EFFECT OF WASTE WATERS ON ALGAL GROWTH
INTRODUCTION:

Algae are among the oldest and toughest life forms on earth. Under natural conditions, they grow as mixed communities in natural water bodies. To study the individual alga, it must be isolated to make it free from rest and cultured as unialgal culture. During the last two decades considerable attention has been devoted to the study of these organisms for obvious basic and applied reasons. These organisms provide a simple and model system for the study of cellular processes at molecular and functional level. Their important ecological and environmental role and their part in biological nitrogen fixation (by Blue-green algae) in agricultural ecosystems are increasing attractions of these organisms to the applied biologist. Algal cultures have been used in various morphological, physiological biochemical and genetic researches.

Famintzin (1871, in Venkataraman 1969) was the first person to report the growth of algae in water culture. Since then several physiologists continued to work on this aspect. To mention few of them are Bold (1942), Singh (1942), Pringsheim (1946 a and b), Venktraman and Saxena (1963) and Olsen (1989). It was during world war II that interest in mass cultivation of micro-algae attracted attention of scientist as a source of proteins and feeds. Many other potential applications for large scale algal cultures have advanced in recent years. To mention some are waste water treatment, aqua-culture as a source of commercially important products and bioconversion of solar energy.

With the advancement of civilisation and subsequent indutrilization the problem of water pollution has been assuming greater dimensions. Eutrophication in generally taken to mean the enrichment of water with all forms of life. The luxuriant growth of algal forms in eutrophic waters of streams, lakes, rivers and ponds is common phenomenon and the role of algae
in the removal of various kinds of inorganic and related substances by their metabolic activities has been studied by several workers during last decade (Nair et al. 1981 Sengar et al. 1985 and Sengar and Sharma, 1987).

In recent years, bioassays have been used to measure the magnitude of pollution using living organisms like fishes, protozoan and algae. These organisms serve as parameters or yardsticks for evaluating the acute toxicity of a micro-chemical pollutants. The main sources of these pollutants are sewage and industrial effluents. Since most aquatic algae grow in a wide variety of polluted and unpolluted waters one can hope that proper knowledge of their pollution biology may provide a useful solution for this problem. Thus algal organisms are gaining much importance recently for testing the water bodies (Palmer, 1969, 1980; Venkatraman, 1969; Kumar et al. 1974; Olaniya et al. 1976 and Schoeman, 1976)

Algal mass culture research for waste water treatment was initiated by Oswald (cf, Venktraman, 1969). Since then a number of studies have been made. Some important works have been reviewed here. Srinivasan (1960,1967) isolated a number of algae from distillery wastes and found that Chlorella, Hydrodictyon, Scenedesmus, and Mastigocladus would grow in presence of distillery wastes. He also developed the technique for mass culture of Chlorella and Hydrodictyon to use in laboratory and pilot plant studies. The BOD reduction in the wastes in presence of algae has been reported. He also tried for growing Scenedesmus spp. in straw board waste which proved to be helpful in the reduction of colour and BOD of the effluent.

The use of algae for the treatment of Synthetic Drug Waste (SDW) has been studied by Khan et al., (1962 a, b), who conducted the experiment on Chlorella and Scenedesmus and observed that the algae can withstand the SDW up to a period of 1 1/4 to 2 months when diluted to a ratio of 1:25 and
1:50 with freshwater and sewage respectively. He also observed the reduction of BOD up to 88.93%.

The biological effects of various industrial wastes on the growth of algae has been studied by several workers like, Rana et al. (1971), Rana and Kumar (1974) and Rana (1977). They have suggested the possible use of these algae in the assessment of pollution. This was followed by Rai and Kumar (1976) who studied nutrient uptake by *Chlorella vulgaris* and *Anacystis nidulans* isolated from effluent of a fertiliser factory and found that, *Chlorella* absorbs more of PO$_4$ from the medium than *Anasystis* and nitrate promoted growth at all concentrations tested.

Nair *et al.* (1981) studied the removal of nutrients from sewage effluents using *Selenastrum obliquus* and observed the luxuriant growth of this alga in the secondary treated domestic sewage. They also observed that the outdoor mass culture of this algae removes nitrogen and phosphorus from the sewage.

Reddy and Chandrashekhar Rao (1982) have made the studies on toxicity of industrial effluents to physiological responses of algae. They observed that acid phosphatase and amylase were completely inhibited in *Scenedesmus incrassatulus* at 10% effluent while other metabolic products were reduced. Reddy *et al.* (1983) have observed the toxicity of oil refinery effluent on physiological responses of algae and found that the growth decreased with the increasing concentrations of effluent in *Scenedesmus incrassatulus* and *Synechococcus aeruginosus* but *S. aeruginosus* showed a higher tolerance. This was followed by the work of Choudhary *et al.* (1987) who tried to grow *Spirulina platensis* in diluted (either with distilled water or with raw sewage) night Digester effluent and observed the good growth also.
Umamaheshwar Rao et al. (1984, 1985) and Umamaheshwar Rao and Mohanchand (1990) have made an extensive work on the growth of different marine algae e.g. *Gracilaria* sps., *Skeletonema costatum* and other marine diatoms in the zinc smelter effluent at different concentrations. They observed the marked retardation of growth of these algae at 20% and 25% levels of the effluent and the concentrations higher than 25% were found to be lethal in case of *Gracilaria*. Whereas 5% level in the case of *Skeletonema costatum* and 0.01 to 0.5% concentrations for the other marine diatoms respectively.

