The Influence of *Azotobacter* on the Growth and Mercury Content of Water Spinach Grown in Mine Tailings

Reginawanti Hindersah,[a,*] Erin April Felicia Silitonga,[b] Gabrieta Christy Adventia Tangke,[b] Betty Natalie Fitriatin,[a] Rija Sudirja,[a] Pujawati Suryatmana,[a] Aliya Zahrah Adawiah,[c] and Sri Handayani[d]

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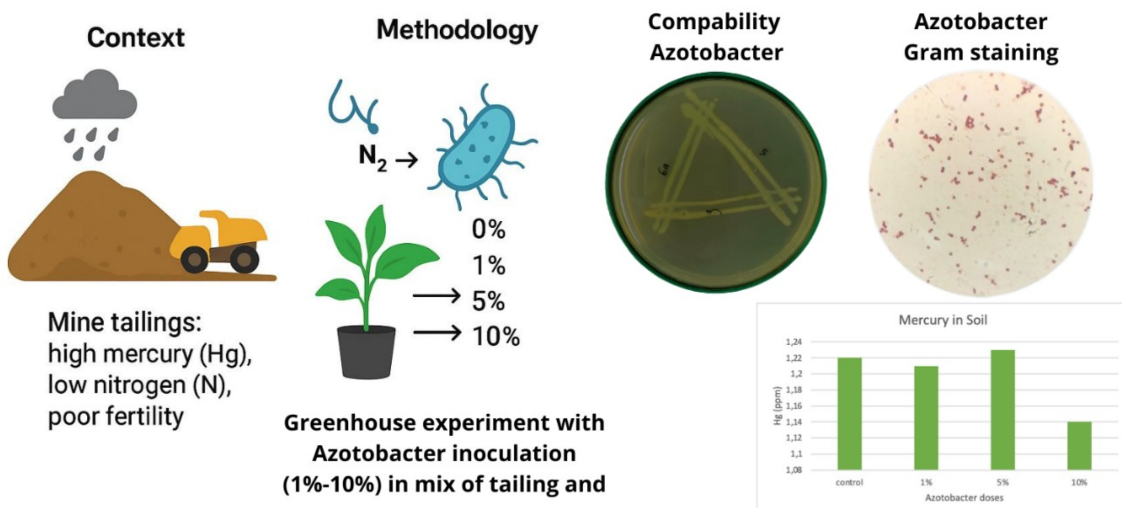
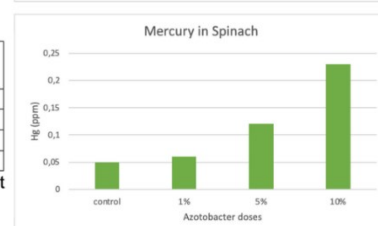
GRAPHICAL ABSTRACT



Azotobacter Treatment	Plant height (cm)	Leaf Number	Stem Diameter (cm)	Root Length (cm)
Control	8.66 ± 1.04 a	4.17 ± 0.81 a	1.71 ± 0.07 a	5.13 ± 0.57 a
1 %	8.85 ± 0.75 a	4.11 ± 0.66 a	1.77 ± 0.03 ab	4.81 ± 0.55 a
5 %	10.29 ± 0.99 b	5.28 ± 0.35 b	1.84 ± 0.04 b	5.39 ± 0.53 a
10 %	10.75 ± 1.16 b	5.96 ± 0.61 b	1.95 ± 0.10 c	5.76 ± 0.69 a

Values ± standard deviation followed by the same letter indicate that the treatments were not significantly different based on the DMRT at $p < 0.05$.



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Growing food crops in gold mine tailings is limited by low nitrogen and mercury contamination. Little is known about the responses of water spinach (*Ipomoea aquatica* L.) to nitrogen-fixing bacteria biofertilizer. This study aimed to analyze changes in growth media properties, growth, biomass of water spinach, and mercury in both tailings-based growth media and intact plants following the application of the nitrogen-fixing *Azotobacter*. A liquid inoculum of *Azotobacter* was analyzed before the experiment. A greenhouse experiment was arranged in a randomized block design to evaluate three inoculant concentrations. Acidity and electrical conductivity of the inoculant were 7.95 and 1.74 mS/cm, respectively, while the *Azotobacter* count was 9.18 on a log scale. Introducing 5% and 10% inoculants increased microbial counts, total nitrogen, and acidity of the growth media, as well as shoot growth and biomass, but did not affect root length. *Azotobacter* did not affect mercury levels in the soil but increased mercury accumulation in intact plants. Mercury levels in soil and plants remained higher than the maximum threshold value. While soil pH and nitrogen levels showed a positive correlation with plant growth, mercury concentration in the soil exhibited a significant negative correlation. Because of high mercury accumulation, the water spinach was not safe for cultivation.

