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Removal of Heavy Metals and Nutrients from Municipal Wastewater using *Salvinia molesta* and *Lemna gibba*

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Abstract

The present study was focused on the investigation of the abilities and efficiencies of *Salvinia molesta* and *Lemna gibba* to remove selected heavy metals (Cr, Cu, Fe, Ni and Pb) and excess nutrients from wastewater taken from the Moratuwa-Ratmalana municipal wastewater treatment plant. The wastewater samples were analysed for pH, Temperature, for N-Nitrates, N-Nitrites, ammonia Nitrogen, Phosphates, and selected heavy metals, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Kjeldhal Nitrogen. Then, the wastewater samples were treated with *S. molesta* and *L. gibba* separately for a period of 7 days and analysed for N-Nitrates, N-Nitrites, Ammonia Nitrogen, Phosphates and five selected heavy metals at 24 hour intervals. BOD, COD and Total Kjeldhal Nitrogen were analysed at 7 days intervals. The average Total Nitrogen removal efficiencies of *S. molesta* and *L. gibba* were 73.3% and 62.1% whereas the average Total Phosphate removal efficiencies of *S. molesta* and *L. gibba* were 72.6% and 77.2% respectively. The average Cr, Cu, Fe, Ni and Pb removal efficiencies of *S. molesta* were 81.6%, 69.8%, 65.2%, 66.3% and 74.8% respectively. The average Cr, Cu, Fe, Ni and Pb removal efficiencies shown by *L. gibba* were 86.9%, 69.7%, 73.1%, 61.8% and 85.7% respectively. The Bio Concentration factors of *S. molesta* for Cr, Cu, Fe, Pb and Ni were 823, 698, 652, 663 and 748 respectively and the Bio Concentration factors of *S. molesta* for Cr, Cu, Fe, Pb and Ni were 870, 698, 731, 618 and 857 respectively. According to the obtained results in the present study *S. molesta* and *L. gibba* can be considered as suitable candidates for the polishing of municipal wastewater.

Keywords: Heavy metals, Lemna gibba, nutrients, Salvinia molesta

1. Introduction

Wastewater contains Biodegradable organics, Nutrients; mainly nitrogen and phosphate compounds that lead to eutrophication, Organic pollutants, heavy metals and dissolved inorganics such as sodium, calcium and sulphates etc. Almost all the human activities generate wastewater. Nature has an assimilation capacity to cope with small amounts of wastewater and pollution associated with it, but nature has no capacity to handle the huge amount of wastewater that is generated every day after being subjected to human consumption and several other uses. Therefore, the treatment of this water and returning clean and safe water into the waterways is essential to ensure the safety of people and the environment. The proper treatment of wastewater is an environmental challenge since the wastewater is required to be treated and disposed safely in an efficient manner. Further, the presence of some components in the water poses a challenge in the wastewater treatment process.

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Enrichment of large quantities of nitrogen and phosphate compounds in wastewater is one of the main causes of eutrophication that negatively affects many natural water bodies. Heavy metals are considered as priority pollutants due to their acute toxicity. Heavy metals in wastewater cause detrimental effects on environment and human health. The heavy metals in wastewater have the potential of bio-accumulation which can cause adverse impacts on environment and human health.

Phytoremediation is the use of both aquatic and terrestrial plants for the treatment of contaminated water and soils (Ali et al., 2013). According to previous studies, some aquatic macrophytes have shown a great promise in wastewater treatment, both in the removal of nutrients (Phosphates, Nitrogen compounds) and heavy metals. *S. molesta* and *L. gibba* are two preferable candidates which had been used for the treatment of wastewater in previous studies. Low cost and easy maintenance make the aquatic plant system preferable to use. In an aqueous solution, metals are available in soluble form. Therefore, the accumulation by the aquatic plants can be achieved much more easily and more efficiently than using terrestrial plants.

S. molesta is a free floating aquatic plant which spreads rapidly by vegetative reproduction. The species is well-known for its phytoremediation potential (Koutika and Rainey, 2015). *L. gibba* is a rooted free-floating aquatic plant consisting of small fronds. Due to the high growth rate and large potential for the uptake of heavy metals and nutrients, members of Genus *Lemna* have appeared as suitable candidates for the phytoremediation of heavy metal contaminated wastewater (Verma and Suthar, 2015).

According to previous studies, the influent of Moratuwa-Ratmalana wastewater treatment plant contains Total nitrogen and total phosphates higher than the upper limits given in the standards. The presence of several heavy metals i.e. Cu, Cr, Pb, Fe, and Ni is also recorded (Danushika et al., 2017). Since the sludge disposed by the treatment plant constitutes of heavy metals, the final disposal of sludge has posed problematic issues. The study is carried out to determine the efficiency of *S. molesta* and *L. gibba* as candidates for polishing of the influent of Moratuwa-Ratmalana wastewater treatment plant. The potential of using *S. molesta* and *L. gibba* for the removal of nutrients (nitrogen compounds, phosphates) and selected heavy metals (Cr, Cu, Ni, Pb, Fe) is determined in the study, thus investigating the capability of these two aquatic macrophytes to be used for the treatment of municipal wastewater.

