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Photosynthetic rate and biochemical composition of green algae *Ulva flexuosa* (Wulfen) J. Agardh as potential indicators of environmental stress in the intertidal zones

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Abstract

Photosynthetic rate and biochemical composition total chlorophyll (TC), total soluble protein (TSP) and total soluble carbohydrates (TSC) were determined for Ulva flexuosa*, a green marine algae collected from Nasese, Lami and Laucala Beach areas in Suva, Fiji Islands.* Ulva flexuosa *from Laucala Beach area had the highest photosynthetic rates, TC and TSP content, while algae from Nasese area had the highest amount of TSC. Algae from Lami area had the lowest amount of photosynthesis rate, TC, TSP and TSC. High photosynthetic rate and biochemical content were mainly due to the presence of high levels of nitrogen in the area. Algae thrived in Laucala Beach and Nasese area where nitrogen content was high. Low photosynthetic rate and biochemical composition were recorded in algae from the Lami area, which was apparently the most polluted area. It appeared that conditions in Lami area were intolerable to* U. flexuosa*. Algae collected from the three sites differed in the measured parameters with respect to the differences in the pH, temperature and nitrogen levels indicating that physiological responses of algae could be used as indicators to monitor the environmental quality of the intertidal zones.*

Keywords: Ulva flexuosa*, photosynthetic rate, biochemical composition, environment*

1. Introduction

The tubular green algae *Ulva* from the phylum Chlorophyta, class *Ulvophyceae*, bright green seaweeds are highly adapted to grow in the intertidal zones and coastal areas especially where there is pollution. One species commonly found in Fiji is *Ulva flexuosa* (Wulfen) J. Agardh. *U. flexuosa* is a long (usually up to 150 mm), tubular, light green algae. Its thallus consists of hollow tubes that are made up of walls one cell thick and axis 1 - 7 mm wide (N'Yeurt, 2001).

Ulva species in general are found at all levels of the shore line and usually they are found in large quantities in areas where fresh-water runoffs occur. They are often referred to as 'fouling algae' as they develop abundantly in coastal zones affected by pollution from municipal or industrial discharge (Littler and Littler, 1984). They can also occur as 'green tides' accumulating in the form unattached green algae in most polluted and eutrophicated marine environments (Back *et al.*, 2000; Nelson *et al.*, 2003; Blomster *et al.*, 2002). Algae from the same genus (Ulva) were reported to form massive green tides that threatened the Olympic sailing events in Qingdao, China in 2008 (Leliaert *et al*., 2009).

Spatial and temporal distribution of *U. flexuosa* is limited by factors such as temperature, pH, salinity and nutrient availability. Mairh *et al*. (1986) noted a favorable growth of *U. flexuosa* at temperatures ranging from 15°C - 30°C and adverse effects on growth together with bleaching at temperatures of 33°C and above.

Algae respond rapidly to water quality changes, hence considerable information concerning the environmental condition of an aquatic habitat can be obtained from their analysis (Lewis and Wang, 1997). Abiotic environmental stresses due to variations in temperature, salinity, pH and nutrient availability limit algal distribution and abundance. Because of their trophic level and rapid growth, algae are rapid responders to environmental stresses within an ecosystem. A variety of physiological, morphological and community parameters of algae can be used to monitor the health of an ecosystem (Lewis and Wang, 1997). Decline or increase in the diversity of algae mostly indicates the environmental quality of a system. Investigating physiological parameters which include functional measures such as the rates of productivity, photosynthesis, nutrient flux and cellular activity require a large amount of time and work hence indications of environmental stresses are not studied at physiological levels (Cairns and McCormick, 1997). However, physiological parameters and processes provide an accurate account of environmental stress response since the physiological responses of most species reveal the mechanisms by which the organisms adjust to cope with the environment.

This paper investigates the difference in photosynthetic rates and the biochemical composition (TC, TSP and TSC) of *U. flexuosa* collected from three different sites and examines the possibility of using its rate of photosynthesis and biochemical composition as indicators of the health of the coastal area.

