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Heavy Metal Uptake Test by Aquatic Plants Tissue Culture Products with Neutron Activation Analysis in Ciliwung River

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***Abstract*— High levels of human activity and the amount of waste generated from these activities often end up in rivers. One of the largest and longest rivers in the Jakarta and West Java regions is the Ciliwung River. The purpose of this study was to test the phytoremediation ability of 5 types of aquatic plants propagated through tissue culture (*Bacopa* sp., *Eichornia diversifolia, Althenanthera reinicky, Ludwigia* sp, and *Murdannia*, sp.). The research method used was tissue culture water plants to be tested in an aquarium filled with water collected from the Ciliwung River for 1 month, and the water was replaced every 5 days. Utilization of water plants from Tissue Culture is a novelty to see heavy metal uptake sharply and precisely because it is sterile and free of water pollution. Analysis of heavy metal uptake in aquatic plants using NAA (Neutron Activation Analysis) Method. The result is Ciliwung River waters contain Aluminum (Al), Brom (Br), Calcium (Ca), Cerium (Ce), Cobalt (Co), Chromium (Cr), Iron (Fe), Lanthanum (La), Manganese (Mn), Magnesium (Mg), Scandium (Sc), Sodium (Na), Strontium (Th), and Zinc (Zn). *Bacopa* sp has shown the ability to absorb large amounts of heavy metal elements with a 100% survival rate, with the highest absorption element being Calcium (Ca) of 2.7191, 76 mg / kg. Murdannia was unable to absorb La and Ce and was the weakest species among the species tested.**

***Keywords*—*Bacopa*, sp; *Eichornia diversifolia*;*Althenanthera reinicky*; *Ludwigia* sp; *Murdannia*, sp.**

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I. INTRODUCTION

The Ciliwung River is the longest river in the Jabodetabek area, and it starts at the foot of Mount Pangrango, West Java, flows downstream, and empties to the Bay of Jakarta [1]. The Ciliwung River traverses many densely populated settlements and residential areas with an approximate length of 370.8 km from upstream to downstream and is divided as follows: the upriver region with a length of 150.7 km spans along with Bogor Regency, Mega Mendung, Cisarua, Ciawi, East Bogor City District, South Bogor City, the middle region with a length of 157 km spans along the region of Sukaraja, Cibinong, Bojong Gede, Cimanggis, East Bogor City, Central Bogor City, North Bogor City, Tanah Sereal, Pancoran Mas Depok, Suma Jaya, Beji and the downriver region with a length of 62.9 km spans along the region of South Jakarta, Central Jakarta, Manggarai, Central Jakarta, West Jakarta, North Jakarta and the West Canal [2].

This river has been a witness of human activity along its banks. Urban human activities always impact the environment and aquatic ecosystems, the higher the intensity of these activities, the higher their impact on the environment. Schweitzer [3] argues that there are always two sides to this impact, a positive impact in the form of increased prosperity and a negative impact in the form of pollution or environmental damage. Pollution of the aquatic environment and water sources can threaten agricultural and livestock activities, especially fish farming. Aquaculture or the fish farming industry utilizes water reservoirs or rivers. Therefore, it is highly vulnerable to water pollution, i.e., antibiotics, pesticides, herbicides, hormones, anesthetics, pigments, minerals, vitamins, aquaculture environmental, toxicological, and health issues [4]. The Ciliwung River carries around 5-17 tons of pollutants per year to the java sea [1]. One way to reduce waste and pollutants is through the phytoremediation process of aquatic plants [5].

The objective of the phytoremediation process is to reduce the effects of toxins, heavy metals, organic pollutants, and other contaminants in the aquatic environment. Phytoremediation is a concept of symbiosis between bacteria and plants [6] since plants provide a cool nuance and provide additional oxygen to the environment at a low cost [7]. This technique is easy to implement; it is environmentally friendly, cost-effective, and efficient; it is environmentally friendly because it also acts as a chelator and a chelating agent [8]. Other studies show that aquatic plants can absorb heavy metals such as Ag, Cd, Cr, Cu, Hg, Pb and Zn, Ni, Mn, Co in waters and other essential micronutrients required by plants for their metabolism [9]. Several researchers also point out that *Potamogeton pectinatus* L is extremely tolerant of severe contamination and potential for phytoremediation [10]. The objective of the current study is to measure and determine the types of heavy metals found in the Ciliwung River and measure the phytoremediation capabilities of tissue culture aquatic plants to absorb heavy metal elements.

