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Phycoremediation Efficacy of *Gloeocapsa gelatinosa* on Coffee, Tea and Sugar Effluent from Bungoma, Nandi and Kakamega Counties: Kenya

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Abstract
Microalgae can phycoremediate wastewater and reduce the excess nutrient content from agricultural effluents due to the algal ability to assimilate nutrients. The conventional treatment of effluents may result to a clear and clean effluent but the final effluent is usually heavily loaded with nitrates and phosphates and other pollutants of public health concern. The study set out to investigate the phycoremediation efficacy of Gloeocapsa gelatinosa in tea, coffee and sugar wastewater from Nandi, Bungoma and Kakamega counties, Kenya. 10ml of serial dilutes of pure G. gelatinosa in test tubes were mixed with 100ml of the wastewater in a beaker from the three types of waste water then incubated at 25^0c and monitored for nutrient absorption and reduction of TDS,BOD, COD, pH and conductivity levels. The BOD/COD track machine were used to determine the BOD and COD levels and pH meter used for estimation of pH, while the phosphate and nitrate contents were determined using the colorimetric method before and after specific algal inoculation. Results showed statistical significant differences ($p<0.05$) in the phycoremediation of TDS in coffee, tea and sugar effluents in days 5,10 and 15.The phycoremediation effect in all the effluents showed an increase in pH levels of the effluents in day 5 before stabilizing at day 15.The phycoremediation efficacy of Gloeocapsa gelatinosa in all the other physicochemical parameters in all the three effluents showed a significant difference in the pollutants reduction ($p<0.05$) in the Gloeocapsa gelatinosa inoculated effluent. The phycoremediation efficacy of Gloeocapsa gelatinosa was better and more significant ($p<0.001$) in tea effluent than in the coffee and sugar effluents on the nitrate, phosphate COD,BOD, pH TDS and conductivity with the phycoremediation efficacy improving with the increase in the incubation period and no further phycoremediation effect noted by day 15. The information generated will be useful in the factories implementation of effective effluent treatments and in community sensitization on public health impacts of the waste water to the nearby ecosystems.

Key words; *Phycoremediation, Gloeocapsa gelatinosa, efficacy, microalgae.*

Introduction

Phycoremediation is a process which involves the use of macro-or microalgae species for effective removal or biotransformation of environmental pollutants, including nutrients and xenobiotics from effluents and carbon dioxide (CO_2) from waste air thereby making the recycled wastewater an ideal habitat for most of the aquatic organisms Olguin (2003).Phycoremediation studies have always employed the use of different algal species in the bioremediation of various agricultural, industrial, domestic and municipal wastewaters. Phycoremediation has found applications in dealing with pollution caused by industrial effluents (Olguin,2003).

Phycoremediation technology it's a cheaper and most effective remedial approach to remove excess nutrients in wastewater like phosphates and nitrates besides producing potentially valuable biomass (Sengar *et al*., 2011). Large-scale phycoremediation of industrial, agricultural, domestic and municipal effluent has been done and reported successfully in many different parts of the world (Sivasubramanian *et al*., 2009; Mostafa *et al.,* 2015; Sivasubramanian *et al*., 2010; Rao *et al*., 2010).Through phycoremediation technology, efficient pH correction, sludge reduction and reduction of BOD and COD can be achieved effectively and thereby mitigating most of the environmental impacts brought about by un treated effluents . During effluent treatment process large amount of valuable algal biomass is also generated by these industries. Microalgae culture especially in the tertiary stage of treatment offers a cost-effective approach to removing nitrates, phosphates and also in reducing BOD and COD levels from different effluents. Different algal species have a high uptake capacity for inorganic nutrients and they can be grown in mass culture in outdoor solar bio-reactors and the process has been known to perform well especially when compared to the conventional processes which are in general, too costly to be implemented in most places and which may lead to secondary pollution especially where chemicals are used in the treatment (Akali *et al*., 2011, Abioye, 2011). Microalgae cultures also offer an elegant solution to tertiary and quaternary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorous for their growth and their capacity to remove heavy metals. The interest algal cultures stems from the fact that conventional treatment processes suffer from some important disadvantages like variable efficiency in nutrient removal, high cost of operation, secondary pollution especially where chemicals are used and lastly the loss of valuable potential nutrients like the nitrates and phosphates (Lim *et al*., 2010). Effluents treatment in Africa are insufficient in ensuring safe water and basic sanitation for the general public and to address this challenge, Hongtao *et al*. (2013) concluded that joint efforts are needed, including; transforming to green economy, innovating technologies, improving operation and maintenance and harvesting energy which can be made possible through phycoremediation processes of wastewaters. In Kenya all industries are required to collect, treat and also discharge safely all the wastewater that are produced within their areas of jurisdiction. Sanitary conditions are a mandatory requirement for all industries in order to prevent the occurrence therein of any conditions liable to be injurious or dangerous to public health. The release of noxious substances into water resources is also prohibited. Every one living in Kenya has been bestowed with the responsibility to create and protect a healthy environment through the Environmental Management and Co-ordination Act.. There is of great concern from the public, and Lake Victoria Environmental Management Programme (LVEMP) over the deterioration of the water quality in rivers discharging into Lake Victoria. Communities living along River Yala have also registered their concern regarding the deteriorating water quality and the sources of pollution have been suspected to be the tea, coffee and sugar factory effluents that discharge into the nearby streams and rivers draining into lake Victoria (Maghanga *et al.,* 2009).

