

## The Use of *Spirodela polyrrhiza* (Duckweed) and *Eichhornia crassipes* (Water Hyacinth) to Phytoremediate Wastewater in Guyana

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### ABSTRACT

*Spirodela polyrrhiza* (orth. var. *S. polyrrhiza*) is a species of duckweed known by the common names common duckweed,[1] greater duckweed,[2] great duckweed,[3] common duckweed, and duckweed. It can be found nearly worldwide in many types of freshwater habitat. *S. polyrrhiza* is an ideal system for biofuels, bioremediation, and carbon cycling due to its aspects of fast-growing, direct contact with media, and smallest genome size (~150 Mb).[4] A comprehensive genomic study of *S. polyrrhiza* was published in February 2014. The results provide insights into how this organism is adapted to rapid growth and an aquatic lifestyle.[5]

*Eichhornia crassipes*, commonly known as common water hyacinth, is an aquatic plant native to the Amazon basin, and is often a highly problematic invasive species outside its native range. Water hyacinth is a free-floating perennial aquatic plant (or hydrophyte) native to tropical and sub-tropical South America. With broad, thick, glossy, ovate leaves, water hyacinth may rise above the surface of the water as much as 1 meter (3 feet) in height. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8–15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals. When not in bloom, water hyacinth may be mistaken for frog's-bit (*Limnobium spongia*)[6] or Amazon frogbit (*Limnobium laevigatum*).

This research was conducted to study the phytoremediation effect of *Spirodela polyrrhiza* and *Eichhornia crassipes* respectively on domestic wastewater in East Bank of Essequibo, Essequibo Islands, West Bank Regions, Region Three, Canal Number Two Polder, Belle West, Guyana, South America. The analysis of the quantity of the influent and effluent wastewater residues (mg/L): Chloride, Sulphate, Phosphate, Potassium and Lead. Data was statistically analysed using: Levene's F Test, Paired –Wise T-Test, Analysis of Variance – two way, and the Fisher's Least Significant Difference Test using R-Studio. Data were analysed at the 95% confidence level with the p-value of 0.05.

Results showed from this experiment indicated that influent wastewater was highly contaminated with Sulphate and was least contaminated with Lead; there was a statistically significant difference between the influent and effluent wastewater residues and also between the measured intervals (day 5, 10 and 15) for *E. crassipes* and *S. polyrrhiza* respectively; both aquatic species showed significant phytoremediation effect from as early as five days when tested. Results also showed that *E. crassipes* showed a great affinity for Sulphate throughout the measured intervals when compared to the other residues whereas *S. polyrrhiza* showed a great affinity for Phosphate throughout the measured intervals when compared to the other residues and the result showed that *S. polyrrhiza* showed a greater phytoremediation effect than *E. crassipes* since it removed > 90% of the residues in effluent wastewater.

**Keywords:** *Eichhornia crassipes*, *Spirodela polyrrhiza*, Phytoremediation, Wastewater, Constructed Wetland Environment, Influent, Effluent.

### Introduction

Guyana is known as “Land of many waters”. Guyana’s population and economic base is concentrated in the low-lying coastal plains, much of which is below sea level; the area is subject to inundation, and is protected by a series of sea walls, which compose a coastal sea defence system; repairs and maintenance of the sea defences are very expensive, thus the system is in a state of disrepair, and coastal areas are sometimes “flooded” by the sea [7].

The use of aquatic and semi-aquatic plants has been one the most operative means to remove heavy metals and other pollutants from the waterways. Aquatic treatment involves passing wastewater through either wetlands or other aquatic plant ecosystems, whether natural or man-made [8]. Removal of contaminants takes place by plant uptake, microbial degradation, filtration, chemical precipitation, and sedimentation. Wetlands systems are designed around emergent aquatic plants (macrophytes) and can be divided into subsurface flow systems and free water surface systems [9].

In this study the two studied species are *Eichhornia crassipes* (water hyacinth) and *Spirodela polyrrhiza* (duckweed). *E. crassipes* belongs to the Pontederiaceae family; this plant has been used for decades as an

ornamental plant due to its attractive appearance by humans; indigenous to Brazil, the Amazon basin, and Ecuador region<sup>[10] [11]</sup>. It has long roots which are generally suspended in water; the root structure can present a suitable environment for the aerobic microorganisms to function in the sewage system; aerobic microorganisms use the organic matter and nutrient present in the wastewater and convert them into inorganic compounds, which can be utilized by the plants<sup>[10]</sup>.

