



PHYTOREMEDIATION OF HEAVY METALS BY WATER HYACINTH IN SEWAGE WASTEWATER STABILIZATION PONDS UNDER HUMID LOWLAND TROPICAL CLIMATIC CONDITIONS

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Abstract

Plant macrophytes in wastewater treatment systems are important for providing various ecological and environmental benefits, e.g. detoxification and removal of toxic heavy metals. In this study, phytoremediation of four heavy metals (Cd, Cu, Pb and Zn) by water hyacinth (*E. crassipes*) in sewage wastewater stabilization ponds under humid lowland tropical climatic conditions in Papua New Guinea was studied using a purposive design and grab sampling technique. The wastewater and plant samples collected were analysed for the heavy metals. In almost all cases, an increasing concentration of heavy metals exceeding the standard (FAO and WHO) minimum permissible levels was measured in both the wastewater and the leaves. The general trend in concentration of the effluent pond was such that Pb>Zn>Cu>Cd in the wastewater and Zn>Cu>Pb>Cd in the leaves, respectively. The high variability in heavy metal concentration ranged from between 57-99% in the wastewaters and 61-63% in the leaves, respectively. The availability in the effluent wastewater probably results from decomposition of plant matters and release of the heavy metals bioaccumulated back into the wastewater. A management option to address high availability and mobility in the wastewater is to remove the plant macrophytes well before senescence and turnover of plant matters.

Keywords: Phytoremediation, heavy metals, sewage stabilization ponds, water hyacinth

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Introduction

Indiscriminate release of untreated residential sewage effluent is one of the major sources of ecosystem pollution (Environmental Protection Agency, 2001; Rono, 2018). Sewage effluents are rich in nutrients, solids and toxic contaminants in quantities capable of causing concerns (negative impacts) in the receiving environment and the ecological services it provides. Environmental pollution related to sewage effluent ranges from eutrophication and algal bloom because of high nutrient (organic or inorganic) loading to contamination of ecosystems (e.g. pollution) because of release and accumulation of environmentally damaging heavy metal pollutants in the form of Cu, Zn, Pb and Cd (Al-Musharafi *et al.*, 2013). High concentrations of inorganic salts (e.g. Na, Ca, K, Mn and NH₄) are also present in sewage WSP and cause further problems, e.g. eutrophication (Lim, 2010).

A wide range of sewage effluent treatment methods are used but are beyond the scope of this study to give a comprehensive review. In the poor economies, interest in natural systems of treatment for specific environmental and sanitary problems (Deng *et al.*, 2007) has emerged in wastewater stabilization ponds, “WSP”. Quite a number of countries consider using WSP because the ponds use natural processes, ecologically sustainable, cost-effective, reliable and easily-operated to treat wastewaters (Abdel-Raouf *et al.*, 2012). The WSP system comprises a string of single anaerobic, facultative and maturation ponds set up in several series in parallel. The sewage system where this study was conducted is an example of this with three ponds laid out parallel to each other. The main purpose of the anaerobic and facultative ponds is to remove biochemical oxygen demand (BOD). In the WSP system, BOD is dependent on the activities of the anaerobic and facultative microbes (fungi and bacteria) in removal of nutrients and heavy metals (Kiran *et al.*, 2007; Pandi *et al.*, 2009; Afkar *et al.*, 2010; Chen *et al.*, 2012).

In WSP, one or more water tolerant vascular plants are dominant because of co-evolutionary mechanisms developed by these types of plants (Michael and Reid, 2018). The roles of these types of plants in domestic sewage WSP are well investigated (e.g. Anushee, 2005; Rezanian *et al.*, 2016a; Michael *et al.*, 2017; Obogu *et al.*, 2018). In the humid lowland tropical climatic conditions, studies dealing with such plants in sewage ponds are lacking in the literature. Therefore, this study was carried out to assess the importance of water hyacinth establishment in sewage WSP under humid lowland tropical climatic conditions to help remove heavy metals. The study hypothesised that (i) if the plant macrophyte removes heavy metals by phytoremediation, there would be less concentration of the heavy metals in the sewage wastewater, and, (ii) if so high concentration of the heavy metals in the leaves.

