

The Ability of Mexican Sword Plant (*Echinodorus palaefolius*) as Copper (Cu) Phytoremediator

*Kemampuan Melati Air (*Echinodorus palaefolius*) sebagai Fitoremediator Logam Tembaga (Cu)*

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Abstract. Water pollution can come from inorganic pollutants like copper (Cu). Copper in high concentrations will cause toxic effects, so water quality treatment efforts are needed. Phytoremediation can be used to treat water polluted with heavy metals whose use will support the Sustainable Development Goals (SDGs). One plant with potential as a phytoremediator is mexican sword plant (*Echinodorus palaefolius*). This study aims to describe the ability of *Echinodorus palaefolius* as a phytoremediation agent to reduce Cu levels in the growing media, describe the effect of variations in Cu concentration on leaf chlorophyll levels and wet biomass. Data were analyzed using the SPSS one-way ANOVA test followed by the Duncan test. Analysis of supporting data in the form of pH and water temperature was done descriptively quantitative. The results showed that the ability of *E. palaefolius* to absorb Cu metal in the planting media, leaf chlorophyll content, and wet biomass was influenced by Cu concentration. The higher the concentration of Cu in the growing media, the greater the absorption of Cu by *E. palaefolius*, the lower the leaf chlorophyll content, and the increase in wet biomass. The results of this study prove that *Echinodorus palaefolius* can absorb Cu metal.

Keywords: chlorophyll content; Cu content; waste water treatment; wet biomass

Abstrak. Pencemaran perairan dapat bersumber dari polutan anorganik berupa logam berat tembaga (Cu). Tembaga dalam konsentrasi tinggi akan menyebabkan efek toksik sehingga diperlukan upaya pengolahan kualitas air. Fitoremediasi dapat digunakan dalam pengolahan air tercemar logam berat yang penggunaannya akan mendukung Sustainable Development Goals (SDGs). Salah satu tumbuhan yang berpotensi sebagai fitoremediator adalah melati air (*Echinodorus palaefolius*). Penelitian ini bertujuan untuk mendeskripsikan kemampuan *Echinodorus palaefolius* sebagai agen fitoremediasi terhadap penurunan kadar Cu pada media tanam, mendeskripsikan pengaruh variasi konsentrasi Cu terhadap kadar klorofil daun dan biomassa basah. Data dianalisis menggunakan SPSS uji ANAVA satu arah yang kemudian dilanjutkan dengan Duncan test. Analisis data pendukung berupa pH dan suhu air dilakukan secara deskriptif kuantitatif. Hasil penelitian menunjukkan bahwa kemampuan *E. palaefolius* dalam menyerap logam Cu pada media tanam, kadar klorofil daun, dan biomassa basah dipengaruhi oleh konsentrasi Cu. Semakin tinggi konsentrasi Cu pada media tanam, maka semakin besar penyerapan Cu oleh *E. palaefolius* dan semakin rendah kadar klorofil daun serta peningkatan biomassa basah. Hasil penelitian ini membuktikan bahwa *Echinodorus palaefolius* memiliki kemampuan untuk menyerap logam Cu.

Kata kunci: kadar klorofil; kadar Cu; pengolahan air limbah; biomassa basah

INTRODUCTION

Water is an essential element in life, especially for humans. The increasing population and various activities will increase the water demand (Hidayati, 2017). This situation will affect the quantity and quality of water, which will decrease. This decline is due to daily human activities that produce waste, causing water to be polluted. Water pollution can come from organic pollutants, inorganic pollutants, residual fuel, oil, and kerosene spills (Rukandar, 2017). Heavy metals are one of the inorganic pollutants. Copper (Cu) metal is one of the heavy metals that, if in high amounts, has a toxic effect on living.

