1.0 INTRODUCTION

Environmental pollution is an increasing hazard to human health and it is more severe in the industrial intense cities. The poor quality of water is due to the addition of industrial pollutants which is a major problem faced by big industrial cities. The uncontrolled discharge of effluents into large water bodies has harmful effects both on water quality and aquatic life. Human beings manipulate the environment or even the entire eco-sphere by changing the global cycles of elements or by releasing chemicals, industrial effluents, pesticides etc., in the environment. Such adapted eco-sphere presents a threat to man's own survival on the earth.

India is one of the largest industrialized nations in the world. A major portion of the industrial waste is collected and dumped in landfills. In addition, large volumes of effluents both in solid and liquid forms are generated by breweries, sugar mills, distilleries, food-processing industries, tanneries, paper and pulp industries pollute the soil.

Environment contaminated with toxic chemicals and their by-products posses a mammoth task of treatment and/or management. These toxic contaminants, primarily of anthropogenic origin, are broadly classified as metal, non-metal, metalloid, inorganic and organic compounds (Cluis, 2004). Organic contaminants comprises of aliphatic, acyclic aromatic and polycyclic aromatic hydrocarbons that include halogenated and non halogenated compounds, pesticides and explosives. Inorganic metal pollutants include metals are Ag, Al, Be, Cd, Cr, Cu, Hg, Fe, Ni, Pb, Sb, Se, Zn, radioactive elements and their derivatives (Meagher, 2000; Allen, 2002). The principle concern associated with a contaminant its toxicites and health risk to humans. It is therefore, essential to contain or mitigate these organic and inorganic contaminants so as to prevent them from contaminating surface and groundwater by dissolution or dispersion (Mc Laughlin ***et al***., 2000) and it is also imperative on our part to develop sustainable organic technologies and biological systems to these requirements.

1.1 Wastewater

Wastewater can be defined as the remaining spent water, from commercial establishments, industries, public institutions, and similar entities. (Sincero and Sincero, 2003). Wastewater enters the environment through either 'point' or 'non-point' sources. Point sources are definite locations, such as pipes, from where wastewater enters water bodies. On the other hand, wastewater that comes from diffuse sources such as the runoff from agricultural fields or parking lots is defined as non-point sources. (Welch and Lindell, 1992).

1.1.1 Effects of Wastewater on Water Quality

Water usage can be categorized in two ways namely : a) Non-consumptive, where water from its natural sources and b) Consuming part is the amount of water that is taken out of a stream or pumped out of underground or surface reservoirs for various purposes. (E.g.) Dumping of domestic sewage, solid waste and industrial effluents is the single greatest non-consumptive use of water, which significantly affects the quality of water.

Physico-chemical parameters are important for deciding the quality of water and they play an important role in determining the distribution of aquatic organisms. Thus physico-chemical parameters are directly or indirectly related to the quality of water.

Studies on water quality are gaining increasing importance in our country. Many exhaustive and comprehensive studies have been carried out by Raka ***et al***., (1999); Singh ***et al.,*** (2001) and Patil and Tijare (2001). The term water quality is intimately related to water pollution. Polluted water is one which has more negative qualities than it has positive ones. Water quality refers to the physical, chemical and biological characteristics of water.

1.1.2 Wastewater treatment

In developing countries, greater capital and technological resources helps in building sound collection, treatment and disposal facilities for most of the pollutants, whereas the situation is pathetic in developing countries. It is difficult to separate industrial growth from environmental pollution, but it can be minimized through cost effective approaches of pollution abatement.

The basic function of the wastewater treatment plant is to speed up the natural processes by which water purifies itself. In earlier years, the natural treatment process in streams and lakes was adequate to perform basic wastewater treatment. As our population and industry grew, increased levels of treatment prior to discharging domestic wastewater became necessary.

1.1.3 Basic Wastewater Treatment Processes

The basic wastewater treatment process consists of physical, chemical and biological methods. These physical processes are employed in many modern wastewater treatment facilities today. Chemicals are used for creating changes in pollutants that increase the removal of these new forms by physical processes. In nature, bacteria and other microorganisms in water consume organic matter in sewage, turning into new bacterial cells, carbon dioxide, and other by-products. The bacteria utilize oxygen present in water for breaking down the organic waste in the sewage. Any excess microbial growth could be removed from the wastewater by physical processes.

1.1.4 Advanced Methods of Wastewater Treatment

As our country in the demand for clean water has grown, it has become more important to produce cleaner wastewater. The demand for cleaner discharges has been met through better and more complete methods of removing pollutants at wastewater treatment plants. In addition to pretreatment and pollution prevention, which helps to limit the types of wastes discharged to the sanitary sewer system. Currently, all WWTPs (Wastewater treatment plants) provide a minimum of secondary treatment. In some plants receiving water, the discharge of secondary treatment effluent would still degrade water quality and inhibit aquatic life. Further treatment is needed. Treatment levels beyond secondary stage are called advanced treatment.

Advanced treatment technologies can be an extension of conventional secondary biological treatment to further stabilize oxygen-demanding substances in the wastewater, or to remove nitrogen and phosphorus. Advanced treatment may also involve physical-chemical separation techniques such as adsorption, flocculation/precipitation, membranes for advanced filtration, ion exchange, and reverse osmosis. These processes can achieve the desired degree of pollution control invarious combinations. As wastewater is purified to higher and higher degrees by such advanced treatment processes, the treated water can be reused for urban, landscape, and agricultural irrigation, industrial cooling and processing, recreational uses and water recharge, and even indirect augmentation of drinking water supplies.

In order to develop an appropriate biotechnology, new concept has been developed specifically for rural communities: i.e., integrated rural ***biotechnology systems***. The development of these systems depends primarily on the climatic conditions of the regions. Two of the premises of sustainable development are that economic growth has to be in harmony with the environment and that a rational and sustainable use of natural resources has to be implemented (Olguin, 2000). In congruence with such premises, industrial development has to change from the degradative to the sustainable style, which requires the adoption of cleaner production systems.

1.2 Bioremediation

The conventional techniques used for remediation involve digging up contaminated soil and removing it to a landfill, or capping and containing the contaminated areas of a site. A better approach than these traditional methods has to be implied to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV-oxidation). They can be very effective at reducing contaminants, but have several drawbacks. Principally due to their technological complexity, the cost for small-scale application and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents. Thus, almost all the conventional methods employed to manage contaminants have only created major problems while incineration pollutes the air, buring its in sea, pollutes the oceans and landfills and tunnels into the soil has become sources for toxic gas and groundwater pollution. Therefore, a need has arisen to evolve a safer, efficient and cost effective method to save water bodies of the world from pollution.

Bioremediation generally utilizes microbes (bacteria, fungi, yeast and algae), although higher plants are used in some applications. Bioremediation continues to be the favoured approach for processing biological wastes and avoid microbial pathogenesis. It also plays an increasing role in concentrating metals and radioactive materials to avoid toxicity or to recover metals for reuse. Recently developed a rapid screening assay identify, that the organism is capable of degrading specific wastes and new gene-probe methods can ascertain their abundance at specific sites (Bonaventura and Johnson, 1997). New tools and techniques for use of ***in situ*** bioremediation, in biofilters and in bioreactors are contributing to the rapid growth of this field. Bioremediation has already proven itself to be a cost effective and beneficial in addition to chemical and physical methods of managing wastes and environmental pollutants. Furthermore, stricter regulatory standards imposed by various countries on decontamination of contaminated sites have created an interest in the bioremediation approach (Hattan ***et al***., 2003).

There has been an unhealthy emphasis on just meeting discharge standards irrespective of the total load or quantum of wastes being disposed. However, this situation is slowly changing. The number of industries that currently discharge wastes have increased significantly. Due to toxic effects often caused by the presence of these wastes, even at very low concentrations, there is now a renewed pressure on regulatory agencies to monitor the treatment and disposal of these wastes. Some of the regulatory agencies are now forced to impose zero discharge on some units where the assimilation capacity of the water body is low.

1.3 Microalgae and Wastewater Treatment

With the increasing environmental problems around the world, algae have been found to be effective organisms for bioremediation. The role of algae in the removal of various kinds of inorganic and related substances have been studied by several workers during the last several years (Oswald, 1992; Olguın, 2000). The ability of cyanobacteria to reduce the pollution load in industrial wastewater has been studied by various authors all over the world (Oswald, 1963; Senegar ***et al***., 1981; Uma and Subramanian, 1990; Manoharan and Subramanian, 1992a; Kotteswari ***et al***., 2007; 2012; Murugesan ***et al,*** 2007; 2012). Blue green algae are ideally suitable to play a dual role in treating wastewater and in the process of effective utilization of different constituents essential for growth leading to enhanced biomass production (Rawat ***et al***., 2011).

Since, the pioneering work of Ludwig ***et al***., (1951) and Oswald ***et al***., (1953a; 1953b), it has been recognized that the core treatment processes within a WSP (Wastewater Stabilization Ponds) are centered on inherent biological interactions-specifically those between heterotrophic microbes and algae. This functional synergism between photosynthetic algal oxygenation and aerobic microbial oxidation is the driving force behind the effective WSP operation, and is depicted in Figure.1.

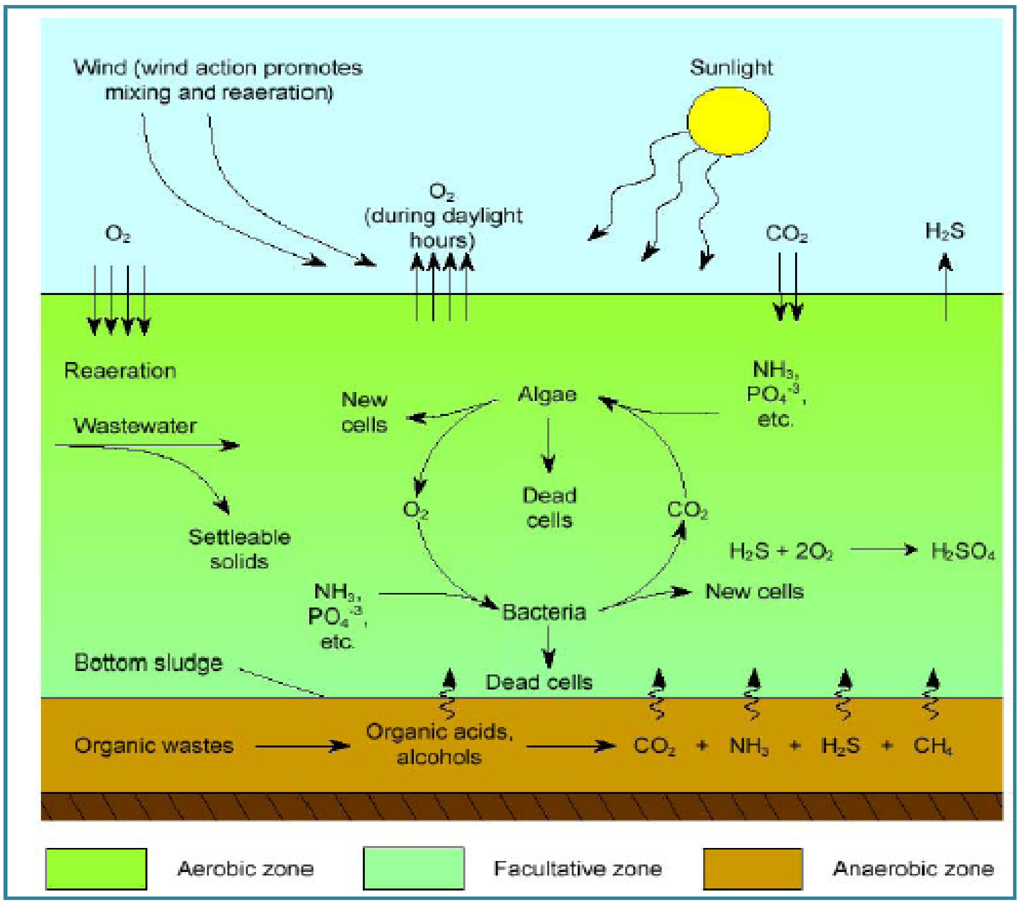

Fig.1 Schematic representation of day time WSP operation, Metcalf and Eddy (1991).

Microalgae are more advantageous than any other organisms for bioremediation purposes because of their

(i) Oxygenating potential

(ii) Capacity to fix CO_2 using sunlight as an energy source

(iii) Simple growth requirements like N and P

(iv) Wide adaptability (from the Antarctic to Sahara desert)

(v) Ability to grow under stress and non-stress conditions and

(vi) Easy scale-up from lab to industrial size photo bioreactors.

As we entered the 21st century, it became clear that a good approach and an important step in managing the waste problem and environmental pollution did not go far enough. The main idea behind this shift is a paradigm change where one now thinks not of the ultimate disposal of a material (i.e., the grave) but the ultimate reuse of all material components of a product of process in any novel manner (i.e., the new cradle). This meant that there would be no waste and consequently the concept of zero-waste processes would be in place and move towards a zero-waste society, where all wastes could be thought of as resources and used in some way. It would be formalized into a new paradigm to approach environmental problems and hence facilitate their solution.

The usefulness of algal systems, more particularly the cyanobacteria, is not only in treating the wastes but also producing a variety of useful byproducts from their biomass is being understood by Subramanian and Shanmugasundaram (1983). Microalgae have vast industrial and economic potential (Rai ***et al***., 2000) as valuable sources for pharmaceuticals (Liu ***et al***., 2000; Park and Lee, 2001), dyes fine chemicals, biofuels and others.

Furthermore, they have the capacity to solve emerging environmental problems, such as the greenhouse effect (Murakami and Ikenochi, 1997; Nagase ***et al***., 1998). Microalgae have many uses. They can serve as bioremediation agents (Oswald, 1992) in the removal of heavy metals (Wilde and Benemann, 1993) and waste treatments (Lee and Lee, 2001; Uma ***et al***., 2002). They can fix carbon dioxide by photosynthesis (Lee and George, 2000; Lee and Lee, 2001) and remove excess nutrients efficiently at a minimal cost (Murakami and Ikenouchi, 1997).

Nutrient removal by using algae is far beneficial to other conventional technologies (de la Noue ***et al***., 1992); Muthukumaran ***et al***., 2005). Biodegradation is cost-effective, environmentally friendly treatment for oily contaminated sites by the use of microorganisms (Bassam Mrayyana, 2005). In response to heavy metals, microorganisms have evolved various measures in a process such as transport across the cell membrane, biosorption to cell wall and entrapment in extra cellular capsules, precipitation, and complexation and oxidation-reduction reactions. It has been proved that they are capable of adsorbing heavy metals from aqueous solutions especially for the metal concentration below 50 mg/l (Lu and Wilkins, 1995).

The ability of cyanobacteria to reduce pollution load in industrial wastewater have been studied over the period of time (Rai and Kumar, 1976; Somashekar, 1984; Sahai ***et al***., 1985; Uma and Subramanian, 1990; Manoharan and Subramanian, 1992a; Kotteswari ***et al***., 2007, 2012; Murugesan ***et al,*** 2007; 2012; Veeralakshmi ***et al***., 2007; Dhamotharan ***et al***., 2008; 2009; Kamaleswari ***et al***., 2009, Vanithasree and Murugesan, 2010; Padmapriya and

Murugesan, 2012; Ravikumar ***et al***., 2012). Cyanobacteria can take up and bioconcentrate a variety of environmental contaminants present in the wastewater (Dash and Mishra, 1999). The capacity of cyanobacteria to remove large amounts of phosphorus from wastewater was demonstrated by several workers (Chan ***et al***., 1979; Tam and Wong, 1990; Dash and Mishra, 1999).

1.3.1 Wastewater as a substrate for algal cultures

Wastewater treatment with microalgae is an environmentally sound approach to reduce nitrogen and phosphorus levels and has been applied for almost 50 years in special reactors termed as high-rate ponds (Oswald ***et al***., 1957). Microalgae mass culture appears to be a feasible way to remove from wastewaters inorganic nutrients, among which nitrogenous and phosphoric compounds, which are well-known primary agents of pollution and eutrophication. In the recent past, various systems and microbial species have been investigated to improve the effectiveness and economic feasibility of such as biotreatment. Moreover, the cultivation of photosynthetic microorganisms on adequately treated effluents can be an economically feasible process, to produce high value proteins for animal feeding and to obtain products of pharmaceutical interest (Borowitzka, 1988). Algal cultivation on wastewaters aims at producing biomass and at the same time, removing organic and inorganic pollutants (Martinez ***et al***., 2000). The use of microalgae can offer a valuable alternative to the conventional purification treatments and provides several advantages among which:

(a) It is not environmentally dangerous, because it rests on the principles of natural ecosystems

(b) Biomass can be recycled reducing the causes of secondary pollution; and

(c) Algal growth on effluent removes heavy metals and xenobiotic substances and allows, under photosynthetic conditions, oxygen to be released, thus enhancing the auto-depuration potential of water bodies.

One of the major and practical limitations in the algal treatment system is the harvesting of algal biomass from the treated water. An efficient removal of algal biomass is essential for recycling of the wastewater. Numerous efforts have been devoted to develop a suitable technology for harvesting microalgae ranging from simple sand filtration for energy-intensive centrifugation (Richmond and Becker, 1986); Oswald (1992). Auto flocculation, i.e., self-aggregation by stopping aeration followed by decantation, particularly for cyanobacteria, has also been practised. In this context, immobilization of algal cells in wastewater treatment has been proposed for circumventing the harvest problem as well as retaining the high-value algal biomass for further processing (De la Noüe and De Pauw, 1988).

1.3.2 Biodegradation by algae

Microalgae play an important role during the tertiary treatment of domestic wastewater in maturation ponds or the treatment of small–middle-scale municipal wastewater in facultative or aerobic ponds (Abeliovich, 1986; Mara and Pearson, 1986; Oswald, 1988; Aziz and Ng, 1993). They enhance the removal of nutrients, heavy metals and pathogens and furnish O_2 to heterotrophic aerobic bacteria to mineralized organic pollutants, using in turn the CO_2 released from bacterial respiration. Photosynthetic aeration is therefore especially interesting to reduce operation costs and limits the risks for pollutant volatilization under mechanical aeration and recent studies have shown that microalgae can indeed support the aerobic degradation of various hazardous contaminants (Safonova ***et al***., 2004).

India, a tropical country has got plenty of sunshine and is rich source for microalgae species. Parameters like nutrient requirement, tolerance to pollution, mixotrophic growth tolerance to extreme temperature and presence of biochemical products of economic importance are to be investigated. Industries in India must switch over to this new technology by conducting meaningful help and field trials.

1.4 Phycoremediation

'Photosynthetic wastewater treatment' by William J. Oswald (1963) is not new for the environment scientists who are engaged in the wastewater management program. Phycoremediation may be defined in a broad sense as, the use of macroalgae or microalgae for the removal or biotransformation of pollutants, including nutrients, organic contents and xenobiotics from wastewater and is felt with great potential and demand. This field has evolved from the early work done by Oswald (1963) for the use of microalgae for tertiary treatment of municipal wastewater to many other applications in which microalgae and macroalgae are cultivated and utilized for specific bioremediation needs. However, the development of more efficient nutrient removal by algal systems requires further research in key areas. Algal growth rate controls directly and indirectly the nitrogen and phosphorus removal efficiency. Thus, maximum algal productivity is required for effective nutrient removal and must be considered as a key area of research. Likewise, lower harvesting costs are required for a cost-effective nutrient removal system. Another key area of research is the use of algal strains with special attributes such as tolerance to extreme temperature, chemical composition with predominance of high value added products, quick sedimentation behaviour, or a capacity for growing mixotrophically.

Phycoremediation can be considered as one of the most inexpensive types of pollution clean up. With regards to bioremediation of heavy metals there are some limitations such as metals being absorbed by the algae and sinking to the bottom. This might be hazarded in shallow water systems because organisms which eat the algae will still be affected by the metal (s). This is the main reason why it can only put into practice in deep water systems. Humans can also be affected by the contaminated water. The contaminated water can leak into groundwater which could be used by humans later. Most systems of water purification do not have sufficient ability to remove metals from contaminated water. Bioremoval capabilities of microalgae have been extensively studied by Lee ***et al***., (1992); Gupta ***et al***., (2000) and Katarzyna Chojnacka (2007). However, there is a need to have more knowledge of the basic mechanism involved in order to develop using inexpensive growth media or obtained as a byproduct from some industry. Becker (1994) had evolved five parameters to determine the algae for heavy metals removal:

1. Rate of growth with algae
2. Concentration of metal in the algae
3. Concentration of heavy metals in the medium
4. Metal removal from medium in required percentage
5. Metal recovery in relation to cost.

Moreover, the massive problem of chlorinated solvent pollution is another potential application of algae. Several studies have successfully been able to manipulate or induce plant species to extract, degrade, or render harmless trichloroethylene, ethylene dibromide, and the heavy metals cadmium and manganese (Gordon ***et al***., 1998; Zhu ***et al***., 1999; Hirschi ***et al***., 2000). The positive effects of pesticides on cyanobacteria have been demonstrated by several researchers (Subramanian ***et al***., 1994; Barton ***et al***., 2004). The effects of herbicides on cyanobacteria (Abou-Wily, 1991) and green algae (Fairchild ***et al***., 1998; Lockert ***et al***., 2006) have been studied by several researchers. Cyanobacteria degrade various aromatic hydrocarbons, including naturally occurring compounds such as naphthalene (Cerniglia ***et al***., (1980) as well as xenobiotics such as lindane (Kurtz and Wolk, 1995).

Thus, phycoremediation comprises several applications:

(a) Nutrient removal from municipal wastewater and effluents rich in organic matter;

(b) Nutrient and xenobiotic compounds removal with the aid of algae based biosorbents;

(c) Treatment of acidic and metal wastewaters;

(d) CO_2 sequestration;

(e) Transformation and degradation of xenobiotics; and

(f) Detection of toxic compounds with the aid of algal-based biosensors.

Microalgae are naturally occurring living organisms and therefore phycoremediation is a naturally occurring phenomenon. The microalgae used in phycoremediation are already preset in nature and are at work consuming unwanted materials. We come into contact with them on a daily basis with no ill effects. After phycoremediation is completed, the environment is virtually restored to its pristine condition.

The green-cell factories of microalgae tackle simultaneously more than one problem, a solution not capable by conventional chemical processes. That is, for example, problems such as pH correction, sludge removal, TDS (Total dissolved solids) reduction, BOD (Biological oxygen demand) and COD (Chemical oxygen demand) removal, etc., can be handled simultaneously by micro-algal treatment (phycoremediation), whereas in conventional methods, separate methods or stages of treatments are required.

The unique feature about phycoremediation is that while it can be used in the treatment of a wide variety of effluents, it can also be highly specific, for example, in the treatment of heavy metal-bearing effluents or dyeing effluents or treatment of RO (Reverse osmosis) rejects.

There is no need to separate the algae from the treated effluents as the process is an ecologically safe and natural process. Since microalgae are heavier than other microbes algal cells can be easily sediment and harvested. In addition, microalgae such as filamentous algae are available with high auto-flocculation capacity.

It is highly compatible with existing operations such as physical, chemical and other biological methods. Phycoremediation technology is robust as it minimizes automation, maintenance and the need for skilled operators. Phycoremediation assures nil sludge generation and as a result there is no disruption of surrounding, non-contaminated areas.

In the era of global warming, climate change and carbon crediting, there is grave concern about increasing carbon dioxide levels in addition to the laborious task of treating pollutant-containing wastes. Of late, microalgae cultures have been proposed for use in the fixation of CO_2, which is of interest in greenhouse gas mitigation and in production of biofuels. Algae considered as green-cell factories are not only good scavengers of toxic chemicals but are also involved in oxygenation of the atmosphere and carbon dioxide sequestration, thereby making them a better candidate among bioremediation systems. The development of this idea was first conceptualized by Oswald and Golueke (1960), who described a large - scale system with dozens of large (40 ha) high-rate ponds, with the algae grown and the biomass harvested by a simple flocculation-settling step, and the concentrated algal sludge anaerobically digested to produce biogas (methane and CO_2).

1.5 Role of Cyanobacteria in Effluent Treatment

Cyanobacteria have long been recognized as having enormous potential for use in biotechnology, especially in agriculture, and now slowly drift is towards their use in effluent treatment, because of the following reasons.

- Cyanobacterial growth does not require energy rich compounds like another non-photosynthetic microorganisms.

- Cyanobacteria have simple growth requirements which use over other photosynthetic bacteria.

- Many cyanobacteria combine photosynthesis and nitrogen fixation. This is another advantage over other eukaryotic photosynthetic organisms.

- Cyanobacterial biomass production is in abundance and this can be used as food for animals (Mosbach, 1987) an important source for the extraction of high value substances like vitamins and drug intermediates (Venkataramanan, 1994) Nitrogen fixation (Stewart ***et al***., 1987) hydrogen production (Mosbach, 1987), light energy photo conversion and amino acid production.

- They are environmental friendly and do not cause toxicity to other biotic components.

- Separation of cyanobacterial biomass is much easier than other microbial biomass due to their size.

During the recent past, studies on cyanobacteria have emphasized their important role in the ecosystem. However, specific algae grow in specific environments and therefore their distribution pattern, ecology, periodicity, qualitative and quantitative occurrence differs widely. It is said that they flourish well either in nutrient rich and warm water or at times in water with apparently low nutrient concentrations, subjected to higher temperature and bright light conditions (Ganapati, 1940; Philipose, 1960; Munawar, 1970; Saxena ***et al.,*** 1974; Fogg, 1975; Subramanian and Shanmugasundaram, 1983. Several reports are now available on the occurrence of algae in polluted waters (Palmer, 1969; Somashekar and Ramaswamy, 1983; Tarar ***et al.***, 1998 and Kamaleswari ***et al***., 2007). However, the diversity in physical, chemical and biological characteristics of industrial effluents is so great that each wastewater habitat requires a separate study. To develop suitable and efficient treatment systems, it is obligatory to understand the mutual influence and interactions between the effluents and organisms, so that manipulations to improve the treatment systems become feasible.

1.6 Wastewater and other carbon sources for heterotrophic growth of microalgae

The autotrophically grown microalgae are commonly used in wastewater treatment (de la Noue and de Pauw, N. 1988; de la Noue ***et al***., 1992; Oswald, 1992; Canizares ***et al***., 1994; Gonzalez ***et al***., 1997; Lee and Lee, 2001; de-Bashan ***et al***., 2002; 2004; Hernandez ***et al***., 2006). The major advantages of these treatments are that additional pollution is not generated when the biomass is harvested and efficient recycling of nutrients is possible (de la Noue ***et al***., 1992). To date, this has hardly been tested under heterotrophic conditions (de-Bashan and Bashan, 2010; Octavio Perez-Garcia ***et al***., 2011). Nonetheless, ***Chlorella*** sp. and strains of ***Scenedesmus*** were isolated from wastewaters, kept in the dark and in oxidation ponds (Post ***et al***., 1994). Use of organic compounds was influenced by the supply of CO_2 to the culture; decrease in the organic matter per unit of cell weight produced was greater when the supply of CO_2 was low (Octavio Perez-Garcia ***et al***., 2011).

1.7 Metal pollutants

Metal pollution is a global concern and the levels of metals in all environments, water, air and soil, are increasing, in some cases to toxic levels, with contributions from a wide variety of industrial and domestic sources. Metal availability is strongly dependant on environmental components, such as pH, redox and organic content and soluble and bio-available metals. Metals in the environment can be divided into two classes: 1) bio-available (soluble, non-sorbed and mobile) 2) non bio-available (precipitated, complexed, sorbed and no mobile). According to Becker (1983; 1994) utilizing planktonic algae has a high potential to absorb heavy metals for the removal of residual metals from wastewaters including the separation of the metal-saturated algae from medium is an economic method resulting in high quality reusable effluent and valuable biomass which could be used for different purposes (production of biogas, fertilizer, fodder etc.,).

1.8 Harvesting of Microalgae-Cost factor

Several researchers have pointed out that cost-effective biomass harvesting is one of the key factors limiting the use of algae in various fields (de la Noue and de Pauw, 1988; Oswald, 1988; Lincoln and Earle, 1990; Andrew ***et al***., 2009). In phycoremediation, this factor could determine the economic viability of the whole system. Bearing this problem in mind, the use of monoalgal cultures involving multicellular species or species with self-aggregation capacity has been investigated. Thus, in contrast with the traditional approach of allowing a mixed culture to establish naturally in the HRAPs (High rate algal ponds), selected algae is inoculated and maintained as a monoalgal culture.

1.9 Key factors – critical to successful application of phycoremediation

Environmental conditions, contamination and nutrient availability and the presence of degrading microorganisms are the most important factors critical to successful application of bioremediation. If biodegradation does not occur, the first thing that must be done is to isolate the factor limiting bioremediation. Initial laboratory tests using soil or water from a polluted site can usually determine whether degrading organisms are present and whether there is an obvious environmental factor that limits bioremediation for extremely low pH or lack of nitrogen and/or phosphorus. Low bioavailability due to absorption and aging is another factor that can limit bioremediation.

1.10 Algal growth

Successful treatment of wastewater with microalgae requires good growth, and understanding of the factors that affect growth is therefore essential. The growth rate of algae and cyanobacteria is influenced by physical, chemical and biological factors (Table.1). Examples of physical factors are light and temperature. Chemical factors can be availability of nutrients and carbon dioxide and biological factors are competition between species, grazing by animals and virus infections. Operational factors mentioned above and basically concerns bioreactor design, mixing and dilution rate.

In addition, phycoremediation has advantages over other conventional physico-chemical methods, such as ion-exchange, reverse osmosis, dialysis and electro-dialysis, membrane separation, activated carbon adsorption, and chemical reduction or oxidation, due to its better nutrient removal efficiency and lower cost of its implementation and maintenance.

All the current technologies that are available for treatment of waste have lots of limitations. Most of them are not cost effective and are inappropriate for ***in situ*** treatment. Some of them are not effective in treating a complex array of different pollutants. Biological treatment appears to offer a solution to these limitations (Mezzomo ***et al***., 2010).

Table.1. Factors that influence algal growth in a High rate algal pond (Becker, 1988)

Abiotic factors	Light (quality, quantity)
Physical and chemical	Temperature
	Nutrient concentration
	O_2, CO_2
	pH
	Salinity
	Toxic chemicals
Biotic factors	Pathogen (bacteria, fungi, viruses)
	Predation of zooplankton
	Competition between species
Operational factors	Mixing
	Dilution
	Depth
	Addition of bicarbonate
	Harvesting frequency

1.11 Use of strains with special attributes

Nutrient removal has been shown to be more efficient by using algae strains with special attributes. Such special attributes include tolerance to extreme temperatures, chemical composition with predominance of high value added products, quick sedimentation behaviour, or a capacity for growing mixotrophically. A ***Phormidium*** strain which is capable to remove nutrients more efficiently than a community of green algae below 10°C was isolated from polar environments (Tang ***et al***., 1997). Tang ***et al***., (1997) also suggested that this strain was appropriate for wastewater treatment in cold climates during spring and autumn. On the other hand, Talbot and de la Noue (1993) reported that ***Phormidium bohneri*** was a good candidate for treating wastewater at high temperatures (around 30°C); additionally, such strain had quick sedimentation behaviour. The capacity for heterotrophic growth has also been reported for photosynthetic bacteria and green microalgae. ***Rhodobacter sphaeroides*** and ***Chlorella sorokiniana*** were found to remove nutrients efficiently under aerobic dark heterotrophic conditions (Ogbonna ***et al***., 2000). Such strains also had a very good potential for wastewater treatment.