Phosphates in water will stimulate the growth of planktons and aquatic plants which provides food for the fish. This may cause an increase in the fish population and improve on the overall water quality. However, if an excess of phosphates enters the waterway, algae and aquatic plants will grow wildly, choke up the waterway and use up large amounts of oxygen. This condition is known as eutrophication or over- fertilization of receiving waters. This rapid growth of aquatic vegetation eventually dies and as it decays it uses up oxygen. This process in turn causes the death of aquatic life because of the lowering of dissolved oxygen levels (Akali *et al*., 2011). High phosphates are toxic to both human and animals and can cause digestive problems Olguin (2003). Other consequences of nitrogen compounds in wastewater effluents are toxicity of non-ionized ammonia to fish and other aquatic organisms, interference with disinfection where a free chlorine residual is required and methemoglobinemia due to excessive nitrate concentrations (above 45 $g\ m^{-3}$) in drinking water (Olguin,2003; Akali *et al*., 2011).

A study by Kumar and Saramma, (2012) on nitrate and phosphate uptake by immobilized cells of *Gloeocapsa gelatinosa* found out that the algae species where efficient in the removal of nitrate. The algal species absorbed 93% of nitrate from the medium within 24 hours, whereas algae-free beads and *G. gelatinosa* cells absorbed 46% and 70% respectively ($r = 0.000$, $P<0.05$).However there was a gradual release of the absorbed nitrate into the medium by all the three algal groups after 24 hours. Phosphate nutrients were also effectively absorbed by the immobilized cells of *G. gelatinosa*. The study showed that immobilized cells could be used for effective nutrient removal from a closed system. In another study by Kotteswari *et al.* (2007) on phycoremediation 88.82% reduction in the phosphate content was recorded on the 15th day of algal treatment while Tam and Wong (1990) reported the removal of over 90% total phosphorus within 10days of algal cultivation. The capacity of cyanobacteria to remove large amounts of phosphorus from waste waters was also demonstrated by several other researchers (Chan *et al.* 1979, Anandaraj *et al.* 2001).

More phycoremediation studies of wastewater with an aim to reduce pollution load has also showed that the pH of the pre-treated waste water was 8.1 in day one and no change was found on the 5^{th} day but from 10^{th} day onward the pH decreased and by the 25^{th} day it had remained at about 7.1 (Sengar *et al*.,2011). A similar study by Aarti *et al.* (2008) showed a marked increase and then reduction in pH values in water samples after the treatment with algae, where the algal treatment retained the pH value around neutral value after treatment of the effluents with G. *gelatinosa*. Another related study on the application of phycoremediation technology in waste water by Rao *et al.,* (2011) reported that on the 7^{th} day of the effluent treatment with algae the pH of the effluent increased from 7.6 to 8 and maintained at the same level thereafter. The current study therefore endeavored to provide an efficient means of treating tea, coffee and sugar effluents devoid of the conventional disadvantages by use of *G. gelatinosa*

Materials and Methods

Study area

Nandi County found on 0° 10' 0.00" N; 35° 08' 60.00" E has a high population of tea factories producing a huge amount of the tea effluent while Kakamega county found on 0° 17' 3.19" N: 34° 45' 8.24" E has a concentration of about three sugar factories with Bungoma found on 0° 33' 59.99" N: 34° 33' 59.99" E has the highest concentration of coffee factories in western Kenya which all produce a considerable amount of effluent.

