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# The Green Tide of Macroalgae in The Water of Arabian Gulf, Saudi Arabia; Removal The Nitrogen Compounds By *Ulva Intestinalis*

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***Abstract***— The green tide of macroalgae was recognized as (*Ulva fasciata, U. intestinalis, U.flexusa, U. lactuca, U. Chaetomorphalinum, and Enteromorpha intestinalis* ) had been regarded in the wintry weather and spring of years, 2016, 2017, and 2018 within the water of Arabian Gulf, Eastern Province, Kingdom of Saudi Arabia. The objective of this study; consider the parameters impact at the blooms of macroalgal and the way can be used this macroalgal to removal the nitrogen compounds like ammonia, nitrite, and nitrate from water aquaculture. Through this period monitor analyses carried out in the coastal water Gulf and the density of macroalgae growth. Indoor carry-out experiments to grow the macroalgae at distinct salinity 5, 10, 15, 20, 25, 30, 35, 40g/l, and remove ammonia, nitrite, and nitrate. The results concluded that the excess of nitrogen compounds and the changed of temperature and salinity were the impact of the principal parameters on green tides seem; the macroalgae (*U. intestinalis)* may be used to remove ammonia, nitrite, and nitrate from water and the removal percentage decrease with increase and decrease the salinity, the best nitrate compounds removal was at range (10-30 g/l) salinity.

***Keywords***— Green tides, macroalgal, *Ulva intestinalis*, removal, ammonia, nitrite, nitrate

## I. INTRODUCTION

Green tides are massive accumulations of green macroalgal biomass regularly create within the coastal water of regions assignment eutrophication. This phenomenon is turning into a major disquiet in the world. The terrible growth of those macroalgae produces unfavorable ecological effects together with a decline of seagrass beds resulting from the lowering of light penetration, gas, and nutrient change [1] and [2]. Similarly, it has a bad impact on fish and invertebrates because dissolved oxygen is consumed throughout the night time, oxygen depletion and release of H2S into the water. The anoxic conditions lose benthos and fish populations for a short time and species variety [3]. The surplus nutrient load is intended to be one of the essential elements in charge of the incidence of "green tides". Nitrogen has been known as the fundamental constraining nutrient [4]. Ulva spp. is commonplace quick-development opportunistic macroalgae of the coastal area and is generally regarded as some of the genera forming green tides. Ulva is the primary colonizers on open coastal areas, and global occurrence [5]. Alternatively, the advantageous impact of Ulva dominance on the reef community is the elimination of extra nutrients within the water. The overgrowth of phytoplankton is stopping [6]. A consequence of environmental stresses is such as nutrient obstacle, high light, excessive and irregular temperatures, dehydration, and salinity [7]. The salinity is thought to be the greatest essential factor [8]. The increased and decreased salinity causes stress for growth and nutrient uptake of macroalgae. Nitrogenous compounds consisting of NH3, NO2, and dissolved organic nitrogen are the primary byproduct of mariculture effluents. Those compounds also are nitrogen sources for seaweed growth [9]. Fed aquaculture creates loads of pollution, as fish excrete 60–70 % of the nitrogen [10], it is the reason of eutrophication and harmful algal blooms, to avoid such effects, mariculture effluent water needs to treat before back to the sea. Using macroalgae as 'biofilters' of effluent water from fishponds and cages has been the latest venture, They studied Reducing the unfavorable effect of intensive aquaculture in Integrated multi-trophic aquaculture systems was an economic [11], [12] and [13]. Seaweeds as a biofilter for systems need to have a number of applicable functions: excessive growth rate, simple cultivation, without problems controlled lifestyle, and resistance to epiphytes and disease-causing organisms [14].

## II. RELATED WORK

The appeared of the green tide of macroalgae may be depend on the found nitrogen compounds in the marine environment and change of water temperature and salinity, so may be used the macroalgae to remove nitrogen compounds from aquaculture water as biofilters.

