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The Effect of Heavy Metal Lead (Pb) on the Growth of Ammonia-Degrading Bacteria and Physical Changes of *Eichhornia crassipes* in Groundwater Phytoremediation

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Abstract*— Water hyacinth (*Eichhornia crassipes*) has been extensively used for heavy metal phytoremediation and stimulating microorganism growth in the effort to break down organic pollutants by the exudate secreted by the plant. This study aims to figure out the growth of the ammonia-degrading bacteria population and figure out the physical changes occurring in water hyacinth during the Pb phytoremediation process. The phytoremediation method was performed under the batch system with the treatments: P1 with water hyacinth for groundwater with 2 ppm of Pb; P2 with water hyacinth for groundwater with 4 ppm Pb; and P0 with no water hyacinth and Pb addition. Observations include the growth of ammonia-degrading bacteria, ammonia concentration, Pb analysis, observation of physical changes, and measurement of biomass of the water hyacinth. Results show that the nitrifying bacteria population growth rate was higher in the 2 ppm Pb treatment than in the 4 ppm Pb treatment. The implication was that there occurred a higher ammonia concentration decrease in P1 by 0.43 mg/L from the initial concentration of 1.21 mg/L. As for the water hyacinth's physical changes, a lower growth rate happened to the 4 ppm Pb treatment, resulted in lower biomass of 75.46 g in the said treatment than in the 2 ppm Pb (79.00 g). The use of water hyacinth in phytoremediation also prompted the bacterial growth to break down organic waste, but high concentrations of heavy metals will influence the growth of the aquatic plant, water hyacinth.

***Keywords*— Phytoremediation; lead; water hyacinth; ammonia-degrading bacteria.**

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I. INTRODUCTION

The ever-increasingly rapid industrial development has caused environmental pollution from the waste produced. Besides organic substances, pollution also comes from heavy metals, especially in the electronic industry and mining waste [1]. Heavy metal pollution to serious problems is toxic and negatively influences the environment [2], [3]. If it heavy metal in the aquatic ecosystem, especially in fisheries and shrimp farms, the result causes deleterious effects in all living organisms, including microbial survival of ammonia-degrading bacteria [4]. These bacteria are required because they can naturally eliminate ammonia pollutants in fisheries that come with feed [5]. The Heavy metal lead (Pb) is toxic, which affects the existence of microorganisms in waters.

Therefore, this research focuses on how heavy metal Pb pollution will interfere with a few defense mechanisms to metabolize and transform heavy metal into a less hazardous form, causing ammonia-degrading bacteria. However, bacteria generally have genes coding for resistance mechanisms of heavy metal [6]. Chemically speaking, Pb is capable of binding to enzymes and proteins [7]. Pb may disturb the growth and development of living things with such a property, including plants [8], [9]. The heavy metal Pb may hinder photosynthesis in that the Pb^{2+} in leaves replaces mineral nutrients such as Fe^{2+} and Mg^{2+}, which play a role in the chloroplast structure formation process [10]. It may also hinder splitting and elongation of cells, slow down cell mitosis, and lower the cell wall elasticity, causing the cell to be prone to rupturing [10], [11]. Hence, Pb accumulation can cause both morphological and physiological disturbances to plants [12]. One of the plants frequently used in mitigating Pb pollution is the water hyacinth (*Eichhornia crassipes*) for the great potential for water pollution control lying in its fast growth and for its ability to accumulate various heavy metals like Zn and Cr at efficiency rates of up to 94% and 84%, respectively [13], [14], [15]. For these reasons, water hyacinth is also often used in heavy metal phytoremediation. In wastewater, this plant can adapt with a high level of tolerance and accumulate heavy metals with a high level of effectiveness [13], [16]. Water hyacinth can absorb Pb in water at a concentration of 180 ppm [17]. Water hyacinth sees considerable application in phytoremediation as its roots can absorb heavy metals into the tissues of organs like stalks and leaves [18]. Such a mechanism is made possible by amino acids like aspartic acid, glutamic acid, and glycine as well as the hydroxyl and carboxylate groups contained in this plant's cells. The amino above acids easily forms chelating compounds that play a role in binding the heavy metals existing in the environment; for the metal absorption to increase in rate, water hyacinth forms reductase molecules on the root membranes [17]. The transportation model within this plant's body is that the metals brought into the roots' cells enter the xylem and phloem transport tissues to be carried to other plant parts [13]. Over the phytoremediation process, another remediation mechanism occurs: breaking down organic pollutants, one of which ammonia in a high concentration in domestic-waste-contaminated water [1]. Plant use in phytoremediation is beneficial to microorganisms for their development as plant roots produce exudates in organic substances that are useful for bacterial growth. Thus, bacteria have an indirect role in the remediation of organic pollutants, as in the decomposition of ammonia into nitrite and nitrate in water [19].