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Keywords: Biofertilizer; Biomass; Mercury; Nitrogen-fixing bacteria

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INTRODUCTION

The operation of artisanal and small-scale gold mining (ASGM) in Indonesia depends on mercury (Hg) for extraction. A key problem with ASGM in Indonesia is the disposal of tailings in agricultural areas without proper management. Dumping tailings on farms decreases fertile land, forcing farmers to grow crops in degraded environments.

Tailings are unsuitable for crop growth due to their unfavorable acidity (pH), unstable physical structure, low nutrient and organic matter levels, and microbial content (Chung *et al*. 2019; Hindersah *et al*. 2023). A significant challenge in cultivating plants in ASGM tailings is the high level of Hg. Plant samples collected around the ASGM show high Hg content and are unsafe for consumption (Saragih 2021). The mobility and availability of Hg in soil are influenced by soil organic matter, clay content, and soil pH (Gai *et al.* 2016; Hussain *et al.* 2022a). Hg bioconcentration in plants grown in alkaline tailings is limited due to low metal immobilization (Hussain *et al.* 2022b).

Adding beneficial microbes along with organic matter supports plant growth and simultaneously reduces metal mobilization. *Azotobacter* are reported to synthesize exopolysaccharides (EPS) to protect the nitrogenase (Gauri *et al*. 2012). The presence of carboxyl, phosphoryl, and hydroxyl groups within the EPS structure provides many binding sites for divalent metal cations, including heavy metals (Kondakindi *et al.* 2024). The *Azotobacter* inhibits Cd uptake by plants, suggesting that the EPS is involved in metal ion complexation (Zhang *et al*. 2024).

Another advantage of *Azotobacter* is its ability to convert inert N_2 to NH_3, catalyzed by nitrogenase, which is sensitive to high nitrogen (N) levels (Halbleib and Ludden 2000). *Azotobacter* is reported to contribute approximately 15 to 20 kg of N per hectare through N fixation (Kour *et al*. 2020). Some *Azotobacter* strains produce phytohormones, such as Indole Acetic Acid (IAA) and Cytokinin (CK), during their life cycles and release them into their growth media (Suryatmana *et al*. 2024). The bacterial EPS enhances soil porosity and promotes plant growth (Gauri *et al.* 2012; Guo *et al*. 2018).

Rhizosphere bacteria have potential to support leafy vegetable growth (Razmjooei *et al*. 2022; Nayak *et al*. 2023). Leafy vegetables require sufficient N for photosynthesis and robust biomass growth (Novo *et al*. 2013) and phytohormones IAA and CK; the balance of these phytohormones influences the development of roots and shoots (Kurepa and Smalle 2022). Soil microbes play a vital role in providing N and phytohormones. Therefore, organic matter amendments provide nutrients and energy for microbial proliferation (Cercioglu 2017). They simultaneously decrease the availability of metals for plant uptake by complexation (Lwin *et al*. 2018). Exogenous *Azotobacter* inoculation may influence the microbial community in the soil, as reported by altering the count of beneficial microbes in the rhizosphere of tomatoes grown with *A. chroococcum* 76A and *Trichoderma harzianum* T22 (Cirillo *et al*. 2023).

A previous study demonstrated that water spinach can grow in manure-amendment tailings (Aprila *et al.* 2023). However, the use of NFB *Azotobacter* in growing food crops on Hg-contaminated tailings has received little attention. A pot experiment was conducted to observe the influence of various doses of *Azotobacter* liquid fertilizer on growth and plant biomass, as well as on total bacteria and fungi, pH, Hg, and total N in soil, and to investigate the correlation between specific soil parameters and plant growth traits.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the field of the Faculty of Agriculture, Universitas Padjadjaran, situated 765 m above sea level, from November 2023 to February 2024. The site is located in the tropics; during the experiment, monthly temperatures ranged from 23.8 to 29.9 °C, with a humidity level of 85% and rainfall of 10.5 mm.

The tailings were collected from artisanal and small-scale gold mining (ASGM) activities in Karanglayung Village, Karangjaya District, Tasikmalaya Regency, Indonesia. The acidity of the tailings was 8.08, with low levels of organic carbon, total N, and potential potassium (Table 1). In the previous experiment, the tailings were mixed with 15% organic matter and grown with water spinach using N-P-K fertilizer (16-16-16) at a level of 2.25 g/plant to enhance the organic matter and nutrient content levels. After harvesting the plant, the growth media became slightly acidic, with a pH of 6.53 and high organic carbon (3.82%); however, the total-N content remained low (Table 1). Diluting the tailings with organic matter reduced the Hg concentration from 330 to 75.1 mg/kg.