## III. METHODOLOGY

### 1-1- **physicochemical parameters**

Macroalgae succession appropriate to some physicochemical characters of some water bodies in the coastal Arabian Gulf water (Saudi Arabia) was studied for three years (2016, 2017, and 2018) in winter and spring seasons. The sampling program included 15 coastal locations, at Dammam, Sihat, Al-Qateif, Al- Awamia and Al Safwa. Water temperature, salinity, dissolved oxygen (DO) and pH were measured weekly in situ at each site utilizing a pH/ISE/conductivity/RDO/DO Meter Thermo Scientific Orion Star A329 Portable. Water samples for water quality factors were gathered 0.25-m depth (below surface water), using a PVC Niskin bottle. Physico-chemical parameters were carried out weekly according to Standard Methods for Examination of the Water and Wastewater [15], which including nitrite ($NO_2$-N), nitrate ($NO_3$-N), ammonia ($NH_3$-N), total phosphorus ($TPO_4$), Sulfate ($SO_4$), sulfide ($H_2S$), and Alkalinity.

**2.1 Seaweed Samples collection**

Green seaweed was freshly collected by hand from the nearshore the water coast of the Arabian Gulf at Dammam, Sihat, Al-Qateif, Al- Awamia and Al Safwa, Saudi Arabia. They washed with Gulf water to get rid of the foreign particles, and epiphytes. They were stored in an icebox and at once transported to the lab and washed with tap water and distilled water to remove the salts on the surface of the samples. They were identified by species based on morphology, [16]. The density of macroalgae determined via gathering the macroalgal in one square meter from the surface area in water Gulf blooming and washing by Gulf water and tap water then left one hour in net to remove the excess of water after that was weighed. These were done for 5 samples in every site and calculated the average weight.

In February of 2017, Ulva spp. macroalgae were the dominant species. Ulva was collected from Al-Qateif coastal water (The surface Gulf water temperature 21.2°C, salinity 37.9). They were transported immediately to the lab in an icebox. They were cleaned and incubator in Gulf water after dilution by distilled water at condition (salinity 30 g/l, temperature 25°C, and 90-100 μmol photons m–2 s–1 with photoperiod 12 hours light: 12 hours dark).

**3.1 Growth experiment**

The effect of salinity on the growth of Ulva spp. (Ulva intestinalis), was carried in eight salinity levels (5,10,15,20,25,30,35,40 g/l), every level was three replicates. The fresh cleaned Ulva thalli dried by the paper tissue, weighed approximately 2g for each replicate. GeO2 was added to inhibit the Bacillariophyceae growing (final concentration of 0.50 mg/L). The natural gulf water (salinity 37.9) was collected from Al-Qateif coastal water, filtrated and sterilized before use. Salinity medium was prepared by adding sterilized distilled water to the sterilized natural gulf water for the wanted salinity and Sodium chloride to get salinity 40 g/l; refreshed every 3 days and stirred 6–8 times/day to mix nutrients well. Light intensity set aproximataly100 μmol photos m–2 s–1 with 12 light:12 dark cycle. The incubator flasks were rotated to the similarity light during the culture period 7-days. At the end of seven days, the algal thalli were weighted after they were dried with a paper tissue.

**4.1The removal experiments**

The fresh thalli of Ulva spp. (Ulva intestinalis) were used to remove the Ammonia, Nitrite, and Nitrate in the laboratory at salinity 30 g/l temperature 25oC and light intensity approximately 100 μmol photos m–2s–1 with 12light: 12dark cycle. The three concentrations and three replicates for every compound were used to remove. The period test was 120 hours. Ammonia concentrates were 2.5, 5, 10 mg/l prepared from Ammonium solution 30% NH3. Also Three concentrations of nitrite 0.25, 0.5,1.0 mg/l from sodium nitrite (NaNO2) and three concentrations of Nitrate 10, 20, 30 mg/l from Sodium Nitrate Extra pure (NaNO3), all nitrogen compounds from Loba Chemie Pvt. Ltd. - Mumbai, India.

**5.1 Pigment concentrations analysis in seaweeds**

Chlorophyll a & b, and cartonidoids as μg/g were determined by spectrometric measurements. 0.5 g cleaned seaweeds thalli was homogenated and extracted in 90% acetone. cartonide was determined according to [17] Parsons & Strickland's (1963) and Chlorophyll in accordance with [18].

**2. Statistical analysis**

The mean of three replicates ± Str obtained as (Standard Error). In addition, the mean values of each analysis were subjected to a one-way ANOVA test at $p<0.05$ using the SPSS Inc. program version 22 to detect significant differences among the target.