Further, Mohanchand and Umamaheswar Rao (1992) studied the response of diatoms to Alum factory waste. They observed that even though the growth of diatoms was exhibited at 0.1% it could tolerate 4-7.5% of alum factory effluent. They also studied the effect of sewage on these diatoms (Umamaheshwar Rao and Mohanchand Rao 1990, Sarojini and Umamashwar Rao 1992) and also on Blue-green algae, *Oscillatoria nigroviridis* (Umamashwar Rao and Premila 1991) and concluded that the growth of diatoms was inhibited by 1% sewage and the favourable growth of *O. nigroviridis* in low saline and sewage polluted habitats respectively.

The treatment of Dairy animal waste with filamentous algae *Lyngbya* and *Tolypothrix* was studied by Hosetti and Patil (1986 and 1989). They observed the concomitant increase of pH and dissolved oxygen, and reduction of BOD and other materials in the medium. During 1986 and 1987 Hosetti et al., used two green algal species e.g. *Chlorella vulgaris* and *Scenedesmus quadricauda* in the treatment of pulp and polyfibre waste water and pharmaceutical waste water. They found good growth of these species in 25% of both the effluent with sewage. These algae could reduce the BOD and nutrient levels and increase the dissolved oxygen. They also studied the toxicity of zinc and cobalt chloride of sewage on *Scenedesmus quadricauda* (Hosetti et al. 1990, 1993) and concluded that the higher levels of these compounds are toxic for the growth.
Ahluwalia et al. (1989) studied the physico-chemical characteristic and effect of some industrial effluents on the growth of a green alga *Scenedesmus* sps. and observed that, the final effluent of ghee factory was neutral and supported algal growth with all the concentrations employed. The effluent from electroplating plant, steel, automobile and fertiliser factories were inhibitory at relatively higher concentrations but lower concentrations of some effluents supported the algal growth.

Lakshmi et al. (1987) made an attempt to grow the BGA *Spirulina platensis* in large scale in the tennary soak liquor. They observed a good growth of the alga when grown in the effluent combined with raw sewage. Mohan et al. (1989) studied the growth of *Anabaena* in the same effluent, at different levels. They found that the alga could grow in effluent concentrations up to 20 ppm beyond which it was lethal.

An assessment of the pollution effect of chromium factory waste on the growth of *Spirulina platensis* has been made by Pal and Chatterjee (1989) who found that the growth was hampered at all the concentrations. The toxicity of the waste, however, decreased when 15% cow dung extract was added.

Sengar et al. (1990) tested the capacity of algae to reduce the pollution load in river water by the cultural experiments for DO, BOD, NH₃-N, Organic-N, NO₃-N, NO₂-N, Hardness, Ca, Mg, K, Fe, Zn and Cu. The maximum increase in DO and complete removal of NO₃-N, NO₂-N, Fe, Zn and Cu was achieved by mixed algal growth.

Saxena and Jabeen (1990) studied the growth and behaviour of *Microcystis aeruginosa* and *Chlorella vulgaris* in distillery effluent and found that maximum growth of *M. aeruginosa* was in 2.5 %, while that of *C. vulgaris* was in 3.0 % effluent concentrations.
The role of * Ankistrodesmus falcatus* and *Scenedesmus quadricauda* in purifying the sewage water has been studied by Patil (1991). He observed that these species either individually or in combination were reducing BOD and nutrient level.

Recently, Manoharan and Subramanium (1992, 1995 and 1996) studied the biochemical aspects of Blue-green alga, *Oscillatoria pseudogeminata* var. *unigranata* Biwas with sewage and effluent. They observed that except carbohydrate and free amino acids other biochemical components such as proteins, lipids etc. show a significant reduction in their content.

The use of micro organisms for the treatment of pulp waste has been suggested in the past. The pulp mill wastes have been proved to contain various lignin degradation products. Paper and news print is one of several industries which have been notified by the Government of India as being highly polluting. Nearly 2.0 to 2.5 mg of raw material is required per mg of paper. However, only 41.8% is recovered as bleached pulp; the remainder is discharged as untreated waste water (Sudhakar et al. 1991).

Generally pulp of paper mill industries release huge amounts of lignin bearing waste water into the ecosystem. They impart brown colours to the receiving water and results in depletion of oxygen and emission of foul odours. Several constituents of pulp and paper industry waste water are known to be toxic to fish and other aquatic organisms (Buswell (1991) in Prasad and Gupta, 1997). Algae are very suitable organisms for the determination of the impact of toxic substances on the aquatic environment. In India many works have been published on the characteristic features of the paper mill waste waters collected inside the factory and combines waste waters let out from the factory.

Discharge of untreated animal and dairy waste directly on land or to natural water bodies creates pollution problems such as ponding, which leads to odour and sickness of soils (Mohanrao and Subramanym, 1972). The dairy waste which is rich in organic and inorganic compounds serve nutrients for the growth of microbes in waste waters. A number of microbes mainly algae are known to be important in the removal of nutrients from animal wastes (Mc Griff and Mc Kinney, 1972).

The dairy waste would be normally white coloured with highly foul smell. This waste water appears to be oily in nature, may be because of the more fat and oil content in it. Datar and Kale (1995) have observed very high amount of volatile solids, oil and grease etc., in the dairy effluent. Hosetti and Patil (1986), Hosetti et al. (1986) and Mohanrao and Subramanym (1972) have worked on reclamation of dairy animal waste by biological methods.

From the available literature it is quite evident that number of investigations have been carried out on the experimental pollution studies. Thus possibility of using unialgal cultures of *Pediastrum simplex* Meyen and *Cosmarium laeve* Rabenh. as means of nutrient removal from dairy waste, paper mill effluent and domestic waste was thought to be interesting. Thus present work has been carried out by using three waste waters such as Paper Mill effluent, Dairy effluent and Sewage water. The effect of these 3 effluents on the growth of two algae viz. *Pediastrum simplex* Meyen of Chlorococcales and *Cosmarium laeve* Rabenh. of Desmidials has been studied.
MATERIALS AND METHODS:

For the culture studies, the methods recommended by Venkatraman (1969) were followed. The general requirements taken for present study are as follows.

