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Role of Bed Surface on the Growth Rate of Algae and Treatability Studies to Control Algae

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Abstract:

Controlling the growth of algae is a major issue, wherever there is adequate light, moisture and simple nutrients sustain. The main objective of the current research was to carry out treatability studies to control the growth of algae using barley, rice and ragi straws and a comparison was made between straws by varying its dosages. This study revealed the fact that decomposing straw is effective in controlling the growth of algae. Barley straw at a dosage of 5 g/L was found to be more effective in controlling the growth of algae with 80% reduction in chlorophyll 'a' concentrations when compare with control tray, at an average temperature 29^0 C, light intensity of 562 µ mol^{-2}s^{-1} photon flux and pH of 7.9. However, barley 2 g/L and rice 5 g/L were also found to control the growth of the algae. ANOVA results express barley 5 g/L was highly significant when compared with the control tray with 99% confidence level. Simulation of Kembalu water treatment plant was done to study the effect of bed surface on the growth of algae. Surface study give an insight about role of surface on the growth rate of algae. In the present study it was observed that growth of Chlorophyace and Euglenophyceae were predominant on concrete surface compared Bacillariophyceae, however ceramic surface promoted the growth of Bacillariophyceae. Higher biomass of Cyanophyceae was found on brick surface.

Keywords: Algae, bed surface, growth rate, inhibition, water quality, growth control

1.0 Introduction:

Increase in human population and modern agricultural practices along with deficient water management have resulted in superficial water bodies as a result phytoplanktonic bloom incidents are turn out to be more frequent and widespread. Presence of algae in the superficial water bodies have direct impact on the water utilities and distribution systems. Algal growth in water treatment plants causes several operational (filter clogging, flow disruption, sedimentation basin operation) and water quality (algal toxins) problems (Dempster, 2006). In addition, presence of algae in water treatment plant has direct impact on the disinfection by products (DBP), total organic carbon (TOC) and water quality (toxins). Water quality of source in terms of nutrients (N&P), dissolved oxygen, silica, carbon dioxide, macro and micro nutrients, pH effects have direct impact on the growth algae (AWWA, 2004). Environmental factors such as sunlight, temperature and water movement also play a crucial role in promoting the growth of algae in rivers and treatment plants.

Controlling the growth of algae in treatment plant is becoming a major issue, design of treatment plant also plays a role in controlling and managing the growth of algae. Design consideration including covered sedimentation tanks and filters, algaecide coating on the walls, application of algaecides like copper sulfate and potassium permanganate helps to control the algae to some extent (Hilal and Hankins, 2004). Operational practices like scrubbing the walls and using strong oxidizing agents like ozone or chlorine dioxide also aid in algae control (Shehat *et al.,* 2002). Some emerging techniques including enhanced coagulation, ozofloatation (dissolved air floatation (DAF) and ultrasonication appears to be effective in controlling algae to some

extent, however these treatment methods are not economically feasible. A lot of research has been going on to control the algae at the source, yet they have met limited success, since some species cause problems during the treatment for example *Synura sp. and Anabaena sp*. release oils during chlorination creating additional taste and odor problems (Vymazal ,1995). Therefore, research on feasible and environmentally acceptable approaches to mitigate and control blooms has important theoretical and practical significance.

Consequently, there is increasing interest on biochemical methods to inhibit the growth of algae, by the decomposition of the straw. Anecdotal evidence of the ability of barley straw to control algal growth was observed as early as 1980 (Welch *et al*.,1990). Newman, 1999 demonstrated the algistatic activity of the barley straw. Gibson *et al.,* 1990 evidenced the growth inhibition of the filamentous algae, the key algal divisions studied were cyanobacteria (*Microcystin aeruginosa)* and diatoms (*Dinobryon sp*). Welch *et al.,*1990; Barrett *et al.,*1996; Martin and Ridge,1999; evidenced the algistatic effect of barley on Chlorophytes (*Cladophora glomerata)* and various desmids. *Chlorella vulgaris, Synedra sp. Scenusdusmus quadricauda* (Ferrier *et al.,*2005) were shown no response to the algistatic activity of algae.The Kembalu water treatment plant is facing huge problem with the nuisance of algae. The plant is made to shut down and frequently cleaned or scarped by applying chlorine. The objectives of the current research is to identify a predominant micro and macro algae causing problem in Kembalu water treatment plant and to compare the effectiveness of barley, rice and ragi straw in controlling the growth of algae. In addition to that influence of bed surface in triggering the growth of algae was studied by simulating treatment plant at lab scale.

