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Plant Effectiveness of *Acorus calamus, Pistia stratiotes,* And *Azolla pinnata* as Hyperaccumulator Candidate of Phytoremediation Agent for Copper (Cu) Absorption

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ABSTRACT

Metals are harmful pollutants because they cannot be broken down by living organisms. An excess of metal can become toxic. The metal in question is copper (Cu). This research aimed to determine the phytoremediation effectiveness of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* as candidates for *Cu* metal absorption. This investigation employed a wholly random factorial design with two factors: plant absorbent materials comprised of three plant species (*Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata*) and absorption concentrations of 2 and 5 ppm. The ANOVA test was used to analyze the data, followed by the average difference test. According to the results of this study, three plants—*Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata*—are capable of reducing *Cu* metal in water. The *Acorus calamus* plant can reduce *Cu* concentrations in water from 2 ppm to 96.85% and from 5 ppm to 96.80%. *Pistia stratiotes* plants can reduce *Cu* level in water from 2 ppm to 96.50% and 5 ppm to 99.94% at concentrations of 2 and 5 ppm, respectively. The *Azolla pinnata* plant can reduce *Cu* level in water from 2 ppm to 98.50% and from 5 ppm to 96.54% at concentrations of 2 ppm and 5 ppm, respectively. The highest BCF value at a concentration of 2 ppm is in the roots of *Azolla pinnata* plants, at 6.77 mg/kg, followed by the leaves, at 8.88 mg/kg. The maximum *BCF* value at a concentration of 5 ppm for *Pistia stratiotes* plants is 2.26 mg/kg for the roots and 2.46 mg/kg for the foliage. The greatest concentration of TF at 2 ppm in *Azolla pinnata* is 1.31 mg/kg, while the maximum concentration at 5 ppm in *Acorus calamus* is 1.98 mg/kg.

Keywords: *Acorus calamus, Cu, Azolla pinnata, Pistia stratiotes*

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INTRODUCTION

Metals are harmful pollutants because they cannot be broken down by living organisms. An excess of metal can become toxic. Copper (Cu) is an instance of such a metal. Copper is a metal element that cannot be decomposed by natural processes and is a very hazardous metal (Cai et al., 2019). The process of treating biological debris with growing media is known as phytoremediation. Phytoremediation is the use of plants and soil microorganisms to reduce the concentration or deleterious effects of environmental

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contaminants. Phytoremediation can be utilized to remove heavy metals, radionuclides, and organic contaminants, among others.

This technique is eco-friendly. Phytoremediation is derived from the Greek word *phyto* (fito) for plant and the Latin word remedium, which means to restore or eradicate. Green plants have a tremendous capacity for absorbing environmental pollutants and completing detoxification through a variety of mechanisms. Phytoremediation is the least expensive method among those available (Muthusaravanan et al., 2018). Metals can be absorbed by all plants, albeit in variable quantities. A number of plants are hyperaccumulators, as they are hypertolerant and capable of accumulating metals in high concentrations. Rhizofiltration describes the capacity of aquatic plants to absorb metals through their roots. This research utilized three plant species: *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata*. The objective of the selection of various plant species is to determine which plant species are more efficient at absorbing *Cu* metal from water.

Research utilizing the *Acorus calamus* plant in reducing *Cu* metal by 82.2% at a *Cu* concentration of 800 mL/L to 400 mL/L used the *Constructed Wetlands* technique (Feng Go et al., 2020). *Acorus calamus* bioaccumulates in absorbing *Cu* and *Fe* metals from 0.15 to 6.30 mg/L and in the roots of *Acorus calamus* contain *Cu* and *Fe* metals from 0.5 to 0.40 mg/L (Ding *et al.*, 2022). A research finding utilized the *Pistia stratiotes* plant in reducing Cu metal by 91.5% for 30 days (Tabinda *et al.*, 2018). Apart from absorbing *Cu* metal, *Pistia stratiotes* also bioaccumulates *Pb* metal at a concentration of 5 mg/L from 2.5 mg/L to 1.5 mg/L for 7 days and *Cd* metal at a concentration of 5 mg/L from 0.5 mg/L to 0.1 mg/L (Ergönül *et al.*,2020).

Research utilizing the *Azolla pinnata* plant to reduce *Cu* metal at a concentration of 50 mg/L is able to absorb Cu by 80% (Bind *et al.*, 2018). Bioaccumulation levels of Fe, Mn, Cu, Zn, Pb, Cd in Azolla are 87,6; 0; 99,1; 98,8; 88,2; 78,3; 77,5 of 76,7% respectively (Xinwei *et al.*, 2018).

