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Phycoremediation Efficacy Of Synechocystis Salina On The Characteristics Of Coffee, Tea And Sugar Effluents From Bungoma, Nandi And Kakamega Counties; Kenya

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Abstract: ***Phycoremediation is an alternative way to remove nutrients and other contaminants from potentially polluted wastewater. The conventional treatment by physical and bacteriological processes results in a clear apparently clean effluent but heavily loaded with pollutants. The study set out to investigate the phycoremediation efficacy of Synechocystis salina in tea, coffee and sugar wastewater from Nandi, Bungoma and Kakamega counties.10ml of serial dilutes of pure S.salina in test tubes were mixed with 100ml of the wastewater in a beaker from the three types of waste water then incubated at 25^{0}c and monitored for nutrient absorption of TDS, BOD, COD, pH and conductivity levels. The BOD and the COD were determined using the BOD/COD track machine and pH meter 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 across the studied period except in coffee effluent where the S.salina had a non significant difference in phycoremediation. The phycoremediation effect showed an increase in pH levels of the effluents in day 5 before stabilized in day 15.The phycoremediation effect of all the other physicochemical parameters in all the three effluents showed a significant difference in the pollutants reduction ($p<0.05$) with the Synechocystis salina inoculation across the study period..The comparison of the phycoremediation efficacy of Synechocystis salina in the three effluents showed an insignificant difference in phycoremediation of $p>0.05$ in the physiochemical parameters except for TDS ($p<0.05$) and that sugar and coffee waste water had high levels of nitrates while tea waste water had high levels of phosphates. The phycoremediation efficacy of Synechocystis salina was much more significant in tea effluent ($p<0.001$) than in coffee and sugar effluents. The study has provided information that can be used as a basis for follow-up to medical facilities in the study area to find out on the potential health effects of nitrates and phosphates elevated levels stemming from contaminated effluent discharge. The information generated will also be useful in Community sensitization on public health impacts on the use and discharge of the waste water to the nearby ecosystems and also help the factories treat the effluents effectively before discharge.***

Keywords: Synechocystis salina; phycoremediation, efficacy

I. INTRODUCTION AND LITERATURE REVIEW

Phycoremediation is defined as the effective process of removal or biotransformation of environmental pollutants by use of algal species, Olguin (2003).The process is cheaper and a 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, 2010; 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 . Duringeffluent 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 nutrients and reducing BOD and COD levels from wastewater. Microalgae 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). The interest in microalgal 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).Physicochemical parameters in wastewater are varied and are known to affect their biotic characteristics. Some of the major parameters include; COD, BOD, pH and TDS. The elevation of the mentioned parameters can be used as an indicator of waste water contamination. In this study, the physicochemical parameters of coffee, tea and sugar effluent were determined before and after algal inoculation. Previous studies have shown that algal species have the potential to remediate and improve the quality of wastewater from different sources.

Phycoremediation studies for improving the quality of effluents from a conventional tannery wastewater treatment plant done in Europe Italy showed that the chemical and biological oxygen demands (COD and BOD) are some of the broad spectrum parameters that in the case of industrial wastewater discharge can be used to unmask a plethora of specific contaminants that can be noxious for the environment when the already treated wastewaters are regularly discharged. Residual COD and BOD values were compatible with their controlled and authorized discharge, which resulted from nonylphenols contaminants (Gregorio *et al.*, 2014).

The effect of sugar industry effluents on nitrate and phosphate levels in Rapti river, India established a significant elevation of nitrate and phosphate levels which resulted in increased eutrophication but decreased dissolved Oxygen levels (DO) and hence lesser population of planktons (Nagendra and Ram, 2011). The results on investigation of the influence of sugar mill effluent on physicochemical characteristics of soils at Haridwar area in India indicated significant increase in nitrate and phosphate levels in soil, consequently modifying the soil properties and quality. Hence soils irrigated with sugar effluents were likely to have increased nitrate and phosphate content, increased pH but decreased moisture content. The high levels were due to the effect of mixing of effluents with the ground water around the sugar mill and also the contribution of animal wastes. consequently these levels were higher than the Indian permissible levels hence water samples downstream the effluents were not suitable for drinking and irrigation purposes. (Vinod and Chopra, 2010; Deshmukh, 2014).Biochemical potential of brewery wastes co-digested with glycerol contained sugars which generated high levels of methane. Its co-digestion with glycerol enhanced biodegradation of brewery wastes hence had the potential to increase further production of methane. Anaerobic treatment of waste waters with bacteria has the potential to generate methane (Costa *et al.*, 2013).Further Investigations on treatment of dairy and brewery waste waters with Bacillus and hydrolytic enzymes produced about 79% (Demirel *et al*., 2010). This method has also been applied during treatment of animal dung to generate biogas (Costa *et al.*, 2013).

