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Synergism of Consortium of Heterotrophic Bacterial Strains with *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch as Biophytoremediator for Heavy Metal Removal in Domestic Wastewater

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ABSTRACT

High levels of organic matter and heavy metals in domestic wastewater can increase the pollution of water bodies. The water pollution results correspond to the degree of public health. Waste must be controlled and processed first using management methods such as biophytoremedyator to decrease the rate of pollution because its advantages do not negatively impact public health and the environment. Biophytoremediation offers a better alternative method to repair environmental conditions by combining microorganisms and plants. This study aimed to analyze the effect of heterotrophic bacterial strains with *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch for heavy metal removal in domestic wastewater. It also investigated the influence of heterotrophic bacterial strains with *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch which were also carried out to observe the BOD and COD levels. The results showed decreased heavy metal levels from the three treatment groups. The consortium treatment group of heterotrophs and *Salvinia molesta* D. Mitch bacteria strains reduced heavy metal levels faster than the other groups by 59%. In addition, it is also able to reduce BOD and COD levels. This study has shown the significant effect of combining heterotrophic bacterial (HB) strains with *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch for heavy metal removal in domestic wastewater.

1. Introduction

The availability of fresh water and nature's gifts control much of the global economy. An adequate water supply is necessary for agriculture, human consumption, industry, and recreation. Natural or added pollutants sometimes strip us of our gift and expose us to a more complex situation. It is well-known that fresh water is necessary for our health (Sharma and Bhattacharya 2017). Also, the problem of water security has been made worse by the instability in rainfall patterns, the fast-growing population, urbanization, and industry. Domestic wastewater is produced by daily tasks such as bathing, cleaning one's hands, home, and car, washing clothes and kitchenware, and defecating and micturition. Domestic wastewater can be divided into four subcategories: yellow (urine), brown (feces with flushed water), black (urine, feces, and bacterial activity), and greywater (containing water from the kitchen, laundry, shower, and handwashing) (Koul *et al.* 2022).

Wastewater treatment involves physical, chemical, and biological procedures to remove pollutants and hazardous materials before final release into a body of water without harming the environment or human health. There are several steps for wastewater treatment, i.e., initial pretreatment using physical process, chemicals intervention, biological approach through the metabolism of the microbial agent, and definitive treatment to improve the quality of wastewater (Rodríguez-Rodríguez *et al.* 2020). The biological steps currently became popular to eliminate organic and inorganic substances. Bioremediation is a convenient solution to the contamination of inorganic pollutants. Green plants can remove pollutants from the soil or water through absorption through the roots and subsequent accumulation in the leaves. Microorganisms can also

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1. Introduction

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Wastewater treatment involves physical, chemical, and biological procedures to remove pollutants and hazardous materials before final release into a body of water without harming the environment or human health. There are several steps for wastewater treatment, i.e., initial pretreatment using physical process, chemicals intervention, biological approach through the metabolism of the microbial agent, and definitive treatment to improve the quality of wastewater (Rodríguez-Rodríguez *et al.* 2020). The biological steps currently became popular to eliminate organic and inorganic substances. Bioremediation is a convenient solution to the contamination of inorganic pollutants. Green plants can remove pollutants from the soil or water through absorption through the roots and subsequent accumulation in the leaves. Microorganisms can also

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detoxify or remove inorganic pollutants from the environment (Wang *et al.* 2021). Bioremediation also provides long-term in-place remediation rather than simply relocating the problem. This method removes heavy metals, metalloids, and other inorganic pollutants from soil or water (Ashraf *et al.* 2019).