In the roots of water, hyacinth anaerobic and aerobic conditions coexist, making it easy to remove nitrogen compounds [20]. The plant will absorb the nitrite and nitrate not decomposed by microorganisms as sources of nutrients. The CO_2 produced from the anaerobic process, on the other hand, is used by the plant for photosynthesis [21].

The effort of maintaining water quality by application of microorganisms is ammonia-degrading bacteria as agents of bioremediation is a biological approach to water quality management that employs bacterial activity in decomposing the pollutant ammonia in the waterway system [21]. Such bioremediation agents must have the metabolism capabilities of oxidation, ammonification, nitrification, denitrification, sulphuration, and nitrogen fixation to directly decompose nitrogen organic and other harmful substances in the water [19]. The biological approach to water quality management is a safe alternative potential to develop. The use and development of bacterial isolates may serve as a solution for better water quality [16]. It is believed that bacterial communities in aquatic plant roots will convert substances through ammonification and nitrification. The application of phytoremediation will reduce the ammonia content in organic waste and increase the nitrate content within. Against this background, this research focused on observing ammonia-degrading bacteria and the effect of heavy metal content on water hyacinth as an agent of phytoremediation.

II. MATERIAL AND METHOD

A. Sampling

The water hyacinth (*Eichhornia crassipes*) sample was extracted from the lake of Hasanuddin University, Makassar. All parts of the plant, including the roots, stalks, and leaves, were cleaned with flowing water [18]. The plant was then reared in a pond for two weeks for acclimatization and for obtaining a plant with relative homogeneity in size and weight and good adaptability [15]. The water used for the phytoremediation treatments was the groundwater from the Experimental Farm of Hasanuddin University. The heavy metal Pb used was in the form of the compound Pb $(NO_3)_2$.

B. Preliminary Analysis of the Groundwater and Water hyacinth Samples

A preliminary analysis was performed to figure out the initial condition of the groundwater in terms of nutritional contents like carbon (C), nitrogen (N), phosphor (P), and potassium (K). The preliminary analysis of the Pb content of the water hyacinth was conducted by the Atomic Absorption Spectrometry (AAS) method. The initial concentration of nitrogen in ammonia in the groundwater sample was measured by the phenate method.

C. Phytoremediation Treatment

The phytoremediation method used was the batch system or the method with standing, unflowing water [18]. Treatments were performed in containers of 10 L of groundwater in three ways: P1, with groundwater containing 2 ppm of Pb and water hyacinth; P2, with groundwater containing 4 ppm of Pb and water hyacinth; and P0, without any Pb and water hyacinth addition. The treatments were then subjected to a phytoremediation process for 12 days. Observations were conducted on the counts of ammonia-degrading bacteria on days 0, 4, 8, and 12. Observations of the final ammonia concentrations and the water hyacinth's physical changes were each conducted on day 12 and water hyacinth biomass on day 12.

D. Enumeration of Ammonia-Degrading Bacteria

The numbers of the growing ammonia-degrading bacteria were counted with the pour plate method. A water sample in each phytoremediation treatment was extracted at 10 mL and then placed into a conical flask filled with 90 mL of sterile water under the serial dilution procedure. The growing medium used was the liquid medium Zobell 2216E with the following composition: $(NH_4)_2SO_4$, 472 g; KH_2PO_4, 7.25 g; Na_2HPO_4, 11.32 g; CH_3COONa, 80 g; $MgSO_4$, 200 g; CaC_{12}, 20 g; $NaHCO_3$, 85 g per liter of distilled water. Elements A and B were then added. This was followed by sterilization by an autoclave. Into each microplate well, the medium and bacterial culture were inserted at 200 µL and 20 µL, respectively, before a 24-hour incubation at room temperature. A change in the medium color indicated the ammonia degradation ability.