1.12 Challenges associated with Algae-based Wastewater Treatment Systems

Algae-based wastewater treatment technology is suited for tropical countries where the temperature is warmer and sunlight is optimum. Environmental factors play a major role in algal cultivation. Maintenance of optimum temperature and lighting in algae ponds is difficult. Apart from these environmental factors, there are number of biological problems and operational problems that can arise in the mass cultivation of microalgae using wastewater. These include contamination and grazing. Control measures for avoiding contamination by bacteria and other algal species are sterilization and ultra-filtration of the culture medium. Grazing by protozoans and diseases causing fungi can eventually be treated chemically.

Some of the key challenges are:

* Land requirements
* Temperature
* Mixing
* Contamination
* Oxygen Depletion
* Light requirements
* Rainfall
* Harvesting
* Grazing

1.13 Sustainability of Microalgal Wastewater Treatment

Scandinavian researchers evaluated three wastewater treatment methods based on two frameworks for quantifying the degree to which a process tends toward a sustainable system. The frameworks are known as the "socio-ecological principles" and "energy analysis". Energy analysis locates every resource in an energy hierarchy of the biosphere: "The position of an item in the energy hierarchy is suggested to correspond to the relative influence of that item, [sic] on the system of which it is a part." (Groenlund, ***et al***., 2004).

The wastewater treatment methods considered were 1) a conventional wastewater treatment system (not further defined), 2) a conventional treatment plant used together with a constructed wetland and 3) a model microalgae-based wastewater treatment plant. After comparing costs, use of renewable and non-renewable energy sources, and other factors such as environmental load and energy yield, they conclude that the microalgae-based treatment violated the socio-ecological principles to a lesser degree than the other treatment methods. Unfortunately, the monetary inputs were larger than for the other treatment methods. They observe that if the microalgae biomass could be used in other ways this would narrow the cost gap and could make the process cost-effective. They suggest identifying byproducts, for example, valuable biochemical and methane collection. They seems to be unaware of the potential use of the algal biomass for animal feed and biofuel (Groenlund ***et al.,*** 2004).

1.14 Pollution effects of Dairy wastes

As dairy wastes contain the entire nutrients essential for bacterial growth and the temperatures encountered in most part of India are ideal for bacteria, favours decomposition of pollutants in anaerobic conditions followed by bad odour. It was reported that at a dilution of 1 : 15 dairy wastes (excluding whey) had bad effects in fish. At a dilution of 1 : 35 they had been found to be toxic in a few hours. Dairy wastes can occasionally carry tuberculosis bacteria and there is a risk of spreading this infection from infected cows to men through milk as well as its waste consist of concentrate strontium – 90 a beta emitting radioisotope that affects the bone.

Dairy effluents decompose rapidly and deplete dissolved oxygen of receiving streams that resulted in strong foul odours if disposed off without any treatment. The effect of effluent generated from a dairy/malt based food industry needs to be addressed by all legislative bodies in view of the fact that unorganized dairy units are not serious in treating the effluents from their units which in one way or another cause pollution of water and soil by disposing on land or into water bodies without treatment.

1.15 Objective and Scope

Cyanobacteria are valuable resources that have an immense potential to solve the nation's energy and environmental challenges. The overall objective of this research is to investigate the use of cyanobacteria in the bioremediation of dairy effluent and to evolve effective and economical biological treatment.

The principle objectives were covered both *in vitro* and *in vivo* experiments

- Collection of dairy effluent from Madhavaram Dairy Plant.
- Isolation, purification and identification of the naturally occurring cyanobacteria from Central Dairy Plant (Aavin), Madhavaram and cyanobacteria with high biodegradation and/or removal capabilities for organic pollutants.
- Maintenance of cyanobacteria in the laboratory.
- Quantification of physico-chemical parameters, of untreated and treated dairy effluent.
- To find out the tolerance of cyanobacteria to evaluate the efficiency of algae for best possible removal of nutrients, removal of organic pollutants and removal of heavy metals from dairy effluent.
- To evaluate the Environmental Impact Assessment studies.
- To evaluate the value added products from algal biomass.
- The significance of variance in the effluent should be checked by statistical analysis.
- Policy and managing dairy effluent.

2.0 MATERIALS AND METHODS

2.1 Description of collection site

Madhavaram dairy is a feeder balancing which procures about 1 lakh liter milk daily from Chennai and Tiruvalluvar Districts for marketing milk and milk products like ghee, paneer, butter, and cheese. It is located at Madhavaram which is 25 km from Chennai. The geographical location of the Madhavaram town lies within North Latitude 23°16’ and East Longitude 73°16’. The location of Madhavaram falls in the northwestern portion of Tamil Nadu, India (Plate.1). The Chennai city receives heavy industrial untreated wastewater, domestic primary treated wastewater and agricultural wastewater, and suffers much at present, from the intense pollution. Dairy effluent is one such pollutant which is organic in nature. It is rich in nutrients, nitrate and phosphate, which is source of enhanced algal growth in natural run off.

2.2 Collection of effluent samples

Effluent samples were collected from Madhavaram dairy plant (Plate.2), from the point of intake to the treatment plant and also from the discharge point of the effluent treatment plant (E.T.P) (Plate.3) which is located outside the premises. Collections were made as when they required. Effluent samples were collected in triplicates in plastic containers using standard methods of collection.

2.3 Products

The central dairy unit is manufacturing varieties of milk such as double toned milk, toned milk, full cream milk and standard milk.

2.3.1 The Manufacturing Process

Extended aeration system (agitation) was applied in the dairy industry and more than 90% of their wastes were discharged with wastewaters into the municipal sewer system. The reasons for pre-treatment are very different from those in other branches of industry. Here the biological stage may have to be protected from hazardous materials and less biodegradable substances may have to be eliminated at the point of origin. In the food industry, emphasis is placed on the load induction by removal of biodegradable insoluble and soluble organic matter either to avoid or reduce municipal load discharges and in particular process instabilities of downstream biological treatment plant.

2.3.2 Effluent Generation

The effluent treatment plant (ETP) consists of two tanks. Each tank 30,000 meters long and has the capacity of 4.5 lakh liters to store. The total effluent generated per day amounts to an average of 3 lakh liter depending on the production.

The waste generated from the dairy industry includes:

- Washing, cleaning of all pipelines, pumps, process equipments tanks, tankers, trucks, filling machines, milk cans, bottles, and floor.
- Startup, product change overhead shutdown and pasteurizers.
- Loss in filling operations through equipments and broken packages.
- Lubrication of casers, stackers and conveyors.

2.3.3 Flow from pasteurization plant

The flow from the pasteurization complex is derived from acid, alkali and water washings of the pasteurization tubing complex and are discharged into a pipeline buried below the gully gratings providing some possibility for a visual appreciation of the quantity and quality of such discharges inside the dairy unit building. The periodicity of rinsing is not rigid and it is obviously coupled with the need for such rinsing as arising from milk quality. However, on a general pattern, about two rinsing operations per day are reported to be of normal occurrence.

2.3.4 The flow from the packing bay

The flow from the packing and loading dock is almost continuous in nature. The spillages of milk from the sachet filling operations and washing of floors emanate at this location and flow out to an open drain on the southern side of the dairy plant to eventually find their way into an existing open tank in the dairy. The chief constituent of the wastewater flows arising from these sections is the emptying of the returned milk at certain times and contributes to an instantaneous upsurge in the wastewater's biochemical oxygen demand and especially the fat content which in turn imposes a severe shock load on the receiving waters.

2.3.5 Characterization of effluent

The characterization of effluent from foregoing sources with regard to their general origin, content, characteristics, segregation potential and specific nature with respect to the biological treatment of the effluent.

Among the biological treatments, trickling filter and activated sludge process involve more economy, high power requirement, more chemical consumption and large area requirement. The efficient and low cost treatment method included fixed film Bioreactor Technology, which possesses the advantage of low power consumption, small chemical consumption, small area requirement and high purification rates (Chaubey, 2001).

2.4 Treatment methods

Primary treatment involves screening (removal of unwanted large sized substances), skimming (removal of oil), coagulation (removal of colloidal substances), and sedimentation (removal of settleable solids). It is followed by the secondary treatment of the two biological methods, where aerobic methods are found to give better results. However, adjustment of pH is done (neutralization) prior to treatment. Among the several conventional aerobic treatment methods, activated sludge process, trickling filters, oxidation ditch, aerobic lagoon and waste stabilization ponds is reported to give maximum BOD reduction rate. Effluent of the dairy plant is highly polluting in nature, which may create environmental problems by affecting ground water quality.

The flow diagram of a typical dairy plant shows, that wastewater from dairy industry falls into 3 categories industrial waste, domestic waste and spent uncontaminated water (Fig.2). The last group includes water used in the refrigeration system for condensate and water used for pre-cooling various pasteurized products. Waste in this category contains various dilutions of the milk that enters the drainage system. In general the waste and their sources may be classified as follows.

- Rinsing and washing from cans, tanks, truck equipments, product pipelines and floors.

- Spillage, freeze-on overflow and leakage caused by improperly maintained equipment and poor operation practices.

- Spoiled or damaged raw or manufactured products or by-product that cannot be salvaged for other uses.

The wastewater of the plant is collected in a raw effluent treatment plant (ETP) for treatment. The raw effluent from in ETP (Amudha ***et al.,*** 1997; Richard ***et al***., 1984) found that wastewater of a dairy plant is highly polluting in nature, which may create environmental problems by affecting groundwater quality.

2.4.1 Biological treatment

Biological treatment appears to be the most promising technique, since dairy effluent have low COD: BOD ratio. The effluent also contain the required nutrients for microorganisms in sufficient quantities. The use of bacteria and other microorganisms to remove contaminants by assimilating them has long been a mainstay of wastewater treatment in the chemical processing industries (CPI). Because they are effective and widely used, many biological treatment options are available today. They are, however, not treated equally, and the decision to install a biological treatment system requires ample thought.

Wastewater generated from milk processing and milk product manufacturing units produce huge amount of dairy waste. Dairy waste is basically biodegradable and produces an undesirable odour and contains an appreciable quantity of oil. If untreated dairy waste is let into water bodies it will pollute the water, thereby causing several diseases to animals and mankind. Hence, it is required to treat this effluent before discharge into the water bodies. Activated sludge process can be employed very effectively for a complete treatment of dairy waste.

2.4.2 Treatment and disposal of Dairy wastes

The various sources of effluent from a dairy plant are listed below:-

- The waste is expelled from various stages of dairy
- Wastes from the pasteurization plant
- Wastes from manufacturing of butter
- Wastes from the manufacture of cheese
- Wastes from the casein plant
- Wastes from the washing plant
- Waste from the water softening plant and the boiler house
- Uncontaminated cooling water
- Quantity of effluent

2.5 Pollution effects of Dairy wastes

Dairy effluent decomposes rapidly and depletes dissolved oxygen of receiving streams immediately, resulting in anaerobic conditions and release of strong foul odours if disposed off without any treatment. The effect of effluent generated from a dairy industry in one way or other cause pollution of water and soil by disposing of land or into water bodies without treatment.

2.6 Conventional treatment methods – A Description

2.6.1 Methods adopted in Dairy effluent treatment

In the dairy industry, primary and secondary treatment methods are quite common in the effluent treatment processes, as they are efficient and dependable. The dairy effluent is predominantly organic in nature and due to its biodegradable constituents, it is amenable to conventional treatment. Fresh dairy waste is highly alkaline and turns to acidic due to the fermentation of lactose in to lactic acid. Due to these properties chemical treatment methods may not be appropriate. It is probably due to this reason that most of the existing dairies have treatment plants based on the activated sludge process. This type of treatment method is not effective in filtering the nutrients from the dairy effluent. Dairy effluent also contains sufficient nutrients for biological growth, biological treatment methods are considered more ideal and economical.

2.6.2 Effluent characteristics

The processing methods vary based on the characteristics of the effluent involved. Except for the use of detergents or (acid, alkali) cleaning agents, no specific chemicals are involved in the manufacturing process. Therefore, effluent consists of milk, milk products and enormous quantities of water. The pH of the effluent is acidic and the organic content is considerably higher. Also the effluent is rich in oil and grease.

2.6.3 Effluent analysis

The various physico-chemical parameters i.e. biochemical oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids, oil and grease, nitrogen, phosphorus, chloride and sulphate were analyzed according to American Public Health Association Standard Methods (APHA, 2005).

2.7 Isolation and identification of Cyanobacteria

The effluent was collected from the Central Dairy Plant (Aavin), Madhavaram, Tiruvallur District (Tamilnadu), India, where the effluent has been released from the factory. Standard microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary, 1959).

2.7.1 Media and Culture conditions

Algal samples were microscopically examined and plated on solid agar medium - BG11 (Rippaka ***et al.***, 1979) and CFTRI medium (Venkataraman and Becker, 1985, Singh ***et al***., 2002). The inoculated plates were incubated in the culture chamber (temperature maintained at 25 ± 2°C fitted with cool white fluorescent tube emitting 2500 lux for 18 hrs a day) and were regularly examined for the growth of cyanobacteria. Colonies appearing on solid medium were picked up and transferred to the liquid medium. By repeated streaking, unialgal cultures obtained and maintained in BG11 (Blue Green medium) (Rippaka ***et al.***, 1979) and CFTRI (Central Food Technological Research Institute), (Venkataraman and Becker, 1985; Singh ***et al.***, 2002) liquid medium and colonies of different morphologies were identified according to morphological characters. Identification of algae was made with the help of the monograph on Cyanophyta by Desikachary (1959) and Anand (1998).

The pure cultures were maintained by subculturing in 250 ml Erlenmeyer flasks- containing 100 ml of sterile BG-11 medium and CFTRI medium and incubated under fluorescent light (2500 lux) at a temperature of 25 ± 1°C. The cultures were harvested at 15 days after inoculation by centrifugation at 4000 rpm for 10 min. The dehydrated living algal pellet was used for the experiment.

2.7.2 Composition of BG-11 medium (Rippaka ***et al.***, 1979)

$NaNO_3$	01.5 g/l
K_2HPO_4	0.04 g/l
$MgSO_4.7H_20$	0.075 g/l
$CaCl_2\ 2H_20$	0.036 g/l
Citric acid	0.036 g/l
Ferric ammonium citrate	0.006 g/l
EDTA disodium salt	0.006 g/l
Solution with trace elements	11 ml

Trace Elements

H_3BO_3	2.86 g/l
$MnCl_2$ $4H_20$	1.81 g/l
$ZnSO_4$ $7H_20$	0.222 g/l
Na_2MO_3 $2H_20$	0.39 g/l
$CuSO_4$ $5H_20$	0.07 g/l
CO $(NO_3)_2$ $6H_20$	0.07 g/l
pH	7.1

2.7.3 Composition of CFTRI medium

(Venkataraman and Becker, 1985 - Modified by Singh ***et al***., 2002)

$NaHCO_3$	4.5 g/l
K_2HPO_4	0.5 g/l
$NaNO_3$	1.5 g/l
K_2SO_4	1.0 g/l
NaCl (Crude)	1.0 g/l
$MgSO_4$. $7H_20$	0.2 g/l
$CaCl_2$	0.04 g/l
$FeSO_4$	0.01 g/l
pH	7-10

2.8 Remediation bioassay

The cyanobacteria were inoculated individually or as mixtures into 20 ml culture medium and incubated for a week until the rich growth was obtained. For each species, as well as for each mixed culture, (20 ml of sterilized cyanobacterial medium in 250 ml conical flasks) were prepared and sterilized. Each of the flasks was inoculated with 2 ml/2 gm of the 1 week dense individual cyanobacterial suspensions, or with multiple species in the case of mixed cultures, incubated under the previously mentioned conditions and left to reach mid-late exponential phase of growth.

2.9 Light intensity measurement

Light intensity during the experiments was measured using a Lux meter (Lutron LX- 01A).

2.10 Microscopic Examination

The microalgal cultures were microscopically examined using an Olympus microscope (CH20***i***) and photomicrograph using Nikon digital camera (Coolpix, E8400).

2.11 Growth measurement

Growth was measured by counting cells using a haemocytometer (Neubauer, improved) and the results were plotted in a semi-logarithmic graph. Growth rate (divisions/day) was arrived at using the formula.

$$\frac{\text{Log } N - \log N_o}{\text{Log}_2 \times t}$$

Where,

N - No. of cells per ml at the end of log phase or mg weight/l

No - Initial count of cells per ml or mg weight/l

t - Days of log phase

For dry weight method, the algal cultures were pelleted by centrifugation at 6000 rpm (Remi cooling microfuge) for 15 minutes. Cells were washed with glass distilled water, again centrifuged and dried in an oven for 24 hours or until constant weight.

2.12 Growth monitoring

The growth rate was monitored to determine the stimulatory or inhibitory effect of pollutants on the tested cyanobacteria (their resistance or sensitivity) in order to define the most resistant and promising phycoremediation species. At each exposure time, triplicates of 2 ml from the three replicates of each sample were aseptically drawn from the control culture (pollutants free) and the six cultures were supplemented with pollutants (5&10ppm). The samples were centrifuged at 6000 rpm for 10 min to harvest cyanobacterial cells. In this case, supernatants were discarded and the chlorophyll-content in the pellets was extracted using the standard acetone extraction method. After extraction, chlorophyll-a was determined spectrophotometrically.

2.13 Analytical Methods

The physico-chemical properties of the dairy effluent i.e., untreated effluent (before treatment) and treated effluent (after treatment) were analyzed by standard methods for the examination of water and wastewater, APHA (2005).

2.13.1 Mineral Analysis

The mineral composition of algae and plants can be determined by atomic absorption spectrophotometer (Perkin–Elmer model 303). Samples were subjected to acid digestion and analyzed according to the procedure described by Farias ***et al***., (2001).

2.13.2 Evaluating the heavy metal reduction potential of Cyanobacteria in Dairy Effluent

The dairy effluent was inoculated with ***Aulosira laxa*** and ***Tolypothrix distorta*** for heavy metal sorption. The algal isolates were grown in untreated effluent without any basal nutrients. 2 gm of algal inoculums was added to each flask. The analysis of heavy metals (Cu, Cr, Zn, Pb, Ni, Cd, Ar and Hg) was done in the 15 days old culture. The heavy metal analysis was done using inductively Coupled Plasma - Optical Emission Spectrometer (ICP – OES, Perkin Elmer 5300 DV). The experiments were conducted in triplicates.

2.14 Biological characteristics

While sample for microbiological examination, the dairy effluent was collected in a pressurized paper wrapped BOD bottle kept in freezer boxes. Further, in the laboratory dairy effluent sample was stored at 4°C; it was processed for bacterial analysis within 24 hr from collection.

The dairy effluent samples were qualitatively and quantitatively analyzed for their micro flora following serial dilution technique. One milliliter of appropriately diluted sample was plated and incubated for taking bacterial counts.

2.14.1 Bacterial count

The bacterial count was taken using Nutrient Agar (NA) medium containing peptone (0.5), beef extract (0.3%), sodium chloride (0.5%) and agar-agar (1.5%). The pH was adjusted to 6.8. The plates were incubated at 37 ± 1°C for 24 hr.

One ml of untreated effluent sample was serially diluted and plated on a petridish containing nutrient agar and incubated at 37 ± 2°C for 24 hours. Well-grown colonies were isolated and purified on an agar plate count by the plate streaking method (Aneja, 1996). Further, purified bacterial strains were identified for its morphology, gram staining and other biochemical test as per Bergey's manual (1993).

2.14.2 Isolation and identification of Bacteria

Bacteria were isolated by standard method using nutrient agar (Difco Manual, 1953). The purified bacterial cultures were identified based on colony characteristics, gram staining methods and by various biochemical studies as given by Bergey and Buchanan (1974).

2.15 Growth conditions and selection of Cyanobacteria

Microalgae selection was done by considering their adaptation (cell density evaluation), followed by nitrogen and phosphorus scavenging and lipid content in the dry biomass. They were initially grown in suitable conditions as described earlier. Adaptation was evaluated by cell density evaluation in Neuberger counting chamber. Microalgae that appeared to be better adapted were scaled up to 2 l (working volume of 1.3 l) and cultivated in the same conditions described above. Samples were withdrawn periodically and centrifuged at 6000 rpm for 15 min. Cells were washed and dried at 60°C until constant weight was achieved for the determination of the growth parameters.

2.16 Treatment of Dairy effluent by Cyanobacteria

Inoculation was made by adding 2 gms of the initial inoculums in each flask containing 3 liters of effluent. The experiment was conducted under controlled conditions (green house) (temperature 26 ± 1ºC) with a light intensity of 4000 lux, for a total duration of 15 days. Samples were periodically (every 3rd day) analyzed for various physico-chemical parameters such as pH, carbonate, bicarbonate, calcium, magnesium, sodium, potassium, nitrate, free ammonia, sulphate, phosphate, silica, chloride, iron, BOD, and COD, using standard methods (APHA, 2005). Growth of algae in dairy effluent measured in terms of carotenoids (Jensen, 1978) and chlorophyll as the biomass components (Arnon, 1949). The effluent was milky and grayish black in colour; the growth of algae was very slow. However, the growth of algae was enhanced after seven days. So, fifteen days duration was provided to get an exponential growth of algae. The cultures were harvested by filtration through filter paper and washed repeatedly with distilled water. The filtered effluent (inoculated and control) were used for various physico-chemical analysis. Cyanobacteria obtained from effluent were used for the estimation of growth (estimation of total chlorophyll and carotenoid).

2.17 Biomass extraction

The biomass of cyanobacteria was washed briefly to remove the adhering medium components. To the biomass 2 ml of acetone was added, kept for 15 min and a mild sonication for 5 min was given. The supernatant was separated from the debris by centrifuging for 15 min at 2000 rpm. Then the extract itself was centrifuged for 15 min at 5000 rpm. The extract was collected in a storage vial, allowed for complete evaporation of solvent and resuspended with 1 ml of sterile water. The vial was stored in a refrigerator until further use.

2.17.1 Estimation of Chlorophyll

The amount of chlorophyll was determined by following the method of Arnon (1949). The plants and algal biomass were ground in 90% acetone and centrifuged at 3000 rpm for 10 minutes. The supernatant was diluted to 10 ml with one ml of distilled water and acetone and taken for measurement of optical density at 645 nm and 663 nm. For the determination of total chlorophyll following formula was employed.

$$\text{Total chlorophyll (mg/g)} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times w} \times V$$

Where, V = volume of the supernatant in ml

W = fresh weight of the sample

2.17.2 Estimation of Carotenoids

The extraction and estimation of carotenoids was carried out with the help of the method proposed by Jensen (1978).

Procedure:

10 gms of algal powder was taken in a test tube; 10 ml of 80% acetone was added to this and macerated in a mortar with pestle. Coloured homogenate was centrifuged and the filtrate was collected. Algal debris was again crushed

with acetone. This procedure was repeated till complete extraction was confirmed. Each time the filtrate was collected in a volumetric flask.

It was dried at 37ºC and then mixed with a solvent mixture containing equal volumes of petroleum ether and 10% methanolic KOH.

This mixture was kept in darkness for 2 hours for saponication. It was diluted with 30% aqueous NaCl in a separating funnel and shaken gently. It was allowed to stand for the complete separation into two layers.

The upper fraction, yellow in colour containing carotenoids was separated and washed with 3% aqueous NaCl. The final volume of petroleum ether extract was noted as V and optical density was taken at 450 nm against petroleum ether as blank.

Calculation:

Carotenoids were estimated by following equation Jensen (1978) and have been expressed as percentage of dried algal powder.

$$C = D \times V \times F \times 10/2500 \times W \times 100$$

Where,

C = Percentage of the carotenoids

D = Optical density at 450 nm

F = Dilution factor (if any)

W= Weight of dried algal powder

2.18 Biochemical analysis

Biochemical constituents such as proteins, total sugars, total lipids of the laboratory cultured algae, treated algae and plants were estimated following procedures as described below:

2.18.1 Estimation of protein

Principle

The carbonyl groups of protein molecules react with the copper potassium of the reagent to give a blue coloured copper potassium biuret complex. This complex together with tyrosine and phenolic compounds present in the protein reduce the phosphomolybdate of the folin reagent to intensify the colour of the solution (Lowry ***et al***., 1951).

Reagents

1N NaOH	4 grams of sodium hydroxide dissolved in 100 ml of distilled water.
0.1N NaOH	0.4 grams of sodium hydroxide dissolved in 100 ml of distilled water.
Reagent A	2 grams of sodium carbonate dissolved in 100 ml of 0.1N NaOH.
Reagent B	500 mg of cupric sulphate dissolved in 1% sodium or potassium tartarate.
Reagent C	50 ml reagent A added to 1 ml of reagent B.
2N Folin Reagent	Folin phenol reagent diluted with double the volume of double distilled water (1 : 1).
Standard	1 mg of Bovine serum albumin (BSA) dissolved in 1 ml of 1N NaOH, so that 1 ml solution contains 1 mg protein.

Procedure

Total protein content was estimated following the method of Lowry ***et al.***, (1951). Known quantity of the algae, plants and fish were homogenized in 10% TCA (trichloroacetic acid) to precipitate the protein. Then, the sample was centrifuged at 3000 rpm for 5 min, and the supernatant was decanted. The precipitate was redissolved in 1 ml of 1N NaOH solution. To this 5 ml of reagent C was added and mixed well, and allowed to stand for 10 minutes. To this solution 0.5 ml of folin-phenol reagent was added and mixed rapidly and allowed to stand for 30 minutes. 1N NaOH solution and 1% bovine serum albumin (W/V) were used as blank and standard solution respectively. The blue colour was developed and read at 520 nm using U.V-spectrophotometer.

2.18.2 Estimation of total free sugars

Principle

Sulphuric acid in anthrone reagent hydrolyses di and oligosaccharides into monosaccharide and dehydrates all monosaccharide into furfural or furfural derivatives. These two compounds react with a number of phenolic compounds and one such compound is anthrone, which produces a complex coloured product. The intensity of which is proportional to the amount of sugar present in the sample (Roe, 1955).

Reagents

Anthrone reagent	:	200 mg of anthrone was dissolved in 100 ml of 95% ice cold sulphuric acid.
Glucose standard	:	100 mg of D-glucose dissolved in 100 ml of saturated benzoic acid.
5% Trichloroacetic acid (Deprotenising agent)	:	5 gm of TCA dissolved in 100 ml of distilled water

Procedure

Total free sugars were estimated following the method of Roe (1955). Known quantity of the algae and plants were homogenized in 2 ml of 5% TCA and homogenate was centrifuged at 2500 rpm for 5 min. To the supernatant 4 ml of anthrone reagent was added and kept in mild water bath for 10-15 min. After that it was allowed to cool in dark at room temperature for 30 minutes. The colour developed was read at 620 nm using U.V-spectrophotometer.

2.18.3 Estimation of total lipid

Principle

This method is based on the sulphophosphovanilin reaction of charbroil and charonn as described by Zollner and Kirsch (1962) which depends on the reaction of lipids with sulphuric acid, phosphoric acid and vanillin to give a red complex.

Reagents

Chloroform: Methanol	:	20 ml of chloroform added to 10 ml of methanol (2 : 1).
0.9% NaCl	:	900 mg of sodium chloride dissolved in 100 ml of distilled water.
Phosphovanillin Reagent	:	800 ml of orthophosphoric acid added to 100 ml of distilled water and shaken well. To this 2 gms of vanillin added.
Standard	:	1 mg of cholesterol added to 1 ml of chloroform: methanol (2 : 1) solution.

Procedure

Total lipid was quantified following the method of Barnes and Black Stock (1973). Extraction of the lipid of the sample was done following the procedure of Folch ***et al***., (1957). Known quantity of algae and plants were homogenized in 5 ml of chloroform: methanol mixture to which 0.5 ml of 0.9% NaCl was added and shaken well. This mixture was transferred to separating funnel and allowed to stand for 12-14 hrs. The lower phase containing lipid was collected in a test-tube and the volume was made up to 5 ml with chloroform. Aliquot of 0.5 ml of lipid sample was taken and allowed to evaporate. The individual dry samples were digested with 0.5 ml of concentrated H_2SO_4 in boiling water for 15 minutes. A volume of 0.2 ml of acid digest was taken to which 5 ml of phosphovanillin reagent was added. The mixture was allowed to stand for 30 minutes. The resultant pink coloured complex was measured spectrophotometrically at 520 nm. Chloroform and cholesterol were used as blank and standard for lipid estimation respectively.

2.18.4 Amino acid analysis by high performance liquid chromatography (HPLC) (Yamamoto ***et al***., 1994).

Principle

The peptides with N-terminal primary amines get derivatized by orthopthaldehyde (OPA) and are separated by reverse phase chromatography (Shimatzu-High Performance Liquid Chromatography LC 6A). Separation of the sample components occurs in the column by interaction between the sample components of the stationary and the mobile phase.

The reverse phase separation involves a sample partition between two immiscible liquid phases, one non-polar station such as C_{18} and the other polar mobile such as acetonitrate.

Separation of any protein mixture or peptides depends upon the strength of the hydrophobic interactions of each component in the mixture with the hydrophobic surface of the column matrix and the elution strength of the organic solvents in the mobile phase. As the concentration of the organic solvent increases the interaction between the peptides or protein and the column matrix diminish and elution of the polar or ionic solutes occurs first, followed of the elution of non-polar solutes. The techniques are reliable with enhanced sensitivity and could detect amino acids using a variable wavelength detector without interference (Yamamoto ***et al***., 1994).

Reagents

Mobile phase A

A stock solution of ethylene diamine tetra acetic acid (EDTA) was prepared by dissolving 200 mg of EDTA, in 5 ml of HPLC grade water. Sodium acetate trihydrate (1.36 gm), EDTA stock (500 µl) and sequencer grade triethylamine (TEA) (90 ml) were added to 500 ml HPLC grade water. The pH was adjusted to 7.20 ± 0.05 with 1-2% acetic acid. To this 1.5 ml of unstabilized, UV grade tetrahydrofuran (THF) was added and the pH was verified.

Mobile phase B

Sodium acetate trihydrate (1.36 gm) was dissolved in 100 ml of HPLC grade water and pH was adjusted to 7.20 ± 0.05 with 1-2% acetic acid. To this 200 ml of methanol and 200 ml of acetonitrile was added and mixed well.

OPA reagent

The reagent for amino acid derivatization was prepared by dissolving 5 mg of anhydrous O-phathaldehyde (OPA) in 2 ml of methanol. Borate buffer (8 ml) and 50 µl of 2-mercaptoethanol were added to it.

Preparation of Sample

Initially the samples were homogenized in 5 ml of chloroform: methanol mixture (2 : 1) to which 0.5 ml of 0.9% NaCl was added and shaken well. This mixture was transferred to separating funnel and allowed to stand for 12-14 hrs to remove all the lipids. The protein precipitated below the lipid layer was collected. Further 6 ml of acetone was added to the sample and kept in hot air oven for 1 hr at 40º C. The solvent was decanted and the sample was allowed to precipitate. The samples were then subjected to acid hydrolysis by treating them with 6N HCl at 100ºC for 5 hr. The dried amino acid sample was dissolved in 50 µl of HPLC grade water.

Procedure

Derivatization of amino acid sample

20 µl of OPA was added to the vial containing 20 µl of the amino acid sample and thoroughly mixed and kept for two minutes. After this, 50 µl of borate buffer was added and mixed well.

Sample application

Filtered and derivatized amino acid sample of 20 µl was injected into the Shimadzu amino acid analyzer containing a C_{18} reverse phase column and samples were analyzed using sodium acetate buffer with THF and TEA and sodium acetate with methanol and acetonitrite as solvent systems.