The primary mechanism of microbial remediation of polluted media is to immobilize and reduce pollutant bioavailability. Heavy metals, for example, cannot be degraded by microorganisms but can be converted to another form due to altered physical and chemical properties (Fester *et al.* 2014). Bioaccumulation and biosorption are essential processes in which microorganisms, or biomass, bind to and concentrate contaminants from their surroundings (Gupta and Diwan 2017; Ashraf *et al.* 2019). The bioremediation process can be improved by utilizing a microbial consortium with specific characteristics for contaminant degradation (Alami 2014; Waluyo 2020). The metabolic interactions of microbial consortia have a significant impact on their application. Synthetic microbial consortia are created artificially by co-culturing select (two or more) species in an (at least initially) well-defined medium. Unlike natural microbial consortia, synthetic microbial consortia can have their metabolic pathways reconfigured and their social interactions programmed to perform the desired function (Li *et al.* 2021).

Some microorganisms can degrade various environmental pollutants if they have enough nutrients to grow. However, they stop when they run out of nutrients. Plant root exudates are the best available food and nutrient source for bacteria in the soil. They are promoting their colonization, and combining the root colonization and pollutant degrading properties of microorganisms with the root exudation capacity of plants aids in removing toxic substances/pollutants from the environment. As previously discussed, many plants and microbes can tolerate high concentrations of heavy metal pollutants (Vangronsveld *et al.* 2009; Ali *et al.* 2020). However, using plants and microbes separately to remediate contaminated soil and water is ineffective and insufficient (Singh *et al.* 2016). In the previous study, when inoculated with *Pseudomonas putida*, plants like *Eruca sativa* improved nickel uptake by up to 46% from the soil. They lowered the level of nickel in the soil (Kamran *et al.* 2016).

The application of a combination of bacterial consortium strains and the aquatic plant is not explored massively yet. This work utilizes bacterial consortium strains and aquatic plants for biophytoremediation of domestic wastewater. Therefore, this study aims to determine the ability of the synergism of heterotrophic bacterial (HB) consortium strains with *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch in the removal of heavy metal pollutants in domestic wastewater.

2. Materials and Methods

2.1. Materials and Equipment

The consortium strain of heterotrophic bacteria was obtained from the Biomedical Laboratory Faculty of Medicine, University of Muhammadiyah Malang, Malang, Indonesia. The aquatic plants used in this study were *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch obtained from Malang, East Java, Indonesia. Other chemicals were obtained from Merck Chemicals. Equipment used in the study included pH meters, thermometers, dissolved oxygen meters, UV-Visible spectrophotometers, and AAS.

2.2. Sample Collection

Samples of domestic wastewater liquid were obtained from the exhaust system at a communal wastewater treatment plant facility from black water and grey water sewerage at 7°55'29.8 "S 112°36'02.2 "E on June 23, 2022, in Tlogomas Area, Malang, East Java, Indonesia. Water samples were randomly taken at a depth range of 0 to 30 cm. Water samples were tested without any additional treatment or preservation except for determining heavy metals using AAS with the addition of HNO_3 1 M reagent.

2.3. Research Procedure

2.3.1. Preparation of Heterotrophic Bacterial Consortium

The HB strain used in this study was gained from our previous study (Waluyo 2020) and had a 10^6 bacteria/ml density. This bacterial suspension blinding was standardized using the Mc. Farland 1 method, equivalent to a 10^8 bacteria/ml bacterial density. The stages of making heterotrophic bacterial suspensions are carried out by providing 4-10 ooze bacteria from nutrient agar plate media that have been incubated for 24 hours, inserted into tubes containing aquades, and then homogeneous. The suspension of the heterotroph bacteria consortium is equivalent in turbidity to the standard solution of Mc. Farland 1 to become a bacterial suspension with a density of 10^8 bacteria/ml, equivalent to the concentration of a consortium of heterotrophic bacteria 100%. A total of 20 sterile tubes were marked with labels of tubes A, B, C, D, and E, with each label consisting of 8 repeats. The concentration of heterotrophic bacterial consortium is made with variations of 0.5%, 1%, 2.5%, 5%, and 7.5%.

2.3.2. Acclimatization of *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch

This stage is done by preparing water samples of as much as 5 liters into each container measuring 35 × 20 × 15 cm containing *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch. *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch (see Figure 1) were acclimatized for seven days. After seven days, the selection of *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch was carried out. The types of plants used in the study were sorted to obtain similar plants in fresh green conditions. They had relatively the same size as *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch. Each plant is put in a prepared container.