II. Materials and Method

The study was conducted in February 2019 at the Research Institute for Ornamental Fish Culture at Jalan Perikanan No. 13 Pancoran Mas Depok. The Neutron Activation analysis (NAA) was performed at the Center for Nuclear Industry Material Technology-BATAN.

A. Aquatic Plant Material

The aquatic plants used in the test were Bacopa sp, Eichhornia diversifolia, Alternanthera reineckii, Ludwigia sp., and Murdannia, sp. These plants are grown from a single cell within vitro propagation (tissue culture technique) developed by Indonesian Research Institute for Ornamental Fish Culture, worked in Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development Laboratories. The reason for using aquatic plants grown from the propagation through tissue culture is that they are grown from single and pure sterile cells and free contaminated with any pollutants either from the soil or the water. The use of aquatic plants from tissue culture gives confidence that the absorption of heavy metals received by aquatic plants is purely from the medium where they grow in this study is Ciliwung river waters. (Figure 1).

a b c

d e

Fig. 1 Aquatic plants propagated by tissue culture (a. *Bacopa sp,* b) *Eichhornia diversifolia,* c) *Alternanthera reineckii,* d) *Ludwigia* sp., e) *Murdannia, sp.*

B. Water Sampling Location

Water from the Ciliwung River is collected at the crossing point of the midsection of the Ciliwung River in the Pancoran Mas Depok area (6°24’26’’S and 106° 49’10’’ E) with an elevation of 184 m (Figures 2 and 3).

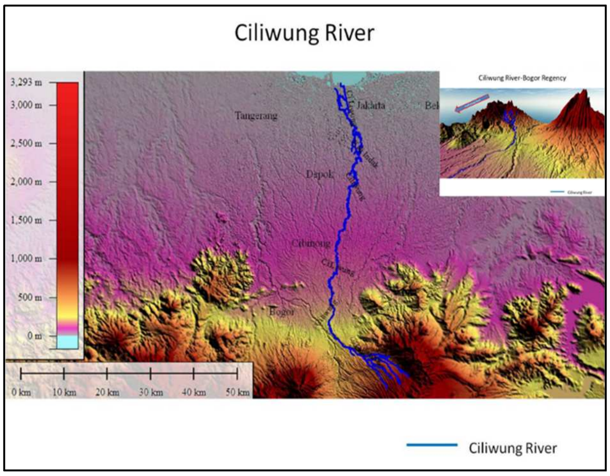

Fig. 2 Ciliwung River flows from the upriver to downriver regions

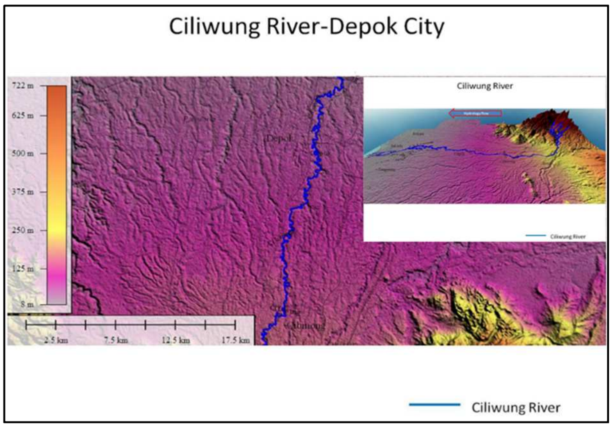

Fig. 3 Ciliwung River area that crosses Depok region

C. Incubation of Aquatic Plant in Ciliwung River Waters

Aquatic plants were incubated in 5 aquariums—each measuring 40 x 40 x 100 cm—and filled with 120 L of Ciliwung River waters. The planting medium in the test was aquarium sand, which was used as substrate and placed at the height of 7 cm from the bottom of the aquarium. In each aquarium, 30 individual test water plants were planted. The aquatic plants' *Bacopa australis, Eichhornia diversifolia,* *Alternanthera reineckii, Ludwigia* sp., and *Murdannia* sp, grown from in-vitro propagation, were put into an aquarium filled with water from the Ciliwung River, and the water in each aquarium was changed every five days. The water was replaced with water collected from the Ciliwung River in the Pancoran Mas Depok area, and observations were made for one month.

D. Neutron Activation Analysis (NAA)

NAA analysis was performed as follows:

1) Ciliwung River waters: Analysis of heavy metal elements in Ciliwung River waters with NAA was conducted when the waters were collected from Ciliwung River.