1) **Glasswares:** All the glassware used in the experiments were made of Borosil. Erlenmayers flasks of 250 ml capacity, plugged with non absorbent cotton were used.

2) **Cleaning of the glasswares:** To prevent chemicals as well as biological contamination of media during experiments, the glassware were cleanly washed with concentrated H₂SO₄ solution. The typical washing procedure followed is as follows: Glasswares were cleaned using detergent and thoroughly rinsed with tap water. This was followed by rinsing with concentrated H₂SO₄ acid. After repeated washing in tap water the final rinsing was done with glass-distilled water.

3) **Sterilisation:** Sterilisation of culture vessels, media and cotton plugs was accomplished by autoclaving at a pressure of 1.1 Kg/cm² for 10 minutes.

4) **Inoculation:** All the inoculations were carried out in an inoculation chamber which was cleaned with alcohol and sterilised by U.V light for 1/2 an hour, prior to every inoculation.

For culturing of alga the *Cosmarium laeve* Rabenh. of desmidials was collected from the moist wall of cement water in the garden of P. G. Dept. of Botany during June 1995. *Pediastrum simplex* Meyen of Chlorococcales (Chlorophyceae) was collected from a pond located near Dharwad. Both species were isolated following the methods recommended by Venkatraman (1969). Since the Chu 10 medium (modified by Chu,1942) has wide
applications, the culturing of these algae was tried in this medium first. The water used for the preparation of media was all glass distilled water. The chemical used were of 'Qualigens ' AR grade. Although the growth was observed in normal Chu 10 medium, it was comparatively more in the following modified medium.

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\begin{align*}
\text{Ca(NO}_3\text{)}_2 & \quad 0.04 \times 2 \text{ gms/l} \\
\text{K}_2\text{HPO}_4 & \quad 0.01 \times 2 \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & \quad 0.025 \times 2 \\
\text{Na}_2\text{CO}_3 & \quad 0.02 \times 2 \\
\text{Na}_2\text{SiO}_3 & \quad 0.025 \times 2 \\
\text{FeCl}_3 & \quad 0.008 \times 2
\end{align*}
\]

and As micro-nutrient solution 2 ml/lit.


\[
\begin{align*}
\text{H}_3\text{BO}_3 & \quad 2.9 \text{ gms/lit.} \\
\text{MnCl}_2 \cdot 4\text{H}_2\text{O} & \quad 1.81 \\
\text{ZnCl}_2 & \quad 0.11 \\
\text{CuSO}_4 \cdot 5\text{H}_2\text{O} & \quad 0.08 \\
3 (\text{NH}_4\text{O}) \cdot 7\text{MoO}_3 \cdot 4\text{H}_2\text{O} & \quad 0.018
\end{align*}
\]

Bacteria free cultures were obtained by repeated sub culturing after U. V. treatment for 30 sec (Kaushik -1987). For the maintenance of algal cultures natural light was given by keeping the glassware on the wooden planks of glass-doored window in the laboratory. These culture stalks were maintained throughout the study period. For the Analysis of effluents as well as sewage the methods prescribed by APHA (1980) and NEERI (1988) were followed.
For the experiments paper mill effluent was obtained from the West Coast Paper Mill Dandelli, situated on western side about 50km away from Dharwad and the dairy effluent was collected from the K.M.F. (Karnataka Milk Federation factory), situated 6 km away from the Dharwad city. Sewage water was collected from the sewage pond of the Karnataka University Campus, Dharwad. Analysis of two effluents and sewage water were made as per the methods prescribed by Anonymous (1980) and Anonymous (1988). To study the effect of these effluents on the above said algae, different combination of the effluents were prepared by diluting with bore well water and the synthetic medium of known concentrations. Dilution in the following proportions were made in order to get different ratios.

| Sterile effluent : Culture Medium Percentage dilution |
|-----------------|-----------------|-----------------|
| 100 : 00        | 100%            |
| 80 : 20         | 80%             |
| 60 : 40         | 60%             |
| 40 : 60         | 40%             |
| 20 : 80         | 20%             |
| 00 : 100        | 0%              |

| Sterile effluent : Sterile Bore well water Percentage dilution |
|-----------------|-----------------|-----------------|
| 100 : 00        | 100%            |
| 80 : 20         | 80%             |
| 60 : 40         | 60%             |
| 40 : 60         | 40%             |
| 20 : 80         | 20%             |
| 00 : 100        | 0%              |
For sewage water also similar dilutions were made in order to get different ratios. Triplicates were maintained for each combinations. For this, 2 ml of inoculum (exponentially growing) was added for each flask and allowed them to grow. The harvesting was made at an interval of 5 days for C. laeve and 3 days for P. simplex. For the harvesting 5 ml of medium with material was drawn aseptically to measure the dry weight.
RESULTS AND DISCUSSION:

EFFECT OF PAPER MILL EFFLUENT ON THE GROWTH OF ALGAE:

Chemical factors of the different waste waters and the bore well water have been tabulated in the Table 37. In general paper mill effluent was alkaline having very high values of EC, carbon-di-oxide, sodium and nitrate. The dissolved oxygen value was comparatively lower than bore well water. Hardness value was very low.