2.3.3. Application for Biophytoremediation

Domestic wastewater samples were measured for their pH and temperature before and during the treatment (about 14 days). The wastewater samples were then simultaneously tested for heavy metal levels (Pb^{2+}, Hg^{2+}, Cd^{2+}, and Cu^{2+}), also Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) parameters. This study had three treatment groups and control (without any treatment). The first treatment group consists of A, B, C, D, and E treatments in domestic wastewater with serial variations of heterotrophic bacteria consortium concentrations of 0.5, 1.0, 2.5, 5.0, and 7.5%, respectively. Then the heavy metal content, BOD, and COD parameters were measured on the seventh day.

The second treatment group was carried out after test measurements of heavy metal levels and BOD and COD parameters in the first treatment group. Then, 5 liters of wastewater were put in each treatment in a container and transferred into a labeled container (20 experimental units with five treatments and four repeats until there were any). Furthermore, added with *Pistia stratiotes* L. as much as 5 grams in each treatment and repeated, then heavy metal levels and BOD and COD parameters were measured.

The third treatment group was carried out after test measurements of heavy metal levels and BOD and COD parameters in the first treatment group. Then 5 liters of wastewater is put in each treatment in a container and transferred into a labeled container (20 experimental units with five treatments and four repeats until there are any). Furthermore, 5 grams of *Salvinia molesta* D. Mitch were added to each treatment and repeated. The heavy metal levels, BOD, and COD parameters were measured.

2.4. Data Measurement

Water quality parameters in pH are observed using a pH meter and temperature using a thermometer. Determination of heavy metals (Pb^{2+}, Hg^{2+}, Cd^{2+}, and Cu^{2+}) was measured using AAS. A 5-day test measured BOD by determining the dissolved oxygen value using the membrane electrode method. Determining dissolved oxygen

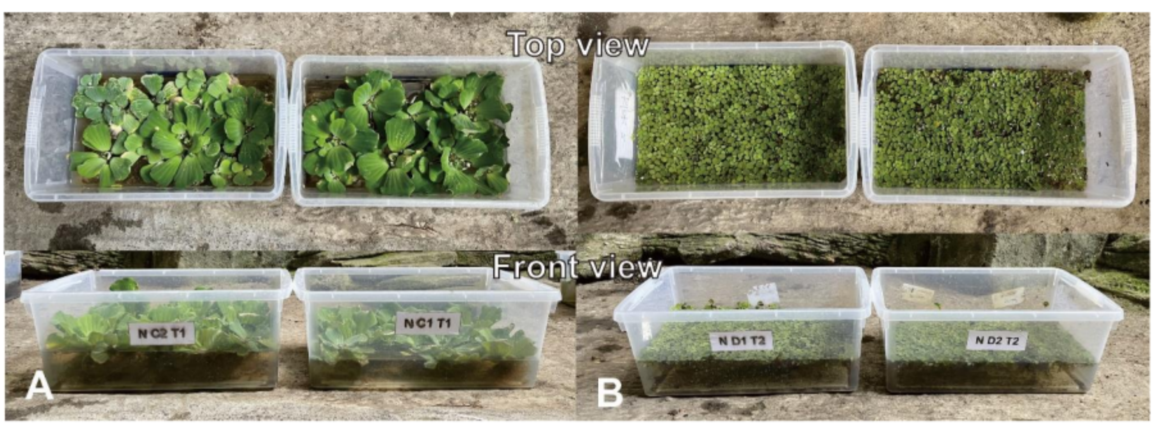

Figure 1. Acclimatization of (A) *Pistia stratiotes* L. and (B) *Salvinia molesta* D. Mitch

by COD was measured spectrophotometrically using the dichromate closed reflux method.