Copper (Cu) is an essential metal available in nature, so it is widely utilized. Copper is a good conductor of electricity and heat, so it is widely used to manufacture electrical wires and

cables (Amiruddin and Lubis, 2018). For humans, copper plays a role in cellular respiration and defense mechanisms against free radicals (Ransun *et al.*, 2021). Plants need copper in chlorophyll formation, photosynthesis, protein and carbohydrate metabolism (Rehman *et al.*, 2019). The presence of copper (Cu) metal in high concentrations will cause environmental toxicity. Accumulation of heavy metals in waters due to the presence of these metals over a long time will decrease the carrying capacity of water.

Water polluted by heavy metal copper (Cu) causes physical changes in water, endangers plant ecosystems and aquatic biota, and harms humans if water, plants, and marine biota polluted with heavy metals are consumed (Bubala *et al.*, 2019). As peak consumers, humans will experience large bioaccumulation of heavy metals in their bodies because heavy metal accumulation occurs through the food chain (Hananingtyas, 2017). For human health, the content of copper metal (Cu) consumed in high concentrations can cause anemia, headache, vomiting, liver organ damage, and the tongue tastes bad (Sekarwati *et al.*, 2015).

Water pollution caused by heavy metals must be addressed using specific techniques to efficiently remove contaminants from water (Zamora-Ledezma *et al.*, 2021). Efforts to treat water polluted with heavy metal copper (Cu) can be made by phytoremediation. The existence of polluted water treatment using this technique will support the Sustainable Development Goals (SDGs), which aim to ensure clean water and proper sanitation and address climate change. Specific goals include improving water quality, managing household waste, wastewater treatment, and safely reusing.

Phytoremediation uses living plants to clean up polluted environments (Sukono *et al.*, 2020). Plants that can accumulate metals in high concentrations, have high growth rates, are resistant to pests and diseases can be used as phytoremediators. Plants that can be utilized as phytoremediators are mexican sword plant (*Echinodorus palaefolius*). *Echinodorus palaefolius* can absorb and decompose pollutants so that it can reduce and eliminate pollutants (Koesputri *et al.*, 2016). This is because *Echinodorus palaefolius* can provide oxygen to the roots in large quantities. After all, it has fibrous roots with large stem cavities that help decompose organic matter (Kasman *et al.*, 2019).

According to research by Caroline and Moa (2015) using mexican sword plant (*Echinodorus palaefolius*) as a lead metal phytoremediation agent, the percentage of lead metal removal was 81.72% in the waste reactor and 86.05% in the control reactor. The results of research by Sari *et al.* (2021) stated that using mexican sword plant for phytoremediation of laundry waste on the 14th day of the phytoremediation process was able to reduce phosphate levels with a percentage of 98.90%. Based on the potential of *E. palaefolius* in absorbing heavy metals, it is necessary to conduct research related to the potential of *E. palaefolius* in reducing copper (Cu) metal. This study aims to describe the ability of *E. palaefolius* to reduce Cu levels and the effect of Cu concentration variations on leaf chlorophyll levels and plant wet biomass.

MATERIALS AND METHODS

The research was conducted experimentally in Green House C10 Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University in November-December 2023. Analysis of Cu levels in water from phytoremediation at the Environmental Laboratory of the East Java Provincial Environmental Service. Leaf chlorophyll levels were analyzed at the C10 Physiology Laboratory, FMIPA, Universitas Negeri Surabaya. The design used in the study was a Randomized Group Design (RAK) with one treatment factor in the form of Cu metal concentrations of 0 ppm, 3 ppm, 5 ppm, and 7 ppm. Repetition was done six times in each treatment so that 24 treatment units were obtained.

The research phase began with acclimatizing *E. palaefolius* in a container containing distilled water for seven days. Planting media was made by adding Cu heavy metal solution ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) with concentrations of 3 ppm, 5 ppm, 7 ppm, and without Cu (0 ppm) as a control into a container containing 5 liters of distilled water. *E. palaefolius* in the treatment stage was weighed as much as 100 grams and put into the planting media containing Cu solution as a simulation of waste with various concentrations. The research treatment was carried out for seven days with measurements of physico-chemical factors in the form of pH and temperature before and after treatment.