EFFECT OF PAPER MILL EFFLUENT ON THE GROWTH OF Cosmarium laeve Rabenh.:

Initially to see the toxic effect of paper mill effluent on the growth of C. laeve an experiment was conducted by selecting the different combinations of sterile effluent and sterile growth medium. From the Fig 1 it is clear that the effluent is slightly inhibitory to the growth of the alga because the growth was generally more in 100% sterile medium (control i.e. in 0% sterile effluent) throughout the experiment and with the addition of the effluent the growth was retarded. Initially on the 5th day growth of the alga was very low in all the combinations of sterile effluent and sterile medium. However, on 10th day growth was more in 40% sterile effluent. Similarly in subsequent days i.e. on 15th, 20th, and 25th day, more growth of the alga in 40% sterile effluent indicates the suitability of this concentration for the growth of the alga. This observation is supported by the study made by Hosetti et al. (1987), who estimated the growth and biochemical activities of Chlorella vulgaris, Scenedesmus quadricauda and E. coli in 25% dilution of effluent from pulp and poly-fibre industry with domestic sewage (1:3). However, lower growth of alga in 60%, 80% and 100% sterile effluent indicate that toxic chemicals along
with dark lignin colour might have retarded the growth in higher concentrations. On the other hand, dilution of these chemicals and dark colour with the addition of growth medium, resulting in higher growth of the alga, is indicating the suitability of paper mill effluent for the growth of the alga.

Thus the alga did grow with the addition of effluent to the medium, which indicates that, paper mill effluent is not inhibitory and this is further confirmed by its growth in all other dilutions, though less than pure medium.

In view of this the second experiment was conducted with the sterile effluent and sterile bore well water dilutions (Fig.2). In this experiment growth could be seen in 40% dilution of sterile effluent with sterile bore well water confirming the results of first experiment. Fig. 2 reveals that initially on 5th day growth of the alga was equally higher in 40% and 60% sterile effluent. However on 10th day onwards growth of the alga was more in 40% sterile effluent and reached the peak on 20th day. Growth decreased on 25th day. From the Fig. 2 it is also clear that growth fluctuation in 20% and 60% sterile effluent is also similar to 40% sterile effluent but growth was comparatively lower than 40% sterile effluent. However, in 80% and 100% sterile effluents growth of the alga decreased on 20th day itself, indicating the unsuitability of the effluent as the concentration increases. Retarded growth in 80% and 100% effluent can be attributed to the higher concentration of chemical and dark lignin colour which might have retarded the growth. On the other hand in 60% and 40% and 20% sterile effluent more growth of alga was probably because of the decreased lignin colour and concentration of chemicals due to dilution by bore well water.

To see the effect of raw effluent on the growth of the C. laeue third experiment was conducted with various combinations of raw effluent and raw bore well water (Fig 3). The results are almost confirming the above findings.
The growth was minimum in 100% raw effluent and it increased with the dilutions. Initially growth of the alga on 5th day was almost uniform in all the combinations. However, from 10th day onwards growth comparatively increased in 40% raw effluent, reached highest on 20th day and it decreased on 25th day. Growth pattern in 20% and 60% raw effluent was similar to 40% raw effluent, but was comparatively low. On the other hand in 80% and 100% raw effluent, growth declined from 15th day only, indicating the influence of higher concentration of raw effluent on the growth of alga. This observation suggests that 40% dilution of raw effluent with raw bore well water is optimum for the growth of the C. laeve. Comparatively lower growth in 80% and 100% raw effluent is an indication that the chemicals in effluents are inhibitory and dark lignin colour of the paper mill effluent might have retarded the growth of the C. laeve. However, more growth in 40% raw effluent indicates that paper mill effluent certainly contains some nutrients favouring the growth of the C. laeve.

Thus from these experiments it can be concluded that C. laeve can grow in the paper mill effluent. However, the effluent is not that favourable for the growth, which is indicated by its more growth in the pure medium (Fig 1). It is quite possible that, though certain nutrients are favouring the growth of the C. laeve, dark colour of the effluent hinders the growth in 100% and 80% effluent. Various dilution experiments, with sterile and raw bore well water, confirms this view as the growth increased with the dilution. The alga can be used for treating paper mill effluent but with dilutions. It is also concluded that paper mill effluent is not toxic to the alga.
Effect of Paper Mill Effluent on the growth of *Cosmarium laeve*
Combinations: Sterile Effluent + Sterile Medium

![Graph showing the effect of Paper Mill Effluent on the growth of *Cosmarium laeve* over different percentages and time periods.](image-url)
Effect of Paper Mill effluent on the growth of *C. laeve*

Combinations: Sterile effluent + Sterile Borewell Water

Figure 2
Effect of Paper Mill effluent on the growth of *C. laeve*
Combinations: Raw Effluent + Raw Borewell Water
EFFECT OF PAPER MILL EFFLUENT ON THE GROWTH OF *Pediastrum simplex* Meyen:

Similar to *C. laeve* different experiments were conducted for *P. simplex* also to see the effect of paper mill effluent on its growth. Fig. 4 reveals the growth pattern of *P. simplex* in various dilutions of sterile effluent with sterile growth medium. Form Fig.4 it is clear that growth of the alga was highest in 100% medium (0% dilution) only than in various combinations of sterile effluent indicating the inhibitory nature of the paper mill effluent on the growth of the alga. However growth in various dilutions, even though it was lower than that in 100% medium, indicates that paper mill effluent is not lethal to *P. simplex*. Initially on 5th day alga slowly adjusted to the sterile effluent. Thereafter on 10th day onwards growth increased in relatively lower concentration of the sterile effluent. The higher concentration of sterile effluent (80%-100%) reduced the algal growth significantly (Fig 4). Thus, this indicates that paper mill effluent supports the growth of the alga at lower concentrations. Therefore, on 10th day onwards growth of the alga increased in all the dilutions of the sterile effluents. Of these, more growth was observed in 20% sterile effluent throughout the experiments. Fig 4 also depicts that, as the concentration of the sterile effluent decreased, growth of the alga increased.