The *A. tropicalis* S5, *A. vinelandii* S6a, and *A. chroococcum* S9 were isolated from gold-mine tailings at Pongkor ASGM, Bogor; these tailings contained 300 mg/kg Hg due to the use of Hg in the amalgamation process. The three isolates displayed no antagonistic interactions based on the compatibility test conducted on Ashby's mannitol agar (Fig. 1). All isolates were found to be capable of fixing the N and synthesizing exopolysaccharides, organic acids, and phytohormones GA and Zeatin (Suryatmana *et al*. 2024).

Table 1. Soil Properties of Tailings Before and After being Mixed with Cow Manure (Grown with Water Spinach Previously)

Properties	Tailings		Mixture of Tailings and Manure	
	Value	Criteria	Value	Criteria
Water pH	8.08	Slightly alkaline	6.53	Slightly acid
Organic C (%)	1.57	Low	3.82	High
Total N (%)	0.16	Low	0.18	Low
C/N	9.81	Low	21.22	High
Potential P_2O_5 (mg/100 g)	42.49	High	134.75	Very high
Available P_2O_5 (mg/kg)	9.45	Average	44.41	Very high
Potential K_2O (mg/100 g)	12.28	Low	72.76	Very high
Exchangeable Cation and Anion (cmol/kg)				
K-dd	0.18	Low	1.27	Very high
Na	0.21	Low	0.28	Low
Mg-dd	6,82	High	7.65	high
Ca-dd	15.03	High	7.32	Average
Al-dd	0.25	-	0.24	-
H-dd	0.19	-	0.81	-
CEC (cmol/kg)[1]	3.51	Low	11.29	Low
Hg (mg/kg)	330	Very high	75.1	Very high
Solid fraction (%)				
Sand	2.48	Tailings Texture: Silty Clay	22.50	Growth media Texture: Clay loam
Silt	56.74		48.79	
Clay	40.78		28.70	

[1]CEC Cation exchange capacity

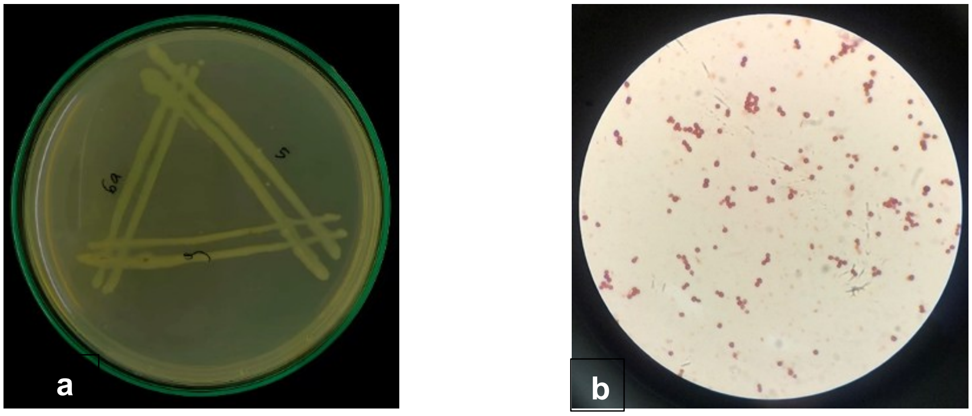

Fig. 1. a: No-inhibition cross sections of the three species of Azotobacter on N-free agar plate; and b: Cocci cell morphology of Gram-negative *A. tropicalis* S5

Mixed Liquid Inoculant Characterization

A mixed culture of *A. tropicalis* S5, *A. vinelandii* S6a, and *A. chroococcum* S9 with a volume ratio of 1:1:1 was prepared in 200 mL of N-free Ashby's mannitol broth (mannitol 20 g, dipotassium phosphate 0.2 g, magnesium sulfate 0.2 g, sodium chloride 0.2 g, potassium sulfate 0.1 g, calcium carbonate 5 g, final pH 7.4 ± 0.2), with an initial concentration of 2% pure culture of each strain. The culture was incubated in a 115-rpm gyratory shaker at room temperature for three days before conducting pH and Azotobacter population analyses, as well as pH and EC analyses. Bacterial counts were performed using the serial dilution plate method on Ashby plate agar (Hindersah *et al*. 2018).

