SEAWEEDS AS BIOFILTER FOR MONITORING WATER QUALITY IN AQUACULTURE SYSTEMS

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Certified that the dissertation entitled **"SEAWEEDS AS BIOFILTER FOR MONITORING WATER QUALITY IN AQUACULTURE SYSTEMS"** is a record of independent bonafide research work *carried out by* **Ms Seema. C** *(MC- 66) during the* period of study from September 2000 to August 2002 under our supervision and guidance for the degree of **Master of Fisheries science (Mariculture)** at the Central Marine Fisheries Research *Institute, Kochi, and that the dissertation has not previously formed* the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that the thesis entitled "**SEAWEEDS AS BIOFILTER FOR MONITORING WATER QUALITY IN AQUACULTURE SYSTEMS**" is an *authentic record of my own research work and that no part thereof has been* presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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विश्व भर में , प्रोटीन उत्पादन में जलकृषि की संभाव्यता बढ़ती जा रही है. समुद्री संवर्धन केलिए अपतट की अपेक्षा स्थलाधारित सुविधाएं अपनाने से परभोजन, अतिक्रमण, मौसम बदलाव, नियमन जैसी कई बाधाएं रोक सकते हैं. लेकिन तटीय समुद्री संवर्धन में भी फोसफेट और अमोनिया, नाइट्रेट और नाइट्राइट जैसे नाइट्रोजनी संयुक्तों का संचयन विषाक्त स्तर तक हो जाता है जिससे पानी जलकृषि के लायक नहीं बन जाता है. इस विषाक्तता को नियंत्रित करके पानी को कृष्य स्तर तक शुद्ध करने में महाशैवालों का प्रमुख स्थान है. उसके अतिरिक्त ये पानी का पी एच कम करके, विलीन ऑक्सिजन की सांद्रता बढ़ाकर और जैवरासायनी ऑक्सिजन आवश्यकता कम करके पानी की गुणता बढ़ाते हैं. अतः समुद्री शैवाल के साथ मछली और झींगा जैसे समुद्री जीवों का बहु संवर्धन द्वारा या पालन टैंक के बहिःस्रावों को समुद्री शैवाल डाले गए परीक्षण टैंक में पुनः परिचालित करके जलकृषि प्रबंधन किया जा सकता है. इन समस्याओं के निर्धारण के लिए वर्तमान अध्ययन किया गया और मछली बहिःस्रावों तथा झींगा बहिःस्रावों में विभिन्न समुद्री शैवालों जैसे *ग्रेसिलेरिया कोर्टिकेटा, अल्वा लैक्ट्यूका* और *अल्वा रेटिकुलेटा* का परीक्षण करने पर प्रोत्साहजनक परिणाम निकले. मछली तथा झींगा बहिःस्रावों में समुद्री शैवाल का उपचार करने पर इन में उपस्थित अमोनिया , जो एक प्रमुख उपापचयज है, की तेज़ कमी हो जाती है. मछली बहिःस्राव में उपचार किए गए *जी. कोर्टिकेटा* तथा *यू. लैक्ट्यूका* दोनों शैवालों ने अमोनिया की सांद्रता क्रमशः 13.29 से 2.1 और 2.33 मैक्रोग्राम आटम/लि तक कम कर दिया. लेकिन हरित शैवाल *अल्वा रेटिकुलेटा* का झींगा बहिःस्राव के साथ उपचार करने पर अमोनिया 44 से 10 मैक्रोग्राम आटम/लि. तक घट गया और झींगों के साथ उपचार करने पर 320 से 22 मैक्रोग्राम आटम/लि. तक घट गया. फोस्फेट की मात्रा की अधिकता बंद जलकृषि व्यवस्था के मछली बहिःस्रावों की मुख्य समस्या है. मछली तथा झींगा बहिःस्रावों में किए गए वर्तमान परीक्षण द्वारा बहुत मंद गति से फोस्फेट निकाल दिया जा सका.

ABSTRACT

Aquaculture poses a great potential for protein production world wide. Land-based facilities reduce many of the obstacles such as predation, poaching, weather conditions and regulation connected with offshore mariculture. Onshore mariculture faces a lot of difficulties due to accumulation of phosphate and nitrogeneous compounds such as ammonia nitrate and nitrite to a toxic level which makes the water unfit for aquaculture. Macroalgae plays a vital role in controlling this toxic wastes to a reasonable and cultivable limits. Also, it improves the water quality by lowering pH, increasing dissolved oxygen concentration and lowering biochemical oxygen demand. This aquaculture management can be made either through a polyculture system of marine organisms such as fish and shrimp with seaweeds or by recirculating the effluents from the culture tank to the treated tank supplement with seaweeds. To understand these problems, the present experiment was planned and encouraging results were achieved from the treatment of fish effluent and shrimp effluent by different seaweeds such as *G.corticata, Ulva lactuca* and Ulva reticulata. Ammonia, a major metabolite available in the fish and shrimp effluent was drastically reduced when treated with seaweeds. Both the seaweeds, *G.corticata* and *U.lactuca* used for the treatment of fish effluent reduced ammonia concentration from 13.29 to 2.1 and 2.33 µg atom/l respectively. On the other hand, the green algae *Ulva reticulata* treated with shrimp effluent and also cultured along with shrimp showed reduction of ammonia from 44 to 10µg atom/l in the earlier case and 320 to 22µg atom/l in the later case. The increase in phosphate content is a major problem in the fish effluent in closed aquaculture system. Removal of phosphate was found to be very slow in the present experiment from both fish and shrimp effluent.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

The term aquaculture denotes the farming of aquatic organisms for food or for commercial purposes. The importance of aquaculture production in providing high protein foods and enhancing economics worldwide is increasing.

World aquaculture production from inland and marine waters in 1999 is 32.9 million tones. Asian region, particularly China dominated world production. Water is the life support system of any aquatic organisms under culture. Thus ensuring the water quality in the culture site is the fundamental management requirement and role of the industry. Over the last two decades, many intensive aquaculture enterprises have suffered severe losses due to disease outbreak, poor farm management and degrading quality of the aquatic environment.

The discharge of low-quality water from land-based mariculture facilities cause environmental and economic concerns, since fish excrete to the water 70-80% of their ingested protein nitrogen. The intensive marine shrimp culture has had a negative influence on water quality of coastal zone.

Effluents from intensive farming contain much organic matter, nitrogen compounds, phosphorus and other nutrients lead to eutrophication. Nutrient load of the water encourages toxic plankton blooms and mortality of the aquatic animals. The aquaculture enterprises will eventually collapse if the water quality is allowed to deteriorate either through self-pollution arising from uncontrolled culture practices or through pollution from external sources.

Biofiltration, the treatment of effluents using live organisms has been found successful for improving water quality in aquaculture systems. The new approach is essentially to use closed systems of farming coupled with the application of biotechnological bacterial products and the integration of compatible candidate species of bivalves, fishes, sea cucumbers and seaweeds in the farming systems.

Since, it does not harm the environment and utilize ecological niches fully and yield maximum, it may be called "ecological aquaculture". These biofilters have proved to be potential removers of particulate and dissolved metabolites from shrimp/fish ponds. This production process has the advantage that it produces high priced products, while sharing many of the production inputs such as feed, water, energy and ponds.

The aquatic vegetation is an important factor in the self-cleansing capability of the water. This vegetation includes microscopic algae as well as macroscopic plants such as duckweeds, water hyacinths, seaweeds etc. Most of the metabolites are removed through assimilation by vegetation and biological conversion. Integration of economically important marine plants in an aquaculture system allows the management of eutrophication problems associated with the present fish mono-aquaculture and coastal agriculture or urban or industrial practices.

Seaweeds can clean the excess nutrient supply and other animal metabolic by-products and simultaneously grow and provide a significant amount of the needed oxygen for the farms through their photosynthetic activity. Seaweeds not only participate in the bioremediation of nutrient enriched coastal waters, but are also a high-value crop that diversifies sources of revenue and labour force of the aquaculture industry.

The polyculture of various species of fish with detritus feeders and plants has been used traditionally in land-based aquaculture Worldwide to exploit more foods naturally available. The excretion of one organisms in such system often supply food to another (Mohan, 1990).

The present study aims to study the impact on water quality when seaweeds are cultured in effluents of fish and shrimp and seaweeds with shrimps in a polyculture system. Water quality parameters like pH, dissolved oxygen, biological oxygen demand, nitrate, nitrite, ammonia, phosphate, silicate, were monitored to assess the change in water quality as a result of uptake of nutrients by the seaweeds.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The most direct aquaculture impact and the one most receiving attention is concern for maintenance of water quality.

Degradation of water quality and effluents by particulate and dissolved nutrients from animal excretion and uneaten food from land-based mariculture has been reported (Krom and Neori, 1989; Neori *et al.*, 1989). These effluents may have a negative impact on the receiving environment (Tomascik and Sander, 1985; Bell *et al.*, 1989)

The technique of biofiltration (treatment of effluent using living organisms) was found successful because it would pave way for additional income besides improving the quality of source water through abatement of aquatic pollution (Oswin and Rahman, 1997)

The work on the use of biofilters in aquaculture system has been attempted by few workers (Cohen and Neori, 1991; Rosenthal, 1991). In these studies, biofilters of bacteria, phytoplankton, suspension feeders (bivalves) and seaweeds, separately or in combination has been incorporated.

In closed systems of fish culture, ammonium and nitrite can accumulate to toxic concentrations. In fresh water culture, nitrifying and denitrifying bacteria are used to detoxify and eliminate these compounds (Meade, 1974). In marine systems, seaweeds are a logical alternative to bacteria because they have capacity to clean the excess nutrient supply and other animal metabolic by-products, and simultaneously grow and provide a significant amount of the needed oxygen for the farms through their photosynthetic activity. By selecting seaweeds of commercial value, additional profits can be made.

Integrated approach can be applied where wastes from one crop are recycled within the farm as inputs for another crop (Ruddle, 1991). Integrated aquaculture, otherwise termed, as "Ecological aquaculture" is energy efficient, recycles wastes, minimizes environmental impact and is integrated with other food production systems

It is generally accepted hypothesis that seaweeds in co-culture systems incorporate the metabolic wastes of animals into algal biomass resulting in a high growth rate (Harlin *et al.*, 1978). The animals in the system presumably benefit from improved water quality. In Israel, the concept of developing an "environmentally clean" aquaculture based on an integrated fish- molluscs- macroalgae system was first proposed by Gordin *et al.* (1981). Such a system was also tested at Eilat in Israel (Gordin, 1982; Gordin *et al.*, 1990, Shpigel *et al.*, 1991, Mukhi *et al.*, 1993).

Systems integrating fish and macroalgae (Mc Donald, 1987), fish or shrimp and oysters in land based facilities (Wang, 1990) or in offshore facilities (Jones and Iwama, 1991) have been conducted.

The combined culture of marine algae and animals has been tested in China and Taiwan. This system is based on the concept that algae actively uptake CO_2, release oxygen to the surrounding environment and utilize metabolic wastes from the culture organisms. As a result algae can create a favourable environment for animal growth (Shan and Wang, 1985).

Folke and Kautsky (1992) proposed polyculture with interacting species, which would recycle nutrients and minerals. Focus is mainly on the use of bivalve molluscs (oysters, mussels and clams), seaweed and mangroves to reduce the environmental effects of fish and shrimp culture.

The filter feeders like molluscs remove the particulate suspended matter and deposit it on the bottom as faeces and pseudofaeces (Inui *et al.*, 1991). Molluscs, which recycle waste products (uneaten food, faeces etc.) keep the surrounding water clean, reduce sedimentation and decrease the risk of algal blooms and turbidity in the water.

Seaweeds such as species of *Gracilaria* have been tested to grow in polyculture system, because they absorb or remove soluble nutrients (Nitrogen and Phosphorus), which are not absorbed by molluscs (Inui *et al*, 1991; Macintosh and Philips, 1992). The advantages of this type of polyculture are decreased feeding requirement, fuller use of ponds, enhanced utilization of water, reduction of costs, increase of profits and a decrease in pollution.

Kappaphycus alvarezii and *Pinctada martensia* were used as experimental materials for integrated cultivation (Pei-Yuan *et al.*, 1996). They found that algae removed wastes released by pearl oysters very efficiently, especially ammonium. The algae treated with pearl oyster wastes grew much faster than those without oyster wastes.

Several workers have attempted integrated cultivation of salmonids and seaweeds. *Gracilaria chilensis* used in tank cultivation of salmonids resulted in 25% Nitrogen and 3.5% Phosphorus removal (Buschman *et al.*, 1994). Mwandya *et al.* (1999) studied the efficiency of macro algae like *Euchema denticulatum, Gracilaria crassa* and *Ulva reticulata* to remove nutrients from fish tanks.

Neori *et al.* (1996) suggested a system of integrated culture of fish *Sparus aurata* and *Ulva lactuca*. L. Seawater was recirculated between intensive fishponds and seaweed ponds. The seaweeds remove most of ammonia excreted by fish and oxygenated the water.

Integrated cultivation of salmonids and seaweeds in open systems was conducted by Petrell *et al.* (1996) to provide information on production, nutrient removal and technical and economic feasibility of different production strategies.

Radhakrishnan (2001) conducted an experiment on the use of seaweeds for removal of nitrogenous wastes in closed lobster culture system. The seaweeds used were *Gracilaria corticata* and *Gracilaria verrucosa.*

A clean technology in aquaculture has been suggested by Torjan *et al.* (1996) which involves a theoretical model linking production of salmon, mussels and seaweeds in floating, enclosed units based on field data, laboratory tests and literature data.

A simulation model was developed for an experimental recirculating mariculture system in Israel (Stephen *et al.*, 1996). Several studies have reported (Chiang, 1980; Chang and Wang, 1985; Lou and Wei, 1986; Tian *et al*, 1987; Chunhan, 1989; Trono, 1989; Wei, 1990) enhanced growth rate of seaweeds and animals in integrated culture.

The advantages of intensive closed system aquaculture are greater control over water quality, more production per unit area and controllable growth rates. The feeding techniques and high loadings generally produce a concentrated wastewater that must be treated by physical, chemical and biological processes before reuse.