Wet processing of coffee is known to produce high quality coffee over the dry processing method. During wet processing, the exocarp and coffee pulp is removed via fermentation, a process that generates ethanol. In presence of methanogenic bacteria, ethanol is broken into ethanoic acid and eventually to methane and Carbon (IV) Oxide. The overall effluent also contains high levels of nitrate and phosphates initially present as nutrient fertilizers (Enden and Calvert, 2002). Whereas the above studies were known to release methane and carbon dioxide gases which are all environmental pollutants the current study aims at reducing the release of methane and carbon dioxide to the environment through the adoption of an algal based wastewater treatment technology which sequesters most of the methane gas and utilizes most of the nitrate and phosphates in the effluent hence reducing its environmental impacts.

Phycoremediation studies by Dominic *et al. (*2009) using *Synechocystis salina* has been successful in treatment of waste water samples where the algal species reduced the phosphate content of the waste water samples by 64.52%. The concentration of the phosphate was also noted to have decreased from 3.1µmol/l to 1.1µmol/l. Nitrate content reduction rate was however reported to have been high at 96.23% having been reduced from 5.3µmol/l to 0.2µmol/l. The pH of the wastewater sample was also reported to have changed drastically when the wastewater was treated with the *Synechocystis salina* (Dominic *et al.*,2009). Kotteswari *et al.* (2007) also reported a pH change of 5.62 to 9.82. While in another study Manoharan and Subrahmanian, (1992) reported a rise in the pH value up to 10th day of the waste water treatment with the algal species. Aarti *et al*., 2008 during his earlier phycoremediation study on waste waster had noted that the carbon dioxide produced by respiration of plants and animals in water had the effect of lowering down the pH of the waste water samples .Carbon dioxide and bicarbonate removed from the water by the photosynthetic process of aquatic plants raises the pH and alters the dissolved oxygen content

II. MATERIALS AND METHODS

STUDY AREA

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Nandi County has a high population of tea factories producing a huge amount of the tea effluent while Kakamega has a concentration of about three sugar factories with Bungoma having the highest concentration of coffee factories which all produce a considerable amount of effluent. The above study areas have no effluent treatment facilities except in few tea factories of Nandi county where conventional treatment of the tea effluents is the norm.

STUDY DESIGN

The intent of the research was to determine the phycoremediation efficacy of *S.salina* on the tea, coffee, and sugar effluents from Nandi, Bungoma and Kakamega counties. The experimental study design used 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 *S.salina* inoculation.

III. 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 ie 16 tea, 8 coffee and 2 sugar, hence after calculation only 26 factories were sampled.

IV. 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, mixed and then 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 *S.salina* phycoremediation efficacy.

V. EXPERIMENTAL LAYOUT

Wastewater treatment ponds from an abandoned paper mill in Webuye (Pan Paper Company) were identified for the sampling of the *S.salina* 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 *S.salina* for use in the tea, coffee and sugar effluent inoculations (APHA 2005). The COD, BOD, Nitrate, Phosphates, pH, conductivity and TDS were analyzed using the elecrocoagulation method, Chemical oxygen demand by reflux method, the trak machine incubator, Nitrate (cadmium Reduction method, Electrometric method respectively (APHA 2005).

DATA ANALYSES AND MANAGEMENT

Statistical Analysis was both descriptive and inferential. Means and standard deviations, graphs and tables, have been applied. Analysis was done using STATISTICA version 9.

