

Assessing the Tolerance of *Sagittaria lancifolia* (Spear Plant) to Linear Alkylbenzene Sulfonates (LAS) Exposure

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Abstract

One of pollutant that commonly found in aquatic environment is detergent. To address this issue, it is critical to implement effective policies and regulations to control detergent pollution and promote sustainable wastewater management practices in Indonesia. One of the approaches to overcome detergent pollution is to use plants as pollutant absorbing agents, or commonly called phytoremediation techniques. Therefore, this study aims to determine the growth response of spear plant (*Sagittaria lancifolia*) in response to the stress of Linear Alkylbenzene Sulfonate (LAS) detergent. This research is an experimental research using a completely randomized design (CRD). There were four treatments tested with three replicates for each treatment. Treatment variations were based on the concentrations used in this study, namely 0 mg/L, 10 mg/L, 30 mg/L, and 50 mg/L. The research was conducted for four months, starting from July to October 2022, and was conducted in the greenhouse of Purwodadi Botanical Garden – BRIN. The result showed that although it experienced some morphological changes such as leaf drying, discoloration, and structural changes, *Sagittaria lancifolia* were able to survive and conduct metabolic activities properly during 28 days of Linear Alkylbenzene Sulfonates (LAS) pollutant stress. Thus, *Sagittaria lancifolia* are able to be used as one of the strategies in restoring the aquatic environment polluted by domestic waste, including LAS detergent.

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Introduction

Water pollution in Indonesia caused by detergents is a serious issue that poses significant environmental and health risks. The primary cause of this pollution is the discharge of untreated or inadequately treated wastewater from households, industries, and agriculture into water bodies (Madhav et al., 2019). Detergents contain high levels of phosphorus and surfactants, which can cause eutrophication, decrease oxygen levels in the water, and harm aquatic life (Suastuti et al., 2015). Additionally, detergent pollution can cause skin irritation and other health problems in humans and animals that use contaminated water sources (Al-Taai, 2021). Furthermore, the consequences of detergent pollution in Indonesia include a decline in water quality, loss of aquatic biodiversity, and reduced economic benefits from fisheries and tourism industries (Mousavi & Khodadoost, 2019). To address this issue, it is critical to implement effective policies and regulations to control detergent pollution and promote sustainable wastewater management practices in Indonesia.

One of the approaches to overcome detergent pollution is to use plants as pollutant absorbing agents, or commonly called phytoremediation techniques. The advantages of the phytoremediation technique are

low cost, low contamination risk, no hazardous waste, and easy treatment (Tang et al., 2020). Many plants that have been studied for their ability to remove the detergent pollution, especially aquatic plant. According to Muthukumaran (2022), aquatic plants such as water hyacinth, duckweed, water lettuce, reed, cattail, bulrush, water fern, and water pennywort have the ability to remediate detergent or LAS pollution in water. Furthermore, *Sagittaria lancifolia* also reported has an ability to remove heavy metals and detergent in the water (Afifudin et al., 2022; Fitrihidajati et al., 2020). The application of aquatic plants in phytoremediation is based on the principle that all plants are able to absorb pollutants in their bodies, but each plant has a different ability and tolerance level (Hidayati, 2020). Detergent contamination of soil and water can have significant morphological effects on plants. Several research has shown that detergents have harmful effects on the germination of plants and the soil flora in the area (Mousavi & Khodadoost, 2019). In another study, phytoremediation using *Pistia stratiotes* and *Echhornia crassipes* also showed chlorosis and necrosis responses at the end of the observation (Fathoni, 2020; Taufiq et al., 2008). Furthermore, Estuningsih et al (2013) also reported that grass in remediating petroleum-contaminated soil shows varying responses, such as plant chlorosis, leaf shriveling, and reduction of plant roots.

Based on several conditions of physiological abnormalities that have been revealed, it is necessary to investigate the physiological responses of plants after exposure to heavy metals. Because in essence, the ability of plants to accumulate and absorb contaminants depends on the type and characteristics of each plant (Hidayati, 2020). Therefore, this study aims to assessing the tolerance of *Sagittaria lancifolia* (lanceleaf arrowhead) to Linear Alkylbenzene Sulfonates (LAS) exposure. Linear alkylbenzene sulfonate (LAS) is an anionic surfactant that is widely used in detergents and cleaners, both in industrial and household applications. Furthermore, this research was conducted to determine the ability of lanceleaf arrowhead to have more ability to deal with the stress of detergent pollutants.