Operating conditions

The following gradient programme was done to achieve the effective separation of amino acids from the sample.

Column: - C_{18} reverse phase, ion exchange chromatography.

Column temperature: 40ºC

Initial Flow rate = 0.450 ml/min

Time	A%	B%	Flow rate
0	100	0	0.450 ml/min
17.00	40	60	0.450 ml/min
18.00	0	100	0.450 ml/min
18.50	0	100	0.800 ml/min
23.90	0	100	0.800 ml/min
24.00	0	100	0.450 ml/min
25.00	100	0	0.450 ml/min

Detector parameters:

Time	Signal A	Reference	Bandwidth
0.0 min	338 nm	510 nm	10 nm
14.80 min	266 nm	370 nm	16 nm

A variety of amino acid standards were injected simultaneously. By comparing the sample retention time with (R_t) with the time of the standard amino acids run at identical condition, the amino acids present in the sample were identified and quantified.

2.18.5 Estimation of vitamins (Margherita Grotzkyj Giorgi *et al.*, 2012)

Chromatographic Conditions

An Agilent 1100 chromatographic system (AOAC, 1995) was used for the analysis and quantification of vitamins in the algal samples. The chemstation software controlled the whole chromatographic system.

To the dry powdered algal biomass, 100 mM perchloric acid: acetonitile (92 : 1 v/v) solution was added and the left in a water bath at 50ºC for 30 minutes. The resulting solution was centrifuged at 6000 x g and the upper layer was used for HPLC analysis. HPLC system (Schidadzu) equipped with UV-detector was under the following analytical conditions for the estimation of vitamins B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_{12}.

Vitamins were separated on a reversed-phase chromatographic column MetaChem Polaris C18-A (5 μm, 250 mm x 2.1 mm i.d.,) fitted with a precolumn (MetaGuard column, C_{18}-A) using combined isocratic and linear gradient elution with a mobile phase consisting of acetonitrile / 100 nm, sodium phosphate buffer (pH 2.1) 0, solvent A) and 0.8 mM sodium octane sulfonate (9 : 1 v/v) (solvent B). Linear gradient profile (A : B) started at 90 : 10 and it was kept constant for the first 4 minutes, then linearly decreased up to 2 : 98 during the next 6 minutes, then it was kept constant in the next 20 minutes and finally linearly increased up to 90 : 10 in the last 5 minutes of separation. Total run time was 30 minutes. This gradient was utilized for temperature studies; subsequently, the timing of gradient was modified to suit the reduced analysis time. The flow rate was adjusted to 1.0 ml / min, injection volume was 3 μL, and column temperature was kept constant at 40°C. Detection was performed with a photodiode array detector monitoring the eluent at 280 nm; however, quantification was performed at the maximum wavelength for each vitamin as follows: 254 nm for ascorbic acid, 254 nm for thiamine, 254 nm for riboflavin, 254 nm for nicotinamide, 254 nm for pantothenic acid, 254 nm for pyridoxine, 280 nm for folic acid, and 230 nm for cyanocobalamin. Identification of resolving peaks in real samples was executed by comparing their spectra with those derived from aqueous standard solutions.

2.18.6 Estimation of vitamin-A (Woollard and Indyk, 1986**)**

Known quantity of the dry powdered algal sample was saponified with ethanolic KOH for 30 minutes and transferred to a separating funnel and repeatedly with n-hexane. The final pooled extract was evaporated to dryness under reduced pressure in a rotary evaporator and vitamin-A level was determined by HPLC as described below under the following analytical condition.

Column	:	STR ODS-II (4.6* 150)
Mobile phase	:	methanol: distilled water (95 : 5 v/v)
Flow rate	:	1.0 ml/min
Temperature	:	40°C
Detection	:	UV / 210 nm

Standards Preparation

The aqueous stock solutions of water-soluble vitamins (B_1, B_2, B_3, B_5, B_6, B_7 as pyridoxal phosphate [5′-PLP], B_9, B_{12}, coline, nicotinic acid, vitamin-C, vitamin-A) were prepared by weighting 10 mg of each vitamin in a volumetric cylinder in 100 mL of ultrapure water (Maxima water, USF Elga, High Wycombe, UK) containing 0.01% of trifluoroacetic acid (TFA). Vitamin B_2 was prepared by weighting 5 mg and subsequently added to the multivitamin solution (final concentration of riboflavin was 50 ng / µl). After brief agitation, the solution was transferred by pouring into an amber-glass bottle for storage at +4°C. The final concentration of each vitamin was 100 ng / µl (except vitamin B_2 which was 50 ng / µl). Vitamin B_9 solution was prepared by weighting 5 mg of powdered Vitamin B_9 in a volumetric cylinder and dissolved in 100 mL of 1M $NaHCO_3$. All solutions were stored in a refrigerator in amber-glass bottles to protect vitamins from light-induced oxidation. Working standard solutions were prepared fresh daily from stock solutions. The final concentration of water-soluble vitamins standards ranged from 0.25 to 25 ng / µl (six concentration levels). Theobromine (internal standard) was used at a concentration of 2 ng / µl.

Sample Preparation

Experiments were carried out to identify a sample preparation procedure that would allow simultaneous detection of seven water-soluble vitamins in biological samples. Aliquots of one spiked plasma sample (final concentration of each water-soluble vitamins: 20 ng / µl) were concomitantly processed following one of these three procedures: deproteinisation with 400 µl pure acetonitrile followed by solid phase extraction. The solid phase extraction method was obtained from Chatzimichalakis ***et al***., (2004). The second methodology involved liquid-liquid extraction method [600 µl n-hexane + 150 µl ethanol:methanol, 95 : 5 v/v] with no solid phase extraction. The third methodology involved deproteinisation with 600 µl ethanol : methanol, 95 : 5 v/v followed by solid phase extraction procedure as per Chatzimichalakis ***et al***., (2004). Experiments were run in triplicate.

1. The experiment was performed following the procedure reported in Chatzimichalakis ***et al***., (2004) with the minor modification of using 400µl of pure acetonitrile in a 1 : 1 ratio (v/v) to fresh or freshly thawed plasma previously spiked with internal standard (theobromine, 2 ng / µl).

2. Further it was carried out by transferring to a glass analysis tube 400 µl of fresh or freshly thawed plasma containing IS. Six hundred micro liters of pure n-hexane were added and the tubes were briefly vortex mixed. The addition of hexane was used to extract lipid-soluble matrix components that may interfere with vitamin analysis. Tubes were then centrifuged at 4,000 rpm for 5 minutes at 4°C. After centrifugation was complete, 150 µl of ethanol : methanol (95 : 5 v/v) was added to the tubes and centrifuged at 23000 rpm for 15 minutes at 4°C. The upper layer (organic phase) was discarded. The lower layer (aqueous phase) was collected and placed in a new, capped micro centrifuge tube. Tubes were placed in a SpeedVac instrument (Thermo Scientific, Fisher Scientific, Loughborough, UK) to

dry. When the supernatant from the aqueous phase was completely dried, samples were resuspended in two HPLC vials containing 0.01% TFA in water. No solid phase extraction procedure was carried out prior to HPLC injection.

3. The supernatant was added 600 μl of ethanol : methanol (95 : 5 v/v) to 400 μl of fresh or freshly thawed plasma. Samples were vortex - mixed briefly (30 seconds) and samples were centrifuged at 15000 rpm for 15 minutes at 4°C. Supernatant was carefully collected and placed in a new capped micro centrifuge tube and placed in a SpeedVac instrument. When dry, samples were resuspended in water:methanol (50 : 50 v/v) before applied to the solid phase cartridge. Solid phase extraction was carried out following the procedure reported by Chatzimichalakis ***et al.***, (2004).

Solid-Phase Extraction Procedure

Solid-phase extraction (SPE) procedure was carried out following the protocol described by Chatzimichalakis ***et al.***, (2004) with no modifications.

2.18.7 Antibacterial Assay

Preparation of sample

The samples were collected and allowed to dry under the shade completely. The dried samples were ground into powder, which was used for further extraction.

Extraction and Drying

About 1 gm of dry sample powder was weighed and macerated with 15 ml of methanol separately and kept overnight in a shaker Doan ***et al.,*** (2000). The extract was collected after filtration using Whatman No.1 filter paper and was stored. Another 15 ml of solvent was added to the residual mixture and incubated in shaker for 24 hours and the extract was collected again using a Whatman No.1 filter paper. This procedure was repeated once again and the extract was evaporated below 40°C, which was used for antibacterial assay.

Yield

The yield obtained from (***Aulosira laxa*** and ***Tolypothrix distorta***) of methanol extract:

Aulosira laxa	:	0.60 g (untreated effluent)
Tolypothrix distorta	:	0.304 g (untreated effluent)
Aulosira laxa	:	0.398 g (Treated effluent)
Tolypothrix distorta	:	1.846 g (Treated effluent)

2.18.7.1 Antibacterial activities of *Aulosira laxa* and *Tolypothrix distorta*

Principle

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's and the agar well diffusion assay method is used for quantifying the ability of antimicrobial agents to inhibit bacterial growth. The assay is performed on Mueller Hinton Agar as it helps in better diffusion of antibiotics.

Materials required

1. Sample untreated effluent ***Aulosira laxa***
2. Sample untreated effluent ***Tolypothrix distorta***
3. Sample treated effluent ***Aulosira laxa***
4. Sample treated effluent ***Tolypothrix distorta***
5. Positive control
6. Microbial Strain - ***Escherichia coli*** and ***Staphylococcus aureus, Bacillus subtilis*** and ***Pseudomonas aeruginosa***
7. Mueller Hinton agar (MHA)

1. Procedure

1. Mueller Hinton Agar (MHA) was prepared, autoclaved and poured into sterile petriplates and allowed to solidify.

2. A sterile swab was dipped into the culture inoculums tube (***Escherichia coli*** and ***Staphylococcus aureus, Bacillus subtilis*** and ***Pseudomonas aeruginosa.***

3. The swab was rotated against the side of the tube (above the fluid level) using firm pressure, to excess fluid was removed. The swab should not be dripping wet.

4. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; the plate was rotated approximately 60 degrees each time to ensure an even distribution of the inoculums.

5. Rim the plate with the swab to pick up any excess liquid.

6. The swab was discarded into an appropriate container.

7. Using gel punch well was punched to load the sample to be tested for antimicrobial activity.

8. An appropriate quantity (20 ***μ***l) of the sample (A, B and C) was added to each well including positive control.

9. The plates were incubated for 18-24 hrs and observed in the zone of inhibition.

2.18.8 Antioxidant activities of *Aulosira laxa* and *Tolypothrix distorta*

Preparation of algal extract

The thoroughly washed samples of cyanobacteria were collected to remove extra moisture. The sample was ground in a mortar and pestle along with a pinch of sand for the preparation of the extract in water and organic solvent viz., methanol (Dubey ***et al***., 2001). The extracts were filtered with Whatmann No.1 filter paper and the final volume of the extract was made up to 100 ml by adding the respective solvent and this was considered as 100 percent extract (w / v). The extract was then diluted to 50 ml solvent.

2.18.8.1 DPPH radical activity

DPPH scavenging activity was measured by the spectrophotometric method (Sreejayan ***et al.,*** 1996). To a methanolic solution of DPPH (200 ***μ***M), 0.05 ml of the test compounds dissolved in methanol was added at different concentrations (100 - 900 **μ**g / ml). An equal amount of methanol was added to the control. After 20 min, the decrease in the absorbance of the test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula (Prasanth ***et al.,*** 2000). The experiment was repeated in triplicate.

$$\text{Inhibition (\%)} = \frac{\text{(Control - test)}}{\text{Control}} \times 100$$

2.18.8.2 ABTS radical cation decolorisation assay (Sun ***et al***., 2007).

In this improved version, ABTS, the oxidant is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-sulfonic acid) - ($ABTS^{2-}$). ABTS radical cation was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use (Sun ***et al***., 2007). For the study, different concentrations (100 - 900 **μ**g / ml) of methanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1.0 ml. The absorbance was read at 745 nm and the percentage inhibition calculated.

$$\text{Inhibition (\%)} = \frac{\text{(Control- test)}}{\text{Control}} \times 100$$

2.19 Environmental Impact Assessment study

Seed germination and fish feed studies were conducted. Apart from this, various biochemical parameters such as pigments were also analyzed.

2.19.1 Effect on seed germination and seedling growth

Seeds of black gram (***Phaseolus mungo***, L (Vampani variety) were obtained from the Government seed Depot, Chennai-32, and was preserved in an incubator at 19ºC. The seeds were surface sterilized with 0.1% $HgCl_2$ for 3 to 5 minutes and then thoroughly washed with distilled water. For each algae and target species and each extract, the mud pots was prepared in triplicate. The mud pots were filled with soil. In each pot 25 seeds of target species and treated dairy effluent were placed to observe the effect of effluent on the germination behavior of seeds. The experiments were done in a greenhouse and were carried out for a period of 7 days. In each pot 10 ml of treated dairy effluent was added. Emergence of radicle was considered as the index of germination. The growth and development of the seedlings were observed by analyzing various biochemical parameters. Chlorophyll (Arnon, 1949), carotenoids (Jensen, 1978) proteins (Lowry ***et al***., 1951), total free sugars (Roe, 1955), and total lipids (Folch ***et al***., 1957) were estimated in untreated effluent, treated effluent with algae and control samples.

2.20. Toxicity testing of dairy effluent through *Hypophthalmicthys molitrix* (Silver carp)

Hypophthalmicthys molitrix (Silver carp) was collected from the Tamil Nadu Fish seed Farm, Poondi using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water (temperature 28 ± 1ºC; pH 8.0; DO ˜ 7.0 ± 0.3 mg/l) with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish feed (aqua fine). The water in the aquarium tank was siphoned off every day to remove the feed and faecal matter remains and made up with fresh aerated water.

Toxicity study was conducted for a period of 7 days in the laboratory. 2gms of ***A. laxa*** and ***T. distorta*** were feed to fishes once in a day. The number of the organisms used in the experiment was maintained and checked every day. From fish, their number, length, width, weight and survival of the organism was taken. At the end of the experiment fish were examined for biochemical parameters.

2.20.1 Preparation of sample for Biochemical analysis

The total carbohydrates, proteins and lipid content of fish grown in control, untreated and treated with algae were estimated following Roe ***et al***., (1957); Lowry ***et al***., (1951) and Folch ***et al.,*** (1957) respectively. ***Hypophthalmicthys molitrix*** (Silver carp) maintained in media containing algal feed, and control was homogenized with 5% and 10% TCA and centrifuged. The supernatant was analyzed for various biochemical analyses as described elsewhere (Section 2.18).

2.21 Statistical analysis

The results of the physico-chemical parameters, heavy metals, bacteriological examination and biochemical composition were analyzed using SPSS 16.0. T-test was used for further testing the similarity or difference between the variations of different biochemical constituents of the organism, to the level of significance set at $P \leq 0.05$. Triplicate assays were performed for each set of test conditions.

3.0 RESULTS

3.1 Description of the Dairy effluent

In the present study, dairy effluent was collected from Central Dairy plant (Aavin), Madhavaram, Chennai. Both untreated (control) and effluent treated with cyanobacteria were analyzed for various physico-chemical parameters, heavy metals and microbiological examination to assess the water quality. The results of the various physico-chemical parameters were presented in the tables (Tables.3 to 10).

3.2 Microflora in the Dairy effluent

Dairy effluent collected from the factory premises was examined for the microbial load. It contains both microalgae and bacteria. The following microalgae species were present in the effluent : ***Chroococcus turgidus, Spirulina platensis, Oscillatoria animalis, Stigonema turfaceum, Scytonema multiramosum***, ***Closterium ehrenbergii, Chlorella vulgaris, Scenedesmus bijuguadus, Pithophora polymorpha, Navicula ambigua*** and bacteria present in the effluent are ***Streptoccocci*** sp. and coli forms.

3.2.1 Isolation of Cyanobacteria from the Dairy effluent

Among the various microalgae the blue green algae alone acclimatized well with dairy effluent. Hence, they were selected for the treatment process. The cyanobacteria present in the effluent were isolated by serial dilution and standard plating techniques. Then they were cultured in BG-11 and CFTRI medium. Except ***Stigonema turfaceum*** and ***Hapalosiphon welwitschii*** other cyanobacteria grew well in the laboratory conditions. ***Chroococcus turgidus, Oscillatoria animalis*** and ***Chroococcidiopsis indica*** grew in both BG-11 and CFTRI medium.

3.2.2 Feasibility study

A feasibility study was conducted by growing various cyanobacteria in the dairy effluent (untreated) which includes the native cyanobacteria present in the effluent as well as the cyanobacterial species from the Algal Culture Laboratory, Pachaiyappa's College, Chennai (Plates. 5 to 7). The algae employed for the study were (Table.2; Plate.4);

Isolates of Cyanobacteria from Dairy effluent

1. ***Chroococcus turgidus*** (Kutz.) Nag.
2. ***Spirulina platensis*** (Nordst.) Gomont.
3. ***Oscillatoria animalis*** Ag.ex. Gomont.
4. ***Scytonema multiramosum*** Gardner.
5. ***Stigonema turfaceum*** (Berk.) Cooke ex Born.et Flah.

Cyanobacteria species from Algal Culture Laboratory

6. ***Chroococcidiopsis indica*** Desikachary.
7. ***Phormidium ambiguum*** Gomont.
8. ***Cylindrospermum licheniforme*** Kutz ex Born.et Flah.
9. ***Nostoc muscorum*** Ag. ex Born.et Flah.
10. ***Anabaena variabilis*** Kutzing ex Born.et Flah.
11. ***Aulosira laxa*** Kirchner ex Born.et Flah.
12. ***Tolypothrix distorta*** Kutzing ex Born.et Flah.
13. ***Calothrix membranacea*** Schmidle.
14. ***Hapalosiphon welwitschii*** W.et G.S. West.
15. ***Fischerella ambigue*** Nag.Gom.

Since, ***Aulosira laxa*** and ***Tolypothrix distorta*** taken from the culture function efficiently in phycoremediation for all experimental purpose this has been taken for the present investigation.

3.2.3 Selection of Cyanobacteria for phycoremediation of Dairy effluent

The green-cell factories of microalgae tackle simultaneously more than one problem, a solution not capable by conventional chemical processes. Microalgae are naturally occurring living organisms and therefore phycoremediation is a naturally occurring phenomenon. The microalgae used in phycoremediation are already present in nature and are at work consuming unwanted materials. The problems such as pH correction, sludge removal, TDS reduction, BOD and COD removal, etc., can be handled simultaneously by phycoremediation treatment, whereas in conventional methods, separate methods or stages of treatments are used. We come into contact with them on a daily basis with no ill effects. After phycoremediation is completed, the environment is virtually restored to its pristine condition. So that in the present study cyanobacteria was selected for dairy effluent treatment.

Cyanobacterial activity and sensitivity usually limit the removal rate of hazardous pollutants in algal-bacterial system. In the present study, various species of freshwater cyanobacteria were inoculated individually and as well as algal consortium into the dairy effluent. The observations were made and the results were represented in the Tables.3 to 10.

It is important to select fast growing and highly resistant cyanobacteria for the feasibility study were carriedout. Among the 15 species of cyanobacteria better growth rates were exhibited by ***Phormidium ambiguum***, ***Anabaena variabilis***, ***Aulosira laxa***, ***Tolypothrix distorta*** and ***Calothrix membranacea*** as they were able to reach the logarithmic phase within 5 days, whereas the other cyanobacteria showed slow growth. Further, screening was made among the five species, for phycoremediation to occur at a faster rate and the organisms with better growth rates were preferred. The cyanobacteria were selected based on the removal efficiency of physico-chemical parameters of short duration of reduction. The success of the phycoremediation is influenced by many factors and one of them is the identification of suitable algal species for remediation trials. Therefore, preliminary ***in vivo*** studies were conducted using two cyanobacteria, ***Aulosira laxa*** and ***Tolypothrix distorta***. The selected cyanobacteria are heterocystous forms and they have more metabolic activity.

The following treatments were employed in order to study the interaction of cyanobacteria with dairy effluent. i) Effluent without cyanobacteria (control) and ii) Effluent treated with cyanobacteria. Experiments were conducted in the duplicates.

3.2.4 Growth rate of Cyanobacteria

The cell suspensions of cyanobacteria were inoculated in dairy effluent without adding any nutritive medium. The cultures were incubated for 15 days; division rates were calculated from the initial and final by dry weight (Table.11a and 11b). The growth patterns of all the cyanobacteria were compared. Growth rates were higher in ***Aulosira laxa, Tolypothrix distorta, Calothrix membranacea, Phormidium ambiguum*** and ***Anabaena variabilis;*** moderate in algal consortium, ***Scytonema multiramosum, Nostoc muscorum, Oscillatoria animalis, Stigonema turfaceum*** and lower in ***Hapalosiphon***

welwitschii, Cylindrospermum licheniforme, Fischerella ambigue, Spirulina platensis. In the case of coccoid forms the cell growth measured by means of division/day the ***Chroococcus turgidus*** and ***Chroococcidiopsis indica*** showed moderate growth (0.0224 and 0.0553). When compared to control, the growth rates of the tested organisms were high. But on the whole, ***Aulosira laxa*** and ***Tolypothrix distorta*** were considered a better option due to the stability of its cell morphology and size in the effluent. On the contrary, the size of ***Stigonema turfaceum***, ***Hapalosiphon welwitschii*** and ***Phormidium ambiguum*** cells diminished with the dairy effluent. Hence, for further lab experiments, two cyanobacteria ***Aulosira laxa*** and ***Tolypothrix distorta*** were selected.

3. 3 Laboratory study

In the present study fifteen genus comprising fifteen species of cyanobacteria falling within seven families were identified. Out of the fifteen species of cyanobacteria two species were selected for further effluent treatment processes. Actively grown filaments of ***Aulosira laxa*** and ***Tolypothrix distorta*** were harvested and the mat was washed and resuspended in 3 liters of the effluent in a container and incubated for a period of 15 days. The results of the dairy effluent, before (control) and after treatment with cyanobateria of various physico-chemical parameters are presented in Tables 12 and 13.

3.3.0 Physico-chemical analysis

3.3.1 Colour

In the present investigation the colour of the untreated dairy effluent was milky and grayish black with a disagreeable odour which may be due to decomposition of organic matter or presence of various aromatic and volatile organic compounds, and it may also be due to microbial activity. When the effluent was treated with cyanobacetria the colour changed to yellowish green on the 3rd day. On the 15th day the colour changed to green (Plates.8 and 9). The odour of the effluent had an offensive smell which eventually changed to algal smell. Photosynthetic activity reduced due to dark colouration and aquatic ecosystem is totally changed. Colour also affects the other parameters like temperature, DO and BOD.

3.3.2 Temperature

Temperature is an important parameter, its effects on certain chemical and biological reactions taking place in water and in organisms inhabiting aquatic media and will depends upon the seasons and time of sampling. In the present investigation, the temperature of untreated effluent is 28°C and treated effluent is 26°C. The temperature of the effluent had a general conformity with atmospheric temperature.

3.3.3 Turbidity

The turbidity depends upon the strength of wastewater. The stronger or more concentrated the waste, the higher is the turbidity. The effluent contains organic and inorganic substances; organic matter induces the proliferation of microbial flora which in turn enhances the turbidity. The turbid water is always associated with water pollution especially water borne diseases. Initially the turbidity was 154.1 NTU (Nephelometric Turbidity Units). From 3rd day onwards there was a significant reduction of turbidity occurred and on the 15th day the maximum reduction of turbidity was observed in ***Tolypothrix distorta*** (86.34%) and minimum in ***Aulosira laxa*** (84.73%). (Tables.12 and 13. Fig.3a).

3.3.4 Total suspended solids

Suspended solids do not mean that they are floating matters and remain on top of the water layer. They are under suspension and remain in the water samples. Total suspended solids play an important role in water and wastewater treatment. Their presence in water sample causes depletion of oxygen level. Initially the total suspended solids were 160 mg/l. On the 15th day total suspended solids was reduced to 38% in ***Aulosira laxa*** and it was 17.47% in ***Tolypothrix distorta*** (Tables.12 and 13; Fig.3b). The value of total suspended solids lies above the permissible limits laid down by BIS (Bureau of Indian Standards) (100 mg/l).

3.3.5 Total dissolved solids

The total solid concentration in the effluent represents the colloidal form and dissolved solids. The probable reason for the fluctuation in value of total solid and subsequently the values of dissolved solids are due to content collision of these colloidal particles. Total dissolved solids were found due to the presence of various kinds of minerals and organic substances, which are generally found in polluted water, may also contribute to the dissolved solids. In the present study, the total dissolved solids of the dairy effluent were 1028 mg/l. From 3rd day onwards there was a reduction of total dissolved solids. The reduction was maximum in ***Tolypothrix distorta*** (90.10%) and minimum in ***Aulosira laxa*** (49.86%) (Tables.12 and 13; Fig.3c). The value of total dissolved solids lies in the permissible limits lies down by BIS (2100 mg/l). Statistically significant variation ($p<0.05$) was observed in total dissolved solids between control and treated effluent.

3.3.6 Total solids

The term solid refers to the matter either filterable or in filterable that remains as residue upon evaporation and subsequent drying at a defined temperature employed for drying and ignition. The total solids are the sum of the values of the total dissolved solids and that suspended solids. The solid variation in proportions with temperature and rarely varied inversely with the water level. In the present study, the concentration of total solids was 1304 mg/l in control. From 3rd day onwards reduction of total solids was observed. On the 15th day the total solids were significantly reduced by cyanobacteria. The maximum reduction of total solids was observed in ***Tolypothrix distorta*** (90.82%) and the minimum was observed in ***Aulosira laxa*** (90.42%) (Tables.12 and 13; Fig.4a). The value of total solids lies above the permissible limits laid down by BIS (100 mg/l). Statistically significant variation ($p<0.05$) was observed in total solids between control and treated effluent.

3.3.7 Electrical conductivity

Electrical conductivity is an essential criterion in determining the suitability of water for drinking. Conductivity is a measure of the capacity of a substance or solution to conduct the electric current. It is a reciprocal of resistance. Presence of salts and contamination with ionic moieties increase conductivity. In the present study, electrical conductivity was observed at 1579 μS/cm in control. On the 15th day conductivity was reduced maximum in ***Tolypothrix distorta*** (52.18%) and minimum in ***Aulosira laxa*** (15.58%) (Tables.12 and 13; Fig.4b). The high level of electrical conductivity in the untreated effluent could be qualified for the use of inorganic chemicals. The value of electrical conductivity lies in the permissible limits is not laid down by BIS (2009).

3.3.8 pH

The pH is a measure of intensity of acidity or alkalinity and measures the concentration of H^+ ions. It is one of the most important physico-chemical parameter. It affects soil quality and also affects microorganism activities.

In effluent treatment, pH is an important criterion for various purposes like, coagulation, disinfection, water softening and corrosion control. In biological treatment methods adjustment of pH is favourable for the microbial activity. In the present study, the initial pH of the untreated effluent was 5.15, the sample is slightly acidic, and the waste becomes acidic due to decomposition of organic matters under aerobic condition. When the dairy effluent was treated with cyanobacteria, ***Aulosira laxa*** and ***Tolypothrix distorta*** there was a gradual increase in pH (8.48) was observed (Tables.12 and 13; Fig.4c). The value of pH lies in the permissible limits laid down by BIS (6-9).

3.3.9 Total Alkalinity

Alkalinity is the capacity of waste to neutralize the strong acid, depending upon the capacity of hydroxyl ions to combine with hydrogen ions. The hydroxide carbonate and bicarbonates determine the total alkalinity. The initial alkalinity level was 550 mg/l in control. When the dairy effluent was treated with cyanobacteria, there was a gradual reduction of bicarbonate on 3rd day onwards. On the 15th day the reduction percentage of alkalinity was high in

Aulosira laxa (70.72%) and low in ***Tolypothrix distorta*** (63.14%) (Tables.12 and 13; Fig.5a). The value of alkalinity lie in the permissible limits laid down by BIS (600 mg/l). Statistically significant variation ($p<0.05$) was observed in total alkalinity between control and treated effluent.

3.3.10 Total Hardness

The presence of alkaline earth metals such as calcium, magnesium, iron and manganese in water determine its hardness. Out of these, calcium and magnesium plays an important role especially in industrial effluents and these metals are present in very high amount. Total hardness of the dairy effluent was found to be 800 mg/l in control. The reduction percentage was maximum in ***Tolypothrix distorta*** (74.89%) and minimum ***Aulosira laxa*** (74.13%). As the hardness is reduced the growth of algae is enriched (Tables.12 and 13; Fig.5b). The value of total hardness was above the tolerance limit of 100 mg/l prescribed by BIS (2009). Statistically significant variation ($p<0.05$) was observed in total hardness between control and treated effluent.

3.3.11 Calcium

Calcium is one of the most abundant elements in natural water imparting hardness. The concentration of calcium in the untreated effluent was 140 mg/l. When the effluent was treated with the cyanobacteria, the high reduction percentage was observed in ***Tolypothrix distorta*** (64.61%) low in ***Aulosira laxa*** (62.35%) (Tables.12 and 13; Fig.5c). The value of calcium lie in the permissible limits laid down by BIS (200 mg/l). Statistically significant variation ($p<0.05$) was observed in the calcium between control and treated effluent.

3.3.12 Magnesium

The concentration of magnesium was 80 mg/l in control, when the effluent was treated with cyanobacteria; the magnesium concentration was reduced gradually on 3rd day onwards. On the 15th day magnesium concentration was reduced in ***Aulosira laxa*** 77.75% whereas in ***Tolypothrix distorta*** it was 70.68% (Tables.12 and 13; Fig.6a). Magnesium is easily consumed by algae compared to other metallic components and thus requires periodic supplementation. The value of magnesium lie in the permissible limits laid down by BIS (30-100 mg/l). Statistically significant variation ($p<0.05$) was observed in the magnesium between control and treated effluent.

3.3.13 Sodium

Sodium is an important cation occurring naturally. Many industrial wastes are rich in sodium. In the present study, the concentration of sodium was 130 mg/l in control. From 3rd day onwards there was a significant reduction of sodium. On the 15th day the sodium level was reduced to ***Aulosira laxa*** 14.52%; whereas in ***Tolypothrix distorta*** the sodium content increased to 10.83% (Tables.12 and 13; Fig.6b). Increased sodium content in effluent responsible for the high salinity. The value of sodium lies in the permissible limits is not laid down by BIS. Statistically significant variation ($p<0.05$) was observed in sodium between control and treated effluent.

3.3.14 Potassium

It is one of the important cations naturally occurring elements; however, the concentrations remain quite lower than the sodium, calcium and magnesium. In the present study, the concentration of potassium was 30 mg/l in control. From 3rd day onwards the concentration of potassium was reduced. The concentration of potassium was reduced to 36.16% in ***Aulosira laxa*** whereas in ***Tolypothrix distorta*** it was 2.84% (Tables.12 and 13; Fig.6c). The value of potassium lies in the permissible limits is not laid down by BIS. Statistically significant variation ($p<0.05$) was observed in potassium between control and treated effluent.

3.3.15 Iron

Iron in its oxidized state is very essential for several enzymatic and redox processes in aquatic organisms. The concentration of iron was found to be 9.9 mg/l in control. On the 15th day the iron was reduced to 96.67 percent in ***Aulosira laxa*** and ***Tolypothrix distorta*** (Tables.12 and 13; Fig.7a). The value of iron lies in the permissible limits laid down by BIS (3.0 mg/l). Statistically significant variation ($p<0.05$) was observed in iron between control and treated effluent.