E. Determination of Ammonia Concentration

Determination of ammonia concentration was conducted by the phenate method based on the formation of the blue-colored complex compound indophenol. A sample of 10 mL of groundwater was pipetted into a 25 mL cell sample, then added with 0.4 mL of phenol solution, 0.4 mL of sodium nitroprusside, and 1 mL of oxidizing solution. The mixture was then homogenized and let to stand for 1 hour.

Measurement was then conducted with a UV-Vis spectrophotometer at a wavelength of 640 nm.

F. Observation of Physical Changes

TABLE I
TYPE OF PHYSICAL CHANGE THAT IS OBSERVED ON WATER HYACINTH.

Part of plant water hyacinth	Physical change
Stalk	Addition of petiole
Root	Extended root
Leaf	Number of leaves
- Leaf appearance	Color, withered
- Leaf widening	Leaf size
Overall plant growth	Normal, dwarf

Physical change observation of the water hyacinth was performed on the plant's physical appearance and the morphology of its parts, including the leaves, stalks, and roots (Table 1).

G. Measurement of Water hyacinth Biomass

Biomass observation was based on the dry and wet weight. After measuring the water hyacinth's wet weight, the dry weight was then measured by first heating the plant in an oven for two days at 65 °C [12]. The relative water content of the water hyacinth was calculated using the following formula:

$$RWC = \frac{FW - DW}{FW} \times 100\% \tag{1}$$

Where RWC = relative water content (%), FW = wet weight (g), FD = dry weight (g). The following is the flowchart of the research stage (Figure 1).