## IV. RESULTS AND DISCUSSION

**Results**

The green tide of macroalgae was collected from different sites that were identified by species based on the morphology to (U. fasciata, U. intestinalis, U. flexusa, Chaetomorphalinum, and Enteromorpha spp.) According to [16], they appeared in the winter and spring of 2016, 2017, and 2018 in the water Arabian Gulf, Saudi Arabia. The density of macroalgae was highest values range (14.36 – 9.47 kg/m2) in February and March in all sites and all years of study. The U. intestinalis was the dominant species in all sites in the winter seasons.

The average of water temperature values through the winter seasons was 21.45 oC, 20.8 oC, 20.5 oC and was 25.3 oC, 24.7 oC, 25.1 oC in spring season 2016, 2017 and 2018 respectively as tabulated in Table 1

Table 1: Physico-chemical parameters in water Arabian Gulf through winter and spring seasons 2016, 2017 and 2018

| Seasons | Temperature °C | DO mg/l | $NH_3$ mg/l | $NO_2$ mg/l | $NO_3$ mg/l | Total $PO_4$ mg/l | pH | Salinity g/l | EC | TDS | Sulfate mg/l | Sulfide µg/l | Alkalinity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Winter 2016 | 1.45± 0.42 | 9.9±0.45 | 0.35±0.09 | 0.03±0.001 | 1.28±0.5 | 2.23±0.6 | 8.11±1.2 | 35.89±3.4 | 54.37±7.2 | 26.81±3.1 | 31.59±5.6 | 43.25±7.2 | 108.79±9.8 |
| Spring 2016 | 25.3±0.7 | 8.53±0.6 | 0.47±0.06 | 0.032±0.00 | 1.33±0.4 | 1.90±0.4 | 8.31±0.20 | 37.62±4.2 | 57.30±4.5 | 27.60±3.5 | 51.2±6.4 | 47.27±5.6 | 142.2±7.5 |
| Winter 2017 | 20.8±0.5 1 | 10.0±0.2 | 0.11±0.03 | 0.01±0.001 | 1.25± 0.3 | 0.76±0.22 | 8.35±1.7 | 38.71±5.5 | 58.04±9.8 | 28.47±4.9 | 35.75±4.9 | 34.25±8.2 | 122.3±5.9 |
| Spring 2017 | 24.7±0.7 | 7.8±0.6 | 0.26±0.02 | 0.2±0.001 | 3.10±0.2 | 1.30±0.31 | 8.41±0.8 | 39.3±3.4 | 59.93±5.1 | 29.34±5.4 | 45.8±3.9 | 37.72±5.4 | 151.2±9.5 |
| Winter 2018 | 20.5±0.8 | 9.2±0.8 | 0.24±0.05 | 0.02±0.001 | 1.93± 0.4 | 0.15±0.03 | 8.17±1.1 | 37.63±3.9 | 56.79±8.6 | 27.78±4.2 | 33.20±9.2 | 33.62±5.2 | 136.5±8.2 |
| Spring 2018 | 25.1±0.2 | 7.6±0.5 | 0.41±0.03 | .019±0.001 | 2.00±0.3 | 0.95±0.5 | 8.32±1.4 | 38.51±2.9 | 58.70±5.5 | 28.72±6.1 | 40.6±5.6 | 41.2±2.1 | 147.6±6.9 |

The values of water dissolved oxygen, DO were reversed parallel with the temperature where there was an increase in winter and decrease in spring seasons, DO was 10 mg/l at winter seasons and 8 mg/l at spring seasons approximately. From Table 1, the average of ammonia values was the highest value in spring season 2016 was 0.47 mg/l and the lowest value in winter 2017 was 0.11 mg/l. Also, the nitrite values were recorded the highest value 0.2 mg/l in spring season 2017 but the lowest value 0.019 mg/l was in spring 2018. The results in Table 1 recorded the highest value of nitrate (3.1 mg/l) in spring 2017and the lowest value (0.019 mg/l) in winter 2016. The highest value of total phosphorus was 2.23 mg/l in winter season 2016 and the lowest value was 0.15 mg/l in winter 2018. The pH values were increased in spring seasons 8.4 and decreased in winter seasons 8.1 approximately. The salinity was recorded the high average values in spring seasons 39.3 g/l and lower average values in winter seasons 35.89 g/l. The salinity values were parallel with total dissolved salt (TDS) values and electric conductivity (EC) values as high and low. The sulfate average values were increased with spring seasons and decrease with winter seasons ranged 33.2- 51.2 mg/l but the sulfide values decrease in winter seasons and increase in spring seasons ranged 32.72 – 47.27 µg /l. The total alkalinity values recorded the lower value of 108.79 in the winter season 2016 but the higher value 151.2 in spring 2017.