EFFLUENT TYPE	SPECIES	DAY	*TDS*	*Ph*	***Nitrates***	***phosphates***	***BOD***	*COD*	*CONDUCTIVITY*
COFFEE	*S. Salina*	Day 0	734.875±234.52	6.5±0.378	21.375±2.129	4.419±1.784		3459±1875.41	1185.25±378.22
		Day 5	306.25±97.795	7.313±0.284	8.575±1.231	2.511±1.345	1147±840.342	2331.13±1108.16	493.625±157.625
		Day 10	243.125±84.607	7.3±0.279	5.838±0.628	2.04±1.097	212.625±113.171	2223.88±1106.56	392.25±136.454
		Day 15	243.125±84.607	7.288±0.273	5.275±0.699	2.039±1.097	212.625±113.171	2223.75±1106.59	392.25±136.454
			P=0.05508067%	P=0.188738	P=0.00000076%	P=0.56334255%	P=0.32303482%	P=0.89454336%	P=0.05504767%
TEA	*S. Salina*	Day 0	505.875±58.987	7.711±0.2941	4.119±0.5463	69.196±5.789		1777.81±264.408	815.625±95.0978
		Day 5	169.1937±25.056	9.017±0.0967	2.274±0.2735	31.6±3.360	684.5±75.238	1119.94±250.018	272.875±40.444
		Day 10	131.9375±22.506	8.698±0.098	1.809±0.2213	30.208±3.238	482.437±82.267	1124.63±219.026	212.812±36.234
		Day 15	131.9375±22.506	8.691±0.0991	1.809±0.2213	30.05±3.247	482.437±82.2668	1124.31±218.956	212.812±36.234
			P=0.00000073%	P=0.000007	P=0.00001556%	P=0.00000057%	P=0.13103330%	P=0.14290637%	P=0.00000074%
SUGAR	*S. Salina*	Day 0	474.5±12.5	6.55±0.45	28±2	5±0.9		4872±1088	765±20
		Day 5	285.5±12.5	8.25±0.25	10±3	2.35±0.35	880±20	2777.5±533.5	460.5±20.5
		Day 10	177±8	8.15±0.15	4.5±1	1.55±0.45	420±80	1250±240	285.5±12.5
		Day 15	177±8	8.15±0.15	4.25±0.75	1.5±0.5	420±80	1250±240	285.5±12.5
			P=0.00010262%	P=0.032014	P=0.00257585%	P=0.03770570%	P=0.02503052%	P=0.04114974%	P=0.00010063%

Table 1: Determination of the phycoremediation efficacies of S.salina on the physicochemical parameters of coffee, tea and sugar effluent

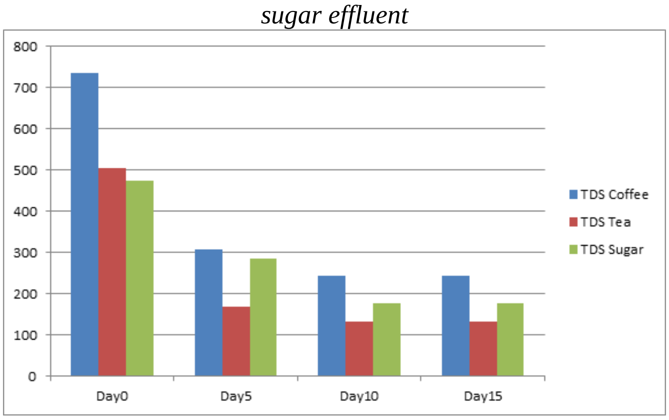

Figure 1: S.salina phycoremediation efficacy on TDS in coffee, tea and sugar effluents

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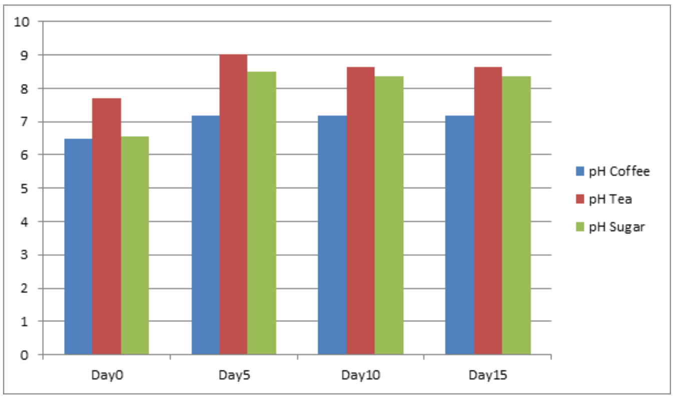

Figure 2: S.salina phycoremediation efficacy on pH in coffee, tea and sugar effluents

