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Utilisation of nitrate and ammonium by algal biomass available in prawn cultivation sites in Chilika Lake, Orissa

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Abstract- Water quality and seaweed flora were studied during prawn farming activities at various sites of Chilika Lake. The brackish water lagoon has become eutrophicated as it receives the waste material of agricultural, aqua cultural and domestic sewage as well as the release of extracellular nitrogenous substances released from the fishing activities. Conservation and proper management of Chilika ecosystem is essential not only for preserving biodiversity but also to save and promote the existence of economically important seaweeds. Four seaweeds *Enteromorpha intestinalis, Chondrous crispus, Gracilaria verrucosa* and *Polysiphonia sertularioides* were examined for their efficiency in uptake of nitrate and ammonium to access the potentiality of these algae for removal of nutrients from aqua culture effluents. *Enteromorpha intestinalis* and *Gracilaria verrucosa* removed nitrate from the medium at considerable higher than those measured for *Chondrous crispus* and *Polysiphonia sertularioides*. At similar temperature and irradiance the Vmax and Ks of nitrate uptake *in E. intestinalis and G. verrucosa* are thrice that of *Chondrous crispus*. The result are discussed in designing protocols for management of water quality in fishing zones for removal of nitrate and ammonia from the fish culture sites in Chilika lake.
Keywords- *Enteromorpha intestinalis*, *Chondrous crispus*, *Gracilaria verrucosa*, *Polysiphonia sertularioides*

Introduction

The intensification of fish culture with fish stocking and feeding rates can lead to severe water quality problems. Due to large scale aquaculture activities and intensive shrimp farming in Chilika lake, situated in the extreme south-east corner of Orissa, it has become highly eutrophic during last several years. Although fish consume most of the feed applied, a fish percentage of the dietary intake is excreted into the water as metabolic waste which includes mostly ammonia and phosphorus. In the marine environment NO3- and NH4+ are the two major nitrogen sources available for assimilation by macro algae. The abundance and rate of utilization of nitrate and ammonia by algae differ markedly. Studies on nitrogen uptake and assimilation by seaweeds have received attention [21, 22, 15, 16, 4, and 6]. Nitrogen uptake ability is critical in the utilization of available nitrogen by the algae. The two major sources of nitrogen available to macro algae, NO3- and NH4+ differ markedly in their abundance rates of utilization by these algae [2]. Nitrate is usually the most abundant form of inorganic nitrogen in sea water, but many seaweed species show uptake preference and grow differently as ammonium or nitrogen. Ammonium is thought to be a more important N-source due to the capability of algal species to take up NH4+ more rapidly than NO3- [1, 5, 23, and 20]. In Chilika Lake, at the prawn cultivation sites algae with marine red algae *Gracilaria verrucosa*, *Polysiphonia sertularioides* and *Chondrous crispus*, association members of Ulvales, *Enteromorpha intestinalis* in particular is a nuisance as they have a theoretical potential to compete successfully with the valuable seaweeds mostly for light and costly nutrient. The prawn farming sites in chilika lake are associated with four seaweeds namely three red algal seaweeds *Gracilaria verrucosa ,Polysiphonia sertularioides ,Chondrous crispus* and one green marine macro algae *Enteromorpha intestinalis* ,which remain densely attached to the nets, bamboo poles used in prawn cultivation. Macro algae species such as Ulva, Porphyra and Gracilaria have been proven to effectively reduce the nutrient load in effluents and assist in maintaining water quality at acceptable levels [13]. The discharge waste water from intensive fish culture may cause environmental concerns. The effluents, which consist of excess feeds and excretory products, can promote eutrophication and result in harmful algal blooms and ammonia conditions [25]. In order to mitigate the environmental impact due to effluent discharge and maintain sustainable prawn farming, various methods have been proposed to address the issue of nutrients discharged from intensive prawn aquaculture [13]. The objectives of the study was to measure the growth of *Enteromorpha intestinalis, Gracilaria verrucosa, Polysiphonia sertularioides* and *Chondrous crispus* in different NO3- and NH4+ concentrations, their capability to uptake NO3- and NH4+ under the influence of light, temperature and substrate concentrations. Irradiance and uptake of inorganic nitrogen are interrelated [12, 19, and 24]. It influences nitrogen uptake and assimilation by providing energy and hydrogen donors either directly through photosynthesis [7] or indirectly through respiration [3].