AAS is an instrument used to determine the content of an element in a compound based on its atomic absorption. Used for the analysis of inorganic compounds or metals. The spectrum measured is in the UV-Vis region. The measured sample must be in the form of a clear solution. The ASS method is based on the absorption of light by atoms. Several studies were conducted by previous researchers as a reference for further research. This research was conducted to test the effectiveness of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* plants as candidates for phytoremediation agents for *Cu* metal absorption. This research aimed to determine the effectiveness of the plants *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* plants as candidates for *Cu* metal phytoremediation agents.

METHOD

This research was conducted in December 2022–March 2023 at the Greenhouse Laboratory of the USU Faculty of Agriculture. The source of the phytoremediation plants was Siombak Lake, Medan Marelan District; chemical analysis was done at the Basic Science Laboratory and the Shafera Enviro Laboratory, Kec. Lucky Field. The tests carried out in this study were to determine the effectiveness of the roots and leaves of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* as candidates for phytoremediation agents for the uptake of *Cu* heavy metal copper.

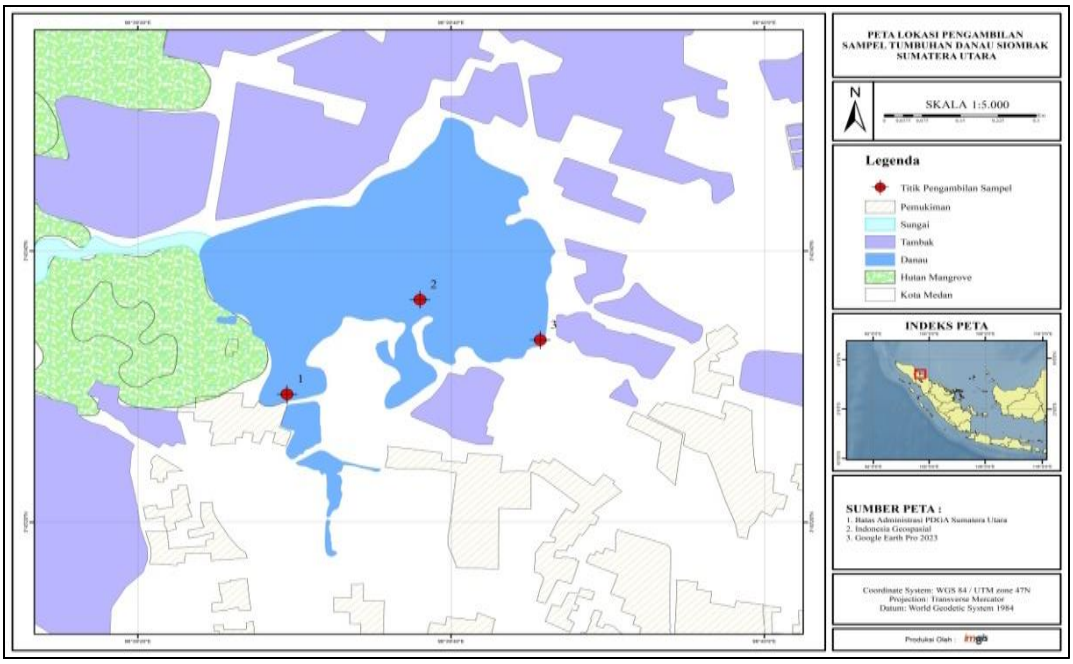

Figure 1. Plant sampling locations

The method used in this study was experimental. This research consisted of 2 stages: the first stage was the RFT (Range Finding Test) experiment, and the second stage was phytoremediation of *Cu*-polluted water using the *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* plants. This research used factor RAL (Completely Randomized Design) by making a standard solution of *Cu* copper heavy metal, testing the levels of *Cu* copper solution, and testing the parts of plant organs for absorbing levels of heavy metal *Cu* copper by compositing the same plant organ parts in repetition I, repetition II, and repetition III.