2.0 Materials and Methods:

Algal cultures were collected from the Kabini river, Mysuru. Lab scale study was conducted at Sri Jayachamarajendra college of Engineering Mysuru, Karnataka.

2.1 Study Area:

Kembalu water treatment plant is located near Kabini river in Nanjangud. Nanjangud is spread over from $12^0$7'12" N longitude to $76^0$40'48" E latitude. The Intake structure is 1.5 Km away from the treatment plant. The capacity of treatment plant is 60 MLD and consists of cascade aerator, flash mixer, settling tank, tube settlers and rapid sand filter units. Geographic depiction of intake point of Kembalu water treatment plant and Kabini river is given in figure 1.

Fig.1: Geographic depiction of intake point of Kembalu water treatment plant and Kabini river

2.2 Sampling and Analysis of Water and Phytoplanktons

Water and algal samples were collected from the treatment plant and river for the phytoplanktons and water quality analysis. For analysis of planktons samples were collected separately by addition of 4% formaldehyde followed by few drops of Lugol's iodine. Identification and enumeration of algae were done by Lackey's drop count method. Water samples were analyzed as per APHA (20^{th} edition) standard methods for examination of water and wastewater.

2.3 Effect of Surface on the Growth of Algae

Inconsistency in the cell count results of treatment plant units and visual inspection during site visit, clearly indicated that surface has influence on the growth of algae. In treatment plant exuburent growth was seen in tube settler and cascade aerator when compared to the indoor filter beds. No single dominant species were seen in the filter beds whereas thick biomass of *Cladophora* and *Spirogyra* was seen on the steps of aerator. Thus based on field visit an attempt is made to study the role of surface on the growth of algae, by simulating treatment units surfaces at lab scale.

The experiment was carried out in five polythene trays (50*30cm). Each study tray had ceramic, concrete, brick and PVC surfaces to simulate various treatment units of Kembalu treatment plant, and one tray with plane surface referred as control, as presented in figure 2. Experimental design for the surface study is given in table 1.

Table 1: surface study set up

Tray No	Surface	Media
1	Brick	Bold's basic media
2	Ceramic	
3	Concrete	
4	PVC grooves	
5	Control	

Each tray contains 5 L water 13 grams(wet weight) of algae which constitutes 0.2304 mg/m^3 of chlorophyll 'a'. Water was added regularly since there was evaporation losses of water, nutrient supply is done by addition of the Bold's basic media. This experimental set up was monitored for 60 days.

2.4 Treatability studies to control the algae.

The experiments were carried out in seven trays having 50x30 cm^2 area, with working volume of 5 L. Trays were inoculated with 13 grams algae which is equivalent to 0.23048 mg/m^3 of chlorophyll 'a' concentration. Initial setup of the experimental is depicted in figure 2. Water and Bold's basal media was added to each tray for supplementing nutrient requirements for algae. The straws were dried and cut into small pieces and added to trays. The straws tested were barley, ragi and rice at dosage of 2 and 5 g/L, experimental design is given in table 2.

Table2: Experimental design of inhibition study

Treatment trays	Inhibition substance	Amount of straw added
1	Barley	2 g/L
2	Barley	5 g/L
3	Rice	2 g/L
4	Rice	5 g/L
5	Ragi	2 g/L
6	Ragi	5 g/L
7	Control	Nil

Analysis of Variance (ANOVA) was performed to check whether the treatment process is statistically significant or not. ANOVA is performed using XL stat 2015.

Fig.2: Experimental setup of inhibition and surface study at zeroth day

2.5 Analytical Methods

Various parameters analyzed were chlorophyll a, growth rate, identification of and enumeration of algae and chemical oxygen demand (COD).

2.5.1 Cell Density and Identification of Algae

The cells were counted under digital microscopy by Lackey's drop count method and counted cell numbers were expressed as cell density (cells/L). Morphological and structural changes in the algae during the inhibition phase were observed under digital microscope (make/model: ADI LAB TECH/ EU3490). The photographs were obtained under 40*10X magnification.