2. Methodology

2.1 Plant acquisition and acclimatisation

S. molesta was collected from a fresh water body near the University. *L. gibb*a was collected from Moratuwa-Ratmalana wastewater treatment plant. Healthy mature plants were selected for the experiment and rinsed with tap water in order to remove adhering mud particles or epiphytes. The plants were left for an adaptation period of 10 days, growing in the containers inside the Green house of Department of Forestry and Environmental Science, University of Sri Jayewardenepura to be adapted for the experimental conditions.

2.2 Sample collection

Wastewater samples were collected from the inlet of the Moratuwa-Ratmalana wastewater treatment plant. Samples were collected weekly. After the collection, the samples were checked for pH, Temperature, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrates, Nitrites, Ammonia Nitrogen, Phosphates and Total Kjeldhal Nitrogen and heavy metals (Cr, Cd, Cu, Mn, Pb, Ni, and Fe).

The pH was measured using the pH meter and the temperature was measured using a thermometer at the time of sample collection. BOD was measured using Winkler method. COD was determined using closed reflux method. Ammonia Nitrogen was measured by comparative Ammonia method. Total Kjeldhal Nitrogen concentration was measured by Micro Kjeldhal method. The concentrations of Nitrates, Nitrites and Phosphates were measured using HACH 890 meter by Powder Pillow method. The concentrations of the heavy metals were measured by Atomic Absorption Spectroscopy (AAS) method. The wastewater samples were collected in polypropylene bottles and were transferred to the green house immediately.

2.3 Experimental design

Eight rectangular shaped glass aquariums with the dimensions of 1'×6"×6" were used to perform the experiment. Six containers were taken and 3l of collected wastewater was added to each tank. To three tanks, 15 g of *S. molesta* was introduced. To other three tanks, 15 g of *L. gibba* was introduced. The other two tanks were filled with distilled water. 15 g of *S. molesta* was added to one tank and 15 g of *L. gibba* was added to the other tank. These two tanks with distilled water were used as control samples in order to compare the relative growth of the plants in wastewater and the control samples. Each experiment was carried out for 7 days. 40 ml of wastewater samples were withdrawn from each tank at 24 hour intervals. The seven day experiment was repeated for six times.

2.4 Sample analysis

The samples withdrawn from the tanks at 24 hour intervals were checked for pH, temperature, nitrates, nitrites, ammonia nitrogen and phosphates. The collected water samples were filtered using Whatmann No.1 filter papers and were analysed using atomic absorption spectroscopy (AAS) method to determine Cr, Cu, Pb, Ni and Fe concentrations at 24 hour intervals. At the end of the experiment, on the 7^{th} day, the samples were checked for BOD, COD and total Kjeldhal nitrogen.

2.5 Analysis of plant materials

On the 7^{th} day, plant materials were harvested and their fresh weights were determined. Then the plant materials were oven dried at 800° C for 48 hours and the dry weights were determined. Then the dried biomass was digested according to dry digestion method (Kalagbor and Opusunju, 2015) and were analysed for concentrations of Cr, Cu, Pb, Ni, and Fe using Atomic Absorption Spectroscopy.

2.6 Calculations

The following parameters were calculated using the mean values of the data obtained through the experiments.

Relative growth

Relative growth values were calculated for *S. molesta* and *L. gibba* using the initial fresh weight and final fresh weight values.

$$\text{Relative growth}=\frac{\text{Final fresh weight (g)}}{\text{Initial fresh weight (g)}} \tag{1}$$

(Thayaparan et al., 2013)

Removal efficiency

The removal efficiencies for total Nitrogen, total Phosphates, COD, BOD and selected heavy metals by *S. molesta* and *L. gibba* were calculated using initial and final values.

$$\text{Removal efficiency}=\frac{\text{Initial concentration -Final concentration}}{\text{Initial concentration}}\times 100 \tag{2}$$

Metal uptake capacity

The uptake capacities for each metal for *S. molesta* and *L. gibba* were calculated.

$$\text{Metal uptake capacity (mg/kg)}=\frac{\text{Metal concentration of the dried biomass }\left(\frac{\text{mg}}{\text{l}}\right)\times\text{Total diluted volume(ml)}}{\text{Dry weight (g)}} \tag{3}$$

(Lokuge, 2016)

Bio Concentration Factor (BCF)

The Bio Concentration Factors for each metal for *S. molesta* and *L. gibba* were calculated.

$$BCF = \frac{\text{Metal concentration of the dried biomass } (\frac{mg}{kg})}{\text{Initial concentration of the metal in external solution } (\frac{mg}{l})} \quad (4)$$

(Uysal, 2013)

2.7 Statistical Analysis

The data were statistically analysed using Minitab 18 software. The significant differences among the parameters were determined by One-way Anova test. The treatment means were compared using Tukey's 95% simultaneous confidence intervals test.

3. Results and Discussion

3.1 Nutrient removal by S. molesta and L. gibba

Total nitrogen

In the present study, the final total nitrogen concentrations in the tanks with *S. molesta* and *L. gibba* were 31.82 mg l^{-1} and 51.81 mg l^{-1} respectively, whereas the initial Total Nitrogen concentration was 136.27 mg l^{-1} *S. molesta* showed a significantly higher potential ($p<0.05$) in the uptake of total nitrogen than *L. gibba* in the present study.

