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Tolerance and Survival of *Scirpus grossus* and *Lepironia articulata* in Synthetic Mining Wastewater

[1]Nur Izzati Ismail, [1]Siti Rozaimah Sheikh Abdullah, [2]Mushrifah Idris, [1]Hassimi Abu Hasan, [1]Nadya Hussin AL Sbani and [1]Omar Hamed Jehawi

[1]Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia, UKM Bangi, Sealngor, 43600, Malaysia

[2]Tasik Chini Research Center, Universiti Kebangsaan Malaysia, UKM Bangi, Sealngor, 43600, Malaysia

Corresponding Author: Nur Izzati Ismail, Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia, UKM Bangi, Sealngor, 43600, Malaysia Tel: +603-89216407

ABSTRACT

Phytoremediation technology involves plants to treat contaminated areas. In this study, before phytoremediation technology can be used to treat mining wastewater, the range of the mixture of Fe and Al concentrations was determined in order to investigate the plant tolerance and survival towards those contaminants. The combined effects of Fe and Al were investigated by observing the visible symptoms of withering and death of plants at the end of exposure day (Day 21). Three-litre pails containing 3 kg of sand and plants (*Scirpus grossus* and *Lepironia articulata*) were exposed to different concentrations of Fe and Al solution mixture (150 mg L^{-1} Fe+50 mg L^{-1} Al until 750 mg L^{-1} Fe+250 mg L^{-1} Al with mass ratio between Fe and Al was 3:1). After 21 days of exposure, it was observed that *L. articulata* could tolerated in a more highly concentrated mixture of Fe and Al (450 mg L^{-1} Fe+150 mg L^{-1} Al) compared to *S. grossus* (300 mg L^{-1} Fe+100 mg L^{-1} Al) with 33.3% survival. Therefore, by determining the tolerance and survival of *S. grossus* and *L. articulate* towards Fe and Al, it is useful in designing the effective phytoremediation technology for the treatment of mining wastewater.

Key words: Phytoremediation, tolerance, mining wastewater, *Scirpus grossus*, *Lepironia articulata*

INTRODUCTION

Phytoremediation is defined as the use of various plants to reduce, remove, degrade, transform, extract or immobilize environmental toxins, which can be either organic or inorganic contaminants (Chirakkara and Reddy, 2014), from soil or water (Pantola and Alam, 2014; Sharma and Reddy, 2004) in order to provide less harmful conditions (Paz-Ferreiro *et al*., 2014), combining the disciplines of soil microbiology and chemistry and plant physiology (Cunningham and Ow, 1996). It has received increasing attention during recent decades as an emerging approach that utilizes the natural properties of plants to remediate contaminated soils due to the unique genetic, biochemical and physiological features through which the plant roots have a natural ability to absorb or extract heavy metals from the contaminated medium and then translocate them to the shoots, thus performing natural phytoremediation (Achakzai *et al*., 2011; Ejaz ul Islam *et al*., 2007; Ziarati and Alaedini, 2014).

Plant tolerance and survival test or also can be called as range finding phytotoxicity test was conducted in order to determine the concentrations of the toxicant that will be administrated to the test organisms (Titah *et al*., 2012). Normally the test is conducted at five different concentrations of contaminant to determine the percentage of survival of plant species towards each concentration of contaminants. Thus, the suitable concentrations that the plant can tolerate to treat the contaminant and still survive can be determined. The aim of this study was to determine the range of iron (Fe) and aluminium (Al) concentrations in which *Scirpus grossus* and *Lepironia articulata* can tolerate and survive. Both *S. grossus* and *L. articulata* are emergent plant. *Scirpus grossus* also known as giant bulrush, greater club-rush, rumput menderong (Malaysia), mensiang and walingi (Indonesia) (Tangahu *et al*., 2013) while, *L. articulata* commonly called as grey sedge, tube sedge and krajood (Wunbua *et al*., 2012; Domyos and Te-chato, 2013). The study was conducted using plastic pail containing sand as a growth media. The tolerant and survival of plants towards Fe and Al were investigate by exposing the plants with different mixture concentrations of Fe and Al.

MATERIALS AND METHODS

Source and propagation of plants: In this study, the native plants *Scirpus grossus* and *Lepironia articulata* from Tasik Chini, Pahang, were used to facilitate the phytoremediation process to remediate Fe and Al. These plants were selected based on the plant analysis collected from mining area in Tasik Chini, Pahang (Ismail *et al*., 2013) and also based on some studies being conducted at Universiti Kebangsaan Malaysia (unpublished data). Both plants were propagated from rhizome in garden soil at the greenhouse conditions. All of the plants used in the experiment were six weeks old before being exposed to the contaminants.