Analysis of copper (Cu) metal content in the planting media after treatment using an Atomic Absorption Spectrophotometer (SSA) with a wavelength of 324.7 nm. Analysis of leaf

chlorophyll content was carried out by weighing 1 gram of leaves, then cutting them into small parts and crushing them using a mortar and pestle. 96% alcohol, as much as 100 ml, was added to the crushed leaves and then filtered with filter paper. Chlorophyll content was tested with wavelengths of 649 nm and 665 nm in a spectrophotometer. Measurement of the initial and final wet biomass of *E. palaeifolius* using digital scales. pH measurements were made using a pH meter and temperature was measured using a thermometer.

Data on the reduction of copper levels in post-phytoremediation water, chlorophyll levels, and wet biomass were analyzed using the SPSS one-way ANOVA test. To determine if there is a significant difference in the data from each treatment, followed by the Duncan test at the 5% level. Data on pH and temperature as parameters of water quality polluted with metals were analyzed descriptively quantitative.

RESULTS

The treatment of various concentrations of Cu affects the decrease in Cu levels in the media; the results are presented in Table 1. Based on the results of the study, it is known that *E. palaeifolius* is able to reduce Cu levels in the planting media; the absorption of Cu levels by *E. palaeifolius* will be more significant, along with the higher concentration of Cu in the planting media. Cu treatment with a concentration of 7 ppm has a higher decrease than Cu treatment with a concentration of 3 ppm and 5 ppm (Table 1).

Table 1. Reduction of copper metal (Cu) levels in the growing media after treatment with various concentrations of Cu

Initial Cu Concentration in Planting Media (ppm)	Final Cu Concentration in Planting Media (ppm)	Reduction of Cu Level in Planting Media (ppm)*	Percentage of Cu Reduction in Planting Media (%)
0	0 ± 0.000	0 ± 0.000 ^a	0%
3	0.035 ± 0.006	2.964 ± 0.006 ^b	98.83%
5	0.077 ± 0.031	4.922 ± 0.031 ^c	98.47%
7	0.107 ± 0.025	6.892 ± 0.025 ^d	98.46%

Notes: *)Numbers followed by different alphabetical notations in the column indicate that the data are significantly different according to the results of the Duncan Test at the 0.05 test level.

The chlorophyll content of *E. palaeifolius* leaves after treatment with various concentrations of Cu can be seen in Table 2. The results of leaf chlorophyll content after treatment were influenced by variations in Cu concentration. The chlorophyll content of *E. palaeifolius* leaves is lower when a higher concentration of Cu is contained in the planting medium. The treatment with a concentration of 0 ppm obtained the highest chlorophyll level of 19.151 ppm, while the lowest chlorophyll level was in the Cu treatment with a concentration of 7 ppm at 12.088 ppm (Table 2).

Table 2. Chlorophyll content of leaves after treatment with various concentrations of Cu

Cu concentration (ppm)	Chlorophyll a (mg/l)	Chlorophyll b (mg/l)	Total Chlorophyll (mg/l)*
0	9.209 ± 2.170	9.942 ± 1.690	19.151 ± 3.800 ^b
3	5.803 ± 1.783	7.422 ± 2.331	13.255 ± 3.797 ^a
5	5.094 ± 1.485	7.147 ± 1.648	12.241 ± 3.073 ^a
7	4.964 ± 2.917	7.124 ± 2.937	12.088 ± 5.717 ^a

Notes: *)Numbers followed by different alphabetical notations in the column indicate that the data are significantly different according to the results of the Duncan Test at the 0.05 test level.

The final wet biomass of *E. palaeifolius* increased after the treatment of various Cu concentrations, as presented in Table 3. Cu concentration treatment affects the wet biomass. The higher the Cu concentration in the growing media, the lower the growth (wet biomass) of *E. palaeifolius*. The largest increase in wet biomass was in the treatment with a concentration of 0 ppm, while in the treatment with a concentration of 7 ppm the increase in wet biomass was the lowest (Table 3).