Second experiment of *P. simplex* with the combinations of sterile effluent and sterile bore well water (Fig 5) is almost confirming the above findings. In this experiments growth of the alga was more in 20% and 40% sterile effluent for almost throughout the experiment period. Initially on 5th day itself growth was higher in these combinations. In subsequent days growth in these combinations increased over control, recorded peak on 15th day and decreased from 20th day onwards. Comparatively decreased growth in 60%, 80% and 100% sterile effluent clearly indicates that higher concentrations of the effluents retards the growth of the alga.
To see the effect of raw effluent on the growth of the alga *P. simplex* was grown in various combinations of raw effluent and raw bore well water. Fig 6 shows the growth pattern of *P. simplex* in various combinations of raw effluent and raw bore well water.

Growth of the alga was similar to that of earlier experiment with that of sterile effluent. The alga could grow in pure bore well water but the enhancement of growth was in the various effluent combinations. Of all the combinations 20% and 40% effluents proved to be more beneficial as the growth was significantly higher in these dilutions than the remaining. Growth, however, retarded after 15th day of the experiment and it is confirmed that higher dose of effluent is inhibitory to the growth of the alga.

Thus, it is also clear here that it may be the dark colour of the effluent, which might have hindered the growth in its higher concentration. On the other hand dilution of the effluent and thus reducing the intensity of colour with raw bore well water as in 20% and 40% raw effluent, growth of the alga was much better than that in higher concentrations. This observation is supported by the observations made by Hosetti et al. (1987) who estimated the growth and biochemical activities of *Chlorella vulgaris* and *Scenedesmus guadricauda* and *E. coli* in 25% dilution of effluents from pulp and poly-fibre industry with domestic sewage (1:3).

Thus from these experiments of *C. laeve* and *P. simplex*, it can be concluded that paper mill effluent is not inhibitory to both these organisms. It is clear here that paper mill effluent contains some nutrients that are favouring the growth of these algae. Of these two algae studied, *C. laeve* appears to be better organism for treating the paper mill effluent. Since *C. laeve* showed better growth in both sterile and raw effluents in 40% (Fig. 2 &
Effect of Paper Mill Effluent on the growth of Pediastrum simplex
Combinations: Sterile effluent + Sterile Medium

Figure 4
Effect of Paper Mill effluent on the growth of *P. simplex*
Combinations: Sterile effluent + Sterile Borewell Water
Effect of Paper Mill Effluent on the growth of *P. simplex*  
Combinations: Raw Effluent + Raw Borewell Water

![Bar chart showing the effect of different mixtures of raw effluent and borewell water on the growth of *P. simplex*. The x-axis represents the number of days, and the y-axis represents dry weight (g). The chart includes bars for 0%, 20%, 40%, 60%, 80%, and 100% mixtures at various time points.]
3) dilution, it is clear that alga can tolerate both sterile as well as raw effluent in equal concentration. While *P. simplex*, on the other hand, showed more growth in sterile and raw effluents in 40% (Fig. 5) and 20% (Fig. 6) dilutions respectively, which indicates that raw effluent is inhibitory to *P. simplex* to some extent.

Thus as compared to *P. simplex*, *C. laeve* can tolerate slightly higher concentration of raw paper mill effluent. Even though *P. simplex* also grows in the effluent its efficiency is comparatively lesser.

**EFFECT OF KMF DAIRY EFFLUENT ON THE GROWTH OF ALGAE.**

Chemical factors of the KMF dairy effluent have been tabulated in the Table 37. In general, the pH was acidic, with very high value of EC, carbon-dioxide, hardness, dissolved organic matter, sulphate, phosphate, sodium, potassium and nitrate. Thus almost all the nutrients were rich in concentration and the dissolved oxygen value was comparatively less. The bore well water used for dilution had comparatively lower values of nutrients like dissolved organic matter and hardness but slightly higher pH and dissolved oxygen.

**EFFECT OF KMF DAIRY EFFLUENT ON THE GROWTH OF C. laeve:**

Initially to see the effect of KMF effluent on the growth of *C. laeve*, an experiment was conducted by selecting the different combinations of sterile effluent and sterile growth medium. Fig. 7 clearly indicates the inhibitory nature of effluent on the growth of the alga because, the growth was generally more in 100% sterile medium (control) throughout the experiment. With the addition of the effluent the growth was retarded. Initially, on 5th day, growth of
the alga was uniformly less in all the combinations. From 10th day onwards growth started increasing steadily and in subsequent days growth of the alga was more in 100% medium (control). However, among different dilution combinations growth of the alga was more in 20% sterile effluent, suggesting the suitability of this combination for the growth of the alga. This observation is supported by the observation made by Hosetti and Patil (1986), who observed the growth of the *Nostoc punctiformae* in 50% dilution of diary animal waste with tap water. However lower growth in 40%, 60%, 80% and 100% sterile effluent is an indication of inhibitory nature of the effluent possibly due to higher fat and oil contents. The visual observation also showed the formation of fat scum on the surface in the flasks which probably hindered the growth. On the other hand dilution of this effluent with the addition of growth medium which resulted in higher growth of the alga is an indication that the low quantity of KMF dairy effluent supports the growth of the alga. Thus the alga did grow with the addition of the effluent to the medium which indicates that KMF dairy effluent is not inhibitory and is further confirmed by its growth in all other dilutions though less than pure medium (control).

In view of this, the second experiment was conducted with the sterile effluent and sterile bore well water dilutions (Fig 8). In this experiment growth could be seen in 40% dilution of sterile effluent with sterile bore well water, confirming the result of first experiment, where growth of the alga was more in 20% sterile effluent and sterile growth medium combinations. From Fig. 8 it is clear that, initially on 5th day itself growth of the alga was more in 40% sterile effluent and in subsequent days also more growth was maintained in this combinations till the end of the experiment.