Materials and Methods

Description of the sewage ponds

This study was conducted at the domestic sewage WSP at the PNG University of Technology, Lae, Papua New Guinea ($6^{\circ}42'55.89''\text{S}$; $146^{\circ}59'59.66''\text{E}$). The ponds were established in the early 1970s. The raw sewage from the residents of the University is released into three ponds laid parallel to one another and are treated biologically before releasing into the environment as effluent.

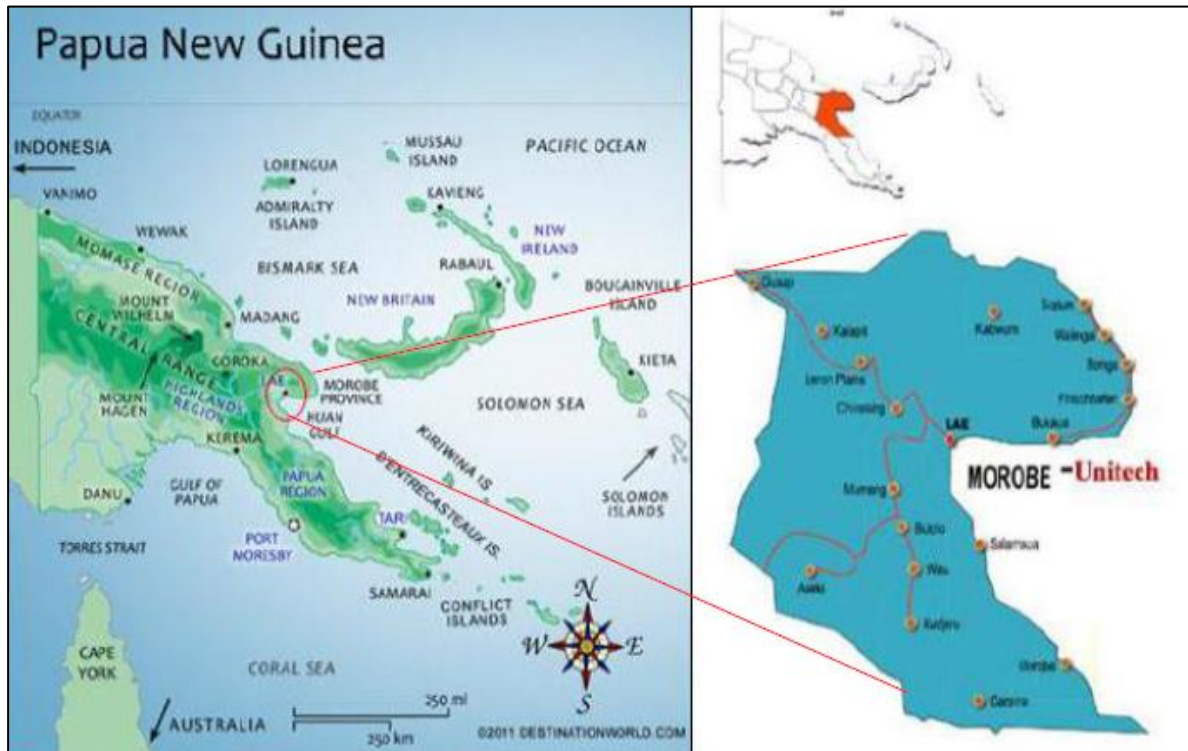


Figure 1. Map showing the location of the study site in Lae, Morobe Province, PNG