Table 1. Experimental design with treatment code

Treatment Code	Plant Treatment	*Cu* Concentration
T0K1	No Plants	2 ppm
T0K2	No Plants	5 ppm
T1K1	*Acorus calamus*	2 ppm
T1K2	*Acorus calamus*	5 ppm
T2K1	*Pistia stratiotes*	2 ppm
T2K2	*Pistia stratiotes*	5 ppm
T3K1	*Azolla pinnata*	2 ppm
T3K2	*Azolla pinnata*	5 ppm

As for repetition, each treatment got three repetitions so 24 experimental containers were needed. The preparation of the absorbent material began with taking the *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* plants from Lake Siombak, Medan Marelan District. The phytoremediation plants entered the acclimatization stage and were left for 14 days so that they could adapt to their new environment. The plants that had been left for 14 days were drained, weighed 100 g for each plant, and replicated. RFT was carried out in duplicate for 7 days at several concentrations of *Cu* pollutant, namely: 3, 5, and 15 ppm. The RFT results that had been carried out were taken at two concentrations for the phytoremediation experiment. Concentrations of 2 and 5 ppm were used for phytoremediation for 14 days using 3 repetitions. 2.512 g of Cu mass was needed to be put into a 100 ml volumetric flask and given distilled water until the boundary mark was homogenized, and making concentrations of 2 and 5 ppm by adding 2 and 5 ml of 1000 ppm mother liquor into each 1000 ml volumetric flask until entering 1000 ml of distilled water into the volumetric flask, then they were homogenized. Observation of the morphological conditions of the treated plant organs was carried out for 14 days.

According to Singha et al., (2019), The procedures for testing *Cu* copper content in plant extracts consist of: The plant organs were dried in the oven at 80°C, after drying, they were cut into small pieces and then blended until slightly smooth. The plant organs that had been blended were then weighed using an analytical balance of ± 1 g. The sample that had been blended was put into an Erlenmeyer volume of 25 ml, and then the code was marked with a label. Dissolve the sample in Erlenmeyer with 5 ml of 5 M HNO3 diluent. Heat the sample that had been dissolved in the fume hood until it boiled and its size becomes ½ of its original size. It was refrigerated for 15 minutes. The cooled solution was filtered using Whatman paper and placed in a 50 ml Erlenmeyer. After filtering, it was rinsed by the Erlenmeyer with 50 ml of distilled water. The dissolved plant organs and solutions were then measured for Cu levels in the solution and plant organs using AAS (Atomic Absorption Spectrophotometry), an instrument used to determine the levels of an element in a compound based on its atomic absorption.

Water Quality Measurement

The degree of power hydrogen (pH) and temperature were measured by a Eutech brand pH meter and thermometer. The pH meter and thermometer sensors were put into a bucket containing *Cu* solution according to the repeated filtration material and absorption time. This condition was left for a while until the number shown was constant.

Calculation of Copper Heavy Metal Bioaccumulation in *Acorus calamus, Pistia stratiotes, Azolla pinnata* Plants

BCG analysis was performed to determine the level of accumulation of the heavy metal as *Cu* in the roots and leaves of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata*. The bioconcentration factor is calculated by the formula (Eid et al., 2019).

$$\text{BCF} = \frac{Kb}{Cw}$$

Information
BCF: Bioconcentration (mg/kg)
Kb: Heavy metal content in roots and leaves (mg/kg)
Cw: Heavy metal content in solution (mg/L)

Plant categories can be divided into 3, namely: Accumulator: if the BCF value is > 1, Accumulators are plants that can accumulate high concentrations of metals in their plant tissues, even exceeding the concentrations in the soil. As a note, if the BCF value is

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< 1, the excluder is a plant that effectively prevents heavy metals from entering the upper area of the plant, but the metal concentration in the roots is still high. As a note, if the BCF is = 1, the category of plants as bioindicators is that plants tolerate the presence of metal concentrations by producing metal binding compounds or changing the composition of metals by storing metals in sensitive parts.

Translocation Factor (TF)

TF analysis is used to calculate the process of translocation of heavy metal copper Cu from roots to leaves. The TF calculation used is:

$$TF = \frac{Concentration\ on\ the\ leaves}{Concentration\ on\ the\ roots}$$

TF values have categories, namely: TF > 1: Phytoextraction mechanism. Phytoextraction is the process of absorption of heavy metals by plant roots which are then translocated to the stems and leaves. TF <1: Phytostabilization mechanism is a process carried out by plants to transform pollutants in the soil into non-toxic compounds without first absorbing these pollutants into the plant's body.

The results of the analysis of the content of the three plants obtained were processed statistically to see the ability of the three aquatic plants to absorb copper (*Cu*). The statistical analysis used was a two-way ANOVA test to see the effectiveness of the three aquatic plants in reducing copper (*Cu*) metal concentrations. If the data obtained was significantly different, then proceed with the Ducan test at the 5% level (Gomez and Gomez, 1995). All statistical analyses using the application statistical packet program (SPPS v25.0) were significant at $p < 0.05$.

