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Consortium of *Marsilea crenata* and *Ludwigia adscendens* for Linear Alkylbenzene Sulfonate Detergent Phytoremediator

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Abstract: Water clover (*Marsilea crenata* Presl.) and water primrose (*Ludwigia adscendens* L.) are plants grow in wetlands, polluted by inorganic or organic materials, including detergent. This study aims to evaluate the capability of *M. crenata* and *L. adscendens* individually or as a consortium to remediate linear alkylbenzene sulfonate (LAS) detergent-polluted water, and to measure the growth and chlorophyll content of these plants. *M. crenata* and *L. adscendens* were grown in a hydroponic system exposed to LAS at 0, 10, 20, and 30 ppm for 10 days. Concentration of LAS (as anionic detergent) in treated media was analysed by UV-Vis spectrophotometer at 652 nm, wet biomass was measured using analytical balance, and chlorophyll content was analysed using spectrophotometer at 649 and 655 nm. Data were analysed statistically using analysis of variance followed by the Duncan test. The results shows that (1) at the end of experiment the concentration of residual detergent increased with increasing LAS in the media and (2) the plants biomass and the chlorophyll concentration decreased with increasing the LAS concentration in media. However, since both *M. crenata* and *L. ascendens* can significantly reduce LAS detergent concentration and can survive in 30 ppm concentration of LAS detergent, both species can be applied as phytoremediator agents.

Key words: Phytoremediation, aquatic plants, *Marsilea crenata, Salvinea molesta*, detergent.

Introduction

Detergent wastes discharged into water bodies from common household generally have not been specifically processed to reduce its effect on the environment. One of the efforts that can be performed to decrease detergent contamination in water is phytoremediation method. Phytoremediation is a method used to minimise pollutant using plants and their parts in cooperation with microorganism in a reactor or directly on the field; thus, it is not harmful to environment (Golrizkhatami et al., 2018). Phytoremediation method involves root systems, which enable rooting plants in translocation, bioaccumulation and degradation of pollutants. Therefore, plants used in phytoremediation should be a hyperaccumulator plant which is able to transport more than one substance, possess large range of tolerance towards contaminant, can grow on any land condition in short time. Aquatic plants are generally used in water remediation, due to their high growth rate and ability to absorb contaminant quickly. For example, *Pistia stratiotes* at a density of 35 mg/cm^2 was able to decrease 99% phosphate (0.07 mg/L), 98% BOD (6 mg/L) and 96% COD (17 mg/L) from the same type of wastewater (Raisaa and Tanghau, 2017).

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Ludwigia adscendens (L.) or commonly called water primrose belong to *Ongraceae* family, which is synonymous with *Jussiaea repens* L. (Hock et al., 2015) is one species of weeds widely found in ponds, ditches, swamps, and farmland. This plant emerges from water with the looming end of stem into the air and has a rapid growth rate. According to Sharma and Sanghi (2012), *L. adscendens* is one of macrophyte plants that potentially accumulates and absorbs heavy metals contaminating water. Rachmadiarti and Sholikah (2019) combined *L. adscendens* and *L. grandiflora* and found that they were able to decrease Cd at average of 6.28 ppm from initial concentration of 15 ppm at detention time of 10 days.

Water clover (*Marsilea crenata* Presl.) belongs to a group of aquatic ferns (Hydropterides) from Marsilea, a genus that grows around rice fields or water irrigation channel. *M. crenata* hashydrophyte thin cuticle, constantly open stomata, water bladder and thin roots. Previous study of *M. crenata* in wetlands of Surabaya, Indonesia showed that the plants had capability to absorb Pb (Rachmadiarti and Trimulyono, 2019). Asrari and Avatefinezhad (2017) reported that *L. adscendens* and *M. crenata* can also absorb both organic and inorganic materials. Based on their potential to absorb a wide range of pollutants, this study was conducted to evaluate the ability of *M. crenata* and *L. adscendens,* both individually or as a consortium to reduce concentration of detergent in contaminated water and to measure plants growth and chlorophyll content.

Materials and Methods

Plants Growth and LAS-Exposure

This study was designed with the help of completely randomized block design with three replications. Two factors of treatment were applied: plants species (*M. crenata* and *L. adscendens,* combination of plants) and LAS detergent concentration (0, 10, 20 and 30 ppm as methylene blue active substance, MBAS) at detention time of 10 days. Before being used in experiment, plants were acclimated in a plastic chamber filled with 20 L Hoagland's medium within green house for a period of 7 days to reduce contaminant concentration in the plants (Rachmadiarti and Trimulyono, 2019).