Experimental Design

The experimental treatments, arranged in a Randomized Block Design, included 10 mL, 50 mL, and 100 mL biofertilizer, corresponding to 1%, 5%, and 10% of 1.0 kg growth media. The biofertilizer was diluted to 200 mL with unsterilized groundwater. The control treatment lacked biofertilizer but received 200 mL of groundwater. All treatments were replicated six times.

Growth Media Preparation and Inoculation

The previously used growth media, consisting of 85% tailings and 15% cow manure in a 1-kg polybag, was air-dried for one month and watered with 100 mL of groundwater. It was left for two days until the water evaporated from the surface of the growth media. *Azotobacter* was applied by mixing the liquid inoculant with the growth media one week before planting. Before application, the inoculant was diluted with unsterilized groundwater to 200 mL and incubated for seven days in the greenhouse. It was watered daily with 5 to 10 mL, depending on the weather, to maintain humidity and prevent excess water on the surface of growth media.

Seed Sowing and Fertilization

The water spinach var. Bangkok LP-1 seeds were sown in three planting holes, each 3 cm deep, with a 5 cm distance between the holes. Each hole was filled with five seeds and covered with a similar growth media. Next, the planting area was watered until moist, using 5 mL to 10 mL of water. The recommended dose of NPK compound fertilizer (16-16-16) at 450 kg/ha, equivalent to 4.5 g/plant, was placed in the hole at a distance of 2.5 cm from the stem of the water spinach in a split application at 14 and 21 HST.

Parameters and Statistical Analysis

At 30 days post-transplant, measurements were taken for the plant's height, stem diameter, number of roots, and both fresh and dry weights of the shoots. Concurrently, the total bacterial and fungal populations in the soil were quantified using the serial dilution plate method (Ben-David and Davidson 2014). Additionally, soil acidity was assessed through the potentiometric method, while total N content in the soil was measured using the Kjeldahl method (AOAC 2012).

The dry weight of intact plants was determined by heating the fresh biomass for 2 days at 70°C until a constant weight was achieved (Hunter *et al.* 2018). A viable bacterial and fungal count was conducted using a serial dilution plate on nutrient agar and potato dextrose agar, respectively (Ameh and Kawo 2017). All counts were performed in triplicate. Because of the limited plant sample, the total Hg in the shoots was determined from four samples of each treatment. The Hg levels in the soil and intact plants were determined using the ASTM D3223-17 method (ASTM 2017) with an Hg analyzer (NIC-MA 3000).

All data were analyzed using analysis of variance at $p < 0.05$. The Duncan's Multiple Range Test (DMRT) was conducted at $p < 0.05$ to check if the treatments significantly affected the specific parameters. Pearson Correlation at $p < 0.05$ was conducted between the independent variables of pH, total N, and total Hg in the growth media, and the dependent variable of plant growth. SPSS version 25 was used for all statistical analyses.

RESULTS AND DISCUSSION

Mixed *Azotobacter* Inoculant Properties

The incubation duration affected the pH, electrical conductivity (EC), and *Azotobacter* population in N-free Ashby's Mannitol broth (Fig. 2). After 72 h of incubation, the acidity of the liquid culture decreased slightly to 7.95, down from 8.4 at 24 hours. Meanwhile, the EC of the broth culture increased from 1.43 mS/cm to 1.74 mS/cm as the incubation period lengthened, in line with the rise in *Azotobacter* viable count from log 4.96 to log 9.18.

The broth's pH slightly decreased due to the excretion of organic acids by *Azotobacter*. After 72 h, the viability of *Azotobacter* increased, resulting in a higher concentration of organic acids in the broth. This study noted that the EC of the mixed *Azotobacter* culture broth rose over time. *Azotobacter* enzymatically converts N_2 into ammonia, which is then reduced to ammonium in the presence of an H^+ source in the liquid culture. A study on the water system found that the specific concentrations of ammonia and ammonium correspond to an increase in EC (Shcherbakov *et al*. 2009).