Biological treatment process appears to be economical, sound and scientifically acceptable, because of its simplicity, ease of operation and maintenance. Mangroves, seaweeds, bivalve molluscs have been used for effluent treatment.

Mangroves offer protection to coastal ponds by removing nutrients, heavy metals, suspended solids and toxic hydrocarbons (Landers and Knut, 1991). Use of mangroves to treat shrimp pond effluent, either by retention of a buffer zone or by replanting mangroves has been tried by Macintosh and Philips (1992). Oswin and Rahman (1997) studied the treatment of waters in effluent treatment plant (ETP) of a coastal shrimp farm (Bismi Aquqfarms, Perunthottam) using seaweeds (*Gracilaria verrucosa*), mussel (*Perna virdis*) and oyster (*Crassostrea madrasensis*) and proved their efficiency in considerably reducing the suspended solids, nitrates and phosphates.

The biological treatment of sewage by algae and bivalves was also studied by Ryther *et al.* (1972 and 1975). In this secondarily treated sewage, mixed with seawater was used to culture unicellular algae. These algae and the remaining dissolved nutrients were removed by bivalves and seaweeds.

Duck weed culture in waste water has gathered much scientific concern, since the removal of Biochemical Oxygen Demand, suspended solids, nitrogen, phosphorus and metals by duckweed from waste water has been reported to be appreciably high. (Reed *et al.*, 1998; Ayyappan, 2001).

Asare (1980) found that animal waste could be used as nitrogen source for cultivated *Gracilaria tikvahiae* and *Neogardhiella baileyi*. In Thailand, seaweeds such as *Gracilaria fisheii* and *Gracilaria tenuistipitata* or colonies of green mussel in drainage canals removed nutrients before the water was discharged (Chandrakrachang *et al.*, 1991; Lin *et al.*, 1992). Mans *et al.* (1994) used bivalve mollusc and seaweeds for treatment of shrimp farm effluents. In this study, concentration of phosphate, ammonium and nitrate showed significant reduction.

Large scale experiments on purification of nutrient rich effluents have been made with seaweeds, principally *Gracilaria* (Ryther *et al.*, 1979). Buschman *et al.* (1994) conducted a study on the use of salmon tank effluents for *Gracilaria chilensis* cultivation. Seaweeds are known to utilize different sources of nitrogen simultaneously but not necessarily at the same rate. Nitrate and ammonia are equally effective in promoting growth of *Chondrus crispus* (Neish and Shacklock, 1971). *Ulva lactuca* is more efficient in assimilating total nitrogen than *Chondrus crispus* when nitrogen is in excess.

Lapointe and Tenore (1981) found that uptake of nitrate by *Ulva fasciata* depends mostly on daily nitrogen supply of that nutrient. Ryther *et al.* (1981) demonstrated a much higher ammonium uptake rate in nitrogen starved *Gracilaria tikvahiae* than in nitrogen sufficient seaweed.

Most of the work on seaweeds as biofilters for marine fish pond effluents is based on ammonium biofiltration (Cohen and Neori, 1991; Jimenez *et al.*, 1994). The use of *Ulva spp* as biofilters has been suggested as an efficient method to recover large amounts of dissolved inorganic nitrogen (Fralick *et al.*, 1979; Vandermeulen & Gordin, 1990; Jimenez *et al.*, 1996)

Fish pond biofilters with green seaweed *Ulva lactuca* L. remained clean and functional for years with minimal maintenance, producing high yields using only fish pond effluents for water and nutrition. (Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Neori *et al*, 1991).

Macroalgae showed many physiological mechanisms for responding to environmental changes and the ability to tolerate environmental disturbances (Kuebler et al., *1991*). The survival of the species is often determined by its ability to acclimate to such environmental changes.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Collection of seaweeds and experimental animals

Species of *Gracilaria corticata* and *Ulva lactuca* were collected from Thonithurai ($9^0 17'$ N and $70^0 11'$E), located on the south east coast of TamilNadu, near Mandapam. Samples were collected during low tide in the morning and transported to the laboratory of Mandapam Regional Centre of C.M.F.R.I. The plants were brushed off epiphytes and cleaned well in running seawater 3-4 times. They were kept under running seawater in fibre glass tanks in the green house. Healthy seaweeds were sorted out and packed in perforated polythene bags and transported to CMFRI, Cochin. They were maintained in the seaweed culture laboratory of marine hatchery complex for 1-2 days to overcome the transportation stress before starting the experiment.

Ulva reticulata was collected from Ashtamudi lake($8^0 45'$-$9^0 28'$N and $76^0 25'$-$77^0 17'$E) near Dalavapuram in Quilon.The plants were cleaned thoroughly in running water to remove the debris. Fresh seaweeds were sorted out and transported to Cochin in perforated polythene bags kept with a bucket of estuarine water.

Indian white shrimp (*Penaeus indicus*), H.Milne Edwards (1837) were collected from the ponds of valappu and brought to the marine hatchery complex in 25 l jerry can filled with water. Fish effluents were collected from the Fisheries Harbour laboratory of CMFRI, Thopumpaddy, where the grouper fishes are maintained in 5 ton capacity fibreglass tank. The shrimp effluent was collected from the shrimp farm (Ajanta) near Valappu.

3.2. Experimental set up

3.2.1 Fish effluent

Experiments were set up in the Marine Hatchery complex to assess the change in water quality of fish effluent when treated with seaweeds. Transparent rectangular Perspex tanks of 100 l capacity were used for these experiments. Two sets of experiments were set up in duplicate with different seaweeds whereas one set was kept as control.

In the control tank the fish effluent (90 l) were kept without any seaweed with adequate aeration. Whereas in the treated tanks 200g of *Gracilaria corticata* and *Ulva lactuca* were kept separately on the nylon mesh just dipped in the effluent (Plate 1). Vigorous aeration was provided to in the treated tank to allow the effluents mixed up uniformly. All the experiments were carried out in duplicate.

3.2.2 Shrimp effluent

In this experiment, shrimp effluent was treated by green seaweed *Ulva reticulata* Two hundred gram of seaweed was placed on the nylon mesh of the tank just dipped in the effluent. Adequate aeration was provided in the treated tank to allow the effluents mixed up uniformly. All the experiments were carried out in duplicate. The shrimp effluent without seaweed was taken as control (Plate.2).

3.2.3 Shrimp with seaweed

In this experiment 4 shrimps of body weight, 20g (fresh weight) were kept in tanks with 90 l water of 23 ppt salinity and 100 g seaweed *Ulva reticulata* with a ratio of 1:5. In the control tank the shrimps of same body weight were kept in the water of 23 ppt (90 l) without seaweed (Plate.3).

This experiment was planned to know the stocking density of seaweed and shrimp in a closed aquaculture system and to evaluate the efficacy of seaweed to remove the waste dissolve toxic material from the system. All the experiments were carried out in duplicate. Adequate aeration was provided from the compressor with a control valve.

3.3. Sampling

All the experiments were continued for 20 days. Regular sampling was done in control and different treated tanks at *initial period i.e before treatment* (BT), 10 days after treatment (10 DAT) and 20 days after treatment (20 DAT).

Plate 1.Experimental set up of fish effluent treated with seaweeds.

Plate 2.Experimental set up of shrimp effluent treated with seaweeds.

Plate 3.Experimental set up for polyculture of shrimp and seaweeds

3.3.1 Monitoring environmental parameters

All the experiments were carried out in the controlled condition in seaweed culture laboratory of Marine hatchery complex. The temperature was maintained at 28^0 C. Hanging tubelights were provided above each tank to get uniform light intensity. Salinity of the seawater was maintained 35 ppt in the fish effluent whereas it was 23 ppt in shrimp effluent. Aeration was provided from the compressor with controlled valve.

3.3.2 Monitoring water quality parameters

Various water quality parameters were estimated at each sampling period from initial to 20 days of treatment from both control and treated tanks.

Dissolved oxygen was estimated by the standard procedure of Strickland and Parson (1968) by using Winkler's reagent. The pH was taken by a digital pH meter. Salinity was recorded by using a refractometer with reference to distilled water. Biochemical Oxygen Demand (BOD) was estimated by incubating the water sample in a BOD incubator for 5 days at 20^0 C under complete darkness.

Ammonia estimation was carried out by the method of Solarzano (1969) where the seawater was treated with alkaline citrate medium with sodium hypochlorite and phenol in presence of sodium nitroprusside which act as a catalyzer. The blue indophenol colour with ammonia was measured at 640 nm by a U 2010 UV-Visible Hitachi spectrophotometer.

Nitrate was estimated by the modified method of Morris and Riley (1963) with some modifications suggested by Grassenhoff (1964) and Wood *et al* (1967). The sample was allowed to get reduced by a buffer reagent and reducing agent for 20 h and then react with sulphanilamide in an acid solution. The highly coloured azo dye was measured at a wavelength of 545 nm in U-2010 UV-Visible Hitachi spectrophotometer.

Nitrite was estimated by Shinn method (1941) modified by Bend Schneider and Robinson (1952). The sample was allowed to react with sulphanilamide in an acid solution. The resulting diazo compound is reacted with N

(1-Naphthyl) ethylene Diamine to form a highly coloured azo dye, which was measured at 545 nm in the above mentioned spectrophotometer.

Phosphate was estimated by the method of Murphy and Riley (1962). The sample was allowed to react with a composite reagent containing molybdic acid, ascorbic acid and trivalent antimony. The resulting complex was reduced to blue colour and measured at 885 nm in spectrophotometer.

Silicate was estimated by the method of Mulin and Riley (1955). The sample is allowed to react with molybdic acid. The resulting complex is reduced with a composite reagent containing oxalic acid, sulphuric acid and metol sulphite and the colour developed is measured at 810 nm in the UV-visible spectrophotometer.

3.3.3 Estimation of photosynthetic pigments

All the pigments such as chlorophyll (chlorophyll a and b) and accessory pigments (Phycoerythrin, phycocyanin and allophycocyanin) were estimated in the seaweeds *Ulva lactuca, Ulva reticulata* and *Gracilaria corticata* by the standard method of Jeffery and Humphrey (1975).

Known quantity of plant material were cut into pieces, ground in a pre-cooled mortar cum pestle with 90% acetone. The extract was centrifuged at 8000 rev/min in a Hitachi refrigerated high-speed centrifuge at 4^0C. (Himac model CR 21 G) for 10 minutes. The clear supernatant was made up to a volume of 6 ml and optical density was measured at 630, 645 and 663 nm by UV-visible spectrophotometer (Hitachi model U-2210). Chlorophyll a (Chl a) was estimated for *Gracilaria corticata* and Chlorophyll a & b (Chl b) were estimated in *Ulva lactuca* and *U.reticulata* by the following formulae were expressed as microgram of chlorophyll per gram fresh weight.

$$\text{Chl a} = (12.7 \times A663) - (2.96 \times A645)$$

$$\text{Chl b} = (22.9 \times A645) - (4.68 \times A663)$$

The accessory pigments such as Phycoerythrin (PE), Phycocyanin (PC) and Allophycocyanin (APC) of *Gracilaria corticata* were extracted with 0.5 M phosphate buffer at pH 6.8. The apical portion of the thallus were cut into small pieces and ground in the above mentioned buffer in a pre-cooled mortar cum pestle. The extract was kept in the refrigerator for 24 h for complete extraction of pigments and then centrifuged at 10,000 rpm for 15 minutes by the refrigerated centrifuge at $4^{0}C$. The volume was adjusted with the buffer to 6 ml and the optical density was measured by the Hitachi spectrophotometer at wavelengths of 498, 614 and 651 nm. The pigments were estimated by the formulae as follows. The pigments content were expressed as milligram of pigment per gram fresh weight.

$$PE = (155.8 \times A498) - (40.4 \times A614) - (10.5 \times A651)$$

$$PC = (151.1 \times A614) - (99.1 \times A651)$$

$$APC = (181.3 \times A651) - (22.3 \times A614)$$

3.3.4 Monitoring the live stock of prawn

The animals were fed daily with pelleted diet, 2% of their body weight. Adequate aeration was provided from the compressor with controlled valve. The live animals were observed daily and replaced if mortality any was recorded.

3.4. Statistical analysis

Results were interpreted by analyzing tha data statistically by Analysis of Variance (ANOVA) for each character and treatments with or without replications.

RESULTS

4. RESULTS

4.1. Treatment of fish effluent with seaweeds

Fish effluents collected from Fisheries Harbour Laboratory of CMFRI, Thoppumpady were treated by two different types of seaweeds. *Gracilaria corticata*, a red algae widely available in the Indian coast and *Ulva lactuca*, a green algae also available in most of the area in Indian coast.

It was observed that the pH values in fish effluents treated with *Gracilaria corticata* and *Ulva lactuca* showed a declining trend on 20 DAT after an initial rise in pH by 1 % on 10th day. The decline was more pronounced with *G. corticata* (22.5 %) than *U. lactuca* (5.27 %) (Fig.1). The analysis of variance did not show any significant difference between the treatment and control tank throughout the experimental period (Table.1).

Dissolved oxygen (DO) content of the treated and control tank showed almost a similar trend. There was an initial increase of DO for 10 days and then declined on 20 DAT. The increase was of DO is only 8.25 % compared to 33 % in treated tank of *G. corticata* but no change was observed in *U. lactuca*. On 20 DAT the DO declined by 38 % in *G.corticata*, 16 % in control tank but only 2 % in *U. lactuca* with reference to 10 DAT. The overall decline was more pronounced in the tank treated with *G. corticata* (Fig.1). Statistical interpretation did not show significant difference between the control and treated tanks (Table.1).

The Biological oxygen Demand (BOD) also showed a similar trend like dissolved oxygen. There was an initial increase in BOD on 10 days of treatment which was more pronounced in *G.corticata* followed by a decline on 20 DAT both in control and treated tanks. The decline on the BOD was maximum in *G. corticata* (61.5 %) and 28.6 % in *U.lactuca* from the initial value. The analysis of variance table showed no significant difference in BOD values between treatment and control tanks throughout the experimental period (Table 1).