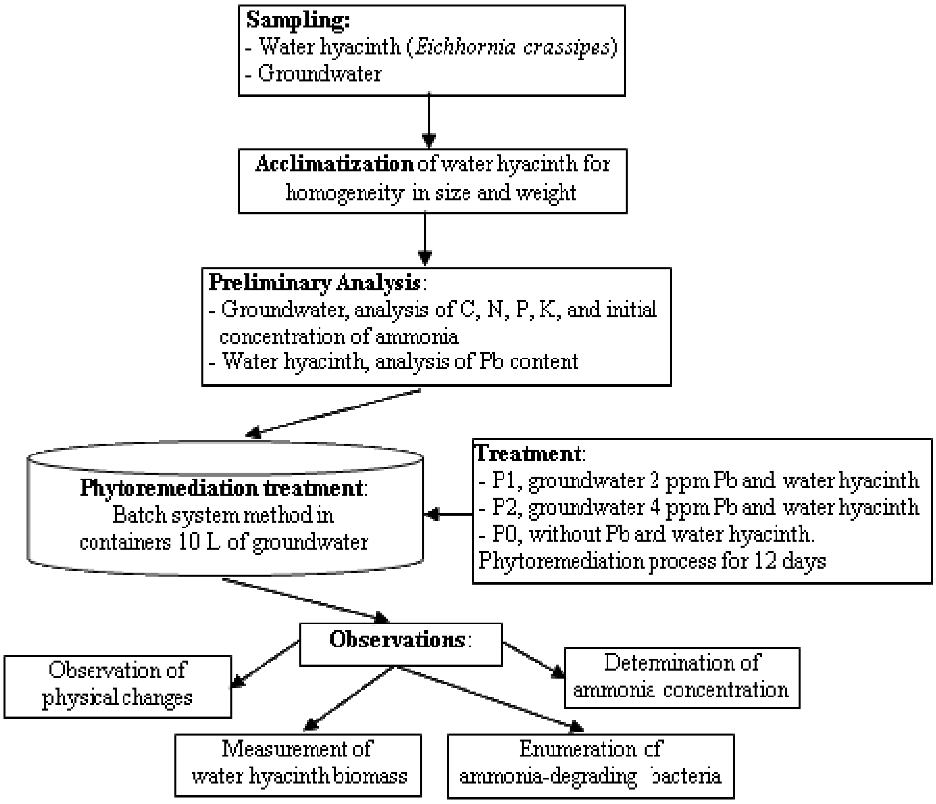

Fig.1 flowchart of the research stage

III. RESULTS AND DISCUSSION

A. Preliminary Test

From the preliminary test of the lead (Pb) content of the water hyacinth before the treatments using the atomic absorption spectrometry (AAS), the Pb content was 0.0004 mg/kg. The acclimatized water hyacinth 's Pb content was attributed to the plant's ability to accumulate heavy metals in its tissues, albeit at small concentrations [13]-[18]. The presence of Pb in the water hyacinth showed that lake water had been contaminated with Pb at an average concentration of 3.906 mg/L [22].

The analysis result shows that the nitrogen's initial concentration in the groundwater in ammonia amounted to 1.21 mg/L. The ammonia compound content in the groundwater sample was relatively high compared to the ammonia content in natural waterways (less than 0.1 mg/L). This indicates organic pollution from domestic waste because, when a closer look is taken, the groundwater sampling location was in the vicinity of a human settlement that produced organic wastes like domestic one [23].

The results of the analysis of the nutritional contents of the groundwater [18], including carbon (C), nitrogen (N), phosphor (P), and potassium (K) (Table 2).

TABLE II
C, N, P, AND K COMPOSITION IN THE GROUNDWATER.

Nutritional content	Value (ppm)
Carbon (C)	777.03
Nitrogen (N)	68.85
phosphor (P)	33.53
potassium (K)	14.17

Plants highly demand major nutrients like C, N, P, and K. The groundwater availability in the groundwater indicates that aquatic plants will grow well and support the phytoremediation process [23].

B. Ammonia Concentration

The final concentrations of ammonia in the groundwater observed on day 12 show some changes. The final concentration in P1 was 0.43 mg/L, in P2 0.78 mg/L, and in P0 1.02 mg/L, meaning that the reductions in ammonia concentrations from an initial concentration of 1.21 mg/L in P1, P2, and P0 amounted to 65.46%, 35.53%, and 15.70% (Figure 2). Ammonia is a form of nitrogen compound in water that is measured as total ammonia (NH_3 and NH_4^+). Unionized free ammonia is toxic to aquatic organisms, while ammonia itself is a source of nitrogen directly useful for aquatic plants. Serving as an aquatic nutrient, ammonia, when available in high concentrations, may lead to eutrophication [23].

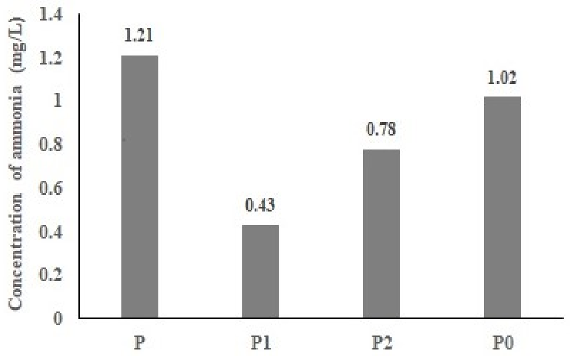

Fig. 2 Ammonia concentration; P is the initial concentration of ammonia in the groundwater in all treatments; value at P1, P2, and P0 are the final concentrations of ammonia in all treatments.

C. Growth of Ammonia-Degrading Bacteria

Observation of ammonia-degrading bacteria growth was performed by the plate count method as indicated by the change in the color of the medium used. In P1, with an addition of 2 ppm of Pb, on day 0, there were 16 x 10^3 cells/mL, and this value grew to 48 x 10^3 cells/mL on day 12. In P2, which involved addition of 4 ppm of Pb, there was an increase up to 21 x 10^3 cells/mL on day 6 from 14 x 10^3 cells/mL, but these figures went down on day 9 until day 12 at a final count of 16 x 10^3 cells/mL. In P0, which involved no Pb and water hyacinth addition, the bacterial growth rate was relatively stable with a slight increase from 12.7 x 10^3 cells/mL on day 1 to 13.4 x 10^3 cells/mL on day 12 (Figure 3).