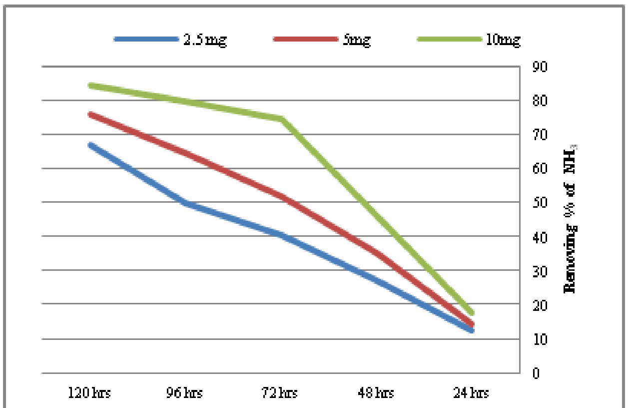


Fig 1: Removing percentage of Ammonia

Fig. 1

The percentage ammonia removal from water by U. intestinalis was recorded as the increase removal percentage with increase time and increase ammonia concentrating. After 120 hours, it was recorded 84.51%, 75.83% and 66.78% at concentrations 10 mg/l , 5 mg/l and 2.5 mg/l respectively in fig 1.

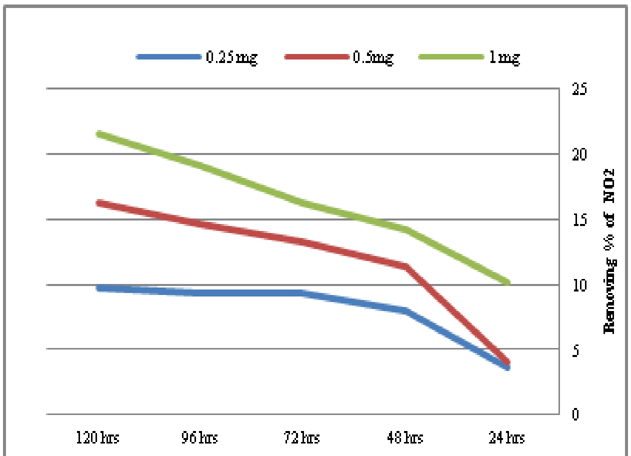


Fig 2: Removing percentage of Nitrite

Fig. 2

From the fig 2 notice that the percentage nitrite removal was increased with increasing the nitrite concentrating in water and the time of the experiment was recorded that 21.1%, 16.3, 9.7 after 120 h with the concentrating nitrite values in water 1 mg/l and 0.5 0.25 mg/l respectively. On the other hand, the percentage removal of nitrate was increased with increased time of experiment and recorded the highest values after 120 h. But decreased the removal percentage nitrate values with increased concentrating the nitrate in water. It was 62.5 %, 74.75%, and 81.71% at nitrate concentrating 20mg/l, 10mg/l and 5 mg/l respectively and cleared in fig. 3.

 

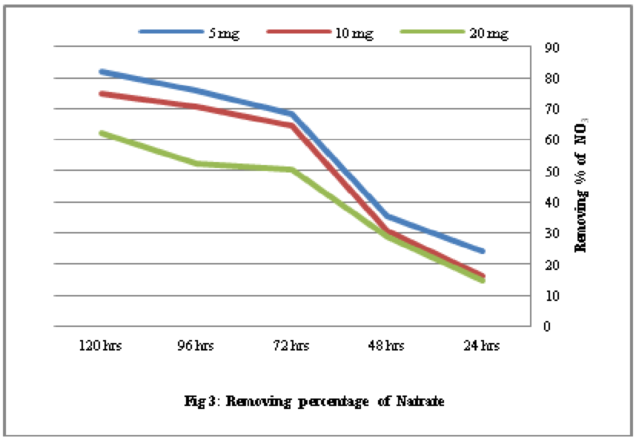


Fig. 3

The growth rate was the highest value at 30 g/l salinity was 22.76% chlorophyll b 4.12 µg/g, chlorophyll-a 2.35 µg/g and cartonide 0.53 µg/g and noticed the decreased significantly with decreased salinity and increased salinity fig5.