RESULTS AND DISCUSSION

Range Finding Test (RFT) of Plant Morphology

RFT is carried out to determine the concentration at which the plant is able to live and whether there is any fatal damage to the plant's organs. RFT was carried out in duplicate for 7 days at several concentrations of Cu pollutant, namely: 3, 5, and 15 mg/L. The results of the RFT that have been carried out are two concentrations for the phytoremediation experiment. Concentrations of 2 and 5 mg/L were used for phytoremediation for 14 days using 3 repetitions.

Table 2. RFT observations of the *Acorus calamus* plant

***Cu* Concentration**	**Initial Observation (0 days)**	**Final Observation (7 days)**	**Description**	**Percentage**
3 ppm	Green and fresh plant, fresh roots	Fresh green plant, not fresh roots	Live plant	100
3 ppm	Green and fresh plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
5 ppm	Green and fresh plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
5 ppm	Green and fresh plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
15 ppm	Green and fresh plant, fresh roots	Yellow green leaves, fresh roots	Live plant	90

15 ppm	Green and fresh plant, fresh roots	Yellow green leaves, fresh roots	Live plant	90

Table 3. RFT observations of the *Pistia stratiotes* plant

***Cu* Concentration**	**Initial Observation (0 days)**	**Final Observation (7 days)**	**Description**	**Percentage**
3 ppm	Fresh green plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
3 ppm	Fresh green plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
5 ppm	Fresh green plant, fresh roots	Slightly yellow leaves, fresh roots	Live plant	90
5 ppm	Fresh green plant, fresh roots	Slightly yellow leaves, fresh roots	Live plant	90
15 ppm	Fresh green plant, fresh roots	Dry yellow leaves, brittle roots	Live plant	50
15 ppm	Fresh green plant, fresh roots	Dry yellow leaves, brittle roots	Live plant	50

Table 4. RFT observations of *Azolla pinnata* plant

***Cu* Concentration**	**Initial Observation (0 days)**	**Final Observation (7 days)**	**Description**	**Percentage**
3 ppm	Fresh green plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
3 ppm	Fresh green plant, fresh roots	Fresh green plant, fresh roots	Live plant	100
5 ppm	Fresh green plant, fresh roots	Green leaves, fresh roots	Live plant	80
5 ppm	Fresh green plant, fresh roots	Green leaves, fresh roots	Live plant	80
15 ppm	Fresh green plant, fresh roots	Dry leaves brittle roots	Live plant	60
15 ppm	Fresh green plant, fresh roots	Dry leaves, unbroken roots	Live plant	60

Table 5. Observation results of plants after phytoremediation treatment

Trial Code	**Observation in 7 days**		**Observation in 14 days**	
	Root	**Leaf**	**Root**	**Leaf**
T1KIU1	Fresh	Green	Fresh	Green
T1K1U2	Fresh	Green	Fresh	Green
T1K1U3	Fresh	Green	Fresh	Green
T1K2U1	Fresh	Green	Fresh	The green ones slightly turned to be yellow
T1K2U2	Fresh	Green	Brittle	The green ones slightly turned to be yellow
T1K2U3	Fresh	Green	Brittle	The green ones slightly turned to be yellow
T2K1U1	Fresh	Green	Brittle	Yellow
T2K1U2	Fresh	Green	Brittle	Yellow
T2K1U3	Fresh	Green	Brittle	Yellow
T2K2U1	Fresh	Yellow	Brittle	Dry yellow
T2K2U2	Fresh	Green	Brittle	Dry yellow
T2K2U3	Fresh	Green	Brittle	Dry yellow

T3K1U1	Fresh	Green	Brittle	Dry green
T3K1U2	Fresh	Green	Brittle	Dry green
T3K1U3	Fresh	Green	Brittle	Dry green
T3K2U1	Fresh	Green	Brittle	Dry green
T3K2U2	Fresh	Green	Brittle	Dry green
T3K2U3	Fresh	Green	Brittle	Dry green

Description :T1 = *Acorus calamus,* T2 = *Pistia stratiotes,* T3 = *Azolla pinnata*
K1 = 2 ppm, K2 = 5 ppm, U1 = Ulangan 1, U2 = Ulangan 2,
U3 = Ulangan 3

RFT results showed that *Acorus calamus* grows well at *Cu* concentration of 3, 5, and 15 ppm. Only at a concentration of 15 ppm, plant roots shows dead roots, and slightly turned to be yellow leaves. Based on research, it stated that the *Acorus calamus* plant is able to survive at a concentration of *Cu* metal of 20 ppm with a percentage of absorption of *Cu* metal of 90–94% (Chand et al., 2021).