3.3.16 Free ammonia

Ammonia of mineral origin is rare in natural waters. Occurrence of ammonia in the water can be accepted as the chemical evidence of organic pollution. In water bodies, it is produced naturally by the reduction of nitrates under aerobic conditions. The concentration of free ammonia was 23.52 mg/l in control. When the effluent was treated with cyanobacteria; the free ammonia concentration was reduced to reduce to 95.91% in ***Tolypothrix distorta*** whereas in ***Aulosira laxa*** it was 85.52% (Tables.12 and 13; Fig.7b). The value of free ammonia lies above the permissible limits laid down by BIS (1.0 mg/l).

Statistically significant variation ($p<0.05$) was observed in free ammonia between control and treated effluent.

3.3.17 Nitrite

Nitrites are an intermediate product, both in the oxidation of NH_3 to NO_2 and in the reduction of NO_3, which occurs in wastewater treatment plants. The presence of even a small quantity of nitrite will indicate the organic pollution and the availability of partially oxidized nitrogenous matter. The value indicated random fluctuation due to differences in oxidation-reduction potential of wastewater. In the present study, the concentration of nitrite was 0.15 mg/l in control. On the 15th day the concentration of nitrite was reduced to 64.28 percent in ***Aulosira laxa,*** whereas**,** in ***Tolypothrix distorta*** the nitrite content was increased to 63.15 percent (Tables.12 and 13; Fig.7c). As increased the light intensity resulting in a proportional increase in nitrate uptake was observed. The value of nitrite lies in the permissible limits laid down by BIS (10 mg/l).

3.3.18 Nitrate

Nitrate represents the highest oxidized form of nitrogen. In wastewater treatment systems, high amount of nitrates denotes the aerobic condition and high stability of the wastes. In the present study, the concentration of nitrate was 8 mg/l in control. On the 15th day nitrate concentration was reduced to 19.16 percent in ***Tolypothrix distorta*** whereas in ***Aulosira laxa*** it was increased to 10.39 percent (Tables.12 and 13; Fig.8a). The value of nitrate lies in the permissible limits laid down by BIS (10 mg/l). Statistically significant variation ($p<0.05$) was observed in nitrate between control and treated effluent.

3.3.19 Chloride

Industries are the important sources of chlorides. Chlorides are highly soluble with most of the naturally occurring cations and do not precipitate, sediment and can not be removed biologically in treatment of wastes. Chloride concentration in wastewater had a random change in the value due to gradual increase/decrease in concentration as well as changes in the quality of water in fluxed. The highest concentration of chloride is the clear cut index of pollution. Beyond 200 mg/l, they give a bad taste to water and corrode the metals. In the present study, the concentration of chloride was 233 mg/l in control. When the effluent was treated with the cyanobacteria, the chloride concentration was reduced to 96.16% in ***Aulosira laxa*** whereas in 60.51% in ***Tolypothrix distorta*** (Tables.12 and 13; Fig.8b). The value of chloride lies in the permissible limits laid down by BIS (1000 mg/l). Statistically significant variation ($p<0.05$) was observed in chloride between control and treated effluent.

3.3.20 Fluoride

Fluoride is an active component that exists in both simple and complex forms. The concentration of fluoride was 0.12 mg/l in control. When the effluent was treated with cyanobacteria the fluoride concentration was reduced gradually on 3rd day onwards. Whereas on the 15th day the reduction percentage was higher in ***Aulosira laxa*** 93.33 percent and lower in ***Tolypothrix distorta*** (66.66%) (Tables.12 and 13; Fig.8c). The value of fluoride lies in the permissible limits laid down by BIS (2.0 mg/l). Statistically significant variation ($p<0.05$) was observed in fluoride between control and treated effluent.

3.3.21 Sulphate

Sulphate indicates the pollution of waste. It is naturally occurring anion. Discharge of industrial wastes and domestic sewage in waters tend to increase its concentration. It is an important constituent of hardness with calcium and magnesium. The concentration of sulphate was 73 mg/l in control. From 3rd onwards sulphate concentration was reduced. Whereas on the 15th day the sulphate concentration was reduced to 52.66% in ***Aulosira laxa*** and in ***Tolypothrix distorta*** it was 45% (Tables.12 and 13; Fig.9a). This is because the precipitation of sulphate content in untreated effluent was more than that in treated effluent. The value of sulphate lies in the permissible limits laid down by BIS (1000 mg/l). Statistically significant variation ($p\leq 0.05$) was observed in sulphate between control and treated effluent.

3.3.22 Phosphate

It occurs in wastewaters as inorganic and organic bound phosphates. The higher concentration of phosphorus is therefore and indication of pollution. The concentration of phosphate was 12 mg/l in control. When the effluent was treated with cyanobacteria, the phosphate concentration was reduced on 3rd day onwards. Whereas on the 15th day the phosphate concentration was reduced to 90.54% in ***Tolypothrix distorta*** and in ***Aulosira laxa*** lacks the level of phosphate was increased to 85.58 percent (Tables.12 and 13; Fig.9b).

Statistically significant variation ($p<0.05$) was observed in phosphate between control and treated effluent.

3.3.23 Silica

Silica is the most abundant element in the earth after oxygen. In water, silica exists as silicate. The concentration of silica was 6.62 mg/l in control. When the effluent was treated with cyanobacteria, the concentration of silica was reduced on 3rd onwards. Whereas, on the 15th day the concentration of silica was reduced to 29.50% in ***Tolypothrix distorta*** and in ***Aulosira laxa*** it was 19.21% (Tables.12 and 13; Fig.9c). Statistically significant variation ($p<0.05$) was observed in silica between control and treated effluent.

3.3.24 Chemical Oxygen Demand

The chemical oxygen demand test (COD) determines, the oxygen required for chemical oxidation of organic matter with the help of strong chemical oxidant. COD is generally considered as a major indicator of organic pollution in effluent. COD test is useful in pinpointing toxic condition and presence of biological resistant substances. The initial analysis of the dairy effluent sample showed very high COD i.e., 370 mg/l in control. On the 15th day the COD was reduced to 69.57% in ***Aulosira laxa*** and in ***Tolypothrix distorta*** it was 67.37% (Tables.12 and 13; Fig.10a). This is because of more oxidizing organic compounds present in untreated effluent than in treated effluent. The value of COD lie in the permissible limits laid down by BIS (250 mg/l).

3.3.25 Biological Oxygen Demand

Biochemical oxygen demand (BOD) is defined as the amount of oxygen required by microorganisms, while stabilizing biological decomposable organic matter in wastewater under aerobic conditions. Since, the test is mainly a bioassay procedure, involving measurement of oxygen consumed by bacteria, it is necessary to provide standard conditions of nutrient supply and pH. BOD reduction showed a trend similar to COD reduction. BOD is the measure of amount of O_2 required by the microorganisms to oxidize the organic content in effluent. The BOD is an important parameter to determine the pollution strength of the effluent and the purifying capacity of the receiving waters. The initial concentration of BOD was 120 mg/l in control. The reduction of BOD occurs on 3rd day onwards. The maximum reduction of BOD in ***Tolypothrix distorta*** was 66.67 percent and minimum in ***Aulosira laxa*** (65.01%) (Tables.12 and 13; Fig.10b). This is because of effluent contains more biologically oxidisable substances. The value of BOD lies in the permissible limits laid down by BIS (50 mg/l).

3.3.26 Oil and grease

The oil and grease content of domestic and certain industrial wastewater and of sludge is an important in the handling and treatment of this material for ultimate disposal. Oil and grease may influence wastewater system. The concentration of oil and grease was 0.012 mg/l in control. From 3rd day onwards oil and grease concentration was reduced. On the 15th day, the oil and grease concentration was reduced to 99.41% in ***Aulosira laxa*** and ***Tolypothrix distorta*** (Tables.12 and 13; Fig.10c). The value of oil and grease lie within the permissible limits laid down by BIS (20 mg/l). Statistically significant variation ($p<0.05$) was observed in oil and grease between control and treated effluent.

3.4 Heavy metals

The dairy effluent was treated with ***Aulosira laxa*** and ***Tolypothrix distorta;*** there was reduction of heavy metals was observed.

3.4.1 *Aulosira laxa*

The results of the heavy metal analyses of the dairy effluent, before and after treatment with ***Aulosira laxa*** are presented in Table.14. The heavy metals like copper reduced to 99.55 percent, total chromium reduced to 70 percent, zinc reduced to 65 percent, lead to 25 percent, nickel reduced to 82.35 percent, cadmium reduced to 95.65 percent, arsenic reduced to 60 percent and mercury reduced to 33.33 percent. The interaction between treatments and the incubation period was also significant. Statistically the values are highly significant ($p<0.05$) (Table.14; Figs.11-13).

3.4.2 *Tolypothrix distorta*

The dairy effluent was treated with ***Tolypothrix distorta;*** there was a reduction of heavy metals observed (Table.14). The heavy metals like copper reduced to 99.47 percent, total chromium reduced to 45 percent, zinc reduced to 75 percent, lead to 50 percent, nickel reduced to 94.11 percent, cadmium reduced to 99.13 percent, arsenic reduced to 80 percent and mercury reduced to 50 percent (Table.14; Figs.11a, b to 13 a, b, c). The interaction between treatments and the incubation period was also significant. Statistically the values are highly significant ($p<0.05$).

As a general conclusion, it is pointed out that the interaction between cyanobacteria and metals are very complex. It depends on several factors that is, the chemical and morphological features of cyanobacterial cells, the chemical and physical properties of metals to be removed, and the operational conditions utilized in the treatment. For these reasons, the selectivity, a character that may have great interest for many industrial applications, as well as the best metal uptake capability shown by some of the cyanobacteria tested in the laboratory must be confirmed to the specific interest in industrial applications. In any case, the promising results so far obtained phycoremediation study open up new perspectives on the use of cyanobacteria for the removal of heavy metals from effluents. As a consequence, the only way to evaluate the potential of a cyanobacterial strain in metal removal is through an experimental evaluation of its performances.

3.5 Bacteriological Examination

Bacteria are the most common microbial pathogens found in wastewater. Infection by the pathogenic microorganisms is the major risk factor associated with the recycling of wastewater. There are wide ranges of microbial pathogen which can be present in the wastewater, with the different types is being highly dependent on the socioeconomic conditions of communities creating wastewater. Bacteriological examination of dairy effluent showed a broad range of bacterial growth. Diverse bacterial species were isolated and characterized. These strains were characterized as a total coli form, faecal ***Streptococci*** and faecal coli forms. The identified bacterial strains of dairy effluent provide ample opportunities for diversified bacterial growth. These identified bacterial strains could be used for degradation of various components of dairy effluent.

Microbiological analysis of treated diary effluent showed a reduction of total coli form, faecal ***Streptococci*** and faecal coli form bacteria, although the plate counts were 17000 ± 1.00/ml for the ***Aulosira laxa*** and 140 ± 1.52/ml for that of ***Tolypothrix distorta,*** the total coli forms were 2100 ± 1.00/g for the ***Aulosira laxa*** and 270 ± 1.00/ml for that of ***Tolypothrix distorta***, faecal ***Streptoccocci*** were 300 ± 1.52/ml for the ***Aulosira laxa*** and 20 ± 1.00/ml for that of ***Tolypothrix distorta*** and faecal coli form counts were 500 ± 1.52/ml for the ***Aulosira laxa*** and 70 ± 2.00/g for that of ***Tolypothrix distorta*** (Table.15).

3.6 Characterization of Dairy effluent using FT-IR spectroscopy

FT-IR spectroscopy, which is a complementary and extensively used method, gives information about the molecular confirmation and hydrogen bonding patterns. As shown in the spectra of figures.14-16, the untreated dairy effluent and cyanobacteria cultured dairy effluent were analyzed by FT-IR spectroscopy shows the molecular modifications present in the effluent after pollutant exposure. There was a variation in the intensity of bands in different regions of test samples analyzed (Table.16, Fig.14). A number of broad bands are observed in the region 3385, 2135, 1646 and 735 cm^{-1} of untreated dairy effluent (Figs.14 and 15) was observed. When the dairy effluent was treated with ***Aulosira laxa*** the following peaks were assigned i.e., 3361, 2136, 1646, 728 and 615 cm^{-1}. Whereas, the dairy effluent was treated with ***Tolypothrix distorta*** changed the spectral assignments in the region of as 3962, 3909, 3892, 3408, 2132, 1656 and 866 cm^{-1}.

In the untreated dairy effluent four bands were observed. The peaks at wave numbers of 735 cm^{-1} corresponds to an aromatic CH group, 1644 cm^{-1} indicate the functional group of amide. The peak 2135 cm^{-1} represents C=-CH (terminal) and 3385 cm^{-1} represents intermolecular H.

When the dairy effluent was treated with ***Aulosira laxa*** five bands were observed. The 3661 cm^{-1} band may be assigned to intermolecular H bonds, the 2136 cm^{-1} band may be assigned to aromatic CH-bending vibrations, 1646 cm^{-1} band may be assigned C=O cm^{-1} stretching vibrations in the amide linkages of the proteins, 728 cm^{-1} band be assigned as aromatic CH bending and 615 cm^{-1} band be assigned as aliphatic Bromo compounds CBr stretch (Table.16; Fig.15a).

When the dairy effluent was treated with ***Tolypothrix distorta*** seven bands were observed. The band 3892 to 3962 cm^{-1} may be assigned to OH stretching vibrations, the 3408 cm^{-1} band may be assigned to intramolecular H-bonds, 2132 cm^{-1} band may be assigned alkynyl C-H stretch, 1656 cm^{-1} band may be assigned C=O cm^{-1} stretching vibrations in the amide linkages of the proteins and 866 cm^{-1} band be assigned as benzene 1, 2, 4 for sub (strong weak) benzene 1, 3, 5 in sub (strong, medium) stretching vibrations (Table.16; Fig.15b).

The common point without any pretreatments was the small intensity of absorbance. In the present study, the detected functional groups which are deposited on the dairy effluent without any pretreatment was an indication of pollution removal by the experimental algae and it can be confirmed by characterization of FT-IR.

3.7 Statistical Significance

The t-test was performed for all the physico-chemical parameters analyzed throughout the study to eliminate the maximum number of sources of extraneous variability. The calculated t-values were always greater than the tabulated t-values at $p \leq 0.05$. Based on this evidence, we could conclude that there was a significant difference in the untreated and treated dairy effluent parameters.

3.8 Value added products from *Aulosira laxa* and *Tolypothrix distorta*

3.8.1 Pigment composition

The quantitative analysis of chloroplast pigments on the initial and on the 15th day of treatment revealed that the total chlorophyll and carotenoid pigments were continued to be synthesized by ***Aulosira laxa*** and ***Tolypothrix distorta*** in control conditions. When the culture was grown in effluent, there was hardly reduction observed in the synthesis of chlorophyll and carotenoid. The concentration of chlorophyll in the laboratory cultured of ***Aulosira laxa*** was 0.510 ± 0.01 mg/g (dry wt), when treated with dairy effluent it was decreased to 0.300 ± 0.01 mg/g (dry wt). Similarly, in the laboratory cultured of ***Tolypothrix distorta*** the concentration of chlorophyll was 0.480 ± 0.01 mg/g, (dry wt) when treated with dairy effluent it was decreased to 0.300 ± 0.01 mg/g (dry wt) (Table.17; Fig.16). Statistically significant reduction in chlorophyll content was observed on effluent was observed in ***Aulosira laxa*** and ***Tolypothrix distorta*** when treated with dairy effluent.

Carotenoid content in ***Aulosira laxa*** and ***Tolypothrix distorta*** was also analyzed to find out the effects of dairy effluent on the algae. As shown in figure.17, it is observed that the dairy effluent treatment lowered the intracellular levels of carotenoid from 0.180 ± 0.08 to 0.080 ± 0.001 mg/g (dry wt) in ***Aulosira laxa*** and 0.190 ± 0.01 to 0.100 ± 0.02 mg/g (dry wt) in ***Tolypothrix distorta*** on the 15th day. ***Tolypothrix distorta*** and ***Aulosira laxa*** showed statistically significant reduction in carotenoid when they are treated with dairy effluent (Table.17; Fig.17).

3.8.2 Biochemical Composition

In the laboratory cultured ***Aulosira laxa*** (control), the carbohydrate content was observed to be 9.71 ± 0.01 mg/g (dry wt), whereas treated ***Aulosira laxa*** it was 18.70 ± 0.01 mg/g (dry wt). Similarly, in the laboratory cultured ***Tolypothrix distorta*** (control) the carbohydrate content to be 7.64 ± 0.02 mg/g (dry wt) and in treated ***Tolypothrix distorta*** it was 20.12 ± 0.01 mg/g (dry wt). ***Tolypothrix distorta*** showed statistically significant in carbohydrate content when they are treated with dairy effluent (Table.18) (Fig.18).

The protein content laboratory cultured ***Aulosira laxa*** was 7.16 ± 0.00 mg/g, whereas effluent treated ***Aulosira laxa*** it was 8.11 ± 0.01 mg/g (dry wt). Similarly, the laboratory cultured ***Tolypothrix distorta*** control was 7.78 ± 0.02 mg/g (dry wt) and in treated ***Tolypothrix distorta*** it was 8.11 ± 0.01 mg/g (dry wt). Laboratory cultured of ***Aulosira laxa*** showed statistically significant in protein content when they are treated with dairy effluent (Table.18) (Fig.19).

Lipid content in the laboratory cultured ***Aulosira laxa*** was 1.31 ± 0.01 mg/g (dry wt) whereas in treated ***Aulosira laxa*** it was 1.51 ± 0.01 mg/g (dry wt). Similarly, in the laboratory cultured ***Tolypothrix distorta*** it was 0.92 ± 0.01 mg/g (dry wt) and in treated ***Tolypothrix distorta*** it was 1.64 ± 0.01 mg/g (dry wt). Treated effluent with ***Tolypothrix distorta*** showed statistically significant in lipid content in dairy effluent (Table.18) (Fig.20). Thus, the results show that phycoremediation of dairy effluent using ***Aulosira laxa*** and ***Tolypothrix distorta*** resulted in a significant increase in carbohydrate, protein and lipid.

3.8.3 Amino acid composition

Results obtained on the amino acid composition of laboratory cultured ***Aulosira laxa*** and ***Tolypothrix distorta*** and the effluent treated ***Aulosira laxa*** and ***Tolypothrix distorta*** are summarized in Table.19. The amounts of a particular amino acid in dried samples of laboratory cultured algae and effluent treated algae varied considerably since there were large differences in the protein content of the samples. Amino acid analysis of the laboratory cultured ***Aulosira laxa*** showed relatively high content of non-essential amino acids amounted to 61.68% and essential amino acids (38.32%) (Table.19) (Fig.21a&b). Almost a similar result was obtained in ***Tolypothrix distorta viz;*** non-essential amino acid amounted to 52.78% (Table.19) and essential amino acids (47.22%) (Fig.21a & b).

Aulosira laxa treated with dairy effluent showed an increase in the level of essential amino acids (53.04%), and the essential amino acid thrionine is the higher percentage compared to other amino acids 3.96% which is followed by lysine is 1.23%. A very low amount of non-essential amino acids which was recorded (46.96%) and in non-essential amino acids proline (8.82%) which is followed by cystine (1.99%) (Fig.22a).

Dairy effluent treated with ***Tolypothrix distorta*** biomass showed a reduction in the level of essential amino acids (47.22%), and the essential amino acid phenylalanine (3.42%) followed by lysine (1.51%) and a very high amount of non-essential amino acids aspartic acid (5.69%) followed by asparagine (5.58%) (Fig.22b). In the present study, the contents of essential amino acid were found to be decreased in ***Tolypothrix distorta*** and increased in ***Aulosira laxa*** in the treated dairy effluent.

3.8.4 Vitamins

Analysis of the vitamin content of ***Aulosira laxa*** and ***Tolypothrix distorta*** shows relatively a high amount of beta-carotene and vitamin-C when compared to biotin, Vitamin B_{12}, pantothenic acid (Vitamin B_5), folic acid, choline, niacin (Vitamin B_3), pyridoxine (Vitamin B_6), riboflavin (Vitamin B_2), and thiamine (Vitamin B_1) (Table.20; Fig.23). In cyanobacteria vitamin profile is unusually even and complete for any single food and is rich in B vitamins, including B_{12}, and beta carotene (precursor to Vitamin A).

The content of B-complex vitamins (thiamine, riboflavin, nicotinic and folic acids), ascorbic acid and carotene was estimated. Comparative analysis showed that the treated ***Aulosira laxa*** and ***Tolypothrix distorta*** had highest vitamin content than that of control. The laboratory cultured ***Aulosira laxa*** and ***Tolypothrix distorta*** contained the lowest quantity of thiamine, riboflavin and carotene and larger amount of nicotinic acid. Comparison of the content of vitamins-C, B_1, B_2, B_{12}, folic acid and β-carotene in laboratory cultured cyanobacteria and in treated cyanobacteria the amount of thiamine, riboflavin, folic acid, and β-carotein per gram dry matter was found to be higher.

3.8.5 Minerals

The eight metallic elements namely, nitrogen, phosphorus, potassium, iron, zinc, copper, sulphur and magnesium were analyzed in cyanobacterial biomass (***Aulosira laxa*** and ***Tolypothrix distorta***). Phosphorus, potassium, iron and sulphur were found to be in higher concentration, followed by nitrogen, zinc, magnesium and copper (Table.21; Fig.24). The concentration of metals including heavy metals in the algal biomass (Table.21) was higher than the laboratory cultured ***Aulosira laxa*** and ***Tolypothrix distorta*** (Fig.24)**. *Aulosira laxa*** showed the highest concentration of phosphorus, potassium, iron and zinc. Whereas the concentration of phosphorus, potassium, iron and zinc was maximum in ***Tolypothrix distorta*** (Fig.24). Moreover, concentrations of elements increased with increasing concentration of the effluent. The mineral concentration is significantly different in the laboratory culture of ***Aulosira laxa*** and ***Tolypothrix distorta. Aulosira laxa*** and ***Tolypothrix distorta*** are claimed to be nutraceutically valuable species due to the presence of good quantity minerals in it.

3.9 Antibacterial activities of *Aulosira laxa* and *Tolypothrix distorta*

The antibacterial activity of the methanolic extracts of the cyanobacteria was studied against Gram positive and Gram negative bacteria. The pathogens used for the present study are ***Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*** and ***Escherichia coli*** and were compared with ampicillin. The antibacterial activity was assessed by measuring, the zone of inhibition in the culture plates treated with algal extracts. The results of the antibacterial activity of algal extracts from control and extracts ***Aulosira laxa*** and ***Tolypothrix distorta*** treated with dairy effluent was used against bacteria were presented in the Table (22).

The present study showed that the methanolic extract of ***Aulosira laxa*** from control showed highest activity against ***Bacillus subtilis*** followed by ***Staphylococcus aureus.*** Whereas, the effluent treated ***Aulosira laxa*** extracts showed the highest activity in ***Bacillus subtilis*** followed by ***Staphylococcus aureus.*** The methanolic extracts did not show the activity against ***Pseudomonas aeruginosa*** and ***E. coli*** in both control and in dairy effluent treated ***Aulosria laxa*** extract (Table.22) (Plate.10).

The present study showed that the methanolic extract of control ***Tolypothrix distorta*** did not show the activity against ***Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*** and ***Escherichia coli.*** Whereas, the effluent treated ***Tolypothrix distorta*** treated with effluent showed higher antibacterial activity in ***Bacillus subtilis, Staphylococcus aureus*** and the activity was low in ***Pseudomonas aeruginosa.*** The methanolic extracts did not show the activity against ***E. coli*** in both control and ***Tolypothrix distorta*** treated dairy effluent (Table.22) (Plate.11).

While comparing with standard antibiotic ampicillin, the methanolic extracts of ***Tolypothrix distorta*** treated with effluent exhibited maximum activity against ***B. subtilis*** (80%) and minimum activity against ***Pseudomonas aeruginosa*** (26.92%).

3.10 Antioxidant activities of *Aulosira laxa* and *Tolypothrix distorta*

The antioxidant activity of the laboratory cultured algae and effluent treated algae were determined by using the DPPH and ABTS assay. Methanolic extract was used in this study and the results were compared with that of the standard antioxidants such as BHT (Butylated hydroxytoluene) and L-ascorbic acid.

Concerning DPPH, the laboratory cultured ***Aulosira laxa*** and ***Tolypothrix distorta*** of methanolic extracts showed 45.28 and 44.35% of inhibition at 900 **µ**g/ml concentration (Figs.25 and 27). Comparatively this was higher than that of the effluent treated algae which show the range of 34.69 and 29.24% of the concentration of 900 **µ**g/m.

The same pattern was observed in the ABTS assay. The laboratory cultured ***Aulosira laxa*** shows more activity i.e., 79.51% and 96.27% in ***Tolypothrix distorta*** at the same concentration (900 **µ**g/ml) (Figs.26 and 28). When compared to the effluent the laboratory cultured algae the effluent treated algae showed lesser activity 67.37% of ***Aulosira laxa*** and 66.24% for ***Tolypothrix distorta***. IC_{50} values were calculated for both the tested algae and the standard synthetic antioxidants BHT and L-ascorbic acid (Table.23). All these results comparatively showed lesser radical scavenging activity when compared to that of the standard antioxidant.

The results show that the algal extracts have effective radical scavenging activity against DPPH and ABTS induced radicals. The obtained data illustrated that ABTS method shows a higher percentage of antioxidant activity than DPPH method. The results shows significant differences between the tested samples. The activities of the laboratory cultured algae and the dairy effluent treated algal biomass extracts were dose dependent and characterized by increasing scavenging activity with a rise in sample concentration.

3.11 Effect of Dairy Effluent (untreated and treated) on Seed Germination

A screening study was carried out with the ***Phaseolus mungo*** (Black gram) using untreated and treated dairy effluent revealed that there was a marked variation in germination percentage of plants when treated with different concentrations of dairy effluent. Specifically, the treated sample showed a remarkable germination percentage (Table.24).

Seed germination percentage was higher in 25 and 50% concentration of dairy effluent and low in 100% concentration. There was a significant reduction in germination percentage in different concentration of dairy effluent and complete inhibition of germination in 100% concentration. The maximum seed germination was recorded at 25% and inhibition at 100% of effluent concentrations, as compared to control. A gradual decrease in germination percentage was noted to increase in strength of the effluent. The strength of effluent above 50% was hazardous to the plant and strength above this range inhibited germination completely. Observations on the effects of dairy effluent on seedling germination revealed that the effluent concentration at 25% may be helpful for better cultivation, luxuriant growth and massive production of the

Phaseolus mungo**.** Lower concentration of dairy effluent showed promoting effect on seed germination, seedling growth and biomass production.

Hence, further work was carried out with the various concentrations of 25% untreated and 100% treated dairy effluent to find the suitable concentration which would increase the seed germination and growth (Plates.12 and 13). When compared to the control, the observation showed that the effect of effluent from 25% to 50% was remarkable. After 7 days of observations in pots trial, it was found that the maximum growth of plant was at 25% treated effluent. This showed that the percentage of seed germination was inversely proportional to effluent concentrations. Lower concentrations of treated dairy effluent showed promoting effect on seed germination, seedling growth, and biomass production in black gram. The magnitude of reduction in seed germination and seedling growth was higher at 100% concentration which may be probably due to higher osmotic pressure.

The effects of the effluent and algal filtrate on the germination of ***Phaseolus mungo*** seeds were studied. Germination of seeds of ***P.mungo*** was found to be more tolerant to effluents treated with algal filtrate, as compared to those of the untreated effluent and control. There was a significant difference between control and effluent than that of algal extract. Higher concentrations of effluent almost inhibited the germination of ***Phaseolus mungo*** seeds (Table.24).

3.11.1 Growth parameters

3.11.2 Shoot length and leaf area

The experimental data revealed that the length of shoot and leaf area of the ***P.mungo*** varied considerably under the influence of different concentration of treated effluent treatments. The shoot length of ***P.mungo*** plants differed with different concentrations on the dairy effluent in the soil. For a lower concentration of irrigated effluent (25%), the shoot length and leaf area of ***P.mungo*** plants were higher than that of control plants. It may be taken as an indication of beneficial range, while for higher concentrations of effluent (75% and 100%) decreasing trend was observed, which confirms the toxic effect of this effluent to ***P.mungo*** plants. The reduction was 100% in ***Phaseolus mungo*** under 100% concentration of dairy effluent.

The shoot length was reduced in 100% dairy effluent concentration in seedlings of ***Phaseolus mungo*** (Black grarm)**.** For 25% concentrations of dairy effluent with algal filtrates, the reduction in shoot length was not so obvious. When the concentrations of the dairy effluent started increasing from 25% onwards, an increase in the shoot length was observed. This could also be related to the fact that some of the nutrients present in the effluent are essential but at above a particular concentration, they become hazardous.

Shoot length ranged from 67.04 ± 0.1 to 145 ± 0.26 cm. Maximum shoot length (145 ± 0.26 cm) was recorded in dairy effluent treated with algal filtrate (***Aulosira laxa***); whereas the minimum shoot length (67.04 ± 0.1) was recorded in under untreated effluent (Table.25) (Plate.12). Leaf length ranged from 10.83 ± 0.05 to 26.40 ± 0.01 cm. Highest leaf length was recorded (26.4 ± 0.01 cm) under effluent treated with algal filtrate (***Tolypothrix distorta***); whereas the lowest leaf length was recorded in (10.83 ± 0.05 cm) untreated effluent (Table.25) (Plate.13).

3.11.3 Pigment and Biochemical composition

The total chlorophyll contents of the seedling of black gram (***Phaseolus mungo***, L.) were found to be 1.486 ± 0.00 mg/g in control and in those treated with ***Aulosira laxa*** filtrate the total chlorophyll content was 1.509 ± 0.00 mg/g and 1.542 ± 0.00 mg/g (dry wt) and in the untreated effluent it was 0.014 ± 5.77 mg/g (dry wt) (Table.26; Fig.29a). A decrease in the chlorophyll content in untreated effluent suggests pollution injury. The carotenoid content of the seedling of ***Phaseolus mungo*** in untreated effluent was 0.190 ± 0.00 mg/g (dry wt) and in those treated with cyanobacteria filtrate it was 0.450 ± 0.00 mg/g (dry wt) and 0.490 ± 0.00 mg/g (dry wt) than the control 0.310 ± 0.19 mg/g (dry wt) (Fig.29b).

The carbohydrates, proteins and lipid content in ***Phaseolus mungo*** control, untreated effluent and treated effluent with algal filtrate were represented in Table.27. The carbohydrate content of control was 12.87 ± 0.01 mg/g (dry wt), in untreated effluent it was 10.54 ± 0.02 mg/g (dry wt), treated with ***Auloira laxa*** it was 15.03 ± 0.01 (mg/g dry wt) and when treated with ***Tolypothrix distorta*** it was 26.44 ± 0.01 mg/g (dry wt) (Fig.30a) respectively.

Protein content of control was 3.40 ± 0.001 mg/g (dry wt), in untreated effluent it was 2.79 ± 0.02 mg/g (dry wt), when treated with ***Aulosira laxa*** it was 3.73 ± 0.00 mg/g (dry wt) and treated with ***Tolypothrix distorta*** it was 4.10 ± 0.00 mg/g (dry wt) (Fig.30b), respectively.

