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Research Article

Nutrient removal efficiency of *Spirogyra sp.* and *Oedogonium sp.* in wastewater from Egerton University, Kenya.

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ABSTRACT

Water sources all over the world are constantly being polluted through disposal of untreated human and animal wastes, agrochemicals and industrial effluents. Aquatic plants such as algae take up nutrients together with other pollutants for their growth. This study aimed at assessing nutrient removal efficiency of *Spirogyra sp*. and *Oedogonium sp*. in wastewater. The two algal species are aquatic flora commonly observed as pioneer photoautotrophs in both lotic and lentic water bodies. They were cultured in wastewater for a period of 90 days during which physico-chemical parameters of the wastewater were measured using multimeter electronic probe while nutrients, mainly Soluble reactive phosphorus (SRP), Total Phosphotus, Ammonium-nitrogen, Nitrates-nitrogen and Nitrite – nitrogen were analysed using standard methods at the beginning of the experiment and monthly after inoculation with a given weight of *Spirogyra sp*. and *Oedogonium sp*. Data collected was analyzed using Statistical Package for Social Sciences Software (SPSS) version 17.0 software. The results showed that the physico-chemical parameters of waste water treated with *Spirogyra sp*. and *Oedogonium sp*. varied significantly **($F_{(df, N)}$ =008641 P<0.05)**. Likewise, nutrient uptake by *Spirogyra sp*. and *Oedogonium sp*. varied significantly **($F_{(df, N)}$= 1.175345 P<0.05).** There was also a significant difference in the change in weight of *Spirogyra sp*. and *Oedogonium sp*. **($F_{(df, N)}$=0.8023 P<0.05)** between day 1 and day 90. *Spirogyra sp*. and *Oedogonium* sp. were effective in uptake of nutrients in waste water. There is need for further characterization of the isolated *Spirogyra* and *Oedogonium sp*.

Key words: Nutrient; efficiency; *Oedogonium sp*.; *Spirogyra sp*.; wastewater

INTRODUCTION

Various water uses such as laundry, bathing and water used in toilets result in production of **w**astewater (Lawton *et al*., 2014). Such water may be treated in sewage treatment plants prior to their disposal into the natural water bodies such as lakes, rivers and oceans (Saunders *et al*., 2012). Some of the wastewater however end up in different water bodies without passing through treatment plants. In addition, run off from agricultural land find their way to natural water

bodies without treatment which often lead to eutrophication problems (Bird *et al*., 2012).

Treated and untreated wastewater as well as runoff from agricultural land contain nutrients and other pollutants that cause adverse effects to the aquatic environment that may disrupt the natural aquatic food chain (Lawton *et al*., 2013). Some of these pollutants include toxic pesticides sprayed to control insect pests, fungi and herbs (Sarkar and Sekh, 2019) as well as lead and cadmium all of which may bioaccumulate in the microorganisms which may later be consumed by fish and shellfish (Alfasane *et al*., 2019). This transfer of biomass through consumption in the food chain leads to bio magnification of the pollutants at higher trophic levels and may cause harm to other organisms when consumed. In addition, biomagnification may threaten the reproduction and survival of carnivores which occupy high levels of food chains (Satpati and pal, 2016).

Metal pollutants like lead, arsenic, mercury and chromium are very harmful, toxic and poisonous even in low concentrations (Ji and Kim, 2017). Although some minerals such as Zinc, copper and iron are useful to human and animal health in small doses, when ingested in high amounts they become fatal (Yaseen and Scholz, 2019).

Eutrophication is a process by which water body slowly becomes rich in plant nutrients (Sulfahri *et al*., 2017). These nutrients lead to excessive growth of algae and other large water plants such as *Salvinia molesta*. From this excessive growth, the huge biomass generated cause high oxygen demand for decomposition of the dead organic materials from this biomass which lead to oxygen deficiency in water bodies (Prabha *et al*., 2016). This high biological oxygen demand leads to death of oxygen dependent organisms in aquatic ecosystems (Ayodhya *et al*., 2013).

Diversity and abundance of aquatic organisms have been adversely affected by increase in water pollution (Brahmbhatt *et al*., 2012). This has given way to high pollution tolerant organism from the orders diptera, oligochaete and hirudinea (Chan *et al*., 2014).

Algae are a major component of aquatic flora that have a great potential to solve energy and environmental challenges around the world through their rapid growth and uptake of nutrients and other pollutants from the water habitat (Zheng *et al*., 2011). Wastewater treatment though growth of Algal culture has been observed to produce a more environmental sound approach in reduction of eutrophication through uptake of nitrogen and phosphorus from wastewater (Kumar *et al*., 2015). Some of the macro algae such as *Spirogyra sp.* and *Oedogonium sp*. amongst others can absorb significant amount of nutrients because through their rapid growth, they need large amounts of nitrogen and phosphorus for proteins synthesis and growth (Oswald and Gotaas, 1957). This study aimed at determining the efficiency of *Spirogyra sp.* and *Oedogonium sp*. in removing nutrients from wastewater.