Table 3. Final wet biomass after treatment with various concentrations of Cu

Cu concentration (ppm)	Final Wet Biomass (gram)*
0	157.33 ± 6.683 ^d
3	142.33 ± 2.805 ^c
5	135.50 ± 3.886 ^b
7	127.00 ± 3.578 ^a

Notes: *)Numbers followed by different alphabetical notations in the column indicate that the data are significantly different according to the results of the Duncan Test at the 0.05 test level.

Environmental physico-chemical factors, including pH and temperature, were measured at the initial and final conditions. The treatment of various Cu concentrations affected changes in the pH and temperature of *E. palaeifolius* growing media. The change was in the form of an increase in pH to neutral at the end of phytoremediation (Figure 1). After the treatment of 0 ppm, 3 ppm, 5 ppm, and 7 ppm Cu concentrations, the average pH values were 7.13, 7.16, 7.20, and 7.19, respectively. The average temperature value in the final condition changed to be lower than the initial temperature with consecutive values of 27.05°C, 27.15 °C, 27.40 °C, 27.35 °C. (Figure 2).

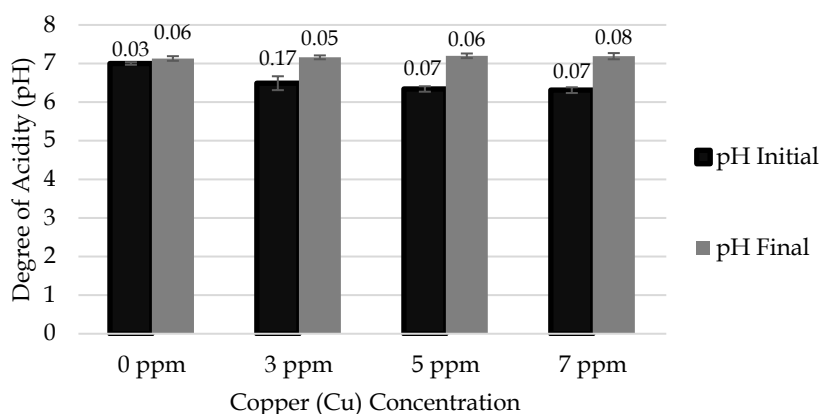


Figure 1. Changes in pH of the growing medium

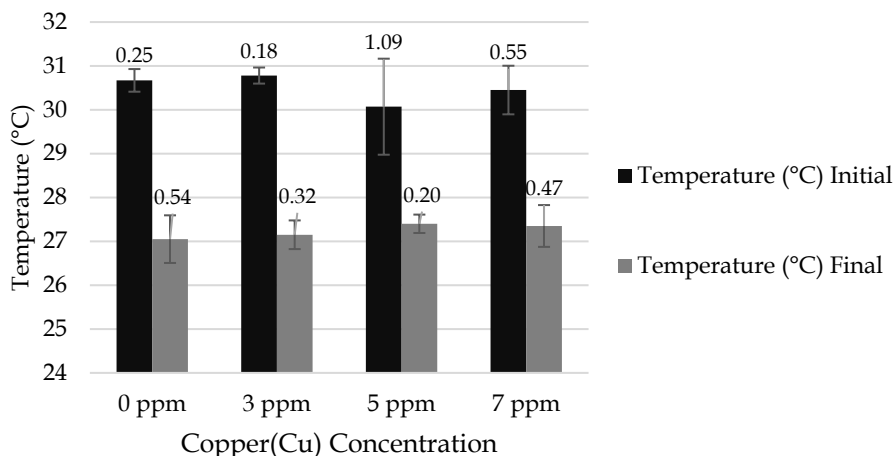


Figure 2. Temperature changes in the growing medium

DISCUSSION

Based on the research conducted, it was found that *E. palaeifolius*, used as a phytoremediation agent, was able to reduce Cu levels in the growing media. This decrease is due to the mechanism in the body of Mexican sword plants. The Cu absorption mechanism carried out by plants begins with the absorption of heavy metals in the form of dissolved ions carried out by the roots and enters with the flow of water. Roots, as the first line of defense that absorbs Cu metal, will limit the entry of metals into the plant body (Dalvi and Bhalerao, 2013). This is done by forming phytochelatin. When meeting with metals, phytochelatin will form

complex compounds, namely phytochelatin-metal, whose function is detoxifying the metal. This formation can occur in various metals, including copper (Gupta and Inoue, 2013).