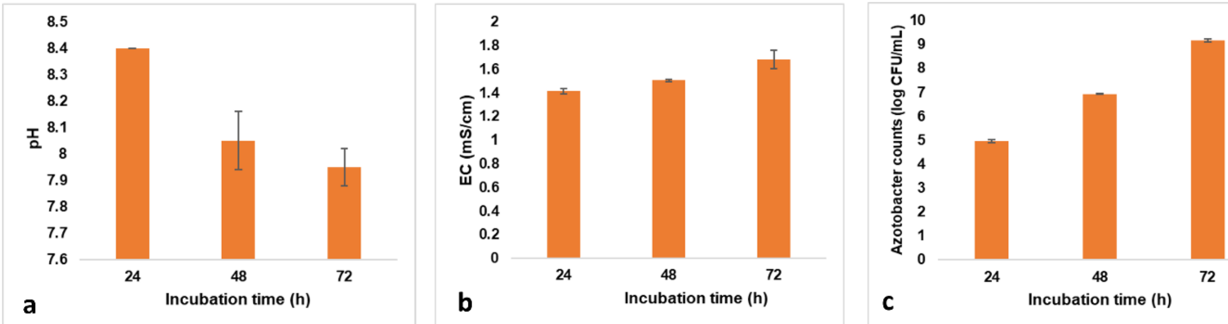

Fig. 2. a: The acidity; b: Electrical conductivity; and c: *Azotobacter* population in the 72-h *Azotobacter* inoculant before being used in the pot experiment

Bacterial and Fungal Viability in the Growth Media

The study demonstrated a slight increase in total bacteria and fungi in the rhizosphere of water spinach following the application of *Azotobacter* inoculant (Table 2). The 5% and 10% inoculants were likely more effective at enhancing bacterial viability than the 1% inoculant. The trend of both microbial increments is associated with the concentration of the *Azotobacter* inoculant added. The increase in bacterial and fungal count taken from the rhizosphere of the plant receiving 10% inoculant was 58.26% and 82.88% higher than the control.

Both microbes serve as indicators of soil health and further influence plant growth and health. Non-pathogenic bacteria play a crucial role in the macronutrient cycle in soil, particularly concerning N and P elements, while specific fungi aid in the solubilization of P and K.

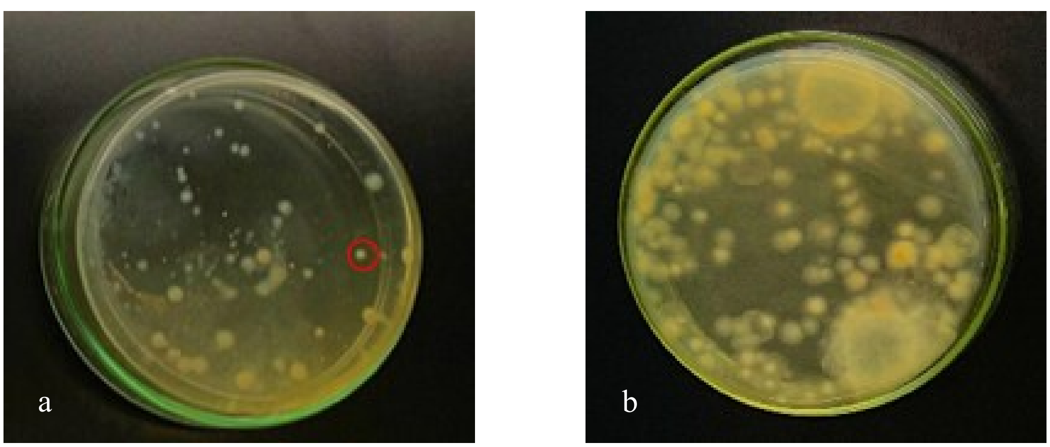

Fig. 3. Colonies of a. bacteria on nutrient agar showed low diversity of morphology; b. Fungi on potato dextrose agar exhibited various morphological properties and diameters

The method used for bacterial and fungal enumeration in this study cannot distinguish between pathogens and non-pathogenic soil microbes, as the count relied on the number of colonies appearing on the agar plate (Fig. 3). However, during the experiment, the plants were not affected by soil-borne diseases, indicating that the pathogenic count in the soil may be low.

Table 2. Effect of Azotobacter-based Biofertilizer Dose on Bacterial and Fungal Population in Planting Medium

***Azotobacter* Treatment**	**Total Bacteria (10^{10} CFU/mL)**	**Total Fungi (10^{5} CFU/mL)**
Control	1.15 ± 0.16 a	1.11 ± 0.22 a
1 %	1.47 ± 0.52 ab	1.50 ± 0.23 b
5 %	1.95 ± 0.40 c	1.73 ± 0.16 c
10 %	1.82 ± 0.24 bc	2.03 ± 0.11 d

Values ± standard deviation followed by the same letter indicate that the treatments were not significantly different based on the DMRT at $p < 0.05$.