Experimental Design and Sampling

As per the aim, a survey was carried out in 2017 to identify plant macrophytes in all the ponds and water hyacinth was widely dominant. Studies show this plant is an ideal plant for phytoremediation of heavy metals (Pilon-Smits, 2005). Based on the pre-assessment, a purposive design and a grab sampling technique were used to sample triplicate plant and water samples from all the ponds. The data presented are based on the one off sampling carried out in June, 2018. In the influent pond (first pond 50 x 30m), the samples were collected 1 m away from each side of the discharge channel. In the second pond (60 x 66m), samples were collected from the centre (30 m away from the point of discharge). The third one (80 x 66m) is the effluent pond and it is from this pond that biologically treated sewage is released into the environment. In this pond, samples were collected 0.5 m away from the point of disposal of the effluents. In all the ponds, the first and second fully opened leaves (Michael *et al.*, 2015) of water hyacinth (henceforth referred to as “plant macrophyte”) were

collected. Similarly, 500 ml of water samples in triplicate were collected from immediately under the plants from which leaves were sampled.

The intension of collecting samples near the point of discharge in the first pond was to assess the “near exact” concentration of heavy metals directly received from the residential wastes, sampling from the middle (lengthwise) in the second pond was to ensure the samples collected were representative of the whole pond. Sampling directly from the point of disposal in the third pond was to estimate how much was disposed as effluent into the environment. The same amount of water in triplicates was sampled from the water source used in the domestic residents and water hyacinth collected from a recreational (non-sewage) pond a few kilometres away from the study site, and used as the positive controls. Note that the pond from which the control water hyacinth leaf samples were collected receives a lot of flood water from the Lae Metropolitan area (main city centre) through a series of flood drains that run into the pond.



Figure 2. Sample photo showing the effluent pond covered with water hyacinth

The influent per day ($\text{m}^3 \text{d}^{-1}$) and approximate composition from the residents into the first pond (henceforth referred to as influent pond) were not measured. The domestic wastes from the residents is stabilized biologically in all the ponds where within the surface is aerobic because of surface-mixing and oxygenation through parenchymatous tissues pumping oxygen into the rhizosphere or anaerobic as a result of the biological oxygen demand (BOD) created by decomposition of the wastes and reduction reactions of wastewater ponding. The BOD was not measured as the study was designed to assess phytoremediation of heavy metals from the ponds and accumulation in the leaves. The water data from ponds one, two and three are presented as P1W, P2W and P3W, and the data from the leaves as P1P, P2P and P3P, respectively.

Laboratory and statistical data analysis

Prior to analysis, all the samples were composited (Al-Musharafi *et al.*, 2013) and analysis done at the University Analytical Services Laboratory (UASL) according to APHA methods (APHA, 1998). Leaf samples were dried at 105 °C for 3 h and a sample 0.2 g each was obtained by weighing in perfluoroalkoxy polymer containers. These were treated with 0.5 ml of hydrofluoric acid and 4 ml of concentrated nitric acid and heated in a microwave for 40 min. These were diluted to 100 ml with Milli-Q water to give a final concentration of 1 g L⁻¹ (Moor *et al.*, 2011). These samples were filtered and analysed for Cd, Cu, Pb and Zn using (ICP-OES) type Perkin Elmer 3300 DV ICP (USA). Blank and certified reference solutions were used as control.

Table 1. Minimum permissible levels (MPLs) of the selected heavy metals in drinking water, irrigation, plants and soil (FAO, 1985; 1989; WHO, 1996).

Heavy Metal	Minimum Permissible Levels			
	Drinking	Irrigation	Plants	Soil
Cadmium	0.003	0.01	0.02	0.8
Copper	2.00	0.05	10.0	36
Lead	0.01	0.10	2.0	85
Zinc	3.00	5.00	0.6	50.

The MPLs of water and plant samples are in ml L⁻¹ (FAO) and mg kg⁻¹ (WHO), respectively.