2.5.2 Chlorophyll "a" Analysis and Growth Rate

The concentration of chlorophyll 'a' pigments is used extensively to estimate phytoplankton biomass. The chlorophyll pigments are extracted by 90% acetone in subdued light and steeped for overnight at 4°C in the dark room or in aluminum foil wrapped container, to avoid degradation and concentrations were estimated by spectrophotometric method (APHA 20th edition, Standard methods for examination of water and wastewater).

$$\text{Chlorophyll a, mg/m}^3 = \frac{\text{Ca* extract volume (L)}}{\text{Volume of the sample (m}^3\text{)}}$$

Where,

Ca is concentrations of chlorophyll *a*

Ca = 11.85(OD664) − 1.54(OD647) − 0.08(OD630)

OD is Optical density.

2.5.3 Growth Rate

The specific growth rate or growth rate (μ) was estimated by the following formula (Ono and Cuello, (2007) and X. Wang *et al.,* 2011).

$$\mu = \ln \frac{E_t/E_0}{\delta_t} = \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{per day}$$

Where, E_t and E_0 are the final and initial chlorophyll a concentrations, respectively, and δt is the cultivation time in day.

2.5.4 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand was determined by the closed reflux- titrimetric method (APHA 2010).

3. Results and Discussion:

The phytoplanktonic analysis showed mixed culture of algae. Among them Bacillariophyceae, Chlorophyceace, Cyanophyceae and Euglinophyceace were seen predominantly. Seasonal variations of phytoplanktons were observed in the stretch of Kabini river as well as in treatment plant. The major species encountered in treatment plant are *Cladophora, Bacillariacceae, Fragilariophyceae, Microcystin, Zygnema, Navicula sp, Cosmerium, Synedra Sp Pleurosigma Sp. and Scendusmus sp*,. Potential filter clogging diatoms *Synedra* and *Fragilaria Sp.* were seen throughout the water treatment plant. In the treatment plant substantial algal biomass was observed in the cascade aerator and tube settler when compared to the indoor filter beds. No single dominant species were seen in the filter beds whereas thick biomass of *Cladophora* and *Spirogyra* was seen on the steps of aerator. Even *Oscillatoria Sp.* were also abundant in the aerator steps. All filamentous green and blue-green algae were accounted more in the aerator and tube settler. Surface plays a vital role in the growth of the algae. In the present study it is observed that growth of Chlorophyace was predominant on concrete surface compared Bacillariophyceae and Cyanophyceae. The effect of bed surface on the growth of Chlorophyace and Euglinophyceace is given in figure 3. However growth of Cyanophyceae was observed to be highest on brick surface due to its filamentous nature. Relatively Euglinophyceace were seen to be more on concrete compared to ceramic, PVC groves and control. The effect of bed surface on the cell density of Cyanophyceae and Bacillariophyceae is given in figure 4.

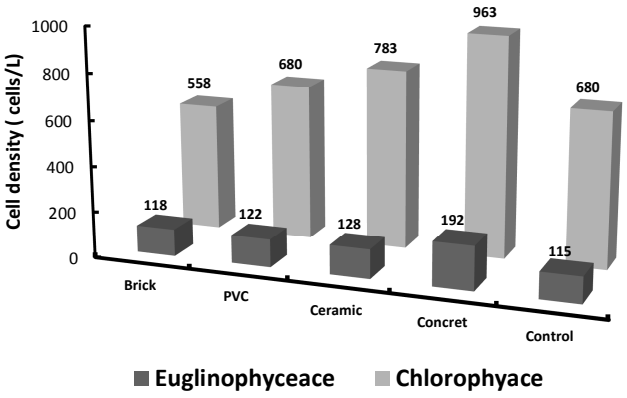


Fig.3: Effect of bed surface on the growth of Chlorophyace and Euglinophyceace

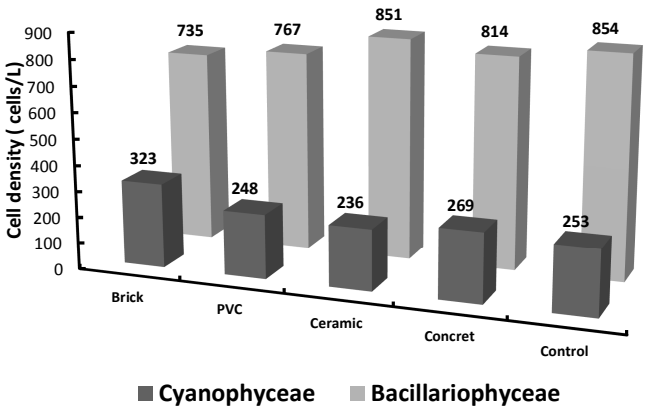


Fig.4: Role of bed surface on the growth of Bacillariophyceae and Cyanophyceae

The concentration of chlorophyll 'a' was also monitored in addition to cell count, to estimate the biomass in the trays. At the end of the experiment it was found that concrete blocks exhibited more growth of algae, as the concentration of chlorophyll 'a' was found to be 10.9912 mg/m^3, highest compared to other trays. The variations of chlorophyll 'a' concentrations during the study period is given in figure 5.