Growth Media Properties

The pH and total N levels of the growth media changed following *Azotobacter* inoculation, but the Hg content remained unchanged (Table 3). Soil inoculation with 5% and 10% liquid inoculant resulted in a significant reduction in pH; meanwhile, all inoculant concentrations increased the total N in the soil by approximately 27.77%. Before the experiment, the pH and total N of the growth media were 6.53% and 0.18%, respectively (Table 1). The total Hg in the soil sharply decreased from 75.1 mg/kg before the experiment to less than 2 mg/kg. All *Azotobacter* inoculant concentrations did not influence the Hg in soil, even though the 10% inoculant had the potency to decrease soil Hg up to 6.55% compared to the control.

Table 3. Effect of *Azotobacter*-based Biofertilizer Dose on pH, N Total, and Hg Content of Growth Media at Harvest Time

***Azotobacter* Treatment**	**pH**	**Total N (%)**	**Hg (mg/kg)**
Control	6.13 ± 0.10 c	0.18 ± 0.03 a	1.22 ± 0.24 a
1%	6.08 ± 0.05 c	0.22 ± 0.03 b	1.21 ± 0.06 a
5%	5.98 ± 0.04 b	0.24 ± 0.02 b	1.23 ± 0.07 a
10%	5.89 ± 0.03 a	0.23 ± 0.02 b	1.14 ± 0.10 a

Values ± standard deviation followed by the same letter indicate that the treatments were not significantly different based on the DMRT at $p < 0.05$

Growing water spinach lowers the pH due to the rhizosphere effect, where the plant's rhizosphere excretes root exudates made up of organic substances, including organic acids (Chai and Schachtman 2022). The breakdown of manure provides simple carbohydrates promote the proliferation of *Azotobacter* and the release of organic acids. The increased viability of *Azotobacter* in the soil may enhance the production of organic acids, resulting in a lower pH. The total N increment following *Azotobacter* inoculation (Table 3) results from enzymatic N fixation, which makes N available for the plant as described by Halbleib and Ludden (2000).

The experimental data showed a sharp decrease in total Hg after the experiment compared to before, partly because Hg was released from the pot through the infiltration of irrigation water. The temperatures in the greenhouse reached up to 34.0 °C; Hg was quickly converted to methylmercury under high-temperature conditions (Zhou *et al*. 2020). In that form, the Hg is released into the air and is no longer available for root uptake.

Plant Phenotypes

Analysis of variance revealed that *Azotobacter* treatment significantly influenced the plant height, leaf number, and stem thickness of water spinach 30 days after sowing;

however, it did not affect the root length. The initial concentration of *Azotobacter* inoculated into the tailing-based growth media impacted the plant height, leaf count, and stem diameter of 30-day-old water spinach, but it did not influence the root length (Table 4 and Fig. 2). Applying 5% and 10% inoculants resulted in significant increases in plant height by 18.8% to 24% and 16.2% to 21.4%, respectively, compared to the control and 1.0% liquid inoculant. The increase in leaf number with 10% inoculants was approximately 40% compared to the control and 1.0% inoculants, while the largest stem diameter was observed in plants inoculated with 10% of these inoculants. Nonetheless, the plant height and leaf number of water spinach treated with 5% and 10% inoculants did not differ.

Table 4. Effect of *Azotobacter*-based Biofertilizer Dose on Some Growth Parameters of 30-day-Old Water Spinach Grown in Tailings-based Growth Media

***Azotobacter* Treatment**	**Plant height (cm)**	**Leaf Number**	**Stem Diameter (cm)**	**Root Length (cm)**
Control	8.66 ± 1.04 a	4.17 ± 0.81 a	1.71 ± 0.07 a	5.13 ± 0.57 a
1 %	8.85 ± 0.75 a	4.11 ± 0.66 a	1.77 ± 0.03 ab	4.81 ± 0.55 a
5 %	10.29 ± 0.99 b	5.28 ± 0.35 b	1.84 ± 0.04 b	5.39 ± 0.53 a
10 %	10.75 ± 1.16 b	5.96 ± 0.61 b	1.95 ± 0.10 c	5.76 ± 0.69 a

Values ± standard deviation followed by the same letter indicate that the treatments were not significantly different based on the DMRT at $p < 0.05$.

Azotobacter provides available N for amino acid and protein synthesis, which is essential for cell formation and enlargement. It fixes gaseous N_2 into NH_3, which is then chemically converted into NH_4^+. Aerobic soil enables nitrifying bacteria to convert NH_4^+ into NO_2^- and then into NO_3^- (Hink *et al*. 2017); both ionic forms of N are accessible for root uptake. The N is crucial for synthesizing various enzymes involved in cell metabolism. The phytohormones excreted by *Azotobacter* may play a vital role in shoot and root growth; a high concentration of auxin relative to cytokinin promotes root growth, while more cytokinin is needed to enhance shoot growth compared to auxin (Kurepa and Smalle 2022). In this study, auxin production by *Azotobacter* may have surpassed that of cytokinin, despite the consortium of the three species producing both phytohormones in the *in vitro* assay (Adawiah *et al*. 2024).