Study design

The intent of the research was to determine the phycoremediation efficacy of *Gloeocapsa gelatinosa* on the tea, coffee, and sugar effluents from Nandi, Bungoma and Kakamega counties. The experimental study design which involved analyzing for the physicochemical parameters namely TDS,COD,BOD, nitrates, phosphates, Conductivity and pH of the tea, coffee and sugar effluents before and after the *Gloeocapsa gelatinosa* inoculation and then determining their public health effects through a structured questionnaire was used.

Sample Size Determination

To calculate the sample size required Taro Yamane1991 method was used. The formula has been set as follow:

$$n = \frac{N}{1 + Ne^2}$$

Where; n=sample size, N=population size, e=the error of sampling; N= 17; e=0.05; Therefore, n= $17/1+17(0.05)^2$; n=16

Therefore, 16 tea ,8 coffee and 3 sugar factories were all included in study.

Sample size was 26 factories i.e, 16 tea, 8 coffee and 2 sugar, hence after calculation only 26 factories were sampled.

Sampling Method
Purposive and random sampling was used whereby all the functional factories had to be identified and then serialized. The numbers were all put in one bucket then mixed and picked randomly for inclusion in the study. A total of four samples from each factory were taken between December 2015 and March 2016 for the *Gloeocapsa gelatinosa* phycoremediation efficacy.

Experimental Layout
Wastewater treatment ponds from an abandoned paper mill in Webuye (Pan Paper Company) were identified for the sampling of the *Gloeocapsa gelatinosa* to be used in the study. Wastewater Samples were collected from ponds of the treatment plant then transported to the Eldoret water and sanitation company (ELDOWAS) laboratories for microscopic identification and cultural propagation of the *Gloeocapsa gelatinosa* for use in the tea, coffee and sugar effluent inoculations (APHA 2005).The color of the effluent was first removed through electro-coagulation method for easy of nitrate estimation and then COD, BOD, Nitrate, Phosphates, pH, conductivity and TDS were analyzed using the Chemical oxygen demand by reflux method, the trak machine incubator, Nitrate(cadmium Reduction method, Electrometric method respectively (APHA 2005).

Data Management and Analyses
Statistical Analysis was both descriptive and inferential. Means and standard deviations, graphs and tables, were all been applied. Analysis was done using STATISTICA version 9.

Results and Discussion
Table 1 below shows the TDS phycoremediation mean results of coffee, tea and sugar effluents using *G. gleocapsa.* From the results coffee had the highest TDS values followed by tea and lastly sugar. The phycoremediation success was 58%,65% and 58% for coffee, tea and sugar effluents respectively. The values after treatment with *G. gleocapsa* reduced with an equal margin in day 5 and day 10 with no further reduction in day 15. Similar results were reported by Kotteswari *et al*., (2012), Ahmad *et al*., (2013), Elumalaei *et al*., (2013) and Rao *et al.(*2011) using *G. gelatinosa* and other different species of *cyanophyceae* for wastewater treatment also observed an average reduction in TDS of up to 60 %. Rao *et al.(*2011) and Ahmad *et al.,* (2013) attributed the above reductions in TDS to the utilization of various nutrients by algae because of their unique mechanism of bioabsorption.

From the current phycoremediation findings in table 1 and figure 2 below using *G.gelatinosa* on the pH of the coffee, tea and sugar effluents, the study established a progressive increase in pH from neutral to alkaline across all the different effluents. During the phycoremediation process, while the other physico chemical parameters were decreasing the pH levels increased initially and thereafter remained around a median of 8 at and after day 10 as shown in figure 2. The reason for the rise in pH levels can be attributed to the reduction of dissolved CO_2 concentrations through photosynthesis which, in turn, raises the pH level (Rao *et al*., 2011).Borowitzka, (1998), and Rao *et al*.(2011) also found out that the inorganic species used mainly by microalgae are usually CO_2 and bicarbonate, the latter requiring the enzyme carbonic anhydrase to convert it to CO_2 for use in photosynthesis process.

