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Translocation Mechanism of Lanceleaf Arrowhead (*Sagittaria lancifolia*) on Copper (Cu) and Phytoremediation Ability

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Abstract

Copper (Cu) is a heavy metal that pollutes the environment. One of the methods to resolve Cu pollutants is phytoremediation; which is the utilization of plants to absorb pollutants. Lanceleaf arrowhead (*Sagittaria lancifolia*) is proven to absorb copper. Metal absorption and translocation mechanism in plants have not been understood in more detail. This study aims to determine the ability of lanceleaf arrowhead (*Sagittaria lancifolia)* and the translocation factor in copper (Cu) phytoremediation. This study is an experimental study with a Completely Randomized Design (CRD). The experiment was conducted with eight treatments and three replications. The treatments were distinguished based on differences in the concentration of Cu used: 0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L. The concentration of Cu in the media and plant was measured for two weeks and four weeks, respectively. The results indicated that the value of the translocation factor at two weeks of exposure ranged from 0.57 to 0.6, while that at four weeks of exposure ranged from 1.5 to 2.33. Furthermore, at two weeks of exposure, the average metal loss ranged from 0.49 to 0.89 ppm, while that at four weeks of exposure ranged from 0.64 to 1.97 ppm. The highest effectiveness value of 66% was obtained in the three ppm treatment with four weeks of exposure. Meanwhile, the lowest effectiveness value of 30% was obtained in the three ppm treatment with two weeks of exposure. In addition, *Sagittaria lancifolia* was influential in the phytoremediation of copper (Cu) and could translocate metal into their tissues at four weeks of exposure.

Keywords: Copper; Lanceleaf arrowhead; Phytoremediation; Translocation

1. Introduction

Water environments are one of the areas prone to pollution (Afifudin & Irawanto, 2021), one of which includes heavy metals. Several types of heavy metals commonly found in waters include copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), vanadium (V), cobalt (Co) and nickel (Ni) (Delshab *et al.,* 2016; Rezaei *et al.,* 2021). Among those heavy metals, copper (Cu) dominantly pollutes waters (Filipus, 2018). Besides wetland waters, Cu has also been reported to contaminate marine waters (Rukmi, 2019; Yunasfi & Singh, 2019). One of the sources of Cu pollution is the electroplating or metal coating industry (Sekarwati, 2014). If Cu accumulates in high concentrations for an extended period in our body, it will disrupt our body's metabolic processes (Siotto & Squitti, 2018). Therefore, resolving copper (Cu) pollution is crucial, especially in water areas, because most of our activities require water (Fitria, 2014).

One of the methods to resolve heavy metal pollution in water areas is utilizing plants as pollutant absorbing agents. This method is called phytoremediation. The advantages of phytoremediation techniques include low cost, low contamination risk, not producing hazardous waste, and accessible treatment (Tang *et al.,* 2020). Many plants have been investigated for the phytoremediation of copper (Cu), including aquatic plant species such as *Juncus effusus, Acorus calamus, Eichhornia crassipes, Sagittaria sagittifolia, Arundina graminifolia, Echinodorus major, Nymphaea tetragoma, Salvinia molesta,* and *Pistia stratiotes* (Lu *et al.,* 2018; Mustafa & Hayder, 2021). However, several organisms also showed their potential for remediation of polluted environments, including microalgae (Musa *et al.,* 2020), fish (Dwipayanti *et al.,* 2021), and *Scenedesmus* sp (Nurhayati *et al.,* 2021). The utilization of aquatic plants in phytoremediation is based on the principle that all plants can absorb pollutants in their bodies but have different abilities and tolerance levels (Hidayati, 2020).

Plants with more ability to accumulate pollutants in their bodies are called hyperaccumulator plants. Some characteristics of hyperaccumulator plants include a high tolerance limit to pollutant stress, significant absorption rate and capacity, and the ability to translocate pollutants into plant parts completely (Hidayati, 2013). However, there is no scientific standard for hyperaccumulator plants, and there is no quality standard yet stating whether these plants are included in the hyperaccumulator category or not (Hidayati, 2013; Nazir *et al.,* 2011). Meanwhile, according to Ali (Ali *et al.,* 2013), hyperaccumulator plants are plants with a translocation factor score of higher than one (> 1).