Regardless of the inoculant concentration, the plant roots did not respond to *Azotobacter* inoculation. *Azotobacter* may not have produced enough auxin to promote root growth. Auxin biosynthesis involves both non-tryptophan and tryptophan-dependent pathways (Tang *et al*. 2023). Tryptophan (Trp) is the least abundant amino acid in the cell, and its synthesis requires significant cellular energy (Barik 2020). *Azotobacter* only synthesizes a low amount of Indole Acetic Acid (IAA) without exogenous Trp, and a pronounced increase of IAA production when exogenous Trp is supplied (Ahmad *et al*. 2005). The tailing-base growth media properties might limit the supply of exogenous Trp due to the low content of Trp in the added manure, since organic waste is a primary source of Trp in soil (Arkhipchenko *et al.* 2006). Consequently, the restricted auxin synthesis may have hindered root proliferation despite the presence of *Azotobacter*.

High silt content (Table 1) in the soil may reduce soil aeration and hinder plant growth, as well as the activity of *Azotobacter* in auxin production. The physical properties of tailings render them unsuitable for food crop cultivation, although the macronutrient level in a mixture of tailings and manure was high (Table 1).

Plant Biomass and Mercury Levels

A difference in biomass was observed between plants treated with 5% and 10% *Azotobacter* consortia and the control (Table 5). *Azotobacter* inoculation increased the total N content of the growth media (Table 3) and promoted plant growth, as indicated by increased plant height, leaf count, and stem thickness (Table 4). Consequently, the increase in plant growth contributed to the rise in biomass (Table 4). These results agree with the increase in heavy metals in plant biomass following N fertilization (Yang *et al*. 2020).

The experiment determined that both 5% and 10% of the *Azotobacter* inoculant levels can increase Hg uptake. *Azotobacter* inoculation lowered the growth media's pH to a slightly acidic range of 5.85 to 5.95 (Table 3), which may have enhanced Hg mobilization. Hg uptake increases in soil with a pH < 6.5 and decreases with a pH >7.5 (Yu *et al*. 2018). The exopolysaccharides produced by *Azotobacter* also reduce the availability of heavy metals through surface complexation and ion exchange (Li *et al*. 2022).

A 5% and 10% *Azotobacter* concentration in the growth media appeared to be less effective at immobilizing Hg than the 1% concentrations. A high concentration of *Azotobacter* inoculant increased the total N (Table 3), which may be linked to the increased Hg uptake. Information on how *Azotobacter* inoculant affects Hg uptake is not yet available. However, Liu *et al*. (2024) reported that Hg uptake increased after inoculation with symbiotic N-fixing Rhizobia, due to an increase in nitrate reductase activity, photosynthesis, and amino acid N content, which consequently led to increased Hg uptake. These findings suggest that *Azotobacter* inoculant in higher concentrations may inadvertently enhance Hg mobility and uptake through pH reduction and increased N uptake. High Hg accumulation in food crops and wide plants grown in gold mine tailings was also reported in Uganda (Ssenku *et al*. 2023).

Table 5. Effect of *Azotobacter* Dose on Biomass and Hg Concentration on 30-day-Old Water Spinach Grown in Tailings-based Growth Media

Treatments	Fresh Weight (g)[1]	Dry Weight (g)[1]	Hg (mg/kg)[1]
Control	2.53 ± 0.24 a	0.37 ± 0.03 a	0.05 ± 0.001 a
1 %	2.58 ± 0.35 a	0.37 ± 0.04 a	0.06 ± 0.001 a
5 %	3.22 ± 0.30 b	0.49 ± 0.08 b	0.12 ± 0.001 b
10 %	3.52 ± 0.38 b	0.50 ± 0.06 b	0.23 ± 0.002 c

Values ± standard deviation followed by the same letter indicate that the treatments were not significantly different based on the DMRT at $p < 0.05$. [1]Intact plant.

Hg concentrations in intact plants were higher than the threshold for Hg contamination in vegetables, which is set at 0.03 mg/kg according to the Indonesian Regulation Number 23 of 2017 regarding "Maximum Limits for Heavy Metal Contamination in Processed Foods". However, as Bradl (2005) noted, Hg levels in the edible parts of plants should not exceed 50 µg/kg. Meanwhile, according to the Food Safety Standards used in China, the maximum Hg concentration is 10 µg/kg (Riedel *et al*. 2014).