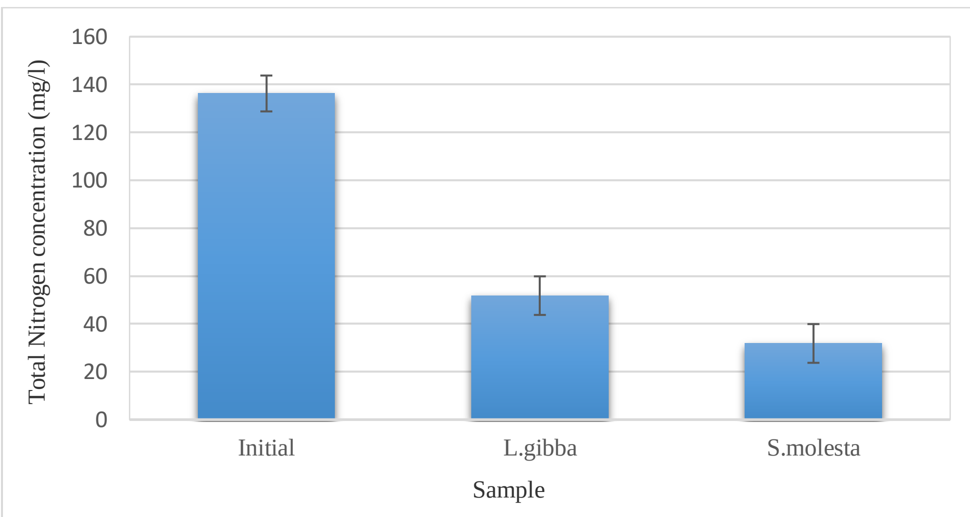

Figure 1. The mean values of initial and final concentrations of Total Nitrogen in the tank with *S. molesta* and in the tank with *L. gibba*. Bars indicate mean±SD (Standard Deviation), where n=6.

According to Ng and Chan (2017), the nitrate concentration in palm oil mill effluent treated by *S. molesta* has increased until day 12 and then has slightly decreased in the end. In a free floating system, the total nitrate concentration is controlled by nitrification and denitrification, in addition to plant and microbial uptake. In the present study also, the nitrate concentration of the tank with *S. molesta* had remained constant from day 3 to day 4. The nitrate concentration in *L. gibba* had increased slightly from day 3 to day 4. These are due to the increase of nitrates as a result of nitrification. However, in the present study, the increase of nitrates by nitrification is not much high. nitrification/ denitrification can be caused by nitrifying bacteria which are attached to flocculates (Korner and Vermaat, 1998). But, the flocculates settled to the bottom of the tanks due to the small depth of the tanks. Therefore, the nitrification/ denitrification by suspended bacteria can be neglected.

In the present study, volatilisation of ammonium ions as ammonia gas can be neglected since the surface of the tank is covered with the dense mat of plant materials. Also, the pH value of the wastewater had remained close to neutral values throughout the experiment. Therefore, only a small amount of ammonium ion may be present as ammonia gas.

The concentrations of nitrites, nitrates, ammonia nitrogen and phosphates in the tank with *S. molesta* had decreased daily in the seven day period. The total nitrate concentration did not show a gradual decrease. The concentration of nitrates had remained constant from day 3 to day 4. The concentrations of nitrites, nitrates, ammonia nitrogen and phosphates in the tank with *L. gibba* had decreased in the period of 7 days. The concentration of nitrates has shown a slight increase from day 3 to day 4.

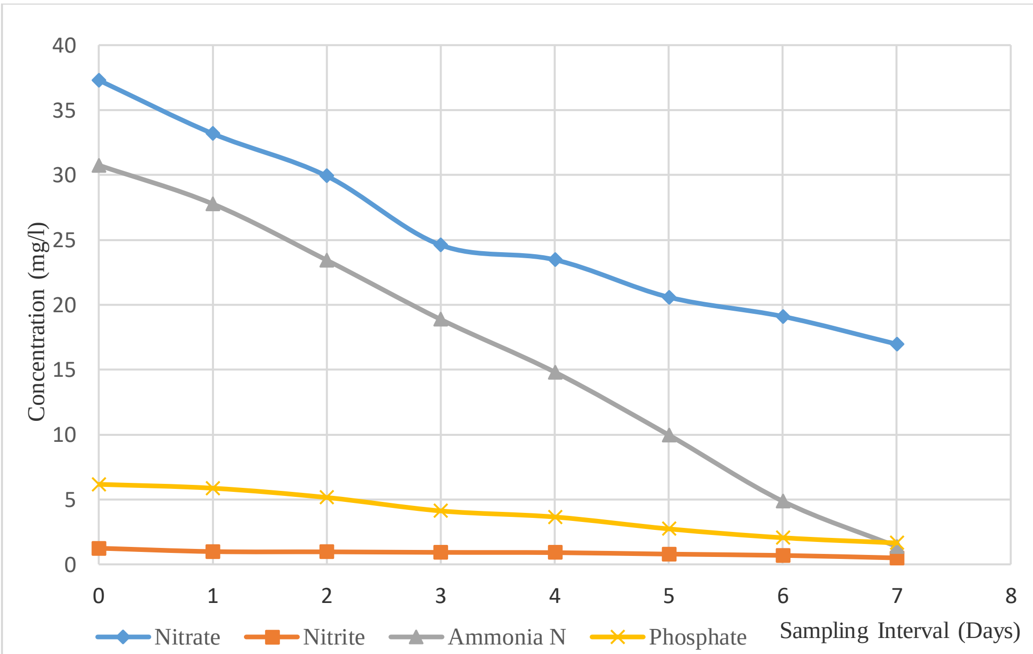

Figure 2. The daily variation of the mean values of concentrations of nitrites, nitrates, ammonia nitrogen and phosphates in the tank with *S.molesta* where n=6.