Lipid content of control was 0.56 ± 0.51 mg/g (dry wt), in untreated effluent was 0.51 ± 0.01 mg/g (dry wt), when treated with ***Aulosira laxa*** it was 0.81 ± 0.01 mg/g (dry wt) and treated with ***Tolypothrix distorta*** it was 0.95 ± 0.01 mg/g (dry wt) (Fig.30c), respectively. The results indicated that the algal filtrate on ***Phaseolus mungo*** in dairy effluent has a stimulatory effect on all the biochemical contents, but had deleterious effects in untreated effluent.

3.11.4 Minerals

When ***Phasleolus mungo*** treated with water (control) the concentration of minerals like nitrogen, phosphorus, potassium, iron, zinc, magnesium, copper and sulphur was lower (Table.28). Where as, the plant treated with untreated effluent the concentration of nitrogen, phosphorus, potassium, iron, magnesium was increased, whereas, the concentration of zinc, copper and sulphur decreased (Table.28). But when the plant was treated with cyanobacterial filtrate (***Aulosira laxa***) the concentration of nitrogen, phosphorus, potassium, iron, magnesium, sulphur increased and the concentration of zinc and copper decreased. The plant when treated with cyanobacterial filtrate (***Tolypothrix distorta***) the concentration of nitrogen, phosphorus, potassium, iron, zinc, magnesium, copper, sulphur was increased higher compared to that of control and untreated effluent (Table.28). Thus the present investigation has revealed that cyanobacterial filtrate treatments had a positive effect on mineral composition in ***Phaseolus mungo*** (Table.28). Application of effluent treated algal filtrates had pronounced effect on mineral composition in ***Phaseolus mungo*** than that of untreated effluent (Table.28 and Fig.31). A progressive increase of minerals in the leaves was observed as a result of increasing concentration of the cyanobacterial filtrate up to 100%.

3.12 Toxicity testing of dairy effluent through *Hypophthalmicthys molitrix*

In the present investigation toxicity testing of fish was conducted (Plate.14). The survival rate of the fishes were exposed to the dairy effluent during the experimental period from 1st to 7th day are furnished in Table No.29.

In effluent treated with ***A. laxa***, the survival rates of fish were noticed 80 percent, 90 percent and 100 percent on the 1rd, 3rd, 5th day and 7th day. Similarly the effluent treated with ***T. distorta*** the survival rate of fishes were 90 percent on 1st and 3rd day and 100 percent on the 5th and 7th day (Table.29).

The toxicity studies revealed that untreated effluent was more toxic ***Hypophthalmicthys molitrix*** as compared to treated effluent. The fish came to surface frequently due to distress in untreated effluent, with quick opercular movements and moving drastically.

The distress to fishes was due to reduction of dissolved oxygen in untreated effluent owing to high Biochemical Oxygen Demand (BOD) where the effluent treated with ***A. laxa*** and ***T.distorta*** witnessed lesser effect. The toxicity test clearly indicates that untreated effluent was more toxic and treated effluent toxicity was reduced somewhat due to marginal reduction of BOD, COD and suspended solids with the slight removal of heavy metals by algae (Table.30).

It was found that the toxicity was reduced by more than 50%. Still it is not safe to discharge the effluent as fishes do not survive for a long duration into it. So the effluent was subjected to further biological treatment. It was confirmed from the toxicity tests that the effluent was completely safe for discharge after phycoremediation, the fish mortality was reduced. The effluent treated with ***A. laxa*** and ***T.distorta*** is non toxic to fish as shown by the percentage of survival furnished in the Table.29.

3.13 Nutritional qualities of live feed organisms

The fish ***Hypophthalmicthys molitrix*** commonly known as silver carp fed with the algae (***Aulosira laxa*** and ***Tolypothrix distorta***) shows slight changes in their growth (length and width) (Tables.31 and 32). The weight of the fish increased slightly during the 7th day (Table.33). Statistical analysis indicates that the length and width of the fish values are not significant ($p>0.05$).

The effect of untreated and treated effluent with algae on the growth of the fish was observed. The average length of the fish in control, untreated effluent and treated effluent with algae was measured the end of the experimental period of 7 days and their average weight was also measured up to 7 days were presented in Table.33.

When the fishes were fed with cyanobacteria alone, the average increase in the length of the fish was 3.83 ± 0.26 cm and 4.3 ± 0.1 cm, with commercial feed (Aqua fine); it was 3.63 ± 0.05 cm. The difference between commercial feed and cyanobacteria feed was statistically significant. When the fishes were fed with cyanobacteria alone, the average increase in the width of the fish was 1.13 ± 0.05 cm and 1.16 ± 0.1 cm, with commercial feed (Aqua fine); it was 1.03 ± 0.05 cm (Table No.32). The difference between commercial feed and cyanobacteria feed was statistically significant. A similar trend was observed in weight gain also. When ***Aulosira laxa*** and ***Tolypothrix distorta*** was used as a feed, the increase in weight gain was nearly 1.12 ± 0.005 g and 1.37 ± 0.01 g, whereas in commercial feed which exhibits 1.48 ± 0.01 g moderate weight gain (Table.33). The difference in weight gains was statistically significant between all the three groups. Based on the observation of the present study it is concluded that the treatment of effluent with cyanobacteria is more effective and can be utilized for aquaculture.

The fish fed with commercial feed shows with decreased carbohydrate level 2.16 ± 0.01 than that of control 2.45 ± 0.01 mg/g (dry wt) (Table.34). The fish, ***Hypophthalmicthys molitrix*** fed with algae (***Aulosira laxa*** and ***Tolypothrix distorta***) showed an increased trend of carbohydrate content 6.15 ± 0.01 mg/g (dry wt) to 7.52 ± 0.01 mg/g (dry wt) (Table.34; Fig.32). The difference in the carbohydrate composition between algae and fish was significant ($p>0.05$).

The fish fed with commercial feed shows with increased protein levels 5.22 ± 0.005 mg/g (dry wt) than that of control 2.16 ± 0.01 mg/g (dry wt) (Table.34). The fish ***Hypophthalmicthys molitrix*** when fed with algae (***Aulosira laxa*** and ***Tolypothrix distorta***) showed an increased protein content (6.44 ± 0.01 to 12.41 ± 0.01 mg/g (dry wt); Table.34; Fig.32). The change in the protein composition between the two organisms was significant ($p>0.05$).

The fish fed with commercial feed shows with decreased lipid level 0.48 ± 0.01 mg/g (dry wt) than that of control 2.05 ± 0.01 mg/g (dry wt) (Table.34). The fish ***Hypophthalmicthys molitrix*** when fed with algae (***Aulosira laxa*** and ***Tolypothrix distorta***) showed an increased lipid content (1.46 ± 0.01 to 2.29 ± 0.005 mg/g (dry wt) (Table.34; Fig.32). The variation in the lipid composition between them was highly significant ($p<0.05$).

3.14 Environmental Impact Assessment

In this study the physico-chemical parameters, heavy metals and microbial examination of dairy effluent were determined and compared with the BIS standard (2490-2009). The concentrations of different parameters are given in Table.35.

The physico-chemical, heavy metal parameters and microbiological examination of dairy effluent were monitored. It showed that the dairy effluent was highly polluted. High values of COD, BOD, TSS, and TDS were present in the effluents indicating the presence of high levels of pollution. (Table.35). It is concluded that, leakages and untreated effluent discharge may have contributed to the level of metal contents in the effluent. The impact of bacterial contamination on the effluent was observed throughout the entire sampling points and its pollution concentration was quite high. Furthermore, from the test that was confirmed, the use of treated dairy effluent for agricultural purposes such as irrigation and aquaculture it have positive effects on crops and fishes.

4.0 DISCUSSION

As the global consciousness on the ecological balance, pollution abatement and environment friendliness increase, the need for technology that caters to the modern trend is also on the rise. Considering the disadvantages of conventional chemical treatment methods to abate pollution, the use of organisms directly or indirectly to remove contaminants from aquatic or terrestrial ecosystem is of many an importance. This innovative remediation technology is potentially applicable to a variety of contaminants.

Phycoremediation is a novel technique that uses algae to clean up polluted water and soil. It takes the advantage of the alga's natural ability to take up, accumulate and degrade the organic constituents that are present in their environment. Algae based effluent treatment systems offer simpler and economically viable technology as compared to the other environmental protection systems. Photosynthesis is effectively exploited by algae to generate oxygen from effluent for remediation of pollution.

The ability of cyanobacteria to reduce the nutrient load of dairy effluent has been studied and compared. This technology is flexible to handle bulk fluctuations in quality and quantity of effluent feed. Moreover, it has been proved by us to be effective in treating an array of effluents.

Dairy effluent contains almost all the nutrient elements required for the growth of algae. These nutrients are present in the form of complete organic compounds in dairy effluent, therefore, they are first to be oxidized into assimiliable forms before being utilized by algae. This oxidation is brought about by the complex symbiosis of algae and bacteria. The O_2 liberated by algae is utilized by bacteria which oxidize the organic compounds of the dairy effluent into simple forms which are suitable for healthy growth and development of algae.

4.1 Biodegradation of Dairy effluent

The indigenous cyanobacteria isolated from dairy effluent in the present study were found to be superior degrades or removers of pollutants. The growth of cyanobacteria and biodegradation of pollutants was affected by the large number of microbial species, which depends on their native environment, pollutant concentrations, exposure (contact) time and application as individual or mixed cultures. Removal was attributed to either biodegradation, or bioaccumulation, or both, with different ratios. Unlike the effect of pollutant on cyanobacterial growth, which was positively correlated with the contact time, the removal efficiency percentage by almost all the investigated species achieved high levels within the first 5 days. Removal efficiency (RE) percentages increased to maximum in most cases on the 10^{th} day, after which it declined slightly up to the 15^{th} day.

4.2 Cell growth

The desired level of water treatment with algal systems can be achieved by optimizing autotrophic growth, and for which the basic principles of algal mass cultures must be applied (Goldman, 1979; Torzillo ***et al***., 2003). Light availability and light/dark regime represent two important factors affecting productivity. The latter is strongly influenced by the mixing which prevents:

(1) Sedimentation and thermal stratification

(2) Formation of nutrients and pH gradients

(3) Depletion of carbon dioxide on the pond surface and

(4) The improvement of the stripping supersaturating oxygen (Richmond, 2003).

However, the effect of mixing on a light/dark regime is expected to be more relevant in dense cultures of microalgae in which a light gradient exists. The effect of mixing of cultures of ***Scenedesmus obliquus*** on growth and N and P removal was investigated by Martinez ***et al***., (2000). They concluded that stirring had a positive effect on biomass productivity during the linear phase of growth, but constant stirring did not influence the removal of either P or N.

Preliminary ***in vitro*** experiments of dairy effluent were carried out using cyanobacteria. In the present investigation phycoremediation was performed using ***Aulosira laxa*** and ***Tolypothrix distorta,*** because they had a better growth rate than the other native cyanobacteria investigated. Phycoremediation assures nil sludge generation and as a result there is no disruption of surrounding.

The results of the physico-chemical parameters of the dairy effluent shows that it was very toxic and if discharged, it can cause serious environmental problems and harmful effects on human beings, animals, plant and aquatic organisms. Analysis of various physico-chemical parameters before and after treatment revealed that the cyanobacteria could effectively improve water quality by removal of pollutants. Both ***Aulosira laxa*** and ***Tolypothrix distorta*** showed high efficiency in the removal of nutrients.

4.3.0 Physico-chemical analysis

4.3.1 Temperature

Temperature in the wastewater is an important parameter, as it affects the chemical and biological reactions of aquatic organisms. Abnormally increased temperature can increase undesirable plankton species and fungi. Temperature is also very important in determining various parameters such as pH, conductivity, saturation level of gases and various forms of alkalinity.

4.3.2 Appearance and Odour

The appearance of industrial effluent depends upon the nature of the product manufactured. Odour present in effluents, are mainly due to dissolved impurities, often of organic nature caused by living and decaying aquatic organisms and accumulation of gases. The most characteristic odour of dairy effluent is offensive smell. When the dairy effluent was treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** the offensive smell was reduced to algal smell.

4.3.3 Colour

Milky and grayish black in appearance of dairy effluent is due to biological reactions of organic and inorganic materials. The discharge of any amount of colouring substances in the form of liquid effluent from industries to water bodies makes the water source highly unsuitable for domestic applications, drinking and recreational purposes. It also affects the photosynthetic activity in aquatic organisms because of reduced light penetration and toxic substances. The development of effective and low cost removal technology for the decolourization of the effluent is important (Inthorn ***et al***., 2002).

In the present study, the dairy effluent treated with cyanobacteria, the colour changed from grayish black to greenish yellow from 5^{th} day onwards. These changes in colour and odour of the dairy effluent may be due to the influence of algae. The water clear and changes at initial stages in green colour. These findings are in accordance with Verma and Madamwar (2002).

Mohan and Bhaskar (2004) studied biological decolourization of two azo dye effluents (direct and reactive dye) by using a commonly available green alga ***Spirogyra*** sp. Previously, several researchers have proved that biosorption processes using algae were highly pH dependent and it is the most important parameter to be considered (Aksu and Tezer, 2005; Kumar ***et al***., 2005 and 2006).

Colour removal of algae was due to three intrinsically different mechanisms viz., assimilative utilization and absorption of chromophores for production of algal biomass, CO_2 and H_2O, transformation of coloured molecules to non-coloured molecules.

Decolourization during the initial stage of inoculation is due to adsorption and during the later stage; it is due to the degradation of organic matter. The algae showed better colour removal due to the bioflocculant activity (Shilo, 1987). It was suggested that hydrogen peroxide generated by cyanobacteria during photosynthesis might have reacted with hydroxyl anion to give perhydroxyl ion, which has strong nucleophilic activity and might have helped in decolourisation in case of distillery effluent (Chauhan and Dikshit, 2006). Colour change might have also resulted due to active oxygen released during photolysis of water by the cyanobacterium as reported by Kalavathi ***et al***., (2001).

Mohan ***et al.,*** (2004) observed that during the initial stages primary mechanism of colour removal from textile effluents is by the adsorption on the surface of the algal cells. Due to metabolic activity, and especially under stress algae secretes exopolymers into the environment. The released polymers possess an excellent complexion capacity which may result in the removal of remaining dye molecules from the aqueous phase by chelation/complexation reactions (biocoagulation).

The results of the present study suggested that ***Aulosira laxa*** and ***Tolypothrix distorta***, has a potential capacity for colour removal and may be further investigated for the development of oxidation pond (Waste Stabilization) for treatment of wastewater of various effluents. Further research work is in order for a better understanding of the molecular processes involved in colour removal by various algal species.

4.3.4 Turbidity

Turbidity is a measure of the light-scattering potential of effluent, caused by the presence of colloidal and suspended material. Effluent filtration can decrease turbidity levels. Bioflocculants are extra cellular macromolecules that are known for their activity to clarify turbid water and these are known to produce by the green algae (Kaplan ***et al***., 1987) and cyanobacteria (Fattam and

Shilo, 1984). Organic suspended solids were found to be responsible for the turbidity in the water reducing high penetration and impairment of photosynthetic activity of aquatic life. In the present study, the amount of turbidity was reduced to 84.73 percent by ***Aulosira laxa*** and 86.34 percent by ***Tolypothrix distorta*** due to a reduction in the concentration of carbonate, bicarbonate, chloride, calcium, magnesium and sodium. Cyanophyceae have a great competitive advantage under conditions of high turbidity.

4.3.5 Total suspended solids

Total suspended solids in the effluent were found to be reduced up to 38 percent of ***Aulosira laxa*** and 17.47 percent by ***Tolypothrix distorta***. The high suspended solids caused steady temperature in the effluent and could affect the productivity by interfering with the light. However, the value of total suspended solids could be related to the huge oxygen production and bubbling through algal photosynthesis that allows re-suspension of algal flakes settled at the bottom of the tanks. Veeralakshmi ***et al***., (2007) reported that the reduction of total suspended solids, in petroleum effluent treated with ***Oscillatoria;*** Kotteswari ***et al***., (2007; 2012 a and b) have reported in dairy effluent when treated with ***Spirulina platensis; Aulosira*** sp and ***Nostoc*** sp. The study confirmed that due to reduction of suspended solids the effluent is suitable for safe disposal on land through irrigation.

4.3.6 Total dissolved solids

Total dissolved solids contain mainly organic and inorganic compounds. In the present study large amounts of suspended material which have been discharged by dairy industry was noted. The higher amount of total suspended solids present in untreated effluent may be due to the presence of higher concentration of biodegradable organic matter in the dairy effluent.

The total dissolved solids present in the effluent was found to be reduced to 49.86 percent by ***Aulosira laxa*** and 90.10 percent by ***Tolypothrix distorta*** is due to the utilization of various nutrients. Similar observations have been made in Textile mill effluent by Govindan and Sundaralingam (1977). The high suspended solids in different industrial effluents were also reported earlier by Sinha (1993); Amudha and Mahalingam (1999) and Sundaramoorthy ***et al***., (2000). Kotteswari ***et al***., (2007) have investigated on the treatment dairy effluent of ***Spirulina platensis*** and ***Nostoc*** sp. (Kotteswari ***et al***., 2012a). This is because a large number of salts were found dissolved in treated dairy effluent. The decrease in total suspensed solids and the total dissolved solids increase, is due to organic substances being broken down into simpler inorganic forms for absorption by growing cyanobacteria (Kanika Sharma ***et al***., 2003).

4.3.7 Total solids

Total solids in the present study reduced to 90.42 percent by ***Aulosira laxa*** and 90.82 percent by ***Tolypothrix distorta***. Vignesh ***et al***., (2006) have reported a reduction of total solids when the tannery effluent was treated with ***Chroococcus turgidus***. Similarly, Ravikumar ***et al***., (2012) have reported a 41.57 percent reduction of total solids when steel effluent treated with ***Oscillatoria*** sp.

4.3.8 Electrical conductivity

Electrical conductivity is related to total dissolved solids (TDS). Electrical conductivity in the present study was reduced to 15.58 percent by ***Aulosira laxa*** and 52.18 percent by ***Tolypothrix distorta***. The higher level of electrical conductivity in untreated effluent could be attributed to the use of inorganic chemicals in dairy manufacturing. In treated effluent electrical conductivity was found to be within the permissible limits as per BIS standards (IS-2009). Vanithasree and Murugesan (2010) have reported the reduction of electrical conductivity in aquaculture effluent was treated with ***Oscillatoria acuminata*** and ***Scenedesmus armatus.*** The present study, confirms the low electrical conductivity could be attributed to decreased concentration of salts.

4.3.9 pH

The pH of the aqueous solution is an important controlling factor in the adsorption process and thus the role of hydrogen ion concentration was examined in dairy effluent. Because many treatment plants use biological systems for effluent treatment, pH must be maintained within a range tolerated by the biomass involved in waste reduction. Strains of cyanobacteria and green algae grew well in dairy effluent. This enhancement of their growth in effluent and their better performance compared to the other strains indicate some substance(s) required for their growth were available in dairy effluent. This was not due to the pH of the effluent (pH 5.15) because they have been known to grow even at pH 6.9 of the medium (Subramanian, 1982). This shows the CO_2 absorptive capacity of ***Aulosira laxa*** and ***Tolypothrix distorta***. Along with nutrients from effluent for their growth metabolism, concurrently they produce oxygen. The byproduct oxygen released during algal metabolism is utilized by the heterotrophic bacteria for biological oxidation of dissolved organics in effluent. These bacteria oxidize the pollutant into simpler compounds which can serve as carbon/energy source for algal species. The pH of the metal solution played an important role in the biosorption of metals. The values of pH in effluent discharged indicate that it is well within the permissible limits of BIS (IS-2009). In the present study interestingly, the pH of the dairy effluent increased from 5.15 to 8.61 when dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta.***

Furthermore, the pH levels are maintained so that there will be no increase in ammonia concentrations. Ammonia concentrations in the effluent and the pH are related by the following equilibrium relationship:

$$NH_3 + H_2O \leftrightarrows NH_4 + OH^-$$

The high pH (above 8.48) would move the equilibrium to the left and raise the ammonia concentration. Hence, pH stabilization during micro algal treatment not only keeps the ammonia levels but also makes the effluent pH level conform to discharge. It is a fact that photosynthesis can cause pH to rise (Garcia ***et al***., 2006).

The typical capacity of cyanobacteria to bring about changes in the pH to suit the requirement was evident from the studies carried out by Manoharan and Subramanian (1993). Hence, the pH effect on the treatment efficiency was investigated. In the present study, pH was found to be increased in dairy effluent treated with cyanobacteria, whereas there was no change of pH in control. In the present study pH was observed to increase from 3rd day onwards. However, Manoharan and Subramanian (1992b, 1993) have found a rise in pH value up to the 10th day of growth of algae in paper mills effluent. The increase in oxygen was associated with the rise in pH, as reported by Kayombo ***et al***., (2002). Several authors have pointed out that the increase in pH due to photosynthetic activity plays an important role in effluent bioremediation, promoting ammonia stripping and phosphorus deposition as insoluble salts (Larsdotter 2006a). Vijayakumar ***et al.,*** (2005) reported an increase of pH in dye effluent, when treated with ***Oscillatoria*** sp. Kotteswari ***et al***., (2007;2012a) reported an increase of pH, when the dairy effluent was treated with ***Spirulina platensis*** and ***Nostoc*** sp. The present study confirmed that ***Aulosira laxa*** and ***Tolypothrix distorta*** were used for improving water quality (pH, suspended solids etc.,) and removal of nutrients and metals from dairy effluent. In the present study, pH was recorded above 8.35, which was below the tolerance limit of 5.5-9.0 prescribed by the Bureau of Indian Standards (IS-2009) and they suggested that this alkaline pH of the effluent could affect the biological property of the receiving water body.

4.3.10 Alkalinity

Alkalinity is a measurement used in the process control of diverse water treatment processes in dairy effluent. Initially there was no carbonate, but fairly high levels of CO_2, bicarbonate were observed. The gradual removal of both CO_2 and bicarbonate was observed from 5th day onwards. The relative proportion of CO_2 and HCO_3 depends on the pH of the medium. It was found that bicarbonate (HCO_3) with 6 to 7 pH is predominant. Thus cyanobacteria may have a competitive advantage over Chlorophytes since, the former are capable of assimilating HCO_3 as a source of inorganic carbon for photosynthesis (Colman, 1989). In general, the removal of these carbon sources effectively by cyanobacteria, as expected, was observed in the present investigation.

In the present study, alkalinity was reduced to 70.72 percent by ***Aulosira laxa*** and 63.14 percent by ***Tolypothrix distorta***. Utilizing the alkalinity produced by the microalga ***Spirulina***, precipitating heavy metals in acid mine drainage has been demonstrated by Van Hille ***et al***., (1999). Kotteswari ***et al***., (2012a) reported in dairy effluent was treated with ***Nostoc*** sp.

Autotrophic algal cells require inorganic carbon for growth; therefore CO_2 gas is usually used in many microalgal photobioreactors (Keffer and Kleinheinz, 2002; Yamasaki, 2003). Since no CO_2 was supplied to the culture in this study, algal cells were forced to utilize dissolved inorganic carbons in the effluent. Anaerobically digested effluent normally contains high level of alkalinity (Hill and Bolte, 2000; Bjornsson ***et al***., 2001) of which major constituent is bicarbonate HCO_3^- ion. In the present study, the alkalinity in dairy effluent was reduced significantly because there was no usage of sodium carbonate during the process.

4.3.11 Total Hardness, Calcium and Magnesium

Effluent treatment processes generally have little effect on the hardness. Hardness in effluent would make it unsuitable for industrial purpose as it may cause scaling of equipments (Goel, 2000). Hardness of water is due to carbonates of calcium and magnesium. In the present study the total hardness was reduced to 74.13 percent by ***Aulosira laxa*** and 74.89 percent by ***Tolypothrix distorta.*** In the present study 62.35 and 64.61 percent of calcium removal and 77.75 and 70.68 percent of magnesium removal were observed in dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** respectively**.** Observations of Munawar (1970) and Kannan (2006) have suggested that Cyanophyceae grow luxuriantly with great variety and abundance in ponds rich in calcium. Uma and Subramaniam (1990) studied the effective use of cyanobacteria (***Oscillatoria*** and ***Aphanocapsa***) in ossein effluent which has a high level reduction of calcium. On the other hand Vijayakumar ***et al.,*** (2005) reported more than 90 percent removal of calcium and magnesium removed from the dye effluent when treated with ***Oscillatoria*** sp. Although, calcium is undoubtedly required for cyanobacterial growth substantial reduction in calcium and magnesium are known to be essential for flocculation and would co-flocculate (Richmond and Becker (1986).

The decrease of hardness was well noticed during 10 - 15 day of growth and after that it almost stabilized. Similar findings were reported by Kannan and Raja Shekaran (1991). Manoharan and Subramanian (1992b) in their study of sewage cyanobacteria interaction have found 25 percent reduction of calcium by the BGA ***Oscillatoria pseudogerminata*** var. Unigramulata Uma and Subramanian (1990) have made similar observations while investigating the feasibility study of ossein effluent using cyanobacteria and Halo bacterium. Kotteswari ***et al***., (2007) reported reduction of calcium and magnesium, in dairy effluent treated with ***Spirulina platensis*** and Ravikumar ***et al.,*** (2012) in steel effluent treated with ***Oscillatoria*** sp.

Although calcium undoubtedly was required for cyanobacterial growth (Fogg 1975); substantial reductions in calcium level cannot be explained for uptake. Divalent cations such as calcium and magnesium are known to be essential for flocculation and would flocculate (Richmond and Becker, 1986) which could explain the observed reductions with calcium and magnesium. ***Calothrix*** and ***Tolypothrix*** alone and in combination with natural microbial population could considerably reduce the magnesium level. Probably because of co-flocculation mentioned earlier.

4.3.12 Sodium and Potassium

When compared to untreated effluent, the treated dairy effluent an increased amount of sodium and decreased amount of potassium was observed. In the present study sodium level was reduced to 14.52 percent in ***Aulosira laxa*** and increased to 10.83 percent in ***Tolypothrix distorta***. Similarly, the level of potassium was reduced to 36.16 percent when dairy effluent was treated with ***Aulosira laxa*** and 2.84 percent in ***Tolypothrix distorta.*** The treated effluent contains increased amount of sodium was due to the additions of nutrients in the treatment process (Somashekar ***et al***., 1984). The high concentration of these metal ions in drinking water causes harmful effects. Hanumantha Rao ***et al***., (2011) reported that the sodium and potassium reduction in leather processing chemical manufacturing effluent was treated with ***Chlorella vulgaris***.

4.3.13 Iron

In the present study when the effluent was treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** reduction of iron seems to be 96.67 percent. Most of algal forms occurring in the polluted fields have a well defined sheath. Only the ensheathed forms of blue-green algae were found tolerating high concentrations of industrial effluents in laboratory culture (Adhikary, 1985; Adhikary and Sahu, 1988). Thus it is fairly convincing that these outermost surface structures play an important role in making ensheathed forms of blue green algae to thrive in adverse conditions. Steven K Wilhelm (1995) has demonstrated that cyanobacteria are capable of responding to low levels of iron availability through alterations in cellular iron requirements and by increasing their ability to scavenge iron from the environment through the activation of siderophore-mediated high-affinity transport systems. Smilar observation were reported by Vignesh ***et al***., (2006) in tannery effluent treated with ***Chroococcus turgidus*** and Ravikumar ***et al***., (2012) in steel effluent treated with ***Oscillatoria*** sp.

4.3.14 Free ammonia

Ammonium levels were very high at the beginning of the experiment, likely because it is a byproduct of anaerobic bacterial hydrolysis and oxidation of organic nitrogen (O'Farrill ***et al***., 2003). High ammonium concentrations may be toxic to algae, thus restraining photosynthesis and oxygen production, which can deplete dissolved oxygen in water (Pearson, 1990). The present study demonstrates a drastic reduction in ammonia nitrogen in effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta***. The values of ammonia removal were 85.52 percent of ***Aulosira laxa*** and 95.91 percent in ***Tolypothrix distorta.*** About 73-82% removal of ammonia nitrogen has been reported in fish form effluent treated with cyanobacterium ***Phormidium bohneri*** (Dumas, ***et al***., 1998). A high efficiency of about 94% removal in ammonia nitrogen was reported from high-rate algal ponds (Picot ***et al***., 1991). However, the pH of the experimental tanks inoculated with ***Spirulina platensis*** was high (>9.0) during the experiment, whereas it was about 8.5 in tanks with ***Nostoc muscorum*** (data not shown). At a lower pH (<8.0), ammonia is predominantly in its ionic form (Wetzel, 1983). Hence, at a higher pH during the culture, some part of the ammonia may have volatilized because of its molecular form. However, ammonia was removed mostly through active uptake by the algae as evidenced from the elevated biomass protein content. Ammonia nitrogen exceeding 50 mgl^{-1} would inhibit algal growth (Ip ***et al***., 1982). The NH_4-N (15mg/l^{-1}) in our study did not reach that level. Therefore the low pH may be the reason for low algal density in stable phase and low nutrient removal efficiencies. Canizares-Villanueva ***et al***., (1995) reported that the effluent dilution is necessary to reduce the toxic effect of the ammonia in the growth of ***Phormidium*** sp. It is similar to that obtained in this work. Once in the high water content; the ammonia was toxic for the microalgae growth. In higher dilutions, the toxic effect of the ammonia was not limited. At high concentration, the presence of free ammonia has been shown to slow microalgal productivity in the “self-inhibition” mode (Rittman and McCarty, 2001).

The toxicity of ammonia is dependent on pH, oxygen concentration and temperature (Cote, 1976). Martinez ***et al***., (2000), who described elimination of NH_4^+ (between 79% and 100%) after 188.25 hrs (about 8 days). There are obvious advantages of eliminating ammonium from effluent using microalgae:

(1) It does not generate secondary pollution of NH_3 and

(2) The microalgal biomass can be harvested and used as a slow-release fertilizer or soil conditioner (de la noue ***et al***., (1992); Mallick (2002); Mulbery ***et al***., (2005).

4.3.15 Nitrite

Nitrite and nitrate removal efficiencies were lower compared with that of ammonia. Nitrite and nitrate may be produced during secondary effluent treatment, when ammonia-N is biologically converted to the oxidized forms by nitrifying bacteria. Nitrogen uptake rates by microalgae depend on the concentration of nitrogen sources, and on environmental factors such as radiance, temperature and water movements (Lobthobban and Harrison, 1994). The uptake rate of nutrients is influenced by several biological factors such as, various types of tissues, age of the plant, its nutritional history or nitrogen status of the thallus and interplant variability.

In case of ***Aulosira laxa*** 64.28 percent reduction was observed at the end of the 15^{th} day, after which the reduction efficiency decreased. This reduced efficiency may be due to the increase in nitrate level caused by nitrification. When the dairy effluent was treated with ***Tolypothrix distorta*** the nitrite content was increased to 63.15 percent, there was an increase in the level of cultivation of cyanobacteria with efficient, that removed all forms of nitrogen as have been reported (Phang ***et al***., 2000; Lodi ***et al***., 2003; Chuntapa ***et al***., 2003). The increase in nitrite was accompanied by a decrease in nitrate in the effluent after treating with cyanobacteria, which is an interesting phenomenon.

4.3.16 Nitrate

Although nitrate itself is not toxic, its conversion to nitrite is a concern in the domain of public health. Nitrogen is biologically removed from effluent in two major ways.

(1) Uptake of nitrogenous compounds by microorganisms and larger organisms growing in effluent in an assimilative way creates a biomass that concentrates the nitrogen and leaves the water with less nitrogen.

