irrigation, Ir + P = Irrigation + Polyculture, Ir + Pb = Iriration + Pb, Ir + Pb + P = Irrigation + Pb + Polyculture)

The abundance of bacteria can be seen in the irrigation treatment on day 0 of 118.66 CFU/ml and day 28 of 380 CFU/ml, in irrigation + polyculture treatment on day 0 of 142.66 CFU/ml and day 28 of 2,363.33 CFU/ml, irrigation + Pb treatment day 0 of 97.5 CFU/ml and day 28 of 1,133.33 CFU/ml. Meanwhile, irrigation + Pb + polyculture treatment on day 0 was as much as 159.33 CFU/ml and on day 28 as much as 1,203.33 CFU/ml. There were significant differences in bacterial abundance on day 0 and day 28 of incubation and in each treatment (Figure 3). The abundance of bacteria increased on day 28. In irrigation treatment, there is an increase in bacterial abundance, although not significant. This is due to the content of organic fertiliser in irrigation water, which can provide favourable conditions for bacterial growth (Han *et al*., 2023).

The abundance of bacteria in irrigation + Pb treatment increased significantly due to the presence of organic matter in water and environmental factors that support bacterial growth. Other than that, certain bacteria can absorb and convert heavy metals for their proliferation in water (Akhtar *et al*., 2021). In contrast to irrigation + polyculture and irrigation + Pb + polyculture treatments, there is a significant increase in bacterial abundance in the two treatments due to plant roots releasing various compounds that can be a food source for bacteria. This causes an increase in bacterial populations (Supreeth, 2022), providing substrates (Paredes-Páliz *et al*., 2016), and creating environmental conditions that support its growth to improve the phytoremediation process (Houida *et al*., 2023). However, irrigation + Pb + polyculture treatment is slightly lower than irrigation + polyculture because plants that accumulate heavy metals can create unfavourable conditions for some bacteria, as heavy metals can be toxic and inhibit bacterial growth (Hong *et al*., 2015).

Results of Next Generation Sequencing (NGS) of Bacteria in Irrigation Water with Pb Enrichment through Phytoremediation Treatment with Local Hydromacrophytes Batch Culture System on the 28th day of the Incubation Period

To determine the difference in bacterial diversity of irrigation water with Pb enrichment in treatment with hydromacrophytes and without hydromacrophytes, we conducted NGS tests to understand the genetic information in the irrigation water. Based on the results of taxonomic annotations, the top 10 taxa of each selected sample can be seen in Figure 4.

Bacterial populations at the phylum level reveal similar spread. From the obtained 100 species of bacteria, 10 dominant bacterial groups were identified, namely *Actinobacteria*, *Spirochaetota*, *Proteobacteria*, *Bacteroidota*, *Cyanobacteria*, *Bdellovibrionota*, *Firmicutes*, *unidentified Bacteria*, *Crenarchaeota*, and *Verrucomicrobiota* (total relative abundance > 95%). *Actinobacteria* have the highest abundance in the two treatments, especially the Ir + Pb + Poly treatment abundance, which is above 50%. *Actinobacteria* abundance is very high due to its role in helping plants in phytoremediation processes, absorbing and converting heavy metals into complex compounds that are not toxic to plants. It also encourages plant growth in contaminated environments, producing bioactive compounds to dissolve heavy metals to be more easily absorbed by plants (Henao & Ghneim-Herrera, 2021).

The abundance of *Spirochaetota* is relatively high in Ir + Pb treatment, plays a role in irrigation water bioremediation, helps bind Pb to cell surfaces, and converts the chemical form of Pb from inorganic to organic form. *Proteobacteria* ranked third in both treatments. Two groups of bacteria only exist in Ir + Pb + Poly treatment: *Cyanobacteria* and *Firmicutes*. In the Ir + Pb treatment, two types of bacteria were also found that were not present in the Ir + Pb + Poly treatment, namely *Spirochaetota* and *Verrucomicrobiota* (Figure 4). The types and functions of bacteria based on the previous references are summarised in Table 4.