The total nitrogen removal efficiencies of *S. molesta* and *L. gibba* were 73.35% and 62.18% respectively. *S. molesta* has shown a significantly greater ($p<0.05$) efficiency in the removal of Total Nitrogen than *L. gibba*. According to Žaltauskaitė et al., (2014), the efficiency of *L. gibba* in the removal of Nitrogen from wastewater is between 42%-62% of total nitrogen, depending on initial nitrogen concentrations. The present study has also shown similar results. According to Mkandawire and Dudel, (2005), the total Nitrogen removal efficiency by Lemna spp. is 50%. In the present study, *L. gibba* has demonstrated an efficiency higher than that.

3.1.2 Total phosphate

Phosphorous is an essential macronutrient for plants which is required for the synthesis of Adenosine Diphosphate (ADP), Adenosine Triphosphate (ATP) and nucleic acids. Therefore, free orthophosphates are specifically absorbed from wastewater by plant systems. In the present study, the initial concentrations of Total Phosphates in the tanks with *S. molesta* and *L. gibba* were 1.65 mg l^{-1} and 1.5 mg l^{-1} respectively, whereas the initial concentration was 6.17 mg/l . The final Total Phosphate concentrations in the two tanks were not significantly different ($p>0.05$).

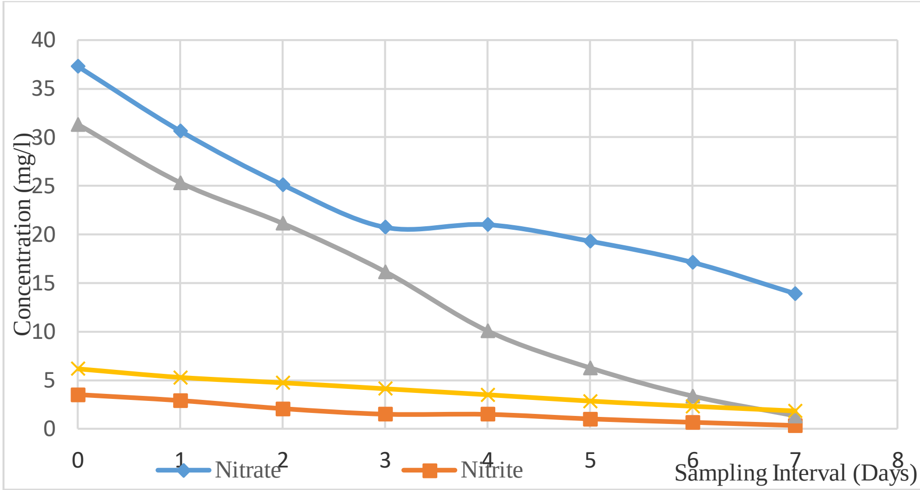

Figure 3. The daily variation of the mean values of concentrations of nitrites, nitrates, ammonia nitrogen and phosphates in the tank with *L. gibba* where n=6.

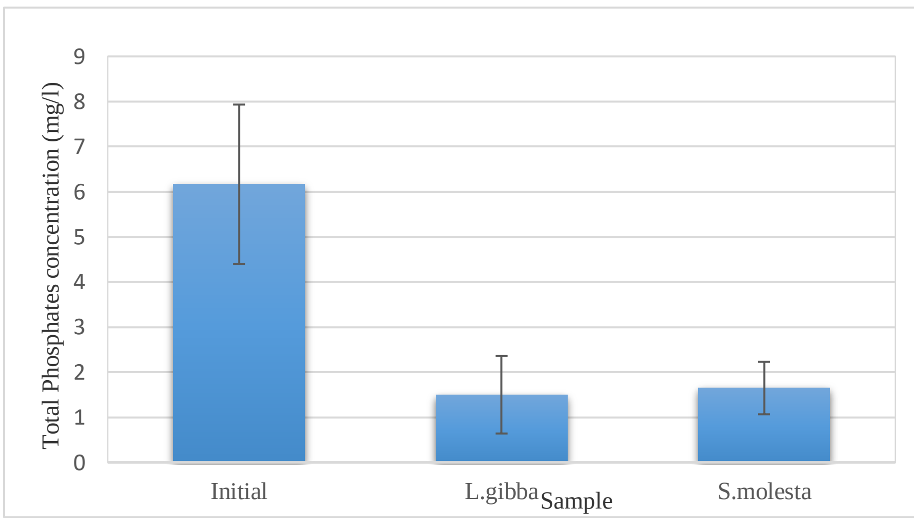

Figure 4. The mean values of initial and final concentrations of total phosphate in the tank with *S. molesta* and in the tank with *L. gibba*. Bars indicate mean±SD, where n=6.

