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Resistance of Yellow Velvetleaf (*Limnocharis flava* (L.) Buch.) Exposed to Lead

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ABSTRACT

The potential use of yellow velvetleaf (*Limnocharis flava* (L.) Buch) for remediation of lead (Pb) polluted water was studied in Green house experiment. The study was also investigated the distribution of lead in plant tissue. Plants of equal size was grown hydroponically and exposed to 0, 1, 5, 10 mgL^{-1} of Pb concentration for 10 days, 20 days, and 30 days. As a comparison, water spinach (*Ipomoea aquatica*) was grown in the same growth media. The results show that, although not as good as water spinach, yellow velvet leaf could grow well in Pb polluted water. The bioaccumulation factor of both plants were >1 with the translocation factor < 1 indicated that both plants are tolerant to Pb metals The highest accumulation of Pb found in root tissue, and then followed by stem and leave tissue. The concentration of Pb in leaves tissues found in yellow velvetleaf and water spinach exposed to 10 mg L^{-1} Pb were 1.30 and 1.90 mg kg^{-1} which is higher than the criteria given by FAO and WHO (5 mg kg^{-1}). Therefore, this fact should be considered if there is any attempt to use water spinach grown in Pb polluted growth media for human consumption or anaimal feeds.
Keywords: lead, Phytoremediation, water pollution, waste water, bioaccumulation, translocation factor

INTRODUCTION

Surabaya River is one of the main sources for providing water requirement of Surabaya communities, the second biggest city in Indonesia. In addition for domestic used, Surabaya River is also a source of irrigation water for rice and vegetable crops. Lately, with increasing industrial and domestic waste discharged to this river. Rachmadiarti *et.al.* [1] observed that its water had contaminated by some heavy metals, including lead (Pb) and Cadmium (Cd) with concentration of 0.01 mg L^{-1} and 0.01 mg L^{-1} respectively.).This is far higher compared to the safe water quality standard given by WHO [2]. The contribution of domestic was to high Mercury (Hg), Cromium (Cr) and Pb in Surabaya river has also been notified by Arisandi [3][4]. In addition, Rachmadiarti *et al.* (1) found that there were abundance of Yellow Velvetleaf (*Lymnocharis flava)* grow at Gayungan and Sepanjang wetland area which received irrigation water from Surabaya River. This yellow velvetleaf is commonly used for vegetable of the surrounding community. In fact this yellow velvetleaf ha concentrations of > 0.05 mg Pb kg^{-1}, this is exceeded the food quality standard given by WHO [2].

Park *et al.* [5] noted that the existence of arsenic and heavy metals in water caused negative impacts on aquatic ecosystems and biota growing in such habitats. It has been widely known that some heavy metals are toxic substances for animal, and hence for human life through food chain [2]. Duruibe [6] noted that Pb cause teratogenic effect. Pb toxicity also inhibits the hemoglobin synthesis; causes kidney malfunction; influence reproductive systems and cardiovascular system; and causes acute and chronic damage to the central nervous system and peripheral nervous system [7]. Reduce of heavy metals concentration in Surabaya River, therefore, is a compulsory. A lot of methodologies have been developed to clean the pollutant from water, but, it seems that the cheapest and easiest method to do this is by the use of plant. This method, which is known as "Phytoremediation" has been used widely, to remediate either contaminated soils or polluted water [8].

The success of phytoremediation depends on growth rate and the ability of the plant to uptake the metals from the growth medium [8]. Plants must produce sufficient biomass and able to accumulate high concentration of heavy metals in their tissue. Gothberg *et al.* [9] reported that water spinach could accumulate Cd in high concentration without any negative effect on their growth. Gothberg *et al.* [10] also demonstrated the ability of *Ipomoea aquatica* Forsk to decrease Pb concentration in polluted water. Abhilash *et al.* [11] used yellow velvetleaf for phytofiltration of Cd polluted in water.