2) Aquatic plant: After 1-month Aquatic plant samples test were dried using freeze drier equipment at temperature -60 0C for 72 hours. The samples were powdered in an agate mortar, passed through a 100-mesh sieve, and placed in a dark bottle. Special codes were given to each sample before storage and subsequently analyzed for heavy metal absorption using the NAA method.

3) Sample Preparation for Neutron Activation Analysis (NAA): Ciliwung river waters sample Preparation for neutron activation analysis is 120 mL water sample is reduced by heating at a temperature of 50-60 ^{0} C to a volume of 1 mL and is transferred into 500 µL LDPE vial (Low-density polyethylene) gradually and dried in a desiccator that equipped with a vacuum pump. Aquatic plant sample (*Bacopa sp, Eichhornia diversifolia, Alternanthera reineckii, Ludwigia* sp., and Murdannia sp) preparation based on [11] method. Irradiation and Counting each element of heavy metal based on Mulyaningsih [11], [2] method. Conditions during irradiation, cooling, and counting are given in Table I.

TABLE I
IRRADIATION, COOLING AND COUNTING TIME OF SAMPLES

Irradiation time	Cooling time	Counting time	Elements
1 minute	± 5 minutes	120 seconds	Al, Ca, Cl, Mg, Mn, Na
30 minutes	2-4 days	15-30 seconds	La, K,
30 minutes	2-3 days	15-30 minute	Br
3 hours	10-20 days	2-3 hours	Sb, Ba, Ce, Cs, Cr, Co, Hf, Eu, Fe, Sc, Sr, Tb, Yb, Zn, Th, Rb

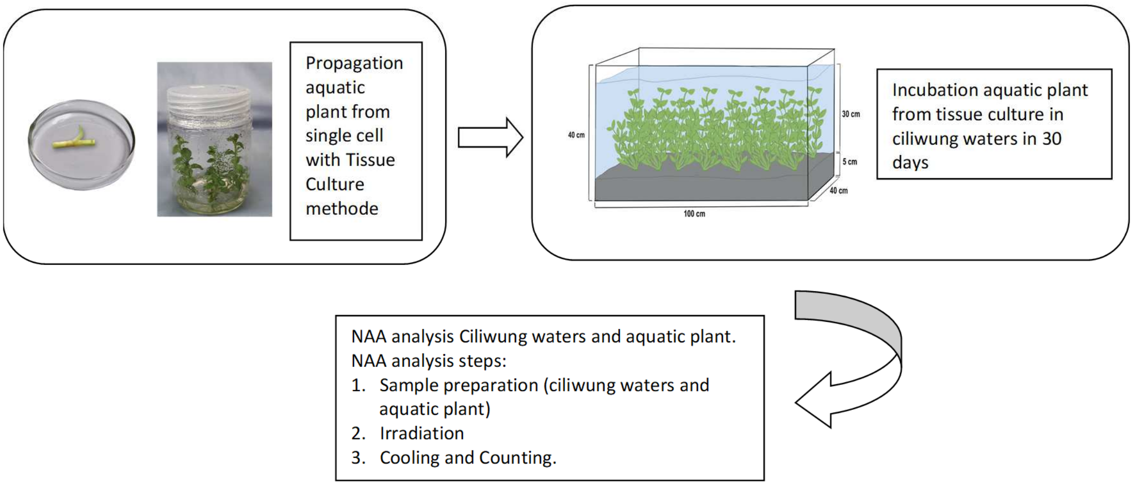

Fig. 4 Graphical abstract research method

III. RESULT AND DISCUSSION

A. NAA Analysis Result in Ciliwung River Waters

The flow of the Ciliwung River is shown in Figures 1 and 2, with the upriver area located in Bogor Regency and the downriver area at the Bay of Jakarta. Results of the NAA analysis of waters from Ciliwung River, wherein 15 heavy metals are identified (Table II). The three metals with the highest concentration in the waters of Ciliwung River are Sodium (Na) with a concentration of 8.75 ± 0.25 milligram/L, Calcium (Ca) with a concentration of 0.712 ± 0.048 milligram/L, and Magnesium (Mg) with a concentration of 0.231 ± 0.25 milligram/L. Thorium (Th) has the lowest concentration at 0.008 microgram/L. [2] reported that 23 types of heavy metals were found in the sediments of the Ciliwung River. The elements (As, Cr, Sb, Zn) have a high content among other elements but are still within the limits of tolerance for pollution and low ecological damage.