MATERIALS AND METHODS

Study Area

The study was carried out in the laboratory at the Department of Biological Sciences in Egerton University. The wastewater was collected from Egerton University Tatton Aquaculture Farm. Egerton University is located approximately 182 kilometers, by road, northwest of Nairobi, the capital city of Kenya. The university lies at 0°22’11.0”S, 35°55’58.0” E (Latitude: 0.369734; Longitude: 35.932779) (Waithaka *et al*., 2019).

Sample collection and isolation

Samples containing filaments of *Spirogyra* and *Oedogonium sp* were collected from Egerton University Tatton Aquaculture Farm along the trenches in between the fish ponds. The samples were taken to the Department of Biological Sciences in sterile polythene papers. *Spirogyra* and *Oedogonium sp* were established from other fragmented filaments using pipette-washing procedure (Kumar and Oammen, 2012). The *Spirogyra* and *Oedogonium sp* were separately grown in 100 mL of Bold’s Basal Medium (BBM) in glass vessels (50 mm × 95 mm) held at 20°C

under a 14:10 hour light/dark cycle. Temporally, slides were prepared and observed under compound and dissecting microscope to ensure that there was no contamination of the culture by growth of other algae and therefore each growth was a monoculture of only the given species. The *Spirogyra* and *Oedogonium sp* were identified using morphological characteristics (Salam *et al*., 2014).

Experimental design

The experiment was set in the laboratory using nine buckets (Delgadillo-Mirquez *et al*., 2016). Three litres of waste water was placed in each of the six buckets. Three litres of distilled water was placed in each of the remaining three buckets which acted as control experiments. Two mL of starter culture of *Spirogyra sp*, was inoculated in 3 buckets containing waste water. This was repeated using *Oedogonium sp.*

Determination of physico-chemical characteristics

Water temperature, Dissolved oxygen, pH and conductivity of the water cultures as well as the controls were determined in each bucket during the experiment using probe meters (Abdel-Raouf *et al*., 2012). The parameters were determined monthly for a period of 90 days.

Determination of nutrients content

Total phosphorus (TP) was determined from unfiltered samples from the buckets through digestion of samples with potassium persulphate followed by ascorbic acid method colorimetrically with the spectrophotometer.

The soluble reactive phosphorus (SRP) was determined using the ascorbic acid method on filtered samples. The nitrite-nitrogen was determined using the reaction between sulfanilamide and N-Naphthyl-(1)-ethylendiamin-dihydrochloride (*Samori et al*., 2013). Nitrate-nitrogen was determined using the sodium-salicylate method with standard solution of the nitrate prepared for the standard calibration curve (Hadiyanto *et al*., 2013). The parameters were determined monthly for a period of 90 days.

Weight of *Spirogyra* and *Oedogonium sp.*

A large plastic scoop was used to sample 1L from each experimental bucket and the contents placed in a container. The volume of each bucket was restored to the initial volume through addition of distilled water.

The sample were weighed and placed in a muffle furnace at 550°C for 2h and the weights determined. Harvest productivity was calculated (Sutherland *et al*., 2014). At end of the experiment the total weight of the two separate algae in the bucket were determined (Dwivedi, 2012).

Data analysis

All statistical analyses were carried out using Statistical Package of Social Sciences Software (SPSS) version 17.0.

The results on Physico-chemical parameters and nutrient uptake by *Spirogyra* and *Oedogonium sp.* were analyzed using ANOVA. The weights of *Spirogyra* and *Oedogonium sp.* were compared using t-test. All statistical results with $P \leq 0.05$ were considered statistically significant.

RESULTS

The pH levels in the buckets containing *Spirogyra sp.* varied from 5.3±0.1 to 7.0±0.2, temperature (9.2±0.2^{o}C - 12.0±0.2^{o}C), dissolved oxygen (3.0±0.3mg/L - 7.0±0.2mg/L) and conductivity (300.0±0.1µS/sec -360.0±0.1µS/sec) (Table 1). In the buckets containing *Oedogonium sp.* the pH ranged from 6.6±0.2 to 8.0±0.1, temperature (13.0±0.2^{o}C - 14.0±0.1^{o}C), dissolved oxygen (3.3±0.2mg/L-5.7±0.2mg/L) and conductivity (259.0±0.3µS/sec - 350.0±0.1µS/sec).