This study is the first of its nature to provide standard data on the phytoremediation of wastewater (grey water) at every five days' interval from emerging run-offs. The principal goal is to provide policy makers and managers with water quality data of useful aquatic plants (*E. crassipes* and *S. polyrrhiza*) that are prevalent in the water ways in Guyana and also can be used to prevent harsh conditions in the aquatic ecosystems and purify wastewater.

The data gathered can be possibly used to reinforce the laws governing the wastewater management in Guyana and other Caribbean countries where enormous discharge of various waste are deposited into the water ways such as trenches, rivers, oceans, lakes and others. We address this goal by introducing *E. crassipes* and *S. polyrrhiza* in the wastewater emerging from households to absorb the residues (pollutants) from the aquatic environments.

### Materials and Methods

This study was conducted along the East Bank of Essequibo, Essequibo Islands, West Bank Regions, Region Three, Canal Number Two Polder, Belle West. The drainage canal which is comprised of artificial waterway, carrying water away from a wetland or from drainage ditches.

#### *Sample collection and tested samples*

Influent wastewater was collected from the main drain where residents' domestic water flowed from engaging in domestic activities. Influent (before phytoremediation) wastewater sample and effluent (at day 5, 10 and 15) wastewater sample were collected and tested.

#### *Wastewater Chemical Analysis*

The following chemical wastewater analysis was conducted below test for the following variables such as: Phosphate test was conducted using Mitchell and Stapp techniques<sup>[12]</sup> (Mitchell and Stapp, 2008). Chloride test was carried out using the Mohr method<sup>[13]</sup> (Sawyer et al., 2000). Sulphate test was carried out using the Water Treatment test<sup>[14]</sup> (Water Treatment, 2017). Potassium test was carried out using the LaMotte test kit<sup>[15]</sup> (LaMotte, 2018). Lead test was carried out using the Atomic Absorption Spectrophotometer. The standard method was used where the gas phase sample absorbed UV or visible light causing transitions to higher electronic energy levels<sup>[16]</sup> (Parsons and Slavin, 1993). The absorption of light was correlated to concentration using the Beer Lambert Law:

$$A = -\log \left( \frac{I}{I_0} \right) = \epsilon bc$$

$\epsilon$ = molar absorptivity (L/mol\*cm),

b=pathlength of sample cell (cm),

c=concentration of compound (mol/L),  $I_0$  = initial intensity and  $I$ =final intensity.

### Statistical analyses

The following analyses were completed in R-Studio to evaluate the data collected: Levene's F-Test: tested is group of influent wastewater residues (mg/L); Potassium, Phosphorus, Sulphate, Chloride and Lead. Paired- Wise T-Test: tested the influent and effluent group of measured residues (mg/L); Potassium, Phosphorus, Sulphate, Chloride and Lead for *E. crassipes* and *S. polyrrhiza* respectively. Two Way Analysis of Variance: tested the various intervals (day5, 10 and 15) for the effluent group of measured residues (mg/L); Potassium, Phosphorus, Sulphate, Chloride and Lead for *E. crassipes* and *S. polyrrhiza* respectively. Fisher's Least Significant Difference: tested the various intervals (day5, 10 and 15) for the effluent group of measured residues (mg/L); Potassium,

### Results

Data were collected from influent and effluent wastewater analysis which was taken from the thirty (30) samples from CWE that were set up in the Belle West Scheme, Canal Polder, West Bank Demerara, Region three (3). The *S. polyrrhiza* and *E. crassipes* were used separately to phytoremediate wastewater from the run-offs (drains) for a total period of fifteen (15) days.

**Table 1:** Summary of the where the differences can be found based on the obtained p-values using LSD

Variable (mg/L)	<i>E.crassipes</i> (Interval-day)			<i>S. polyrrhiza</i> (Interval-day)		
	0-5	5-10	10-15	0-5	5-10	10-15
Lead	0.00*	0.00*	0.94-	0.00*	0.00*	0.93-
Chloride	1.00-	0.00*	0.00*	Nd	Nd	Nd
Sulphate	0.00*	0.00*	0.00*	0.00*	0.59-	0.24-
Phosphate	0.25-	0.99-	0.00*	0.00*	0.00*	0.00*
Potassium	0.14-	0.53-	0.00*	0.00*	0.10-	0.00*