Mohedano et al., (2012) showed a final total phosphate concentration of 5.2 mg l^{-1} on day seven, whereas the initial concentration was 215 mg l^{-1} in which the swine waste was treated by *L. gibba*. In this study, the effluent has been sent through a system consisting of a storage pond and two duckweed ponds. Therefore, the removal potential is higher than the present study as the effluent has gone through the Duckweed pond twice in a single treatment and because of the increased surface area for the treatment.

The total Phosphate removal efficiencies of *S. molesta* and *L. gibba* were 72.63% and 77.29% respectively which were not significantly different (p>0.05). According to Žaltauskaitė et al., (2014), the total phosphate removal efficiency from municipal wastewater by *L. minor* is 100%. However, Mkandawire and Dudel, (2005) shows that the efficiency of Total Phosphate removal by *Lemna* spp. is between 50-60%. In the present study, *L. gibba* has demonstrated a greater efficiency than that.

BOD and COD

The mean initial BOD value was 259.22 mg l^{-1} whereas the mean final BOD values in the tank with *S. molesta* and in the tank with *L. gibba* were 73.75 mg l^{-1} and 85.29 mg l^{-1} respectively which were not significantly different (p>0.05). The mean initial COD value was 530.19 mg/l whereas the mean final

COD values in the tank with *S. molesta* and in the tank with *L. gibba* were 200.80 mg l^{-1} and 111.89 mg l^{-1} respectively which were significantly different ($p<0.05$). The COD reduction potential demonstrated by *L. gibba* was higher than in that of *S. molesta* in the present study. In the present study, the BOD removal efficiencies of *S. molesta* and *L. gibba* were 71.51% and 67.24% respectively which were not significantly different ($p>0.05$). The COD removal efficiencies of *S. molesta* and *L. gibba* were 61.985% and 78.957% respectively. *L.gibba* demonstrated a significantly higher efficiency ($p<0.05$) in the removal of COD.

According to Mkandawire and Dudel (2005), the BOD removal efficiency and COD removal efficiency of *Lemna* spp. are 60% and 30-40% respectively. But, in the present study, *L. gibba* has shown greater efficiencies than that in the removal of both BOD and COD. Kumari and Tripathi (2014) reports BOD and COD removal efficiencies by mixed culture of *Eichhornia crassipes* and *Salvinia natans* accompanied by aeration in municipal wastewater as 84.5% and 83.2% respectively. The efficiencies are greater than the BOD and COD removal efficiencies of *S. molesta* and *L. gibba* recorded in the present study. The effect of aeration and the enhancement of removal caused by the use of mixed culture of *Eichhornia crassipes* and *Salvinia natans* can be the reasons for higher efficiencies.

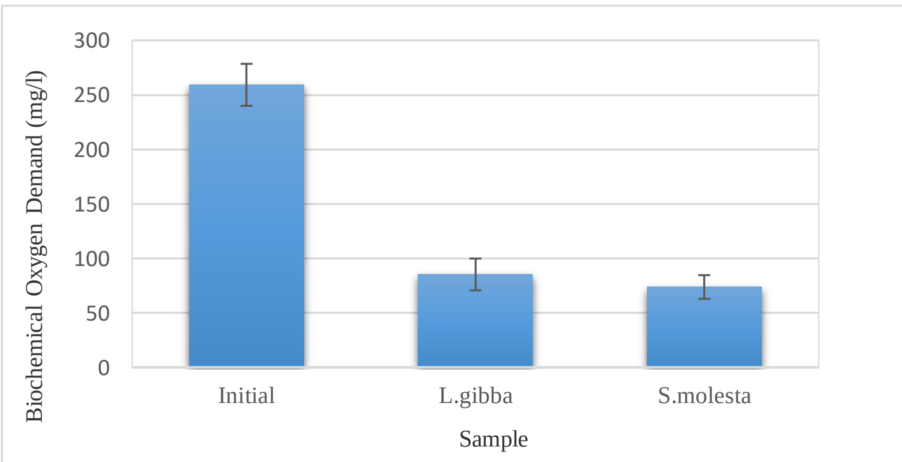

Figure 5. The mean values of initial and final values of BOD in the tank with *S. molesta* and in the tank with *L. gibba*. Bars indicate mean ± SD, where n= 6.

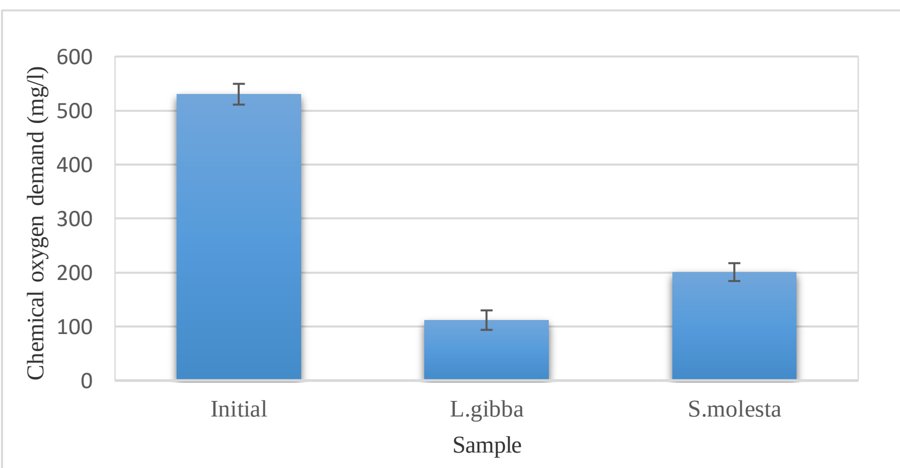

Figure 6. The mean values of initial and final values of COD in the tanks with *S. molesta* and *L. gibba*. Bars indicate mean±SD, where n=6.