(2) Oxidation of ammonium to nitrate, nitrite, and NO eventually forms gaseous nitrogen that evaporates into the atmosphere.

Nitrate content in the effluent was found to increase up to 10.39 percent by ***Aulosira laxa*** whereas in ***Tolypothrix distorta,*** it was reduced to 19.16 percent. Travieso ***et al***., (1996) reported that increasing the light intensity can lead to a higher microalgal activity and an increased removal of nutrients from effluent. Microalgae also have the ability to take up various kinds of nitrogen Hong and Lee (1993) and Kim and Lee (2000).

In the present investigation, in dairy effluent, all forms of nitrogen were observed in appreciable quantities and cyanabacteria removed more than 55 percent of inorganic nitrogen from the effluent. However, ***Aulosira laxa*** and

Tolypothrix distorta were marginally better in removing all forms of nitrogen. Studies on ***Spirulina*** (Boominathan ***et al.,*** 2007); ***Anabaena*** (Mallick and Rai, 1994); ***Oscillatoria*** (Manoharan and Subramanian, 1992a and 1993) concluded that cyanobacteria can efficiently eliminate inorganic nitrogen compounds from effluents. Padmapriya and Murugesan (2012) have reported that the 50 percent reduction of nitrate in distillery effluent treated with ***Oscillatoria*** sp. The removal of inorganic nitrogen compounds by cyanobacteria is governed by growth conditions and physiological conditions of the state of the organism like pH, light, temperature, inoculums size and subtracted concentration.

4.3.17 Chloride

Chlorides are generally considered as one of the major pollutant in the effluents which are difficult to be removed by conventional biological treatment methods. In the present study chloride level was reduced to 96.16 percent by ***Aulosira laxa*** and 60.51 percent by ***Tolypothrix distorta.*** Uma and Subramanian (1990) observed nearly 50 percent removal when ossein effluent, which has very high levels of chloride, was treated with ***Oscillatoria*** and ***Aphanocapsa*** respectively. Manoharan and Subramanian (1992a&b and 1993a) also made similar observation observed in various effluents when treated with ***Oscillatoria*** sp; ***Oscillatoria brevis*** in dye effluent by Vijayakumar ***et al.***, (2005); oil refinery effluent (Boominathan ***et al.***, 2007) and sugar mill effluent (Gopalakrishnan, 2007). Similarly, Murugesan ***et al***., (2007) had reported, in oil refinery effluent treated with ***Spirulina platensis*** and in ***Chroococcus*** sp and in pharmaceutical effluent by Ravikumar ***et al***., (2012). In the present remediation study the amount of chloride was lower than that prescribed by BIS (IS-2009) in the effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta***. An excessive amount of chloride content (200 mg/l) will create problems of reuse of the water systems (Middledrop ***et al***., 1990).

4.3.18 Fluoride

Fluoride enters aquatic systems as a result of its addition to domestic water. Because of the limited effluent concentration, data available for fluoride is limited. Hence, it is recommended that data base on effluent has expanded. In the present study fluoride content was reduced to 93.33 percent when the dairy effluent was treated with ***Aulosira laxa*** and 66.66 percent by ***Tolypothrix distorta***. Saha (1993) has established that in the BARC method, the reduction of fluoride from 8 to 2 mg/l is achieved by forming a water soluble stable complex which contains fluoride, calcium and aluminium. Biosorption process is dependent on the aqueous phase pH and the functional groups on the algal cell walls and their ionic states (on particular pH) (Venkata Mohan ***et al***., 2003).

The algal cell wall contains a high amount of polysaccharide and some of them are associated with proteins and other components (Ilhami ***et al***., 2005). These biomacromolecules on the algal cell surfaces have several functional groups (amino, carboxyl, thiol, sulfydryl and phosphate groups) and biosorption phenomena depend on the protonation or unprotonation of these functional groups on the surface of the cell wall (Ilhami ***et al***., 2005). The ionic form of fluoride in solution and the electrical charge of the algal cell wall components (i.e., functional groups carrying polysaccharides and proteins) depend on the solution pH. The results of the present study were similar to

Kotteswari ***et al***., (2007) in dairy effluent treated with ***Spirulina platensis.*** Venkata Mohan ***et al.,*** (2007) reported adsorption studies performed on the algal ***Spirogyra*** IO2 sp as biosorbents revealed the ability of algal species to remove fluoride from the aqueous phase. In the present study, fluoride levels in the dairy effluent were lesser (0.4 mg/l) than that of the Bureau of Indian Standards (IS-2009) (2.0 mg/l).

4.3.19 Sulphate

In the present study sulphate content in the dairy effluent was reduced to 52.66 percent by ***Aulosira laxa*** and 45 percent by ***Tolypothrix distorta***. A sulphate process could be employed for the treatment due to the high degree of metal removal due to lower solubility of metal sulphides**,** low retention times owing to the high reactivity of metal sulphide systems, better thickening and dewatering properties of the sludge's compared with hydroxides and relatively lower interference from chelating agents and complexing agents. The slowly degradable sulphate converted to sulphide which subsequently combine with the metal and form metal sulphide. This sulphide becomes toxic as well as corrosive in the degrading system. Moreover, the metal sulphide develops dark colour and foul smell. They are less subject to leaching more even at pH 5, than metal hydroxides which are amphoteric in nature.

Dash and Mishra (1999) studied the reduction of sulphate when the paper mill effluent was treated with blue green alga ***Westiellopsis prolifica.*** Kotteswari ***et al***., (2007) reported a sulphate reduction in the dairy effluent was treated with ***Spirulina platensis*** and ***Aulosira*** sp by Kotteswari and Murugesan (2012). Similarly, Rajasulochana ***et al***., (2009) had reported sulphate reduction in oil refinery effluent treated with ***Scenedesmus obliquus.***

4.3.20 Phosphate

Phosphate removal by the algae during phycoremediation is due to the utilization of phosphorus for growth. The phosphorus is used in the cells mainly for the production of phospholipids, ATP and nucleic acids. Algae assimilates phosphorus as inorganic orthophosphate, preferably as H_2PO_4or HPO_4^{2-}, and the uptake process is active, i.e., it requires energy (Becker, 1994). Moreover, microalgae are able to assimilate phosphorus in excess, which is stored within the cells in the form of polyphosphate (volutin) granules. These reserves can be sufficient for prolonged growth in the absence of available phosphorus (Fogg, 1975; Oliver and Ganf, 2000).

Biological phosphorus removal by microalgal biomass is dependent on both the algal solids concentration and the amount of phosphorus that can be accumulated in the biomass.These phosphates were removed by photosynthetic assimilation and calcium phosphate precipitation because of high pH levels caused by intense algal photosynthetic activity (Hammouda ***et al***., 1994). Moreover, microalgae are able to assimilate phosphorus in excess, which is stored in the cells as polyphosphate granules, and magnesium and potassium are co-transported along with phosphate (Fogg ***et al***., 1973; Bitton, 1990). All the above findings confirm that cyanobacteria can absorb phosphate in excess amount as it is required and this could be the reason for maximum removal of all forms of phosphate from the effluent.

In the present study of dairy effluent, phosphate uptake was 85.58 percent when the effluent was treated with ***Aulosira laxa*** and it was 90.54 percent in ***Tolypothrix distorta***. The capacity of cyanobacteria to remove large amounts of phosphorus from industrial effluents has been demonstrated by several workers (Mittal and Senegar, 1989; Monoharan and Subramanian (1992a and 1993); Dash and Mishra (1999); Vijayakumar (2005); Jing Shi ***et al***., (2006); Boominathan ***et al***., (2007); Dhamotharan ***et al***., (2009) and Murugesan ***et al***., (2012). Tam and Wong (1990) have reported over 90 percent removal of total phosphorus within 10 days of algal cultivation. Tam ***et al***., (1994) reported that the phosphate removal depends on the type of effluent, the type of algae and their growth conditions, and most importantly on the relationship between the amount of biomass applied and the hydraulic loading of the effluent.

The major phosphate reserve of cyanobacteria is polyphosphate, which accumulates as discrete granules in the cytoplasm of the cell wall when phosphate is in excess (Fay, 1983). The total phosphorus and soluble reactive phosphorus concentrations suggest that these are consumed by the algal biomass more slowly than they are produced, as is seemingly the case for total phosphorus in stabilization ponds in Mexico, according to studies carried out by Lloyd ***et al***., (2003).

In the present study, the removal of phosphorus was not as effective as that of nitrogen. Variable phosphate removal efficiencies were found in fish form effluent treated with ***Phormidium bohneri*** (Dumas ***et al***., 1998). Better removal of nitrogen (99.3%) than phosphorus (48.1%) was found in the algal ponds as studied by Li ***et al***., (1991). In an aerobic treatment system with ***Spirulina platensis***, the removal of phosphorus could be by biological assimilation and chemical precipitation (Laliberte ***et al***., 1997). Biological assimilation generally takes place during the growth phase, and once the biomass concentration reaches a threshold level, chemical precipitation may predominate. It is possible that the phosphates were mostly removed by biological uptake rather than chemical precipitation because of the increasing culture densities.

There are two major ways to remove phosphorus from the effluent:

(i) Direct cellular absorption under aerobic conditions and

(ii) Sedimentation to anoxic conditions.

Phosphorus removal in the present study under aerated conditions can be explained by its interaction with the nitrogen in the water. Because nitrogen is the limiting nutrient factor in the medium, the phosphorus concentration will still be high even after ammonia exhaustion. This leads to saturation in the phosphorus cellular absorption mechanisms (Chevalier and De la Node, 1985).

4.3.21 Silica

In the present study silica content was reduced to 19.21 percent when the dairy effluent was treated with ***Aulosira laxa,*** whereas in ***Tolypothrix distorta*** it was reduced to 29.50 percent. Kotteswari ***et al***., (2007) reported a 80.77 percent reduction of silica when the dairy effluent treated with ***Spirulina platensis.*** Dhamotharan ***et al***., (2008) reported a 54.23 percent reduction of silica when the sewage effluent was treated with cyanobacteria.

4.3.22 BOD and COD

BOD indicates the quality of pollutant present in the effluent can be decomposed by bacteria under aerobic condition; hence the BOD value increases with increase in organic content. The high content of organic matter results in high value of COD of effluent because chemical oxygen demand measures the recalcitrant (non-biodegradable) organic matter in biologically treated industrial effluents (Malaviya ***et al.***, 2001). BOD and COD values were also considered as an index for the survival of living organisms in industrial effluents. Higher values of BOD and COD may be due to the presence of organic and inorganic load in the effluents which was decamp and increasing temperature.

In the present study, the COD valueded in the effluent was found to be reduced by 69.57 percent when the dairy effluent was treated with ***Aulosira laxa*** and 67.37 percent by ***Tolypothrix distorta*** treated effluent. The BOD valueded in the untreated effluent was found to be reduced by 66.66 percent when dairy effluent treated with ***Aulosira laxa*** and 66.67 percent in ***Tolypothrix distorta***. It is thus becomes evident that reduction in COD was less as compared to a reduction in BOD. Therefore, it is obvious that the degradation sought was through biological activity and not through a chemical agent. The dairy waste contains lots of complex organic compounds**.** These compounds have different biodegradability. Depending on their ratios, the waste streams at various outlets, the amount of BOD have varied widely compared to the COD values. That could be the reason that in some of the values where BOD has not increased to that extent as compared to the increase in the CODs observed. This observation was in conformity with Baruah ***et al.,*** (1996); Amudha and Mahalingam (1999) and Kumar Senthil ***et al***., (2001) based on experiment related to different industrial effluents. COD measures the equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. The use of acclimatized algal cultures in considerably reducing BOD and COD with different effluents including tannery was also reported (Govindan, 1985). Reduction of BOD and COD levels might be occurred due to the removal of dissolved organic compounds and derivatives to some extent from the effluent during the treatment process (Verma ***et al***., 1988). The use of algal cultures for reducing COD from different types of effluent has been reported (Govindan, 1984, 1985; Manoharan and Subramanian, 1992a and 1992b, 1993). Similar kind of studies was carried out by various authors. Vignesh ***et al***., (2006) in pharmaceutical effluent treated with ***Chroococcus turgidus;*** Kotteswari ***et al***., (2007) in dairy effluent treated with ***Spirulina platensis;*** Murugesan ***et al***., 2007 at oil refinery effluent treated with ***Spirulina platensis.***

Among all the nutrient reduction parameters, reduction of COD, an approximation of carbon levels, was the greatest. This may be attributed to the fact that carbon is a macro nutrient necessary for algae growth. When an organic carbon source is present in the medium and light is used for energy, algal growth is considered as mixotrophic growth. The mixotrophic growth rate is believed to be approximately the sum of both autotrophic and heterotrophic growth rates, which represents the fastest way to grow algal biomass (Lee ***et al***., 1989).

4.3.23 Oil and grease

Oil and grease interfere with biological life forms in the surface waters (Metcalf and Eddy, 1991). In effluent treatment much of the oil and grease floats and is removed by mechanical skimming. When oil coats the suspended particles, it becomes more difficult to treat. In the present study, oil and grease were reduced to 99.41 percent when the dairy effluent treated with ***Auloisra laxa*** and ***Tolypothrix distorta*** it was 99.41 percent**.** Analysis of dairy effluent showed the presence of oil and grease which was far below the permissible limits of BIS (IS-2009). If the surface water is contaminated with oil and grease oxygen transfer from the atmosphere is reduced. If these fatty substances emulate, they create a very high oxygen demand on account of their bio-degradability. Murugesan ***et al***., (2007) reported reduction of oil and grease when the oil refinery effluent was treated with ***Spirulina platensis.***

4.4 Heavy metals

Microorganisms specifically algae play an important role in the removal of heavy metals from effluent. They have the ability to uptake and accumulate the heavy metals such as copper, cadmium and lead from the effluent to a level far exceeding the metal level in the water.

4.4.1 Copper

Copper is an essential component of most living organisms. It is extensively used in numerous industrial activities and it has some derivatives that are mildly poisonous. Its main danger to the environment results from the huge quantities accumulating in every habitat.

Algae have been shown to respond differently to pollutants. Jensen ***et al***., (1974) demonstrated that ***Skeletonema costatum*** responded with equal sensitivity to copper ions, but differed markedly in response to zinc. It may therefore be necessary to test many species to find those most sensitive in most tests. Many cyanobacteria require combined inorganic nitrogen sources for aerobic growth. Such an obligate requirement for growth may not be exhibited by hetrocystous forms which thrive in a low nitrogen environment and fix nitrogen. ***Nostoc muscorum*** can grow in the presence or absence of inorganic nitrogen sources. This organism therefore provides an opportunity to investigate the effects of nitrogen sources against copper toxicity. The results suggested that nitrate could protect the organism from copper toxicity to a limited extent since under nitrogen fixing condition the organism was more sensitive than in the nitrate supplemented medium (Fogg, 1974).

In the present study, the copper reduction occurs up to 99.55 percent when the dairy effluent treated with ***Aulosira laxa*** and 99.47 percent by ***Tolypothrix distorta.*** Similar observations were made by Dashora and Gupta (1978) with copper. The results thus suggested that the nutritional status of an organism may be an important factor while determining heavy metal toxicity.

The effectiveness of copper uptake by cyanobacteria grown in the dairy effluent was studied. The results showed that a high percentage of copper removal (99.47%) was achieved by ***Tolypothrix distorta*** reared in the dairy effluent. Similar results were also obtained by Chan ***et al***., (1981) in a mixture of electroplating and sewage effluent. In the present study, copper levels in the dairy effluent were lower than the amount prescribed by BIS (3.0 mg/l).

4.4.2 Chromium

Chromium is used primarily in metallurgy, automotive paint, tannins of leather, catalyst industries and is not usually considered a health hazard in its metal forms. However, chromium (VI) compounds are toxic, especially to the eyes and are carcinogenic. Levels of toxicity in drinking water are low. Hence, there is no motivation for cleanup. Hexa valent chromium may lead to the lung carcinoma. Excess chromium interferes with the effluent treatment to a great extent (Law, 1977; Gottawalder, 1985).

Throughout the world, there are two main systems of recycling of chrome liquors are practiced in tanneries. One system involves the direct recycling of chrome liquor, and the other system involves cycles of chrome liquor following precipitation (or adsorption) and then resolubulizing. The chromium concentration in the effluent was reduced to 70 percent in ***Aulosira laxa*** and 45 percent in ***Tolypothrix distorta*** treated with dairy effluent. It was observed that as the pH increases, the flock formed is more which carries the suspended particles and the removal of chromium along with other contaminants increases till its optimum pH. Rehman and Shakoori (2003) reported green alga (***Chlamydomonas*** sp) in chromium - containing industrial effluents indicated that the alga has acquired efficient means of resisting, tolerating or processing Cr^{6+}. Cervantes ***et al***., (2001) described that the potential tools for bioremediation of Cr pollution using algae and it seems clear that different species of algae accumulate metals to various degrees.

From the analysis, it is found that the ***Aulosira laxa*** is the most economical in the removal of chromium and other toxic substances from effluents. In the present study, chromium level in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was lower than the amount prescribed by BIS (2.0 mg/l).

4.4.3 Zinc

Zinc is an essential element for sustaining all life and too much zinc can be harmful. Zinc is an essential element for many enzymatic activities (Cheblowski and Coleman, 1986) in plants. Zinc ions are present in different types of industrial effluents, being responsible for environmental pollution. Biosorption is a process in which solids of natural origin are employed for binding heavy metals. It is a promising alternative method to treat heavy metal wastes mainly because of high metal binding capacity.

In the present study, the zinc reduction occurs up to 65 percent in ***Aulosira laxa*** and 75 percent in ***Tolypothrix distorta*** treated with dairy effluent. Similar observations were made by Rana and Kumar (1974) with zinc. However, zinc at toxic concentrations affects the growth and metabolism of green plants (Shrotri ***et al***., 1981). In the present study, the level of zinc in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was lower than the amount prescribed by BIS (1.0 mg/l).

Gaur and Dhankhar (2009) demonstrated ***Anabaena variabilis*** is a good capacity of Zn^{+2} ions biosorption highlighting its potential for the effluent treatment process. Cristina ***et al.,*** (2011) also reported the maximum extent of Zn removal (836.5 and 429.6 mg Zn/g biomass), by ***Scenedesmus obliquus*** and (ACOI), respectively, indicate that both microalgae strains have an absorption capacity compared to other biosorbents.

4.4.4 Lead

Lead is a toxic metal, but is an extremely valuable component in numerous industrial applications, such as car batteries, glazed ceramics and bullets. It causes serious damage to the nervous system (especially in young children) and causes blood and brain disorders. ***Gloeocapsa*** sp, ***Nostoc paludosum***, ***N. piscinale***, ***N. punctiforme***, ***N. commune***, ***Oscillatoria agardhii***, ***Phormidium molle*** and ***Tolypothrix*** removed 90–96% of Pb from 1 mg/l^{-1} solution (Drungkokkruad, 2002).

In the present study, the lead reduction occurs up to 25 percent when the dairy effluent treated with ***Aulosira laxa*** and 50 percent by ***Tolypothrix distorta***. Inthorn ***et al***., (2002) reported that the cyanobacterium ***Calothrix marchica*** was able to remove Pb from effluent, and the cells (Ruangsomboon ***et al***., 2007) demonstrated that the cells of this cyanobacterium, covered with a mucilaginous sheath, were able to remove Pb ions also owing to the contribution of polysaccharide material. The accumulation of heavy metals in capsular polysaccharide was also reported for ***Phormidium laminosum***, which showed the capability of adsorbing lead. In the present study, the level of lead in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was lower than the amount prescribed by BIS (1.0 mg/l).

4.4.5 Nickel

Nickel also plays numerous, essential role in the biology of microorganisms and plants, but some nickel compounds, including nickel sulfide fumes and dust are possibly carcinogenic. It has high value and therefore, after removal, the product is recycled. In the present study the nickel reduction occurs up to 82.35 percent when the dairy effluent treated with ***Aulosira laxa*** and with ***Tolypothrix distorta*** 94.11 percent. Azeez and Banerjee (1991) reported the toxicity of nickel in ***Anacystis nidulans*** and ***Spirulina platensis*** grown in artificial culture medium and also the accumulation of this metal by these cyanobacteria. In the present study, the level of nickel in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was lower than the amount prescribed by BIS (3.0 mg/l).

4.4.6 Cadmium

Cadmium is a common, toxic pollutant because it is part of many industrial and domestic applications, such as batteries, pigments, coatings, electro-plating, stabilizer in plastics, and in alloys with low melting temperatures. Removal of cadmium is a top priority in many developed countries. Immobilization of the cyanobacterium ***Spirulina maxima*** with the microalgae ***Tetraselmis chuii***, both with a high cadmium uptake capacity, was done by adherence of the cell surface. Regarding the immense problem of cadmium pollution, bioremoval is still a minuscule technique in effluent treatment. The mechanism of the effectiveness in removing heavy metals from effluent by microalgae relates to their large surface area and high binding affinity (Roy ***et al***., 1993). Cristina ***et al***., (2010) reported both the wild and the commercial strains of ***Desmodesmus pleiomorphus*** (i.e., L and ACOI 561) are able to remove Cd from aqueous solution-hence those strains were used for bioremediation of metal-polluted waters or effluents. In the present study, the cadmium reduction occurs up to 95.65 percent when the dairy effluent was treated with ***Aulosira laxa*** and 99.13 percent in ***Tolypothrix distorta***.

Sudarat Chaichalearam ***et al***., (2006) reported immobilized cells of ***Scytonema*** sp. and ***Hapalosiphon hibernicus*** on synthetic fiber (SF) have the potential to remove cadmium in wide spread low cadmium contaminated natural water in rivers or channels and can be applied for use in shrimp ponds. Reddy ***et al.,*** (1997) studied the effect of cadmium on the growth of blue green algae ***Chloroglobea fritschii*** and two species of ***Synechocystis. C.fritschii*** could tolerate up to 6 ppm of Cd but concentration above 2 ppm has shown a marked decrease in the growth. Biosorption by algae thus demonstrated itself to be a useful alternative to conventional systems for the removal of toxic metals in solution. Inthorn ***et al***., (2001) have reported that the ***Scytonema schmidlei***, ***Anabaena cylindrica*** and ***A. torulosa*** removed 96 to 98% of Cd from 1 mg/l solutions of this metal. In the present study, cadmium level in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was lower than the amount prescribed by BIS (2.0 mg/l).

4.4.7 Arsenic

Arsenic is a naturally occurring metalloid that generally exhibits a concentration range of 1-10 $\mu g/l^{-1}$ in freshwaters unimpacted by geogenic or anthropogenic sources of arsenic (Williams, 2001). Algae could accumulate arsenic if it is present in solution. The higher the concentration of arsenic in solution, the greater the accumulation will be. The toxicity and adverse health effects of arsenic are widely known (Lin ***et al***., 1998). In the present study the arsenic reduction occurs up to 60 percent when the dairy effluent treated with ***Aulosira laxa*** and 80 percent when treated with ***Tolypothrix distorta***. In the present study, arsenic level in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was higher than the amount prescribed by BIS (0.2 mg/l).

The cyanobacteria were shown to reduce arsenate. This is important for two reasons. Reduced arsenate increases mobility and potential toxicity (Jain and Ali, 2000). Also, the presence of arsenate in anoxic environment may be a biomarker of an organism's exposure to arsenic. Toxic effects were not evident when comparing cyanobacterial growth, though extractions indicate accumulation of intracellular arsenic by the cyanobacterium.

4.4.8 Mercury

Mercury is used in many electrical and electronic applications; however, the main source of pollution is from coal-fired power generating facilities and cement production, and smelters. As such, the environment has a constant supply of polluting mercury. Mercury is harmless in an insoluble form, such as mercuric sulfide, but it is extremely poisonous in soluble forms, such as mercuric chloride and organic compounds. Mercury is a highly toxic metal which, once released into water, accumulates in the food chain, damaging fish, shrimps and poisoning people who eat them. In the present study, the mercury reduction occurs up to 33.33 percent when the dairy effluent treated with ***Aulosira laxa*** and 50 percent in ***Tolypothrix distorta***. Inthorn (2001) reported ***Tolypothrix tenuis*** and ***Calothrix parietina*** showed a very high capability of Hg removal. Amber Cain (2004) reported, two strains of cyanobacteria (***Spirulina platensis*** and ***Aphanothece flocculosa***) were able to remove mercury (II) from solution. In the present study, mercury levels in the dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** was higher than the amount prescribed by BIS (0.01 mg/l).

The degree of purification of aquatic environments from heavy metals is dependent on the functional state of the culture (duration of cultivation, activity of producing exopolysaccharides). The algae, in the active phase of growth (15^{th} day) bind 21-99 percentage of heavy metals from solution. The prolonged cultivation of the cyanobacteria promotes hyper production of mucous exopolymers. It can be used for the removal of heavy metals from effluent due to its high rate and capacity of adsorption and absorption. The results indicate that the algal culture has a high tolerance (100 ppm) and can be effectively employed in the phycoremediation of effluent. Although these results based on laboratory experiments suggest the possibility of treating effluents using cyanobacteria, further experiments need to be carried out with the objective of optimizing the conditions which would allow creating an efficient algal biosystem for metal removal from industrial effluents.

Effluent treatment by algae has to be intensified so as to develop innovative technologies, which will definitely pave a way for cleaner as well as a greener environment for the generations to come. Improved methods for evaluating the degree of pollutant biodegradation in complicated environmental settings need to be developed; with simpler, less expensive, and more rapid assay procedures. Although complete mass-balance analyses are desirable, they are seldom obtainable. In light of this deficit, the durability and effectiveness of bio-remediative technologies will require other indicators.

4.5 Bacteriological Examination

In the present investigation, two different genera of bacteria were isolated and identified. In the literature, very few reports on isolation and identification of bacteria from different effluent samples. Jain ***et al.,*** (2001) have isolated three different bacterial strains from the distillery sludge to treat the predigested distillery effluent. Boominathan ***et al.,*** (2007) have isolated nine different species of bacteria from dairy effluent.

In the present study, the removal efficiencies of total coli form and faecal coli form bacteria were similar to Gersberg ***et al***., (1986) to wetlands treatment of domestic effluent. Total and faecal coli form concentrations followed the same trend of total ***E. coli*** from the same wetlands (Ibekwe ***et al***., 2002). Wetlands treatment achieved a 2 log (99%) decrease in total coli form bacteria and a 3 log (99.9%) decrease in faecal coli form bacteria between the raw wash water pond and the average wetland effluent. This is significant both from the standpoint of surface runoff as well as potential airborne pathogens released during the spray irrigation of raw wash water on disposal lands.

The lesser number of bacterial populations was apparently due to the environmental stress caused by the high level of pollutants, which allowed only a restricted number of species that tolerate such conditions. Sree kumar and Soundarajan Krishnan (2010) have successfully isolated a new strain of spore forming ***Bacillus*** that is capable of fermenting lactose from dairy effluent. Most of the isolated genera are potential pathogens. Some of these bacteria have been previously reported to be present in effluents (Cho and Kim, 2000) and oil polluted sites (Macnaughton ***et al***., 1999; Whiteley ***et al***., 2000).

However, (Sulaiman ***et al***., 2002) isolated only two bacterial genera such as ***Derxia*** and ***Beijerinckia*** were isolated from dye effluent drenched soils. Due to the environmental stress caused by the high level of pollutants, only a restricted number of species have tolerated such conditions. On the basis of this fact it is suggested that the indicator species could be used for pollution abatement programmes. Taking the above facts into consideration a survey was undertaken in dairy effluent to explore the nature of bacterial population as a tool in treating dairy effluent.

4.6 Characterization of the dairy effluent using FT-IR spectroscopy

The FT-IR spectrum of untreated and treated effluent showed several distinct and sharp adsorption bands of different wavelength. The FT-IR spectra of loaded dairy effluent indicated some shifts in some of characteristic bands. Change in the spectrum depicts changes in functional groups of biomass after treatment. This implied the possibility that biosorption could be taking place through an ion exchange process rather than complexation.

The FT-IR spectra a very strong adsorption band around 3892-3962 cm^{-1} found in these samples may be due to the presence of OH stretching of amines and amides and polymeric association which was normally found in hydroxyl compounds. The adsorption band around 3361-3408 cm^{-1} correspond to intermolecular hydrogen bonds. The peak appeared between 2132-2336 cm^{-1} was due to stretching vibration of –C-CH (terminal) vibrations. The FT-IR analysis of biosorbent specifically the 1644-1656 cm^{-1} band indicated the existence of the amide I band of the amide bond in poly-N-acetyl glucosamine (chitin) and the protein peptide bond present in biomass considered to be due to the combined effect of double bond stretching vibrations (mainly C=O) and hydrogen bonding (Li ***et al***., 2008). The absorption at 866 cm^{-1} probably due to benzene 1, 2, 4 (trisub) bands, the band between 728-735 cm^{-1} corresponded to aromatic CH bending vibration of sugar. The peak at 615 cm^{-1} representing aliphatic bromo compounds.

The result indicates that the chemical interactions such as ion-exchange between the various functional groups of biomass and metal ions were mainly involved in pollution in the effluent. The changes in the functional groups and the surface properties of pretreated dairy effluent were confirmed by FT-IR spectra. Thus, analyses of functional groups, break through time, desorption and reutilization of metals and organic compounds, can help in carrying out an efficient pollutant removal process. The common point without any pretreatments was the small intensity of absorbance. The treated effluent with cyanobacteria showed similar changes in the peak intensity. This indicates that most of the organic substances responsible for pollution were successfully removed by cyanobacterial treatment.

4.7 Value added products from *Aulosira laxa* and *Tolypothrix distorta*

4.7.1 Pigment composition

4.7.1.1 Chlorophyll and carotenoid pigments

Cyanobacteria contain the highest concentration of chlorophyll. The chlorophyll molecule is divided into porphyrin and phytol moieties (parts). Cyanobacteria grow fairly well in dairy effluent and generally this growth is measured by the chlorophyll and carotenoid as a biomass component (Sallal, 1983).

In the present study, ***Aulosira laxa*** and ***Tolypothrix distorta*** were found to grow fairly well in the dairy effluent treated cultures. The present study confirms the adverse effect of dairy effluent on total chlorophyll and carotenoids. The organism may grow in mixotrophic condition for the reduction of chlorophyll and carotenoid in the effluent. In cyanobacteria, phycobilliproteins (PBPs), the primary light-harvesting antenna for PS-II is attached to the stromal surface of thylakoid membranes. In response to stress conditions the composition and function of PBPs in cyanobacteria changes. Although, all cyanobacteria are photoautotrophic, many can utilize simple Dissolved Organic Carbon (DOC) compounds for heterotrophic growth or for mixotrophic growth in the light (Fogg ***et al***., 1973, Sahu and Adhikary, 1982; Al-Hasan ***et al***., 2001; Shanthy Sundaram and Soumya, 2011). All environmental stress is affecting the production of active oxygen species in plants, causing oxidative stress (Smirnoff, 1993; Hendry, 1994; Bartosz, 1997). Recently, Ranjithkumar ***et al.,*** (2011) studied mixotrophic condition grown in microalga ***Chlorella vulgaris*** in acidic confectionery effluent.

4.7.2 Biochemical Composition

The biochemical constituents of cyanobacteria depend on the nature of the strains, the physiological state of the culture and the environment (Vargas ***et al***., 1998; Subhashini ***et al***., 2003; Maslova ***et al***., 2004; Rosales ***et al***., 2005). Rosales ***et al***., (2005) reported about the physiological competence of ***Synechococcus*** sp. in hyper saline medium. They observed high cell contents of chlorophyll-a, carotenoids, proteins and carbohydrates at 100 ppm and in good nutrient conditions.