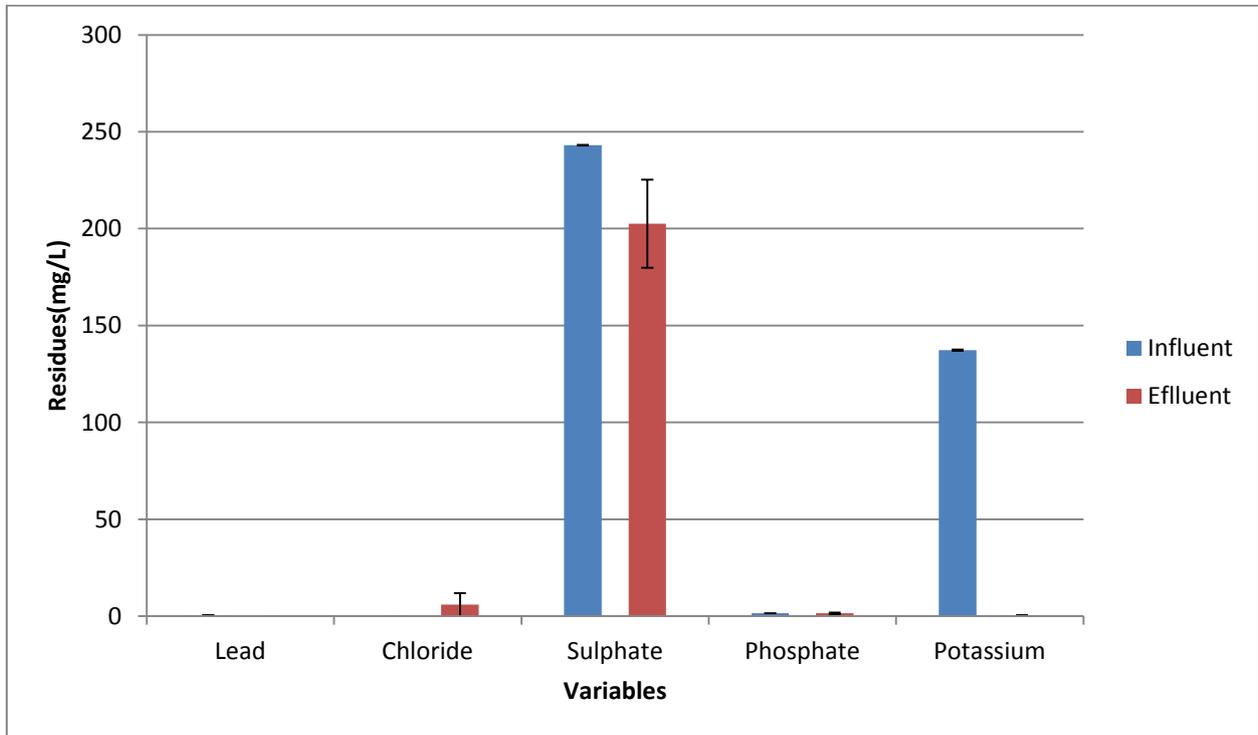
\*indicates significant difference; - indicates not significant; Nd indicates not detected

As seen in the table 1 which summarises where the differences can be found based on the obtained p-values using LSD between the various days intervals. Lead showed a statistical significance throughout the studied period for *E. crassipes* and *S. polyrrhiza* from day 0-5 and 5-10 except for 10-15. Sulphate also showed a significant difference throughout all three intervals for *E. crassipes* but not with *S. polyrrhiza*. *S. polyrrhiza* showed a statistical significance of Sulphate from day 5 to 10 and 10 to15. Phosphate and Potassium were phytoremediated from day 5-10 and 10-15 by *E. crassipes*. However, statistical significance of phytoremediation using *S. polyrrhiza* was seen from 0-5, 5-10 and 10-15 for Phosphate and Potassium.