Parameter Correlation

Table 6 illustrates the correlation between the growth media's pH, total N, and total Hg with plant growth. Both pH and total N showed a positive correlation with the plant growth variables, whereas total Hg exhibited a negative correlation with all plant growth parameters. This suggests that higher total Hg levels led to a decrease in plant growth.

Table 6. Correlation Matrix of Pearson Correlation Coefficients between Growth media and Plant Variables

Independent Variable	PC Coefficient	Dependent Variable				
		Plant Height	Leaf Number	Root Length	Fresh Biomass[1]	Dry Biomass[1]
pH	PC	0.714**	0.695**	0.446*	0.591**	0.695**
	Sig.	0.000	0.000	0.029	0.002	0.000
Total N	PC	0.323	0.407*	0.157	0.427*	0.464*
	Sig.	0.123	0.049	0.463	0.038	0.023
Total Hg	PC	-0.062	-0.036	-0.005	-0.072	-0.201
	Sig.	0.773	0.869	0.983	0.738	0.345

[1]Intact plant; Scale of correlation coefficient: ***very high correlation (0.80 ≤ r ≤ 1.00); **High correlation (0.60 ≤ r ≤ 0.79); *moderate correlation (0.40 ≤ r ≤ 0.59).

The pH of the plant growth media influences nutrient availability. The slightly acidic pH in this experiment (5.89 to 6.13) provided the plants with sufficient N, P, and K while preventing metal and heavy metal toxicity. High pH levels caused mercury to bind to organic matter and clay minerals (Hussain *et al.* 2022b), making it less available for plant uptake. Mercury (II) oxide (HgO) showed high mobility in highly acidic soils (pH < 4), and became stable and less soluble between pH 4 and 10.1 (Yang *et al.* 2007).

Macronutrients, including N, K, Ca, Mg, and S, are readily available at a pH range of 6.0 to 6.5 (Ferrarezi *et al*. 2022). Meanwhile, phosphate desorption decreases at pH 5 to 6 and increases in more acidic or alkaline conditions (Barrow 2017). Consequently, P is available for water spinach plants at a pH range of 5.89 to 6.13. The slightly acidic soil promotes root growth. Acidic conditions can enhance the mobility of toxic elements, such as aluminum (Al^{3+}) and Mg^{2+}, which can restrict root growth and function (Wang *et al*. 2020).

Total N correlates with leaf number and biomass, as N dictates photosynthesis, amino acid and protein synthesis, and consequently cell formation and enlargement (Lemaire and Gastal 2019). Therefore, increasing the N fertilizer dosage has been reported to enable leafy vegetables to produce high yields due to an increase in N and chlorophyll contents (Ncama and Sithole 2022; Gülüt and Şentürk 2024).

The Pearson correlation verified that an increase in Hg in the soil correlates with reduced plant growth. Mercury primarily hinders plant growth by disrupting vital physiological processes. High levels of Hg in the soil reduce chlorophyll content, essential nutrient levels, and leaf development while inducing the synthesis of antioxidative components (Sahu *et al*. 2011). The reduction in photosynthetic rate caused by Hg is reported to result from a decrease in chlorophyll a, chlorophyll b, and carotenoids (Mani *et al*. 2024). The observed negative correlation between Hg in the growth media and plant growth aligns with findings that increasing Hg concentrations (up to 2.5 mg/L) in the growth solution inhibit root and shoot biomass production in rice (Du *et al*. 2005). Meanwhile, the growth rate of water hyacinths was inhibited (up to 52%) at 50 mg/L of Hg in the growth media (Malar *et al*. 2015).

The high Hg content in the intact plant confirms that the mixture of tailings and manure was unsafe for growing food crops. Elevated Hg levels may require further study to determine how to manage the tailings-based growth media in long-term experiments by adding organic matter.

CONCLUSIONS

1. The population of *Azotobacter* in the liquid inoculant was 10^9 CFU/mL with the acidity of 7.95 and EC of 1.74 mS/cm at three days after inoculation.
2. Water spinach effectively responded to *Azotobacter* inoculation; however, its growth was reduced. Introducing 5% and 10% *Azotobacter* inoculant enhanced plant growth and biomass, total N, and fungal and bacterial counts in the rhizosphere, while decreasing the pH to below 6. It enhanced Hg accumulation in intact plants above the threshold.
3. The pH and total N in the growth media were positively correlated with plant growth parameters, but Hg content negatively correlated with plant growth.

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