4.7.2.1 Carbohydrates

Carbohydrates are the primary source of energy and is vital for all body functions and muscular exertion. The carbohydrates in cyanobacteria are straight chains of glucose molecules that are nearly identical to those found in glycogen, the major carbohydrate stored in the liver to regulate serum glucose levels in the body.

Differences in carbohydrate content of cyanobacteria treated with effluent and control were observed. A maximum amount of total carbohydrate was found in treated ***Tolypothrix distorta*** (20.12 ± 0.01 mg/g dry weight) and the minimum in the laboratory cultured ***Tolypothrix distorta*** control (9.71 ± 0.01 mg/g of dry weight). The carbohydrates formed a major organic component of these species. The two species of cyanobacteria from a sulfur spring showed a high concentration of carbohydrates than others. The influence of effluents on the biochemical composition of cyanobacteria had been studied by Manoharan and Subramanian (1992a) in paper mill effluent on the physiology and biochemistry of the ***Oscillatoria pseudogeminata*** var. ***unigranulata.*** They found that the total carbohydrate content of ***O.pseudogeminata*** of paper mill effluents showed more than two fold increase in its level when confirmed that of the control. Hosmani and Anitha (1998) have reported a similar type of results for carbohydrate and protein contents in ***Microcystis aeruginosa***, (84.44 and 22 mg/ml of carbohydrates and protein, respectively). The investigation carried out by Walach (1987) indicates that the carbohydrate synthesis increased with decrease in nitrogen availability under constant carbon availability. Besides food value, extracellular and intracellular carbohydrates of cyanobacteria are involved in some other properties. The cellular carbohydrate content serves to facilitate the buoyancy changes in the bloom forming ***Microcystis aeruginosa*** was reported by Kromkamp and Mur (1984). Another characteristic feature of cyanobacteria observed is that their ability to secrete carbohydrate and protein extracellularly and their potential role in metal removal and in food and packaging industries (Shah ***et al***., 2000). Kawaguchi and Decho (2000) and Kawaguchi ***et al***., (2003) have reported of the extracellular polymeric secretion by ***Schizothrix*** sp., ***Synechocystis*** sp. and ***Oscillatoria*** sp. found in the Exuma Cays and High has borne cay in the Bahamas, which contained acidic polysaccharides and proteins. Growth promoting and inhibiting effect of carbohydrates secreted as extracellular substances by some species of cyanobacteria was reported by Safonova and Reisser (2005).

4.7.2.2 Proteins

Treated ***Tolypothrix distorta*** contain 8.11 ± 0.01 mg/g (dry weight) of protein, in control lowest protein content viz., (7.16 ± 0.01) was observed in ***Aulosira laxa*** (control)**.** The presence of a combined nitrogen source resulted in an increase in the protein content of the cells and a decrease in the levels of lipids, although biomass productivity was not affected significantly. Similar results were found by Sánchez ***et al***., (2001) in algae treated with olive mill effluent. Subhashini ***et al.,*** (2003) observed significant variations in protein content among the four isolates of ***Anabaena azollae.***

4.7.2.3 Lipids

Lipids provide the most concentrated source of energy for the body. They function as carriers for Vitamins A, D, E and K and are important for the conversion of plant Beta-carotene into Vitamin A. The lipids found in cyanobacteria are commonly known as glycolipids, meaning they are composed of a sugar portion and a lipid portion. The lipid moiety acts as an excellent transport vehicle for vitamins and minerals across blood-cell barriers.

Tolypothrix distorta treated with effluent showed higher concentrations of total lipid of 1.64 ± 0.01 mg/g (dry weight) and the minimum in the laboratory culture of ***Tolypothrix distorta*** (0.92 ± 0.02 mg/g (dry weight). Fatma ***et al.***, (1994) analyzed four strains of ***Spirulina*** sp. for their protein and total lipid contents and values obtained were within the range of 43–55% and 2.7-6.8%, respectively.

Aulosira laxa and ***Tolypothrix distorta*** has led to a new supplement that contains more protein and chlorophyll than any other food source. This non-toxic cyanobacteria, which grows only in the wild, is far superior to any other algae on the market and has become the focus of much attention in the health food industry.

4.7.2.4 Amino acids

Cyanobacteria species can accumulate economically valuable amino acids in their cells or release them in the medium. Amino acids are used extensively in the food industry, medicine and in the chemical industry as starting material for the manufacture of cosmetics (Hillol Chakdar ***et al***., 2012). Cyanobacteria contain all nine essential amino acids, both semi-essential amino acids, and is a suitable source for arginine, known to build and tone muscle tissue. Thus, the cyanobacteria apart from their phycoremediation capabilities also have nutritional value. There was an increase in amino acid levels not only in quantity but also in quality in ***Oscillatoria*** and ***Westiellopsis*** treated with Dye effluent by Vijayakumar ***et al***., (2007). Contrary to this observation, Boominathan (2005) reported a decrease in the level of amino acids in both qualitative and quantitative when ***Oscillatoria*** and ***Aphanocapsa*** was treated with dairy effluent.

The presence of various amino acids reported in ***Aulosira laxa*** and ***Tolypothrix distorta*** has already been reported by various workers in different species of cyanobacteria (Dokhan, 1953; Karanth and Madaiah, 2011; Manoharan, and Subramanian, 1993). Rzhanova, (1968) found arginine as the dominant amino acid in ***Phormidium uncinatum*** followed by hisditine, lysine, leusine and α-alanine in fairly good quantity. Similar observation with higher content of histidine has been reported (Boominathan (2005); (Vijayakumar ***et al***., 2007). A noteworthy observation is that most of the amino acids recorded in treating cyanobacteria showed higher levels than the control. Perumal Senthil ***et al***., (2012) have reported higher quantity of amino acid content in cyanobacteria grown in rubber industry effluent.

Pronounced reductions in the total free amino acid contents of the two investigated cyanobacteria were recorded due to the treatment with dairy effluent. Variation in amino acid contents in ***Aulosira laxa*** was more pronounced than those of ***Tolypothrix distorta***. These results were quite in agreement with the data obtained by Canizares-Villanueva ***et al.***, (1995) during their studies on the planktonic cyanobacteria; ***Spirulina maxima*** and ***Phormidium*** sp. They reported that the amino acid contents of these cyanobacteria grown in synthetic media were higher than those grown in the effluent. The reduction in amino acids content could be due to the relatively high salinity of the industrial effluent. In this context, several investigators (Mohamed and Shafea, 1992, Galal, 1998, Abdel-Mawgoud, 2005) have related the reduction in free amino acids to high salinity treatments. The accumulation of proline in the cyanobacteria could be in part attributable to the relatively high salinity of Kima factory effluent. In this context, El- Shimy and Ismail (2007) have shown that proline was the major intracellular amino acid of ***Anabaena oryzae*** in response to osmotic stress by salinity. Proline accumulation under salinity stress may be related to its action as an osmotic regulator or to the regulation of the cellular water structure, which protects against the reduction of the hydration of cytoplasmic constituents (Nakamura, 1979). Mostafa Mohamed El-Sheekh ***et al.,*** (2011) investigated the treatment of cyanobacteria with different concentration of effluent stimulated the biosynthesis of the few amino acids and inhibited others.

The factors related to significant increase in the level of some essential amino acids need to be further studied in detail in order to exploit them commercially.

4.7.2.5 Vitamins

The presence of vitamin B_{12} in algae has earlier been shown in ***Anabaena cylindrica*** (Brown ***et al***., 1956; Okuda and Yamuguchi, 1960).

Differences in vitamin content can be accounted has due to salt stress. During the present investigation in addition to B_{12} vitamin B_1, B_2, B_3, B_5, B_6, vitamin-A, vitamin-C, biotin, folic acid, chloine and nicotinic acid were also observed. The presence of folic acid and niacin was also reported in the extracts of ***Anabaena*** (Aaronson ***et al***., 1977). Vitamin B_{12} was reported in ***Anabaena flos-aquae***, ***Chroococcus minutus***, ***Oscillatoria jasorven,*** biotin in ***Anabaena hassali,*** pantothenic acid; A, B_1, B_2, B_{12}, vitamin-C, vitamin-E, pantothenic acid in ***Spirulina platensis;*** A, B_1, B_2, B_{12}, C in ***Spirulina maxima,*** vitamin, B_1 and B_{12}, biotin and nicotinic acid in ***Nostoc*** sp., (Misra and Kausik, 1989).

Cyanobacteria are potential sources for large scale production of vitamins of commercial interest such as vitamins B-complex group. In both the strains of cyanobacteria, the presence of vitamin-A, vitamin-B complex group, vitamin-C, biotin, folic acid, chloine and nictonic acid was detected. Both ***Aulosira laxa*** and ***Tolypothrix distorta*** were rich in vitamin-C followed by choline and vitamin-A when compared the two strains, vitamin-B complex, vitamin-C and choline was richer in ***Tolypothrix distorta***. ***Aulosira laxa*** and ***Tolypothrix distorta*** contains β-carotene an analogue of vitamin A and has the potential to produce two molecules of vitamin-A. However, when the biomass of algae were compared, algae grown in untreated dairy effluent with cyanobacteria was generally greater than that of cyanobacteria grown in culture medium.

The present study revealed that the cyanobacteria ***Aulosira laxa*** and ***Tolypothrix distorta*** offer a wide scope for the accumulation of large quantities of various vitamins. Thus, the results suggest that ingestion of ***Aulosira laxa*** and ***Tolypothrix distorta*** rich in vitamins might have the chemo preventive potential. Therefore, ***Aulosira laxa*** and ***Tolypothrix distorta*** could be easily incorporated into daily dietary practices since they are relatively non-toxic.

4.7.2.6 Minerals

Minerals are important to the overall functioning of mind and body. They have two general body functions - building and regulating. They build the skeleton and all soft tissues and regulate heart beat, blood clotting, internal pressure of body fluids, nerve response and oxygen transport from the lungs to the tissues. Cyanobacteria contain a full spectrum of chelated (organically bound) minerals.

The cyanobacteria are rich in minerals as they form an integral part of the cell. In the present study, higher concentration of magnesium was observed in all the species as they formed a key mineral of the chlorophyll molecule. Venkataraman and Becker (1985) reported some minerals in different species of ***Spirulina.*** There is a wide variation in mineral composition between the species of ***Spirulina***. A similar trend was also noticed among ***Oscillatoria*** species. Subhashini ***et al***., (2003) analyzed four micronutrients, namely, copper, manganese, ferrous and zinc in ***Anabaena*** species. Of the four isolates of ***A.azollae, A.azollae–***AM recorded maximum copper and manganese content in the concentration of 233 and 1217 μg/ml, respectively. ***A. azollae –*** AF recorded maximum ferrous and zinc content in the concentration of 2365 and 900 μg/ml, respectively.

Heavy metals are important environmental pollutants. Cations uptake and their toxicity were extensively studied in cyanobacteria by many investigators (Ahluwalia and Kaur 1989; Khare and Bisen, 1991; Pandey ***et al***., 1996). Heavy metals such as copper, ferrous and zinc are the essential micronutrients required for the growth of cyanobacteria and in higher concentration, they may have an inhibitory effect on the growth (Kannan and Subramanian, 1992; Reddy ***et al***., 2002). Less toxicity of copper was due to their use as essential elements for various metabolic processes. At acidic pH, heavy metals were more toxic to the growth; EDTA was more effective chelator than citrate and glutamine which played a protective role against the metal toxicity. The potassium and phosphorus were the richest mineral component after all the species of the present study. The ability of cyanobacteria to accumulate heavy metals from the polluted water bodies and transform them into nontoxic form has been successfully utilized in bioremediation processes (Samal ***et al***., 2004; Hernandez and Olguin, 2002).

4.8 Antibacterial activities of *Aulosira laxa* and *Tolypothrix distorta*

The antimicrobial production of ability is significant not only as a defensive instrument for algae but also as a good source of novel bioactive pharmaceutical compounds (Ghasemi ***et al***., 2007). The results obtained from the present study, reveals the antibacterial agents produced by the two selected cyanobacteria against different species of bacteria. Antimicrobial activity depends on both cyanobacterial species and efficiency of extraction of their active principles. Concerning the antibacterial effects, the results showed that the methanolic extracts of ***Aulosira laxa*** and ***Tolypothrix distorta*** of control and treated with effluent shows the highest activity against ***Bacillus subtilis*** and lowest activity in ***Pseudomonas aeruginosa.*** It was also found that the extracts had a moderate activity in ***Staphylococcus aureus*** and no activity in ***Escherichia coli.***

These results are in harmony with those obtained by Volk and Furkert (2006). They found that some microalgae have high biological activity against ***Bacillus subtilis***, ***Bacillus thuringiensis, Bacillus megaterium, Escherichia coli, Pseudomonas aeruginosa, Candida tropicalis*** and ***Saccharomyces cerevisiae***. The present investigation also proves that methanol is the preferable solvent for extracting the antibacterial agents from ***Aulosira laxa*** and ***Tolypothrix distorta.*** The same results were also obtained by Ozdemir ***et al***., (2004). He also found that extracts of ***Spirulina*** sp obtained by different solvents exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms.

Kaushik ***et al.,*** (2009) have reported that cyanobacteria species, ***Anabaena*** sp showed antibacterial activities against ***S. aureus***, ***E. coli***, ***P.aeruginosa***, ***S. typhii*** and ***K. pneumoniae***, which implied that, the extracts of cyanobacteria may have contain diverse bioactive compounds which are responsible for the antibacterial activity (Kempf and Bremer, 1998). Moreover, it has been shown that many bioactive compounds were excreted into the environment due to the stress of survival of cyanobacteria (Nicholson ***et al.,*** 2000; Soltani ***et al***., 2006).

Many investigators have mentioned that the methanol extracts of ***Nostoc muscorum*** revealed antibacterial activity on ***Sclerotinia sclerotiorum*** by Ishida ***et al***., (1997)**.** The methanolic extracts of cyanobacteria were investigated by Kumar ***et al.,*** (2006) for ***in vitro*** antimicrobial activity against ***Proteus vulgaris, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus*** and ***Rhizopus nigricans*** using the agar cup diffusion method. The antimicrobial activity of methanolic extract of ***Spirulina platensis*** was due to the presence of γ-linolenic acid reported by Demule ***et al***., (1996). Antimicrobial active lipids and active fatty acids are present in a high concentration in these algae. The antibacterial activity of the extracts could be due to the presence of different chemical agents that may include flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group (Yu ***et al***., 2009). Zeeshan ***et al***., (2010) reported the presence of certain metabolites such as tannin, alkaloids, protein, and flavonoids in the extract of cyanobacteria.

In the present investigation, it was found that the extracts of ***Aulosira laxa*** and ***Tolypothrix distorta*** showed better antibacterial activity against ***Bacillus subtilis*** and ***Staphylococcus aureus***. However, there is a need to carry out extensive studies to identify the promising strains. Therefore, the basic knowledge can be applied in pharmaceutical and agricultural practices. To obtain a large scale production of cyanobacterial biomass, it has to be preconditioned for the isolation and characterization of the biologically active compounds. Further studies on fractionation, separation and preliminary characterization of active compound are in progress.

4.9 Antioxidant activities of *Aulosira laxa* and *Tolypothrix distorta*

Biotic and abiotic environmental stress are known to affect plants by causing excess accumulation of ROS (Reactive Oxygen Species), which can react with certain biomolecules, and alter or inactivate the biochemical activities (Choudhary ***et al.,***2011). To survive under stress conditions, each microalgal cell possesses a complex array of enzymatic and non-enzymatic antioxidant defense systems.

The microalgae have known to contain the secondary metabolites such as catechin, flavonol, glycosides, phlorotannins and phenolic compounds (El-Baky ***et al.,*** 2009) and working as an effective natural antioxidant. The methanolic extracts of these algae might contain these constituents which have a higher combined antioxidant activity. The antioxidant activity of methanolic extracts from ***Aulosira laxa*** and ***Tolypothrix distorta*** was higher. This was correlated with the results of Suhail ***et al.,*** (2011). The synergistic action of a wide spectrum of antioxidants may be more effective than the activity of a single antioxidant.

As the radical system used for antioxidant evaluation may give different results, two or more radical systems are required to assess the radical-scavenging activity of an antioxidant (Yu ***et al***., 2002). In the present study, DPPH and ABTS assays were used to assess the antioxidant activity of the extracts. The radical scavenging activity of the extract was higher in the ABTS assay than the DPPH assay at a particular concentration. In contrast, some antioxidant compounds which show ABTS scavenging activity may not have DPPH scavenging activity, as found in phenolic compounds from sage (***Salvia***

officinalis) (Wang ***et al***., 1998). In the DPPH assay, the antioxidant effect was likely to be due to the hydrogen donating ability of the extract (Conforti ***et al***., 2005). The ABTS assay is a measure of the activity of the antioxidant in scavenging proton radicals through a donation of electrons (Mathew ***et al***., 2004). Furthermore, factors such as stereo selectivity of the radicals and the solubility of the extract in different testing systems may also affect its capacity to quench different radicals (Benedetti ***et al***., 2004).

In this study, we examined the antioxidant potentials of cyanobacteria ***Aulosira laxa*** and ***Tolypothrix distorta*** grown in dairy effluent. Using dairy effluent as a medium the cost for culturing algal biomass was reduced. The results observed with the methanolic extracts regarding the free radical scavenging potential are advantageous because of their richness in hydrophilic antioxidants and it can be rightly considered as an antioxidant supplement for fish inhabiting contaminated areas and aquaculture farms to elevated pollutant stress.

4.10 Seed germination

Germination is a complex phenomenon involving many physiological and biochemical changes and leading to the activation of the embryo (Bewley and Black, 1985). The germination rate was found nearly high in seeds treated with 25% concentration of dairy effluent. But at higher concentration of effluent i.e. above 25% the seed germination was inhibited. This might be due to the presence of elevated amounts of total dissolved solids. These solids may inhibit the uptake of necessary elements like phosphorous, magnesium etc., by plants (Thabaraj ***et al***., 1964). Retardation of plant growth is also due to the presence of high concentration of toxic heavy metals and other toxic compounds (Sahai ***et al***., 1987; Dollar ***et al***., 1972). But, the presence of potassium, sodium, calcium etc., in the diluted form (25% concentration) of effluent influences the growth of plant in less concentration (Rajannan and Oblisami, 1979; Agarwal ***et al***., 1980).

The osmotic pressure of the effluent at higher concentration of total salt was making imbibitions more difficult and retarded germination (Nagda ***et al.,*** 2006). Suppression of seedling growth by effluent dilutions above 25% observed in the present study is in confirmation with the earlier studies of Anoop Singh ***et al.,*** (2002) in ***Triticum aestivum*** L., Rajendra ***et al.,*** (2010) in Soya beans, Paddy (Dhanam, 2009), Ragi (Sundaramoorthy and Lakshmi, 2000) and Wheat (Priya Koushik ***et al.,*** 2005).

The toxicity causes changes in enzymatic activity (Gomes Filho and Sodck, 1988), hampers protein metabolism (Yupsanis ***et al***., 1994) and affects plant growth regulators balance (Khan and Rizvi, 1994). Salinity interacts with certain plant and environmental factors during germination. Reduction in the amount of water absorption takes place, which results in retardation of seed germination due to, enhanced salinity. The salt concentration, outside of the seed is known to act as limiting factor and it might be responsible for delay in germination (Adraino ***et al.,*** 1973). The other possibility of reduction in germination percentage of higher concentration of effluent may be due to the presence of excess amounts of ammonia in the effluent, causing depletion of the Tricarboxylic acid cycle, which reduces the respiration rate and subsequent germination (Kirkby, 1968). Panaskar and Pawar (2011) have reported that textile effluents were not inhibitory at low concentrations but with the increase in concentration growth of seedlings was affected. Inhibition of seed germination may be due to high levels of dissolved solids, which enrich the salinity and conductivity of the absorbed solute by seed before germination (Gautam ***et al.,*** 1992; Singh ***et al.,*** 2006).

Several workers have studied the effect of different industrial effluents on seed germination. Yadav and Meenakshi (2007) reported that the percentage of germination was higher at 25% effluent than in control of wheat, guar and radish but higher concentration of effluent affected germination percentage. Om ***et al***., (1994), reported combined effects of wastes of distillery and sugar mill observed inhibition of seed germination, seedling growth and biomass in okra; distillery effluents on seed germination in onion by Zalawadia ***et al***., (1996); sugar industry effluent on seed germination in ***Solanum melongena*** and ***Lycopersicon esculentum*** by Arora ***et al.,*** (2006). Ramana ***et al***., (2001) observed a similar kind of inhibitory effects of mustard, cauliflower and radish.

In the present study, germination was significantly reduced in higher effluent treatments but in lower concentrations of germination was affected, it was not completely suppressed. Similar observations have been made by Mahmood ***et al.,*** (2005) for seed germination in corn, Jamal ***et al.,*** (2006a,b,c,d); wheat, ***Vigna*** sp. and ***Prosopis juliflora*** and Shafiq ***et al***., (2008). Suppression of seedling growth by effluent dilutions more than 10% was observed in the present study, which is in accordance with the earlier studies of Anoop Singh ***et al***., (2002) in ***Triticum aestivum L.,*** Tomer ***et al.,*** (2002) in sunflower, Trivedi and Goel (1984), Singh ***et al.,*** (1985) in three varieties of rice. Panse and Sukhatme (1978), Pandey and Soni (1994) also suggested that the interaction between the various constituents of the effluent and native microbes might be responsible for the inhibition of seedling growth. In the present investigation, the conspicuous increase in plant growth as well as the biomass content was higher in both treated effluent with algal filtrates compared to control and that of untreated effluent, was observed from 3^{rd} day onwards.

The results obtained in the experiment regarding the inhibitory effects of dairy effluent on seed germination and seedling growth emphasize that various metallic and nonmetallic elements act as nutrients but at the higher concentration they show toxic effects on seed germination and seedling growth.

It is concluded that all growth characters (shoot length and leaf area) were positively affected by untreated effluent at a concentration of 25 to 100%. Foliar application of algal filtrate was accompanied by stimulating growth characters significantly compared to untreated effluent. The promotion was depended on increasing algal filtrate concentrations. The increase in such characters was significant when algal filtrate was used at concentrations higher than 50%. These results are in coinciding with those obtained by Omran ***et al***., (2003) and Madian (2004) on Red Roomy grapevines.

The results of the present study indicate that the properly treated dairy effluent with algae did not exert any adverse effect on the germination or on biochemical parameters of plant growth. The higher content of biodegradable organic matter can serve as a good source of nitrogen and phosphorus, as an essential nutrient for plant growth, enhancing the plant productivity.

From the results of this study, it can be concluded that, the application of dairy effluent treated with the cyanobacteria meet all the requirement of plants and is economical. This gives good yield while saving a lot of water and labour. The above results confirmed that the use of treated dairy effluent for agricultural purpose provided not only water to the plants but also increased the nutrient availability to the plants and efficiency of the fertilizer applied.

4.11 Pigment and Biochemical composition of *P.mungo*

Black gram (***Phaseolus mungo***) which were grown in control, untreated effluent and effluent treated with algal filtrates, were analyzed for the biochemical parameters. The results showed that there was a significant change in, chlorophylls, carotenoids, total carbohydrates, proteins and lipid contents treated with effluent in plants when compared with control.

4.11.1 Chlorophyll and carotenoid composition of *P.mungo*

In the untreated dairy effluent an overall decrease in chlorophyll content was recorded as compared to control. Changes in total chlorophyll concentration indicate that the chlorophyll synthesizing capacity of the crop has diminished affecting the overall photosynthetic process. Similar observations have been reported in earlier investigations (Krupa ***et al***., 1993; Gouia ***et al***., 2003). Klimentina ***et al***., (2006) has mentioned that Cd present in the effluent has reduced plant length, biomass and leaf pigments. Cadmium can harness photosynthetic activity, chlorophyll content, plant growth and induce oxidative stress (Zhou and Huang, 2001; Yi and Ching, 2003; Zhou ***et al***., 2003).

The total chlorophyll and carotenoid contents of the seedling of black gram were found to be maximum in control and in effluent treated with algal filtrates than the untreated effluent. This observation was in conformity with those of Sahai and Neelam (1987) on ***Phaseolus radiatus***, L and Jabeen and Saxena (1990) on ***Pisum sativum***. A decrease of chlorophyll content in the effluent treated plant is due to toxic effect of effluent on chlorophyll biosynthesis (Sahai and Neelam, 1987). Significant increase in all these parameters was observed in effluent treated with algal filtrates suggesting that algal filtrates enhance the growth of plant and the growth decreases due to nutrient depletion from algae (Tam and Wong, 1990). On the basis of results obtained, it can be concluded that cyanobacteria utilize the organic and inorganic matter present in the effluent as nutrients. The decrease of pollution in effluent treated seedlings suggests pollution injury. Changes in pigment concentration by effluent treatment are also likely to affect carbohydrate metabolism. Hence, decrease in chlorophyll content and carbohydrate content supports the above view. Decrease in chlorophyll and carbohydrate content in rice and ragi seedlings of paper mill effluent has also reported by Mishra ***et al***., (1991) which supports our observations.

Enhancement of chlorophyll could be due to high nutrient uptake, synthesis and translocation probably facilitated by optimum availability of iron and magnesium and also due to the reduction in phenol content in the treated dairy effluent (Nagda ***et al***., 2006). The significant fall in the chlorophyll content under the higher percentage of effluent concentration might have been due to the inhibitory effect of toxicants of effluent on chlorophyll synthesis in exposed plant. This was supported by the previous work on the effect of dairy effluent on finger millet and maize (Pandit ***et al.***, 1996). Similar observations are also reported by Kulkarni and Dharwadkar (1998) in wheat seed; Gautam ***et al***., (1992), in Rabi and kharif crops. The increase in carotenoid content might be due to enhanced influence of nitrogen and other inorganic element present in the effluent. Changes in pigment concentration by effluent treatment are also likely to affect carbohydrate metabolism.

4.11.2 Biochemical composition of *P.mungo*

4.11.2.1 Carbohydrate

Studies have been shown that stress induces increase in soluble sugar content (Rong Guo ***et al.,*** 2007). The present data are in agreement with that shown by El-Nakip (2004) who reported a significant increase in the contents of total soluble sugars when various crop seedlings were treated with culture filtrates of the selected cyanobacteria. The marked increase of carbohydrate in ***Phaseolus mungo*** plants as a result of seed pre-treatment with algal filtrates was attributed to the increased growth and to the presence of regulators present in these filtrates (Haroun and Hussein, 2003).

The sugar content showed decreasing trend at higher concentrations of the dairy effluent (untreated). It may be due to transportation of most of the nitrogen absorbed by the plants (Thamizhiniyan ***et al***., 2000; Thamizhiniyan ***et al***., 2009). The another view for the decrease of sugar content at higher concentration of the effluent might be due to the excessive nutrient uptake that caused the imbalance and eventually cut to depletion of carbohydrate reserve (Tanaka ***et al***., 1964). Similar findings have been reported Mishra ***et al***., (1991) in rice and ragi seedlings of paper mill effluent which supports our observations.

4.11.2.2 Proteins

In the present study, an increase in protein content was observed in both concentrations. The crude protein concentration of corn increased quantitatively with increased N fertilizer with dairy effluent application (Leary, 1990). The enhancement of protein content of crop plants might be due to increased rate of amino acid synthesis which may be attributed to the higher rates of both RNA-ase and transaminase activity (Singh, 1991). Studies have shown that stress induces the decline in protein contents in plants but increase in soluble sugar content (Lenin and Thamizhiniyan, 2009). A significant increase in the protein content of pea plant observed may be due to the potassium and nitrate in their optimum quantity. In the lower concentration of the effluent (Jonker, 1964) the reduction in the rate of nitrogen absorption and amount of nitrogen present in the plants, the total physiological activities were found to decrease resulting in a gradual reduction in protein content of the plants treated with higher concentrations of the effluent (Kannan ***et al***., 2008). Under the environmental stress conditions, the energy forming molecules may be disturbed and subsequently carbohydrates and protein metabolites of the membrane are altered. Studies have shown that stress induces the decline in protein contents in plants but there was an increase in soluble sugar content (Rong Guo ***et al.,*** 2007).

Manu ***et al.***, (2012) reported that the dairy effluent at lower doses and treated effluent samples showed better results in biochemical parameters of maize. A similar reduction in protein content was reported by Bhattacharjee and Mukherjee (1994) in ***Vigna unguiculata*** increasing environmental pollution brings much more attention to toxic effect of both essential and non-essential elements.

4.11.2.3 Lipids

Algal biomass is a potentially inexpensive source of energy which has high lipid content. As the dairy effluent is hostile to the natural environment, the algae is used not only to break down the toxic substances but also to produce biomass, which is produced at a rate of 17g/l, which contains a lipid content of 30% (from 1:1 ratio of effluent and water) (Balasubramanian ***et al***., 2012). Significant increase in all these parameters were observed in effluent treated with cyanobacteria extract suggesting that cyanobacteria enhance the growth of plant and the growth decreases due to nutrient depletion from algae (Tam and Wong, 1990).

On the basis of results obtained, it can be concluded that cyanobacteria utilize the organic and inorganic matter present in the effluent as nutrients. After nutrient depletion, cyanobacteria start undergoing degeneration leading to release of several substances, etc., back into the soil. The decrease of pollution in effluent treated seedlings suggests pollution injury.

In the present study, it was observed that changes in total chlorophyll, carotenoids, total carbohydrates, proteins and lipids in effluent treated with cyanobacteirial filtrates than that of control and untreated effluent of black gram seedlings. This observation was in conformity with those of, Sahai and Neelam (1987) on ***Phaseolus radiatus***. L and Jabeen and Saxena (1990) on ***Pisum sativum***.

These results of the present investigation revealed that the treated effluent can be effectively utilized for irrigating agricultural crops if proper combinations of treatment techniques are judiciously employed to ensure effectiveness and environmental safety. The above results confirmed that the use of treated dairy effluent for agricultural purposes, provided not only water to the plants but also increased the nutrient availability to the plants enriched the soil.

4.11.3 Minerals

Green revolution has greatly increased the food crop production in India, but continuous cultivation of high yielding varieties has led to depletion of native micronutrients in soil. Singh (2009) reported that as much as soils in India are affected with deficiency of Zn, Fe, Mn, Cu, B, Mo and S respectively. Besides this, hidden hunger of micronutrients is widely noticed leading to even entire failure of crops and reduced content of micronutrients in the plant parts.

Available N, P and K in soil significantly increased due to the cyanobacterial inoculation compared to the non inoculated treatments. The highest available N, P and K contents were recorded in the plant, while the lowest values were observed in the plant treated with untreated effluent. In general, nitrogen, phosphorus, potassium and boron percentages in the leaves of most treatments including the control were within the optimum level (Abd El-Migeed ***et al,*** 2004). A progressive increase in percentage of N, P and K in the leaves was observed as a result of increasing concentration of the algal filtrate up to 75% thereafter the increase was only marginal. The present results in similar to that of Gobara ***et al.,*** (2002) on Red Roomy grape vines.

The cyanobacteria filtrate to increase potassium levels in the leaves compared with those sprayed with water. These results of the present study are in agreement with those reported by Hegab ***et al.,*** 2005) and Stino ***et al***., (2009). In the present study, it is clear that treatment of ***Aulosira laxa*** and ***Tolypothrix distorta*** significantly raised potassium content than the control. This may be due to the encouragement of K-absorption of soil rather than utilization in plant tissues.