TABLE II
NAA ANALYSIS RESULT OF HEAVY METAL ELEMENT IN CILIWUNG RIVER WATERS

No	Heavy Metal Element	Unit	Concentration
1.	Aluminum (Al)	Micro gram /L	18,86±0,83
2.	Bromine (Br	Micro gram /L	0,30±0,02
3.	Calcium (Ca)	Milli gram/L	0,712±0,048
4.	Cerium (Ce)	Micro gram /L	0,07±0,01
5.	Cobalt (Co)	Micro gram /L	0,09±0,01
6.	Chromium (Cr)	Micro gram /L	0,100±0,015
7.	Iron (Fe)	Micro gram /L	44,86±1,52
8.	Lanthanum (La)	Micro gram /L	0,037±0,005
9.	Manganese (Mn)	Micro gram /L	65,31±2,80
10.	Magnesium (Mg)	Milli gram/L	0,231±0,041
11.	Scandium (Sc)	Micro gram /L	0,017±0,001
12.	Sodium (Na)	Milli gram/L	8,75±0,25
13.	Strontium (Sr)	Micro gram /L	1,38±0,18
14.	Thorium (Th)	Micro gram /L	0,008±0,001
15.	Zinc (Zn)	Micro gram /L	0,62±0,02

B. NAA Analysis Result in Aquatic Plant Phytoremediation

The metal absorption from Ciliwung River waters by aquatic plants is shown in Table 3. The aquatic plants absorbed a total of 15 types of heavy metals from Ciliwung River waters. *Bacopa* sp. absorbed the most Calcium (Ca) at 27191.76 mg/kg, followed by *Ludwigia* sp, which absorbs Calcium (Ca) at 20410.79 mg/kg. Tabassum-Abbasi [12] states that Bacopa monnieri is a hyperaccumulator in phytoremediation and decreases nitrogen, phosphorous, zinc, copper, nickel, and manganese levels up to 50%. Another case of *Bacopa monnieri*, can reduce fecal coliform and fecal streptococci until > 99% [12]. In the research that was carried out [13], *Bacopa monnieri* is capable as phytoremediation and helps in the reclamation of textile waste with high oxidative antioxidant capabilities along with a high level of tolerance to the ecosystem.

According to Saleh [14], *Ludwigia stolonifera* removal of the three toxic metals Pb, Cd and Cr had the same properties as *Bacopa monnieri*. *Murdannia*, sp absorbed the most amount of Sodium (Na) at 14480.00 ± 163.82 mg/kg, but this species cannot absorb Cerium (Ce) and Lanthanum (La). The same goes for *Alternanthera reineckii*., which also cannot absorb Lanthanum (La). The aquatic plants that can absorb Lanthanum (La) are *Bacopa* sp. (2.84 mg/kg), *Ludwigia* sp (2.04 mg/kg), and *Eichhornia diversifolia*. (2.51 mg/kg). Moreover, *Eichhornia* sp. can also absorb Calcium (Ca) as much as 14726.89 mg/kg. Ting [15] point out that Eichhornia crassipes can filter water from a number of heavy metals such as lead (Pb), chromium (Cr), zinc (Zn), manganese (Mn), and copper (Cu).