3.2 Heavy metal removal by Salvinia molesta and Lemna gibba

Chromium (Cr)

The final Cr concentrations of the two tanks were not significantly different ($p>0.05$). The Cr uptake capacity of *L. gibba* was significantly greater ($p<0.05$) than that of *S. molesta*. The Bio Concentration Factor (BCF) of *S. molesta* and *L. gibba* were not significantly different ($p>0.05$) and both were lower than 1,000. The initial Cr concentration was higher than tolerance limits for industrial and domestic wastewater discharged into marine coastal areas (1 mg l^{-1}). The final concentrations of Cr in both tanks were lower than the tolerance limit. But, toxicity symptoms were not observed in the harvested plant materials.

Table 1: The mean values of initial Cr concentration in wastewater samples, final Cr concentration after being subjected to the treatment by plants, the metal uptake capacities of the two species and the Bio Concentration Factors (BCF).

Characteristic	Control *Salvinia molesta*	Control *Lemna gibba*	*Salvinia molesta*	*Lemna gibba*
Initial concentration (mg l^{-1})	-	-	1.58 ± 0.08^{A1}	1.58 ± 0.08^{A1}
Final concentration (mg l^{-1})	-	-	0.29 ± 0.07^{B1}	0.21 ± 0.05^{B1}
Metal uptake capacity (mg kg^{-1})	-	-	64.32 ± 2.44^{B2}	68.55 ± 2.64^{A2}
BCF	-	-	823.2 ± 40.78^{A3}	870.0 ± 28.25^{A3}

The significant differences are indicated by superscripted letters.

The Cr removal efficiencies of *S. molesta* and *L. gibba* are 81.66% and 86.99% respectively, which were significantly different ($p<0.05$). *L. gibba* demonstrated a higher Cr removal efficiency than *S. molesta* in the present study.

Copper (Cu)

The final Cu concentrations of the two tanks were not significantly different ($p >0.05$). In addition, the Cu uptake capacities of *S. molesta* and *L. gibba* were not significantly different ($p>0.05$). The BCF values of the two plants for Cu were not significantly different ($p>0.05$) and the values were lower than 1000.

The Cu removal efficiencies of *S.molesta* and *L.gibba* were 69.81% and 69.78% respectively. The efficiencies were not significantly different ($p>0.05$).

Table 2: The mean values of initial Cu concentration in wastewater samples, final Cu concentration after being subjected to the treatment by plants, the metal uptake capacities of the two species and the Bio Concentration Factors (BCF).

Characteristic	Control *Salvinia molesta*	Control *Lemna gibba*	*Salvinia molesta*	*Lemna gibba*
Initial concentration (mg l^{-1})	-	-	0.302 ± 0.13^{A1}	0.302 ± 0.13^{A1}
Final concentration (mg l^{-1})	-	-	0.097 ± 0.07^{B1}	0.094 ± 0.05^{B1}
Metal uptake capacity (mg kg^{-1})	-	-	10.283 ± 3.78^{A2}	10.408 ± 4.14^{A2}
BCF	-	-	698.029 ± 72.86^{A3}	698.421 ± 64.80^{A3}

The significant differences are indicated by superscripted letters.

Iron (Fe)

The final Fe concentrations of the two tanks were not significantly different ($p > 0.05$). The Fe uptake capacities of *S. molesta* and *L. gibba* were not significantly different ($p>0.05$). In addition, the BCF values of the two plants for Fe were not significantly different ($p>0.05$) and the values were lower than 1000. The Fe removal efficiencies of *S. molesta* and *L. gibba* were 65.27% and 73.10% respectively. The efficiencies were not significantly different ($p>0.05$).

Nickel (Ni)

The final Ni concentrations in both tanks were not significantly different ($p>0.05$). The Ni uptake capacities of *S.molesta* and *L.gibba* were not significantly different ($p>0.05$). The BCF values of the two plants for Ni were not significantly different ($p>0.05$) and the values were lower than 1000.

Table 3: The mean values of initial Fe concentration in wastewater samples, final Fe concentration after being subjected to the treatment by plants, the metal uptake capacities of the two species and the Bio Concentration Factors (BCF).

Characteristic	Control *Salvinia molesta*	Control *Lemna gibba*	*Salvinia molesta*	*Lemna gibba*
Initial concentration (mg l^{-1})	-	-	0.124 ± 0.02^{A1}	0.124 ± 0.02^{A1}
Final concentration (mg l^{-1})	-	-	0.044 ± 0.01^{B1}	0.036 ± 0.02^{B1}
Metal uptake capacity (mg kg^{-1})	-	-	4.008 ± 1.79^{A2}	4.408 ± 1.74^{A2}
BCF	-	-	652.7 ± 53.71^{A3}	731.0 ± 120.16^{A3}

The significant differences are indicated by superscripted letters.