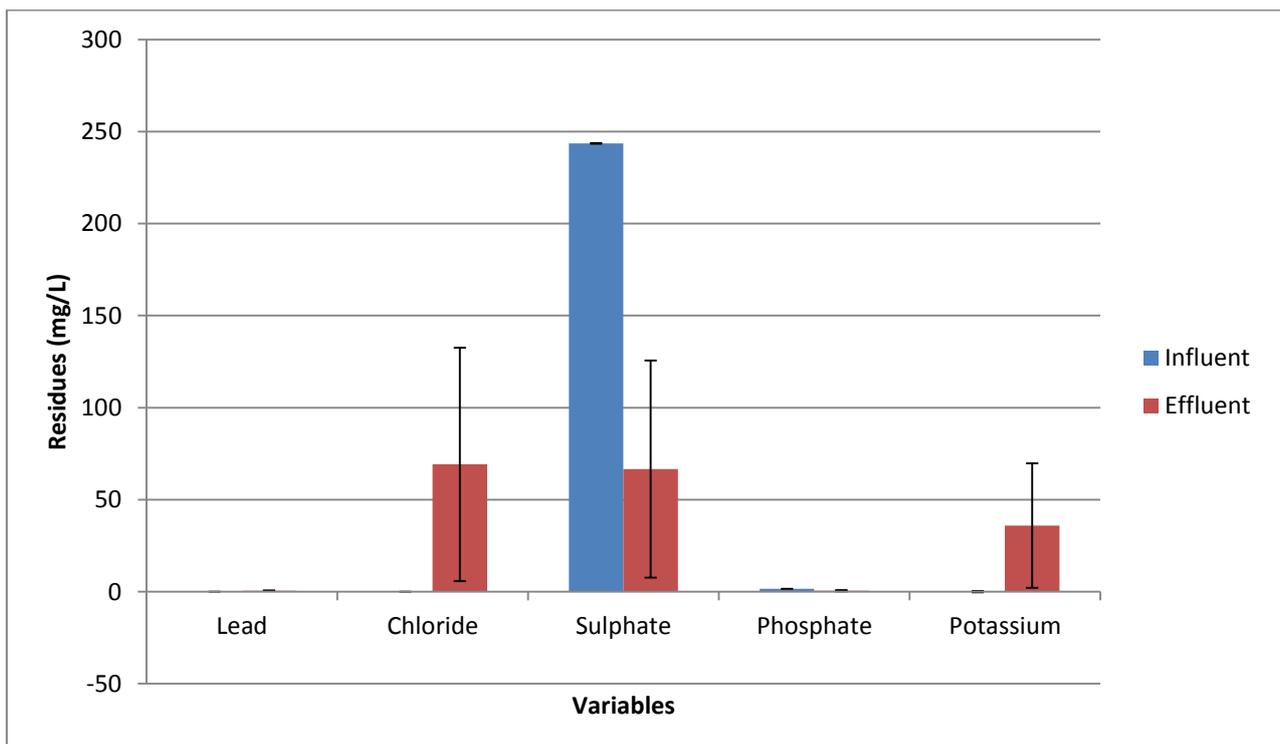
**Table 2:** Summary of percentage of phytoremediated residues

Variables (mg/L)	Influent	Total # of Phytoremediated Residues (Effluent)		% of Phytoremediated Residues (Effluent)	
		<i>E. crassipes</i>	<i>S. polyrrhiza</i>	<i>E. crassipes</i>	<i>S. polyrrhiza</i>
Lead	0.06 ± 0.00	0.08 ± 0.02	0.54 ± 0.02	0	0
Chloride	Nd	5.98 ± 5.98	69.18 ± 63.4	0	93.2

<b>Potassium</b>	137.3 ± 0.3	97.79 ± 24.79	35.86 ± 33.81	80.7	99.2
<b>Phosphate</b>	1.53 ± 0.00	1.45 ± 0.43	0.485 ± 0.35	87	92.1
<b>Sulphate</b>	243.5 ± 0.16	202.5 ± 22.76	66.64 ± 58.96	43.5	97.3



**Fig.1:** The Influent vs. Effluent Concentraion of Residues for *E. crassipes*



**Fig.2:** The Influent vs. Effluent Concentration of Residues for *S. polyrrhiza*

Figure 1 and 2 presented the concentrations of influent residues compared to the concentrations of effluent residues phytoremediated by *E. crassipes* and *S. polyrrhiza* respectively per variable. Table 2 highlighted the percentage of residues phytoremediated by each plant species respectively. Both plants displayed a great affinity for Potassium, Sulphate and Phosphate. *E. crassipes* phytoremediated more than 80% of potassium and phosphate residues. It also showed a great affinity for 43.5% of sulphate residues. Lead and Chloride were not phytoremediated by *E. crassipes*. *S. polyrrhiza* on the other hand displayed a greater affinity for potassium, phosphate and sulphate. Even though chloride was detected in the later intervals of the experiment, 93.2 % were removed. However, no lead residue was phytoremediated.