The experiment reported here was aimed to explore the potential of yellow velvetleaf for remediation of Pb polluted water. To do this, first the experiment was done to study the resistance of yellow velvetleaf in lead polluted water. The growth of yellow velvetleaf was compared to that of water spinach, which has been known as tolerance to high metal concentration. Then, since water spinach and yellow velvetleaf are also used for

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human consumption and fish culture, the study also directed to partitioning of lead in plant organs. This was done to asses any potential hazard if these plants are used for human consumption, or animal and fish culture.

MATERIALS AND METHODS

To study the resistance of yellow velvetleaf to lead, the plant was grown in medium contain Pb solution of : The medium was the added with Pb solution (depend on the experimental treatments), i.e: (1) 0 Pb as the control; (2) 1.0 mgL^{-1} of $Pb(NO_3)_2$ solution; (3) 5.0 mgL^{-1} of $Pb(NO_3)_2$ solution; and (4) 10.0 mgL^{-1} of $Pb(NO_3)_2$ solution. As a comparation the same treatment was done for water spinach. These treatments were arranged in a randomized block design with 3 replications.

Yellow velvetleaf and water spinach were collected from wetlands at Gayung Sari (Surabaya, East Java, Indonesia). Initially, the plants were grown in a plastic chamber containing 20 L of Hoagland's media in a glass house over 5 days. This was done to reduce previous environmental contamination and adapt the plants to glasshouse conditions. Subsequently, plants of yellow velvetleaf were choice with 90 gram biomass and stems of water spinach were cut in which every cutting stem has 2 nodes (10 cm length for water spinach). Each plant was grown for 10 days in a plastic chamber containing 20 L distillation water containing Hoagland's solution [12].

These acclimated pants were used for further study. Yellow velvetleaf with peduncles length of 20-25 cm and roots length of 8-10 cm; and water spinach with roots length of 3-4 cm and stem length of 20-25 cm were selected. The plants were cleaned with distillated water and then it was put in glass aquarium of 40 cm length, 30 cm width, and 35 cm depth which contain of 5 L distilled water and Hoagland's solution. The pH of the medium varied from 5.4 – 6.7 at the beginning of the experiment, and 6.5 – 7.0 at the end of the experiment. The plants were illuminated sunlight 12.12 hours light dark cycle at a photon flux density at 598 candles. The plant samples (destructive sampling) from each glass aquarium were harvested at 10, 20 and 30 days after planting, after which biomass yield and metal content were measured.

Harvested plants were divided into three parts: roots, stem/peduncles and leaves. Each part was oven dried for 2 h at 80^{o} C for 48 hours and weighed to determine its dry weight. Dried plant tissues were ground with a mill. Powdered samples weighing 0.5 g were digested with 5 ml of HNO_3 and diluted to 50 ml with de-ionized double distilled water. Pb analysis was done with the method of extraction described by Göthberg *et al.*, [11]. Media samples (50 ml) and digested plant samples were analyzed for Pb by Inductively coupled plasma (Teledyne Leeman Labs). The total accumulation and partitioning of the metals by the plants were calculated. Plant biomass yield was measured on dry weight basis.

Relative growth rate (RGR), expressed as grams per kilogram per day, and was calculated as:

$$RGR = (\text{final dry - initial dry weight}) / \text{initial weight x days} \quad (1)$$

The lead bio-concentration factor (BCF) for lead of the root system and the aerial parts (stem/peduncles leaves) was calculated according to the formula of Wang *et al.* [12], i.e.:

$$BCF^{a} = C_{roots}/C_{medium} \quad (2)$$

$$BCF^{b} = C_{aerial}/C_{medium} \quad (3)$$

where, $C_{roots\,=}$ concentration of lead in the roots (mg Pb kg^{-1}dry weight)
C_{aerial}= sum of the concentration of lead in stems or peduncles (mg Pb kg^{-1}dry weight) and
C_{medium} = concentration of lead in growth medium (mg Pb L^{-1})

The translocation factor (TF) was calculated to evaluate the translocation of Pb from the water spinach and yellow velvetleaf roots to aerial parts (stem/peduncles and leaves) the above two equation (1) and (2), the TF as follows:

$$TF = BCF^{b}/BCF^{a} \quad (4)$$

Analysis of variance (ANOVA) using SPSS 16 software package was conducted to analyzed the data. If there was a significant differences, the Tukey test (p =0.05) was performed.