2.5. Data Analysis

Analysis of variance with a 5% confidence level was performed on the data using SPSS v25 software. The data were subjected to the Duncan Multiple Range Test (DMRT) with a confidence level of 5% due to the significant effects of treatments.

3. Results

3.1. BOD and COD

Adding *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch into the domestic wastewater caused a decrease in the BOD level, as shown in Figure 2. Based on Figure 2, it is known that there was a decrease in BOD level from the initial data when compared after the addition of *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch. The BOD content in the heterotroph bacterial strain consortium treatment group was highest at 7.5% at 60 mg/L and the lowest at 2.5% at 32 mg/L. The BOD content in the heterotroph bacteria strain group and *Pistia stratiotes* L. was highest at concentrations of 2.5% and 5.0% at 23 mg/L and the lowest at a concentration of 1% at 17 mg/L. BOD content in the heterotrophs and *Salvinia molesta* D. Mitch bacteria strain groups was highest at 2.5% and 7.5%, namely 20 mg/L, and the lowest at a concentration of 5% at 17 mg/L. Overall, BOD level reduction was increased optimally at 0.5% and 7.5% concentrations after additional *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch.

Chemical Oxygen Demand (COD) level is correlated to its BOD level to understand water quality. Figure 3 shows that the COD level was initially over the quality standard of 100 mg/L. Based on Figure 3, it is known that there was a decrease in COD values from the

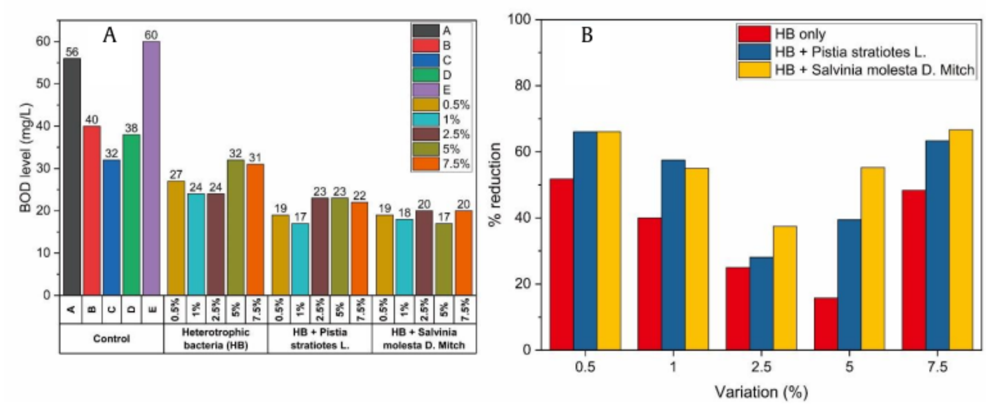

Figure 2. (A) BOD 5-day test result and (B) effect of each treatment on BOD level