Lanceleaf arrowhead (*Sagittaria lancifolia*) is an ornamental plant indicated to absorb Cu highly and can be categorized as a hyperaccumulator plant. It has been proven by several studies that revealed the effectiveness of lanceleaf arrowhead in remediating some pollutants, including crude oil, chromium (Cr), detergent, and LAS (Adistiara & Kustiyaningsih, 2019; Dowty *et al.,* 2001; Fitrihidajati *et al.,* 2020; Lindau *et al.,* 2003; Serang *et al.,* 2018). Another plant from the *Sagittaria* genus, namely S. *montevidensis*, was subsequently detected as presenting a natural phytoextraction ability for potassium and calcium elements and also exhibiting rhizofiltration potential for phosphorus, manganese, aluminum, vanadium, sulfur, iron, arsenic, copper, magnesium, zinc, sodium, lead, cadmium, nickel, and chromium (*Demarco et al.,* 2019). Moreover, *S. latifolia* reduced imidacloprid by 79.3% (*McKnight et al.,* 2022) and significantly increased the diesel removal ratio, from 21 ~ 36% to 54 ~ 85% in soil (Zhang *et al.,* 2015).

S. lancifolia is a plant that easily adapts to various water conditions. So far, the lanceleaf arrowhead is just an ornamental plant that has not been utilized in phytoremediation (Dewi *et al.,* 2018). The advice to using non-food plants in phytoremediation arises from the assumption that people eating vegetables with heavy metals can negatively impact health (Agil *et al.,* 2017). Therefore, this study aims to determine the lanceleaf arrowhead (*Sagittaria lancifolia*) translocation mechanism on copper (Cu) and their phytoremediation ability in more detail.

2. Materials and Methods

It is an experimental study with a Completely Randomized Design (CRD). The experiment was conducted with eight treatments with three replications. The variation of Cu concentration included 0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L. Furthermore, Cu levels in water and plants were measured for two weeks and four weeks. The tools used in this study included a reactor in the form of a 5-liter plastic tub, measuring cup, glass bottle, funnel, measuring pipette, bulb, analytical balance, ruler, scissors, oven, and AAS (Atomic Absorption Spectroscopy) instrument. Meanwhile, the materials used in this research included $CuSO_4$ solution, aquades, filter paper, aquatic plant lanceleaf arrowhead (*Sagittaria lancifolia*), standard solutions of Cu, and HNO_3. The picture of the lanceleaf arrowhead is shown in Figure 1.

Figure 1. Lanceleaf arrowhead (*Sagittaria lancifolia*)

Several parameters observed in this study included (a) metal concentration in the media, (b) metal concentration in plant roots and shoots, (c) translocation factor, (d) absorption rate, and (e) removal efficiency. Furthermore, this research was conducted in several stages: acclimatization, preparation, and experiment.

The calculation of the parameters was as follows:

a) Translocation Factor (Alharbi *et al.,* 2019; Zhang *et al.,* 2018)

$$TF = \frac{Ms}{Mr}$$

Description:
Ms: Metals concentration in the shoot
Mr: Metals concentration in the root

b) Absorption Rate (Indrasti *et al.,* 2006)

$$AR = \frac{(\,Sw\ x\ Ms\,) - (\,Rw\ x\ Mr\,)}{(\,Sw + Rw\,)\ x\ t}$$

Description:
Sw: Shoot weight
Rw: Root weight
Ms: Metal concentration in the shoot
Mr: Metal concentration in the root
t: Period

c) Removal Efficiency (Khoso *et al.,* 2021; Pashaei *et al.,* 2018)

$$RE = \frac{I0 - I1}{I0} \times 100$$

Description:
I0: Initial metals concentration in media
I1: Final metals concentration in media

2.1 Preparation

The concentration preparation was based on variations in the concentration of Cu used, namely 0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L. The primary liquor used was $CuSO_4$, with a concentration of primary liquor diluted with the formula (Afifudin & Irawanto, 2021):

M1 x V1 = M2 x V2

Description:
M1: Initial solution concentration
M2: Desired solution concentration
V1: Initial water volume
V2: Water volume after dilution

2.2 Experiment

The first stage, acclimatization, began by taking Lanceleaf arrowhead with a size of ± 45 cm from the collection in a field. Then the plants were left in the reactor for one month without additional greenhouse pollutants. Acclimatization aims for the plant to adapt to new environmental conditions to minimize bias in research and provide the plants' specific conditions (Afifudin *et al.,* 2022; Heathcote *et al.,* 2018; Purwanti *et al.,* 2020).

After acclimatization for one month, each plant sample was put in each reactor containing Cu with different concentrations and two liters of distilled water media. The plant was observed periodically at each parameter. Furthermore, Cu concentration in the plant was measured by AAS (Atomic Absorption Spectroscopy) instrument.