The species found in the Ir + Pb + P sample are more numerous than Ir + Pb (Table 5), reinforced by the data in the Venn diagram of Figure 5, demonstrating that the presence of hydromacrophytes is a habitat for the growth and development of beneficial bacteria. Besides that, the presence of bacteria also helps plants remediate irrigation water polluted with Pb and heavy metal waste. The presence of plants in the water can create microhabitats that can affect bacterial populations (Sondang *et al*., 2021).

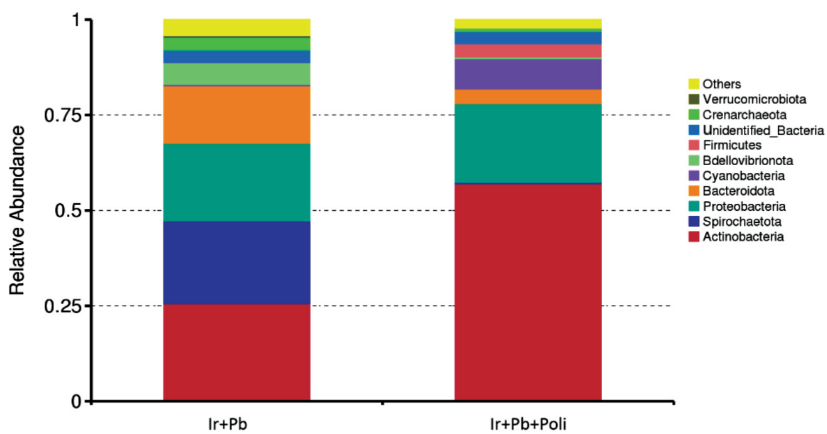


Figure 4: Relative abundance of bacteria in irrigation water with Pb enrichment in phytoremediation process in several treatments with batch culture system on day 28 after incubation period (Ir + Pb = irrigation + Pb, Ir + Pb + Poli = irrigation + Pb + polyculture)

Table 4: Group of bacteria that play a role in helping the *Phytoremediation* process of irrigation water polluted with heavy metal Pb

No.	Name of Bacteria	Function	Literature
1	*Actinobacteria* (> Ir + Pb + P)	• Degradation of organic compounds such as plant residues and leaf litter. • Reducing heavy metals. • Produces phosphatase enzymes, helping dissolve phosphates.	(Lacombe-Harvey *et al.*, 2018) (Li & Gong, 2021) (Balakrishnan *et al.*, 2020)
2	*Spirochaetota* (Ir + Pb)	• Decomposes organic matter and helps cycle nutrients. • The higher the heavy metal, the more *Spirochaetota*.	(Chislock *et al.*, 2021) (Xu *et al.*, 2020)
3	*Proteobacteria* (Ir + Pb = Ir + Pb + P)	• Decomposing organic matter through the breakdown of complex compounds into simple ones. • Plays a role in the nitrogen cycle by nitrifying or denitrifying.	(Ley *et al*., 2023) (Gu *et al*., 2022)
4	*Bacterioidota* (> Ir + Pb)	• Degrades complex organic compounds such as carbohydrates, proteins, and lipids. • Overcoming heavy metal exposure through the production of excretory compounds that can bind to or reduce metal toxicity.	(Ma *et al*., 2023) (Febria *et al*., 2023)
5	*Cyanobacteria* (> Ir + Pb + P)	• Photosynthesis can produce energy from sunlight. • Plays a role in the nutrient cycle by fixing nitrogen in the atmosphere.	(Zimina *et al*., 2022) (Kumar *et al*., 2015)
6	*Bdellovibrio* (> Ir + Pb)	• Predatory against other gram-negative bacteria. • Potential in the bioremediation process to reduce heavy metal pollution.	(Cavallo *et al*., 2021) (Palaniswami, 2018)
7	*Firmicutes* (Ir + Pb + P)	• Able to live well in the presence or absence of oxygen, also able to live in extreme environmental conditions.	(Lye *et al*., 2022)
8	*Crenarchaeota* (> Ir + Pb)	• It lives in an environment rich in heavy metals and has a role in the nitrogen and carbon cycles in water.	(Wittenborn *et al*., 2023)
9	*Verrucomicrobia* (Ir + Pb)	• Able to live in extreme conditions such as high heavy metals organic polymer decomposers, can survive a wide range. • It can bind, precipitate, and change the chemical form of heavy metals and make them easier to remove from the environment.	(Nixon *et al*., 2019) (Yu *et al*., 2019)