2. Materials and Methods

2.1 Algal Material

Samples of *Ulva flexuosa* were collected from the intertidal flat along Nasese, Lami and Laucala Beach areas, Suva, Fiji (Figure 2.1) and analyzed in terms of photosynthesis, TC, TSP and TSC over a 12 month period. The algal samples were hand picked randomly growing on various substrates in the inter-tidal zones. Observations of the three sites and *U. flexuosa* in each site were made before the algal collection. Possible means of pollution were also identified within each area. The samples were collected whenever the algae were available simultaneously at all three sites. Based on the availability of the algae at all three sites at the same time, a total of five samplings were done. Temperature and pH of seawater were determined on site during each sampling using an Ecoscan hand-held pH meter (Eutech Instruments Pte Ltd, Singapore). Samples of seawater were also taken concurrently from the three sites for determination of inorganic nitrogen content (NH_4^+ and NO_3^-).

The collected algae were transported to the laboratory within an hour and were used for determining the photosynthetic rate and the biochemical content. The algae were washed in sterile seawater in a series of ten washes (two litres per wash) to remove debris and as many diatoms and other attached epiphytic algae.

2.2 Rate of Photosynthesis

Photosynthetic rate (µmoles/ min/ g of fwt) was determined by measuring oxygen evolution using a Clark-type oxygen electrode fitted with a DW 2 chamber (Hansatech, Kings Lynn, Norfolk, England) as described by as described by Walker (1990). Algal thalli was held with a thin wire hook and suspended into the cylindrical reaction chamber that was filled with artificial seawater (ASW) medium prepared according to Brand (1984). Oxygen evolution was initiated by illumination using the standard high intensity light source LS2 (Hansatech, Kings Lynn, Norfolk, England).

2.3 Biochemical Content

TC (µg/ mL/ g fwt) was extracted and measured using the spectrophotometric method described by Kreast and Grant (1976). TSP (mg/ g fwt) was determined with Bradfords reagent with bovine serum albumin serving as the standard (Bradford, 1976) while the TSC (mg/ g fwt) was measured using the phenol-sulphuric acid method after Dubois *et al.* (1956) using glucose as the standard.

2.4 Statistical Analysis of Results

The data were analyzed using Graph Pad Prism Version 4 (GraphPad Software). Differences in the physical variables among the sites, TC, TSP and TSC were tested using one way ANOVA while the growth and photosynthesis rates were tested with two ways ANOVA. For both types of testing, Bonferroni's Test was used as a post hoc test for multiple comparisons of means. A significance level of $p \leq 0.05$ was adopted.

3. Results and Discussion

3.1 Physiographical observations of the sites and *U. flexuosa*

3.1.1 Nasese area

Algae in this area were found growing mainly on the rocky platforms, rocks, on the sides of the seawall and on some foreign objects that were the result of dumping of rubbish such as on pieces of clothes, bamboos, coconut shells etc. *U. flexousa* appeared bright green in colour and were about 50 – 100 mm in length and 2 - 3 mm in width.

3.1.2 Lami area

The intertidal area consisted of sand and rubble and is reclaimed. It was located near the main highway and connected to the industrial area. An estuary ran through the industrial area. In addition, it had a rubbish dump (no longer in operation) in its vicinity as well as the main harbour (Suva harbour with Kings Wharf). The intertidal zone had several old metal scraps, which may have been washed up from the nearby dumpsite and also is a result of onsite dumping. *U. flexuosa* was found growing mainly on few rocks, on metal scraps, driftwood and used automobile tires etc. The algae were dark green in colour, much thinner and the growth forms were smaller (20 – 40mm in length and 1 - 1.5mm width).

3.1.3 Laucala Beach

The shoreline was rocky and made up of rocky reef platform. The area was reclaimed, situated away from the main highway but was connected to a residential area. It also had an industrial area in its vicinity. In addition, it was close to a sewage treatment plant. Similar to Lami, the site had several pieces of scrap metals, tires and other rubbish on which *U. flexuosa* was found growing. The algae appeared very dark green in colour, had a width ranging from 1 – 2 mm and were of smaller lengths (20 – 50mm).