The increase in the growth rate of the ammonia-degrading bacteria in P1 shows that the water hyacinth, through its exudates in the form of organic substances, supported microbial growth [1]. This was attributed to the bacteria's tolerance to the Pb concentration of 2 ppm, which allowed them to grow. However, this was not the case in P2 where the ammonia-degrading bacteria's growth rate declined. This was because 4 ppm was a toxic, growth-inhibiting amount to the bacteria [21]. In comparison, the treatment with no Pb and water hyacinth addition demonstrated neither conspicuous bacterial growth for the lack of nutrients from the water hyacinth's metabolites nor growth inhibition by Pb [24]. There was no significant increase in growth rate in P0 from day 0 to day 12 because nutrient supply was absent in the form of organic substances from the exudates of the water hyacinth.

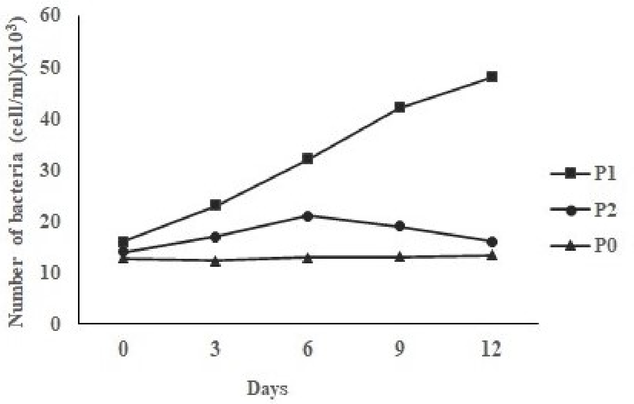

Fig. 3 Ammonia-degrading bacteria population growth in P1 (groundwater containing 2 ppm of Pb and water hyacinth), P2 (groundwater containing 4 ppm of Pb and water hyacinth), and P0 (groundwater not containing Pb and water hyacinth).

From the observation, it was found out that a high Pb concentration of 4 ppm hinders the growth of the bacterial population. As stated by [25], heavy metals are toxic to bacteria and can be lethal. Conversely, the relatively low Pb concentration of 2 ppm in P1 showed a rise in the number of bacteria. High concentrations of heavy metals are toxic to microbes due to the inhibition of enzyme functions in the cytoplasm by metal ions [26]. By contrast, microbes can adapt to the environment contaminated by heavy metals at certain concentrations by heavy metal reduction mechanisms. The change in metal element mobility rendered by microbes from inorganic metals encompasses reducing inorganic metals and change from inorganic to organic, specifically referred to as methylation and demethylation [24]–[27].

The microbial growth in the phytoremediation treatments was influenced by the release of oxygen by the aquatic plant's roots, elevating oxygen level around the root hairs and thus allowing the aerobic nitrifying microbes to live [17]. In these treatments, ammonia was absorbed by the water hyacinth 's roots, and with the help of the microbes living in the roots, the ammonia in the form of ammonia ions was undergoing nitrification into nitrite and nitrate [19]. The reduction of pollutant concentrations in waste water utilizing aquatic plants is a cooperation between the plants and the associated microbes.

D. Physical Changes and Biomass of Water hyacinth

The physical differences throughout the water hyacinth growth in the 4 ppm Pb treatment and the 2 ppm Pb treatment on day 12 (Figure 4). The water hyacinth in the 4 ppm Pb treatment had a slower growth rate and smaller leaves. This was because lead can cause morphological and physiological disorders to plants and cause disturbances to leaf development [12]. In the 2 ppm Pb treatment, the water hyacinth demonstrated growth closer to normality. Water hyacinth normally grows when absorbing such heavy metals as Pb, Hg, Cr, As, Zn, and Ni at a concentration of 5 ppm, while at greater concentrations than 10 ppm, it will wither and even die, depending on the type of the metal [28].