RESULTS AND DISCUSSION

The growth of yellow velvetleaf and water spinach at various medium conditions is presented in Table 1. At 10 days observation there was a negative growth of yellow velvet leaf. It seems that this phenomenon was only adaptation period for yellow velvetleaf to grow in new environment, not because of the existence of Pb in the medium. As shown in Table 1, negative growth of yellow velvetleaf occurred also for that grown in the control treatment. Furthermore, there was a positive growth at 20 and 30 days observations. The biomass of yellow velvetleaf grown in control treatment was not significantly different compared to that of grown in Pb contaminated solution.

Different phenomenon was observed on water spinach. It seems that water spinach was very easy adapted to the new environment so that water spinach did not require adaptation periods. At 10 days observation, the existence of Pb in the solution did not influence the growth of water spinach. However, at 20 and 30 days Pb significantly reduced water spinach growth. The growth of water spinach at various Pb concentrations was not significantly different.

Table 1 the growth of water spinach and yellow velvetleaf at different Pb concentration

Plants	Pb Concentration (mg L^{-1})	Initially Biomass (gram)	Fresh biomass at …. days (g/plant)		
			10 days	20 days	30 days
Yellow velvetleaf	0	100	93.10 a	115.10 a	128.10 a
	1	100	93.57 a	108.57 a	120.24 a
	5	100	94.13 a	108.13 a	121.13 a
	10	100	92.15 a	106.15 a	122.48 a
Water spinach	0	100	157.50 b	229.67 c	231.00 c
	1	100	150.00 b	169.00 b	177.00 b
	5	100	147.33 b	167.50 b	172.80 b
	10	100	140.67 b	159.80 b	165.33 b

*) means followed by the same letters, at the same column are not significantly different (p=0.05)

The result presented in Table 2 showed that yellow velvetleaf and water spinach have a different growth pattern. Until 10 days after planting yellow velvetleaf had a negative growth rate, after which the relative growth rate increased with plant ages. Water spinach, on the other hand, had no negative growth rate, and RGR increased up to 20 days old, then RGR decreased from 20 – 30 days after planting.

Furthermore, data in Table 2 showed that at early growth periods (0 – 20 days after planting) RGR of yellow velvetleaf was lower compared to that water spinach. However, at the periods of 20 – 30 days after planting, RGR of yellow velvetleaf was higher that water spinach. At 30 days after planting the medium had been full covered by water spinach, but this did not occur with yellow velvetleaf (Figure 1). Thus there was a competition for water spinach.

Table 2 Means Relative growth rate (RGR) of yellow velvetleaf and water spinach grown in the Pb contaminated solution.

Plants	Pb Concentration (mg L^{-1})	RGR of plants (g/ days)		
		10 days	20 days	30 days
Yellow velvetleaf	0	-0.0069 a	0.0151 b	0.028 ab
	1	-0.0060 a	0.0086 b	0.021 ab
	5	-0.0059 a	0.008 b	0.021 ab
	10	-0.0079 a	0.006 b	0.022 ab
Water spinach	0	0.0575 b	0.1297 b	0.130 ab
	1	0.0500 b	0.069 b	0.077 ab
	5	0.0470 b	0.068 b	0.073 ab
	10	0.0407 b	0.058 b	0.065 ab

*) means followed by the same letters, at the same column are not significantly different (p=0.05)

From the result presented in Table 1 and 2 it can be concluded that yellow velvetleaf could grow well in lead polluted solution. However, compared to water spinach the growth rate of yellow velvetleaf was much slower. Water spinach has been known as aquatic plants [8], and therefore it was not surprise that this plant grew better compared to yellow velvetleaf.