G. gelatinosa in table 1 and figure 3 below reduced the nitrates from coffee, tea and sugar effluents with a phycoremediation success of 65%,42% and 77% and a significant statistical phycoremediation difference of $p<0.0001$,$p=0.009$ and $p=0.002061$ for coffee, tea and sugar effluents respectively. Tam and Wong (1990) in a related study established a phycoremediation efficacy of 88% on effluent treated with *G .gelatinosa*.

Phycoremediation efficacy of *G. gelatinosa* on phosphate content of coffee, tea and sugar effluents was found to have statistical differences of $p>0.05$, $p<0.05$ and $p=0.060094$ respectively. Figure 4 below summarizes the phycoremediation trend as depicted in table 1. Other studies by Kumar and Saramma, (2012),Tam and Wong (1990) established phycoremediation efficacies of 88.82% and 90% reduction in the phosphate content on the 15th day of *G, gelatinosa* cultivation.

Phycoremediation efficacy of *G. gelatinosa* on the BOD from the coffee, tea and sugar effluents showed a reduction from the initial high levels to lower levels though there was no significant variation noted ($p>0.05$) with *G. gelatinosa* in coffee and tea effluents respectively. A non-significant phycoremediation difference of $p=0.139059$ was however noted across day 5,10 and 15 of phycoremediation of sugar effluent.

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Table 1. Determination of the phycoremediation efficacies of, *G. gelatinosa* on the coffee, tea and sugar effluent

Effluent type	Species	Day	TDS	pH	Nitrates	Phosphates	BOD	COD	Conductivity
COFFEE	*G.gelatinosa*	Day0	734.875±234.52	6.5±0.378	21.375±2.129	4.419±1.784		3459±1875.41	1185.25±378.22
		Day5	367.625±117.21	7.65±0.298	11.012±1.372	2.932±1.463	1147±840.342	2345.5±1135.38	592.875±189.063
		Day10	306.875±111.228	7.65±0.298	8.088±0.96	2.383±1.103	213±113.088	2267.25±1132.77	487.375±181.279
		Day15	308.875±111.228	7.65±0.298	7.588±1.012	2.382±1.103	213±113.088	2267.25±1132.77	487.375±181.279
			P=0.167579	P=0.037072	P=0.000000	P=0.700559	P=0.323294	P=0.905673	P=0.163441
			58%		65%	46%	81%	35%	59%
TEA	*G.gelatinosa*	Day0	505.875±58.987	7.711±0.2941	4.106±0.54	69.071±5.764		1777.813±264.408	815.625±95.0978
		Day5	192.5188±32.818	9.04±0.106	2.794±37	34.922±3.530	652.625±74.701	1362.375±250.23	332.875±50.896
		Day10	174±28.764	8.731±0.1082	2.426±0.3199	32.754±3.335	502.125±78.178	1219.375±244.147	280.5±46.333
		Day15	174±28.764	8.716±0.1049	2.361±0.3152	32.656±3.369	502.063±78.181	1219±244.173	280.5±46.333
			P=0.000000	P=0.000007	P=0.009754	P=0.000000	P=0.289965	P=0.352515	P=0.000000
			65%		42%	53%	23%	31%	66%
SUGAR	*G.gelatinosa*	Day0	474.5±12.5	6.55±0.45	28±2	5±0.9		4872±1088	765±20
		Day5	320.5±1.5	8.45±0.15	11.5±2.5	2.8±0.2	880±20	3590.5±779.5	518±2
		Day10	196±1	8.4±0.3	6.5±0.5	2.35±0.35	543±117	1724.5±459.5	315.5±1.5
		Day15	198±1	8.35±0.25	6.5±0.5	2.35±0.35	543±117	1724.5±459.5	315.5±1.5
			P=0.000017	P=0.029373	P=0.002061	P=0.060094	P=0.139059	P=0.097016	P=0.000017
			58%		77%	52%	38%	65%	59%