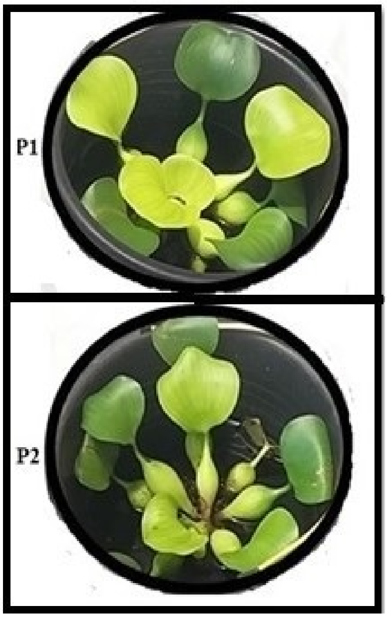

Fig. 4 Physical change of water hyacinth on day 12 in PI with 2 ppm of Pb (A) and P2 with 4 ppm of Pb (B).

There is the availability of nutrients in the phytoremediation treatment, but it is difficult to be absorbed by plant roots because the toxicity of heavy metal Pb, which damages the root cells, causes disturbance absorption of essential nutrients [29]. Besides, the presence of heavy metal Pb will interfere with the cell division and photosynthesis process [30], [31]. High concentrations of Pb can inhibit chlorophyll production in chloroplasts which causes chlorosis symptoms in plants [29]. In general, heavy metal stress causes growth problems as indicated by the parameters of root length increase, stem growth, and number of leaves and root growth [30].

Under a normal condition, plant cells always produce reactive oxygen species (ROS) as it constitutes a part of the metabolism process [32]. However, the accumulation of heavy metals in plants can trigger oxidative stress due to some ROS like hydrogen peroxide (H_2O_2), hydroxyl radical (OH), superoxide anion ($O2^{-\cdot}$), and oxygen (O_2) [12], [33]. Under a stressful condition caused by heavy metal contamination, plants will attempt to survive by increasing the activity of antioxidant enzymes like peroxidase, ascorbate peroxidase, superoxide dismutase, and catalase [33], [34]. Plants will experience heavy metal poisoning if the heavy metals accumulate in a larger amount than the plant tissues' detoxification capacity [34].

The most concrete symptom of Pb toxicity is the inhibition of plant growth. Plant biomass can be used as an indicator for depicting plant growth against Pb toxicity [12], [35]. The wet weight before and after the experiment in the 4 ppm Pb treatment and the 2 ppm Pb treatment [28] (Table 3). Meanwhile, the water hyacinth's biomass and relative water content at the end of the experiment (Table 4).

TABLE III
WET WEIGHT OF THE WATER HYACINTH BEFORE AND AFTER THE EXPERIMENT

Type of treatment	Wet weight of the water hyacinth (gram)	
	Day 0	Day12
Pb 2 ppm	59.03	79.00
Pb 4 ppm	60.28	75.46

TABLE IV
BIOMASS AND RELATIVE WATER CONTENT OF THE WATER HYACINTH ON DAY 12.

Type of treatment	Plant parts	Wet weight (g)	Dry weight (g)	Relative water content (%)
Pb 2 ppm	Leaves	14.23	1.57	88.97
	Stalks	41.83	1.64	96.08
	Roots	22.94	1.81	92.11
	Total	79.00	5.02	93.65
Pb 4 ppm	Leaves	13.69	1.52	88.90
	Stalks	39.64	1.61	95.76
	Roots	22.13	1.78	91.96
	Total	75.46	4.91	93.49

The change in the water hyacinth's wet weight in the 2 ppm Pb treatment after 12 days from 59.03 g to 79.00 g was greater than that in the 4 ppm Pb treatment (from 60.28 g to 75.46 g). The water hyacinth's dry weight and relative water content after 12 days in the 2 ppm Pb treatment were 5.02 g and 93.65%, respectively. While in the 4 ppm Pb treatment 4.91 g and 93.49%, respectively. The water hyacinth's biomass in the 4 ppm Pb treatment was smaller than that of the water hyacinth in the 2 ppm Pb treatment. The biomass of water hyacinth was found to be down 33.28% due to exposure to 1000 ppm of Pb. a rate greater than was found in control (0 ppm Pb) [12].