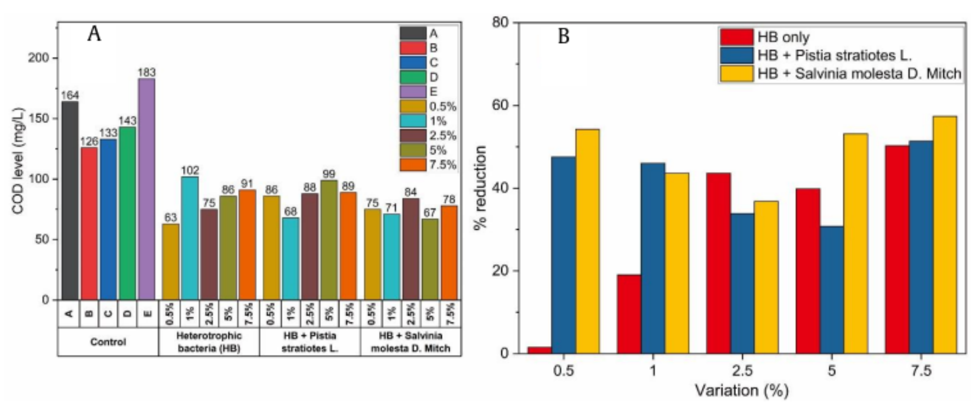

Figure 3. (A) COD test result and (B) effect of each treatment on the COD level

initial condition when compared after the addition of *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch. COD values in the heterotroph bacterial strain consortium treatment group were highest at a concentration of 1% at 102 mg/L and the lowest at a concentration of 0.5% at 63 mg/L. COD level in the heterotrophic bacterial strain group and *Pistia stratiotes* L. was highest at a concentration of 5% at 99 mg/L and the lowest at 1% at 68 mg/L. COD level in the heterotroph bacteria strain group and *Salvinia molesta* D. Mitch was highest at 2.5% at 84 mg/L and the lowest at a 5% concentration at 67 mg/L. Furthermore, it can be seen that a concentration of 0.5% is not enough to reduce the value of COD. The effective concentration to reduce COD is a concentration of 7.5%.

3.2. Heavy Metal Removal

The data in Figure 4 and Table 1 showed the heavy metal removal performances of observed bioremediation (heterotrophic bacteria only) and biophytoremediation (by adding *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch) compared to the control. Figures 4 (A) and (D) show the performance of Pb^{2+} removal. Compared to the control, adding *Pistia stratiotes* L. did not significantly affect the removal of Pb^{2+} statistically. On the contrary, the presence of *Salvinia molesta* D. Mitch has a significant effect (P>0.05) on the reduction of Pb^{2+} at 2.5% optimum concentration of HB. The removal of Cd^{2+} showed satisfactory results for the combination of heterotrophic bacteria at a concentration of 0.5% and *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch, but on the other variation of concentration did not have a significant effect, as shown in Figure 4 (B) and (E).

The combination for biophytoremediation has shown a more significant reduction of Cu^{2+} on *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch, as shown in (Figures 4C and F). Based on Table 1, the biophytoremediation performance on Hg^{2+} also showed satisfactory results on some concentrations (1.0, 5.0, and 7.5%) of heterotrophic bacteria. However, we could not obtain a satisfactory result on each treatment due to the limitation of the AAS instrument, and the exact result was probably under the measurement range. However, the result showed the optimizing ability of the synergism between heterotrophic bacteria and *Pistia stratiotes* L. or *Salvinia molesta* D. Mitch for Hg^{2+} removal in the wastewater.

4. Discussion

4.1. BOD and COD

There was a decrease in BOD levels for three treatments. Adding *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch statistically significantly decreased BOD levels. This process indicated that the source of organic matter from the wastewater itself, microorganism, and plants decreased and decomposed gradually (Sekarjannah *et al.* 2023). Furthermore, the drop was caused by the decomposition of organic materials in wastewater that the *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch absorbed. The addition of heterotrophic bacteria and the microorganism that live on the plant's root also break down the organic compound through aerobic metabolism as a substrate.

On the other hand, plants facilitate the bacteria as a growth medium and provide oxygen for metabolism in bacteria to decompose the organic matter (Ilmannafian *et al.* 2021). The natural process of domestic wastewater treatment using proposed biophytoremediation techniques is synergized for organic degradation by bacteria and plant roots. It has shown its ability to decrease the BOD level. As the COD level was more than the BOD level, it is known that the sample contained a high amount of organic compounds that were difficult to be biodegraded (Mahajan *et al.* 2023). All treatments showed decreased COD levels in domestic wastewater because heterotrophic bacteria and studied water plants could reduce the organic compounds. Microorganisms utilized the available oxygen in the sample to increase the degradation of organic compounds (Tanjung *et al.* 2019).

At the initial exposure to wastewater, plants experience stress conditions that generate exudate, increasing the biodegradable waste in the wastewater sample (Ilmannafian *et al.* 2021). The reduction in COD was obtained because *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch underwent the process of photosynthesis. *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch's photosynthesis activity increases water's dissolved oxygen, thus stimulating aerobic conditions in the domestic wastewater in favor of aerobic bacterial activity to reduce BOD and COD levels (Abdul Aziz *et al.* 2020).