Table 6. The Measuring of the roots length and leaves of plants after phytoremediation

		Before phytoremediation		After phytoremediation	
Plant name	**Concentration**	**Root length (cm)**	**Number of leaves (strands)**	**Root length (cm)**	**Number of leaves (strands)**
Acorus calamus	2 ppm	5.7	7	4.3	7
Acorus calamus	5 ppm	5.9	7	3.6	7
Pistia stratiotes	2 ppm	6.4	6	4.7	5
Pistia stratiotes	5 ppm	6.7	6	2.7	4
Azolla pinnata	2 ppm	2.5	8	2.2	6
Azolla pinnata	5 ppm	2.6	8	1.2	5

RFT results on *Pistia stratiotes* plant showed that it grows well at a concentration of 3 ppm; at a concentration of 5 ppm. The damage begins to occur in the form of yellow leaves at the tips. At a concentration of 15 ppm, the damage is more severe, namely dry and rotting leaves with the damage percentage of 50%. RFT results on *Azolla pinnata* plant showed that it grows well at *Cu* metal concentration of 3 and 5 ppm, but at a concentration of 15 ppm, the damage begins to occur in the form of dry and rotting leaves, with a damage percentage of 60%.

From the morphological condition of the plants at the time of phytoremediation for 14 days, it can be said that the *Acorus calamus* plant at a concentration of 5 ppm still shows a wilted condition and has a yellow green color, while *Pistia stratiotes* and *Azolla pinnata* at a concentration of 2 ppm have started to show discoloration, threshing, changes in water, and decay since the *Pistia stratiotes* and *Azolla pinnata*, at a concentration of 2 ppm, are actually starting to become ineffective in carrying out absorption.

Discussion

Based on the results of calculating the value of the bioconcentration factor (BCF), at a concentration of 2 ppm, the roots of the three plants have values ranging from 2.61 to 6.77 mg/kg, and the leaves of the three plants have values ranging from 2.19 to 8.88

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mg/kg. The highest BCF value in *Azolla pinnata* is in the roots with a value of 6.77 mg/kg, and the highest is in the leaves with a value of 8.88 mg/kg. While the value of the bioconcentration factor (BCF) is 5 ppm, the roots of the three plants have values ranging from 1.20 to 2.26 mg/kg, and the leaves of the three plants have values ranging from 0.86 to 2.46 mg/kg. The highest BCF value in *Pistia stratiotes* is in the roots with a value of 2.26 mg/kg, and the highest is in the leaves with a value of 2.46 mg/kg. The results of calculating the BCF values of the three plants prove that the three plants can accumulate heavy metals at concentrations of 2 and 5 ppm. If categorized according to Baker (1981), three plants are classified as accumulators of the heavy *Cu* metal with a BCF value of > 1. Accumulators are plants that can store high concentrations of metals in their plant tissues, even exceeding concentrations in water. The results of calculating the translocation factor (TF) value of *Cu* metal from roots to leaves in three plants at each concentration ranged from 0.55 to 1.98 mg/kg. The highest TF value of 2 ppm is in *Azolla pinnata* with a value of 1.31 mg/kg, and the highest TF value is 5 ppm in *Acorus calamus* with a value of 1.98 mg/kg. If categorized according to Baker (1981), three plants have a TF > 1 mechanism. Phytoextraction is the process of absorption of *Cu* metal by plant roots which is then translocated to the stems and leaves.

Root Organs

The higher the *Cu* content and the longer the absorption time grow, the more the root growth disturbs. The longest root is at a *Cu* concentration of 2 ppm, while the lowest is at a concentration of 5 ppm for 14 days. In general, *Cu* metal stress causes intracellular and extracellular damage, which results in growth disturbance (Singha et al., 2019).

Leaf Organs

The translocation of metals into the leaves can trigger damage to the cuticle and stomata of the leaves, which has an impact on the disruption of the respiration process and the process of photosynthesis, resulting in symptoms of leaf necrosis and chlorosis. The damage to plant organs that causes yellow color in leaves and roots is thought to be triggered by high levels of *Cu* absorbed by plants such as *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata*. The concentration of *Cu* metal is generally needed by plants in small amounts as a source of micronutrients. However, if *Cu* metal is present at high concentrations that are considered to exceed the quality standard limits, it will be the main trigger for plant cells to be disrupted and to experience lysis (Singha et al., 2019).

***Cu* Level in The Solvent and Plant Organs**

Acorus calamus, *Pistia stratiotes*, and *Azolla Pinnata* are plants that can reduce *Cu* levels in water, and can absorb Cu in roots and leaves. Accordong to Tabinda *et al.*, (2018), *Pistia stratiotes* can reduce *Cu* metal by 91.5% for 30 days. It proves that Pistia stratiotes can be used as a phytoremediation plant by absorbing *Cu* metal.