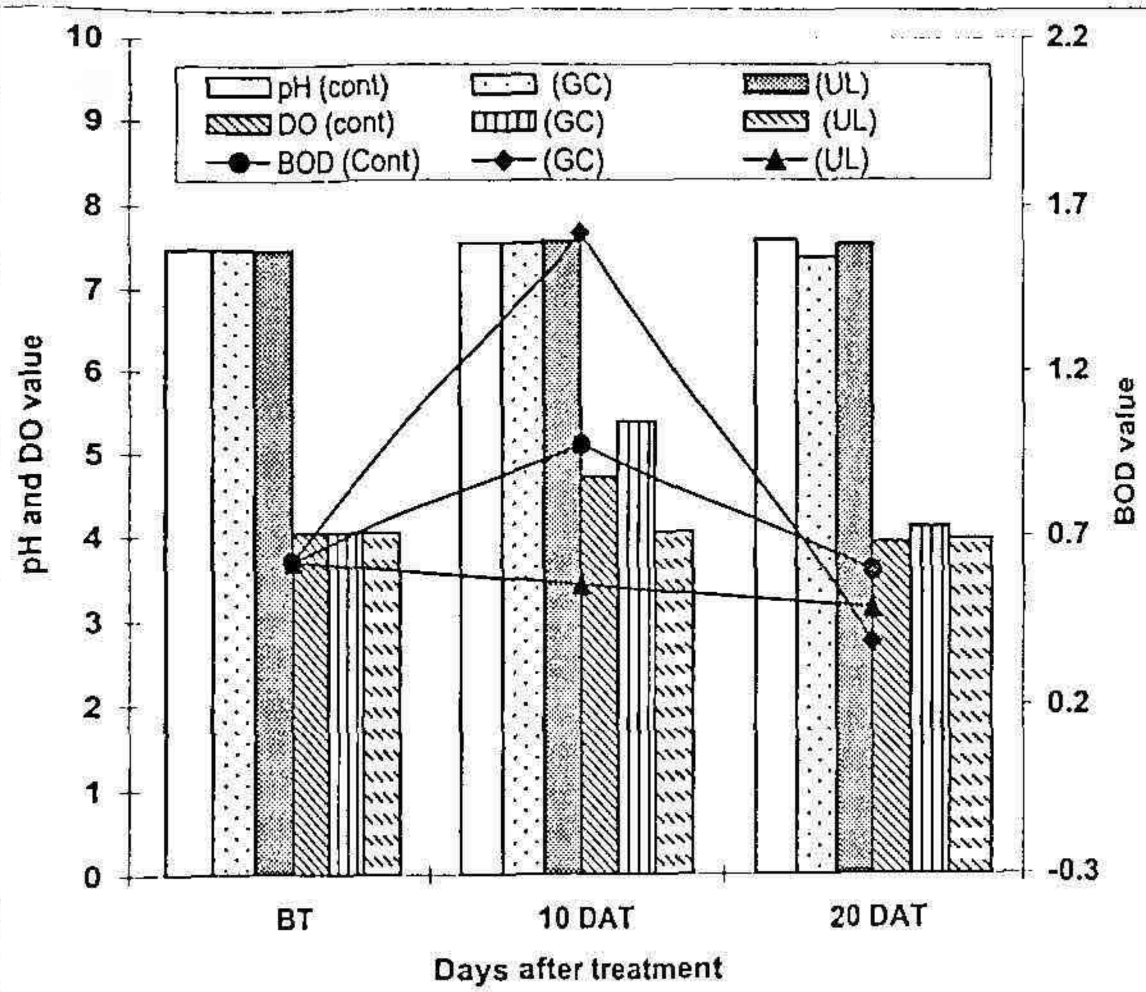

Fig.1. Water quality parameters of fish effluent treated with seaweeds.

ANOVA pH					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	0.009672	2	0.004836	1.297317	0.367907
PERIOD	0.009372	2	0.004686	1.257079	0.377054
Error	0.014911	4	0.003728		
TOTAL	0.033956	8			
ANOVA DO					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	0.105622	2	0.052811	0.182562	0.839705
PERIOD	1.567222	2	0.783611	2.708853	0.180397
Error	1.157111	4	0.289278		
TOTAL	2.829956	8			
ANOVA BOD					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	0.154156	2	0.077078	0.704264	0.546968
PERIOD	0.511489	2	0.255744	2.336751	0.212682
Error	0.437778	4	0.109444		
TOTAL	1.103422	8			

Table 1. Anova table for the water quality parameters of fish effluent treated with seaweeds.

Ammonia, an important parameter of water often showed a very high value in the effluent. In the present experiment, the ammonia concentration declined gradually to a greater extent in the treatment tanks. In the control tank, initially there was an increase in ammonia concentration by 39 % but then it declined to 34 % in the next 10 days. The decline of ammonia in treatment tanks ranged from 82 % in *U. lactuca* to 84 % in *G. corticata* from BT to 20 DAT, where as they were 63 to 70 % decline over control after 10 DAT and 73- 75 % after 20 DAT (Fig.2). Analysis of variance clearly indicated the significant difference in ammonia values between the treatment and control tanks and also among the days of treatment throughout the experimental period (Table.2).

The nitrate concentration showed a different trend in the treated and control tanks. In the initial period of treatment from BT to 10 DAT, the Nitrate concentration increased to a very high value and then declined marginally in the treated tanks. In the control tank, the nitrate content increased further at 20 DAT. While comparing with control at 20 DAT, the nitrate content was found to be declined from 54 and 72 % in *G.corticata* and *U.lactuca* respectively (Fig.3). Statistical interpretation showed highly significant difference in nitrate values between the treatments and control, period of treatment and period / treatment throughout the experimental period (Table 3).

The nitrite content of the fish effluent exhibited an initial increase in both control and the tanks treated with *G. corticata* where as the treatment with *U.lactuca* showed a gradual decline. In *G.corticata* the nitrite content declined to a greater extent by 63.7 % on 20 DAT over the initial value where the decline was marginal in control tank (20.6 %). In *Ulva* the nitrite content declined to 96 % after 20 DAT (Fig.4). The analysis of variance showed a significant difference in nitrite values between the treatment and control tanks throughout the experimental period (Table 4).

Phosphate is a general problem in the closed aquaculture system. The level of phosphate increased due to the feed given to the fish. In the present experiment, there was a gradual decline in the phosphate content treated with seaweeds. But the decline was very slow. It was observed that the phosphate declined by 26.6 % at 10 DAT in *G.corticata* tank and then increased marginally.

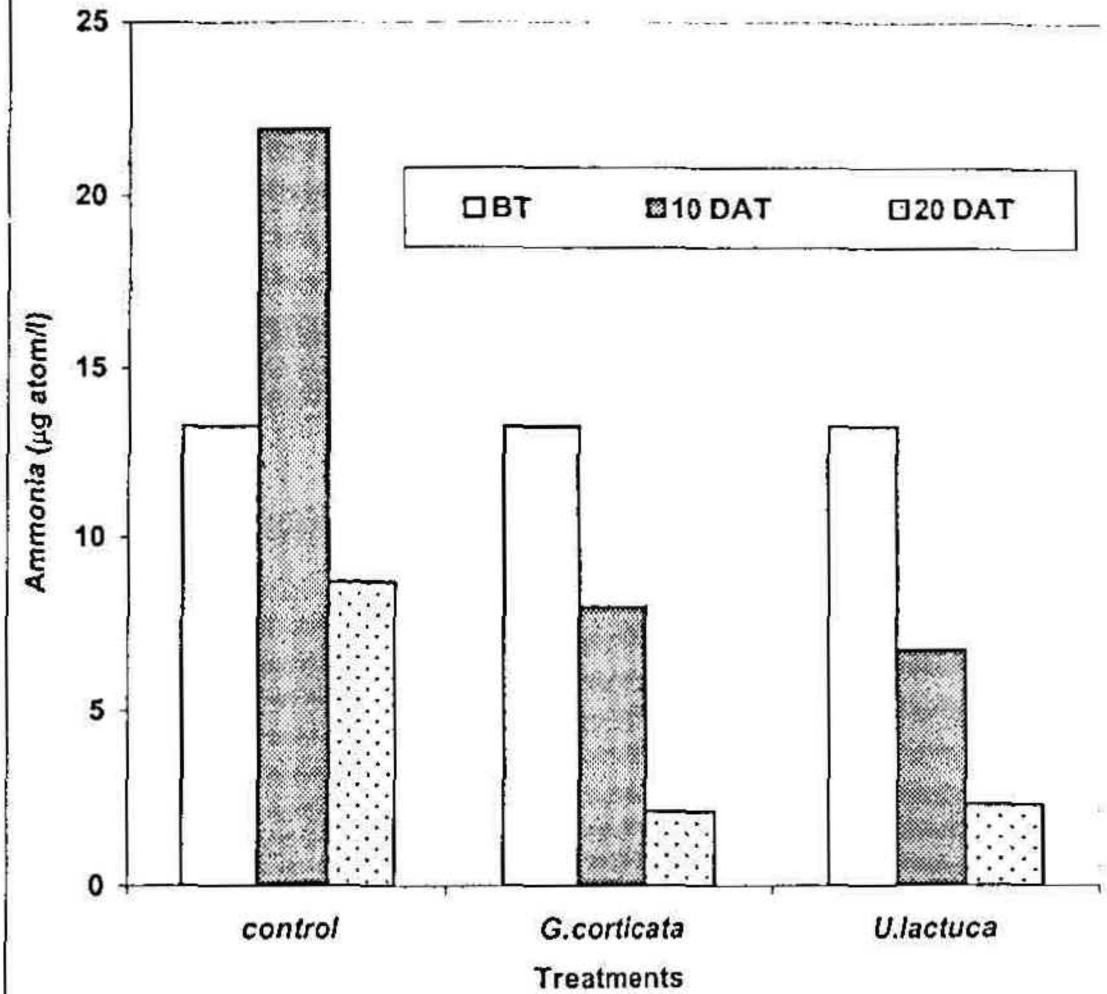

Fig.2. Ammonia content of fish effluent treated with seaweeds

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	197.138	2	98.569	50.225	0.000
TREATMENT	284.523	2	142.262	72.488	0.000
PERIOD*TREATMENT	143.150	4	35.787	18.235	0.000
Error	17.663	9	1.963		

Table 2. Anova table for the ammonia content of fish effluent treated with seaweeds

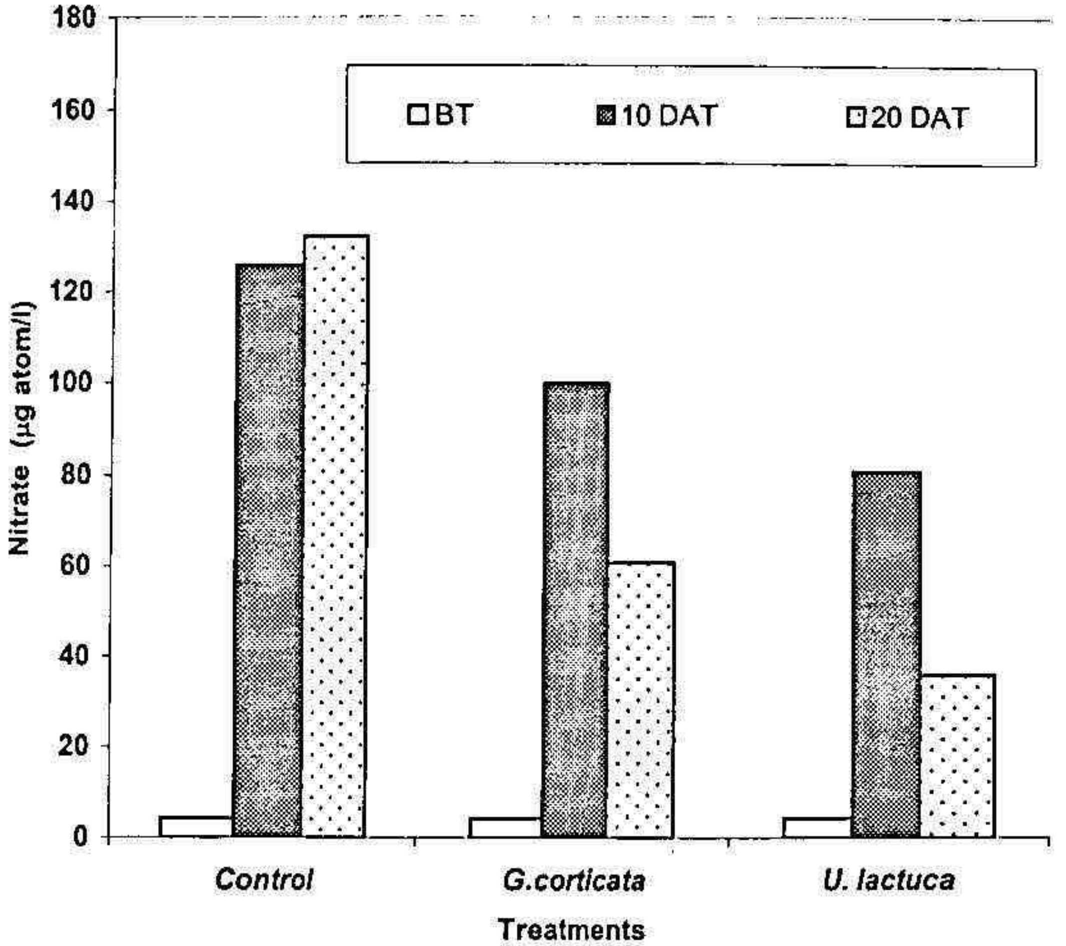

Fig.3. Nitrate content of fish effluent treated with seaweeds.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	6969.893	2	3484.947	87.07	0.000
TREATMENT	30992.805	2	15496.403	390.005	0.000
PERIOD*TREATMENT	5071.607	4	1267.902	31.910	0.000
Error	357.605	9	39.734		

Table 3. Anova table for the nitrate content of fish effluent treated with seaweeds.