The decline in plant biomass is attributed to heavy metals, which can increase lipid peroxidation, decrease the protein level, inhibit photosynthesis, and decrease the mitosis index [36], [37]. Lipid peroxidation was reported to interfere with the membrane structure and damage the protein and DNA components [38]. Malondialdehyde (MDA) is produced by lipid peroxidation when the plant is experiencing oxidative stress [12]. The decrease in the protein level of water hyacinth occurs because of the increase in the catabolic enzyme and protease activity which actively destroys the protein molecules due to heavy metal accumulation [39]. In the photosynthesis process. Lead accumulation in the leaf tissues can inhibit chlorophyll synthesis as the existing Pb ions can replace Mg ions [40]. Lead causes an impediment to the development and growth of roots. Furthermore, this is correlated with the decrease in the mitosis index [11]. Pb, which hinders many enzymes with a sulfhydryl group, can lower the plant biomass [37]–[41].

Based on Table 3, the relative water content in the 4 ppm Pb treatment was smaller than that in the 2 ppm Pb treatment. This shows that Pb can inhibit water absorption by the leaves and the roots. Lead accumulation in the leaves can prompt the closing of the stomata. hence the binding of materials for photosynthesis like CO_2 and H_2O can be hampered [10], [42]. Meanwhile. lead accumulation in the roots can suppress the development and the number of lateral roots. suppress the development and density of root hairs and suppress root growth [11].

IV. Conclusion

The water hyacinth (Eichhornia crassipes) application in phytoremediation influenced the bacteria growth in the groundwater contaminated with heavy metals and the organic waste ammonia. The nitrifying bacteria growth rate in the treatment with water hyacinth and Pb content of 2 ppm was increased. This was linked to the decreased ammonia concentration in the treatment. According to the results of the physical change observation of the water hyacinth. The 2 ppm Pb treatment showed greater normality in growth as well as larger leaves. As a result, the biomass increase was also larger reaching 79.00 g from the initial biomass of 59.03 g, because the heavy metal Pb was able to influence the plant's morphology and physiology. After all, high concentrations of Pb will interfere with the cell division, photosynthesis process, and damage root, which causes disturbance absorption of essential nutrients, leading to the growth of water hyacinth with poor health.

Nomenclature

Heavy metal is defined as metallic elements with a relatively high density of more than 5 g/cm3 compared to water, which is noted for its potential toxicity and adversely affects the environment and living organisms.

Lead (Pb): Lead is a chemical element with the symbol Pb (from the Latin plumbum) and atomic number 82, can be found in all parts of our environment. Much of it comes from human activities, including burning fossil fuels, mining, and manufacturing.

Ammonia is a compound composed of nitrogen and hydrogen in water is either un-ionized ammonia (NH_3) or the ammonium ion (NH_4^+). It is toxic to organisms at concentrations exceeding 0.02 mg/L (U.S. EPA).

Water hyacinth (*Eichhornia crassipes*) is a free-floating macrophyte that shows fast growth rate characteristics, adaptability to a wide range of environmental conditions. It is found at the surface of rivers, lakes, canals, and ponds.

Ammonia-degrading bacteria is the ammonia-oxidizing bacteria group that convert ammonia to nitrate through the oxidation process is known as nitrification, and is a primary activity within the nitrogen (N) cycle.

Groundwater is water located beneath the earth's surface in soil pore spaces and in the fractures of rock formations with their origin in the water cycle; an unconsolidated deposit is called an aquifer.

Phytoremediation is a bioremediation process that uses of plants to remove and destroy contaminants in the soil and groundwater. This method relies on associated microorganisms to improve the functionality and recover contaminated by toxic metals and organic.

Exudate is fluids secretion emitted through the roots of plants. This secretion acts as a cometabolite to facilitate microbial pollutant degradation or inhibit harmful microbes and promote the growth of self and kin plants.

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