Table 7. Average *Cu* levels in water treated with plants and absorption concentrations

Plant Treatment	*Cu* concentration	Average *Cu* Levels in Water	*Cu* Levels in Roots	*Cu* Levels in Leaves	*Cu* Levels in Plants
No Plants	2 ppm	2	0	0	0
No Plants	5 ppm	5	0	0	0
Acorus calamus	2 ppm	0.063	0.260	0.143	0.403
Acorus calamus	5 ppm	0.160	0.193	0.383	0.576
Pistia stratiotes	2 ppm	0.070	0.183	0.153	0.336
Pistia stratiotes	5 ppm	0.153	0.347	0.377	0.724
Azolla pinnata	2 ppm	0.030	0.203	0.267	0.470
Azolla pinnata	5 ppm	0.173	0.270	0.150	0.420

The results of the analysis showed that the significance of *Cu* levels between plant species treatments is < 0.05. It means that there are significant differences in *Cu* levels in each plant species. It also explains that the types of plants tested have different levels of absorption of *Cu* level from water.

Acorus calamus

At a concentration of 2 ppm, the *Cu* content in water treated with *Acorus calamus* became 0.063 mg/L, thereby reducing *Cu* levels in water by 96.85%. At a concentration of 5 ppm, the absorption of *Cu* levels in water by *Acorus calamus* became 0.160 mg/L, thereby reducing *Cu* levels in water by 96.80%.

Pistia stratiotes

At an absorption concentration of 2 ppm, *Pistia stratiotes* reduces the amount of *Cu* content in water to 0.070 mg/L, which is 96.50% less than before. At a concentration of 5 ppm, *Pistia stratiotes* is able to absorb 0.153 mg/L of *Cu* from the water. It cuts the amount of *Cu* level in the water by 99.94%.

Azolla pinnata

At a concentration of 2 ppm, the *Cu* content in water treated with *Azolla pinnata* became 0.030 mg/L, thereby reducing *Cu* level in water by 98.50%. At a concentration of 5 ppm, the *Cu* content in the water with *Azolla pinnata* becomes 0.173 mg/L, thereby reducing the Cu content in the water by 96.54%.

Cu* Metal Bioaccumulation Factor (BCF) in *Acorus calamus*, *Pistia stratiotes*, *Azolla pinnata

Calculation of *Cu* metal bioaccumulation uses the formula of Ghosh and Singh (2005). Bioaccumulation is calculated to determine the ability of *Acorus calamus*, *Pistia stratiotes*, *Azolla pinnata* to accumulate heavy metals through the level of bioaccumulation factor (BCF).

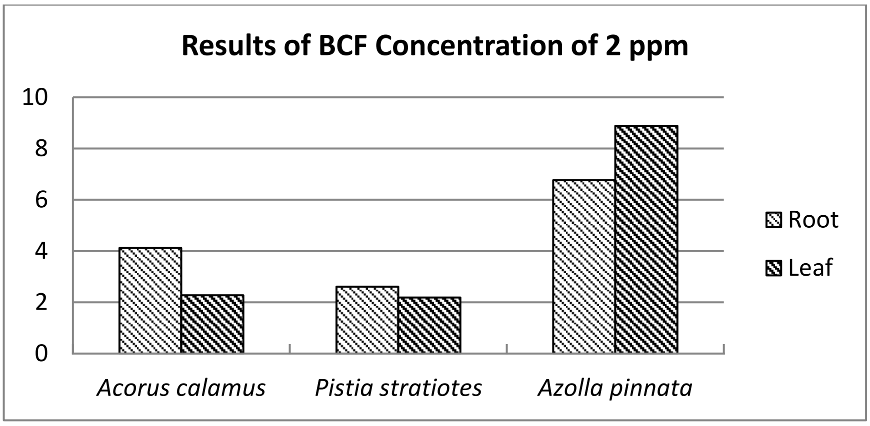

Figure 2. Diagram of the BCF results at 2 ppm of *Cu* concentration

The calculation results of *Cu* metal bioaccumulation in the roots and leaves of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* at a concentration of 2 ppm shows that the BCF value of the roots is greater than the BCF value of the leaves. The BCF value of the roots shows almost the same value for each plant. The highest root BCF value is at a concentration of 2 ppm, namely in *Azolla pinnata* plant with a value of 6.77 mg/kg, and the lowest BCF value is in *Pistia stratiotes* plant with a value of 2.61 mg/kg. Furthermore, the highest leaf BCF value is at a concentration of 2 ppm, namely in the *Azolla pinnata* plant with a value of 8.88 mg/kg and the lowest in the *Pistia stratiotes* plant with a value of 2.19 mg/kg. In detail, the BCF values for each plant can be seen in Figure 2.