The treatment average was obtained by taking the mean of the three replicates. To compare the treatment means, significant differences (p<0.05) between treatments means of the samples was determined by two-way ANOVA using statistical software JMPIN, AS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513. The variability in concentration of the heavy metals shown in Figure 3 was estimated as:

$$CI_{wp} = ([SC_{wp} \div \Sigma(TC_{wp})]100),$$

where CI_{wp} is increase in concentration, SC_{wp} is sample concentration and TC_{wp} is sum of the total concentrations of a metal of a water or plant sample of the ponds. A sample calculation for Zn from water samples of the influent pond as per Tables 2-4 is:

$$\begin{aligned} CI_{wp} &= [P1W \div \Sigma(P1W+P2W+P3W)]100, \\ &= [0.5 \div \Sigma(0.5+4.3+7.2)]100, \\ &= 4.1\% \text{ (see Figure 3).} \end{aligned}$$

Results

In the influent pond, the heavy metal concentrations in the sewage wastewater were below the MPLs except the Cd concentration (Table 2). Compared to the concentrations in the wastewater, the concentrations in the leaves were above the MPLs of plants. A very high concentration of Pb followed by Zn, Cu, and Cd were

found in the leaves (Table 2). Compared to these, the concentrations in the control plants were higher, ranging from 13 mg L⁻¹ (Cu) to 204 mg L⁻¹ (Zn). Similarly, the concentrations in the control water samples were high; Pb>Cd>Zn>Cu (Table 2).

Table 2. The concentration of heavy metals in wastewater and leaves in the influent pond. An asterisk (*) indicates significant differences (p<0.05) between the treatment and control.

Heavy metal	Concentrations (ml L ⁻¹ /mg kg ⁻¹)			
	Water sample	Control water	Plant sample	Control plant
Cd	0.7±0.2*	1.1±0.5	1.9±0.3*	33.00±0.5
Cu	0.5±0.3*	0.5±0.4	4.5±0.2*	12.65±0.3
Pb	0.0±0.4*	63.6±0.3	30.8±0.2*	136.04±0.2
Zn	0.5±0.2*	0.5±0.5	7.2±0.4*	203.86±0.4

The same control water and plant samples data are used in Tables 3 and 4.

Table 3. The concentration of heavy metals in the wastewater and leaves in the second pond. An asterisk (*) indicates significant differences (p<0.05) between the treatment and control.

Heavy metal	Concentrations (ml L ⁻¹ /mg kg ⁻¹)			
	Water sample	Control water	Plant sample	Control plant
Cd	0.7±0.2*	1.1±0.5	17.00±0.4*	33.00±0.5
Cu	0.9±0.3*	0.5±0.4	28.88±0.3*	12.65±0.3
Pb	0.0±0.5*	63.6±0.3	4.62±0.4*	136.04±0.2
Zn	4.3±0.6*	0.5±0.5	48.61±0.6*	203.86±0.4

In the middle pond, the concentrations in the wastewater were such that Zn>Cu>Cd>Pb (Table 3). In this pond, only the Zn and Cd concentrations exceeded the MPLs (Table 1). In the leaves of the plant macrophyte, all the concentrations were above the MPLs, with Zn>Cu>Cd>Pb. Comparatively, these concentrations were lower than those of the control (water and leave) samples. The concentrations in the effluent pond (pond 3) are shown in Table 4. The concentrations in the wastewater were Pb>Zn>Cu>Cd and in the plant macrophyte leaves were Zn>Cu>Cd>Pb, respectively. All of these exceeded the standard MPLs.

Table 4. The concentration of heavy metals in wastewater and leaves in the effluent pond. An asterisk (*) indicates significant differences (p< 0.05) between the treatment and control.