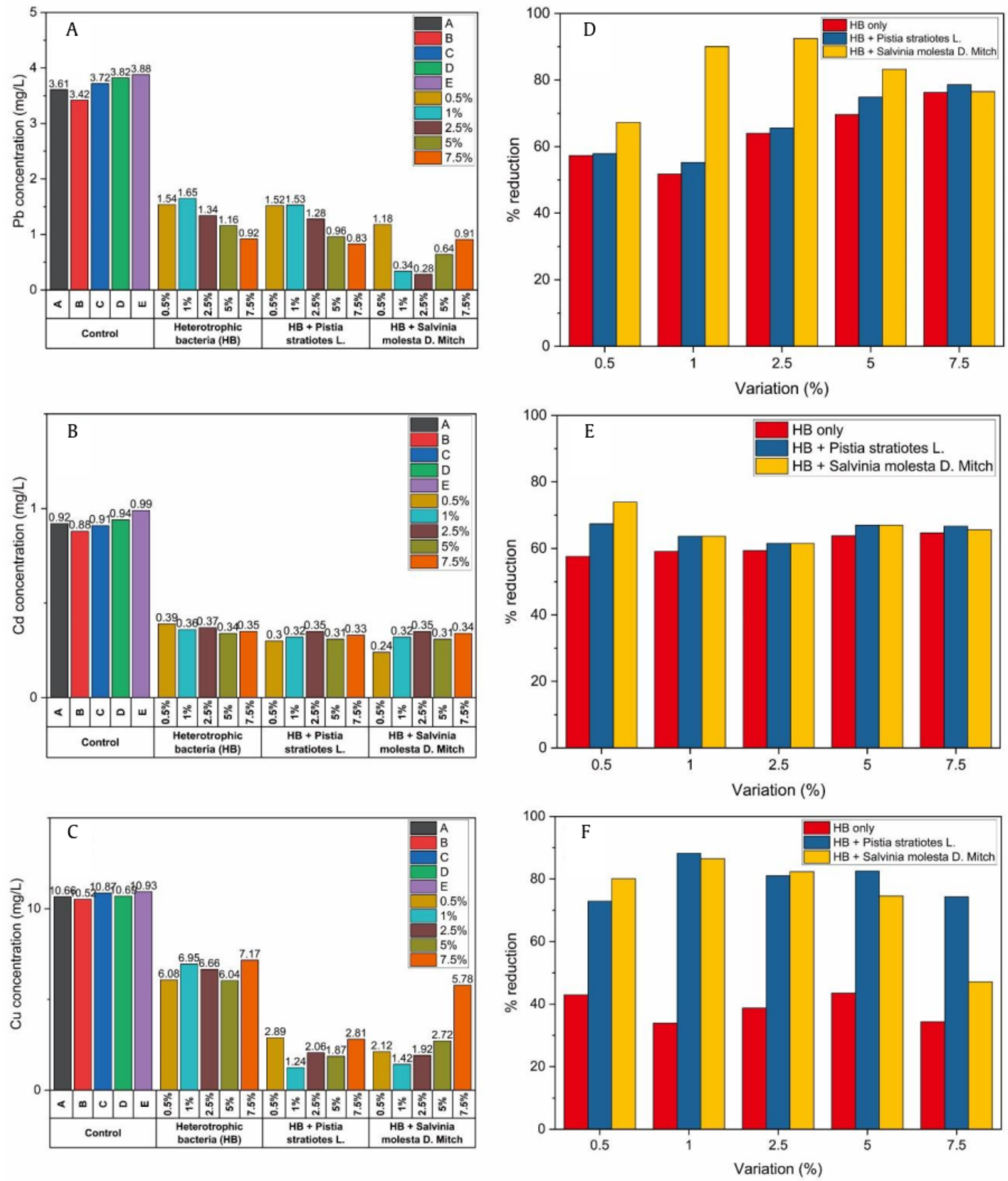

Figure 4. Heavy metal removal performances of each treatment

Table 1. Hg^{2+} removal results at various concentrations of heterotrophic bacteria and after the addition of *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch

Control (mg/L)	HB concentration (%)	Group of treatment		
		HB only (mg/L)	HB + *Pistia stratiotes* L.	HB + *Salvinia molesta* D. Mitch
2.63	0.5	ND*		
2.22	1.0	2.00		
2.47	2.5	ND*	ND*	
2.19	5.0	1.37		
2.41	7.5	1.87		

Initial: before the addition of *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch, ND: not detected, *Due to the limitation of instrumental sensitivity (probably under LoD)

4.2. Heavy Metal Removal

The result showed that the wastewater was positively containing heavy before any treatment. The first group of treatments that only utilized heterotrophic bacteria removed Pb^{2+}, Hg^{2+}, Cd^{2+}, and Cu^{2+} up to 63.80%, 23.25%, 60.90%, and 38.71%, respectively. Based on Figure 4, it can be seen that *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch could enhance heavy metal uptake rather than using heterotrophic bacteria only. Combining heterotrophic bacteria and *Pistia stratiotes* L. could enhance the uptake of Pb^{2+}, Hg^{2+}, Cd^{2+}, and Cu^{2+} up to 66.45%, 100%, 65.25%, and 79.79%, respectively. The ability of *Pista stratiotes* L. to enhance bioremediation is also confirmed in previous studies. The *Pista stratiotes* L. could reduce Cu^{2+} in domestic wastewater by 54–92% in 14 days and 70% of Cd^{2+} (Kumar *et al.* 2018; Novita *et al.* 2019; Schwantes *et al.* 2019). A satisfactory result is also obtained from combining heterotrophic bacteria and *Salvinia molesta* D. Mitch. This combination of heterotrophic bacteria and *Salvinia molesta* D. Mitch enhanced the uptake of Pb^{2+}, Hg^{2+}, Cd^{2+}, and Cu^{2+} up to 81.93%, 100%, 66.35%, and 74.12%, respectively. In the previous studies, *Salvinia molesta* D. Mitch could remove 64% of Pb^{2+} (Ranjitha *et al.* 2016) and 69% of Cu^{2+} (Abeywardhana *et al.* 2018). Both combinations performed better performance than only utilizing heterotrophic bacteria. Combining the aquatic plants and pollutant-degrading microorganisms could help to enhance the environmental quality from toxic substances or pollutants.

Free-floating plants play the role of bio-accumulators because of their ability to accumulate high concentrations of heavy metal in their biomass (Bonanno *et al.* 2017). Heavy metals are actively transported in free-floating aquatic plants like *Pistia stratiotes* L. and *Salvinia molesta* D. Mitch that occurs from the roots, from where metals are transferred to other parts of the plant body. Passive transport is correlated with the direct contact between the pollution medium and the plant body. In passive transport, heavy metal accumulation is mainly in the upper parts of the plant body (Lanka and Murari 2022).

The metal uptake by *Pistia stratiotes* and *Salvinia molesta* could be influenced by several factors, such as pH, temperature, and the ionic condition of the aqueous systems. The accumulation of heavy metals is mainly deposited in various parts of the plants (Uddin *et al.* 2021). Heavy metals that are present in contaminated water form positively charged ions. Those ions then bind on the plant cell wall due to the negatively charged binding sites. Heavy metals are then promoted into the cell by the charge gradient across the membrane after the binding process. After entering the *Pistia stratiotes* and *Salvinia molesta* roots, heavy metal ions could be stored in the root or moved to the shoot mostly through the xylem and may include the phloem. The complex molecule formation with several ligands like organic acids, phytochelatins, or metallothioneins mainly facilitates the transportation of heavy metals.

Conflict of Interest

The authors declare no conflict of interest.

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