TABLE III
NAA ANALYSIS RESULT OF HEAVY METAL ABSORPTION BY AQUATIC PLANT TISSUE CULTURE PRODUCT IN CILIWUNG RIVER WATERS

No	Heavy Metal Elem.	The concentration of Heavy Metal Element in Aquatic Plant Species (mg/kg)				
		1	2	3	4	5
1.	(Al)	13992,3 ±957,9	9558,8 ±363,0	3104,13 ±125,2	7972,2 ±212,92	2468,5 ±135,3
2.	(Br)	6,9 ±0,48	11,23 ±0,45	21,66 ±0,97	12,04 ±0,50	8,85 ±0,66
3.	(Ca)	27191,8 ±1510,9	14726,9 ±634,33	12829,55 ±1203,89	20410,8 ±817,36	14119,7 ±814,17
4.	(Ce)	6,25 ±0,37	4,18 ±0,28	2,00 ±0,02	3,83 ±0,31	-
5.	(Co)	8,14 ±0,18	4,71 ±0,06	2,86 ±0,10	4,19 ±0,05	1,95 ±0,05
6.	(Cr)	23,16 ±0,79	14,91 ±0,39	17,34 ±0,76	19,90 ±0,45	6,76 ±0,21
7.	(Fe)	13680 ±307,2	7860,3 ±101,9	9994 ±319,8	6332,1 ±87,14	1837,0 ±45,43
8.	(La)	2,84 ±0,21	2,51 ±0,18	-	2,04 ±0,20	-
9.	(Mn)	612,47 ±12,95	346,93 ±7,16	260,21 ±8,26	241,17 ±5,00	142,33 ±3,19
10.	(Mg)	17793,8 ±1112,4	9536,3 ±394,8	4188,6 ±297,4	7200,0 ±237,2	4377,5 ±507,7
11.	(Sc)	5,45 ±0,12	2,74 ±0,03	0,82 ±0,03	2,63 ±0,03	0,66 ±0,01
12.	(Na)	4750,00 ±71,25	3053,99 ±35,53	4435,00 ±66,53	3853,45 ±44,84	14480,00 ±163,8
13.	(Sr)	125,73 ±14,41	104,21 ±8,03	100,70 ±9,37	134,90 ±9,48	95,88 ±7,57
14.	(Th)	0,70 ±0,04	0,56 ±0,02	0,16 ±0,02	0,42 ±0,02	0,11 ±0,01
15.	(Zn)	94,09 ±2,88	116,60 ±1,81	96,22 ±3,66	98,05 ±1,72	132,00 ±3,17

Note: 1 = *Bacopa,* sp; 2 = *Eichhornia diversifolia*; 3 = *Alternanthera reineckii* ; 4 = *Ludwigia*, sp ; 5 = *Murdannia*, sp

The adaptability of the aquatic plants tested in contaminated water showed that Bacopa sp. has 100% survivability, with zero mortality and excellent growth when placed in Ciliwung River waters. Furthermore, other types of *Bacopa*, such as *Bacopa monnieri*, are known to have the ability to reduce can reduce oxidative stress in rat brains accumulate [12] and absorb pesticides such as *oxamyl, hexachlorocyclohexanes* (α-HCH, β-HCH, and γ-HCH), *dichlorodiphenyl trichloroethane,* and *dichlorodiphenyl dichloroethylene* [16].

The lowest viability level is observed for *Alternanthera reineckii*, which only reached 26.67% survivability. Other aquatic plants, i.e., *Eicchornia diversifolia* and *Ludwigia* sp. had survival viability of approximately 80%. This indicates that *Bacopa australis* can accumulate water pollutants from the Ciliwung River waters while being kept in the aquarium. Additionally, other species of Alternanthera *philoxeroides* can accumulate chromium contaminated [17], along with other metal wastes such as vanadium, chromium, and cadmium [18]. *Alternanthera bettzickiana* also absorb heavy metals such as lead (Pb), which are found abundantly in its stems, leaf, and much in the roots [7]. Other species from the *Ludwigia* family, such as *Ludwigia octovalvis* can remediate oil or hydrocarbon spills up to a maximum of 79.8% in the experiments conducted by [19]. It is capable of bioremediation of oil spill, but *Ludwigia octovalvis* is also observed to be a bio sorbent of arsenic via their root [19]. Based on our research, *Ludwigia*, sp has an absorption of zinc capacity (98,05±1,72 mg/kg) from Ciliwung River waters. Similar reported by [20] at *Ludwigia prostrata* Roxb potential to absorb Cu, Pb and Zinc (0.94 and 19.17 mg/ kg). Another interesting report by Titah [21] regarding the phytoremediation ability of other *Ludwigia* species is *Ludwigia octovalvis* (Jacq.) Raven can bind 10 types of rhizobacteria at their roots, and these rhizobacteria can absorb arsenic up to> 1,500 mg / L.

IV. CONCLUSION

There are fifteen types of heavy metals polluting the Ciliwung River waters. Aquatic plant from tissue culture products is very adaptive and can absorb of water pollutants with high adaptability. *Bacopa* sp, *Eichornia diversifolia,* and *Ludwigia* sp are shown to have an optimal ability to absorb heavy metals. Moreover, *Bacopa* sp. is incredibly capable of adapting to adverse conditions with 100% survivability and can absorb all the heavy metals in the Ciliwung River waters. Aquatic plant from tissue culture product is extremely suitable to be used as a biosorption and phytoremediation agent in water pollution.

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