The standard solution of copper (Cu) was diluted with HNO_3 of 1%. The solution was prepared in concentrations of 0.05, 0.1, 0.25, 0.5, 1, 1.25, 1.5, 2, 2.25, 2.5, and 3 ppm. Ten grams of root and shoot dry powder samples were put into separate crucible porcelains and burnt at low heat until dissolved. Then, two ml of nitric acid were put into crucible porcelains and reheated at 500 °C up to 600 °C. Wait for the sample until no more white smoke is formed. Remove the sample from the heat, let it cool down, and transfer it back to the 25 ml volumetric flask. Samples were filtered with ash-free filter papers and observed with an AA 6200 Atomic Absorption Spectrometer (AAS) to measure the concentration of Copper (Cu) in the roots and shoots of *Sagittaria lancifolia* (Agil *et al.,* 2021). Meanwhile, the measurement of the copper content in water sample was carried out with the same method, but 50 mL of the water sample was taken to be tested with AAS preserved with 65% nitric acid, and the AAS wavelength was set to 249.2 nm (Tang *et al.,* 2020).

2.3 Data analysis

Analyzing several parameters such as translocation factor, absorption rate, absorption power, and effectiveness value was done qualitatively by calculating, observing, and describing data and facts sequentially. Meanwhile, the Cu content in the media was analyzed using the 2-samples paired T-test to find the difference in metal content in the media before and after treatment.

3. Results and Discussion

All plants basically have the ability to absorb pollutants but in varying amounts and resistance levels. Several species from the plant families have been tested for the ability to phytoremediation of Cu. Based on previous studies, several plants have more resistance to pollutants or hyper-tolerant properties, especially heavy metals at high concentrations. Based on preliminary studies on the ability of lanceleaf arrowhead to absorb copper (Cu), the maximum tolerance limit of Cu that spear leaves can tolerate is five mg/L (Afifudin & Irawanto, 2021). Meanwhile, the average concentration of Cu in the media or environment is 0.05 - 0.5 mg/L (Ed Bloodnick, 2021). However, these studies did not reveal Cu metal's absorption and translocation mechanism in plants. This study shows that lanceleaf arrowhead can remediate copper because this plant can absorb and translocate the heavy metal to their tissue. Furthermore, this study also proves that copper is essential for plants.

3.1 Cu Content in Media and Plants

Measuring Cu concentration in water (planting media) aims to determine the amount of metal remaining. At the same time, measuring Cu content in plants seeks to determine the amount of metal absorbed and the mechanism of translocation.

Table 1. Cu Content in Media

No	Concentration Variation	Exposure Time	Cu Concentration (mg/L)	
			Before	After
1	Control	2 weeks	0	0.02 ± 0.01
2	1 mg/L	2 weeks	1	0.51 ± 0.08
3	2 mg/L	2 weeks	2	1.38 ± 0.02
4	3 mg/L	2 weeks	3	2.11 ± 0.05
5	Control	4 weeks	0	0.01 ± 0.01
6	1 mg/L	4 weeks	1	0.36 ± 0.08
7	2 mg/L	4 weeks	2	0.83 ± 0.07
8	3 mg/L	4 weeks	3	1.03 ± 0.06

In this study, the concentration of copper before treatment was not measured. It was assumed that the concentration of Cu in media was the same as the concentration variation of experimental treatment, namely 0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L. As shown in Table 1, in the control treatment, it was assumed that the initial metal contained in the water was 0 mg/L. However, after observing for two weeks and four weeks, Cu content in the water was 0.02 mg/L and 0.01 mg/L. Meanwhile, the normal range of Cu content in the media (environment) was 0.05 – 0.5 (Ed Bloodnick, 2021). So, it could be concluded that Cu concentration in the initial media in this study was considered moderate. Table 1 also showed that more metals were absorbed along with the length of exposure. It was indicated by the less metal content remaining in the media. It proved that the *Sagittaria lancifolia* could still absorb Cu metal relatively well even though it had been exposed to the metal for an extended period. Furthermore, the statistical analysis results showed p-value of 0.016, indicating a difference between the levels of Cu in the media before and after treatment in plant exposure. This difference proves that Lanceleaf arrowhead had absorbed Cu in the media. Moreover, based on Table 1, at a concentration variation of 3 mg/l, during a two-week exposure, the metal remaining in the media was 2.11 mg/L, while at a four-week exposure, the remaining metal was 1.03 mg/L. So, it could be assumed that the metal in the media would be absorbed entirely in 6 to 7 weeks.