In the Ir + Pb + P treatment, 1,437 species of bacteria were found. In comparison, in Ir + Pb treatment, 1,108 species of bacteria. With 545 species found in Ir + Pb and Ir + Pb + P therapy (Table 5 and Figure 5), some of the same types of bacteria such as *Proteobacteria* and *Bacteroidota* were observed in both treatments. It is known that these two types of bacteria have an essential role in breaking down toxic compounds (Viggor *et al*., 2020)and overcoming heavy metal exposure (Febria *et al*., 2023). Hence, in both Ir + Pb treatment and Ir + Pb + Ptreatment, this type of bacteria was discovered.

Bacterial diversity can be seen from the calculation of the Shannon and Simpson index, which shows Ir + Pb + P samples are lower than Ir + Pb (Table 5). This is because, in Ir + Pb + P samples, there is one dominant species. *Actinobacteria*, so the diversity is more melancholy than Ir + Pb (Figure 4). It is known that *Actinobacteria* have a vital role in helping hydromacrophytes carry out the phytoremediation process. Thus, their presence is dominant in this treatment, as revealed by Lacombe-Harvey *et al*. (2018), who stated that *Actinobacteria* are gram-positive bacteria that play a role in the degradation of organic compounds such as plant residues and leaf litter.

The index of bacterial richness and diversity can be seen in the ChaoI and ACE values, which show that the value of Ir + Pb + P treatment is higher than Ir + Pb. The high value of Chao1 and ACE in Ir + Pb + P treatment shows increased bacterial diversity and species richness. The study's results Sodhi *et al*. (2021) explained that the presence of hydromacrophyte plants causes higher bacterial diversity, reflected in increased Chao1 and ACE values. This suggests that irrigation water with hydromacrophyte crops supports more diverse and rich bacterial communities, having a variety of ecological functions, including pollutant degradation and water quality maintenance.

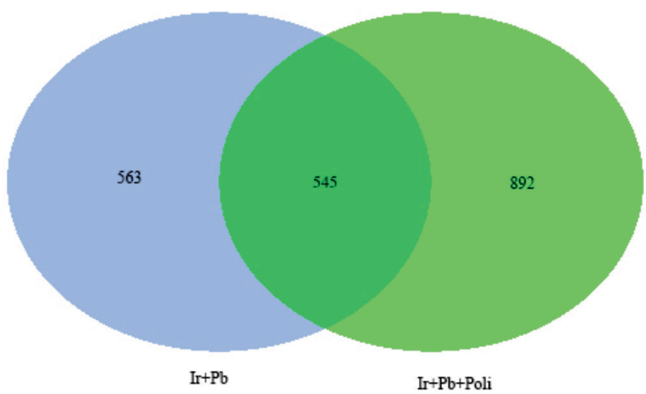


Figure 5: Venn diagram of the number of bacterial species found in Ir + Pb samples (irrigation + Pb) and Ir + Pb + P samples (irrigation + Pb + polyculture)

Table 5: Alpha diversity indices

Sample	Observed Species	Shannon	Simpson	Chao1	ACE
Ir + Pb	1,108	5.310	0.915	1,109.204	1,113.476
Ir + Pb + P	1,437	4.855	0.824	1,484.150	1,485.150

(Ir + Pb = irrigation + Pb. Ir + Pb + P = irrigation + Pb + polyculture).