Synthetic mining wastewater: The types of heavy metals were selected based on the soil mining analysis done taken from mining area in Tasik Chini, Pahang (Ismail *et al*., 2013). From the analysis of mining soil, it was found that the mass ratio of Fe to Al was 3:1. The salts used were iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) (Friendemann Schmidt, UK) and aluminium sulfate ($Al_2(SO_4)_3.16H_2O$) (R and M Marketing, UK). Stock solutions of iron (III) chloride hexahydrate and aluminium sulfate were prepared by dissolving the salts separately with distilled water. Both stock solutions were diluted and mixed according to the concentrations needed as in Table 1.

Tolerance and survival studies: Studies were conducted in a greenhouse of Universiti Kebangsaan Malaysia (UKM). A 3-L pail was used as a batch reactor. Each pail was filled with

Table 1: Experimental detail for both plants and their function

Concentrations of Fe (III) and Al (III)					
PC	C1	C2	C3	C4	C5
0 mg L^{-1} Fe + 0 mg L^{-1} Al	150 mg L^{-1} Fe + 50 mg L^{-1} Al	300 mg L^{-1} Fe + 100 mg L^{-1} Al	450 mg L^{-1} Fe + 150 mg L^{-1} Al	600 mg L^{-1} Fe + 200 mg L^{-1} Al	750 mg L^{-1} Fe + 250 mg L^{-1} Al
■ ■ ■	■ ■ ■	■ ■ ■	■ ■ ■	■ ■ ■	■ ■ ■
▲ ▲ ▲	▲ ▲ ▲	▲ ▲ ▲	▲ ▲ ▲	▲ ▲ ▲	▲ ▲ ▲

PC: Plant control, C1-C5: Contaminant 1 to Contaminant 5, ■: *Scirpus grossus*, ▲: *Lepironia articulata*

3 kg of sand and 780 mL of synthetic wastewater (to simulate subsurface condition for water level) containing Fe and Al at the concentrations shown in Table 1, labelled as contaminants one (C1) to five (C5) together with Plant Control (PC).

Three plants were planted in each pail and the growths of each plant in each pail were observed at the end of 21 days and their conditions are described as healthy, withered or dead. To maintain subsurface condition, watering of plants was done every 2 days. At the end of the observation day, the percentage of plants which had survived throughout the study period was calculated using Eq. 1:

$$\text{Survival}\,(\%) = \frac{\text{No. of healthy plants}}{\text{Total No. of plants}} \times 100 \qquad (1)$$

RESULTS

Each plant in each pail was observed for their conditions as healthy; the whole leaves were green, withered; the leaves were changing color to yellow or bronze or dead; the whole leaves were bronze and dried. Table 2 shows pictures of plant growth for *S. grossus* and *L. articulata* on

Table 2: Pictures of plant growth of *Scirpus grossus* and *Lepironia articulata* on day 0 and 21

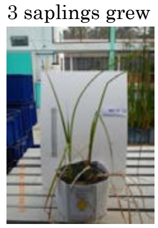
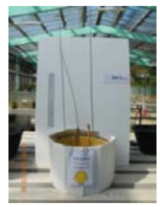
Concentrations of Fe (III) and Al (III)	Species and time of exposure (days): *Scirpus grossus* 00	*Scirpus grossus* 21	*Lepironia articulata* 00	*Lepironia articulata* 21
PC 0 mg L^{-1} Fe + 0 mg L^{-1} Al	 All plants were healthy	 All plants were healthy and 7 saplings grew	 All plants were healthy	 All plants were healthy and a sapling grew
C1 150 mg L^{-1} Fe + 50 mg L^{-1} Al	 All plants were healthy	 2 plants were healthy, 1 plant was dead and 3 saplings grew	 All plants were healthy	 All plants were healthy and a sapling grew
C2 300 mg L^{-1} Fe + 100 mg L^{-1} Al	 All plants were healthy	 2 plants were healthy, 1 plant was dead and 4 saplings grew	 All plants were healthy	 2 plants were healthy and 1 plant was dead

Table 2: Continue

	Species and time of exposure (days)			
Concentrations of Fe (III) and Al (III)	*Scirpus grossus*		*Lepironia articulata*	
	00	21	00	21
C3 450 mg L^{-1} Fe + 150 mg L^{-1} Al	 All plants were healthy	 All plants were dead	 All plants were healthy	 1 plant was healthy and 2 plants were dead
C4 600 mg L^{-1} Fe + 200 mg L^{-1} Al	 All plants were healthy	 All plants were dead	 All plants were healthy	 All plants were dead
C5 750 mg L^{-1} Fe + 250 mg L^{-1} Al	 All plants were healthy	 All plants were dead	 All plants were healthy	 All plants were dead

Day 0 and day 21. Based on the observations, on day 7, one *S. grossus* plant was withered at C1, while one *L. articulata* plant was withered at C2. All *S. grossus* and *L. articulata* plants were withered at C3 and C4, respectively. Plant withering was followed by the death of plants on day 14. Similar results were obtained until day 21 with regard to the deaths of plants, except that more saplings of both species grew.