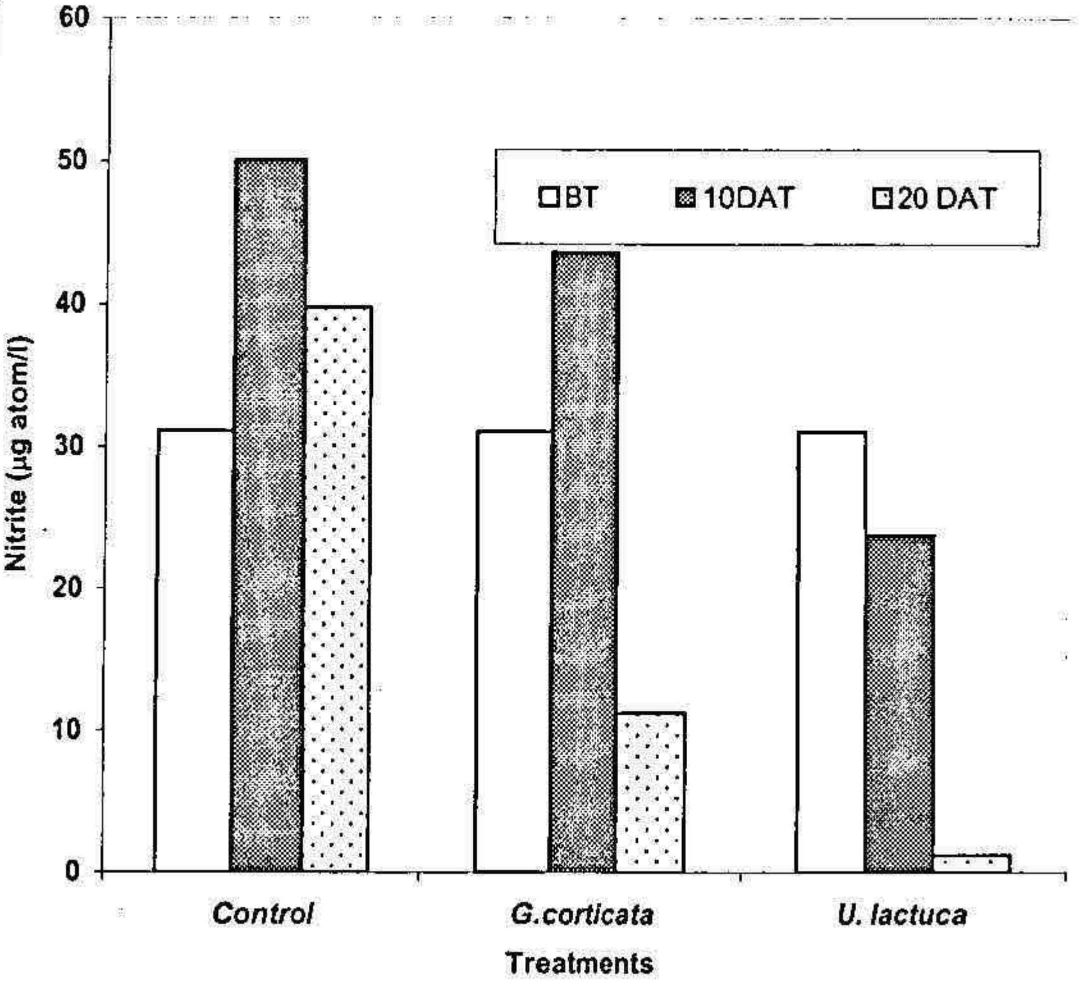

Fig.4. Nitrite content of fish effluent treated with seaweeds.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	1404.436	2	702.218	112.614	0.000
TREATMENT	1457.121	2	728.561	116.838	0.000
PERIOD*TREATMENT	947.091	4	236.773	37.971	0.000
Error	56.121	9	6.236		

Table 4. Anova table for the nitrite content of fish effluent treated with seaweeds.

But the overall decline was found to be 14.6 % over control. In *U.lactuca*, the phosphate declined by 22-25 % on 10 DAT and 20 DAT. The overall decline was more than 16 % than control. In control tank, the phosphate content declined marginally and then increased (Fig.5). The analysis of variance showed a significant difference in phosphate values between the treatment, period and periods and treatments (Table 5).

The silicate value in control and fish effluent tank treated with seaweeds showed generally an increasing trend in all experiment. While comparing with control the silicate value was found to be less by 26 % in the tank treated with *U.lactuca* and 26.2 % in *G.corticata* (Fig.6). The analysis of variance showed a significant difference between periods, however no significance was found between treatments and between treatments and periods (Table 6).

The qualitative changes in the physiological parameters are many, but here emphasis is given only to the change of photosynthetic pigments of seaweeds. The pigment concentration such as chlorophyll a, phycoerythrin and phycocyanin and allophycocyanin were found to decrease in *G.corticata* treated with fish effluent within 10 DAT. The accessory pigments were found in reasonably high concentration declined drastically in the initial 10 days but recovered marginally after 20 DAT. The chlorophyll concentration showed a gradual decline with in the experimental period (Fig.7). Whereas in *U. lactuca* an increase in chlorophyll a and b concentration was observed during the first 10 days of treatment. Further it declined to a marginal extent (Fig.8).

4.2. Treatment of shrimp effluent with seaweed

The pH of the shrimp effluent showed a gradual increase from initial period to 20 days in control and treated tanks. The increase was found to be 2 – 3 % in control tank and 1 – 4 % in treated tanks (Fig.9). No significant difference was found in the pH value of control and treated tank (Table 7).

The dissolved oxygen showed a gradual increase from 4.09 to 4.31 ml O_2/l in treated tank compared to 4.23 ml O_2/l in the control tank. The DO in the control tank showed a marginal decline on 10 DAT (1.5 %) and then increased to 5 % in the next period of treatment.

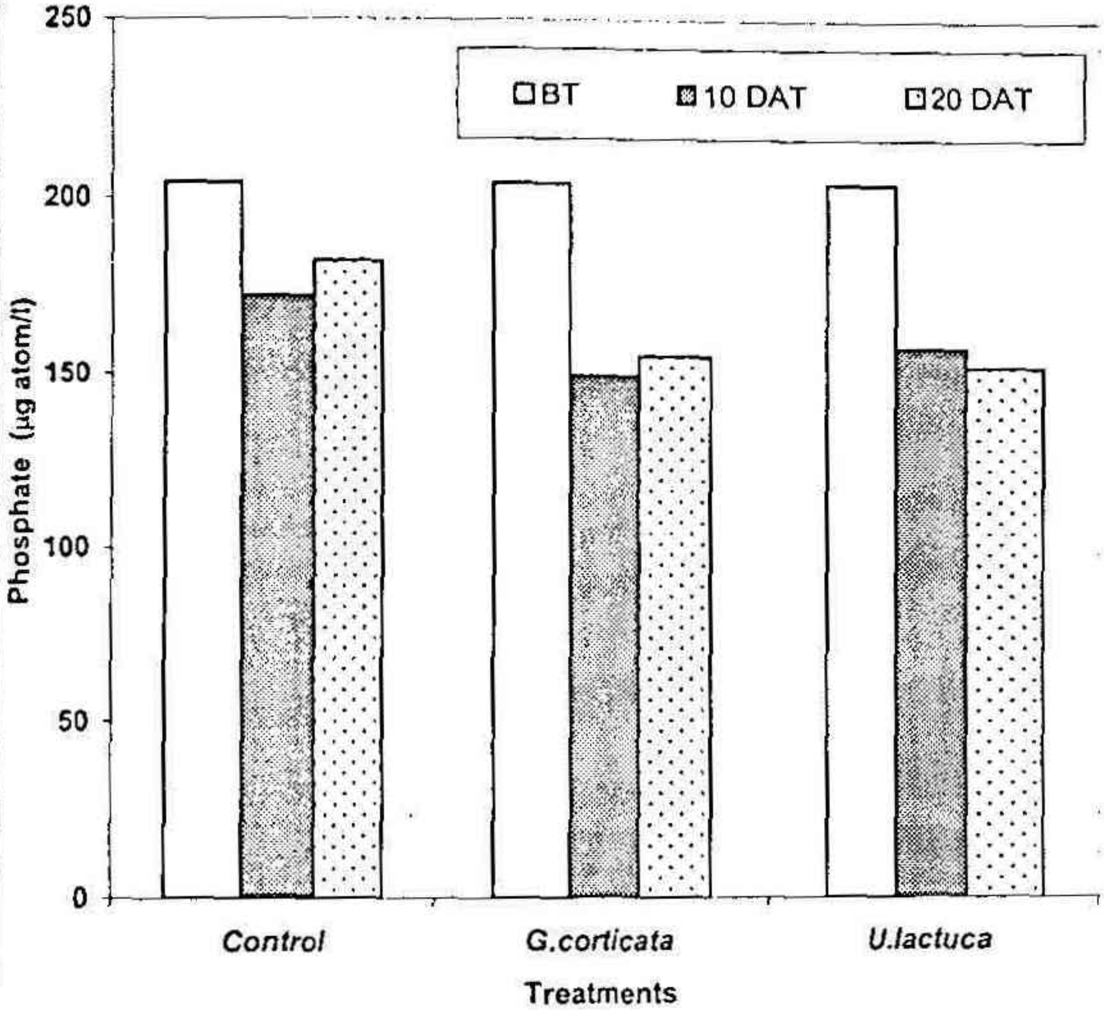

Fig.5. Phosphate content of fish effluent treated with seaweeds.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	989.267	2	494.633	15.877	0.001
TREATMENT	7195.391	2	3597.696	115.483	0.000
PERIOD*TREATMENT	624.624	4	156.156	5.012	0.021
Error	280.382	9	31.154		

Table 5. Anova table for the phosphate content of fish effluent treated with seaweeds.

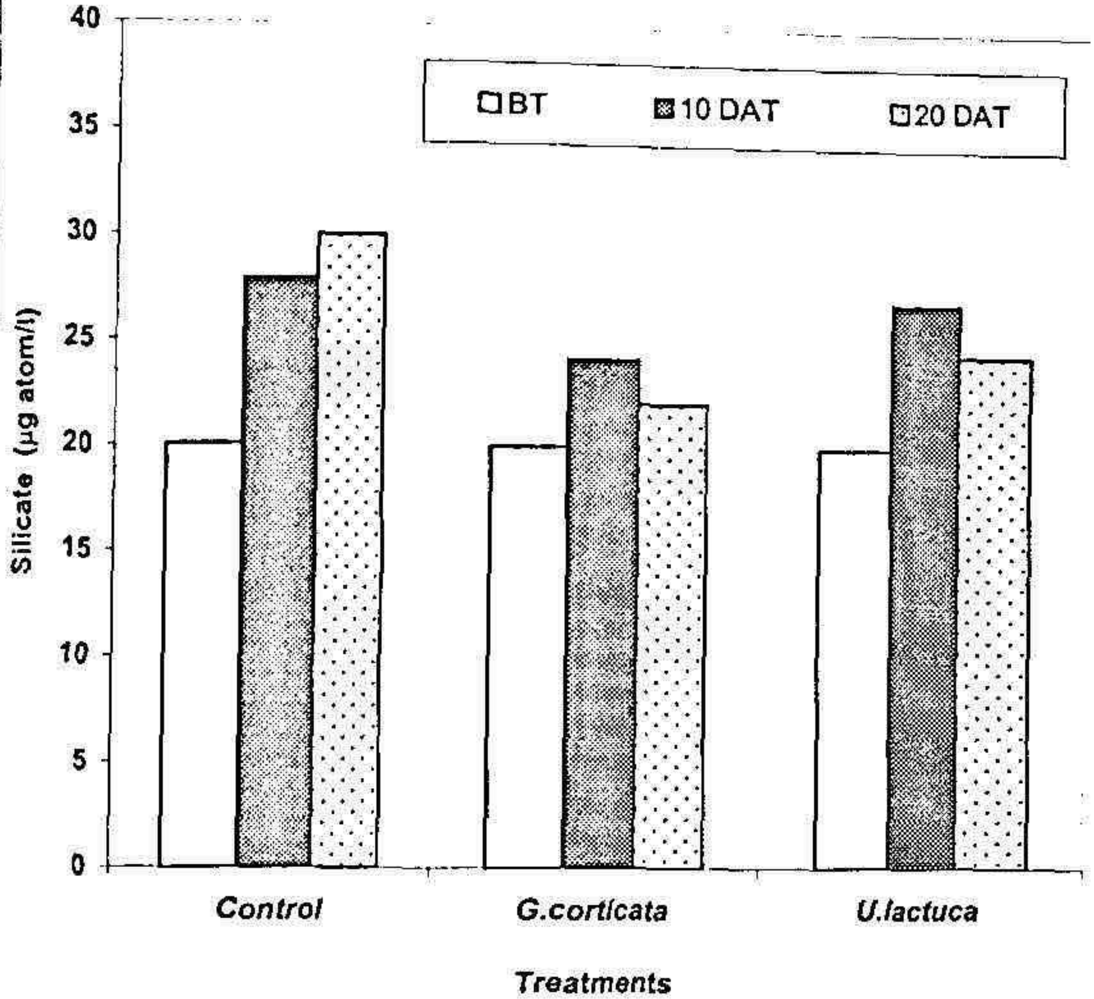

Fig.6. Silicate content of fish effluent treated with seaweeds.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	21.515	2	10.757	2.008	0.190
TREATMENT	207.601	2	103.801	19.371	0.001
PERIOD*TREATMENT	11.399	4	2.850	0.532	0.716
Error	48.227	9	5.359		

Table 6. Anova table for the silicate content of fish effluent treated with seaweeds.