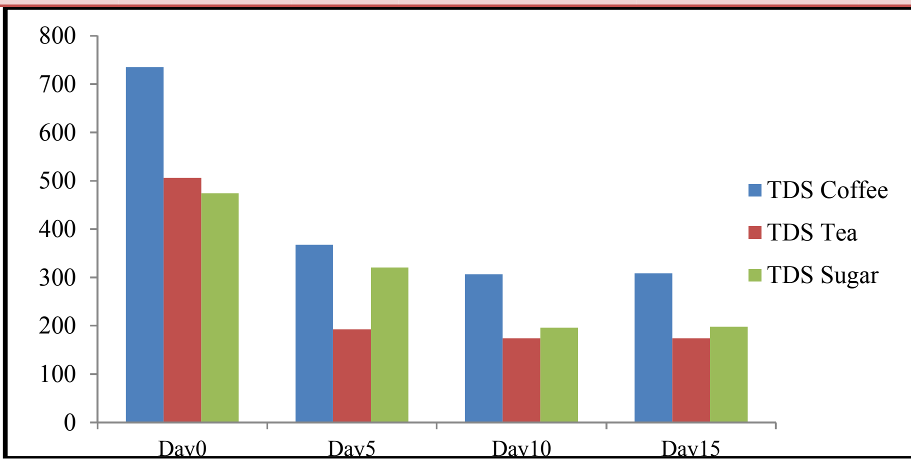

Fig 1. *Gloeocapsa gelatinosa* phycoremediation efficacy on TDS in coffee, tea and sugar effluents.

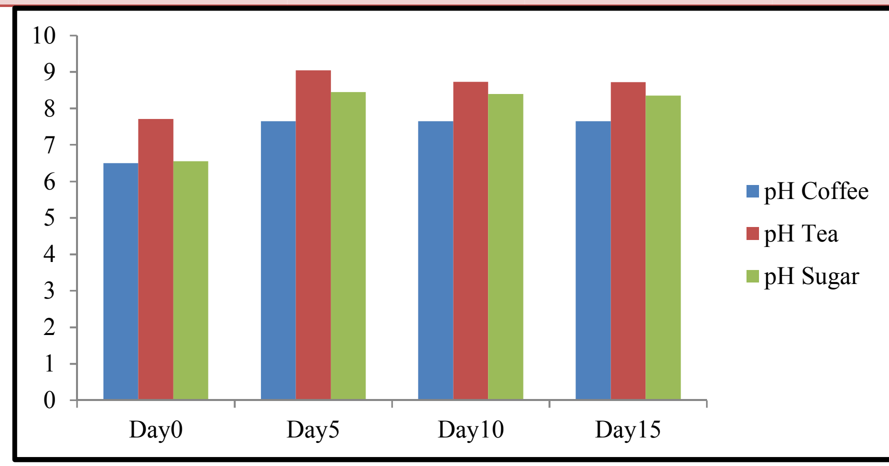

Fig 2. *Gloeocapsa gelatinosa* phycoremediation efficacy on pH of coffee, tea and sugar effluents.

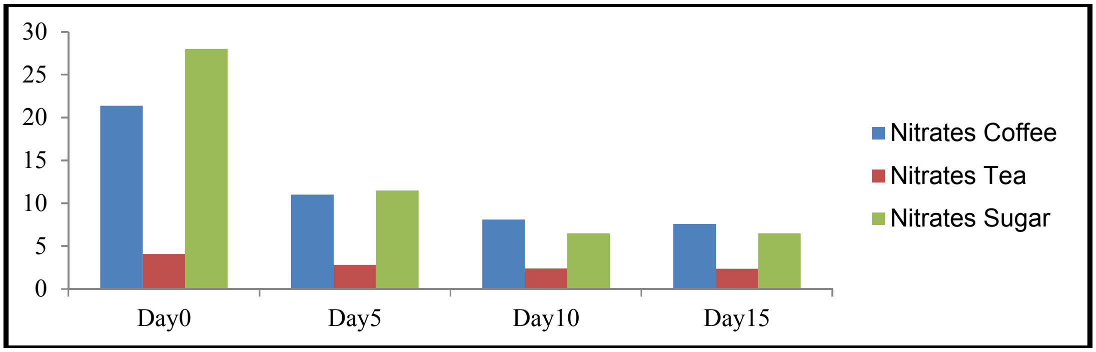

Fig 3. *Gloeocapsa gelatinosa* phycoremediation efficacy on nitrates of coffee, tea and sugar effluents