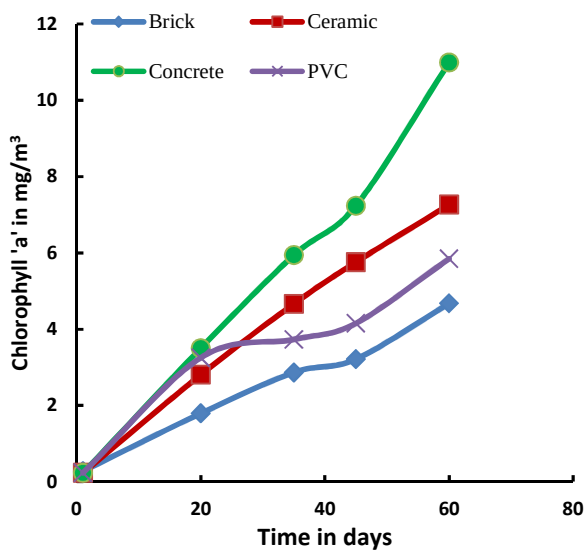


Fig. 5: Role of bed surface on the chlorophyll 'a' concentration

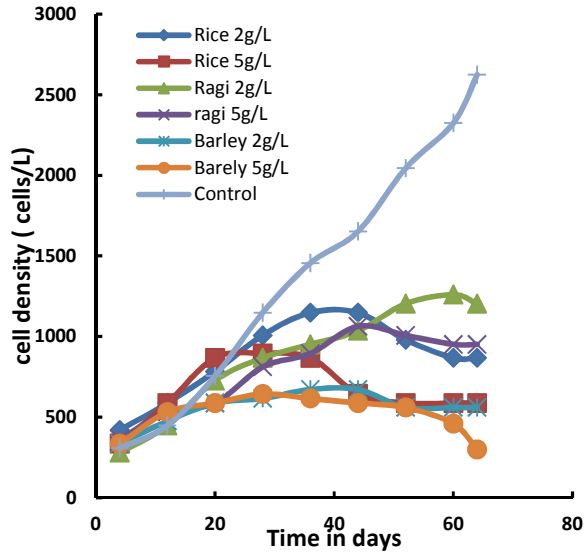


Fig. 6: Algal growth trend before and after straw activation

It is to be stated that, in addition to surface, area of exposure to sunlight also plays significant role as Chlorophyceace were dominant in treatment plant whereas diatoms were more in river water**.** The algistatic activity of the straw is evidenced by the comparing the cell density values of treatment tray and control tray. This effect is mainly due to release of Phenolic compounds and straw contents in the water in presence of sunlight. Addition of straw into the water leads the decomposition of straw in well aerated environment. Decomposition of straw releases humic substance and other hemi cellulosic compounds. When sunlight strikes over humic substances in presence of dissolved oxygen, results in production of hydrogen peroxide. Low levels of hydrogen peroxide have been proven to inhibit algae growth. Peroxides only last a few minutes; it needs the constant flow of fresh hydrogen peroxide into the water in order to keep algae under control. When there is decomposing barley straw in the water, the peroxides are continuously produced (given sufficient sunlight and oxygen) and thereby growth of algae is controlled. This study evidenced the fact that addition of barley straw to the water does not kill algae already present rather it prevents the growth of algae (algistatic). Algistatic activity is achieved when straw is decomposed, on immersion of straw in the water in a well-aerated environment. During initial days of the study period growth was seen in all the treatment trays and average growth rate was 0.12 mg/m^3 in terms of chlorophyll 'a' concentration.