MATERIAL AND METHODS

Four macro algae namely *Enteromorpha intestinalis* (L) Greville, *Gracilaria verrucosa*(Hudson) Papenfuss, *Polysiphonia sertularioides* (Grat). J.Ag and *Chondrous crispus* were used as the experimental algae to study nitrate and ammonium uptake under controlled conditions. The macro algae were collected from Kalijai area of Chilika Lake and thoroughly washed in Chilika water and packed in polythene jar of two liter capacity. The jars were kept inside a thermo cool box containing ice and were taken to the laboratory. The algal samples brought to the laboratory were preconditioned for 2 days In culture vessel containing sterilized Chilika water with a photon irradiance of 40 µE m-2 s- at 16:8 Light: Dark(L:D) cycle. After the precondition period algal samples were transferred to vessels with culture medium for further studies and growth, nitrate and ammonium uptake under different substrate concentration, temperature and light incubation. Modified Erdschreiber medium [18] was used as the culture medium for the growth of experimental algae. Nitrate uptake by the experimental algae was determined by estimating the different levels of nitrate in the culture used per unit time after inoculation with the experimental algae. The medium was inoculated with 800 gm of algae and allowed to grow under laboratory conditions. At regular intervals the algal samples were harvested and the growth in the culture was determined as increase in fresh weights. From fresh weight measurement daily growth rates (r) of the algae were calculated by using the following formula [8].

r= [(Nt / No) 1/t-1].100%

Where ‘ r ’stands for Daily growth rate, No for the initial biomass and Nt for the biomass at day t.

Ammonium chloride (Analar Grade) at desired concentration was used as the source of NH4-N in these studies. Differences in the levels of NH4-N in the medium inoculated with the algae were calculated after specific periods of growth to determine the uptake rate. Ammonia in the media was estimated by phenol hydro chlorine method [17]. The kinetic constants were derived from the Line weaver-Burk plot [9].

RESULT

Fig. (1) Depicted that the daily growth rate of the studies of organism was not affected at 6µ mole/L of ammonium concentration. Subsequent increases in concentration of NH4-N from 6µ moles/L onwards, the daily growth rate declined. The fig. (2) .shows the daily growth rate of E. intestinalis, G. verrucosa, P. sertularioides and C. crispus at different concentration of NO3-N (1.5 to 18 µ moles/L).All the employed concentration enhanced the daily growth rate of G. verrucosa, P. sertularioides and C. crispus. In case of E. intestinalis being maximum in comparison to G. verrucosa and P. sertularioides. However the daily growth rate of C. crispus is maximum at 6m moles/L of nitrate and then declined slightly at higher concentration. The nitrate uptake of NO3-N in the presence of different concentration of NH4-N in laboratory experiment, NO3-N was provided at a concentration of 20 µ moles/L while the levels of NH4-N varied from 1.5 to 20 µ moles/L Fig. (3). The results shows that in the presence of NH4-N level at 1.5 µ mole/L NO3-N uptake by all the experimental algae was not affected. The uptake of NO3-N was maximum in *Enteromorpha intestinalis* however increasing the concentration of NH4-N in the medium from 6 µ mole/L resulted in drastic decrease in the rate of NO3-N uptake by the entire experimental algal organism. However at 20 µ moles/L of NH4-N a short recovery in the NO3-N uptake was obtained. The effect of NO3-N concentrations from 1.5 to 18 µ moles/L on nitrate uptake of *E. intestinalis, G. verrucosa, P. sertularioides* and *C. crispus* was shown in Fig. (4) and Table 1. The result shows that nitrate uptake increased with increasing substrate concentration. Maximum uptake of NO3-N was obtained in *Enteromorpha intestinalis*. At 25 c and 1800 µwcm-2, the value of Ks was calculated as 16.91 with a Vmax of 137 µ mole h-1 g-1 dry weight for *Enteromorpha intestinalis*. The calculated value of Ks 16.47, 9.75 and 17.01 with a Vmax of 117, 81 and 42 µ mol h-1 g-1 dry wt. for*, G. verrucosa, P. sertularioides* and *C. crispus* respectively was shows in table-1. The effect of NH4+-N concentrations from 1.5 to 18 µ moles/L on ammonium uptake by *E. intestinalis, G. verrucosa, P. sertularioides* and *C. crispus* was shown in Fig. (5) and Table 2. Results of uptake experiments with N-starved E. intestinalis, G. verrucosa, P. sertularioides and C. crispus shows that the higher the concentration of NH4-N , the more rapid is the uptake rate up to 18 µ mole/L. Maximum rate of uptake being observed with E. intestinalis and minimum uptake with *C. crispus*.

