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Study the Accumulation of Nutrients and Heavy Metals in the Plant Tissues of *Limnocharis flava* Planted in Both Vertical and Horizontal Subsurface Flow Constructed Wetland

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Abstract. A laboratory-scale study was conducted on subsurface flow constructed wetland systems operated in vertical and horizontal mode. Each system comprises of one planted system and one control system. The planted systems namely VP and HP were planted with *Limnocharis flava*, while the control systems namely VC and HC were left unplanted. The systems operated identically at a flow rate of 0.029 m^3/d and hydraulic retention times of 19.7 hours and 24.1 hours in VSSF and HSSF systems, respectively. The aim of this study is to determine the amount of nutrients (NH_3-N and PO_4-P) and heavy metals (Fe and Mn) uptake by the *Limnocharis flava* plant tissues. This is done by measuring the amount of nutrients and heavy metals accumulated and retained in the plant's roots and aerial part (stems and leaves). The results indicated that HSSF systems exhibit a higher uptake of nutrients and heavy metals as compared to VSSF system due to the higher HRT for HSSF system. The study also shown the contribution of macrophyte in the sense of the uptake of pollutants is significant in this study.

Keywords: Plant uptake, Macrophytes role, Constructed Wetlands.

1. Introduction

The use of constructed wetlands to treat wastewater is relatively new development in Malaysia. The abundance of wetland plant species and good conditions for plant growth in Malaysia provided an ideas environment for wastewater treatment. Plants are plays an integral part of the effluent treatment processes in constructed wetlands (Brix, 1997). The plant transfer oxygen through their root and rhizome systems to the bottom of treatment basins. It also provides a medium beneath the water surface for attachment of microorganisms that perform most of biological treatment (Mashhor et al., 2002). Several processes are envisioned as being effective in pollutants reduction such as phytoextraction, phytoaccumulation and rhizofiltration (Kadlec, 1999).

Phytoextraction and phytoaccumulation refers to plant uptake of toxicants, which is known to occur and has been studies in the storm water and mine water wetland context (Kadlec, 1999). However, in many cases the contaminant is selectively bound up in below ground tissues, roots and rhizomes, and is not readily harvested. For example, metals are taken up by plants, and in many cases stored preferentially in the roots and rhizomes (Sinicrope et al., 1992). Rhizofiltration is the adsorption or precipitation onto plant roots (or absorption into the roots) of contaminants that are in solution surrounding the root zone (Neate, 2003). It is based on a combination of principle of phytoextraction and phytostabilization specially suited to remove metals and radio nuclides from polluted water. Contaminants are absorbed and concentrated by plant roots, then precipitated as their carbonates and phosphates (Salt et al., 1995).

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This research focused on determining the amount of nutrients (NH_3-N and PO_4-P) and heavy metals (Fe and Mn) that accumulate in the plant tissues. The *Limnocharis flava* was used as wetland plant for the vertical subsurface flow (VSSF) and horizontal subsurface flow (HSSF) constructed wetland systems. The wastewater used in the study is landfill leachate. In the VSSF constructed wetland system, the wastewater is fed to the system on the whole surface area through a distribution system and seep through towards the bottom of the system in more or less vertical path. While, in the HSSF constructed wetland system, the wastewater is fed through the inlet and flows under the surface of the bed in more or less horizontal path until it reaches the outlet zone.

2. Methodology

2.1. Experiment Set up

In this study, four laboratory scale constructed wetland systems have been constructed, which consist of two vertical subsurface flows (VSSF) and two horizontal subsurface flows (HSSF) constructed wetland systems. Each system comprises of one planted system and one control system. The planted systems namely VP and HP were planted with *Limnocharis flava*, while the control systems namely VC and HC were left unplanted. Each of the VSSF and HSSF system consists of a wetland reactor, a feeding tank and settling tank. The wetland reactor and operation characteristics are summarized in Table 1. The wetland reactors were constructed using acrylic with the dimension of 0.58 m length, 0.31 m wide, and 0.33 m depth.