Although the growth of yellow velvetleaf was not good as water spinach, the capability of yellow velvetleaf to remove Pb from solution was comparable with that of water spinach (Table 3). At 10 and 20 days after transplanting, the removal of Pb by yellow velvetleaf was lower compared to that of water spinach. However, at 30 days after planting, the removal of Pb by yellow velvetleaf did not significantly different with the removal by water spinach. Looking the data presented in Table 1 and Table 2 the lower Pb removal by yellow velvetleaf could be suggested due to lower growth rate of yellow velvetleaf (compared to water spinach),

Table 3 Percentage removal Pb by Yellow velvetleaf and water spinach

Plants	**Pb concentration (mg L^{-1})**	**Percentage removal Pb by plants**		
		10 days	20 days	30 days
Yellow velvetleaf	1	64.00 a	71.00 a	94.00
	5	65.00 a	75.00 a	95.00
	10	63.00 a	74.00 a	98.00
Water spinach	1	76.00 b	92.00 b	97.00
	5	77.00 b	91.00 b	98.00
	10	82.00 b	98.00 b	98.00 NS

*) means followed by the same letters, at the same column are not significantly different (p=0.05)

Although there was a negative growth of yellow velvetleaf (Table 1 and 2), the existence of plants was able to decrease Pb concentration in the growth medium. This data indicated that the plant was still able to remove Pb from the solution. In the hydroponic system, passive absorption which occurs together with evapotranspiration would be more dominant. The measurement done at 10 days after transplanting, the water lost due to evapotranspiration from yellow velvetleaf varied from 4.2 (10 mg L^{-1} Pb treatment) to 4.7 cm (5 mg L^{-1} Pb treatment). The evaporation at the same period was only 3.6 cm, and the evapotranspiration from water spinach varied from 4.9 (10 mg L^{-1} Pb treatment) to 5.1 cm (0 mg L^{-1} Pb treatment)

The experimental result presented in Table 4 show that the highest Pb concentration of both yellow velvetleaf and water spinach was found in root tissues. The mechanism behind the higher Pb concentration in the roots may include binding of the positively charged toxic metals ion to negative charges in the cell walls [13]. Gothberg [11] suggested that in most plants, a larger proportion of the metal was retained in the roots and thereby prevented from interfering with sensitive metabolic reaction in the shoots. This is probably an internal mechanism to avoid toxic metal concentration in the shoot.

Table 4 Pb concentration in various plant tissues (measured for 5 mg L^{-1} Pb and 5 mg L^{-1} Pb treatments)

Plants	**Days after transplanting**	**Pb concentration in plant tissues exposed to 5 mg L^{-1} Pb (mg kg-^{1})**			**Pb concentration in plant tissues exposed to 10 mg L^{-1} Pb (mg kg-^{1})**		
		Roots	Stems	Leaves	roots	Stems	Leaves
Yellow velvetleaf	10	1,92 a A	0.237 a B	0.24 B	3,13 a A	0.098a B	0.182 B
	20	1.36 b A	0.923 b B	0.287 B	4.66 b A	0.07 b B	0.463 B
	30	0.25 c A	1.33 b B	0.297 B	0.29 c A	0.20 b B	0.40 B
Water spinach	10	2.26 a A	0.157 a B	0.197 B	2.66 a A	0.44 a B	0.77 B
	20	2.71 b A	0.41 b B	0.577 B	5.12 b A	0.39 b B	0.4108 B
	30	2.63 c A	0.05 b B	0.16 B	5.35 c A	0.07 b B	0.273 B

*) means followed by the same letters for the same time of measurements, at the same column (small letters) and rows (capital letters) are not significantly different (p=0.05)