## Discussion

The threshold limit for drinking water is as follows: Potassium <10mg/L (WHO, 2009), Lead <0.01 mg/L (WHO, 2010), Sulphate <20.0 mg/L (WHO, 2004), Phosphate <0.01 mg/L (EPA, 2000) and Chloride < 2.0 mg/L (WHO, 2003). Since these threshold limits are established for drinking water, it can be clearly seen that if these wastewater residues contaminate the consumable water, individuals will be exposed to serious health defects.

Untreated wastewater generally contains: high levels of organic material, numerous pathogenic microorganisms, as well as nutrients and toxic compounds which entails environmental and health hazards and; consequently, must immediately be conveyed away from its generation sources and treated appropriately before final disposal. The ultimate goal of wastewater management is the protection of the environment in a manner commensurate with public health and socioeconomic concerns <sup>[17]</sup>.

Their influent wastewater samples were significantly high in Sulphate and Potassium <sup>[18]</sup>. The results gathered from this research are in agreement with some of research that has been conducted: *E. crassipes* showed effective phytoremediation effect from day 5 to 15 for potassium. Phosphate and sulphate was seen in the later intervals where >20 % of the residues are removed. Gupta, and colleagues in 2015 who also designed CWE in Jhaakhand, measured and used a few aquatic plant species together particularly *E. crassipes* at day 5, 10 and 15. They found that *E. crassipes* showed a statistical significance at p-value <0.01 and reduced the pH of wastewater, Nitrate, and Sulphate respectively. They also studied Cl, Fe, Cu, Mn, Pb, K, F and concluded that Nitrate and Sulphate were drastically reduced after a three weeks' period, adding to this, the 50% of Sulphate was removed at 10 to 15 <sup>[19]</sup> (Gupta *et al.*, 2012). In this experiment, it can be clearly seen that phytoremediation started at an earlier period (before day 5), thus the phytoremediation showed significant difference between the intervals. This result also supported Chandra and colleagues in 2004 also who reported that a major reduction in Sulphate of the tannery effluent were removed using *E. crassipes*. <sup>[20]</sup>

*E. crassipes* also seem to grow rapidly throughout the 15 days period. This was seen by another research which shows that *E. crassipes* grew rapidly only at the first three weeks of the investigation and the remaining period of the plant growth was reduced <sup>[20] [22]</sup>. The worked on Tapioca wastewater using *E. crassipes* plant showed that after 28 days period in CWE, *E. crassipes* has a decrease in plant height in the treatment of Tapioca in high waste contaminated materials, plant was difficult to adapt to the environmental conditions of poor water quality <sup>[23]</sup>.

Lead was not seen to be biologically significant in respect to phytoremediation throughout the 15 days period in this study. At day 5 the residues increased in the wastewater and then it slowly phytoremediated for both species. This occurred because of high quantity of Lead residues which were present within the dry biomass in the plant; translocated in the plant's tissues<sup>[24]</sup> was released into the influent wastewater for both species respectively; seen when conducted the biomass test (*E. crassipes*= 38.9 mg/L and *S. polyrrhiza*= 14.1 mg/L). There is no other research that was conducted in Guyana to support this action of these studied species but other research shows significant phytoremediation effect of removing heavy metals especially Lead from wastewater using *E. crassipes* and *S. polyrrhiza* respectively.

The overall results showed that *S. polyrrhiza* phytoremediated effectively. Chaudhary and Sharma are in agreement of *S. polyrrhiza* removal of nutrients, soluble salts, organic matter, heavy metals and in eliminating suspended solids, algal abundance and total and faecal coliform densities. Pateli and Kanungo posited that *S. polyrrhiza* showed a significant removal of pollutants from domestic wastewater with special reference to nutrients for a period of seven days this supported the results of this research since the phytoremediation process was studied from day 5 to day 15. They posited that *S. polyrrhiza* systems are one of the options that have been widely applied for the combined handling of wastewater with the nutrients used for poultry and aqua-cultural projects<sup>[25]</sup>. The well and stated that aquatic plants especially *S. polyrrhiza* have shown their efficiency in absorbing nutrients from various sources of polluted water<sup>[26]</sup>.

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