Heavy metal	Concentrations (ml L ⁻¹ /mg kg ⁻¹)			
	Water sample	Control water	Plant sample	Control plant
Cd	1.9±0.2*	1.1±0.5	29.00±0.2	33.00±0.5
Cu	4.5±0.2*	0.5±0.4	50.4±0.4*	12.65±0.3
Pb	30.8±0.3*	63.6±0.3	13.43±0.3*	136.04±0.2
Zn	7.2±0.4*	0.5±0.5	90.2±0.2*	203.86±0.4

Discussion

Compared to the MPLs of various environmental factors (Table 1), concentrations of all the heavy metals exceeded the standard MPLs of water, plants or even soil (Tables 2-4). The general trend in variability in concentrations from the influent (pond 1) to the effluent pond (pond 3) in the wastewater and in the leaves is shown in Figure 3. The direct discharge from the domestic residents into the influent pond was not sampled for analysis because of heavy presence of faeces and odor. In this pond, there was an increasing concentration of heavy metal in the wastewater and in the plant leaves but the concentrations were higher in the leaves than in the wastewater (Figure 3). The high accumulation in the leaves is a strong indication that the plant macrophyte was able to remove most of the metals from the wastewater. For example, the Pb concentration in the wastewater was beyond the detection limit and high in the leaves, showing that Pb was removed completely and bioaccumulated in the leaves. In a similar study, Liao and Chang (2004) reported bioaccumulation in the order Cu>Pb>Cd>Zn in a constructed wetland by water hyacinth compared to the Zn>Cu>Cd>Pb of this study. In addition, the findings of other studies using water hyacinth (e.g. Hussain *et al.*, 2010; Worldemichael *et al.*, 2011; Sreelekha *et al.*, 2016) are limited to laboratory or glasshouse conditions, making extrapolation difficult.

The changes in heavy metal concentration in the second pond showed a similar trend as in the first pond. The concentrations in the leaves were more than in the wastewater (Table 3), showing that fairly high quantities of the metals were removed from the wastewater. Even for Zn, 4 mg L⁻¹ was present in the wastewater, whereas in the leaves was 49 mg kg⁻¹ (Table 3), an increase in concentration from a 5% to nearly 62% (Figure 3). The most critical pond is the third pond where the effluent is discharged into the environment. In this pond, there was high availability of Pb and Zn compared to Cd and Cu in the wastewater (Table 4). In the leaves of the plant macrophyte, a high amount of Cu and Zn were present, indicating that a fair amount of these metals are available in the pond wastewater, likely to be disposed into the environment.

An interesting find is the variability in metal concentration in the ponds' wastewater-plant system. The study showed an increasing concentration of heavy metals in all the ponds. In both the water and plant leaves, the heavy metals accumulated from the influent pond (Table 2) to the effluent pond (Table 4), showing that the heavy metal that reaches the influent pond are effectively taken up by the roots of plant macrophyte and bioaccumulated in the leaves, consistent with Woldemichael *et al.* (2011) where a 91% efficacy of Cr removal was reported. When marshland plants mature and senesce, turnover of organic matter from them still end up back into the wastewater. Decomposition of these plant matters release the heavy metals bioaccumulated back into the wastewater, the most probable reason high amounts of heavy metals were still available in the

wastewater. The high availability is a major concern as it presents high risks of mobility and can result in environmental pollution.