Methods

This research is an experimental research using a completely randomized design (CRD). There were four treatments tested with three replicates for each treatment. Treatment variations were based on the concentrations used in this study, namely 0 mg/L, 10 mg/L, 30 mg/L, and 50 mg/L. The research was conducted for four months, starting from July to October 2022, and was conducted in the greenhouse of Purwodadi Botanical Garden - BRIN. This research involved several steps, which includes acclimatization, preparation of solutions and plant samples, implementation of phytoremediation, and data analysis. These stages will be explained in more detail below:

The first step is acclimatization, in this study, samples of fresh spear leaf plants (*Sagittaria lancifolia*) measuring approximately ±45 cm were used. The plants were taken from the aquatic pond at Purwodadi Botanical Garden, then acclimatized in the nursery greenhouse for two weeks. The purpose of acclimatization is to help plants to adapt gradually to the environment and regenerate damaged bodies (Afifudin & Irawanto, 2021). The acclimatization process is carried out by adding 7.5 mL of NPK fertilizer at the beginning of the acclimatization stage, with a ratio of NPK fertilizer and water of 10:1.

The second step is preparation, in this study, the preparation of LAS solutions with varying concentrations of 0 mg/L, 10 mg/L, 30 mg/L, and 50 mg/L. The solution was made using 100% LAS main solution and then diluted using the following formula:

$$M1 \times V1 = M2 \times V2 \quad (1)$$

Description:

M1: Initial solution concentration

M2: Desired solution concentration

V1: Initial solution volume

V2: Volume of the dilution set solution

Furthermore, for plant preparation, the acclimatized plants were moved into a container (reactor) and given 2 liters of water that had previously been contaminated with LAS based on the concentration variations to be tried.

Next is phytoremediation implementation, the implementation of phytoremediation research was carried out for 28 days by observing several parameters, including the number of plant leaves, plants biomass (wet weight and dry weight) and plant morphological changes. Furthermore, the measurement of the leaf number was carried out before and after treatment. The measurement of wet weight and dry weight was carried out after treatment. Meanwhile, the observation of plant morphology was carried out four times during the research time.

After conducted the research, data analysis on the parameter of the number of leaves was carried out by paired T-2 Paired sample test, with the aim of determining the difference between the number of leaves before and after treatment. Then, the parameter of plant biomass was analyzed by one-way Anova

test with the aim of determining differences in each treatment. Meanwhile, on the parameter of morphological changes in plants, it was carried out by descriptive analysis, which is by describing the data and the facts sequentially with tables and pictures.

Results and Discussion

The current investigation places significant importance on observing the growth response of plants, as this is crucial in determining their reaction to exposure to high levels of pollutants. Typically, plants will exhibit changes in both morphology and physiology when subjected to such pollutants. The growth response of plants in this research is being assessed by monitoring parameters such as the number of leaves, morphological changes in plants, and their biomass, which is measured through the wet weight and dry weight of the plants.

Number of Plant Leaves

Leaves play a crucial role in the metabolic process of plants as the primary site for photosynthesis and synthesis of food. According to [Ardiansyah \(2017\)](#), the number of leaves on a plant directly correlates with the amount of space available for the plant to produce its food. As such, an increase in the number of leaves on a plant can lead to a higher rate of photosynthesis and ultimately, a greater yield of food production.

Table 1. Plant leaves

Concentration Variation	Number of leaves	
	Before	After
Control	3	7
LAS 10 mg/L	4	7
LAS 30 mg/L	4	6
LAS 50 mg/L	4	6

[Table 1](#) indicates that the average number of leaves has increased before and after undergoing phytoremediation treatment. This increase demonstrates that there is no observable impact of LAS contaminant exposure on the number of leaves in *Sagittaria lancifolia* plants. To validate this observation, a T-2 paired sample statistical test was conducted to compare the number of leaves before and after phytoremediation treatment. The results of the test shows a sig value of 0.648 for the number of leaves before treatment, which indicates no significant difference between the number of leaves before and after phytoremediation treatment. This finding aligns with [Table 1](#), which displays an increase in the number of leaves of only 2-4 leaves for each treatment.