Pb concentration in root tissues of water spinach was relatively constant with plant age. In root tissues of yellow velvetleaf, Pb concentration decreased from 10 to 20 days plat age, then again increased at 30 days after planting. Looking the data presented in Table 1, it seems that the decrease of Pb concentration from 10 to 20 days plant age was due to dilution effect. The results in Table 4 also show that except for water spinach at 30 days after planting, Pb concentration in stem tissues did not significantly different with that in leave tissues. At 30 days after planting, Pb concentration in leaf tissues of water spinach was higher than that in the stem tissues. The concentration of Pb in stem and leaf tissues of yellow velvetleaf did not significantly changes with plant age. For water spinach, on the other hand, Pb concentration in stem and leave tissues increased as plant growing older.

To study the capability of plants to extract metals from the water and the aptitude of plants to transfer metals from roots to shoots the term bio-concentration factor (BCF), and the as the translocation factor (TF) introduce by Yoon *et al.* [13] was used. The results presented in Table 5 show that until 20 days plant age, TF of both yellow velvetleaf and water spinach are less than 1.0, indicating that the metal ion was largely retained in roots. This result supports the previous data which show that the highest Pb concentration was in the root tissue.

Following the criteria proposed by Yoon et al. (14) it can be suggested that both yellow velvetleaf and water spinach were accumulator plants. According to Yoon *et al.* [14] plant can be classified as the excluder and accumulator if the value of TF<1 and BCF>1. However, value of TF and BCF water spinach better than yellow velvetleaf. The proper disposal of the harvested plant parts is the final and most important step in any kind of plant-based remediation technology. The higher BCF and TF values of tested species enable them to accumulate large amount hazardous metals in the harvested parts and if not disposed properly the accumulated heavy metals may back to the system or can enter into the food chain. Bioaccumulation of the trace metals in water spinach and yellow velvetleaf tissues was not a concern as because the measured levels were lower than the permissible levels set by FAO and WHO (0.5 mg kg^{-1}) for consumption, except of yellow velvetleaf which are exposed to 10 mg L^{-1} Pb.

Table 5 TF each water spinach and yellow velvetleaf

Plants	**Pb concentration (mgL^{-1})**	**Translocation Factor (TF)**			**Bio-concentration Factor (BCF)**		
		10 days	20 days	30 days	10 days	20 days	30 days
Yellow velvetleaf	1	0.32 a A	0.19 a A	2.06 b B	3.42 a A	3.92 ab B	18.74 b C
	5	0.25 a A	0.23 a A	1.74 b B	4.48 a A	8.56 c B	21.79 b C
	10	0.90 a A	0.10 a A	2.08 b B	4.90 a A	9.54 c B	15.06 b C
Water spinach	1	0.39 a A	0.27 a A	0.32 a B	4.82 a A	2.35 a A	3.12 a A
	5	0.16 a A	0.36 a A	0.08 a B	3.09 a A	4.41 b	2.75 a A
	10	0.47 a A	0.56 a A	0.06 a B	3.76 a A	1.86 a	3.28 a A

*) means followed by the same letters, at the same column (small letters) and rows (capital letters) are not significantly different (p=0.05)

CONCLUSION

Yellow velvetleaf, like water spinach, was a potential plant to remediate water polluted by Pb . The bioaccumulation factor of both plants were >1 with the translocation factor < 1 indicated that both plants are tolerant to Pb metals. The highest Pb concentration in both plant were in the roots, then followed leaves, and stem. The concentration of Pb in leaves tissues found in yellow velvetleaf and water spinach exposed to 10 mg L^{-1} Pb were 1.30 and 1.90 mg kg^{-1} which is higher than the criteria given by FAO and WHO (5 mg kg^{-1}). Therefore, this fact should be considered if there is any attempt to use water spinach grown in Pb polluted growth media for human consumption or animal feeds.

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