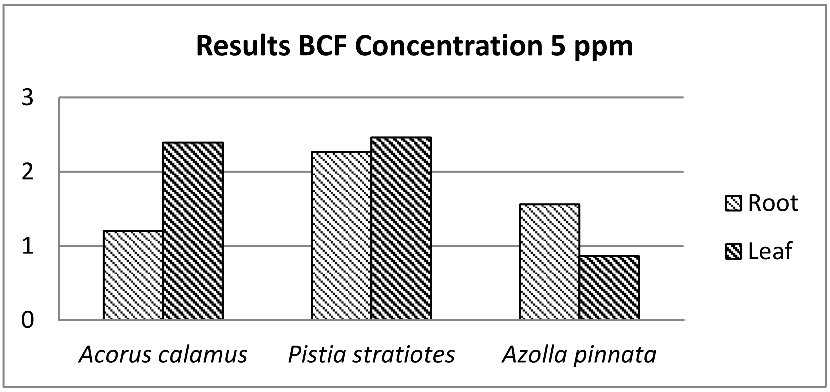

Figure 3. Diagram of the BCF results at 5 ppm of *Cu* concentration

The results of *Cu* metal bioaccumulation in the roots and leaves of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* at a concentration of 5 ppm shows that the BCF value of the leaves is greater than the BCF value of the roots. The BCF value of the roots shows almost the same value for each plant. The root with the highest BCF value at a concentration of 5 ppm is Pistia stratiotes, with a value of 2.26 mg/kg, and the root with the lowest BCF value is *Acorus calamus*, with a value of 1.20 mg/kg. Furthermore, the highest leaf BCF value is at a concentration of 5 ppm, namely in *Pistia stratiotes* with a value of 2.46 mg/kg and the lowest in *Azolla pinnata* with a value of 0.86 mg/kg. In detail, the BCF values for each plant can be seen in Figure 3.

Translocation Factor (TF) of *Cu* Metal in *Acorus calamus*, *Pistia stratiotes*, *Azolla pinnata*

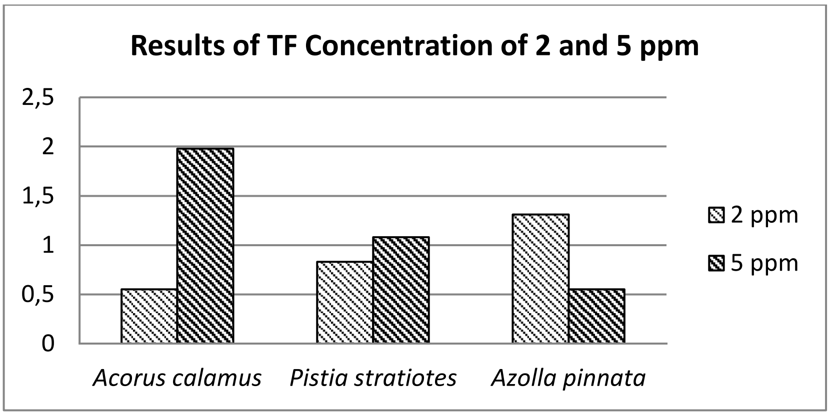

Figure 4. TF diagram of the three plants at concentrations of 2 and 5 ppm

The calculation of TF is used to calculate the translocation of *Cu* metal from roots to leaves using Baker's formula (1981). Calculation results for *Cu* metal shows almost the same value at each station. The highest TF value from root to leaf is 2 ppm in *Azolla pinnata* with a value of 1.31 mg/kg, and the lowest in *Acorus calamus* is 0.55 mg/kg. The TF value from root to leaf at a concentration of 5 ppm is in the *Acorus calamus* plant with a value of 1.98 mg/kg, and the lowest is in the *Azolla pinnata* plant, which is 0.56 mg/kg. It can be seen in Figure 4.