3.2 Temperature, pH and Nitrogen content

The average temperature was almost the same for all three sites while the pH was the highest in Nasese area while Lami had the lowest (Table 1). There was no significant difference in the pH at Laucala Beach and Nasese area. The levels of both forms of nitrogen (NH_4^+ and NO_3^-) in the water samples obtained from the three sites were much higher. While samples from Lami area had relatively low NH_4^+ (1.9 µmoles/L), it was still 4 times higher than that in the artificial

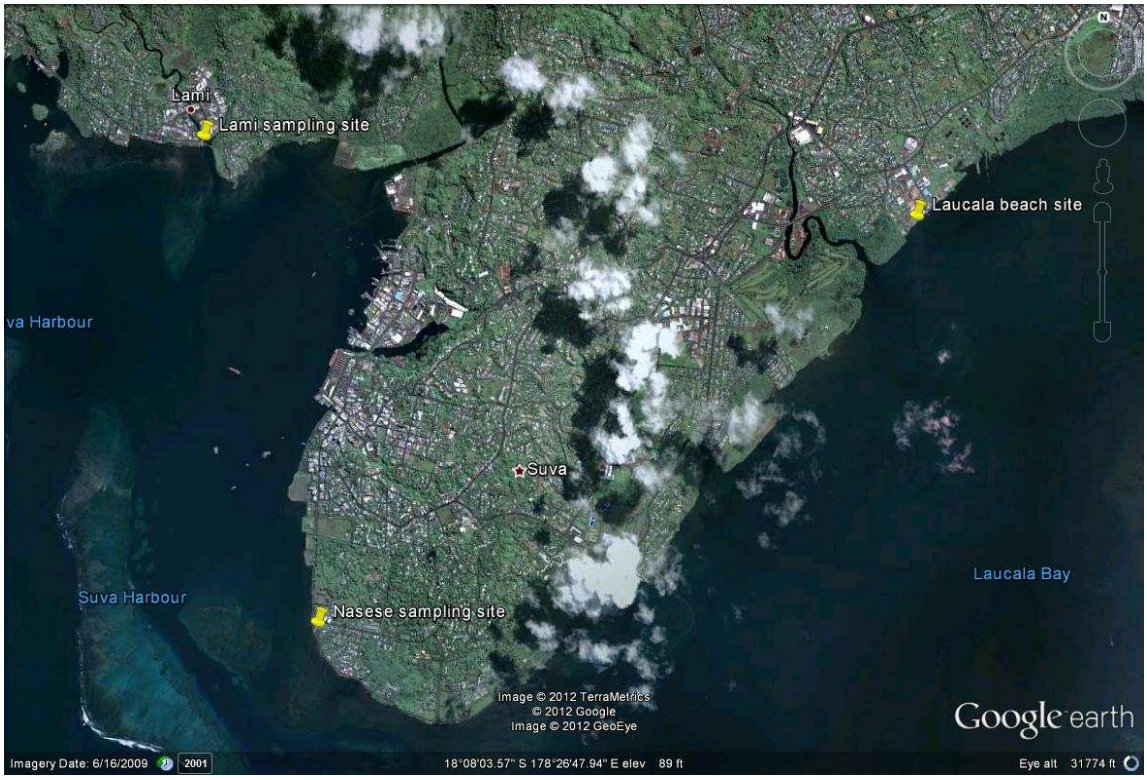

Figure 2.1. Google earth image of the locations of the Nasese, Lami and Laucala Beach sampling sites on Viti Levu, Fiji. Samples from the three sites were used for the comparative study. Source: Google Earth, 2012.

seawater medium. Those from the other two sites were about 10 times higher. The NO_3^- values in the water samples from the three areas were approximately twice the concentration found in the ASW.