Biomass of Plant

To assess the biomass of plants, researchers typically measure their wet weight and dry weight. Wet weight, also known as fresh weight, refers to the weight of the plant at the time of harvest, prior to any wilting or water loss ([Lussy et al., 2022](#)). It reflects the metabolic activity and photosynthetic output of the plant, as the products of photosynthesis are utilized to build cells and can thus influence the wet weight of plants ([Ardiansyah, 2017](#)). In contrast, the dry weight of plants indicates the total biomass that can be assimilated by the plant. As [Pratiwi et al. \(2021\)](#) explain, the dry weight of a plant describes the accumulated products of CO₂ assimilation during its growth and development. Both the increase in wet weight and the accumulation of dry matter can be used to calculate plant growth. As such, a healthy plant will generally have a higher dry weight due to its optimal growth and development.

Table 2. Plant biomass

Concentration Variation	Wet Wight (g)	Dry Weight (g)	Biomass (%)
Control	35,1	2,15	94%
LAS 10 mg/L	28	2,5	91%
LAS 30 mg/L	34,3	3	91%
LAS 50 mg/L	36,9	3,2	91%

[Table 2](#) presents the result of measuring the biomass of *Sagittaria lancifolia* plants. The wet weight and dry weight of the plants were measured 28 days post-harvest. The control treatment exhibited the highest plant biomass percentage at 94%. This can be attributed to good plant growth, as well as the suitable temperature and pH conditions of the medium for plant growth ([Caroline & Guido, 2015](#)). On the other hand, [Table 2](#) indicates that the percentage of plant biomass in the treatment with pollutants was lower than in the control treatment. This suggests that an excessive amount of pollutants in the medium can impede plant growth and result in reduced plant biomass ([Hardiani, 2008](#)).

Furthermore, the dry weight of plants is not only affected by their wet weight, but also by the number of leaves they have, as leaves are where photosynthesis products accumulate. The amount of dry weight produced by a plant is determined by a balance between photosynthesis and respiration processes. Photosynthesis increases dry weight by taking in CO₂, whereas respiration decreases it by releasing CO₂. If respiration is more active than photosynthesis, the dry weight of the plant can decrease, and vice versa (Ardiansyah, 2017).

Morphological Changes of Plant

The observation of plant morphology aims to determine the adaptation and morphological changes of *Sagittaria lancifolia* plants to exposure to LAS contaminants. This is important because morphological changes in plants during stress can help plants adapt to a changing environment. Furthermore, one type of adaptation developed is anatomical adaptation to roots, stems, and leaves (Akmalia, 2021). The description of changes in plant morphology after exposure to LAS for 28 days is presented in table 3.

Table 3. Morphological changes during experiment

Concentration Variation	Detention Time			
	Week-1	Week-2	Week-3	Week-4
S ₀	-	-	-	-
S ₁	-	2 leaf tips experience chlorosis and necrosis	-	1 leaf experience necrosis
S ₂	-	-	1 leaf experience chlorosis	1 leaf experience necrosis
S ₃	5 leaf experience chlorosis and necrosis	tips and 1 leaf experience necrosis and 3 leaves are dry	1 leaf bases experience chlorosis and necrosis, 1 leaf are dry	-

Table 3 shows that in the first week, most of the plants showed no morphological changes, either in the roots or crown. However, at variations of LAS concentration 50 leaves experienced physiological abnormalities such as chlorosis and necrosis. Then, after entering the second week onwards, the plants began to show some response to stress. This is characterized by the presence of several leaf blades that experience physiological abnormalities such as chlorosis and necrosis and dryness until it indicates death. As stated by A'yuningsih (2017) that detergent stress can cause changes in plant morphology, anatomy, biochemistry and physiology. As for some examples of physiological abnormalities in *Sagittaria lancifolia* plants can be seen in Figure 1.