DISCUSSION

Macro algal productivity in marine ecosystem is often limited by the availability of nitrogen. These algal communities contribute significantly to primary production in both coastal and estuarine ecosystems; hence it is expected to play an important role in energy transformation and nutrient recycling in this system [11]. In the present investigation the kinetics of nitrogen uptake by the experimental algae exhibited a specific pattern of N-uptake in culture irrespective of available N-source. When NH4-N was the nitrogen source, a slight increase was observed in the uptake rate as against NO3-N. *Enteromorpha intestinalis* absorbed more NH4-N than, *Gracilaria verrucosa* and *Polysiphonia sertularioides* [14]. Studies on biomass production in relation to nitrogen nutrition in the experiment revealed a linear relationship between biomass production and nitrogen uptake irrespective of the N- source in the medium. The positive correlation between the growth and nitrogen uptake is an indication of the limiting nature of nitrogen for the studied algae. Under similar experimental condition *Enteromorpha intestinalis* always showed higher biomass production and higher NO3--N and NH4+-N uptake rates. Based on nitrogen up take, the studied algal organisms would be suitable as candidates for integrated prawn or fish farming. The uptake of nitrate by all the species of studied organisms on the other hand increase with nitrate concentration. The result is interesting in that, the organism can also obtained ammonium which is considered metabolically more energy efficient than the utilization of nitrate because ammonium can be directly incorporated in to amino acids [10]. This is the first in study of NO3--N and NSH4+-N uptake rates of four common marine macro algae that became abundant in the prawn farming sites of Chilika Lake. Above all *Enteromorpha intestinalis* would be the most efficient algal organism for integrated prawn or fish farming basing on the enhanced biomass and NO3--N and NH4+-N uptake rates in comparison to *Gracilaria verrucosa*, *Polysiphonia sertularioides* and Chondrour crispus.

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Table 1- Maximum nitrate uptake rates by Enteromorpha intestinalis, Gracilaria verrucosa, Polysiphonia sertularioides, Chondrous crispus at 25ºc and 1800µW. cm-2

Macroalgal Organism	**V_{max} (µ.mol.g.dw^{-1}.h^{-1})/NO_3^-**	**K_s**
Enteromorpha intestinalis	137± 1.85	16.91
Gracilaria verrucosa	117±2.08	16.47
Polysiphonia sertularioides	81±1.01	9.75
Chondrous crispus	42±2.32	17.01

Table 2- Maximum ammonium uptake rates by Enteromorpha intestinalis, Gracilaria verrucosa, Polysiphonia sertularioides, Chondrous crispus at 25ºc and 1800µW. cm-2

MACROALGAL ORGANISM	**V_{max} (µ.mol.g.dw^{-1}.h^{-1})/NO_3^-**	**K_s**
Enteromorpha intestinalis	172±2.15	4.83
Gracilaria verrucosa	180±2.68	4.2
Polysiphonia sertularioides	134±2.85	3.35
Chondrous crispus	112±2.09	3.11