Acclimated plants were rinsed using distilled water and moved into 40×30×35 cm^3 glass aquaria filled with 5 L of distilled water and Hoagland's solution with different concentrations of LAS detergent. Approximately 100 g of *M. crenata,* 100 g of *L. adscendens* and 50 g of each plants species for consortium used as initial biomass. The levels of anionic detergent, pH and BOD of planting media were measured. The experiments were conducted in 12:12 light-dark circle daily with 389-candles photon flux density. At the end of exposure time (10 days), all plants were harvested and their biomass measured using analytical balance (Rachmadiarti and Trimulyono, 2019).

Detergent Concentration Measurement in Plants Media

Concentration of LAS detergent was determined using the methylene blue methods and expressed in ppm as MBAS. Five mL of water sample was filled into reaction tube, added 1 spoonful of NaH_2PO_4 and shook until it was homogenised. After that, 1 mL methylene blue was mixed. The solution was extracted by adding 5 mL chloroform, causing separation of water and chloroform phase. Water phase was separated from chloroform phase, added 1 mL rinsing solution, and re-extracted. Remaining water phase was separated from sample. Sample was then centrifuged for 2 minutes at 1500 rpm, then analysed using Genesys UV-Vis spectrophotometer at wavelength 652 nm.

Chlorophyll Concentration Measurement in Plants

Chlorophyll concentration was measured from 1 g of plant's leaf sample. Leaves were collected from each plants species and cut into small pieces, then crushed into smooth paste. Paste was extracted using 100 mL 95% alcohol and filtered using filter water until 100 mL of filtrate was obtained. Chlorophyll concentration was measured using spectrophotometer at wavelength 649 and 655 nm, which was previously calibrated using 95% alcohol.

Data Analysis

Parameters observed in the study were (1) wet biomass, (2) anionic detergent concentration in media and (3) chlorophyll concentration in leaves. Data were analysed using one-way ANOVA and followed by Duncan test at 95% confidence interval using SPSS 21th edition statistical software.

Results and Discussion

Absorption of LAS detergent by plants

The results showed that at the end of experiment the concentration of residual detergent increased with increasing LAS in the media (Table 1). The higher the LAS concentration given at initial of experiment, the higher the detergent residual in planting media. Plants species showed no significant difference in detergent absorption (Table 1). The highest reduction (86.2%) was found in media with 30 ppm of detergent. Previous study conducted by Rizky et al. (2019) using *Lemna* and *Azolla microphyla* demonstrated similar results. The decrease of detergent concentration in media could be due to microbes in plants root that is able to optimally absorb detergent, at least during 10 days of treatment period (Kopriva and dan Koprivova, 2003). As the detergent concentration increased, work done by root microbes increased too, to facilitate diffusion into plants tissues, because pollutants are metabolised by microbes into simpler forms. This caused higher concentration of detergent to be translocated to other parts of plants bodies, but inducing other effects, such as reducing chlorophyll concentration in the leaves (Kopriva and dan Koprivova, 2003) as demonstrated in our study (Table 4). The SO_3^{2-} and Na^+ present in LAS detergent are absorbed by plants via roots and localised to certain parts of cell to prevent disruption of plants metabolism (Kopriva and dan Koprivova, 2003). Since both *M. crenata* and *L. ascendens* can significantly reduce LAS detergent concentration and can survive in 30 ppm concentration of LAS detergent. Hence, both species finds application as phytoremediator agents.

The pH value of initial LAS solution used as planting media ranged from 7.5 to 7.8 units (Table 2). These values were in accordance with the Indonesian standard of minimum limit of waste water pH (6.5-9), which can be disposed in the environment. The LAS pH value dropped between 7.3 and 7.73 after being used as the planting media for 10 days (Table 2). The BOD values of LAS solutions used as planting media ranged from 8.59 to 11.55 mg O_2/L. The BOD value substantially decreases after 10 days. This indicates that the organic compound in media are most likely used as nutrients for both aquatic plants. Thus, the application of *M. crenata* and *L. adcendens* can be used to reduce the pH value and BOD concentrations in the LAS solution.

Biomass of Plants Grown in Planting Medium with LAS Detergent

The study presented that the plant's biomass decreased with increasing the LAS concentration in media (Table 3). No difference was found among biomass of plants species, either individually or as a consortium. However, significant differences were found in the experiment comparing different detergent concentrations (Table 3).

At detergent concentration of 30 ppm, plants have reached the saturation point (Risky et al., 2017). At saturation point, nutrient absorption by plants was reduced, causing disruption of metabolism and decreased their biomass. At visual observation during the experiment, we noted the emergence of new buds and new roots of both species, although their biomass decreased. This means that plants were able to adapt with LAS detergent contamination in medium.