As shown in Table 2, there was a Cu content in the roots and shoots of the plant, even in the control treatment or without metal addition. It proved that Cu is an essential metal for plants in their metabolic processes (Djo *et al.,* 2017; Sağlam *et al.,* 2016). Table 2 also revealed that higher metal concentration in the media was directly proportional to Cu content in the plant roots and shoots. According to (Lestari & Pratama, 2020), the absorption rate of Cu by the plant was affected by different densities and high levels of pollutants in the environment. Furthermore, the metal concentration in the shoots was relatively lower than that in the roots at a two-week exposure. Meanwhile, at a four-week exposure, the metal concentration in the shoots was higher than that in the roots, indicating that the lanceleaf arrowhead had translocated the metal entirely in its body. This result proved that the lanceleaf arrowhead has the characteristics of a hyperaccumulator plant (Hidayati, 2013).

3.2 Translocation Factor

The calculation of the translocation factor aimed to determine the ability of the plant to translocate metals from the roots to all of its areal tissues (Mleczek *et al.,* 2017; Rezaei *et al.,* 2021) because the accumulation of Cu was different in various parts of the aquatic plant (Lu *et al.,* 2018). According to Ali *et al.,* (2013), hyperaccumulator plants are those with a metal concentration in the shoots and roots (Translocation Factor) of greater than 1 (> 1).

Table 2. Cu Content in the Plant

No	Concentration Variation	Exposure Time	Cu Concentration (mg/L)	
			Root	Shoot
1	Control	2 weeks	0.008 ± 0.00	0.005 ± 0.00
2	1 mg/L	2 weeks	0.28 ± 0.06	0.16 ± 0.04
3	2 mg/L	2 weeks	0.45 ± 0.07	0.27 ± 0.03
4	3 mg/L	2 weeks	0.76 ± 0.07	0.43 ± 0.07
5	Control	4 weeks	0.01 ± 0.01	0.01 ± 0.01
6	1 mg/L	4 weeks	0.24 ± 0.05	0.36 ± 0.05
7	2 mg/L	4 weeks	0.42 ± 0.07	0.98 ± 0.06
8	3 mg/L	4 weeks	0.79 ± 0.06	1.22 ± 0.08

Table 3 presents that the score of the translocation factor from the treatment with a contact time of 2 weeks was 0.6. This score was less than 1, meaning that the lanceleaf arrowhead had not translocated the pollutants entirely in their body within two weeks. However, after two weeks, the score of the translocation factor in each treatment was 1.5, 2.33, and 1.54. Based on these results, it could be concluded that copper had the highest potential to be transferred from the plant's roots to the tissues. Furthermore, the lanceleaf arrowhead had translocated metals from its roots to all of its tissues (Figure 2), meaning that this plant was an effective plant for copper transmission from the roots to the leaves (Rezaei *et al.,* 2021). Besides, with its ability, this plant could be included in the hyperaccumulator plant group.

The score of the translocation factor (TF) of > 1 indicates that more Cu is allocated to the shoots than to the roots, while the translocation factor (TF) of < 1 suggests that more Cu is allocated to the roots (Anisa, 2020). Furthermore, the translocation factor measures plant defense mechanisms that tend to limit inorganic contaminants from roots to avoid the translocation of trace elements to vital plant organs, especially seeds. TF > 1 indicates that plants not only tolerate contaminants but also utilize contaminants for something useful, which is usually a characteristic of hyperaccumulators. Thus, TF > 1 is a determining factor for categorizing plant species for phytoremediation (Antoniadis *et al.,* 2017).

3.3 Phytoremediation Ability

This study determined the phytoremediation ability of the plant under copper (Cu) exposure by calculating the absorption rate and removal efficiency. The calculation of the metal uptake rate of the plant was based on the dry weight of the metal (mg/kg) that had been accumulated in the plant (shoots and roots) and the dry weight of the plant (mg). Meanwhile, this study's metal removal efficiency calculation was based on the decrease in the concentration of Cu in water (mg/L).