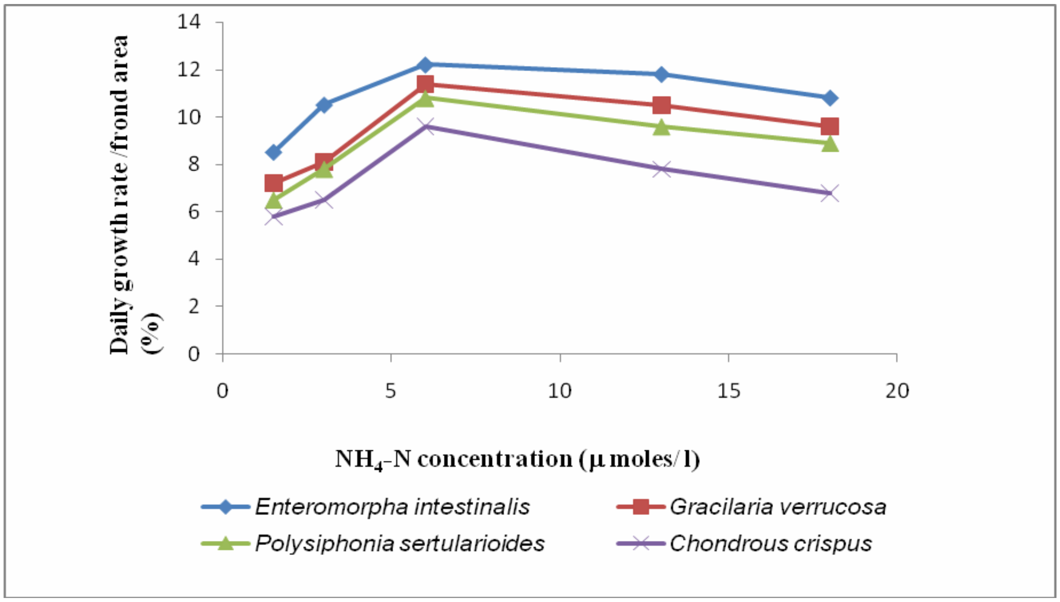

Fig.1-Daily growth rate of *Enteromorpha intestinalis*, *Chondrous crispus*, *Gracilaria verrucosa* and *Polysiphonia sertularioides*.NH4-Nconcentration (m moles/L)

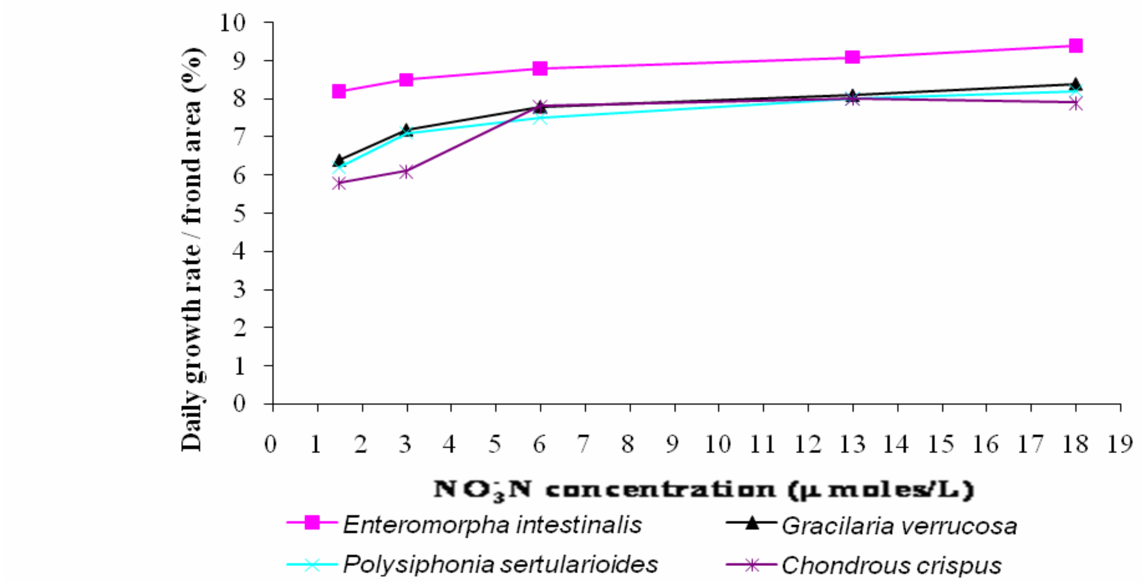

Fig.2-Daily growth rate of *Enteromorpha intestinalis*, *Chondrous crispus*, *Gracilaria verrucosa* and *Polysiphonia sertularioides*.