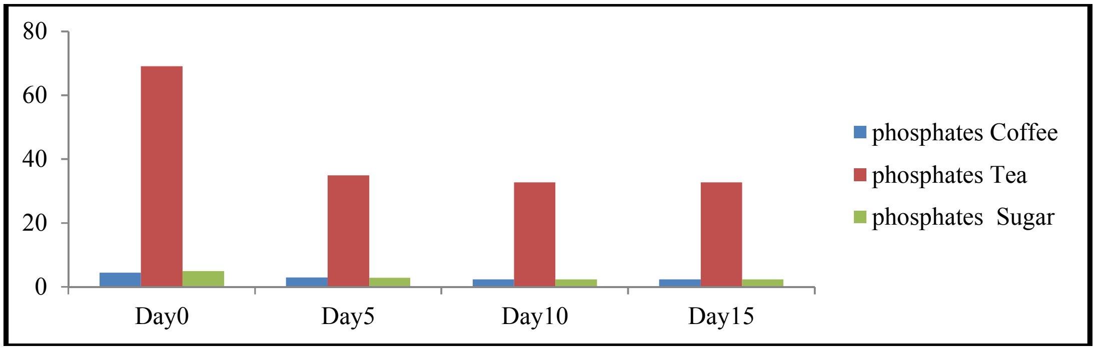

Fig 4. *Gloeocapsa gelatinosa* phycoremediation efficacy on phosphates of coffee, tea and sugar effluents.

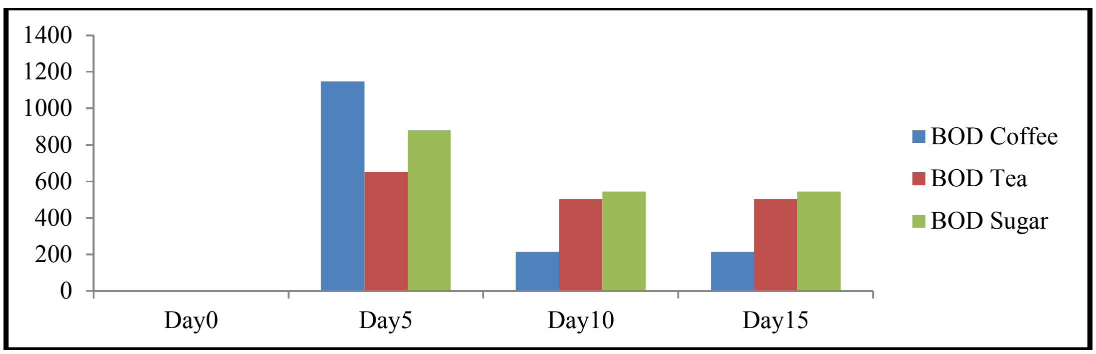

Fig 5. *Gloeocapsa gelatinosa* phycoremediation efficacy on BOD of coffee ,tea and sugar effluents