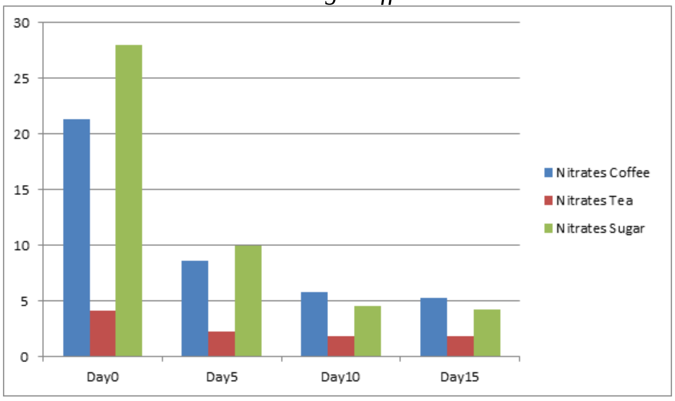

Figure 3: S.salina phycoremediation efficacy on nitrates in coffee, tea and sugar effluents

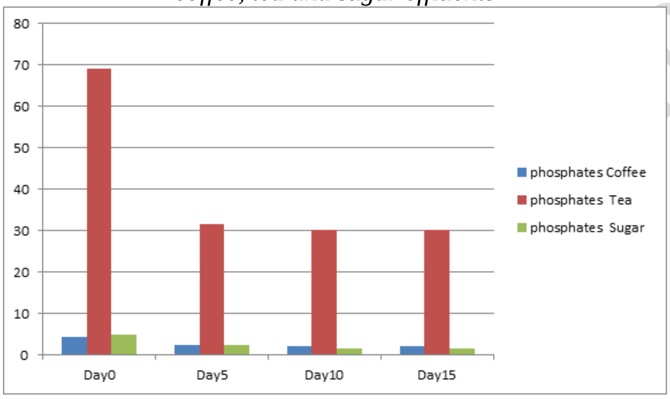

Figure 4: S.salina phycoremediation efficacy on phosphates in coffee, tea and sugar effluents

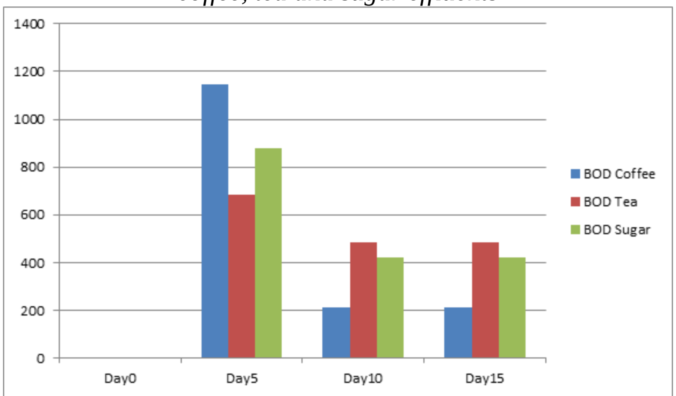

Figure 5: S.salina phycoremediation efficacy on BOD in coffee, tea and sugar effluents

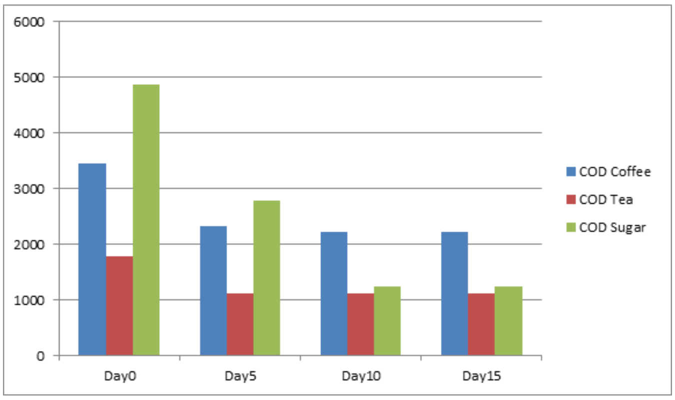

Figure 6: S.salina phycoremediation efficacy on COD in coffee, tea and sugar effluents

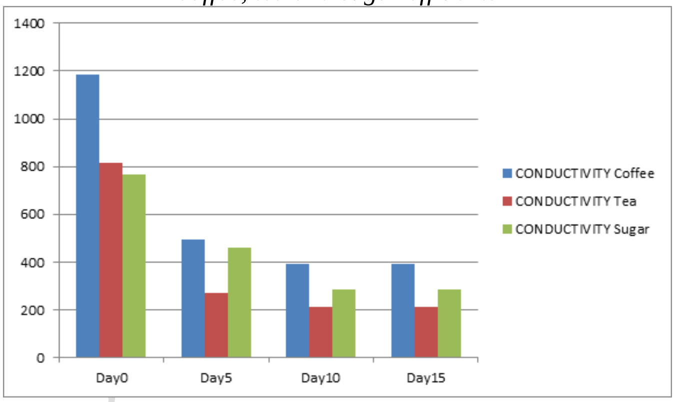

Figure 7: S.salina phycoremediation efficacy on conductivity in coffee, tea and sugar effluents