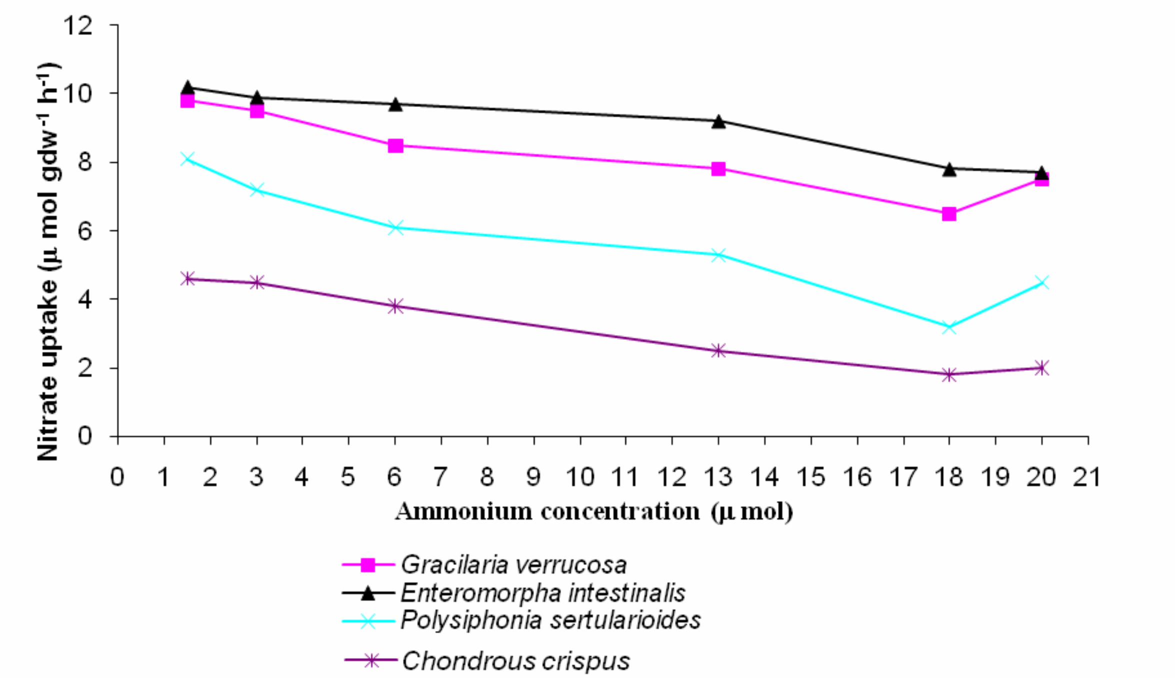

Fig: 3- Nitrate uptake by *Enteromorpha intestinalis*, *Chondrous crispus*, *Gracilaria verrucosa* and *Polysiphonia sertularioides* in presense of ammonium