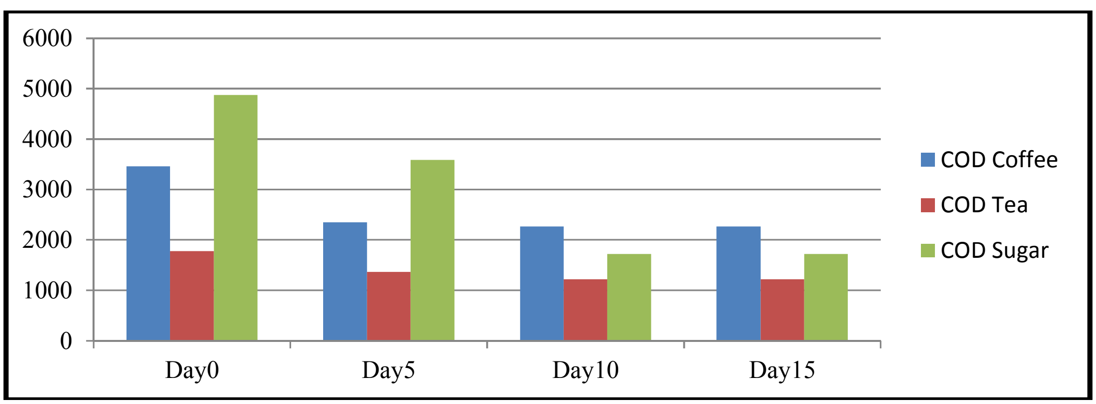

Fig 6. *Gloeocapsa gelatinosa* phycoremediation efficacy on COD of coffee, tea and sugar effluents

Phycoremediation of COD content in the coffee, tea and sugar effluents showed that there was no any significance difference in the COD reduction in days 0,5,10 and15 ($p>0.05$) using *G.gelatinosa* in both coffee and tea effluents and also there was no significant variation in phycoremediation of COD using the *G.gelatinosa* $p=0.09716$ but the reduction trend was evident as shown in BOD and COD figures v, and vi, for coffee, tea and sugar effluents respectively. The progressive reduction in COD and BOD was due to high algal growth rate and intense photosynthetic activity. Chemical oxidations of carbon present in organic pollutants releasing carbon dioxide was also responsible for the reduction of COD and BOD values, and similarly faster biodegradation and bioconversion of organic matter due to algae might be the additional reason (Elumalai *et al*. 2013; Sharma and Khan (2013)).

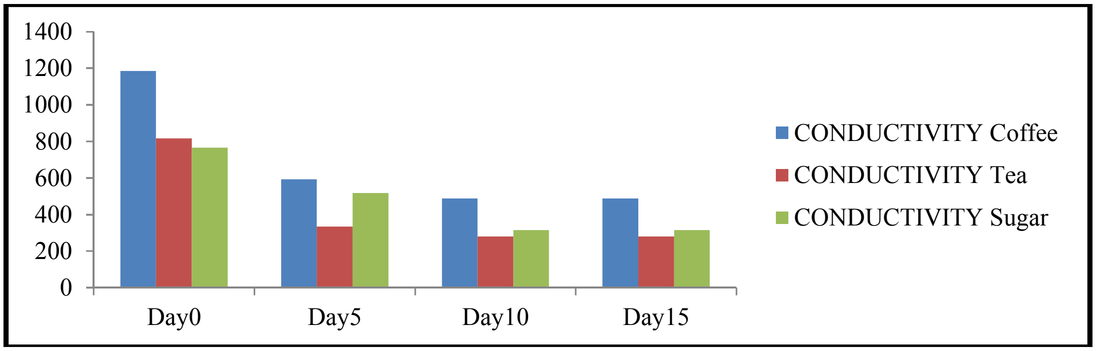

Fig 7. *Gloeocapsa gelatinosa* phycoremediation efficacy on conductivity of coffee, tea and sugar effluents

The mean electrical conductivity of the coffee, tea and sugar effluent in table 1 for day 0 were 1185.25±378.22mg/l, 815.625±95.0978mg/l and 518±2mg/l, respectively but after phycoremediation using *G. gelatinosa* the conductivity values reduced sharply as indicated in figure vii above. A previous study done by Khemka and Saraf (2015) and Yu *et al*. (2005) reported that electrical conductivity highly depended on the presence of ions in the effluents, their concentration, valency and mobility with this reduction attributed to the ability of algal species to consume the nutrients during the algal growth.

Conclusion

The phycoremediation efficacy of *Gloeocapsa gelatinosa* was better and more significant ($p<0.001$) in tea effluent than in the coffee and sugar effluents on the nitrate, phosphate COD,BOD, pH TDS and conductivity with the phycoremediation efficacy improving with the increase in the incubation period and no further phycoremediation effect was noted by day 15. The information generated will be useful for the factories implementation of effective effluent treatments and in the community sensitization on public health impacts of the waste water to the nearby ecosystems.

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