However decline in the growth rate was observed from 30th day and least cell count was recorded by the end of study period. Which clearly indicates that straw activation is achieved by the end of 30th day of straw addition, at an average temperature 29^0 C, at an light intensity of 562 µ $mol^{-2}s^{-1}$ photon flux. The figure 6 shows the algal growth trend before and after straw activation. Nutrient deficiency may not be a reason for the suppressing the growth of algae in treatment trays, since Bold's basic media was added to support nutrient values. The barley dosage of 5 g/L is being more effective algistatic agent when compared to the other treatment straws. Rice 5 g/L is also showed effectiveness in controlling the growth of algae for some extent, where as ragi straw was not so effective in inhibiting the growth. The figure 7 and 8 illustrate the effect of inhibition substance on the growth of algae.

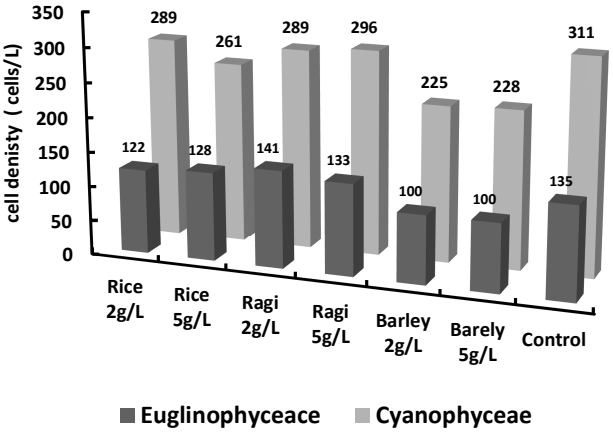


Fig.7: Effect of straw on the growth of Euglinophyceace and Cyanophyceae

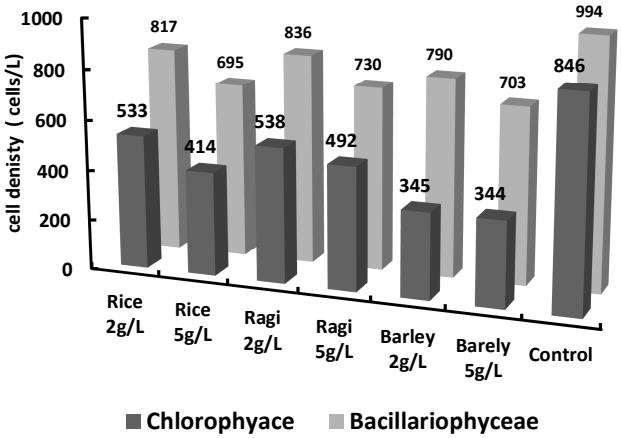


Fig.8: Effect of straw on the growth of Chlorophyace and Bacillariophyceae

However it was evidenced that same species were not affected by the decomposition of the straw. Even after 12 weeks of study period there were few diatom species present such as *Navicula* and *Nistzchia,* however size shrinkage was seen in *Nistzchia Sp.* Cell shrinkage might be the reason for the survival of *Nistzchia Sp,*. Few cells *of Cosmerium* were also seen in all the treatment trays, however their biomass was less in concentration. Control tray was dominated by the Chlorophyace (*Scenusdusmus*, *Zygnema, Spirogyra* and *Cladophora*), Cyanobacteria (*Microcystin* and *Oscillatoria*) and Diatoms (*Fragilariophyceae, Synedra and Navicula)* whereas Euglenophyceae were relatively less in concentration.

Fig.9: After 20days of the inoculation (before activation of the straw)

Fig.10: After 60 days of the inoculation (after activation of the straw)

Figure 9 and 10 gives the visual changes in the experimental trays after and before activation of the

straw. Microscopic examination disclosed that *Cladophora* species were dead after the 12th week of the experiment. No further experiment was done to understand the status of cladophora. The spiral coils of *Spirogyra sp*., were separating and resulting in the death of the *Spirogyra.*The concentration of chlorophyll pigments is used extensively to estimate phytoplankton biomass. Initial concentration of 0.2304 mg/m^3 was inculcated in all the trays, initially there is not much growth is seen in the trays since cells were in lag phase. After 20 days of the inoculation the concentration of the chlorophyll a was comparable in all the trays, barley 5 g/L has 2.9047 mg/m^3 and control tray has 3.34859 mg/m^3 of concentration. Figure 11 illustrate the effect of inhibiting substances on the growth of algae, in terms of chlorophyll 'a' concentration.After the 30 days variations in the concentration is seen. At the end of the treatment barley 5 g/L showed less amount of chlorophyll 'a' when compare to other trays. barley 2 g/L was also successful in reducing the chlorophyll content in the trays, whereas ragi 2 g/L dosage was not enough to control the growth of the algae.