VI. DISCUSSION OF RESULTS

The physicochemical parameters analyzed in table 1 above were mainly TDS, COD, BOD, pH, conductivity and nitrate and phosphate nutrients. From the study it was found that most of the parameters were high above the acceptable values. The physicochemical parameters were first measured before the inoculation of the algal species, with most of the results being above the normal standards. Then later the samples were inoculated with each of the three algal species and the results of each physicochemical parameter noted after 5 days, 10 days, and 15 days. From the analyzed wastewater samples total dissolved solids (TDS) from all the different effluents were found to be above the normal allowable limit with the mean and standard errors of 734.875±234.52,505.875±58.987 and 474.5±12.5of TDS from coffee,tea and sugar effluents respectively at day 0.

Upon phycoremediation with *S.salina* the TDS values from coffee reduced from a mean of 306.25±97.795 mg/lin day 5 and 243.125±84.607in day10 and finally the values were found to stabilize at a mean of243.125±84.607mg/l in day 15as shown in fig i , with an insignificant difference of p=0.055080 and 67% phycoremediation success,

Phycoremediation of TDS mean values in sugar effluent using *S.salina* was found to decrease from 285.5±12.5mg/l to177±8mg/l and finally to 177±8mg/l for days 5,10 and 15 respectively as shown in fig i with a significant difference of p=0.000102 and a phycoremediation success of 62%.Tea wastewater when subjected to phycoremediation using

S.salina showed TDS mean value reductions from169.1937±25.056mg/l to131.9375±22.506056mg/l and then to131.9375±22.506056mg/l in days 5,10 and 15 respectively as indicated in fig I and a significant difference of $p<0.0001$ with a phycoremediation success of 73%..

These reductions in TDS were also reported in another study by Kshirsagar (2014) who showed reduction percentages of TDS from 37.59, 34.40, 42.17 and 24.86 % in sewage, mixtures 1, 2 & 3, respectively, by *C. salina* compared to untreated water samples. Kotteswari *et al*., (2012), Ahmad *et al*., (2013) and Elumalaei *et al*., (2013) using different species of *cyanophyceae* and *chlorophyceae* 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 abovereductions in TDS to the utilization of various nutrients by algae. The unique mechanism of bioabsorption of different types of dissolved solids in wastewater according to Nanda *et al.*(2010), was responsible for the reduced TDS to lower levels, and upon quantification this projected a high reduction levels because there could have been a conversion of the total suspended solids already present in the effluent into dissolved materials mainly for algal uptake and final assimilation by the algae.

The table 1 above on the mean electrical conductivity of the coffee effluent in day 0 was 1185.25±378.22mg/l, but after phycoremediation using *S.salina* the values reduced to 493.625±157.625mg/l,392.25±136.454mg/l,392.25±136.454mg/l for days 5,10 and 15 respectively. The above values didn't vary significantly ($p>0.05$). The mean electrical conductivity of the tea effluent was significantly different ($p<0.0001$) with a mean of 815.625±95.0978mg/l recorded in day 0 before phycoremediation . The means of *S.salina,* was found to be 272.875±40.444mg/l,212.812±36.234mg/l 212.812±36.234mg/l for days 5,10 and 15 with a statistical significant difference of $p<0.0001$.

phycoremediation of conductivity of sugar effluent were found to vary significantly across all the days ($p<0.0001$) with the conductivity means reducing from 460.5±20.5mg/l,285.5±12.5mg/l,285.5±12.5mg/l for days 5,10 and 15 for *S.salina.* The decline trend in conductivity was summarized in fig vii for coffee, tea and sugar effluents respectively.

The current study was therefore found to be consistent with a previous study done by Khemka and Saraf (2015) who reported that electrical conductivity of dairy waste water medium highly depended on the presence of ions, their concentration, valency and mobility. Yu *et al*.(2005) showed that phycoremediation reduced EC value (dSm-1) from initial phase to stationary phase (18th day) and this he attributed to the ability of algal species to consume the nutrients during the algal growth. The results were comparable with the decrease of EC from 0.55 – 1.45 dSm-1 to 0.27 – 1.36 dSm-1.