**Discussion**

The variety of temperature in wintry weather and spring seasons was (20 -25 oC) in water Arabian Gulf, Saudi Arabia approximately, in this period observed the green tide seemed and abundant growth of macroalgae, specially Ulva Spp., in wintry weather than spring, this result agreed with [19], they noticed that Ulva spp. Was the global bloom grow in temperature range 10-30°C, with the very best increase rates commonly going on at 15-20°C. U. lactuca is the simplest macroalgae that can reason blooms, [20].

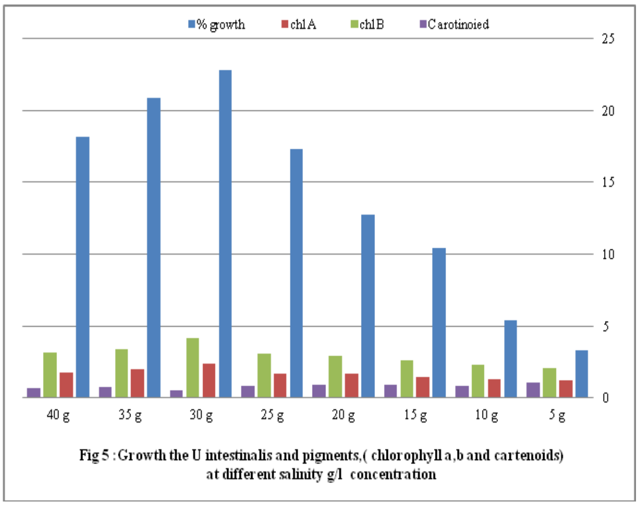


Fig. 4

The occurrence of green tides is resulting from U. lactuca having powerful physiological flexibility and affordability [21]. Likewise, U. Intestinalis, Enteromorpha intestinalis develop in various temperatures, and the best bloom at 15-20°C, moreover, gross photosynthesis is higher at 20 - 25°C [22] and [23]. The dissolved oxygen (DO) changed into 10 mg/l at winter season and 8 mg/l at spring season approximately parallel with reduced and increased the temperature in wintry weather and spring seasons. He decided that Do in open water conditions are in a natural condition, it is rarely found in oxygen-poor [24]. They stated that the source of DO in water comes from the diffusion of oxygen from the air, the flow of water through rainfall and photosynthetic activity by aquatic plants and phytoplankton [25]. Commonly, the reduced nitrogen compounds (NH3, NO2, NO3) in winter than spring season parallel with the increased growth rate of the macroalgae (Ulva spp.) in winter than spring season where the growth increased of Ulva spp. exhausted the nitrogen compounds, it also can maximize nutrient absorption and do the fragmentations easily to vegetables [26]. The range of nitrate was good water quality condition for macroalgae growth in this period; he stated that the nitrate content that describes good water conditions for macroalgae growth is 0.09 to 3.5 mg/l, for this reason, the variety of nitrate content in those waters is still in the safe limits of waters fertility [27]. The increased the phosphate concentrations in wintry weather season explained The phosphate attention can still be tolerated through macroalgae growth increased, he states that the need of phosphate for algal growth might be lower if nitrogen is in the form of salt ammonium and vice versa if nitrogen is inside the form of nitrate [28]. The phosphate awareness need for algal growth ranges from 0.018 - 0.090 mg/l and the highest limit is 8.90 - 17.8 mg/l (P-PO4) if nitrogen is within the form of nitrate. The pH values have been increased in spring seasons 8.4 and reduced in wintry weather seasons 8.1 approximately, the pH range at the examiner sites remains within the growth tolerance restrict of macroalgae, Ulva spp., according to [29]. The most suitable pH for inducing sporulation for Ulva mutable ranged from 8 - 8.5. The salinity recorded the excessive average values in spring seasons 39.3 g/l and decrease average values in winter seasons 35.89 g/l, the salinity values were the alike trend with total dissolved salt (TDS) values and electric conductivity (EC) values as increased and reduced, they were laid low with decreased the temperature and the rain in winter so decreased the values of salinity by way of dilution from rainwater but in spring turned into elevated the temperature and high the evaporation so the salinity increased. Salinity can have an effect on the release of spores by using influencing turgor pressure and pore diameter of sporangia [30]. The growth of macroalgae will be significantly decreased at salinity below 5 ppt [31]. Ulva can develop optimally with salinity more than 20 ppt and the best increase is at 35 ppt. Previous researchers reported the U. lactuca growth rate, dependent on water temperature and light [32]. The very best nitrogen biofiltration performance was (88%) and fastest growth rate (412 g/m2/day) at 25°C in the summer and the lowest biofiltration performance (3%) and growth rate (81 g/m2/day) in winter at 20°C for Ulva spp. Further, they mentioned the highest growth rate and biofilter performance of Ulva clathrata (26.5°C) [33]. The sulfate, the sulfide, and the total alkalinity average values in winter and spring season were suitable for macroalgae, Ulva spp. grow and aquatic fauna.