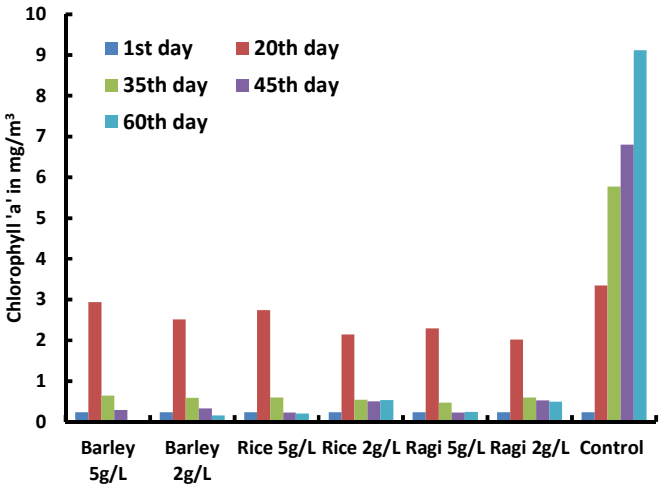


Fig.11: Chlorophyll a concentrations in treatment trays from initial days to 60 days

Analysis of variance (ANOVA) is performed to check whether the inhibition process is statistically significant or not. The F value is the ratio of the mean regression sum of squares divided by the mean error sum of squares, which is given in the Table 3.

Table 3: ANOVA summary

standard weighted-means analysis					
ANOVA Summary		**Correlated samples k=5**			
Sources	Ss	Df	Ms	f	P
Treatment	91.865	4	22.9664	9.52	0.0010
Error	28.9622	12	2.4135		
Ss/Bl	4.5673	3			
Total	125.39	19			

Its value will range from zero to an arbitrarily large number. By rule of thumb, an F-value >4.00 is usually statistically significant. For the current study F value was found to be 9.52, which indicates the treatment process is statistically significant when compared to the control trays. The F-value for each population indicates that the inhibition process is significant. barley 5 g/L was highly significant when compared with the control tray with 99% confidence level. However rice 5 g/L and barley 2 g/L were shown a significance level of 0.01, where as ragi straw was found to be not significant in controlling the growth of algae A p-value helps you determine the significance of your results. The p-value is a number between 0 and 1. A small p-value (typically ≤0.05) indicates strong evidence against the null hypothesis, indicating there is significant difference between populations. Null hypothesis indicates Chlorophyll 'a' in control trays is same as Chlorophyll 'a' in treatment trays. If the p value is less than 0.05, treatment is said to be statistically significant so we reject the null hypothesis. A large p-value (>0.05) indicates weak evidence against the null hypothesis, indicating there is no much variation between treatment and control tray, so you fail to reject the null hypothesis.

Table 4: ANOVA results

Treatment trays	p value	Significance
Barley 5g/L	p<0.01	**highly significant
Barley 2g/L	p<0.05	* significant
Rice 5g/L	p<0.05	* significant
Ragi 5g/L	p>0.05	Insignificant

COD is a measure of organic content in the water. COD exertion due to straw decomposition is illustrated in figure 12 and 13. The COD trend shown for treatment trays is only due to straw content since COD due to algal count is corrected. The COD values in the treatment trays and control tray were comparable in the initial days. After 20th days rise in COD values were accounted for the treatment trays, which might be due to release of straw components

adding up organic load . Algal cells were the contributors of the organic content in the Control tray. After 35 days COD values tends to be stagnant in the treatment trays and at the end of the 45th day COD values starts declining in the treatment trays, which might be due to death of the algae or the denitrification process in trays.