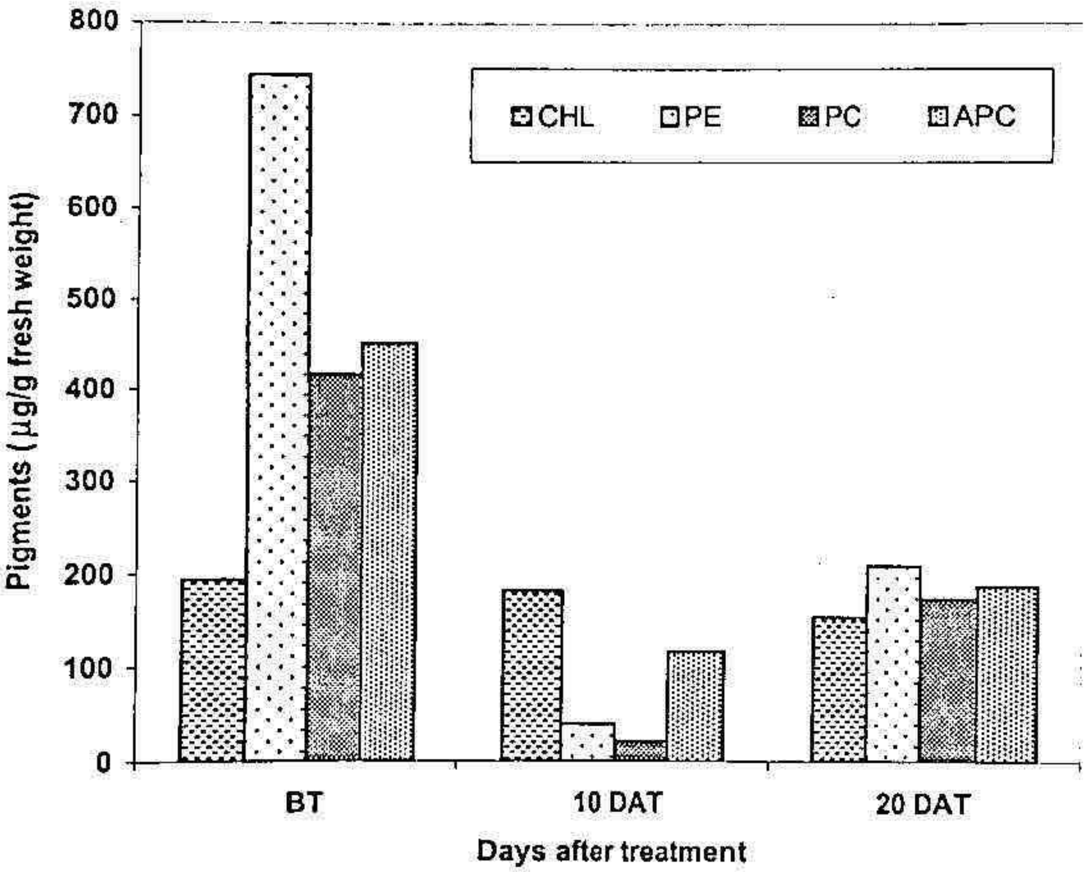

Fig.7. Pigment constituents of *G.corticata* treated with fish effluent.

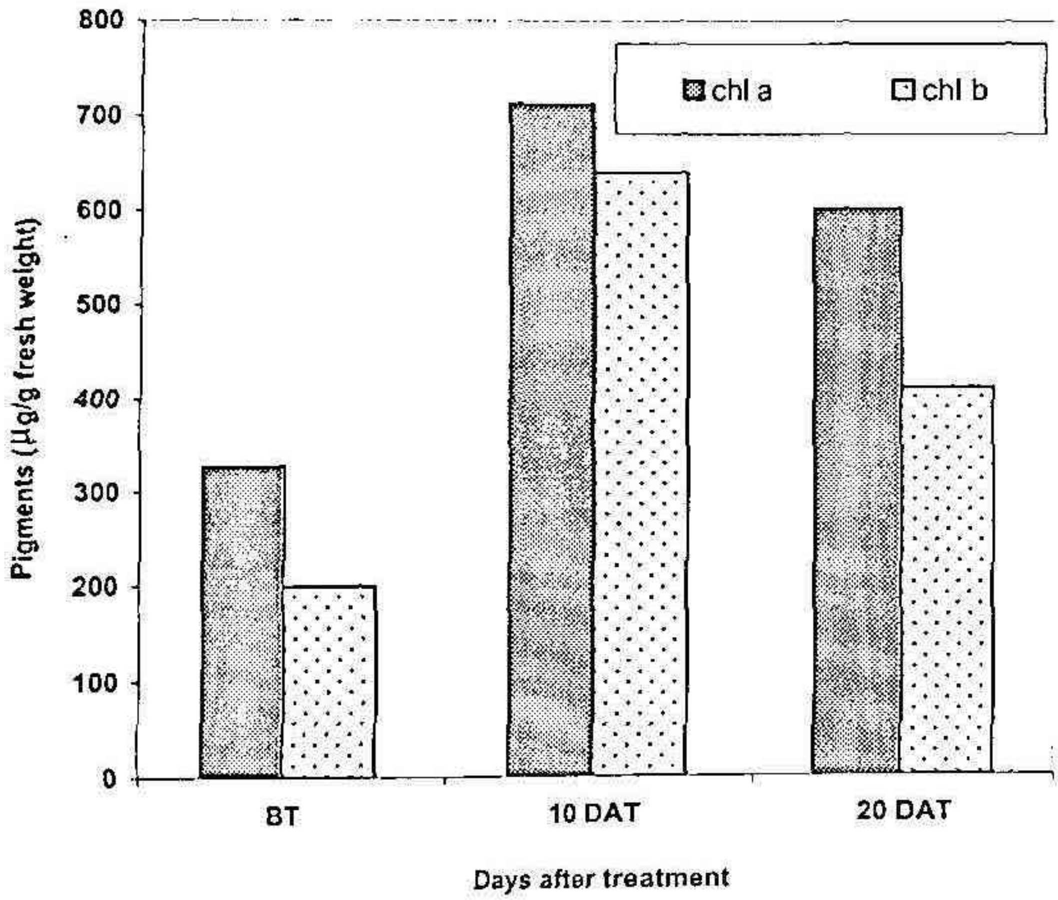

Fig.8. Pigment constituents of *U.lactuca* treated with fish effluent.

In the treated tank, the DO did not show any decline (Fig.9). Analysis of variance did not show any significant difference throughout the experiment (Table 7).

The Biochemical Oxygen Demand (BOD) showed a gradual decline in the control tank from 1.29 to 1.0 ml O_2/l. The effluent treated with *U. reticulata* showed an initial decline of BOD from 1.29 to 1.08 ml O_2/l on 10 DAT and then increased to 1.41 ml O_2/l (Fig.9). The statistical interpretation (ANOVA) did not show any significant difference between control and treatment (Table 7).

The ammonium concentration was found to increase gradually from initial period to 20 days of treatment. The increase was maximum in control (117 %) than the treated tank (38 %) on 10 DAT. The increase of ammonia concentration was 36.4% over control on the same day. On 20^{th} day, the ammonia increased to very high level in the control compared to the treated tank (Fig.10) The Analysis of variance clearly indicated that ammonia values are highly significant different between treatments and the periods (Table 8).

Unlike fish effluent, the shrimp effluents showed a different trend in nitrate concentration. The nitrate content declined gradually from 0 – 20 days of treatment. The concentration of nitrate declined to 60 – 68.9 % in the treated tank on 10 DAT and 20 DAT, whereas it was 45.5 – 47 % in the control tank during the same period. The overall difference of nitrate from control to the treated tank was 41.4 % at the end of experiment (Fig.10). The statistical analysis (ANOVA) showed highly significant difference between treatments, periods and between periods and treatment in the experiment (Table 8).

There is a gradual decline of Nitrite concentration both in control and treated tank in the shrimp effluent. The decline was 76-96 % from the initial value to 10 DAT and 20 DAT respectively in the tank treated with *U. reticulata*. In the control tank, the nitrite content declined by 65 % on 10 DAT and 88.4 % on 20 DAT. The overall nitrite concentration in the treated tank showed almost 70 % reduction of nitrite from the control (Fig.10). The Analysis of variance clearly indicated that nitrite values are highly significant different between treatments, between periods and between periods and treatment (Table 8)

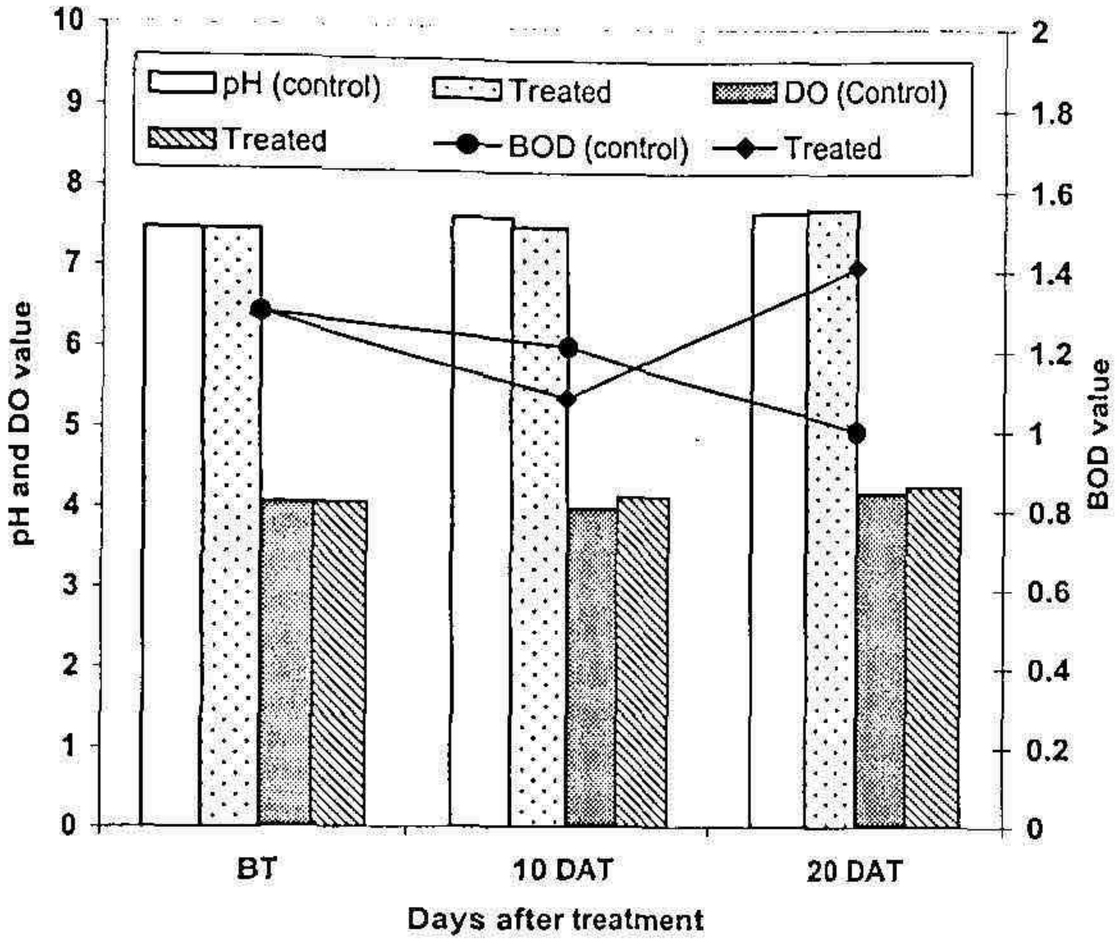

Fig.9. Water quality parameters of shrimp effluent treated with seaweed.

ANOVA pH					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	0.018317	2	0.009158	1.450506	0.306292
PERIOD	0.124567	3	0.041522	6.576331	0.02519
Error	0.037883	6	0.006314		
TOTAL	0.180767	11			
ANOVA D.O					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	0.075117	2	0.037558	4.042152	0.077312
PERIOD	0.205625	3	0.068542	7.376682	0.01945
Error	0.05575	6	0.009292		
TOTAL	0.336492	11			
ANOVA B.O.D					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	0.132817	2	0.066408	0.729072	0.520669
PERIOD	0.449758	3	0.149919	1.645909	0.275958
Error	0.546517	6	0.091086		
TOTAL	1.129092	11			

Table 7. Anova table for the water quality parameters of shrimp effluent treated with seaweed.

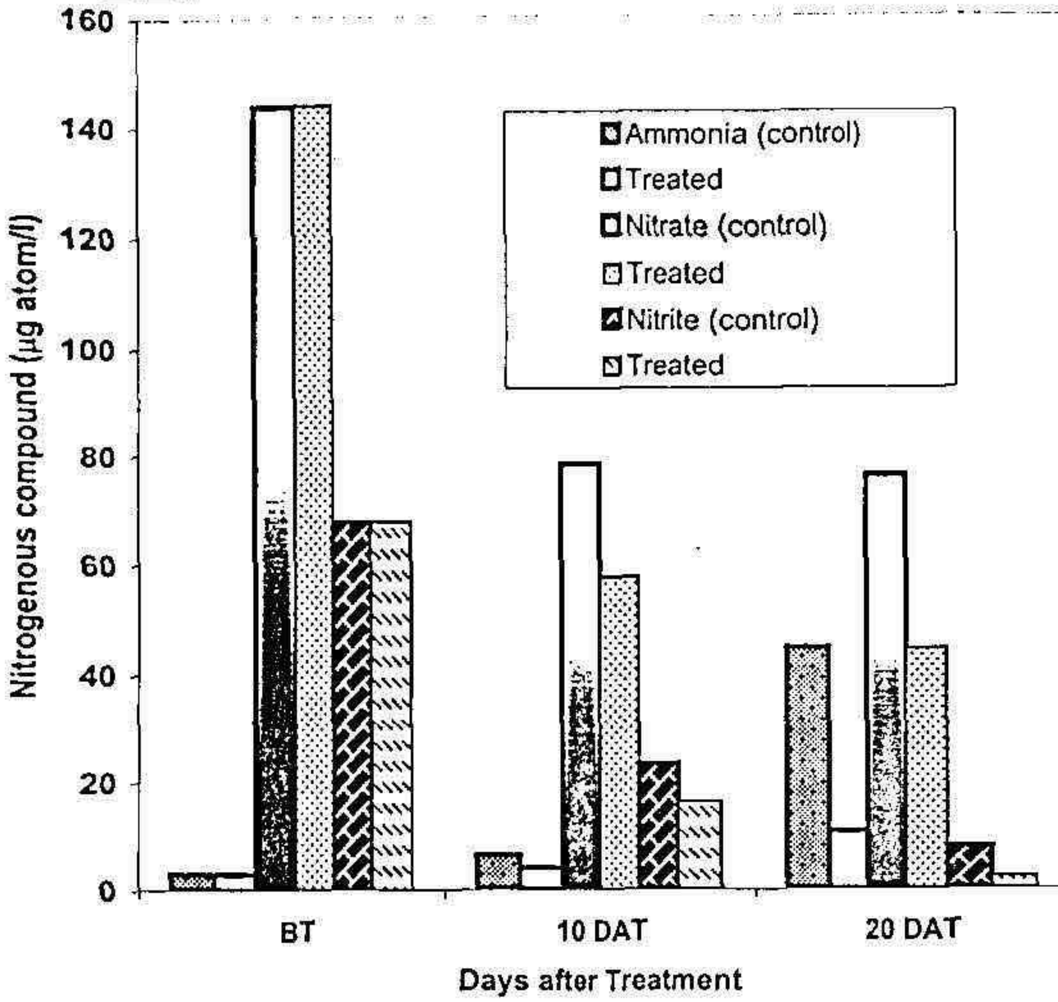

Fig.10. Nitrogenous compound of shrimp effluent treated with seaweed.