3.3 Photosynthesis

Highest rates of photosynthesis (Figure 3.1) were observed in *U. flexuosa* from the Laucala Beach area. The observed rates are significantly higher ($p \leq 0.05$) than the rates in Nasese and Lami areas. The average recorded photosynthetic rates for all the samples were in the range of 1.05 – 1.3 µmoles/ min/ g of fwt. Algae in the Lami area had slightly lower photosynthetic rates than the algae at Nasese but the difference was not significant ($p > 0.05$). In two out of the five analyses done, algae at Nasese and Lami had similar rates. Overall, photosynthetic rates of algae from Lami and Nasese were similar, while that from Laucala Beach was much higher.

3.4 Total Chlorophyll

Algae from Laucala Beach recorded the highest level of TC (Figure 3.2) in all five analyses ($p \leq 0.01$) compared to Nasese and Lami areas. Algae from Nasese and Lami had the average TC in the range of 115 – 140 µg/ mL/ g fwt which was lower than that in algae from Laucala Beach. There was no significant difference between the TC recorded in Lami and Nasese area ($p > 0.05$).

3.5 Total Soluble Protein

TSP (Figure 3.3) overall was highest in algae from Laucala Beach (between 6.5 – 7.5 mg/ g fwt) followed by algae in Nasese area. TSP recorded in both areas was significantly different ($p \leq 0.05$). Algae from Lami showed significantly low ($p \leq 0.05$) TSP contents ranging from approximately 1.5 – 2.5 mg g fwt, corresponding to the lowest photosynthesis rates.

Table 1. Physical variables (mean ± SE) measured from the three sampling sites during the course of the experiment. Different superscripts differ significantly; Bonferroni's test at a significance level of $p \leq 0.05$.

	Lami	**Laucala Beach**	**Nasese**	**One way ANOVA** (*p*-value)
Average Temperature (^oC)	25 ± 0.4^a	26 ± 0.25^a	25 ± 0.25^a	0.114
Average pH	7.7 ± 0.07^b	8.4 ± 0.09^{ac}	8.6 ± 0.04^{ac}	0.000
Average NH_4^+ (µmoles/L)	1.9 ± 0.10^a	5.1 ± 0.14^{bd}	4.5 ± 0.21^{cd}	0.000
Average NO_3^- (µmoles/L)	3.7 ± 0.13^a	2.9 ± 0.17^{bd}	2.9 ± 0.13^{cd}	0.003

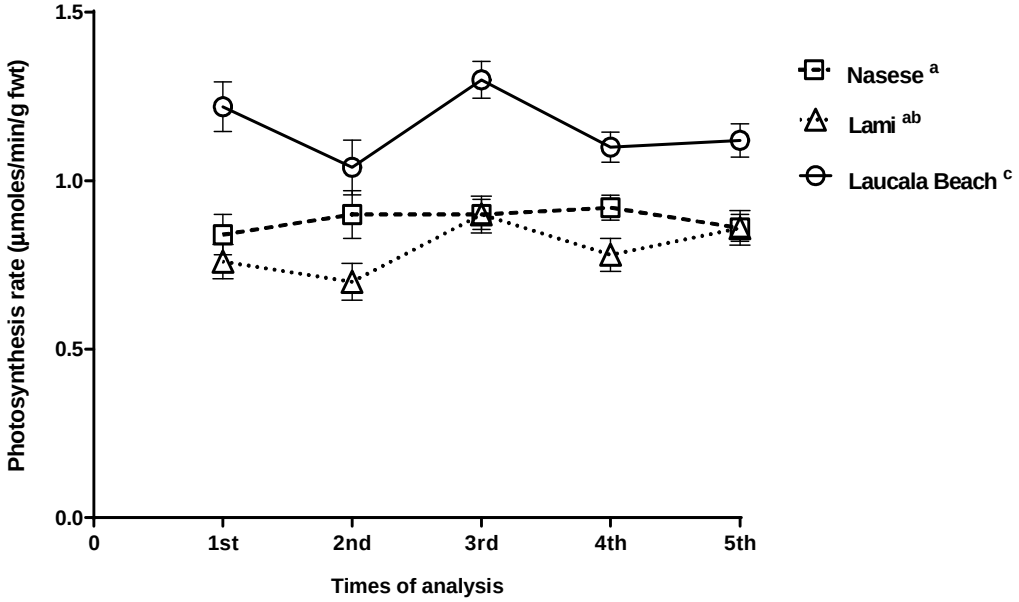

Figure 3.1 The net photosynthetic rates (µmoles/min/g fwt, mean ± SE) of *U. flexuosa* from three sites. Different superscripts differ significantly; Bonferroni's test at a significance level of $p \leq 0.05$.