The phycoremediation efficacy of nitrate in coffee waste water varied significantly ($p<0.0001$) across all the days. *S. salina,* *r*educed the nitrates from 8.575±1.231mg/l,5.838±0.628mg/l,5.275±0.699mg/l with a significant difference of $p<0.0001$ and a phycoremediation success of 76%.

The phycoremediation of nitrates in tea effluent showed reductions from an initial amount of 4.119±0.5463mg/l, to 2.274±0.2735mg/l, 1.809±0.2213mg/l, 1.809±0.2213mg/ for *S.salina, with* a phycoremediation success of 56%,.However there was significant difference in phycoremediation using the *S.salina* of p=0.0001. In the sugar effluent the phycoremediation efficacy varied significantly across all the days with a statistical significance difference of p=0.002575, and phycoremediation success efficacy of 85%, for *S.salina.* The reduction trend in nitrates in coffee tea and sugar effluents was clearly displayed in figure iii

Phycoremediation of nitrates from industrial effluents by Kshirsagar (2013; 2014) showed nitrate reductions using *C. vulgaris* of 70%, 60%, 93.43% and 89.84 %, for days 0,5,10 and 15 respectively, corresponding to 40% 53.33%, 93.38% and 86.53 % by *C. salina* respectively, which compared well with the current results. Dominic *et al. (*2009) also established that Nitrate content reduction rate was high at 96.23% using *Synechocystis salina* with the values having been reduced from 5.3µmol/l to 0.2µmol/l. Yoshida, *et al*. (2006), Sreesai and Pakpain (2007) showed that the nitrate content of the effluent samples inoculated with *Synechocystis salina* reduced significantly with the time of incubation where he noted that the Initial value of nitrate was 64.0 mg/L and decreased to 14.2 mg/L. With maximum reduction occurring on day 12 and onwards which was recorded at 77.8%.

Phycoremediation efficacy of *S.salina* was determined on the phosphate content of the coffee, tea and sugar effluents, whereby a significant difference in phosphate removal was noted in sugar and tea effluents ($p<0.05$).However phycoremediation of phosphate from the coffee effluent was not statistically significant in days 0,5,10 and 15 ($p>0.05$) using *S.salina,* with a phycoremediation success of 55%.Figure iv showed the trend in phosphate absorption across the three effluents. Similar phycoremediation efficacy studies by Dominic *et al. (*2009) using *Synechocystis salina* showed that phosphates content in effluent samples reduced by 64.52%, while Gonzalez *et al.* (1997) studying on industrial waste water found that the microalgae were able to remove 50% of TP effluent samples. Whereas Weerawattanaphong, (1998) reported a maximum TP reduction of 77%. After 8 days of phycoremediation. In the reduction of BOD through phycoremediation of the coffee, tea and sugar effluents using the *S.salina* the BOD values were shown to reduce from the initial high levels to lower levels though there was no significant variation noted in day 5,10 and 15($p>0.05$). A significant phycoremediation difference was however noted in sugar effluent treated with the *S.salina,* p=0.03

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 *S.salina* in both coffee and tea effluents. However there was a phycoremediation effect of *S.salina* on the COD content of the sugar effluent with a statistical significance difference of p=0.041149.The coffee, tea and sugar BOD and COD phycoremediation trend efficacies were shown in figures v and vi respectively.

Phycoremediation study by Sharma and Khan (2013) showed a progressive reduction in both COD and BOD values and this was found to be consistent with the above current study as also depicted above in graphs i and ii. 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).

pH of all the three effluents upon phycoremediation was reported to increase between day 0 and day 5 and then decrease slightly in day 10 in all the effluents before stabilizing between day 10 and day 15 where no further increase or decrease was recorded. The phycoremediation effect was significant ($p<0.05$) in all the days of remediation, however the effect of *S.salina* on the coffee effluent was not statistically significant ($p>0.05$). From the current phycoremediation findings 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 in most of the algal species used in the treatments. Figure ii showed the pH trend in coffee, tea and sugar effluents respectively. 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). A similar study by Borowitzka, (1998), and Rao *et al*.(2011) 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.

VII. CONCLUSION/RECOMMENDATIONS

The study has provided information that can be used as a basis for follow-up to medical facilities in the study area to find out on the causes of waterborne diseases. The information generated will also be useful in Community sensitization on public health impacts on the use and discharge of the waste water to the nearby ecosystems and also help the factories treat the effluents effectively before discharge.

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