The cyanobacteria filtrate increase nitrogen content in the leaves when compared to water and untreated effluent (control). Moreover, the present study clearly shows that increase in concentration of algal filtrate had a beneficial effect on nitrogen content in the leaves, while the effluent has significantly decreased nitrogen content in the leaves at the optimum level. These could be attributed to the nitorgenase as well as nitrate reductase activities of the algae associated with the leaf surface of plants (Mahmoud and Adam, 1999); or the amino acids and peptides produced in the algal extracts or other compounds that stimulate growth. Cyanobacteria can fix about 25 kg N/ha/ season. Apart from nitrogen fixation, inoculation with cyanobacteria is also reported to reduce considerably the total sulphides and ferrous iron content of the soil (Aiyer, 1965). The cyanobacterial ability to mobilize insoluble forms of inorganic phosphates is evident from the finding of Kleiner and Harper (1977) who reported more extractable phosphates in soils with a cyanobacterial cover than in nearby soils without cover. Cyanobacteria is also known to increase soil fertility by enhancing the available N and P levels and exhibited an economical view that it can compensate about 50% of the recommended doses of N, P, K (Singh and Bisoyi, 1989; Mahmoud ***et al***., 2007).

There was a difference among the treatments on phosphorus percentage in the leaves on black gram. Phosphorus percent in the leaves were around the optimum level (0.006 – 0.18%). (Bharagava and Chadha, 1988). In the present study, the concentration of phosphorus in the leaf tissue has increased with increase of algal filtrate.

Iron is required in very small amounts which is usually available in very small amounts in the soil. The highest micronutrients (zinc and iron) uptake was recorded in plant treated with filtrate of both algae, which was significantly superior over control. This might be attributed to increased growth components. These results are supported by the findings of Basavarajappa (1992). They noticed a combination of sulphur and micronutrients had marked influence on micronutrient (zinc and iron) uptake and opined that this could be due to an interaction effect of sulphur, iron and zinc. These results are further supported by the findings of Khandagave ***et al.,*** (1996).

Zinc is one of the essential micronutrients required for optimum crop growth. Plants take up zinc in its divalent form. In the present study, the zinc concentration was less in the effluent treated with ***Aulosira laxa***. High levels of soil phosphorus are also commonly responsible for zinc deficiency. On the contrary, the application of magnesium can enhance zinc availability and uptake by the roots. Whereas, the treated effluent with ***Tolypothrix distorta*** showed an increase in the concentration of zinc. Use of zinc as fertilizers is rapidly absorbed by the leaves, and is translocated to the grain.

Magnesium is a major constituent of the chlorophyll molecule, and it is therefore actively involved in photosynthesis. Mg is a co-factor in several enzymatic reactions that activate the phosphorylation processes. In the present study, the concentration of magnesium in the leaf tissue has increased with increase of algal filtrate.

It is well known that copper is toxic to green plants even in relatively low concentrations. Copper serves as an activator of numerous plant enzymes and plays a role in the development of plant pigments that influence colour. Nevertheless, the amount of copper transportation of soil by plant depends on the plant's ability in metal transportation on soil and root interface and also on the total amount of copper in the soil (MashhadiAkbarBoojar and Goodarzi, 2007). In the present study, the concentration of copper in the leaf tissue has increased with increase of algal filtrate.

Sulphur is a basic element of nature and is one of the most abundant elements in the earth’s crust. Like nitrogen, sulphur is an essential component in the growth of all living things. Sulphur is a building block of proteins, enzymes and vitamins and is a key ingredient in the formation of chlorophyll. It is one of the major nutrients essential for plant growth, root nodule formation of legumes and plant protection mechanisms. The sulphur uptake by ***Tolypothrix distorta*** was significantly superior over control. This might be attributed to increase in growth and growth components. Whereas, sulphur uptake was inferior in ***Aulosira laxa***. The present findings are in line with the findings of Basavarajappa (1992).

Of all the macronutrients, sulphur is perhaps the nutrient which has attracted the most attention in soil science and plant nutrition due to its potential defensive characteristics to pests, good nutritive potentiality to crops and its relative immobility in the soil-plant system. The benefits from sulphur fertilization of crops can be traced to its role in protein development, to improvement of nitrogen use, (Khan and Mazid, 2011).

The stimulatory effects of algae as biofertilizer on some growth parameters of lettuce are in accordance with the results obtained by Rani and Sathiamoorthy (1997). In addition, a similar trend was observed by Galal ***et al.,*** (2000). Such increases might reduce chemical fertilizers and consequently reduce pollution and health hazard as reported for pearl millet Verma (1996). In the present study, results are in agreement with those obtained by Mahmoud and Amara (2000) and Das ***et al***., (2001).

Algal biomass recovered from such systems has a variety of potential on and off-farm uses. The production of benthic algae for manure treatment offers considerable potential for on-farm recycling of nutrients. Most efforts in using algal production for effluent treatment have been focused on treatment of municipal waste effluents using suspended microalgae (Benemann and Oswald, 1996). Combining conventional cropping systems with an algal treatment system could facilitate more efficient crop production and farm nutrient management, allowing dairy operations to be environmentally sustainable on fewer areas. Yet, the potential for high protein yields and nutrient uptake rates justifies consideration of micro algae production system for nutrient recovery from dairy effluent. These kinds of economic considerations must be deal with if dairy effluent treatment is to be mandated in the future. Recent studies focused on the use of the dried biomass as an organic fertilizer demonstrated that it was equivalent to a commercial organic fertilizer with respect to plant mass and nutrient content (Mulbery ***et al***., 2005).

It is worthy to mention that the algal filtrates of ***Aulosira laxa*** and ***Tolypothrix distorta*** were of more efficient, in improving the growth of ***Phaseolus mungo***. Hedge ***et al***., (1999) stated that the application of algal biofertilizer is useful for the reclamation of marginal soils. Zaccaro ***et al***., (2001) documented that, the biofertilizers are likely to assume greater significance as complement and/or nutrient supplies to cereal crops because of high nutrient turnover in the cereal production system, exorbitant cost of fertilizers and the greater consciousness of environmental protection.

In India, considerable progress has been made in the development of cyanobacteria based biofertilizer technology. It has also been demonstrated that this technology can be a powerful means of enriching the soil fertility and improving rice crop yields. However, the technology needs to be improved further for better exploitation under sustainable agricultural systems. It is important to obtain a much more detailed understanding of cyanobacterial population dynamics over the whole annual cycle in agricultural systems. Extensive field studies aimed at developing region specific high quality inoculums are also needed. Understanding the biology of drought resistant cyanobacteria may be useful in terms of extending this approach to dry crops. The use of algae as biofertilizer provides a cyclic nutrient-supply system with inherent ecological advantages.

4.12. Effect of dairy effluent on fish toxicity

Effect of dairy effluent on Silver carp (***Hypophthalmicthys molitrix***) toxicity was conducted for a period of 7 days. When ***Hypophthalmicthys molitrix*** were introduced to experimental tanks, various stressful behaviors like erratic swimming, increased activity, inconsistent jumping were observed in ***Hypophthalmicthys molitrix*** exposed to effluent (above 25% concentration). While in control, fish were observe to be normal. These behavioral responses of fish are in response to toxicants present in the sample at different duration of exposure and the prevailing specific environmental conditions as opined by Bobmanuel ***et al***., (2006). This also signifies respiratory impairment, an outcome of the impact of the effluent on the gills of fish as observed by Adewoye ***et al***., (2005). Carlson and Drummond (1978) stated that, such coughing response is a useful tool for evaluating the quality of industrial and municipal effluents. While in control, the opercular movements of fish were normal and it was clearly seen when compared with experimental fish. There was a gradual loss of equilibrium and eventually 100% mortality at 24 hr occurred at higher concentrations (above 25%) of effluent. This could be a consequence of depletion of energy in the body of the exposed animals and an indication of impairment of carbohydrate metabolism wherein organisms that could not tolerate the contaminants enter into a state of coma and subsequent death (Ogundiran ***et al***., 2010). The responses recorded for the fish in this study were similar to those reported by Danielly de paiva magalhaes ***et al***., (2007) and Chukwuand and Okhumale (2009).

The mortality rate of ***Hypophthalmicthys molitrix*** remained directly proportional to the duration of exposure, concentration and toxicity factor; it was similar to that observed in catfish hybrid by Gabriel and Okey (2009) and TUa (Pool ***et al***., 2009). The acute toxicity units (Pool ***et al***., 2009) obtained for influent were higher than effluent, thus showing that influent is more toxic to carp as compared to effluent. It is observed that the treated dairy effluent impart toxicity in silver carp fish and therefore the present level of treatment of effluent prior to discharge appears insufficient.

4.13 Nutritional qualities of live feed organisms

Cyanobacteria comprises high protein which is one of the main food source for fishes. The ***Hypophthalmicthys molitrix*** fed with algae (***Aulosira laxa*** and ***Tolypothrix distorta***) showed better growth. There was not much significant increase in growth (length, width and weight).

In the present study, the gradual increase in the ingestion rate and assimilation rate to increase of biomass of the animal size was observed. The fish would take a long time to attain satiation if fed with smaller live food organism, and this would result in poor growth due to inefficient feeding and waste of energy. The feeding rate, relative to the body weight decreases as fish size increases; however, the rates of food consumed increase per individual (Wang ***et al***., 1989). In the present study, the ***Hypophthalmicthys molitrix*** consumed significantly more algae and increased slightly in its length, width and weight.

Feeding management plays a major role in the success of fish culture. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well balanced nutritionally complete and cost effective compounded feeds. Microalgae and fish plays a vital role in the hatchery phase of many aquacultures as feed for larval and juvenile crustaceans and fish. Several algae is being investigated as a potential source of protein for both livestock and human consumption.

Carbohydrates are considered to be the first degraded under stress condition of animals. According to Dhavale and Masurekar, (1986), decreased level of carbohydrate constituents in tissues of toxicant exposed animals may be due to the prevalence of the hypotoxic condition in the tissues as a result of pollutant stress. During the hypoxic conditions, there is an increased carbohydrate metabolism to release energy resulting in the extra expenditure of carbohydrate constituents. The observed result in the present study is in accordance with the findings of Valarmathi and Azariah (2002), who have suggested that the decreased level of tissue carbohydrates in the toxicant exposed animals, seemed to induce the glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy demand under stress condition or the toxicant may have an effect of glycogenesis by inhibiting the activity of carbohydrate metabolism.

Fishes derive much of their energy from proteins. The growth of fish in terms of muscle formation depends on the high percentage of protein intake. A high percentage of protein utilization in fish is mainly due to the bronchial deamination of protein into amino acids and excretion of ammonia. The protein requirements of cultivable fish depends on size, age, stocking density, oxygen supply and the presence of toxicants.

The increase in protein content under effluent stress noticed in the present study may be attributed to the utilization of amino acids in various catabolic reactions. The reduction in protein content might be due to the blocking of protein synthesis or protein denaturation or interruption in the amino acid synthesis by metals (Jha, 1991). The food utilization decreases when the animals are under stress condition, which leads to the depletion of protein content in tissues. Murugesan ***et al***., (2010) reported nutritional evaluation and culture of freshwater live food organisms in ***Catla catla.*** To summarize, the present study suggests that protein content increases with increasing food level.

The lipid requirement of any cultivable fish depends largely on its digestibility, the quality and amount of essential fatty acids present in the diet. The variation in the lipid content of algae was significant ($p<0.05$). The above results revealed that the fluctuations of biochemical constituents were higher in the tissues of fish exposed to effluent concentrations. Gilbert and O'Connor (1970) reported that lipids are vital to embryogenesis, providing two third of energy by oxidation. In the present study also, the increased lipid content might be due to activity of enzymes of lipid metabolism. The study also showed that cyanobacteria (***Aulosira laxa*** and ***Tolypothrix distorta)*** and (***Hypophthalmicthys molitrix)*** Silver carp in combination act as an ideal food supplement for fish.

The advantages of live feed are superior, since it contains nutritional value and promotes normal growth and good pigmentation. They supply optimum lipid and protein for fast-growing fish and shrimp larvae. They improve stress resistance in finfish and shellfish larvae. Live food organisms induce visual and chemical stimuli in larvae and enhance their feeding. Live food is a source of exogenous digestive enzymes that improve digestion of prey in early-stage fish larvae when their gut is not fully functional.

Most commercial fish feed ingredients are costly and their non availability poses problem for fish farmers to take up aquaculture practice. So a lot of research has been undertaken to find out suitable alternate cheaper ingredients of wide availability to boost culture. For commercial use of algae as fish feed, suitable commercial methods are to be developed to protect the fatty acid profile of algae. Standardization of mass culture of some specific algae which have the ability to synthesize long-chain n3-fatty acids are an important aspect in aquaculture.

The present investigation recommends that algae (***Aulosira laxa*** and ***Tolypothrix distorta***) can be exclusively used as an important alternate or supplementary feed for commercial seed production of ***Hypophthalmicthys molitrix***. According to the scientific literature, live microalgae with high nutritive value and appropriate physical properties can provide a healthy rearing environment to the aquaculture system.

4.14 Environmental Impact Assessment

Environmental qualities are undergoing degradation by the increasing amount of wastes being produced. Wastes are complex in nature depending on the sources of generation and its environmental fate once generated. The industrial wastes are a common source of water pollution (Oyediran, 1997). Sustainable utilization of the world's resources and conservation resources for further generation require prevention and control of pollution and degradation.

The following are the recommendations;

Periodic assessment, collection of data and analysis of results and systematic reporting of environmental pollution to appropriate agencies are recommended. The need to re-inform industries using central drainage systems channeled for proper treatment of effluents while conforming to minimum safety standards before final discharge becomes inevitable. Also, mathematical models should be generated for predicting the level of pollution at different points. These models can be formulated after repeated sampling and analysis of water and effluents.

The residents of these areas should be educated on various ways of water treatment before use while the Government should provide alternate potable water for their domestic uses. It is to be concluded that BIS standards for pH, BOD, TDS should be maintained while discharging the effluent. Discharges of such nutrient containing effluent directly affect the water quality and it should be prevented.

In the present study, it could be concluded that the untreated dairy effluent was more toxic. However, treated effluent from dairy industry could be used for irrigation purpose. When they are grown in effluent treated with algal filtrate the plants showed optimal growth than that of untreated effluent

In the present investigation the irrigation of effluent treated with algal filtrate found to be beneficial and it had a significant effect on germination growth and development of black gram than control soil where only water (without application of effluent) was used for irrigation. In the present investigation, beneficial impact on the general welfare of the crops was gradually increased with increasing in dairy effluent treated with algae in black gram. It can be recommended for the study that without dilution of the effluent treated with algal filtrate could be used for irrigation in agricultural field to enhance the productivity of crops.

Finally, development and application of clean technology; which involves treatment of dairy industrial discharges, for safe reuse in agriculture should be encouraged.

The effluent treated with algae could be used for invigorating the seed and for irrigating crop or the nursery and aquaculture depending upon the availability of the effluent specific to site as the case may be. The effluent on proper treatment can also be materialized as cash by proper sale of the products. Thus, the present work fresh up idea of utility of waste materials.

In the present study, phycoremediation technology using cyanobacteria was assessed for environmental impact assessment. The fish growth study revealed that ***Aulosira laxa*** and ***Tolypothrix distorta*** resulted in a gain in fish weight and could be used a cheaper source of feed. Thus, the cyanobacteria were not only effective in effluent treatment, but also safe to the environment. Thus, EIA carried out in this study to examine the safety credentials of the cyanobacteria proves that the organism can be used in implementing phycoremediation technology which in turn implies that both the organism and technology are safe.

4.15. General study and Policy recommendations for maintenance, monitoring the dairy effluent including following conclusion could be drawn by Phycoremediation Technology using cyanobacteria at large scale.

I. General study of monitoring and maintenance of Dairy effluent ETP (Effluent treatment plant).

- Keep necessary equipments and first aid kit at the appropriate place on ETP site and it should be safety clean environment.
- Maintain and follow up record notebook for ETP to minimize the error, monitor treatment process, reduce manpower and control expenditure.
- Monitor regularly to prevent the spills and leaks of effluent pumping motor pumps, pipe lines, valve etc.

- Measuring effluent quantity is pooling into collection sump and disposing effluent for treatment inlet and outlet flow by flow meter.
- Minor changes in the milk production and the waste handling process can produce substantial reductions in wastewater volume and pollutant load.
- Testing discharged treated effluent parameter according to APHA standard (2005) in regular intervals with resource consents.
- The recycling of treated water for various purposes (gardening, toilet uses etc.,) is the primary factor and also it can be reduced wastewater volume.
- Adopt a regular inspection and maintenance routine whole ETP site.

II. Policy Recommendation

- Take appropriate corrective measures when problems do arise. It is necessary to strengthen research and development activities in the Dairy industry.
- Central Dairy Plant should have the pretreatment process within the plant boundary and thus waste generated from the plant can treat to reduce the pollution level of BOD, COD, TDS, K^+, $Ca_2{}^+$, $Mg_2{}^+$, NO_3, $PO_4{}^{3-}$ etc.
- Central Dairy Plant should also analyze the physico-chemical parameters to know its assimilation power and to control the environmental contamination of the lake water and vicinity.
- Making existing laws more stringent.
- A quality management system and an environmental management system that conforms to the principles and practices of ISO-9001 and ISO-14001 are adopted and implemented.
- The awareness programme is to be initiated by the NGO's and the DOE to discourage the investors to invest in polluting technology.

III. Phycoremediation technology supported substantial capacity for dairy effluent treatment

- The knack of cyanobacteria to improve and ratified multiple problem concurrently in the dairy effluent treatment at the same time but it cannot possible by conventional chemical process.
- Improve pH from acidic to alkaline condition and stabilizing the same.
- The process can be reduced drastically both inorganic and organic chemicals by cyanobacteria used as nutrients for their growth.
- Achieve a consistently high level of environmental performance and eco-friendliness from an ecological perspective.
- Compatibility with existing operations.
- Operation process can be done by batch-wise, semi-continues and a continues manner.
- Phycoremediation process donating oxygen to environment.
- Effluent treated cyanobacteria biomass can be used as commercial benefits (biomass extracted lipid, protein, carbohydrates, pigments other by-products).
- Select to remove particular microbial contaminations under consideration.
- Treated effluent can be used as fertilizer to the agriculture field.
- This technology is simply eco-friendly and cost effective as it saves power and lots of chemicals.
- Robust to minimize power consumption not required skilled operator for treatment process modifications.

Stimulating the natural process of phycoremediation offers an opportunity for reducing the environmental impact of various pollutants. This forms an effective economic and biological treatment of polluted waters. Many micro and macroalgae are being used in various bioremediation techniques especially in polluted waters. The intimate association which the algae have with the aquatic habitat makes them an interesting tool for such studies. Both ***Auloisra laxa*** and ***Tolypothrix distorta*** employed were found efficient in phycoremediation. The removal efficiency of these algae was very high. It shows that ***Aulosira laxa*** and ***Tolypothrix distorta*** were more efficient than other cyanobacteria for phycoremediation purposes.

These findings are important regarding the practical use of such species in large-scale biotreatment of effluents, where reduction efficiency, coupled with the fast flow rate, is considered as the main parameters from the feasibility and economic point of view. Therefore, the cyanobacteria species investigated in this study are highly recommended for beneficial bioremediation applications for ***in-situ*** and off-site removal of pollutants. The most promising species should help in the optimization of the self purification and remediation of effluents before discharging into surface aquatic systems, providing a low-cost and naturally renewable technology.

The cultivation of cyanobacteria in dairy effluent has demonstrated the capability of biomass production, organic, inorganic and heavy metals removal. Therefore, cyanobacteria can be used as an alternative tool to assist in the dairy effluent treatment, reducing the environmental impact caused by their pollutants and also to use in the produced biomass in (animal feed and fertilizers), vitamins, amino acids and polysaccharides.

There are two main drawbacks of employing microalgae in the phycoremediation of effluent treatment:

1. There is limited knowledge of the technical details of biological treatment systems that result in relatively higher cost compared to traditional chemical treatments that are more straight forward and cheaper (i.e., mixing a chemical with the effluent and collecting the sediment). Considering the amount of effluent to be treated, any slight increase in the cost of operation makes the implementation of new technology difficult to sell.

2. The relatively low proportion of removal of some contaminants, especially phosphorus, and the long retention time in the treatment plants (days compared to hours), raise the cost and increase the reluctance of engineers to get involved in systems that, by nature, are not very precise and depend on many unpredictable environmental parameters. All these make biological treatment of effluent, especially with cyanobacteria, a niche technology.

At best, under current operational procedures, this technology might be an auxiliary technology, to be combined with other biological technologies as alternative tool to traditional chemical technologies. Currently, there are major advantages to ‘greener’ technologies. The public, even in more affluent developing countries, is constantly demanding green technologies for most aspects of civil life.

From a scientific standpoint, phycoremediation technology has many advantages.

1. Its main advantage is that it controls and protects the dominant and always useful microorganism within the polymer itself. Many excellent isolates for effluent treatment, especially genetically engineered ones, are not necessarily environmentally competitive when applied directly to the effluent, resulting in frequent treatment failures when applied in suspension.

2. It is possible to mix in one bioreactor different micro algal immobilization systems using different microorganisms to simultaneously treat several contaminants in the effluent. This will be useful, especially for recalcitrant compounds that require specialized microalgae for degrading the pollutant.

3. The immobilized microorganism has better plasmid stability within the polymeric matrices, allowing successful use of genetically modified microalgae designed for specific cleaning purposes and avoiding the common failure of such genetically modified microorganisms in environmental systems.

From a practical view, micro algal systems use solar energy and need relatively small amounts of other inputs for operation. They are relatively easy to handle on a large scale because they have been used by compound producing industries for a very long time. These systems produce no health hazards, are environmentally friendly (promoting the image of the company that uses them), produce no secondary pollution, and their end products can be converted to additional by-products (like fertilizers or biofuel) that may further reduce costs. The compactness of these systems produces less sludge and is simpler to maintain than large fluidized beds.

Effluents are major contributors to a variety of water pollution problems. Some of these problems include eutrophication, which can stimulate the growth of algae, increase water purification cost, and interfere with the recreational value of water, health risks to humans and livestock, excessive loss of oxygen and undesirable changes in aquatic populations. Since, large amounts of effluents pass through the treatment systems on a daily basis, there is a need to remedy and diminish the overall impacts of these effluents in receiving water bodies. In order to comply with effluent legislations and guidelines, the effluents must be treated before discharge. This can be achieved through the application of appropriate treatment processes, which will help to minimize the risks to public health and the environment. To achieve unpolluted effluents discharge into receiving water bodies, there is the need for careful planning, adequate and suitable treatment, regular monitoring and appropriate legislation. There is also a need to ensure that effluent standards and limitations, as set by regulatory bodies are not compromised. This is will enhance science-based decisions and ensure the sustainability of the environment and the health of plants and animals.

Further studies on phycoremediation are needed for scaling up this study to field level. The present investigation strongly suggests that these cyanobacteria can be utilized for phycoremediation. Other algae may also be scanned for phycoremediation potentials**.** This phycoremediation technique can be used to clean up the industrial effluents.

5.0 SUMMARY

Phycoremediation is a process of removal of pollutants by biotransformation, including nutrients and xenobiotics from effluent and CO_2 from waste by the use of algae. Algal species are relatively easy to grow, adapt and manipulate within a laboratory conditions and appears to be an ideal organism for use in remediation studies. The ultimate goal is to identify suitable algal strains that can target the pollutant in a cost effective, environment friendly manner.

In the present study, dairy effluent was collected from Madhavaram Dairy plant (Aavin), Chennai, Tamilnadu state, and were subjected to phycoremediation experiments with cyanobacteria and the following investigation have been carried out in detail:-

- Isolation, purification and identification of the naturally occurring cyanobacteria from the effluent of Central Dairy Plant, Madhavaram carried out and were maintained in culture for removal of inorganic and organic pollutants.

- Since ***Aulosira laxa*** and ***Tolypothrix distorta*** showed higher efficiency in the removal of nutrients they were utilized for phycoremediation of dairy effluent.

- Physico-chemical parameters of untreated and treated dairy effluent were quantified.

- Value added products from ***Aulosira laxa*** and ***Tolypothrix distorta*** algal biomass were enumerated.

- Antibacterial and antioxidant activities of algal biomass were evaluated.

- Environmental Impact Assessment studies of dairy effluent on seed germination and fish.

- Toxicity testing of dairy effluent through fishes (***Hypophthalmicthys molitrix***) was evaluated.

- Policy and management of dairy effluent were presented.

The cyanobacteria present in the effluent were isolated and feasibility studies were carried out. Of the 15 species tested for the application in phycoremediation ***Aulosira laxa*** and ***Tolypothrix distorta*** were found to be well adapted in the dairy effluent, and they have been selected as a suitable organism for effluent treatment. This is the first report on treatment of dairy effluent using ***Aulosira laxa*** and ***Tolypothrix distorta.***

In the present study, dairy effluent was analyzed for various physico-chemical parameters based on standard methods of APHA (2005).

In the light of the global realization for the removal of environmental pollutants using less expensive biological means, an attempt has been made to explore whether the algal groups have the potential to rapidly, efficiently and effectively decolourize the dairy effluent and whether it may be employed as an alternative to more costly materials.

The cyanobacteria ***Aulosira laxa*** and ***Tolypothrix distorta*** used in dairy effluent effectively reduced up to 90% of total solids, iron, free ammonia, oil and grease, copper and cadmium.

COD which is an indicator of pollution is high in dairy effluent. By application of phycoremediation it is reduced by 69.57% by ***A. laxa*** and 67.39 by ***T. distorta.***

Heavy metal (Cu, Cr, Zn, Pb, Ni, Cd, Ar and Hg) which are toxic and pollute the environment was found above the admissible level in dairy effluent. Removal of such toxic metals from dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** had resulted in effluent having toxic metals within the environmentally acceptable limits (BIS). Because of the metal uptake capability of these algae they were used with specific interest in industrial applications.

Field studies (green house) on application of phycoremediation technology using ***Aulosira laxa*** and ***Tolypothrix distorta*** in treatment of dairy effluent had resulted in better growth rate, potential, for the reduction of nutrients and heavy metals from the effluent.

The accumulation of sludge generated by conventional chemical treatment methods has been the major problem for the industry. In the present study it was found that effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** drastically reduced the sludge levels.

The results of physicochemical parameters together with biological monitoring provided converging lines of evidences for evaluation of pollution in dairy effluent. The result suggested that ***Aulosira laxa*** and ***Tolypothrix distorta*** would be making a perfect remediation and restoration in numerous systems because both the species were not hazardous.

Bacterial examination in dairy effluent indicates that the nature of bacterial population can also be utilized for treating dairy effluent.

Through FT-IR spectroscopy to study the elimination of organic substances responsible for pollution has been identified. And this is the first exercise in application of FT-IR spectroscopy in phycoremediation process.

Phycoremediation has a dual role. The algae not only clean up the effluent but also the algal biomass obtained at the end of the experiment is used for aquaculture, agriculture, gardening, and soil enrichment. The effluent treatment techniques are judiciously employed to ensure effectiveness and environmental safety. This would minimize the use of freshwater for cooling, washing and cleaning purposes and also help in zero discharge and in the control of pollution.

The present investigation focusing on value added products and it is the first information related to the phycoremediation utilization of end products in particularly from dairy effluent. The high value biochemicals found in treating ***Aulosira laxa*** and ***Tolypothrix distorta*** rich in nutrients and scavenging properties. So, it can be used as potential in therapeutics, cosmetics and nutraceuticals.

The effluent treated with cyanobacteria was applied to black gram (***P.mungo***) and it has resulted in stimulating growth characters and biochemical contents of the plant also significantly increased when compared to untreated effluent. This gives good yield and save a lot of water and labour.

The physico-chemical parameters of treated effluent were within the standards of its discharge BIS (2009), and hence the treated effluent was used for irrigation.

The treated dairy effluent provides an effective and environmentally acceptable option for waste disposal, which not only recycles valuable nutrients into the soil to plant system but also improves soil quality.

Effective, well planned effluent treatment systems can provide solutions for safe the restoration of the environment and to create pollution free atmosphere.

Harnessing solar energy to grow the algal biomass in the effluent could provide a viable solution to nutrient management problems in dairy farms and the algal filtrate could be used as a slow releasing fertilizer. The present investigation revealed the beneficial role of cyanobacterial biofertilizers and they are recommended to be used as sustainable natural nitrogen resources for different crop plants in agriculture. They are non-polluting, inexpensive, renewable resources and in addition that they have the ability to use freely available solar energy, atmospheric nitrogen and water.

The dairy effluent after phycoremediation revealed no toxic effect of ***Hypophthalmicthys molitrix*** (silver carp) which indicated their toxicity was fully reduced after phycoremediation and therefore the effluent can be discharged into inland surface waters without harming the aquatic biota.

Experiments conducted on algal filtrate of dairy effluent treated with ***Aulosira laxa*** and ***Tolypothrix distorta*** had proved that they can be used as bio fertilizer in ***Paseolus mungo*** and fish feed ***Hypophthalmicthys molitrix*** silver carp this has been tested successfully in the present study and also it is the first report.

In the present study, variation in nutritional composition of fish was observed. Even though algae is the cheapest source of protein, lipid and other nutrients and their culture are easy; they are yet to be considered as a major food item for fish. The present study, recommends that to avoid such an impact on the aquatic environment the treated dairy effluent was found to be suitable for management of aquaculture.

Phycoremediation technology saves power and a lot of chemicals. The process generally 70–90% less costly than other technologies as there is virtually little investment in "capital equipment". Furthermore, the only energy requirement for the process is solar energy, which is abundantly available in our country. It is necessary to strengthen research and development activities in the treatment of effluent in the dairy industry. To achieve and sustain this world best environmental management practice was recommended.

The present study concluded that phycoremediation by cyanobacteria are technically efficient and economically feasible treating dairy effluent and also for removing and recovering single and multiple metal ions from solutions in dairy effluent. This application of phycoremediation in the selection and simultaneous removal of metal ions from effluent, offers great potential for large-scale exploitation.

A good phycoremediation approach will involve the strategic use of all native algae in an engineered way to achieve the best possible detoxification levels.

Phycoremediation technologies have been successfully employed in the field and are gaining more and more importance with increased acceptance of eco-friendly remediation. The study summarized that the algal reduction potential can be employed as a useful technique for the phycoremediation of environments contaminated with organics and/or metals.

The effluent treatment technique through phycoremediation developed in the laboratory may result in the design of a large scale, Outdoor pilot scale, studies is necessary before the design and operational guidelines are developed for algae through effluent treatment. The information generated would help to scale up the process and moreover, assessing the economic feasibility of the technology. The system when standardized would not only be economical but also eco-friendly and sustainable.

Based on the results obtained from the present investigation it may be concluded that ***Aulosira laxa*** and ***Tolypothrix distorta*** along with natural population of microbes played a major role in the removal of several pollutants from the effluent of the dairy industry. The results are fairly encouraging for future investigations towards expanding/deviling phycoremediation technology.

Further intensive studies on various other specific parameters are needed for utilization of the recommended algal species in the field level. The outcome of the present study on phycoremediation potentialities of cyanobacteria has drawn clear lines of evidences that algae can be employed for the future pollution abatement.

Favourable environmental and economic aspects and largely unharnessed potentials make cyanobacteria ***Aulosira laxa*** and ***Tolypothrix distorta*** a promising future resource.