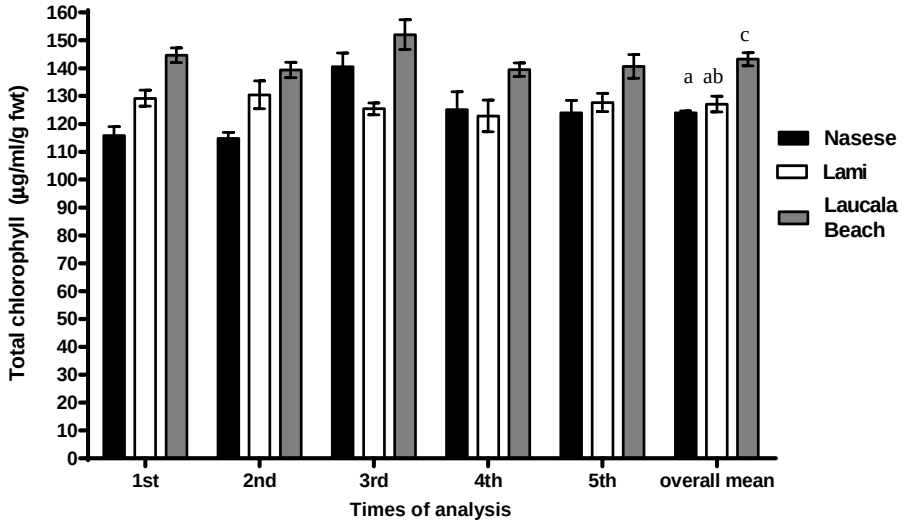

Figure 3.2 The total chlorophyll content (µg/ml/g fwt, mean ± SE) in *U. flexuosa* from three sites. Different superscripts differ significantly; Bonferroni's test at a significance level of $p \leq 0.05$.

3.6 Total Soluble Carbohydrates

TSC measured was highest in *U. flexuosa* at Nasese (Figure 3.4). Algae from Laucala Beach area had moderate amounts of TSC in the range of 6.7 – 7.5 mg/ g fwt. Algae collected from Lami had the lowest TSC in the range of 5.3 – 6.3 mg/ g fwt. There is a significant difference in the TSC content of algae from the 3 sites ($p \leq 0.05$).

4. Discussion

U. flexuosa in the Laucala Beach area showed the optimum growth. This is evident from the measured high photosynthesis (Figure 3.1), high TC (Figure 3.2) and high TSP content (Figure 3.3). Algae from Nasese area had moderate photosynthetic rate, TC and TSP but the highest levels of TSC (Figure 3.4) while algae from Lami area had low photosynthetic rate, TC, TSP and TSC contents.

Favorable growth of algae in Laucala Beach area was obviously due to the high nutrient (nitrogen) content (in the form of NH_4^+) (Table 1) in the area since it is situated near the residential areas and a sewage treatment plant. The high nitrogen content is reflected in the high TSP of algae from this area. High TSP content (Figure 3.3) results in biomass accumulation as well as an increase in proteins that takes part in photosynthesis hence increasing photosynthetic activity (Figure 3.1). However, in two of the analysis, algae from Nasese have similar TSP