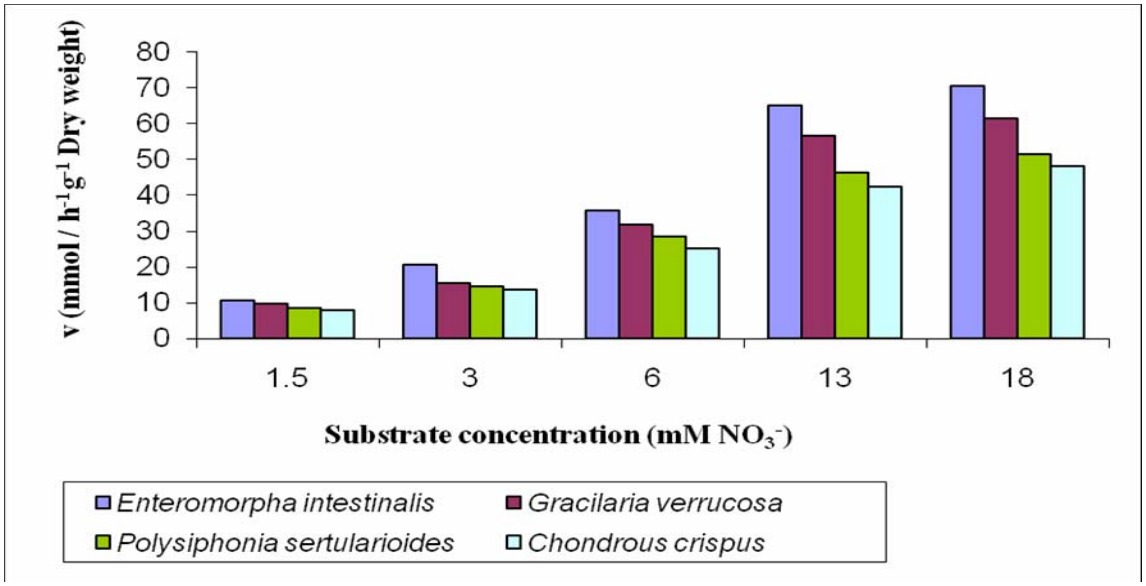

Fig. 4: Effect of substrate concentration on Nitrate uptake by *Enteromorpha intestinalis*, *Gracilaria verrucosa*, *Polysiphonia sertularioides* and *Chondrous crispus*

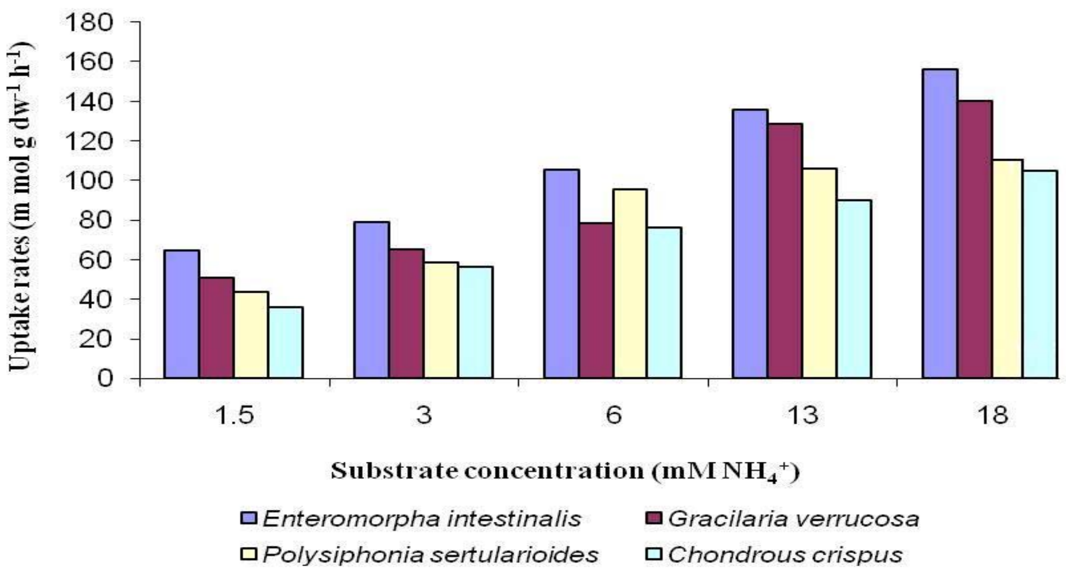

Fig. 5: Effect of substrate concentration on Ammonium uptake by *Enteromorpha intestinalis*, *Gracilaria verrucosa*, *Polysiphonia sertularioides* and *Chondrous crispus*