The cysteine part of phytochelatin will release two hydrogen ions belonging to the thiol group ($-SH$) and then bind to Cu metal. This causes the formation of chains with the length or shortness depending on the metal concentration. The peptide chain will get longer along with the higher concentration of heavy metals that meet phytochelatin (Amalia *et al.*, 2023). This formation will become a complex compound and cause Cu to be carried into plant tissues. Furthermore, the complex compound will be brought or translocated directly to plant tissues through the xylem and phloem (Thakur *et al.*, 2016).

Metals that have entered the plant body will be localized by hoarding heavy metals in specific organs, such as the roots, to protect cells from damage and maintain cell function (Atabayeva *et al.*, 2015). Heavy metals are hoarded in the roots because the amount of chelating substances in the roots is more significant than in other plant tissues (Yeh *et al.*, 2015). In addition, the roots of *E. palaefolius* are fibrous roots that allow heavy metals to be absorbed into the roots.

The treatment of various Cu concentrations affects the decrease in Cu levels in the planting media. A significant decrease in Cu levels occurred in the planting media based on the provision of increasing Cu concentrations. Cu levels in *E. palaefolius* planting media that decreased based on variations in concentration from highest to lowest are presented in Table 1, namely in the concentration treatment of 7 ppm, 5 ppm, 3 ppm, and 0 ppm (control). This shows that the decrease in Cu levels in the planting media is getting higher along with the higher concentration of Cu given to the planting media. This decrease proves that *Echinodorus palaefolius* is included in hyperaccumulator plants that can absorb Cu metal in water.

Echinodorus palaefolius roots that are directly in contact with planting media polluted by copper metal (Cu) can absorb the metal. The roots will absorb Cu metal, which causes a high concentration of Cu in the roots, and then the metal will be carried to the stem and leaf tissues (Rachmadiarti and Trimulyono, 2019). Fiber roots in *E. palaefolius* not only have the ability to absorb metals but there is an interaction mechanism between the roots and heavy metals that form root exudates. Root exudates are plant metabolites that are released to the surface of the roots or rhizosphere to overcome environmental stress or to increase the absorption of plant nutrients, the result of which is low molecular weight organic acids. These results can accumulate metals in water (Rahmaisanti *et al.*, 2022). The degradation of pollutants in the planting media is also assisted by rhizosphere bacteria present in the roots of *E. palaefolius*. The presence of rhizosphere bacteria helps in decomposing organic and inorganic elements contained in the place of growth, which are then utilized as a source of nutrients (Sumarta and Ronny, 2023).

The results of the analysis of the mean total chlorophyll content of *E. palaefolius* leaves after treatment with various concentrations of Cu showed the effect of Cu concentration on leaf chlorophyll content. The differences in leaf chlorophyll levels obtained from the treatment of various Cu concentrations are presented in Table 2. There was a decrease in leaf chlorophyll levels along with an increasing concentration of Cu in the growing medium. Chlorophyll content after treatment with a Cu concentration of 7 ppm was lower than leaf chlorophyll levels at Cu concentrations of 3 ppm and 5 ppm. The control treatment with a concentration of 0 ppm obtained the highest leaf chlorophyll content. The concentration of heavy metal Cu in high amounts disrupts plants' absorption of minerals and microelements. This situation results in the inhibition of gas exchange and photosynthesis due to damaged chloroplast and thylakoid structures (Zhang *et al.*, 2018).