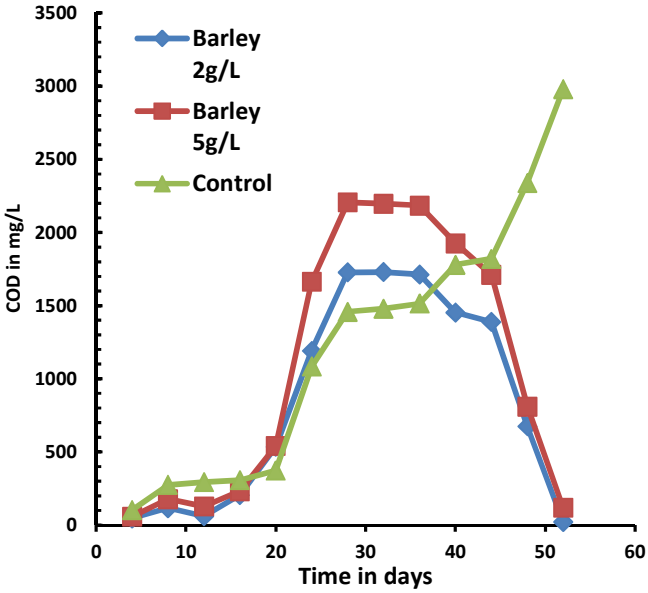


Fig.12: Effect of straw decomposition on the COD at 30^0c in Barley trays

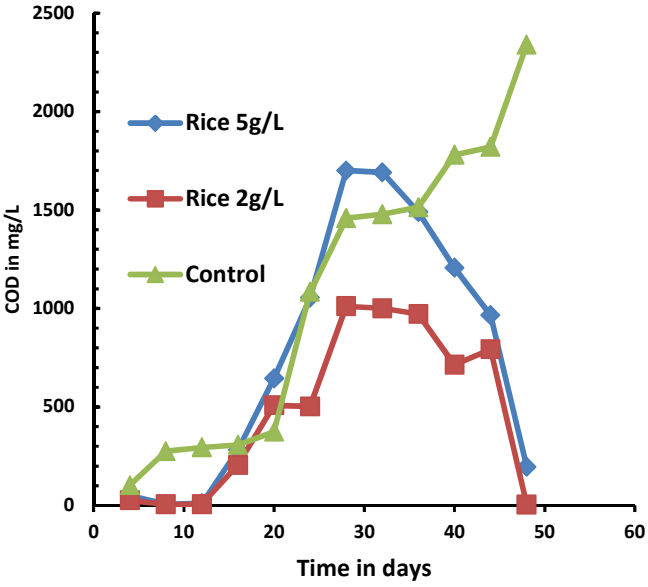
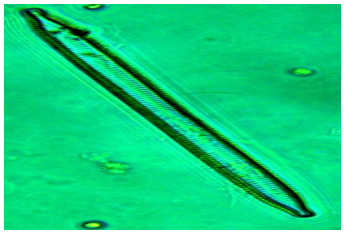


Fig.13: Effect of straw decomposition on the COD at 30^0c in rice trays

The process of denitrification needs readily available external carbon sources as electron acceptors. The organics in the water were likely used to meet the needs of denitrification, resulting in a reduction in COD. However trend for COD kept on increasing continuously throughout the study period which clearly indicates the growth of algal cells in Control trays