There was a significant difference in the physico-chemical parameters between *Spirogyra sp.* and *Oedogonium sp.* **(**F=1.008641 P=0.0440).

Table 1: Physico-chemical parameters of the waste water

Par		*Spirogyra sp.*			*Oedogonium sp.*		
	Day 1	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90
pH	7.5±0.1	7.0±0.2	6.1±0.3	5.3±0.1	6.6±0.2	8.0±0.1	7.9±0.1
Temp (^oC)	18.0±0.1	12.0±0.2	11.0±0.1	9.2±0.2	13.0±0.2	14.0±0.1	13.5±0.3
DO (mg/L)	7.2±0.2	7.0±0.1	4.0±0.2	3.0±0.3	5.7±0.2	4.0±0.1	3.3±0.2
Cond (µS/sec)	300.0±0.2	300.0±0.1	350.0±0.2	360.0±0.1	259.0±0.3	350.0±0.1	348.0±0.3

Par; Parameter, Temp; temperature, DO; Dissolved oxygen, Cond; conductivity

Cultural characteristics of *Spirogyra* and *Oedogonium sp.*

Filaments of *Spirogyra sp.* and *Oedogonium sp,* showed various cultural characteristics as indicated on Table 2 below.

Table 2: Cultural characteristics of *Spirogyra* and *Oedogonium sp.*

Characteristic	*Spirogyra sp.*	*Oedogonium sp*
Filaments	Light green to green and unbranched	Fine unbranched filaments
Cell diameter	70–100µm	18–32µm
Cell plane	Transverse	Transverse
No. of chloroplasts per cell	5–7 spiral chloroplasts with numerous pyrenoids	Typically one
Types of spores	Meiospores and zygospores	Aplanospores and zoospores

Nutrient uptake by *Spirogyra* and *Oedogonium sp.*

The total phosphorus in *Spirogyra sp.* ranged from 350.0±0.1µg/L to 600.0±0.2 µg/L, Soluble reactive phosphorus (259.0±0.3µg/L-500.0±0.2µg/L), NO_2-N (2.4±0.2µg/L-6.0±0.1µg/L) and NO_3-N (0.1±0.2µg/L-0.2 ±0.1µg/L) (Table 3). In *Oedogonium sp.* total phosphorus ranged from 345.0±0.2µg/L to 450.0±0.2µg/L, Soluble reactive phosphorus (195.0±0.2µg/L - 200.0±0.2µg/L), NO_2-N (3.5±0.1µg/L-7.0±0.2µg/L) and NO_3-N (0.4±0.2µg/L-0.5±0.1µg/L). There was a significant difference in nutrient uptake by *Spirogyra sp.* and *Oedogonium sp.* between day 1 and day 90 **(**F=1.175345 P= 0.0350153).

Table 3: Nutrient uptake by *Spirogyra* and *Oedogonium sp*

Par		*Spirogyra sp.*			*Oedogonium sp.*		
	Day 1	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90
TP (µg/L)	700.0±0.2	600.0±0.2	400.0±0.1	350.0±0.1	450.0±0.2	350.0±0.1	345.0±0.2
SRP (µg/L)	600.0±0.1	500.0±0.2	300.0±0.2	259.0±0.3	200.0±0.1	200.0±0.2	195.0±0.2
NO_2 (µg/L)	14.0±0.2	6.0±0.1	2.5±0.2	2.4±0.2	7.0±0.2	4.0±0.1	3.5±0.1
NO_3 (µg/L)	0.7±0.2	0.2±0.1	0.1±0.2	0.1±0.3	0.5±0.2	0.45±0.1	0.4±0.2

Par; Parameter, TP; total phosphorus, SRP; Soluble reactive phosphorus

Weight of *Spirogyra* and *Oedogonium sp.*

The weight of *Spirogyra sp.* in day 30 was 22.9±0.2g while that of *Oedogonium sp.* was 21.9±0.1g, day 60; *Spirogyra sp.* (23.77±0.2g) and *Oedogonium sp.* (23.83±0.1g) and day 90; *Spirogyra sp.* (29.6±0.2g) and *Oedogonium sp.* (29.63±0.1g) (Figure 1). There was a significant increase in the weight of *Spirogyra sp.* and *Oedogonium sp.* (df=3 P=0.0356).