The Ni removal efficiencies of *S. molesta* and *L. gibba* were 66.39% and 61.87% respectively. The efficiencies were not significantly different ($p>0.05$).

Table 4: The mean values of initial Ni concentration in wastewater samples, final Ni concentration after being subjected to the treatment by plants, the metal uptake capacities of the two species and the Bio Concentration Factors (BCF).

Characteristic	Control *Salvinia molesta*	Control *Lemna gibba*	*Salvinia molesta*	*Lemna gibba*
Initial concentration (mg l^{-1})	-	-	0.217 ± 0.04^{A1}	0.217 ± 0.04^{A1}
Final concentration (mg l^{-1})	-	-	0.072 ± 0.01^{B1}	0.081 ± 0.01^{B1}
Metal uptake capacity (mg kg^{-1})	-	-	7.258 ± 1.79^{A2}	6.783 ± 1.74^{A2}
BCF	-	-	663.9 ± 69.63^{A3}	618.7 ± 51.32^{A3}

The significant differences are indicated by superscripted letters.

Lead (Pb)

The final Pb concentrations of the two tanks were not significantly different ($p>0.05$). The Pb uptake capacities of *S. molesta* and *L. gibba* were not significantly different ($p>0.05$). The BCF values were lower than 1000 and the BCF value of *L. gibba* was significantly higher ($p<0.05$) than that of *S. molesta*. The Pb removal efficiencies of *S. molesta* and *L. gibba* were 74.85% and 85.74% respectively. The Pb removal efficiency by *L. gibba* was significantly higher ($p<0.05$) than that of *S. molesta*.

Table 5: The mean values of initial Pb concentration in wastewater samples, final Pb concentration after being subjected to the treatment by plants, the metal uptake capacities of the two species and the Bio Concentration Factors (BCF).

	Control *Salvinia molesta*	Control *Lemna gibba*	*Salvinia molesta*	*Lemna gibba*
Initial concentration (mg l^{-1})	-	-	0.292±0.07^{A1}	0.292±0.07^{A1}
Final concentration (mg l^{-1})	-	-	0.073±0.02^{B1}	0.043±0.01^{B1}
Metal uptake capacity (mg kg^{-1})	-	-	10.917±2.83^{A2}	12.45±3.05^{A2}
BCF	-	-	749.0±34.78^{B3}	857.4±26.61^{A3}

The significant differences are indicated by superscripted letters.

According to Al-Khafaji et al. (2017) the Cr, Ni and Pb removal efficiencies of *Lemna min*or are 32.26%, 74.48%, and 79.1% respectively. However, in the present study, *L.gibba* has reported higher efficiencies for the removal of Cr and Pb and lower efficiency for the removal of Ni. According to Verma and Suthar (2015), Pb removal efficiency of *L.gibba* at pH 7 and 2 mg l^{-1} Pb concentrations is 93.8% which is higher than the present study. Dhir and Srivastava (2011) report Cu, Ni and Cr removal efficiencies in a multi-metal solution by *Salvinia natans* respectively as 73.8%, 56.8%, and 41.4%. In the present study, *S.molesta* has shown higher efficiencies for the removal of Ni and Cr and lower efficiencies for the removal of Cu.

Bio Concentration Factor (BCF)

Bio Concentration Factor (BCF) is an index that provides information about the potential of the plants for the uptake of heavy metals. If the BCF value exceeds 1,000, the particular plant can be considered as a useful candidate for the uptake of heavy metals (Zayed et al. (1998). In the present study, none of the BCF values exceeded 1,000. According to Thayaparan et al. (2013), the BCF values of *Azolla pinnata* for Pb had increased with the increase in the initial Pb concentration and the BCF value is higher than 1,000 when the initial Pb concentration exceeds 4 mg l^{-1}. Lokuge (2016) reports a reduction in BCFs of Cr, Cd, Ni and Pb when they were presented in altogether. According to the author, the competition of the metals for the uptake sites and the interactive effects of the metals are the reasons for the reduction of the BCF values.

According to Ranjitha et al. (2016), *S. molesta* has shown its potential in the uptake of Cu, Cr, Pb and Cd and any change in growth regulation is not observed. According to the observations of the present study also, *S. molesta* and *L. gibba* had grown healthily with the accumulation of these heavy metals. Toxicity symptoms caused by heavy metals were not observed in the harvested plant materials. However, the tolerance limits of the plants for the heavy metals are not discovered. Zayed et al. (1998) reports the BCF values of Duckweeds as 500 to 800 for Cu, 400 to 700 for Cr, and 50 to 450 for Pb at low supply concentrations (1 mg l^{-1}). In the present study, similar BCF values are recorded for Cu, but higher BCF values are recorded for Pb and Cr.

3.3 Plant material analysis

The relative growth values of the two control samples were not significantly different ($p>0.05$). The relative growth values of *S. molesta* and *L. gibba* were significantly different ($p<0.05$). The relative growth value of *S. molesta* was the highest. The relative growth value of *L. gibba* was the second highest. The relative growth values of both control samples were lower than the *S .molesta* and *L. gibba* samples.