Table 1: Concentration of residual detergent in planting media after treatment with LAS

Plants species	*LAS detergent concentration in planting media at initial detergent concentration (ppm)*			
	0	*10*	*20*	*30*
M. crenata	0.00±0.00[a]	1.84±0.25[b]	2.64±0.25[c]	3.51±0.42[d]
L. adscendens	0.00±0.00[a]	1.68±0.33[b]	2.68±0.29[c]	3.27±0.16[d]
M. crenata + L. adscendens	0.00±0.00[a]	0.84±0.17[b]	1.46±0.10[c]	2.07±0.15[d]

* Different superscript letters indicated significant difference of residual detergent concentration based on Duncan test ($p = 0.05$), a<b<c<d.

Table 2: Measurement of chemical factor in planting media of plants consortium

Parameter	*Time*	*Concentration of LAS (ppm)*			
		0	*10*	*20*	*30*
pH *6.5-9	Initial	7.16±0.06	7.5±0.000	7.7±0.000	7.8±0.10
	Final	7.03±0.06	7.3±0.06	7.63±0.06	7.73±0.06
BOD *6.5-9	Initial	6.12±0.41	8.59±0.33	10.45±0.17	11.55±0.25
	Final	3.39±0.24	5.56±0.23	6.70±0.21	7.56±0.21

* = Standard of Indonesian Government (Regulation No. 82/2004).

Table 3: Biomass of *M. crenata, L. adscendens and* consortium of *M. crenata, L. adscendens* at the end of experiment

Plants species	*Wet biomass at LAS detergent concentration (g)*			
	0	*10*	*20*	*30*
M. crenata	156.67 ± 5.77^{d}	140.00 ± 0.00^{c}	133.33 ± 5.77^{b}	123.33 ± 5.77^{a}
L. adscendens	146.67 ± 5.77^{d}	136.66 ± 5.77^{c}	130.00 ± 5.77^{b}	120.00 ± 0.00^{a}
M. crenata + L. adscendens	156.67 ± 5.77^{d}	143.33 ± 5.77^{c}	126.67 ± 5.77^{b}	116.67 ± 5.77^{a}

* Different superscript letters indicated significant difference of wet biomass based on Duncan test (p = 0.05), a<b<c<d.

Table 4: Chlorophyll concentration of *M. crenata* and *L. adscendens* leaf after treated with various LAS detergent concentrations

Plants species	*Chlorophyll*	*Chlorophyll concentration at LAS detergent concentrations (ppm)*			
		0	*10*	*20*	*30*
M. crenata	a	14.73 ± 0.33^{c}	14.44 ± 0.22^{c}	13.72 ± 0.25^{b}	11.85 ± 0.43^{a}
	b	11.29 ± 0.15^{c}	9.94 ± 0.36^{b}	8.48 ± 0.40^{a}	8.31 ± 0.51^{a}
	Total	26.19 ± 0.11^{d}	24.35 ± 0.14^{c}	22.13 ± 0.51^{b}	20.31 ± 0.24^{a}
L. adscendens	a	14.54 ± 0.22^{c}	13.98 ± 0.90^{c}	12.56 ± 0.36^{b}	11.14 ± 0.04^{a}
	b	9.79 ± 0.16^{c}	8.72 ± 0.16^{b}	8.15 ± 0.14^{a}	7.47 ± 0.16^{a}
	Total	24.30 ± 0.17^{d}	22.68 ± 0.24^{c}	20.68 ± 0.25^{b}	18.59 ± 0.20^{a}

* Different superscript letters indicated significant difference of chlorophyll-*a* concentration based on Duncan test (p = 0.05), a<b<c<d.

Chlorophyll Concentration of Plants Grown in Planting Media Containing LAS Detergent

This study showed that the concentration of chlorophyll of all plants decreased with increasing concentration of detergent in media (Table 3). However, no significant difference was found between plants species in the experiment comparing different detergent concentrations.

Wang et al. (2015) reported that detergent can disrupt the formation of chlorophyll by altering Mg and N entering into the cells which were critical in formation of chlorophyll. High concentration of LAS also inhibited chlorophyll synthesis (Liu et al., 2004) producing various effects to plants, e.g. rolling leaves, burnt-like leaves, dry leaves and lethality to plants. Liu et al. (2019) reported that LAS detergent produced chlorophyll damage to *M. spicatum*.

Conclusions

The results of the study showed *M. crenata* and *L. ascendens* capable to reduce the LAS detergent concentration in media polluted by detergent. However, the capability to reduce the detergent decreased with increasing LAS detergent concentration in media. Therefore, for practical purposes the replacement of *M. crenata* and *L. ascendens* is necessary periodically if the symptoms such as rolling leaves, burning leaves and dry leaves appear.

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