Figure 1 illustrates the survival percentage for *S. grossus* and *L. articulata* at the end of the observation period. Both plants show different survival abilities at different concentrations of Fe+Al. Both plants survived during 21 days of observation with a 100% survival rate in the PC condition without the addition of Fe+Al. The ability of *L. articulata* to survive can reach up to C3, while *S. grossus* can survive only up to C2; the respective survival rates for the two plants were 33.3 and 66.7%. It was observed that *L. articulata* could survive in a higher concentration of the mixture of Fe+Al compared to *S. grossus*.

DISCUSSION

According to Olutayo *et al*. (2009), excessive Fe content will cause the bronzing or yellowing symptoms observed as withering in this study, while the most common responses of shoots to Al toxicity are cellular modifications in leaves, reduced stomatal openings, decreased photosynthetic activity, chlorosis and foliar necrosis (Vitorello *et al*., 2005). Yellowing that can be generalized over the whole plant, localized over individual leaves or isolated between some leaf veins

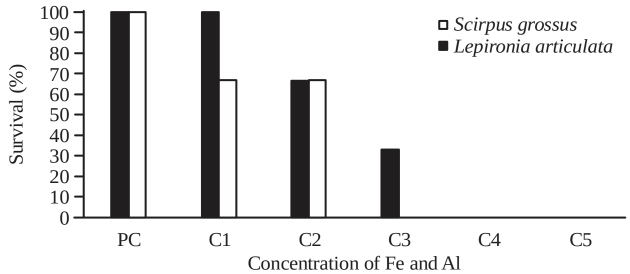


Fig. 1: Percentage survival of *Scirpus grossus* and *Lepironia articulata*

(interveinal chlorosis) is known as “chlorosis”, while the death of plant tissue sometimes occurs in spots is known as “necrosis”. Both symptoms can be due to the deficiency or excess of nutrients (Stevens *et al*., 2002). In this case, it is due to the excess of Fe and Al. Olutayo *et al*. (2009) reported bronzing or yellowing symptoms in two rice cultivars grown on potentially Fe-toxic soils. Besides that, Snowball and Robson (1991) reported that the first specific foliar symptom of Al toxicity in wheat is yellowing along the margin near the tip of the oldest leaf. Within a few days, brown lesions form in these chlorotic regions and work inwards from the margins, resulting in the formation of indentations. The progression of these toxicity symptoms is rapid. Old leaves become drought stressed and withered and at times collapse in the center.

In comparison, Tangahu *et al*. (2013) found that the *S. grossus* plant withered on Day 35 at a concentration of 800 mg L^{-1} Pb. The concentration of lead that caused *S. grossus* to wither was higher than the concentration of the mixture of iron and aluminium, which was only 450 mg L^{-1} Fe+150 mg L^{-1} Al. This was due to the application of single and binary combinations of heavy metals. Vuorinen *et al*. (1999) reported that the combination of iron and aluminium had a more toxic effect on grayling (Thymallus thymallus) and brown trout (Salmo trutta), whose percentage mortality increased significantly when both metals were present compared with exposure to a single metal. To date, no other observations regarding the toxicity of these metals for *L. articulata* have been conducted by other researchers.

CONCLUSION

As the concentration of the mixture of Fe and Al increased, the withering symptoms and plant deaths of *S. grossus* and *L. articulata* also increased, thus reducing the survival of *S. grossus* and *L. articulata*. *Lepironia articulata* can survive at a higher concentration of the mixture of Fe+Al compared to *S. grossus* which was 450 mg L^{-1} Fe+150 mg L^{-1} Al with 33.3% survival. In contrast, *S. grossus* could not (0% survival). For future studies of phytotoxicity, it is suggested that the concentration of Fe+Al for *S. grossus* and *L. articulata* should be up to 450 mg L^{-1} Fe+150 mg L^{-1} Al and 600 mg L^{-1} Fe+200 mg L^{-1} Al, respectively.

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