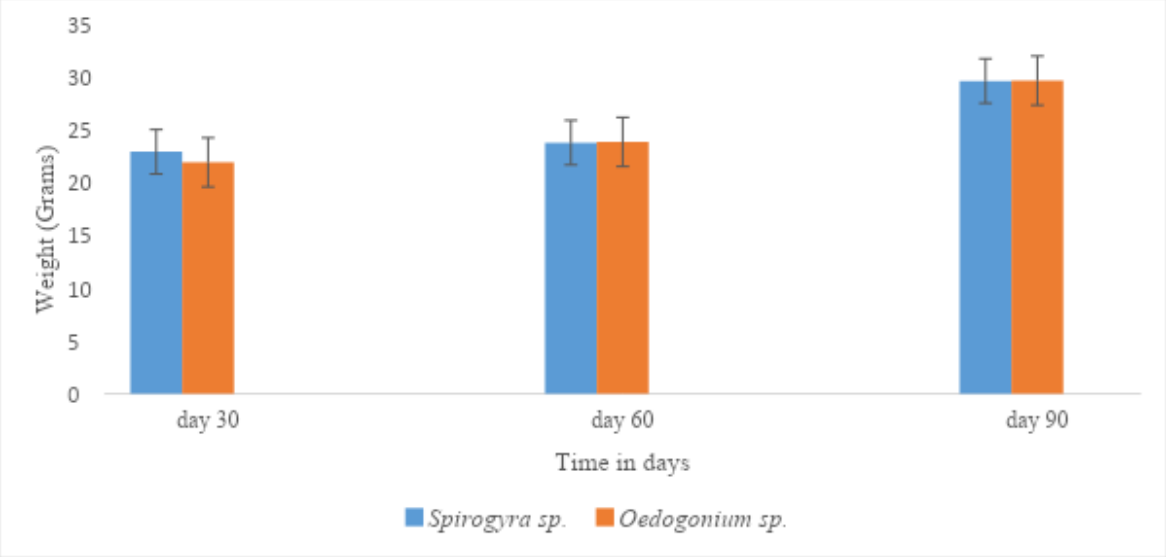

Figure 1: Weight of *Spirogyra* and *Oedogonium sp.*

DISCUSSION

The *Spirogyra* and *Oedogonium* sp. isolated in this study presented typical characteristics of the isolates. According to Kim *et al.* (2014) cultural characteristics are still an important tools for identifying algae species. Further, Lee *et al.* (2015) asserted that the genetic codes within algae species dictates their cultural characteristics.

Some of the physico-chemical characteristics of the waste water samples inoculated with *Spirogyra* and *Oedogonium sp.* such as pH increased with time. Similar results were obtained in a similar study by Scott *et al.* (2010). The degradation of organic materials which subsequently dissolved in the water samples may have led to the increase in pH (Craggs *et al.*, 2012). However, the temperature of the samples reduced with time. This could have been caused by absorption of heat by the degraded organic materials (Neveux *et al.*, 2014). This results concurred with those of a previous study by Roberts *et al.* (2013). Use of similar biochemical activities by *Spirogyra* and *Oedogonium sp.* could have been a contributing factor. In addition, the dissolved oxygen reduced from day 1 to day 90. This concurred with a previous study carried out on utilization of microalgae for integrated biomass production and physico-remediation of wastewater (Mishra, 2017). Chen *et al.* (2012) asserted that increase in algal growing mass leads to reduced dissolved oxygen. Batista *et al.* (2015) further maintained that oxygen uptake by microbes in the decomposition of dead algal mass results in reduced dissolved oxygen in aquatic systems. The temporal increase in conductivity for the samples inoculated with *Spirogyra* and *Oedogonium sp.* may be attributed to the release of inorganic elements from the biodegradation of organic materials within the water samples (Cai *et al.*, 2013). Although the same trend was observed in a study carried out by Craggs *et al.* (2014), the values obtained in the current study was higher. This could be attributed to differences in the algal species used in the study (Dwivedi, 2012).

The reduction in the nutrients contents in the culture media, though minimal points to the uptake of these nutrients by the two algal organisms in the culture, *Spirogyra* and *Oedogonium sp.* The results partially agreed with a study on assessment of phyco remediation efficiency of *Spirogyra Sp.* using sugar mill effluent (Kumar *et al.*, 2016). This may be attributed to presence of the similar dissolved substances in the waste water samples. In addition, Ali *et al.* (2013) suggested that same algal species take the same nutrients from water.

Weight of *Spirogyra* and *Oedogonium sp*. increased with time. However, the weights obtained in the present study were higher than in a previous study carried out by Fouilland (2012). Differences in the dissolved nutrients in the test water samples could have affected the results (Sarkar and Sekh, 2019).

CONCLUSIONS

The *Spirogyra* and *Oedogonium sp*. isolated in the current study had typical characteristics of the species. The isolates influenced the physicochemical characteristics of the water samples. In addition, the isolates absorbed nutrients from the water samples.

Recommendation

There is need for further characterization of the isolated *Spirogyra* and *Oedogonium sp*.

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