Relationship between Physicochemical Quality of Irrigation Water Phytoremediation Process with Pb Enrichment Using Local Hydromacrophyte Batch Culture System with Bacterial Abundance

Water quality after phytoremediation for 28 days was significantly better than day 0. This is indicated by the low quality of all physical quality parameters of water chemistry except DO, which shows higher values. The biplot analysis (PCA) results showed that day 0 and day 28 after the incubation period in each treatment showed a relationship between physicochemical quality and bacterial abundance (Figure 6). Four kinds of day 0 treatment demonstrated a high content of TOM, Pb, nitrate, TDS, TSS, and conductivity. This condition contributed to the low abundance of bacteria characterised by common TPC values. This is supported by research (Rahbari *et al*., 2021), which states that high concentrations of Pb can be toxic to aquatic bacteria and inhibit enzyme activity and biochemical processes from reducing bacterial abundance and activity. Note that high TDS levels also cause osmotic pressure that can harm bacteria. In contrast, high conductivity can indicate ion concentrations that can affect ion balance in bacterial cells (Linda *et al*., 2020). Nitrate levels trigger the growth of aquatic plants or algae that can compete with bacteria for nutrients and sunlight, potentially reducing the availability of nutrients for bacteria (Talapatra & Ghosh, 2023).

The abundance of bacteria increased on the 28th day of incubation. The highest abundance occurred in irrigation and polyculture treatment. This is followed by irrigation + Pb + polyculture treatment, as we know that the presence of hydromacrophytes can improve water quality while increasing bacterial abundance (Ortúzar *et al*., 2020). The abundance of bacteria in two treatments without hydromacrophyte plants is relatively low because the limited availability of nutritional sources can inhibit bacterial growth (Calfee *et al*., 2022). The presence of hydromacrophytes can create favourable environmental conditions for bacterial growth in irrigation water (Ahmed *et al*., 2023). Hydromacrophytes can provide additional substrates for bacteria. Without hydromacrophytes, a lack of substrate can inhibit bacterial growth (Álvarez *et al*., 2019).

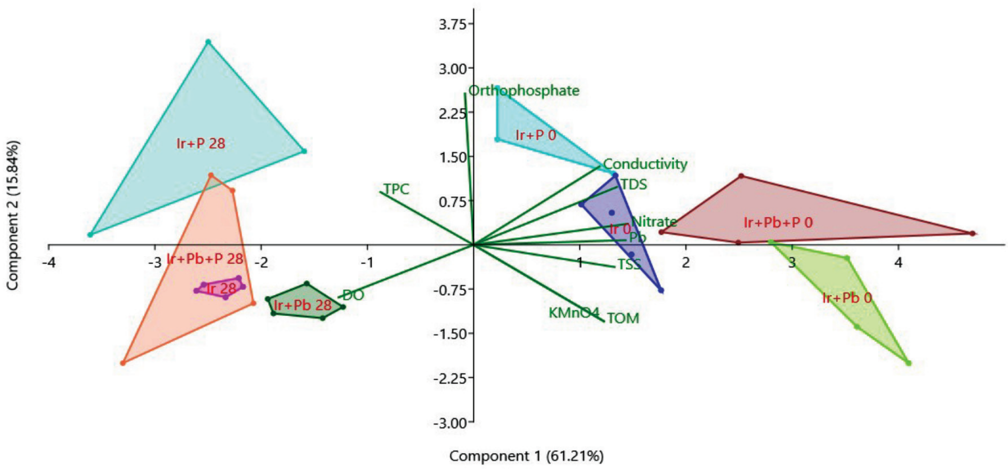


Figure 6: Principal Component Analysis (PCA) to evaluate the relationship between physicochemical parameters of the phytoremediation process of irrigation water with Pb enrichment using local hydromacrophytes polyculture batch culture system with bacterial abundance on (Ir 0 = irrigation-day 0, Ir + P 0 = irrigation + polyculture-day 0, Ir + Pb 0 = irrigation + Pb-day 0, Ir + Pb + P 0 = irrigation + Pb + polyculture-day 0 and Ir 28 = irrigation-day 28, Ir + P 28 = irrigation + polyculture-day 28, Ir + Pb 28 = irrigation + Pb-day 28 and Ir + Pb + P 28 = Irrigation + Pb + polyculture-day 28)

Irrigation + polyculture treatment is the most effective in improving water quality because hydromacrophytes can filter and absorb pollutants and create an environment that supports bacterial growth. The presence of bacteria helps plants in reducing pollutants (Bonaventure *et al*., 2023). Irrigation + Pb + polyculture treatment also effectively improves water quality, especially its ability to reduce Pb toxicity by absorbing these heavy metals. The presence of bacteria plays a role in the phytoremediation process. However, Pb enrichment affects bacterial waste because heavy metals can have a toxic effect on certain bacteria (Zhang *et al*., 2017).