4.0 Conclusion:

Surface study gives an insight about effect of bed surface on the growth of algae. In the present study it is observed that growth of Chlorophyceae was predominant on concrete surface followed by Cyanophyceae and Bacillariophyceae. The filamentous algae such as *Cladophora, Spirogyra, Zygnema, Oscillatoria* and other *Cyanobacteria* are found more on the concrete and brick, they adhere to the rough surface and grow like pelt. However growth of Cyanophyceae was observed to be high on bricks, this is due to filamentous nature and surface roughness of the brick. Relatively Euglinophyceace were seen to be more on concrete compared to ceramic, PVC groves and control tray. In conclusion it can be stated as in addition to structure of surface, area of exposure (to sunlight) also plays significant role. It could be concluded from this study that the growth of algae can be controlled by the straws this inhibition activity is due to synergistic effects of various Phenolic compounds and straw content released in the water in presence of sunlight. In the current study it was found that barley straws and rice straws are the best solutions to mitigate algal growth. Barley straw at a dosage of 5g/L was found to be more effective in controlling the growth of algae with 80% reduction in chlorophyll 'a' concentrations when compare to control, at an average temperature 29^0 C, light intensity of 562 µ $mol^{-2}s^{-1}$ photon flux and pH of 7.9. However, barley 2 g/L and rice 5 g/L were also found to control the growth of the algae. ANOVA results express barley 5 g/L was highly significant when compared with the control tray with 99% confidence level. Use of straws to control the growth of algae represents an inexpensive, effective and environmentally acceptable method. However, further research is needed on the effect of straw content on the natural ecosystem of the aquatic body and fate and transport of the straw content.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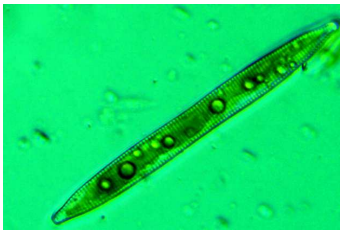
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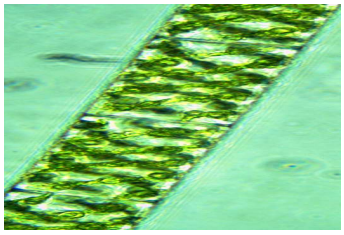
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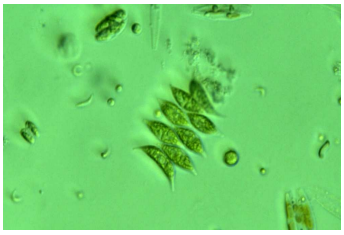
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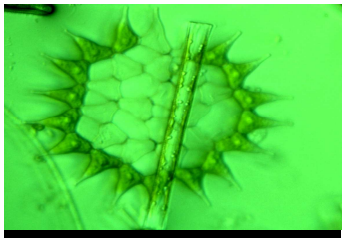
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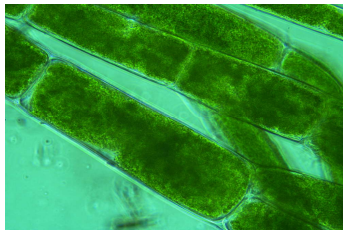
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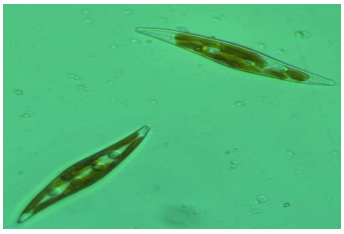
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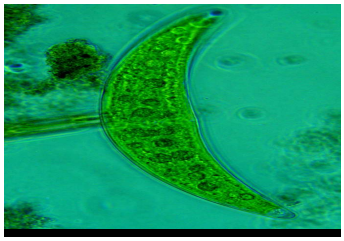
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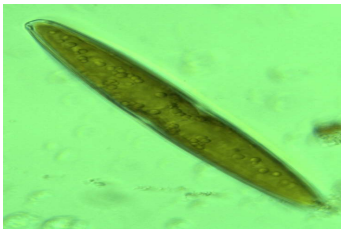
I


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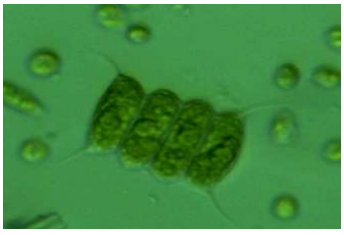
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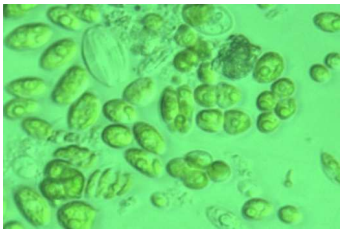
L


M


N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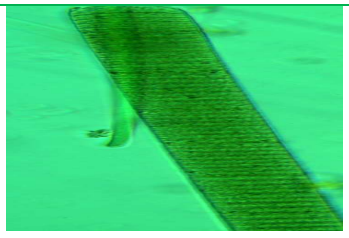
P

ANNEXURE 1: Microscopic photographs of algae sample, **A:** *Bacillariaceae* (x 40), **B:** *Nistzchia* sp. (x 40), **C:** *spirogyra sp.* (x 40), **D:** *Scenedesmus dimorphus* (x 40), **E:** *Pediastrum* simplex (X 40), **F:** *Cladophora* sp. (x 40), **G:** *Pleurosigmataceae* sp (x 100), **H:** *Closterium sp.* (x 40), **I:** *gyrosigmataceae sp* (x 40), **J:** *Scenedesmus bijugatus* (x 40), **K:** *Scenedesmus quadricauda* (x 40), **L:** *Synedra sp.* (x40), **M:** *Cosmerium* (x 40), **N:** *Cymbellales* (x 40), **O:** *Chlorococcales* (x 40), **P:** *Oscillatoria* (x 40).

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