 

This results of the removal ammonia percentage in fig1 agrees with the results of many researchers in this field; [34] they reported, 40–90 % of ammonia removal efficiency for U. lactuca and 76 % for U. rigida from fish wastewater, U. lactuca cultivated in effluents of fish pond could efficiently remove 85–90 % of total ammonia-nitrogen [35]. From the fig 2 observed that the percentage of nitrite removal increased with increased nitrite concentrating in experimental water and increased the time of the experiment. These results agree with [36] they reported removal efficiencies of 22% for nitrite. On the other hand, the percentage removal of nitrate was cleared in fig.3 and in fig.4 the growth rate, chlorophyll-b, chlorophyll-a and cartonide of U. intestinalis were noticed significantly decreased with decreased salinity and increased salinity. The ability to remove NO2 and NO3 increased with time, showing an adaptation to the presence of these nitrogen sources. Similar results have been reported for U. pertosa and Gracilaria, which exhibited higher NH4+ uptake than NO3 and NO2 [37]. Seaweeds are noted to have the greatest preference for NH3-N uptake compared to other nutrients [38]. The majority of macroalgae uptake rates of NH4+ than NO3 under ordinary environmental situation because NH4+ can be directly included in the composition of amino acids [39]. The low salinity was a negative effect on the growth and nutrient uptake of Ulva spp. [8].The decrease in the concentration of the three forms of nitrogen (NO2, NO3, and NH3) in removal experiments shows that seaweed is very required nitrogen nutrients at the period of its growth [40]. They showed that a higher nutrient level can increase pigment content and photosynthesis in the U. intestinalis this explains the increased chlorophyll a, b and cartonid in my results [41]. Chlorophyll a in U. intestinalis grown in high nutrient supply was reported to be higher than those in low nutrient supply. The nitrogen supply in medium culture can influence pigment content, protein and carbon uptake in many species of seaweeds. As previously observed by [42], the removal performance of U. lactuca depends on the nutrient's accessibility, increased with a higher concentration of nutrients. Also, they reported that the nutrient uptake became higher at intermediate salinity and lower at low (five ppt) and high (40 ppt) values, and a stronger effect of salinity on nitrate uptake [43]. The waste components of aquaculture effluents are nitrogenous compounds, these are the principal nitrogen sources for macroalgae [9].

## V. CONCLUSION AND FUTURE SCOPE

The results concluded that the excess of nitrogen compounds and the changed of temperature and salinity were the impact of the principal parameters on green tides bloom; the macroalgae (U. intestinalis) may be used to remove nitrogen compounds as (ammonia, nitrite, and nitrate) from water and the removal percentage decrease with increase and decrease the salinity, the best nitrate compounds removal was at range (10-30 g/l) salinity. In the future, we need more study in the different species of macroalgae to use the removal of nitrogen compounds in marine and freshwater aquaculture.

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