Table 3. Translocation Factor

No	Concentration Variation	Translocation Factor	
		2 weeks	4 weeks
1	Control	0,6	1
2	1 mg/l	0.6	1.5
3	2 mg/l	0.6	2.3
4	3 mg/l	0.6	1.5

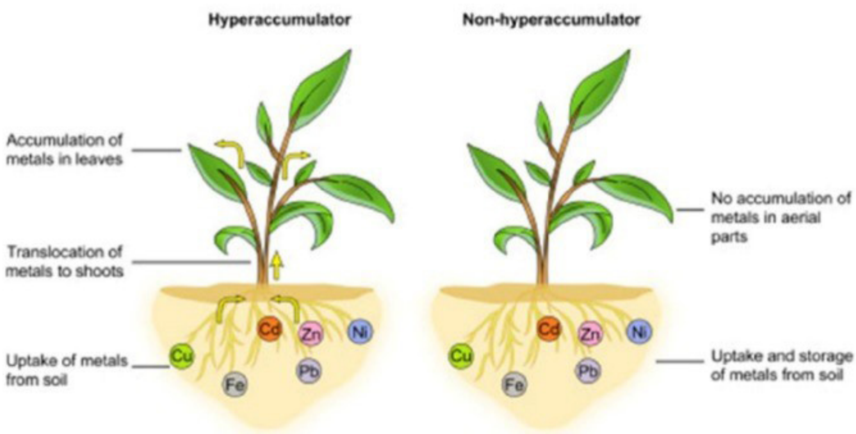

Figure 2. Difference between hyperaccumulator and non-hyperaccumulator plants (Seeda *et al.,* 2020)

Table 4. Phytoremediation ability

No	Concentration Variation	Exposure Time	Absorption Rate (mg/kg.day)	Removal Efficiency (%)
1	Control	2 weeks	0.02 ± 0.02	0%
2	1 mg/L	2 weeks	1.27 ± 0.38	49%
3	2 mg/L	2 weeks	2.38 ± 0.20	31%
4	3 mg/L	2 weeks	3.42 ± 0.56	30%
5	Control	4 weeks	0.06 ± 0.08	0%
6	1 mg/L	4 weeks	1.65 ± 0.20	64%
7	2 mg/L	4 weeks	6.53 ± 0.49	59%
8	3 mg/L	4 weeks	12.11 ± 0.73	66%

Table 4 shows that the higher the concentration of heavy metals and the longer the exposure time, the higher the absorption rate. Following Table 2, the higher the concentration of Cu metal and the longer the contact time, the more metal the plants absorbed. It was most likely due to pressure differences between two types of media: tissue and plant growth media (in this context, water) (Caroline & Arron, 2015). This concentration difference stimulated mass transfer (Cu) due to diffusion and osmosis. In this case, the mass of substances in a high concentration medium moved to a low concentration medium (Haryati *et al.,* 2012). Furthermore, root pressure and transpiration rate also affected the absorption rate (Anisa, 2020).

Table 4 also presents the value of removal efficiency. If we look at the treatment with a contact time of 2 weeks, the removal power decreased as the concentration of Cu metal increased. The decrease in copper removal efficiency by *Sagittaria lancifolia* and the increase in copper concentration might be due to copper toxicity, affecting the plant's physiological processes and health (Abbas *et al.,* 2019). However, in the treatment with an exposure time of 4 weeks, the removal power increased at the highest concentration (3 mg/L). The relatively high copper removal efficiency reported in this study also demonstrated the ability of lanceleaf arrowhead (*Sagittaria lancifolia*) to mediate on both nutrient-poor and nutrient-poor media, as indicated by the increased removal efficiency (Tang *et al.,* 2020). Furthermore, Afifudin *et al.,* (2022) also reported that *Sagittaria lancifolia* did not respond poorly under heavy metal copper stress.

The results of other studies also supported phytoremediation, one of which was conducted by Imron *et al.,* (2019), stating that L. *minor* was a potential phytoremediation agent to remove dyes from wastewater. Amalia *et al.,* (2019) also stated that Pistia stratiotes could reduce lead concentration in water. Besides, Fida *et al.,* (2021) stated that Tabebuya could absorb lead-like pollutants (Pb). In addition, Wardani *et al.,* (2017) stated that 15 plants of L. flava with direct contact with wastewater in 13 days could decrease heavy metal accumulation value. Irawanto & Prastiwi, (2019) also found that aquatic thematic collections were used as applications for environmental phytoremediation. Al-Ajalin *et al.,* (2020), in his study, suggested that future related studies should accommodate the importance of several environmental conditions to the interaction between pollutants, plants, medium, and microbes, as well as the impact of those interactions on the pollutant removal efficiency.

4. Conclusion

Lanceleaf arrowhead (*Sagittaria lancifolia*) proved effective for the phytoremediation of copper (Cu). Furthermore, four weeks of treatment indicated that the lanceleaf arrowhead (*Sagittaria lancifolia*) had properly translocated metals from the roots to the shoots.

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