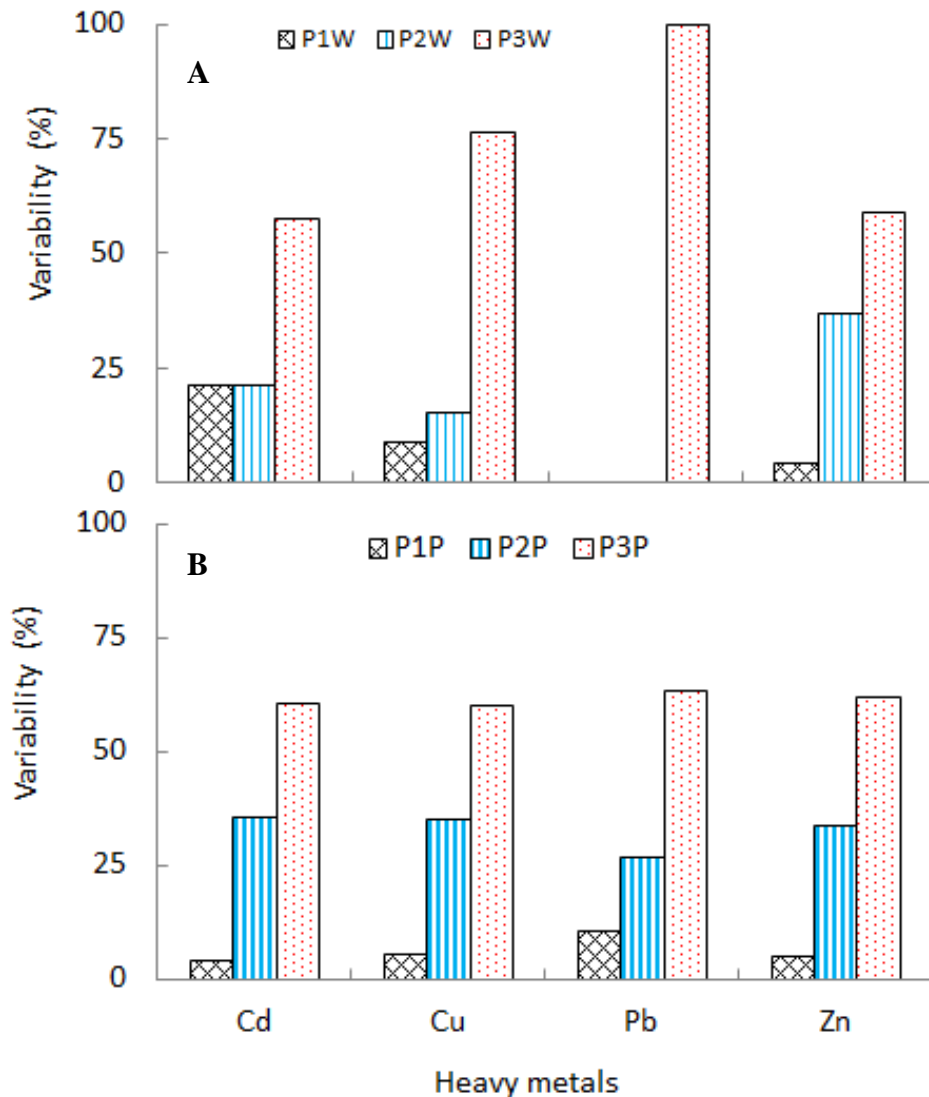


Figure 3. Variability in concentration of heavy metals in the wastewater (A) and in the plant macrophyte leaves (B) from the influent to the effluent pond. The legends have been described previously.

In sewage WSP, the total concentrations in the effluent pond are expected to be less than what were measured in the influent pond because of biochemical processes (microbial metabolism, rhizoremediation, absorption and absorption) that occur. The results shown (Tables 2-4) are contradictory to this expectation. The total concentration of Cd in the influent pond's plant macrophyte leaves, for example, was 1.9 mg kg^{-1} . This concentration has increased to 29.0 mg kg^{-1} in the effluent pond, an increase by 61% (Figure 3). Similarly, the total Zn concentration in the leaves of the influent pond was 7.2 mg kg^{-1} , however, has increased to 90 mg kg^{-1}

¹ in the effluent pond, an increase by 62%. These high increases in availability of heavy metals show there are other sources of these metals apart from the sewage. The possible sources would be industrial wastes in drains or domestic dumps that end up in the ponds through underground flow or surface runoffs (Roldan, 2002). Phytoremediation by *E. crassipes* means reduce chances of the heavy metals mobility into the environment (Roldan, 2002). The evidence of an increasing concentration, even in small quantity, calls for proper management of the WSP systems, particularly to prevent the heavy metals that accumulate in the plant tissues to be released back into the wastewater following senescence and dead. Availability of heavy metals is a major concern as bioaccumulation is detrimental to important receiving ecosystems and public and environmental health (Madyiwa *et al.*, 2002).