ANOVA AMMONIA					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	1601.052	3	533.684	1318.796	0.000
TREATMENT	1984.886	2	992.443	2452.444	0.000
PERIOD*TREATMENT	1863.906	6	310.651	767.656	0.000
Error	4.856	12	0.405		
ANOVA NITRATE					
PERIOD	33624.344	3	11208.115	1632.868	0.000
TREATMENT	1206.356	2	603.178	87.875	0.000
PERIOD*TREATMENT	902.551	6	150.425	21.915	0.000
Error	82.369	12	6.864		
ANOVA NITRITE					
PERIOD	16584.627	3	5528.209	31342.755	0.000
TREATMENT	141.150	2	70.575	400.131	0.000
PERIOD*TREATMENT	55.362	6	9.227	52.313	0.000
Error	2.117	12	0.176		

Table 8. Anova table for the nitrogenous compound of shrimp effluent treated with seaweed.

There was a marked difference in the phosphate concentrations of the control and treated tanks of the shrimp effluent. The phosphate concentration increased marginally by 6.5 % and 8.8% in 10 DAT and 20 DAT in the treated tank, whereas the increase was 30.7 % and 46.6 % in the control tank. At the end of experiment, the phosphate concentration was found to be 16.2 % less in treated tanks from control (Fig.11). Statistical interpretation (ANOVA) showed a significant difference in phosphate concentration between control and treated tanks (Table 9).

The silicate concentration in shrimp effluent treated with *U. reticulata* showed a gradual decline from initial to 20 days of treatment, whereas it was in increasing trend in the control tank. The maximum decline of 15 % was observed in the treated tanks at the end of experiment on the other hand, in the control tank, the silicate content increased by 45 % on 20 DAT (Fig.11). The Analysis of variance showed highly significant difference between treatments, periods and between periods and treatment (Table 9).

Photosynthetic pigments such as chlorophyll a and b were analysed from the seaweed *U. reticulata* during the experimental period. The chlorophyll content was found to increase in the initial 10 days of treatment and then declined after that. The chlorophyll a content was found to be higher than chlorophyll b. The increase in chlorophyll a concentration was 235 % and chlorophyll b was 230 % on 10 DAT. Further, both chlorophyll a and b declined by 20 and 16% respectively on 20 days of treatment (Fig.12)

4.3. Treatment of shrimp with seaweed

In the experiment carried out to find out the stocking density of seaweed and shrimp for the aquaculture management showed the following water quality parameters.

Shrimp effluent was treated with a green seaweed *Ulva reticulata* showed a gradual decline in the pH value both in treated and control tanks. The decline was 3-5 % in both (Fig.13). The Analysis of variance showed no significant difference between treatments, but there was some significant difference between periods (Table 10).

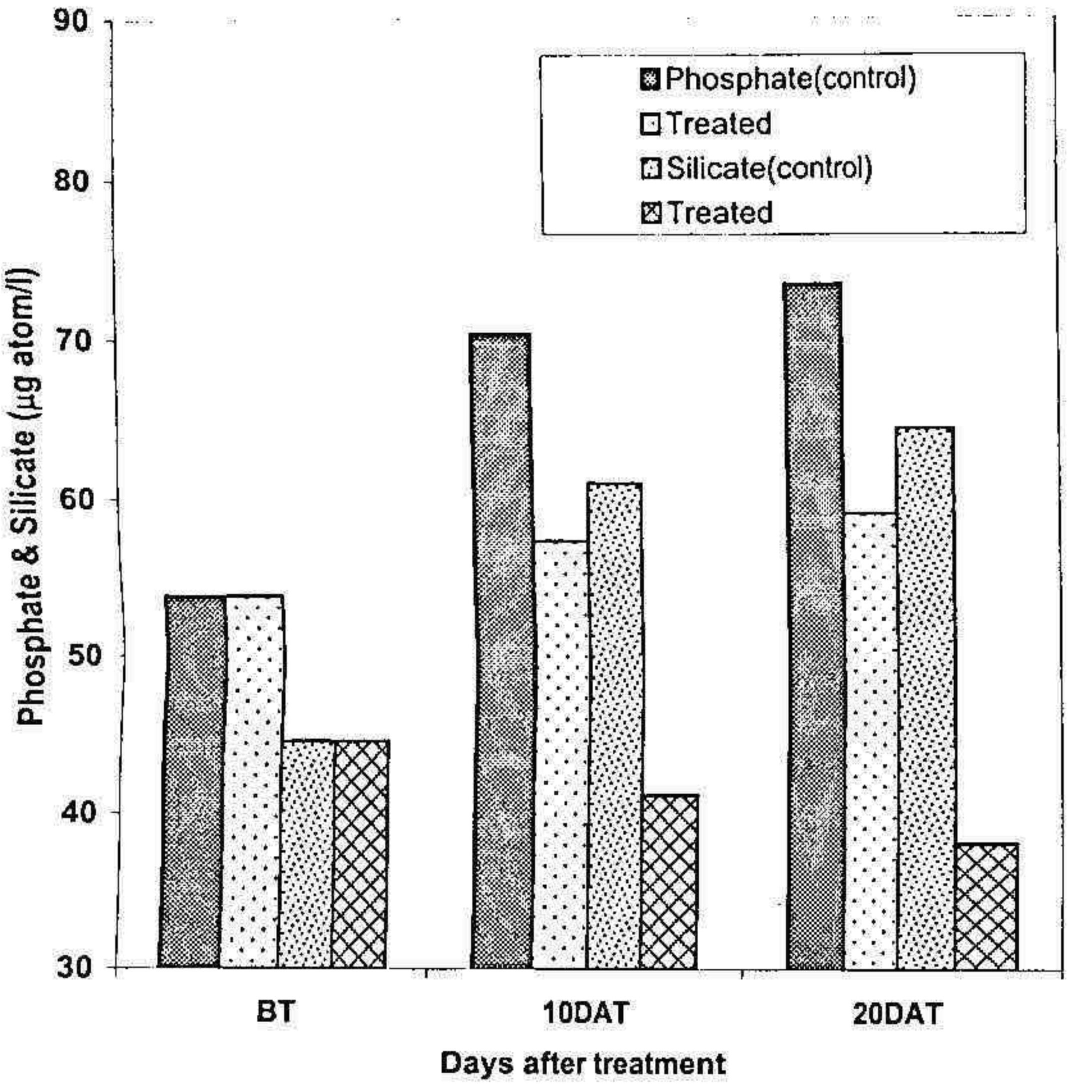

Fig.11. Phosphate and silicate content of shrimp effluent treated with seaweed.

ANOVA PHOSPHATE					
Source	**Sum-of-Squares**	**df**	**Mean-Square**	**F-ratio**	**P**
PERIOD	1054.396	3	351.465	181.626	0.000
TREATMENT	712.356	2	356.178	184.061	0.000
PERIOD*TREATMENT	257.171	6	42.862	22.150	0.000
Error	23.221	12	1.935		
ANOVA SILICATE					
PERIOD	33.807	3	11.269	16.754	0.000
TREATMENT	2804.555	2	1402.277	2804.835	0.000
PERIOD*TREATMENT	1401.819	6	233.637	347.359	0.000
Error	8.071	12	0.673		

Table 9. Anova table for the phosphate and silicate of shrimp effluent treated with seaweed.

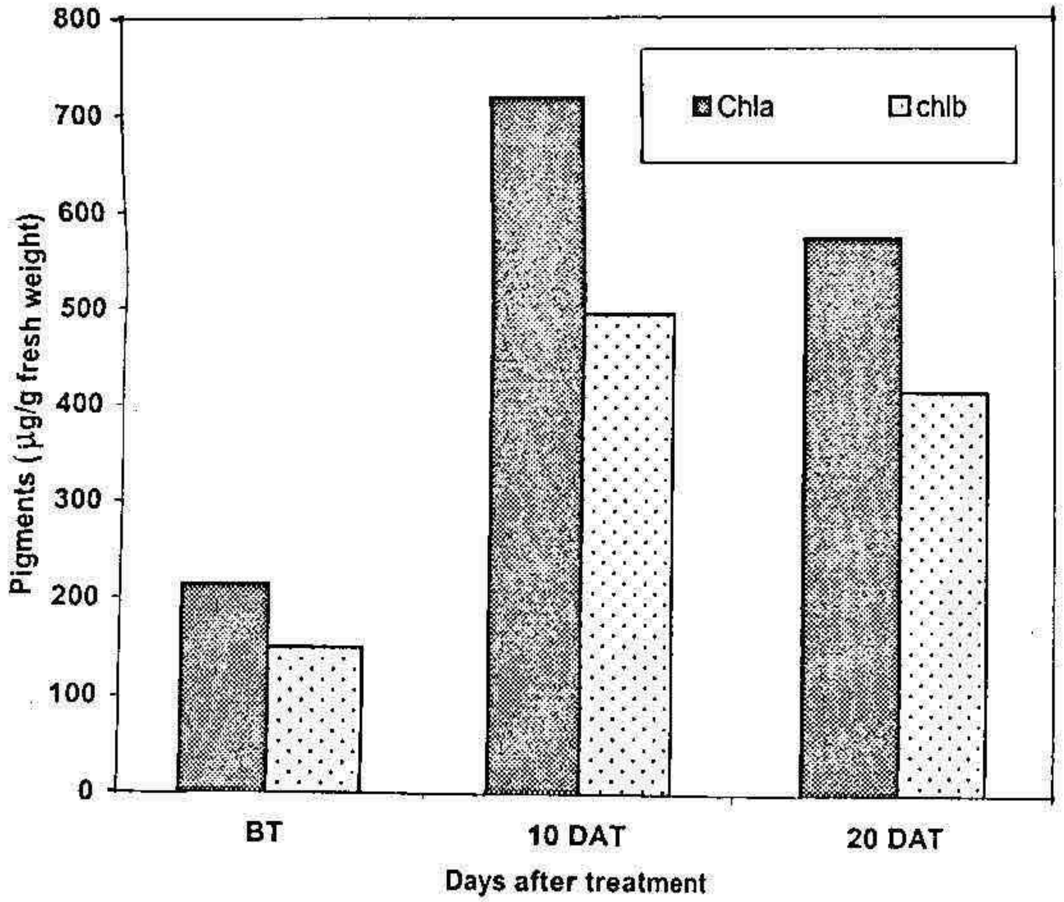

Fig.12. Pigment constituents of *U.reticulata* treated with shrimp effluent.

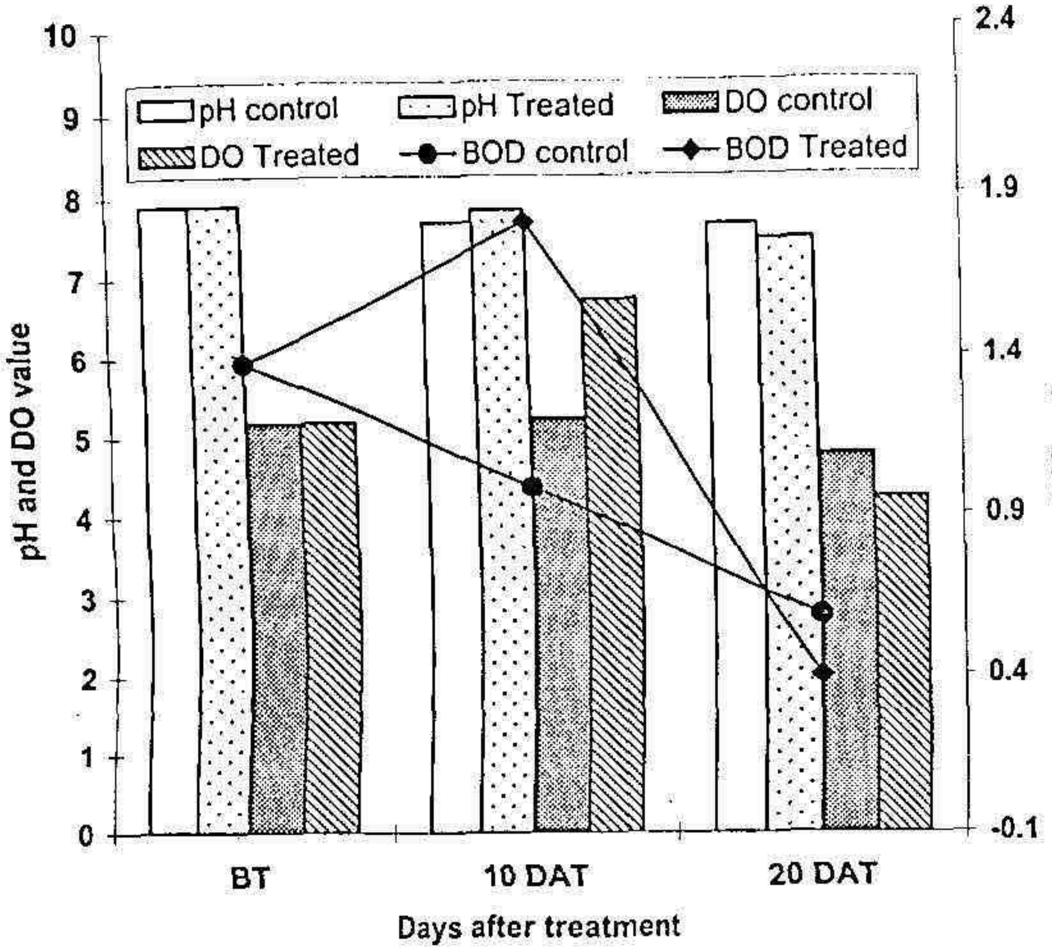

Fig.13. Water quality parameters in the culture system of shrimp and seaweed.