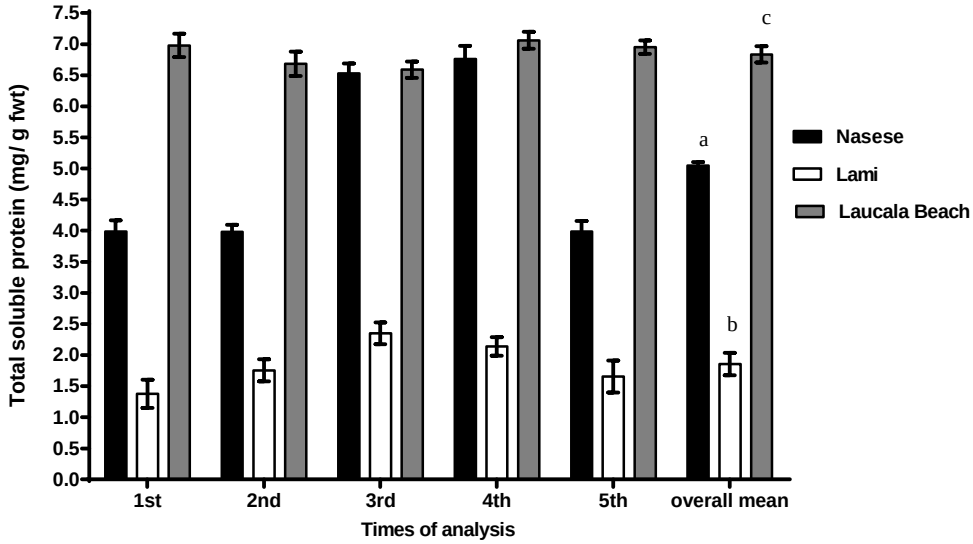

Figure 3.3 The total soluble protein (mg/g fwt, mean ± SE) content in *U. flexuosa* from three sites. Different superscripts differ significantly; Bonferroni's test at a significance level of $p \leq 0.05$.

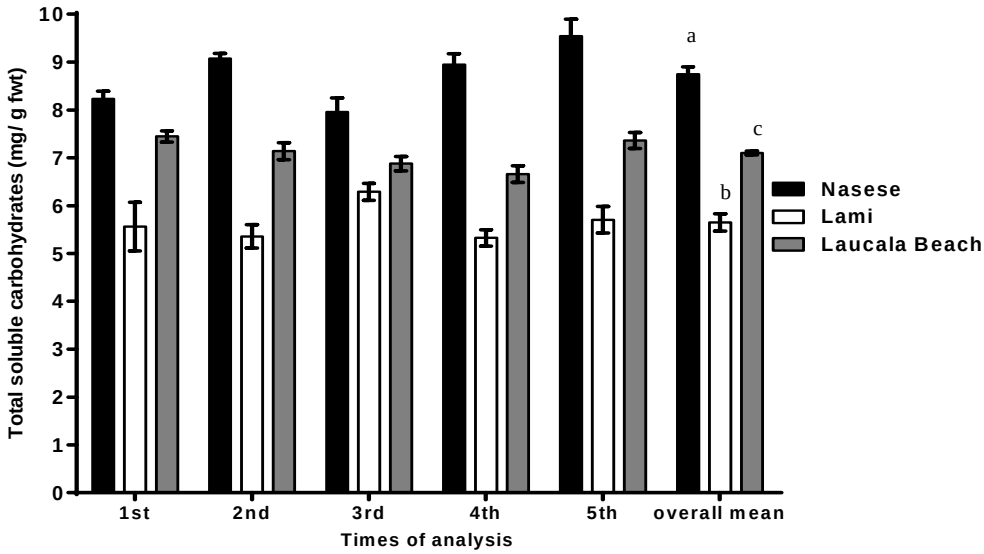

Figure 3.4 The total soluble carbohydrate (mg/ g fwt, mean ± SE) content in *U. flexuosa* from three sites. Different superscripts differ significantly; Bonferroni's test at a significance level of $p \leq 0.05$.

(high content) as the algae from Laucala Beach. This is because nutrient content could be high at Nasese as well since it does have drainage outfalls that open up in its intertidal area. On an average, water from the Nasese area had less NH_4^+ than the Laucala Beach water, although NO_3^- contents were the same.