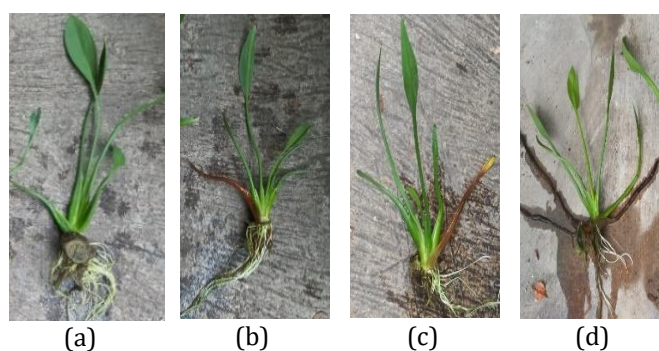


Figure 1. Plants morphology after four week treatment.
 Description: (a) Control, (b) LAS 10 mg/L, (c) LAS 30 mg/L, (d) LAS 50 mg/L.
 (Sourch: Personal Documentation)

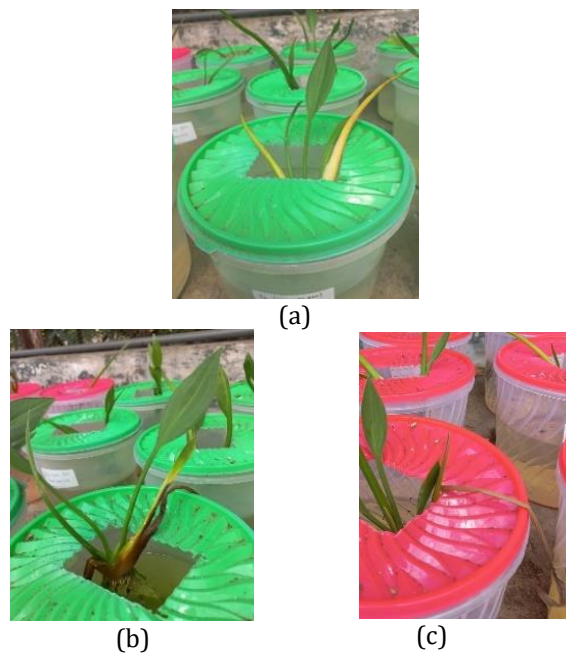


Figure 2. Morphological changes in *Sagittaria lancifolia*.
Description: (a) Chlorosis, (b) Necrosis, (c) Dry leaf.
(Source: Personal Documentation)

In [Figure 2](#), it is apparent that the plants experienced morphological changes. These morphological changes are thought to be caused by plant stress due to exposure to LAS. In the picture, it can be seen that the leaves of the plant have changed color and structure, this is one of the characteristics of plants experiencing chlorosis and necrosis. Chlorosis is a form of yellow to pale color change in plant organs and is generally found in leaves and stems due to disruption of the chlorophyll formation process ([Rizkiaditama et al., 2017](#)). While necrosis is a form of change in the parts and color of the plant to brownish to dry and brown spots on the leaves ([Baroroh et al., 2018](#)).

The changes in color and structure are thought to be caused by the toxicity of LAS contaminants that are chemically overdosed. The contaminants absorbed by plant cells and tissues are the first indicators of toxicity. In addition, detention time can affect leaf color changes to the chlorophyll loss in the leaves ([Rizkiaditama et al., 2017](#)). Therefore, in this observation, the longer the detention time shows the more morphological changes in plants. Furthermore, the effect of detergent on the photosynthetic activity and chlorophyll content in intact bean leaves was investigated, and it was found that detergent for domestic use had a negative impact on the photosynthetic activity and chlorophyll content in the leaves ([Iovanic et al., 2010](#)).

Conclusions

The result of this study showed that although it experienced some morphological changes such as leaf drying, discoloration, and structural changes, *Sagittaria lancifolia* were able to survive and conduct metabolic activities properly during 28 days of Linear Alkylbenzene Sulfonates (LAS) pollutant stress. Therefore, this plant deserves to be used as one of the strategies in restoring the aquatic environment polluted by domestic waste, including LAS detergent.

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