ANOVA pH					
Source	**Sum-of-Squares**	**df**	**Mean-Square**	**F-ratio**	**P**
TREATMENT	0.0098	1	0.0098	0.718826	0.458814
PERIOD	0.15925	3	0.053083	3.893643	0.146824
Error	0.0409	3	0.013633		
TOTAL	0.20995	7			
ANOVA D.O					
Source	**Sum-of-Squares**	**df**	**Mean-Square**	**F-ratio**	**P**
TREATMENT	0.005	1	0.005	0.012127	0.919265
PERIOD	2.6701	3	0.890033	2.158703	0.271818
Error	1.2369	3	0.4123		
TOTAL	3.912	7			
ANOVA B.O.D					
Source	**Sum-of-Squares**	**df**	**Mean-Square**	**F-ratio**	**P**
TREATMENT	0.027613	1	0.027613	0.237127	0.65965
PERIOD	1.576338	3	0.525446	4.512363	0.123784
Error	0.349338	3	0.116446		
TOTAL	1.953288	7			

Table 10. Anova table for the water quality parameters in the culture system of shrimp and seaweed.

Dissolved oxygen values in the shrimp effluent treated with *U. reticulata*, showed an increase in both control and treated tank for the initial 10 days. The increase was more pronounced in treated tank by 29 % but was only 1 % in the control tank. Further the DO content declined in both ranging from 7-18 % in control and treated tanks (Fig.13). The Analysis of variance showed no significant difference between treatments, but significant difference existed between periods (Table 10).

The BOD concentration in the culture tank of shrimp and seaweed showed a gradual decline in control tank from 1.38 to 0.59 ml O_2/l giving an overall decline of BOD by 135 % . In the treated tank of *U. reticulata* the BOD increased from 1.38 to 1.82 ml O_2/l (24%) in the first period of treatment and then declined to 355% in the second period (Fig.13). Analysis of variance clearly indicates that no significant difference exists between treatments and between periods (Table10).

The ammonia concentration in the control tank having 20g body weight of shrimps, showed an increase from 4.93 to 320 μg atom/l within 20 days of culture period. This showed a very high input of toxic material to the system. On the other hand, the tank having shrimp (20g body weight) and seaweed, *U. reticulata* (100g) showed an increase of ammonia concentration from 4.93 to 22.13 μg atom/l, which was very low compared to the control (Fig.14). The Analysis of variance showed very high significant difference in the control and treated tanks (Table 11).

The Nitrate content in the control tank with shrimp alone, showed a drastic increase from initial to 10 days of treatment. Further, there is a marginal increase by 16 %. In the treated tank, the nitrate concentration increased marginally for the initial period of treatment (10 DAT), but during the later stage, it increased to a higher value. The overall nitrate concentration in the tank treated with *U. reticulata* showed 14.4 % reduction from the control at the end of experiment (Fig 14). The statistical interpretation of nitrate by ANOVA showed a significant difference between treated and control tanks (Table 11).

The Nitrite concentration of the water kept for experiment was very low (0.39 μg atom/l). It increased to 47.74 μg atom/l in presence of 20 gram body weight of shrimp. The concentration of Nitrite remained same till the end of experiment.

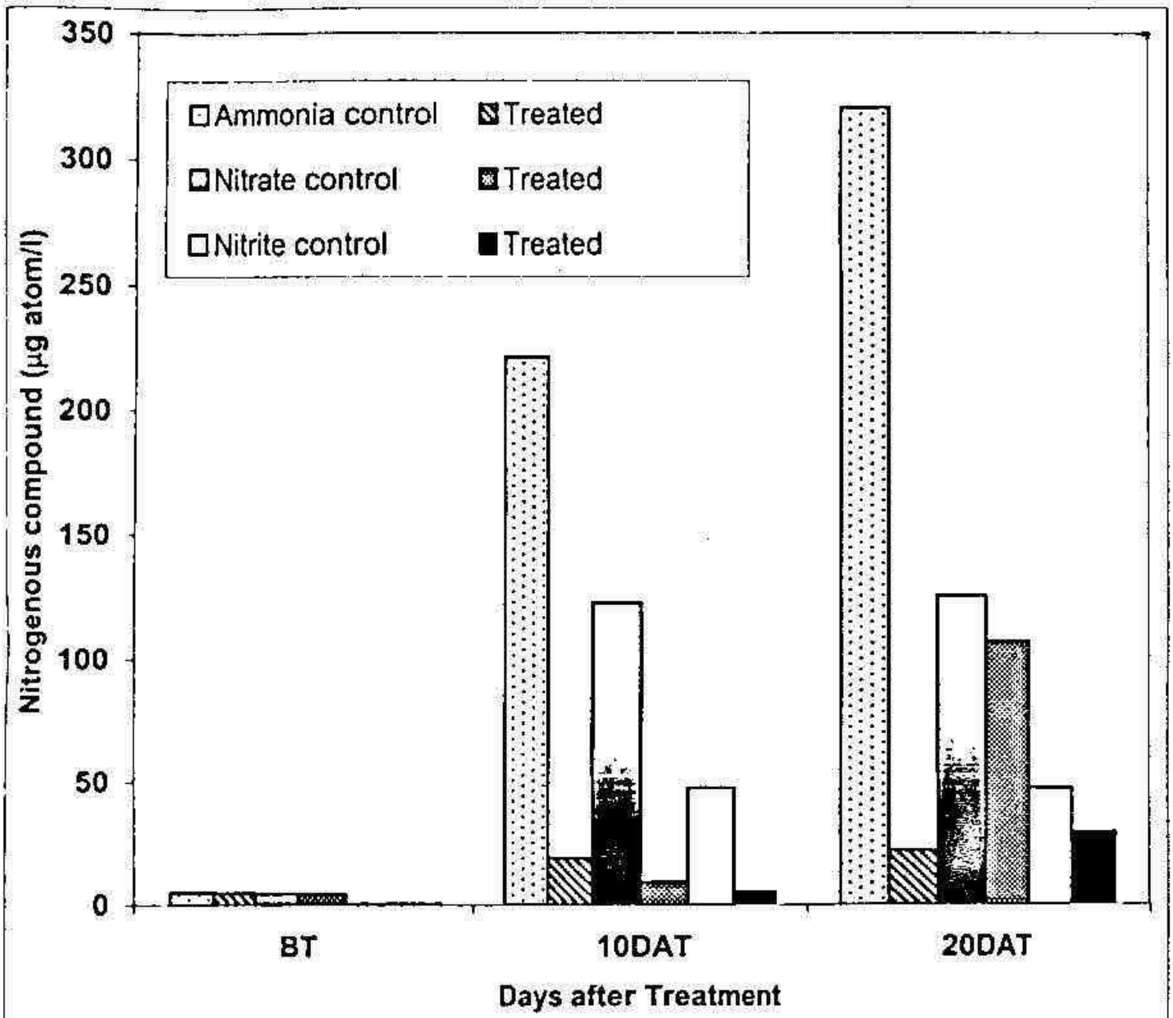

Fig.14. Nitrogenous compound in the culture system of shrimp and seaweed.

ANOVA AMMONIA					
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	63201.933	3	21067.311	6776.956	0.000
TREATMENT	86319.909	1	86319.909	27767.484	0.000
PERIOD*TREATMENT	51349.370	3	17116.457	5506.041	0.000
Error	24.869	8	3.109		
ANOVA NITRATE					
PERIOD	30324.749	3	10108.250	2708.425	0.000
TREATMENT	1141.764	1	1141.764	305.927	0.000
PERIOD*TREATMENT	12955.780	3	4318.593	1157.133	0.000
Error	29.857	8	3.732		
ANOVA NITRITE					
PERIOD	3402.099	3	1134.033	3570.563	0.000
TREATMENT	0.806	1	0.806	2.536	0.150
PERIOD*TREATMENT	2811.805	3	937.268	2951.039	0.000
Error	2.541	8	0.318		

Table 11. Anova table for the nitrogenous compound in the culture system of shrimp and seaweed.

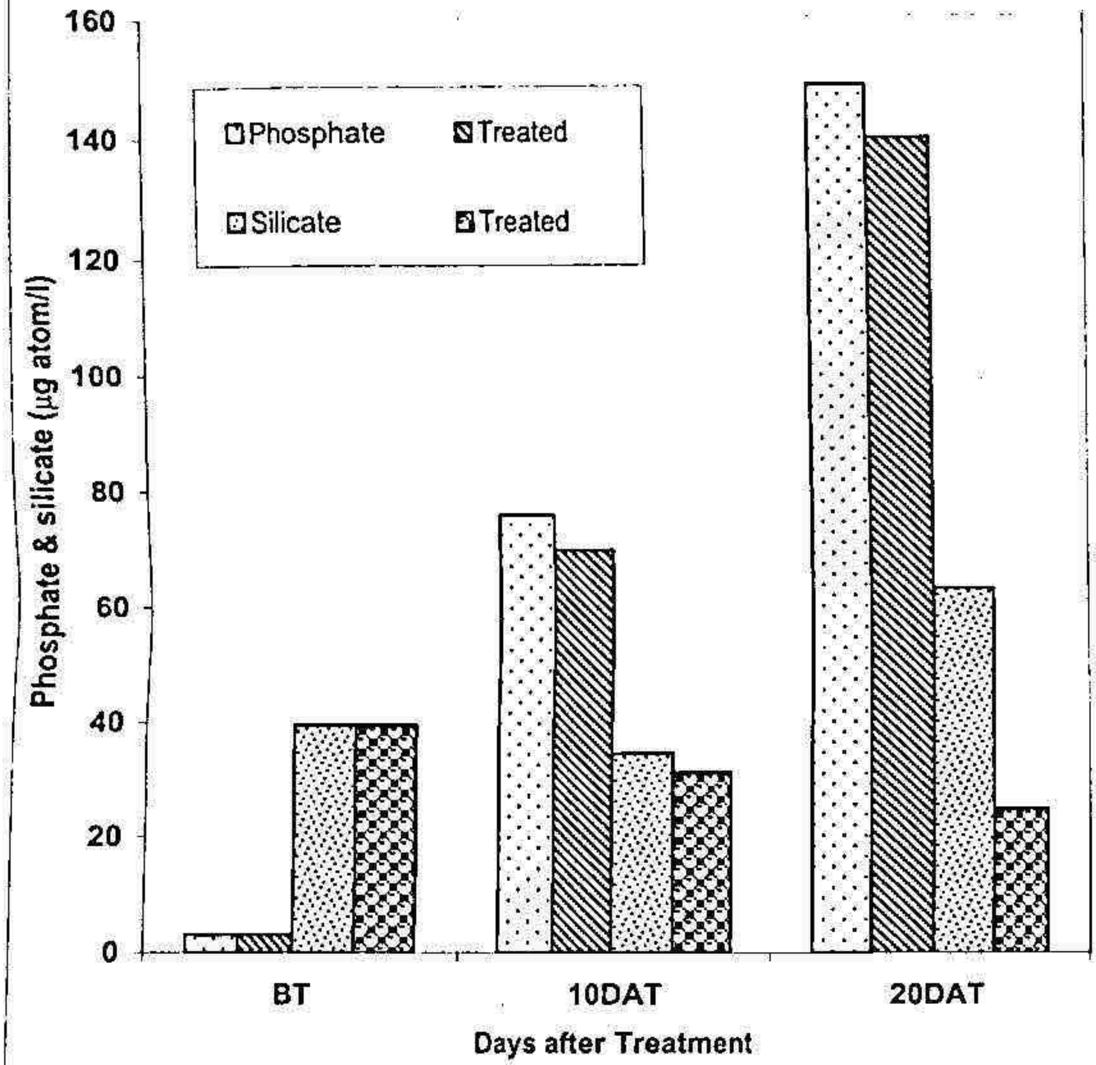

Fig.15. Phosphate and silicate content in the culture system of shrimp and seaweed.

TABLE-17	ANOVA PHOSPHATE				
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PERIOD	58611.749	3	19537.250	27722.733	0.000
TREATMENT	41.538	1	41.538	58.941	0.000
PERIOD*TREATMENT	844.994	3	281.665	399.673	0.000
Error	5.638	8	0.705		
TABLE-18	ANOVA SILICATE				
PERIOD	248.921	3	82.974	70.993	0.000
TREATMENT	1573.511	1	1573.511	1346.312	0.000
PERIOD*TREATMENT	1328.791	3	442.930	378.976	0.000
Error	9.530	8	1.169		

Table 12. Anova table for the phosphate and silicate in the culture system of shrimp and seaweed