From Fig. 8 it is also clear that growth fluctuation in 20% and 60% sterile effluent is also similar to 40% sterile effluent but, growth was comparatively lower than 40% sterile effluent. However, in 80% and 100% sterile effluents growth of the alga decreased on 20th day and in 100% sterile
effluent it decreased on 15\textsuperscript{th} day itself indicating the inhibitory effect of the effluent as the concentrations increase. Retarded growth in 80% and 100% effluent can be attributed to the higher concentration of fats and oils and white turbid colour of the dairy effluent. On the other hand in 60%, 40% and 20% sterile effluent more growth of alga was probably because of the decreased concentrations of fats, oils and turbidity due to dilutions by bore well water. Lower growth in pure bore well water but enhanced growth with addition of effluent clearly proves the supply of nutrients to the growth of the alga. However higher concentration proved inhibitory.

To see the effect of raw effluent on the growth of \textit{C. laeve}, third experiment was conducted with various combinations of raw effluent and raw bore well water (Fig 9). The results are almost confirming the above findings. The growth was minimum in 100% raw effluent and it increased with the dilutions. Initially on 5\textsuperscript{th} day itself growth of the alga was more in 40% sterile effluent and reached the peak on 15\textsuperscript{th} day and it decreased on 20\textsuperscript{th} day. Growth pattern in 20% and 60% raw effluent was similar to 40% raw effluent, but was comparatively low. On the other hand in 80% and 100% raw effluents growth was very less as compared to other dilution combinations and in 100% raw effluent growth declined from 15\textsuperscript{th} day only, indicating the influence of higher concentration of raw effluent on the growth of alga. This observation suggests that 40\% dilution of raw effluent with raw bore well water is optimum for the growth of the \textit{C. laeve}.

Thus from these experiments it can be concluded that \textit{C. laeve} can be successfully grown in the KMF dairy effluent. It contains nutrients favouring the growth of \textit{C. laeve}. However, may be because of turbidity, oil and fat contents, higher concentration retards the growth. Dilution of the effluent promotes the growth proving the suitability of \textit{C. laeve} for purifying dairy effluent.
Effect of K. M. F. dairy effluent on the growth of *Cosmarium laeve*
Combinations: Sterile effluent + Sterile Medium

![Bar chart depicting the growth of *Cosmarium laeve* over different days with varying concentrations of K. M. F. dairy effluent.](chart.png)
Effect of K. M. F. dairy effluent on the growth of C. laeae

Combinations: Sterile Effluent + Sterile Borewell Water

Figure 8
Effect of K. M. F. dairy effluent on the growth of *C. laeve*
Combinations: Raw effluent + Raw Borewell Water

Figure 9
Similar to *C. laeve*, different experiments were conducted for *P. simplex* also to see the effect of KMF effluent on its growth. Fig. 10 reveals the growth pattern of *P. simplex* in various dilutions of sterile effluent with sterile growth medium. From Fig. 10 it is clear that growth of the alga was more in pure medium than in various concentrations of effluents. This indicates the inhibitory nature of KMF effluent for the growth of *P. simplex*. But its growth in all combinations, though less than pure medium, indicates that KMF dairy effluent is not lethal to the alga. Initially, on 5th day, growth of the alga was less in all the combinations. In subsequent days growth increased and reached peak on 15th day. Among the various combinations highest growth was observed in 20% and 40% sterile effluent. On 5th day itself growth in these combinations was higher. This indicates that *P. simplex* would grow at relatively lower concentration of the sterile effluent. Relatively higher concentration of the sterile effluents (80%-100%) reduced the algal growth significantly (Fig 10). Thus this proves that KMF dairy effluent supports the growth of the alga.

Second experiment of *P. simplex* with the combination of sterile effluent and sterile bore well water (Fig. 11) is almost confirming the above findings. From the Fig. 11 it is clear that growth of the alga was more in 40% sterile effluent and less in 100% bore well water as well as 100% sterile effluent. On 5th day growth of the alga was equally more in 20% and 40% sterile effluent. On 10th and 15th day growth was more in 40% sterile effluent. But on 20th day growth in these combinations declined. However on 20th and 25th days growth was more in 20% sterile effluent. This indicates that even though magnitude of growth of the alga was less in 20% sterile effluent as compared to 40% sterile effluent, it lasts for longer time. Comparatively decreased growth in 80% and 100% sterile effluent clearly indicates that higher concentration of the effluent
retards the growth of the alga. This is further confirmed by very less growth of alga in 100% and 80% sterile effluent. Thus lower concentration of KMF dairy effluent favoured the growth of the alga.

To see the effect of raw effluent on the growth of the alga, *P. simplex* was grown in various combinations of raw effluent and raw bore well water. Fig. 12 depicts the growth pattern of *P. simplex* in various combinations of raw effluent and raw bore well water. In this experiment (Fig. 12) highest growth of the alga in 20% sterile effluent almost confirms the findings of second experiment. From the Fig. 12 it is clear that growth of the alga was more in 20% raw effluent and less in 100% bore well water as well as 100% raw effluent. Although initially the growth response was negligible on 5th day subsequently growth suddenly increased in all the combinations with comparatively more growth in 20% raw effluent. In 20% raw effluent growth reached peak on 15th day and declined from 20th day onwards. From Fig. 12 it is also clear that growth fluctuation in 40% and 60% raw effluent is also similar to 20% raw effluent, but growth was comparatively lower than 20% raw effluent. However, in higher concentration of raw effluent growth was very less as compared to their lower dilution combinations.