Table 8. Water Quality Parameters after phytoremediation

Concentration Parameter	Beginning	No Plant		*Acorus calamus*		*Pistia stratiotes*		*Azolla Pinnata*	
		K1	K2	K1	K2	K1	K2	K1	K2
Degree of Acidity (pH)	6.54	7.05	7.18	7.51	7.81	7.33	7.68	7.59	7.86
Temperature (°C)	28.05	28.12	28.89	28.77	29.57	29.27	30.2	29.77	30.70

Water Quality Parameters

Power of Hydrogen (pH)

The plant condition that has a high water temperature and pH, is characterized by the physical condition of the plants, which turn yellow, wither, and begin to fall off. The higher the absorption of Cu levels by aquatic plants, the higher of pH water (Sigcau et al., 2022). According to metal toxicity, plant roots, particularly those of the Acorus calamus plant, have absorbed or bound *Cu*, which is what has caused the decrease in the pH level. Optimal *Cu* absorption is accompanied by an increase in the degree of pH. According to Regulation of the Minister of Health 492 of 2010, concerning water quality, the pH value included in this requirement is 6.5–8.5. From the results of research in the field, all degrees

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of pH in the three plants still meet Regulation of the Minister of Health 492 of 2010 (Permenkes, 2010).

Temperature

Normal temperature conditions in water areas that support photosynthesis and plant survival are 22°C – 25°C. The highest temperature in the treatment of aquatic plants that had the lowest *Cu* level in this study is 28.12°C with absorbed Cu levels of 0.17 mg/L (Paz-Ferreiro et al., 2014). The temperature of the three aquatic plants in the field ranges from 28.27°C – 29.80°C, the water temperature for plant growth is around 22°C – 30°C. At this temperature, it is possible for the three plants to survive and absorb to the maximum extent. According to Regulation of the Minister of Health Number 492 of 2010, the temperature that meets the requirements is 27°C – 28°C, from the results of measurements of water temperature in the field at three plants, it still meets the temperature requirements of Regulation of the Minister of Health 492 of 2010 (Permenkes, 2010).

Changes in Plant Biomass to *Cu*

The wet weight value of *Acorus calamus*, *Pistia stratiotes*, and *Azolla pinnata* has increased from the initial wet weight of the plants before treatment, which is 100 g. The highest values for wet weight and dry weight for *Acorus calamus* occurr at a concentration of 2 ppm, with a wet weight value of 100.32 g and a dry weight of 5.20 g. The highest wet weight and dry weight values for *Pistia stratiotes* occurr at a concentration of 2 ppm, with a wet weight value of 100.06 g and a dry weight of 4.47 g.

Table 9. Changes in plant biomass and uptake of *Cu*

Plant Type	*Cu*	Wet Weight (g)	Dry Weight (g)	Water content (%)
Acorus calamus	2 ppm	100.32	5.20	94.81
Acorus calamus	5 ppm	87.70	4.77	94.56
Pistia atratiotes	2 ppm	100.06	4.47	95.53
Pistia stratiotes	5 ppm	79.80	4.20	94.73
Azolla pinnata	2 ppm	100.05	3.47	96.53
Azolla pinnata	5 ppm	75.06	3.13	95.83

The highest values of wet weight and dry weight of Azolla pinnata occurr at a concentration of 2 ppm with a value of 100.05 and a dry weight of 3.47 g. Based on the statistical test of diversity, the treatment given to plants shows a significant effect on biomass, as can be seen in Table 4.8. Meanwhile, the concentration of metal pollutants affects plant growth. Plants that experience pollution with lower concentration values have a better rate of photosynthesis and produce large amounts of carbohydrates, resulting in an increase in the specific gravity of the leaves, which is directly proportional to their dry weight (Irawanto & Munandar, 2017).

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CONCLUSION

Concentration of 2 ppm, the roots of the three plants have a value ranging from 2.61 to 6.77 mg/kg, and the leaves of the three plants have a value ranging from 2.19 to 8.88 mg/kg. The highest BCF value in *Azolla pinnata* plant is in the roots with a value of 6.77 mg/kg, and the highest in the leaves with a value of 8.88 mg/kg. While the value of the bioaccumulation factor (BCF) is 5 ppm, the roots of three plants have values ranging from 1.20 to 2.26 mg/kg, and the leaves of the three plants have values ranging from 0.86 to 2.46 mg/kg. The highest BCF value in *Pistia stratiotes* is in the roots with a value of 2.26 mg/kg, and the highest in the leaves with a value of 2.46 mg/kg. The value of the translocation factor (TF) of the heavy *Cu* metal from roots to leaves in three plants at each concentration ranges from 0.55 to 1.98 mg/kg. The highest TF value of 2 ppm is in *Azolla pinnata* with a value of 1.31 mg/kg, and the highest TF value is 5 ppm in *Acorus calamus* with a value of 1.98 mg/kg. The *Acorus calamus* is a plant that can absorb *Cu* stably; the *Pistia stratiotes* plant is able to reduce *Cu* at a concentration of 5 ppm; and *Azolla pinnata* is able to reduce *Cu* at a concentration of 2 ppm.

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