The dry weights of the two control samples were not significantly different ($p>0.05$) and the dry weights of *S. molesta* and *L. gibba* were not significantly different ($p>0.05$). The fresh weights of *S. molesta* and *L. gibba* were significantly different ($p<0.05$). The dry weights of the two control samples were not significantly different (p>0.05). The final fresh weight of *S. molesta* plants was the highest because of its high moisture content. However, the dry weight of *S. molesta* was lower than the dry weight of *L. gibba*.

Table 6: The mean final fresh weights, mean relative growth values and the mean dry weights obtained from the tank with *Salvinia molesta*, the tank with *Lemna gibba*, the control tank with *Salvinia molesta* and the control tank with *Lemna gibba*.

	Control *Salvinia molesta*	Control *Lemna gibba*	*Salvinia molesta*	*Lemna gibba*
Fresh weight (g)	17.828 ± 2.16^{C1}	16.479 ± 1.37^{C1}	34.918 ± 3.67^{A1}	24.197 ± 3.67^{B1}
Relative growth	1.188 ± 0.14^{C2}	1.099 ± 0.09^{C2}	2.191 ± 0.37^{A2}	1.613 ± 0.24^{B2}
Dry weight (g)	0.536 ± 0.20^{B3}	0.543 ± 0.12^{B3}	1.091 ± 0.19^{A3}	1.135 ± 0.35^{A3}

n=6. The significant differences are indicated by superscripted letters.

In the control sample, distilled water was used as the substrate for the plant growth. At the end of the experiment, on the 7th day, a brown coloration in leaves was observed in the control sample of *S. molesta*. But, *S. molesta* plants grown in the wastewater tank did not show a discoloration. The most likely reason for the brown colour observed in the control sample of *S. molesta* is the lack of nutrients.

However, in the control sample of *L. gibba*, no discoloration was observed. The *L. gibba* plants showed an increase in growth even in the control sample as well as in the wastewater sample. Therefore, the potential of *L. gibba* species to survive in adverse conditions can be identified.

According to Zayed et al. (1998), growth reduction of Duckweeds is reported only at 10 mg l^{-1} Ni concentration. Also, Ranjitha et al. (2016) reports that there is no change in the growth rate of *S. molesta* when exposed to Cr, Cu, Cd and Pb containing wastewater where the particular heavy metals were present in concentrations lower than 2.5 mg l^{-1}. Therefore in the present study, the low concentration of heavy metals may be the reason for the absence of growth reduction in the plants grown in wastewater tanks.

Srivastav et al. (1994) report the relative growth values of *Salvinia* spp. at 1 ppm of Cr concentration as 1.15 and at 1 ppm Ni concentration as 1.17. Also he reports the relative growth values of *Spirodela* spp. at 1 ppm of Cr concentration and at 1 ppm Ni concentration respectively as 1.16 and 1.13. In the present study, *S. molesta* and *L. gibba* plants grown in wastewater and control sample of *S. molesta* recorded greater relative growth values. The most likely reason is the low concentrations of heavy metals in wastewater. Only Cr was present in a concentration higher than 1 ppm in the wastewater used in the present study. The moisture content of fresh *S. molesta* may be the reason for the high relative growth value in control sample.

4. Discussion

According to the obtained results, *S. molesta* and *L. gibba* have proven their potential for the removal of nutrients and heavy metals from municipal wastewater. *S. molesta* has performed well in the removal of Total Nitrogen, Phosphates, BOD and COD reporting the removal efficiencies respectively as 73.35%, 72.63%, 71.51% and 61.98%. Also, *L. gibba* demonstrated a significant removal of Total Nitrogen, Phosphates, BOD and COD reporting the removal efficiencies respectively as 62.18%, 77.28%, 67.24% and 78.96%. *S. molesta* was more efficient in the removal of Total Nitrogen and *L. gibba* showed a greater removal efficiency for COD.

The average Cr, Cu, Fe, Ni and Pb removal efficiencies of *S. molesta* were 81.66%, 69.81%, 65.26%, 66.39% and 74.85% respectively. The average Cr, Cu, Fe, Ni and Pb removal efficiencies shown by *L. gibba* were 86.99%, 69.77%, 73.10%, 61.87% and 85.74% respectively. *L. gi*bba performed greater removal efficiencies in the removal of Cr and Pb when compared to *S. molesta*. But, the Bio

Concentration Factors recorded by *S. molesta* and *L. gibba* in the uptake of selected heavy metals were always lower than 1000 in the present study. Visual toxicity symptoms were not observed in the plant materials grown in the wastewater tanks. However, both *S. molesta* and *L. gibba* can be considered as suitable candidates for the removal of nutrients and these heavy metals (Cr, Cu, Fe, Ni and Pb) from wastewater even at low heavy metal concentrations.

5. Conclusion

This study was carried out to investigate the nutrient and heavy metal removal potential and the efficiencies of *S. molesta* and *L. gibba* for the removal of nutrients and selected heavy metals (Cr, Cu, Fe, Ni and Pb) from municipal wastewater samples collected from the influent of Moratuwa-Ratmalana wastewater treatment plant.

These aquatic plants have the potential of phytoaccumulation of nutrients and heavy metals from water. This strategy is a low cost and eco-friendly technology for the treatment of wastewater. The invasive nature of *S. molesta* and the fast growth rate of *L. gibba* are the practical issues that may arise in the practical use of these plants.

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