Thus it is clear here that, similar to *C. laeve*, in higher concentration raw KMF effluent hinders the growth of the *P. simplex* also. It is also clear here that it may be the fat and oil contents and white colour of the KMF dairy effluent which might have hindered the growth. This observation is supported by the observation made by Hosetti and Patil (1986) who observed the growth of *Nostoc functiformae* in 50% dilution of dairy animal waste with tap water.

Thus from these experiments of *C. laeve* and *P. simplex* it can be concluded that KMF dairy effluent is not toxic to both these organisms. It is
Effect of K. M. F. dairy effluent on the growth of *Pediastrum simplex*
Combinations: Sterile effluent + Sterile Medium
Figure 11

Effect of K. M. F. dairy effluent on the growth of *P. simplex*
Combinations: Sterile Effluent + Sterile Borewell Water
Effect of K. M. F. dairy effluent on the growth of *P. simplex*
Combinations: Raw Effluent + Raw Borewell Water

![Bar chart showing the effect of K. M. F. dairy effluent on the growth of *P. simplex* over different combinations of raw effluent and raw borewell water. The x-axis represents the number of days, ranging from 5 to 25, and the y-axis represents dry weight (g) ranging from 0 to 0.003. The chart includes different combinations with percentages 0%, 20%, 40%, 60%, 80%, and 100%, showing variation in growth over time.]
clear here that KMF dairy effluent contains some nutrients that are favouring the growth of these algae.

Thus, of the two alga studied, *C. laeve* appeared to be a better organism for treating the KMF dairy effluent. Since *C. laeve* showed better growth in both sterile and raw effluents in 40% dilution (Figs. 8 and 9), it is clear that this alga can tolerate both sterile as well as raw effluent at equal concentration (40% dilution). While, *P. simplex* on the other hand, showed more growth in sterile and raw effluents in 40% (Fig. 11) and 20% (Fig. 12) dilutions respectively, which indicates that raw effluent is slightly more inhibitory to *P. simplex* as compared to sterile effluent. Thus compared to *P. simplex*, *C. laeve* can tolerate slightly higher concentration of raw effluent. Even though *P. simplex* also grows in the effluent its efficiency is comparatively lesser.

**EFFECT OF SEWAGE ON THE GROWTH OF ALGAE:**

Chemical factors of the sewage have been tabulated in the Table 37. In general, the pH was alkaline with very high values of EC, carbon-di-oxide, hardness, dissolved organic matter, calcium, sodium, potassium and sulphate. Thus almost all the nutrients were high while the dissolved oxygen value was comparatively less. The bore well water had comparatively lower values of factors like dissolved organic matter and hardness and slightly higher dissolved oxygen.
EFFECT OF SEWAGE ON THE GROWTH OF C. laeve:

To begin with the experiment was conducted in the sterile sewage and sterile medium combinations. Fig. 13 indicates that the growth of C. laeve was comparatively high in all the combinations than the 100% sterile medium (control). However the growth was highest in 20% and 40% sterile sewage, suggesting the suitability of this dilution for the growth of the alga. In the beginning on 5th day growth of the alga was very low in all the combinations but on 10th day onwards growth was triggered and from 15th day onwards more growth was observed in 40% sterile sewage. Growth reached the peak on 15th day and decreased on 20th day. From Fig. 13 it is also clear that growth fluctuation in 20% and 60% sterile sewage is also similar to 40% sterile sewage, but the growth was comparatively lower than 40% sterile sewage. However, in 80% and 100% sterile sewage growth was comparatively low which indicates the unsuitability of the sewage as the concentration increases.

On the other hand more growth in 40% sterile sewage indicates the suitability of the sterile sewage as concentration decreases. Growth was more than the pure medium in all the combinations indicating that the alga is favoured by certain nutrients present in the sewage.

In view of this second experiment was conducted with combination of sterile sewage and sterile bore well water. This experiment (Fig. 14) confirms the above findings in sterile sewage and sterile medium experiment. From the Fig. 14 it is clear that in general growth of the alga was more in 60% sterile sewage in all the harvests. From the 5th day itself growth of the alga was more in this combination which indicate the suitability of this combination. Growth in this combination reached peak on 15th day and decreased on 20th day onwards. However in 80% and 100% sterile sewage growth reached peak on 20th and decreased on 25th day. Comparatively lower growth in 80% and 100% sewage clearly indicates that the higher concentrations of the sewage retards...
the growth of the alga. On the other hand more growth in 60% sterile sewage indicates the suitability of sterile sewage for the growth of the C. laeve after dilutions.

To see the effect of raw sewage on the growth of C. laeve, third experiment was conducted with the combinations of raw sewage and raw bore well water. Fig. 15 clearly confirms the above findings, as the growth of the alga was more in 60% raw sewage. On 5th day growth of the alga was very less in all the combination with comparatively more growth in 40% and 60% raw sewage combinations. On the 10th day onwards growth was triggered in all the combinations with more growth in 20%, 40% and 60% raw sewage. However on 15th day of all the combinations, growth of the alga was more in 60% raw sewage and it maintained on 20th and 25th day also. Lower growth in 80% and 100% raw sewage indicates the unsuitability of raw sewage at higher concentration. Thus Fig. 15 also reveals that as the concentration of the raw sewage increased growth of the alga decreased. This indicates that growth of C. laeve was supported at relatively lower concentration of raw sewage.