Table 1: Reactor characteristics

Total reactor height	0.33 m
Total surface area	0.178 m^2
Total planting area	0.141 m^2
Weight of gravel used per reactor	35.6 kg
Weight of soil per reactor	27.45 kg
Average gravel size	10-25 mm
Average void volume per reactor	0.016 m^3
Flow rate	0.029 m^3/d
Hydraulic Retention Time (HRT) per cycle	
• HRT_{VSSF}	19.7 hours
• HRT_{HSSF}	24.1 hours

Both VP and HP reactors were planted with *Limnocharis flava* with density of 15 peduncles (stem) per reactor. The *Limnocharis flava* was chosen in this study because of its availability, where it can be commonly found throughout the state of Perlis, Malaysia. It was also chosen due to the fact that it has long fibrous roots that can provide oxygen supply to the media and promote uptake of contaminants. After the transplantation, the wetland reactors (VP and HP) were loaded with tap water to establish the emergent plant. The duration takes 7 days for the acclimatization process, where the readiness of the plant for the actual experimental procedure was illustrated by the healthy leaves and stem and also by the growth of new leaves and inflorescence. The diluted leachate (25% concentration) was fed into the wetland reactors by 60L feeding tank. The flow rate of leachate from the feeding tank into the wetland reactors were set to be 0.029 m^3/d. And this resulted to the hydraulic retention time (HRT) of 19.7 hours for VSSF and 24.1 hours for HSSF.

2.2. Analysis of Plant Tissues

Analysis of the plant tissues were conducted initially before the treatment procedures begin and after the termination of the experiment. This analysis was conducted to determine the uptake of nutrient (ammonia nitrogen, NH_3-N and orthophosphate, PO_4-P) and heavy metals (iron, Fe and manganese, Mn) by the plant tissues. The method used for the analysis of the plant tissue was dry ashing method (Bureau of Nutritional Sciences Ottawa, 1983), where two replicate samples from the planted (VP and HP) reactors were selected and harvested. The plants were cleaned by washing them with tap water followed by distilled water and sorted into leaves, stems and roots component. The plants samples were then placed in a porcelain crucible and ashed by heating it overnight in a muffle furnace at 500°C. The ash residue was then cooled and 1 g of each samples (leaves, stems and roots) were weighted and dissolved in 5 mL of 20% hydrochloric acid (HCl) for digestion. The solutions were then shaken for 4 hours with orbital shaker. It was later filtered through an acid-washed filter paper into a 50 mL volumetric flask. The solutions were then diluted to volume with deionised water and mixed well. The concentrations of the elements of interest were then determined according to United State Environmental Protection Agency (USEPA) approved methods, by using HACH DR 2800 spectrophotometer.

3. Result and Discussion

The analysis of plant tissues were conducted to study the extents of phytoaccumulation or phytoextraction of nutrients (NH_3-N and PO_4-P) and heavy metals (Fe and Mn) in the plant tissues which were segregated into three main components which are leaves, stems, and roots. The results of the plant tissues analysis as shown in Fig. 1 shows that there was an accumulation of nutrients and heavy metals in the tissues of *Limnocharis flava* planted in both vertical (VP) and horizontal (HP) subsurface flow constructed wetland systems. The accumulation of pollutants (nutrients and heavy metals) shows that the contribution of macrophytes in the sense of the uptake of pollutants are significant in this study, apart from providing a large surface area for attached microbial growth, supplying reduced carbon through root exudates and micro-aerobic environment and a via root oxygen release in the rhizosphere, and stabilizing the surface of the bed (Gersberg et al., 1986; Tanner, 2001; Gagnon et al., 2006).