The technology use to manage the sewage wastewater is dependent on the life style of the residents and the climatic conditions of the sewage WSP. Life style of residents affects the type of wastes and climate affects the natural and biological processes associated with phytoremediation that occur to treat wastes (Aizari *et al.*, 2017). Abdedi and Najafi (2001) showed that wastewater is reused in agriculture and the practice has benefits (Nadafi *et al.*, 2005). In poor economies, this option is not considered and continues to raise the concern that heavy metal availability and mobility from sewage ponds is still a health hazard (Nadafi *et al.*, 2005). One study showed availability of sewage WSP is common in the tropics but the performance is low due to lack of proper management (Abdel-Raouf *et al.*, 2012). Proper management, e.g. regular maintenance, helps reduce the high availability of suspended solids, organic matter and nutrients which are additional sources of pollution (Lim *et al.*, 2010).

In line with the turnover of organic matter and release of the heavy metals back into the wastewater, removal of the plant macrophytes from the ponds before senescence and dead is one major management option available to help reduce availability in the effluent. The plants removed from the ponds can be dried, burnt and ashes buried to minimise air pollution, or the whole plant materials allowed to dry out and used as organic matter. The regular maintenance would be such that a good population of young shoots remain in the ponds and continue to grow, preferably mature enough to accumulate high concentration of the heavy metals, which will then be subsequently removed in a similar manner. Studies elsewhere indicate separation of metal-saturated algae as an economical method of removing heavy metal in ponds (Kiran *et al.*, 2007; Bhat *et al.*, 2008; Pandi *et al.*, 2009). Removal of the plants together with sediments around them would result in surface mixing, thus aerobic decomposition and odour but the benefit of the regular maintenance, especially to help reduce heavy metal availability, would offset this community disadvantage. Suspended solids rich in nutrients which are source of pollutants co-exist with live plants (Rono, 2018), and removal of the plant macrophytes

would help reduce solids attached to sediments around the live plants, further reducing the heavy metals in the pond water-plant systems (Rezania *et al.*, 2016a; Rezania *et al.*, 2016b).

We have seen wetland plant macrophytes use paracymatous tissues to transport oxygen and oxygenate the rhizosphere even under anaerobic conditions (Michael *et al.*, 2017; Michael and Reid, 2018; Michael, 2018). In sewage WSP, presence of dominant plant macrophytes with modified physiological features, such as *E. crassipes*, especially under tropical conditions is an advantage to help treat domestic wastes by phytoremediation and tissue accumulation. These types of plants help regulate biochemical and biophysical processes (oxygen penetration, temperature control, raise redox and maintain pH at circumneutral levels) (Adewumi and Ogbiye, 2010), important for treatment of sewage using natural and biological means (Anushee, 2005). Regulations of the processes are important for microbial concentrations in sewage ponds that depend on nutrient loading, temperature, pH and sunlight to rhizoremediate heavy metals. In most cases, anaerobic decomposition work against these and lead to release of CO₂, contributing to greenhouse gas emission. Decomposition under low O₂ further leads to reduction reactions (Michael *et al.*, 2015; Michael, 2018), raising the pH to levels critical to most microbes. All these point out the need for regular maintenance which would sufficiently aerate and mix the wastewater in the sewage ponds besides the community disadvantage of bad odour.

Conclusion

In contrast to the positive phytoremediation of heavy metals by *E. crassipes*, decomposition of dead plant matter seems to release the heavy metals bioaccumulated back into the wastewater system, making them readily available for possible mobility into the environment. Removal of the plant macrophyte on a regular basis would help reduce the availability and mobility in the effluent and prevent environment pollution. These results have implications for management of sewage WSP dominated by plant macrophytes, especially under humid lowland tropical climatic conditions.

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