Thus from the above experiments it can be concluded that C. laeve can grow in the sewage water, which is indicated by its more growth in sterile sewage than in 100% sterile medium (Fig. 13). It is quite possible that though certain nutrients are favouring the growth of the alga higher concentration of these hinders the growth in 100% and 80% sewage. Various dilution experiments with sterile and raw bore well water confirms this view as the growth increased with the dilutions. The alga can be used for treating sewage but with dilutions.
Effect of Sewage on the growth of *Cosmarium laeve*
Combinations: Sterile Sewage + Sterile Medium

Figure 13
Effects of Sewage on the Growth of *C. laeve*

Combinations: Sterile sewage + Sterile Borewell Water

---

**Figure 14**

Effect of Sewage on the growth of *C. laeve*

Combinations: Sterile sewage + Sterile Borewell Water

- 0%
- 20%
- 40%
- 60%
- 80%
- 100%
Effect of Sewage on the growth of *C. laeve*

Combinations: Raw Sewage + Raw Borewell Water
EFFECT OF SEWAGE ON THE GROWTH OF P. simplex:

From the initial experiment on P. simplex with the combinations of sterile sewage and sterile growth medium (Fig. 16), it is clear that the growth in general was very much reduced in 100% sterile sewage. On 5th day growth of P. simplex was very less in all the combinations. On 10th day onwards growth increased in all the combinations, with more growth in 100% sterile medium (control). However, on 15th day onwards growth of the alga was more in 40% and 60% sterile sewage than 100% sterile medium (control). Growth declined on 20th day onwards in all the combinations. Thus on 15th day growth of the alga was comparatively high in 40% and 60% sterile sewage than the 100% sterile medium which indicates that alga slowly adjusted to the sterile sewage from 15th day onwards. Its lower growth in 80% and 100% sterile sewage indicates that higher concentration of sterile sewage is inhibitory to the growth of the alga. Thus from Fig. 16 it is clear that relatively higher concentration of sterile sewage hindered the growth of the alga and relatively lower concentration of sterile sewage favoured the growth of the alga.

In view of this second experiment was conducted with the combination of sterile sewage and sterile bore well water. Fig. 17 reveals the growth pattern of P. simplex in various combinations of sterile sewage and sterile bore well water. Fig. 17 clearly confirms the findings of first experiment.

From Fig. 17 it is clear that in general growth of the alga was very less in 100% sterile sewage. With the dilutions by the addition of sterile bore well water the growth response was more. It can thus be understood that the alga grows in sterile sewage with dilutions. Initially on 5th day to the 15th day growth of the alga was more in 60% sterile sewage. However, on 20th day growth of the alga was more in 40% sterile sewage than the higher concentrations indicating that the alga is not favoured by higher doses of the sewage.
In view of this third experiment was conducted with raw sewage and raw bore well water combinations (Fig. 18). Interestingly, in general, growth of the alga was more in 20% raw sewage than in all the combinations. In 100% raw sewage growth of the alga was very less. From Fig. 18 it is clear that on 5th day itself growth of the alga was more in 20% raw sewage and reached the peak on 20th day. Growth decreased on 25th day. From Fig. 18 it is also clear that growth fluctuation in remaining combinations is also similar to 20% raw sewage but, growth was comparatively lower.

Thus from this experiment it is clear that with the dilution by addition of raw bore well water the growth response more. Thus from Fig. 16 it is clear that *P. simplex* grows well in the sewage water. However, the sewage is not that favourable for the growth which is indicated by its more growth in 40% and 60% sterile sewage than in 100% sterile medium on 15th day. It is quite possible that though certain factors, present in the sewage, are favouring the growth of the alga, higher concentrations of these factors might have hindered the growth in 80% and 100% sewage. Various dilution experiments with sterile and raw bore well water confirms this view as the growth increased with the dilutions. Thus the alga can be used for treating sewage but with dilutions.

From the experiments on *C. laeve* and *P. simplex* it can be concluded that sewage is not inhibitory to both these organisms. It is clear here that sewage contains some nutrients that are favouring the growth of these algae. Of these two algae studied, similar to paper mill effluent and KMF dairy effluent, for treatment of sewage also, *C. laeve* appears to be a better organism. Nair *et al.* (1981) also made similar observation using *Scenedesmus obliquus*. In their studies, *S. obliquus* was found to grow well in secondary treated domestic sewage. *C. laeve* also showed its better growth in slightly higher concentration of sterile sewage (Fig. 14) and raw sewage (Fig. 15). While *P. simplex*, on the other hand, even though showed its better growth in 60%
Effect of Sewage on the growth of *Pediastrum simplex*

Combinations: Sterile Sewage + Sterile medium
Effect of Sewage on the growth of *P. simplex*
Combinations: Sterile Sewage + Sterile Borewell Water

Figure 17
Effect of Sewage on the growth of *P. simplex*  
Combinations: Raw Sewage + Raw Borewell Water
sterile sewage (Fig. 17), in raw sewage its growth was reduced in higher concentrations (Fig. 18), which indicates that raw effluent is inhibitory to \textit{P. simplex} to some extent. Thus \textit{C. laeve}, as compared to \textit{P. simplex}, can tolerate slightly higher concentrations of raw sewage.

To conclude, the experiments with three types of waste waters on the growth of two algae, \textit{C. laeve} and \textit{P. simplex}, showed hopeful results. None of the waste waters were inhibitory to the level of killing the algae. They do grow in exclusive waste waters but luxuriant growth would be in various dilutions. However, the growth response was different. Rana and Palaria (1987) studied pollution abatement of certain waste waters using biological and chemical methods and concluded that inorganic and organic nutrients may be reduced by growing pollution tolerant strains of algae. Thus, these experiments proved that \textit{C. laeve} is the best organism favoured by all the three waste waters.

However, all these effluents, though not toxic, were inhibitory for the growth of \textit{P. simplex}. Thus it is concluded that \textit{C. laeve} is more suitable for the treatment of waste water.
<table>
<thead>
<tr>
<th>Factors</th>
<th>Bore well water</th>
<th>KMF dairy effluent</th>
<th>Paper mill effluent</th>
<th>Sewage water</th>
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<tr>
<td>pH</td>
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All values are in mg/L except pH and EC.