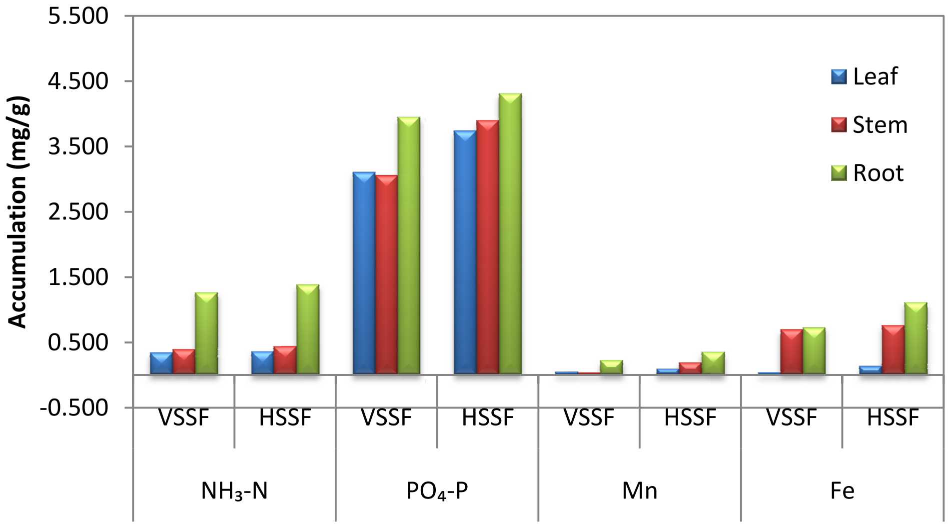

Fig. 1: Accumulation of pollutants in plant tissues of *Limnocharis flava* after 45 days of treatment

As shown in Fig. 1, it can be observed that *Limnocharis flava* planted in both reactors (VP and HP) exhibit the highest uptake on PO_4-P. Where, the highest amount of PO_4-P was observed to be accumulated within the plant's root, with 4.313 mg/g and 3.950 mg/g for VSSF and HSSF reactors, respectively. The amount of PO_4-P decreased towards the plant's leaves and stems with 3.10 mg/g (leaves) and 3.05 mg/g (stems) for VSSF and 3.738 mg/g (leaves) and 3.900 mg/g (stems) for HSSF. The high uptakes of PO_4-P are coherent with the needs for nutrients in order for it to survive. Apart from being undisputedly great in the uptake of PO_4-P, *Limnocharis flava* has shown a momentous result in the uptake of other parameters such as NH_3-N, and Fe. NH_3-N uptake was also high with the uptake of 1.263 mg/g and 1.388 mg/g for VSSF and HSSF, respectively. Both observed in the root tissues where nitrogen removal is known to be influenced by the presence of plant directly through assimilation (Brisson and Chazarenc, 2008).

The ability of *Limnocharis flava* to uptake heavy metals was also proven in this study, which coherent with the findings by Kosopolov et al., 2004; Ujang et al., 2005; Yalcuk and Ugurlu, 2008. Where, the highest amount of Mn was determined in the root for both VSSF and HSSF, with 0.223 mg/g (VSSF) and 0.362 mg/g (HSSF), respectively. While, the highest amount of Fe was determined in the root for both VSSF and HSSF, with 0.728 mg/g (VSSF) and 1.117 mg/g (HSSF), respectively. In which it was consistent with the findings by Janet et al. (1992), Peverly et al. (1995) and Ain Nihla, (2006). Greenway and Simpson (1996), Polprasert et al. (1996), Greenway (1997) and Scholes et al. (1998) also reported that roots of the wetlands play very important role in wastewater purification followed by stem and leaves. These roots have been reported to be the most beneficial for rhizofiltration of the metal contaminants. The high accumulation of heavy metal in the *Limnocharis flava* tissues were due to the plant had many shorter roots and were able to create aeration zones for heavy metal uptake (Krishnan, 2002).

This study also shows that Mn uptake by plant tissues was less than Fe. Study by Thien (2005), Noor Ida Amalina (2006) and Ain Nihla (2006) also reported that the amount of Fe uptake by plants was higher compared to Mn in the plant tissues. Fe^{2+} was the micronutrient for the plants that was required in higher concentration than Mn^{2+} (Kamal et al., 2004). Additionally, plants require a small amount of Mn, high level of Mn interfere with enzyme structure and nutrient consumption. Also, as it can be noticed in Fig. 1, HSSF systems exhibit a higher uptake of nutrients and heavy metals as compared to VSSF system due to the higher HRT for HSSF system. These findings have shown the significant and positive effect of macrophytes on pollutants removal (Tanner, 2001). Whereby, the roles of macrophytes as an essential component of constructed wetland have been well established (Brix, 1997; Stottmeister et al., 2003; Brisson and Chazarenc, 2008).

4. Conclusion

The suitability of *Limnocharis flava* as potential macrophytes to be used in leachate treatment system have also shown and proven in this study. The presence of the nutrients (NH_3-N and PO_4-P) and heavy metals (Fe and Mn) in the *Limnocharis flava's* tissues have proved the ability of this plant to uptake pollutants, where the highest accumulation was obtained in the root tissue for both nutrients and heavy metals. The *Limnocharis flava* have been successfully proved to play an important role in expediting the treatment process through various mechanisms such as phytoextraction, phytoaccumulation and rhizofiltration.

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