According to Afandi *et al.* (2014), the toxic properties of heavy metals begin with the destruction of the chloroplast structure, which inhibits the photosynthesis process, causing the essential organic carbon needs of plants to be reduced, resulting in cell death. Enzyme work will be blocked as a result of chlorophyll biosynthesis in chloroplasts that are disrupted due to the presence of heavy metals. Chlorophyll formation is also influenced by the minerals Mg and Fe. Mg and Fe minerals that enter the plant are reduced due to the presence of heavy metals in large quantities, resulting in changes in the number of chloroplasts. Fe has a function to compose enzymes, chlorophyll, and proteins, whereas Cu metal, which has toxic properties, will be in line with Fe deficiency (Elawati *et al.*, 2018).

The final wet biomass of *E. palaefolius* after treatment with various concentrations of Cu increased. Cu treatment with a concentration of 0 ppm had the highest increase in wet biomass compared to the treatment of 3 ppm, 5 ppm, and 7 ppm (Table 3). The highest increase in wet biomass in the 0 ppm treatment was due to the absence of Cu in the growing media, so it did not inhibit growth. This shows that the smaller the concentration of copper (Cu) given, the greater the increase in wet biomass; otherwise, if the concentration of copper (Cu) given is greater, the smaller the increase in wet biomass.

The concentration of Cu metal given in the growing media can be tolerated by *E. palaefolius*, as evidenced by the increased wet biomass of *E. palaefolius*. There are differences in the increase of wet biomass due to variations in Cu concentration in the growing medium. Plants still grow despite being in a metal-contaminated environment. However, the growth rate decreased as the Cu concentration increased. This causes differences in wet biomass in each treatment. Anggraini *et al.* (2016) stated that when the rate of photosynthesis decreases due to heavy metal stress, the food substance produced also decreases. This will result in slower plant growth. Plants living in water or soil polluted with heavy metals can decrease growth, performance, and yield (Srivinas *et al.*, 2023). In some treatments, *Echinodorus palaefolius* plants were found to have yellowing leaves and dead leaves with changes in leaf color to brown. However, this was followed by growth and reproduction characterized by the emergence of new leaves and shoots. This shows that *E. palaefolius* can grow in a polluted environment.

Physical and chemical factors observed during the treatment phytoremediation process as supporting data were pH and temperature. The phytoremediation process carried out for seven days resulted in an increase in pH in the planting media, from a pH that tends to be acidic to a neutral pH (Figure 1). The pH value of water that changes during the treatment process of polluted water shows that the treatment process affects the pH value, followed by a decrease in heavy metal levels in the water. The ability to absorb organic and inorganic compounds through chemical processes by environmental factors carried out by aquatic plants can improve the pH value of water in the planting medium (Herlambang and Hendriyanto, 2015). Changes in pH value are also influenced by the process of photosynthesis that utilizes CO₂, which results in H⁺ ions being reduced so that the pH of polluted water increases (Marlany *et al.*, 2023). Plants can grow optimally at pH 7 and the more acidic the pH of the environment is, the more it will inhibit the absorption of heavy metals and cause death (Pang *et al.*, 2023).

The temperature of the growing media after the seven-day phytoremediation process decreased, ranging from 27.05°C -27.40°C (Figure 2). The process of photosynthesis and carbohydrate metabolism in plants is influenced by temperature. Temperatures too high can inhibit metabolism, damaging enzymes, cells, and tissues (Seydel *et al.*, 2022). The optimal pH and temperature values for the growth of mexican sword plant (*Echinodorus palefolius*) are in the pH range of 4.5-7 and a temperature of 25-35°C (Setiyanto *et al.*, 2016).

CONCLUSION

Echinodorus palaefolius is proven to be used as a Cu phytoremediator. The ability of *E. palaefolius* to absorb Cu metal in the planting media, leaf chlorophyll content, and wet biomass was influenced by the treatment of variations in Cu metal concentration. The higher the concentration of Cu in the planting media, the greater the absorption of Cu by *E. palaefolius*, the lower the leaf chlorophyll content, and the increase in wet biomass.

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