There is an increase in water quality in irrigation treatment and irrigation + Pb, although not significant, which is caused by the natural deposition process (Chornyy *et al*., 2022), oxidation and reduction by bacteria so that heavy metals are more easily flocculated. Besides that, bacteria can also biotransform lead, turning it into a less toxic form (Ali *et al*., 2023). The recommendation for an effective treatment to improve irrigation water quality is to involve polyculture hydromacrophytes symbiotic with bacteria in improving water quality. Consequently, the results of the biplot analysis (PCA) are confirmed by data from the Pearson correlation coefficient analysis.

From the data in Table 6, it can be seen that the physicochemical quality parameters of irrigation water such as TDS, TSS, conductivity, nitrate, TOM, and Pb play an important role in determining the abundance of bacteria during the phytoremediation process. In general, these parameters showed a negative correlation with bacterial abundance except DO, which was positively correlated. There is a strong negative correlation between TDS, TSS, and conductivity with bacterial abundance. This demonstrates that the higher the TDS, TSS, and conductivity, the lower the bacterial abundance (Table 6).

This correlation is caused by plants' ability to absorb and accumulate pollutants, which, in turn, affects the diversity and activity of microbes in the system (Sekar & Ansari, 2018). There was a moderate positive correlation between DO and bacterial abundance ($R = 0.447$, $p = 0.048$). This indicates that higher DO levels are correlated with higher bacterial abundance (Table 6), indicating that the presence of DO is significant for the growth and survival of microorganisms in the system (El-Liethy *et al*., 2022).

Apart from that, there is also a strong negative correlation between nitrate, TOM, and Pb with bacterial abundance, which shows that the higher the levels of nitrate, TOM, and Pb, the lower the bacterial abundance, meaning that the higher levels of nitrate, TOM, and Pb can inhibit growth and survival life—microorganisms in the system (Xiao *et al*., 2023). There was no significant correlation between orthophosphate and bacterial abundance (Table 6), indicating that the presence of orthophosphate did not significantly impact the growth and survival of microorganisms in the system. Therefore, this data can be used to optimise the environmental conditions of irrigation waters in the phytoremediation process by controlling physicochemical factors that influence the abundance of bacteria needed in the phytoremediation process.

Table 6: Pearson correlation coefficient between TPC and several water quality parameters

	TDS	**TSS**	**Conductivity**	**DO**	**Nitrate**	**Orthophosphate**	**TOM**	**Pb**
Pearson correlation (R)	-0,638	-0,543	-0,508	0,447	-0,730	0,385	-0,469	-0,666
Significance	0,002	0,013	0,022	0,048	0,000	0,094	0,037	0,002

Conclusions

Phytoremediation of irrigation water using local hydromacrophytes through a batch culture system effectively improved the physicochemical quality of irrigation water and bacterial abundance. Local polyculture-grown hydromacrophytes effectively reduced TSS, TDS, conductivity, BOD, COD, TOM, and Pb concentrations, increasing DO and increasing bacterial abundance in irrigation water after day 28 of the incubation period. The research result indicated the potential of polyculture hydromacrophytes collaborating with bacteria as phytoremediation agents, effectively improving irrigation water quality. In addition, this study also showed the role of bacteria in the bioremediation process of irrigation water. The increase in bacterial abundance could result from hydromacrophytes's phytoremediation effects. Hence, the results of this study provide a further understanding of the role of local hydromacrophytes in irrigation water phytoremediation and its implications for the health of agricultural ecosystems.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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