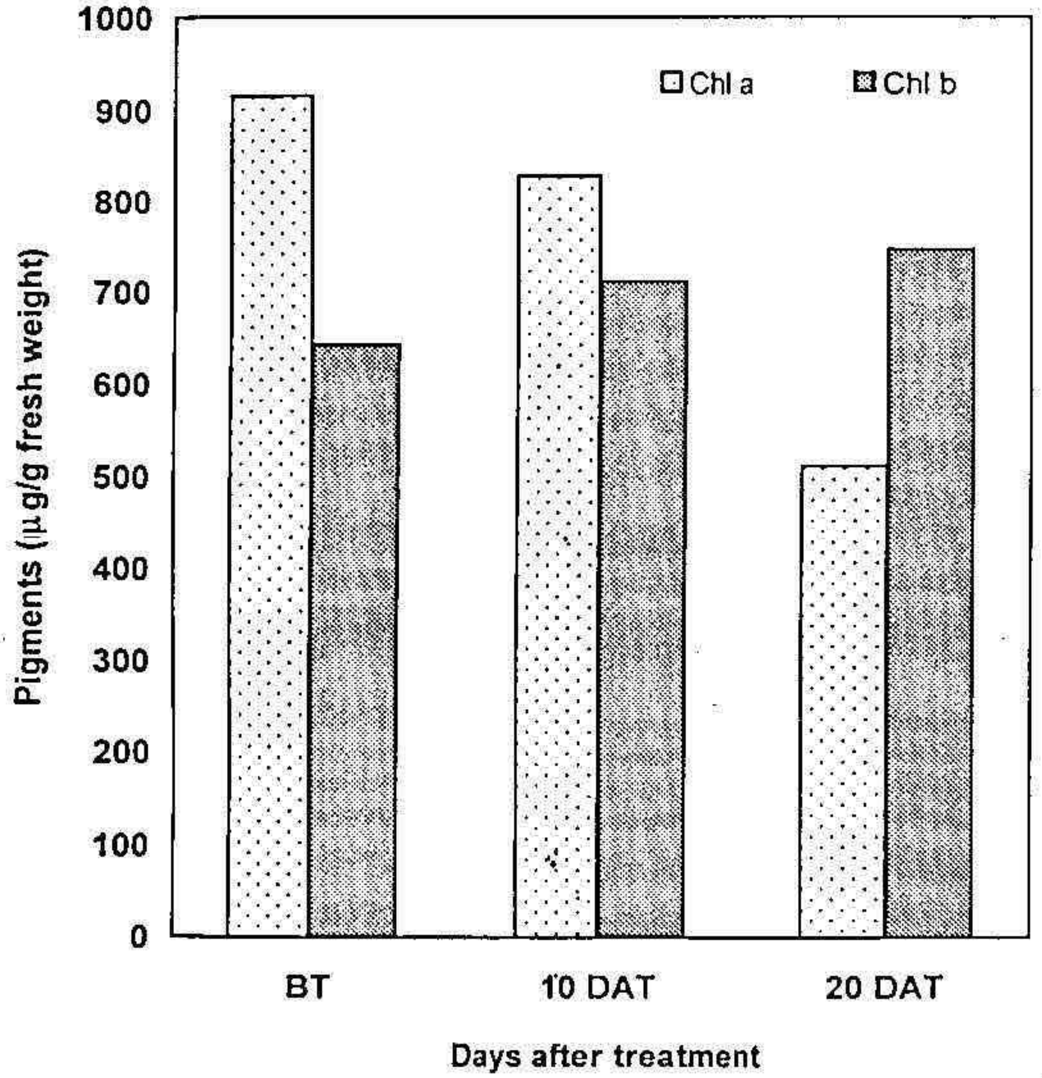

Fig.16. Pigment constituents of *U.reticulata* in the culture tank of shrimp and seaweed

In the tank treated with seaweed, the nitrite concentration was found to be very low, i.e. 89.5% less than that of control on 10 DAT. Further, the nitrite increased from 5 to 29 µg atom/l from 10 DAT to 20 DAT. The overall nitrite concentration was found to 37.7 % less compared to the control at the end of experiment (Fig.14). The Analysis of variance showed highly significant variation period of treatment, but no significance was observed among the treatment (Table 11).

The phosphate concentration increased to a greater extent on 10 DAT and 20 DAT respectively in both control and treated tanks. The difference in phosphate was found to be 6 – 8 % from the control in different days of treatment (Fig.15). The statistical interpretation (ANOVA) showed highly significant difference between period and treatment (Table 12).

The silicate concentration showed a gradual decline in the tank treated with *U. reticulata* where as in the control tank, the silicate declined by 12.8 % at 10 DAT and then increased by 85 % in 20 DAT. In the treated tanks, the decline was 20.5 % and 36 % at 10 DAT and 20 DAT respectively. The overall silicate content in the treated tank was found to be 62 % less than the control at the end of experiment (Fig.15). Significant difference was found in the silicate content between control and treated tanks (Table 12).

The pigment concentration of *U. reticulata* cultured along with shrimps was found to be comparatively higher than the experiment conducted on *U. reticulata* in shrimp effluent. The chlorophyll a declined gradually from 9.5 – 44 % at the end of experiment, whereas chlorophyll b showed a reverse trend. It increased from 10.7 – 13.9 % at 20 DAT (Fig.16).

DISCUSSION

5.DISCUSSION

The concept of ecological sustainability in aquaculture refers to the maximization of internal feed back (recycling) within the culture system which minimizes the wasted output of resource, such as nutrient, water and energy in the effluent water. Macro algae can aid considerably in establishing and stabilizing the system, helping to institute the micro organism community removing toxic wastes such as ammonia, nitrates and nitrites, assisting in buffering the pH, uptake of carbon dioxide, supply of oxygen and balancing the trace elements.

In the present experiment, the water quality parameters such as pH, BOD and dissolved oxygen were found to be in optimal level for both fish and shrimp *experimental set up. The pH value in fish effluent did not show wide variation* in the control and treated tank, but there is a marginal decline of pH in the treated tanks. *G.corticata* showed a decline of pH by 22.5% which may account for the efficient *utilization* of dissolved carbon in the effluent. *Earlier report stated that G.corticata* showed high photosynthetic activity in the initial period, when kept under laboratory condition (Reeta and Kulandaivelu, 2000).

The dissolved oxygen in the treated tank of fish effluent by *G.corticata* increased to a value of 5.45 ml O_2/l confirms the high photosynthetic activity of the plant. The higher carbon dioxide demand for *Gracilaria,* was confirmed by Bidwell et al (1985). On the other hand, the effluent treated with *Ulva* did not show much changes in the DO content. The increase in DO content in the control tank of fish and shrimp may account for the phytoplankton bloom in the enriched effluent when they were supplied with optimal light and air. The control and management of DO, suspended solids and algal densities in water column are vital to the proper management of aquaculture system.

The Biochemical oxygen demand in the fish effluent was found to be comparatively lower than shrimp effluent. The high concentration of BOD in the treated tank of fish effluent by *G.corticata* may account for the encouraging bacterial *growth with a high DO content.* Being an agarophyte, *Gracilaria corticata* is considered as a very good substrate for bacterial communities. In the shrimp effluent, the requirement of BOD was found to be reduced in the treated tanks on 10 DAT, but then increased by 30.5%. This may be accounted for the heterotrophic bacteria present in the effluent and also adhered to the thallus of the seaweed. It was also understood that the effluents are kept in a tank without any additional input of carbon, leading to carbon limitation and thereby reducing dissolved oxygen content. In the culture system biochemical oxygen demand declined gradually in the control tank and in the treated tank after a marginal increase in 10 DAT. The highly enriched water of the culture system might have helped in blooming of phytoplankton and diatoms.

Ammonia, a major metabolite, in a higher concentration was found to be toxic to the aquaculture system. The ammonia concentration in fish effluent reduced when treated with *G.corticata* and *U.lactuca*. The increase in dissolved oxygen content in the effluent might have helped the aerobic bacteria to reduced ammonia to nitrate. In the shrimp effluent, there is a gradual increase of ammonia in the control tank, which increased from 2 to 44 µg atom/l, whereas in the treated tank, it was from 2 to 10µg atom/l showing a reduction of 75% from the control tank during the same period. This result indicated that seaweed has high efficiency to remove toxic ammonia from the system. It is necessary to mention here that the shrimp effluent collected from the pond at Valappu, has got a continuous exchange of water to the natural system, unlike the fish effluent. Due to this, many living organisms still exist in the effluent, which released ammonia to the system. In the polyculture system of shrimp and seaweed, the ammonia concentration increased from 4 to 320µg atom/l in the control tank, whereas 4 to 22µg atom/l in the treated tank. The ammonia concentration in the control tank may lead to toxicity of the system. Nutrients have been efficiently removed from mariculture effluents by seaweed biofilters was observed by Ryther *et al.* (1975); Gordin, (1990); Cohen and Neori.,(1991) and Buschman *et al*, (1994).

Integrated aquaculture improves water quality of the system by removing the toxic waste. Seaweeds in an aquaculture system allows the management of eutrophication problems associated with the present fish mono-aquaculture and coastal agriculture or urban or industrial processes.

Nitrate concentration in the fish and shrimp effluent increased gradually in control as well as treated tank, but in the treated tank, the concentration of nitrate was found to be lower than control. In fish effluent *U.lactuca* was found to be a better nitrate remover from the system than *G.corticata*. The increase in nitrate content in the system is due to the reduction of ammonia to nitrate and nitrite and seaweeds could effectively utilize this nitrogen resource from the system. The high value of nitrate in the 10 DAT both in fish and shrimp effluent may account for the reduction of nitrate uptake inhibited by presence of ammonia. This results are in conformity with Harlin *et al*, (1997). The nitrate content in the culture system of shrimp and seaweed showed an increase in nitrate content from 4 to 123 µg atom/l. In the initial period of treatment, whereas it was 4-9 µg atom/l in the treated tank. But in the later part of treatment, the nitrate concentration increased even in the treated tank. This showed that *U.reticulata* was not very effective in utilizing the inorganic nitrogen after 10 days of treatment.

The nitrite content was found to be efficiently reduced in the treated tank of fish effluent when treated with *Ulva lactuca*, compared to *G.corticata*. This work is in conformity with Neori *et al* (1998) and Harlin (1997). The nitrite concentration reduced gradually in the shrimp effluent both in control and treated tanks. But the decline was more pronounced in the tank treated with *U.reticulata*. The reduction in nitrate in control tank may be due to the growth of microalgae in the system. In the closed polyculture system of shrimp and seaweed, the nitrate concentration was found to be less in the treated tank, showed that seaweeds helps in removing the nitrogeneous toxic material from the system.

The removal of phosphate either from the effluent or from the aquaculture system by seaweeds was found to be quite low. This results were in conformity obtained from seaweed biofilters (De Boer *et al*, 1978; Neori, 1996). It was also stated that macroalgae grown in artificially enriched media are typically supplied with nitrogen and phosphorus at a molar ratio of 10-13:1, but in effluents even though the ratio increases to 16:1, the efficiency of removal of phosphorus is low (Vandermeulen & Gordin, 1990; Frielander *et al.*, 1991; Ugarte and Santelices 1992; Israel *et al.*, 1995).

The silicate content in the fish effluent showed an increase in the control tank from 20 to 30 µg atom/l whereas in the treated tank, the increase is from 2 to 23 µg atom/l. The increase in silicate content in control tank may account for the bloom of diatoms in the enriched effluent, whereas in the treated tanks, the nutrient absorbed by the seaweed might not have encouraged the growth of diatoms. In shrimp effluent, the silica content increased in the control tank, whereas declined to a marginal extent in the treated tank. Similar observation was also made when seaweeds were cultured along with shrimp. There is no concrete evidence on the removal of silicate from the shrimp effluent by seaweeds, but the present experiment confirms that, seaweeds particularly *U.reticulata* showed some influence on the silicate content of the water. Reports have also confirmed that the major determinant of pond water quality was the diatom dominated algal bloom, which may also correspond to the change in silicate content (Wyban *et al.*, 1988).

The photosynthetic pigment of *Gracilaria* in fish effluent showed high value of accessory pigment than chlorophyll. On 10^{th} DAT, the accessory pigment declined to a marked extent which coincide with the high photosynthetic efficiency or carbon assimilation. This results was also observed by Brian and Clifford (1984). He explained that when the nitrate uptake is higher compared to carbon fixation, the C:N ratio reduce and this increases the phycoerythrin content. The second period of treatment, the accessory pigment increased may be to overcome the stress of carbon limitation and optimizing the photosynthetic activity. The pigment constituent of *U.lactuca* showed an increase in chlorophyll a and b concentration on 10 DAT and then declined marginally on 20 DAT. Chlorophyll a was found to be higher than chlorophyll b. This increase in chlorophyll content accounts for the increase in pigment content and the photosynthetic activity in nutrient rich water. This results confirms the work carried out in the coastal water with more run off from the land enriched with nitrogen and phosphorus (Nakahara, H. 1978; Wallentinus, 1988; Zavodinik, 1987).

Similar observation was found in *Ulva reticulata* in shrimp effluent. The polyculture of shrimp and seaweed showed a very high level of chlorophyll content all through the culture period.

SUMMARY

SUMMARY

Marine macroalgae (seaweeds) are widely distributed around the world in a variety of habitats and have attracted the attention world over due to their potential as a source of food to fish, cattle and man, as a source of medicine and fertilizer and as a waste water purifier.

Algae play a key ecological role, because by photosynthesis, they introduce to the nutrient loaded water new energy in the form of organic carbon. The water quality management of the aquaculture system can be done by two methods, either by polyculture or by a recycling system where the effluents can be treated by seaweeds.

The efficiency of macroalgae to remove toxic metabolites in the aquaculture system has been attempted in the present study. Fish and shrimp effluents were treated by seaweeds like *G.corticata, U.lactuca* and *U.reticulata* respectively.

Water quality parameters such as pH, BOD and DO, nutrient parameters such as ammonia, nitrate, nitrite, phosphate and silicate and pigment characterization such as chlorophyll, phycoerythrin, phycocyanin and allophycocyanin were monitored at regular intervals of treatment from both control and treated tanks.

The pH values did not show wide variations in different treatments. The change in pH value of fish effluent treated with *G. corticata* corresponding to the increase in dissolved oxygen explained the efficient utilization of dissolved carbon in the system.

The Biochemical Oxygen Demand in fish effluent was observed to be comparatively lower than shrimp effluent. The possibilities of other microorganisms in the shrimp effluent collected afresh from the pond might have helped in increase of BOD content.

Nitrogenous compounds such as ammonia, nitrate and nitrite, which were found to be in a higher concentration was found to be effectively removed from the effluents to the cultivable limit by different seaweeds.

In the polyculture system, both the commodities should generate a good revenue for the aquaculture industry. Seaweeds have been shown to reduce efficiently the level of eutrophication in the integrated system. Thus seaweeds of economical importance can be used as a nutrient trap in the aquaculture system to improve water quality and also a good yield of the by product.

Although species of *Ulva* is an ubiquitous chlorophyte showing high efficiency in water quality management of fish and shrimp effluent, the biomass produced from the seaweed has got less importance. Thus, valuable Rhodophytes especially different species of *Gracilaria* can be a better substitute for water quality management in fish and shrimp effluent treatment.

In the polyculture system, atmost care should be given in the stocking density of marine organisms with seaweeds. Excess seaweed may lead to anoxia of the aquaculture system especially during night, when the respiration rate increases. So, mechanical aeration need to be provided to oxygenate the water. Effective and periodic harvest of seaweeds is important for balancing the stocking density and to maintain the water quality parameters in the system.

It can be concluded that locally available and commercially important macroalgae can be applied to the mariculture activities for water quality management and also to generate income both from fish or shrimp and seaweed. The scientific input from this study will give a new dimension in water quality management to the aquaculture industry.

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