The carbohydrate content was highest in algae from the Nasese area, which indicates a more healthy growth than Laucala Beach and Lami. Though rate of photosynthesis and TSP and chlorophyll were high at Laucala Beach, the carbohydrate content was lower than the Nasese area. This could be because of increased transfer of photosynthetically incorporated carbon to synthesis of amino acid skeletons at the expense of sucrose synthesis, which results during nitrate reduction and ammonia incorporation (Platt, 1977; Elrifi and Turpin, 1986). Nitrogen plays an important role in the photosynthetic carbon flow in both higher plants and algae (Elrifi and Turpin, 1986). However the nutrient levels can fluctuate on short time scales, hence it is difficult to relate the physiological changes found during the experiment in terms of nitrogen availability. *Ulva* usually grows where there is high nitrogen level in the water.

A fluctuation in nutrient content at Laucala Beach was evident as *Ulva* did not occur continuously over the period of research; it cropped up, flourished a few times but algal occurrence was not seen on every visit made to the site. Similar patterns were observed at Nasese. The site at Lami remained bare (without *Ulva* growth) for a longer period of time.

According to the results obtained, algae from

Lami area were the unhealthiest with lowest photosynthetic rate, TSP and TSC. TC though, was similar to what was obtained in Laucala Beach and Nasese. This was obvious as the algal growth observed in the area was low and whatever growth occurred was after a longer interval compared to the other two sites. Lami area also had lower nitrogen content than the other two sites which could be a factor contributing to low growth. The area does not have a nearby residential area that would contribute to high nitrogen levels. In another experiment, the growth of *U. flexuosa* germlings in ASW medium showed increased growth at similar concentrations of ammonium and nitrate (Prakash, 2008). Hence it can be said that apart from low nitrogen levels, algae here could be affected by other means of pollution resulting from the industrial areas. Industrial chemicals such as Polychlorinated biphenyls (PCBs) and antifouling compounds from the nearby industrial area and Suva harbour could also be affecting *Ulva* growth in the area by lowering the nutrient content. The mechanism of how it lowers the nutrient content is unclear. In addition, heavy metals are one of the major sources of pollution in the Lami area (Tabudravu, 1998; Chand *et al.*, 2010, 2011; Chand and Prasad, 2013) and *Ulva flexousa* has previously shown to bioaccumulate heavy metals in Lami area (Tabudravu, 1998). The order of metal toxicity in algae are Hg > Cu > Cd > Ag > Pb > Zn, but it varies with algal species and environmental conditions (Rai *et al.*, 1981). The toxic effects generally include growth cessation in extreme cases, inhibition of photosynthesis, reduction of chlorophyll content, increased cell permeability and disruption of enzyme activity (Lobban and Harrison, 1994). Presence of heavy metals in the area could be possibly resulting from the antifouling agents as well, which inhibits reproduction and growth of *Ulva*. The effect of industrial chemicals and heavy metals on photosynthesis and biochemical composition of *U. flexuosa* however needs further investigation.

When looking at the possible means of pollution in all the three sites, Lami area appears to be the most polluted in terms of other pollutants. It does have the highest NO_3^- level compared to the other two sites but much a much lower NH_4^+. *Ulva* in this area complements the results obtained as its photosynthesis; TSP and TSC were lower than the other two sites. In addition the growth of algae was very low throughout the duration of the experiment and the algae did not appear healthy when compared to the other two sites. During two visits to Lami, most of the existing algae appeared bleached (whole frond bleaching). Bleaching and loss of colour was also observed in Nasese but loss of colour here could be accounted by the sporulation (release of spores) process, as only part of the thalli picked appeared bleached while part of it remained green and healthy. Nasese area also had significant amount of pollution via small amount of sewage discharge and runoffs from the drainage outfalls, soap and detergent contaminated water from the household drainage. Yet, the algae here appeared healthy and were in larger growth forms. Significant growth in Nasese can be related to the increase in alkalinity, which probably resulted from the household drainage. HCO_3^- is vastly available in seawater at high pH (Lobban and Harrison, 1994). In addition it had high nutrient supply in the form of NH_4^+ from the drainage outfall which optimized the growth conditions for *U. flexuosa*. In Laucala Beach area, the presence and flourishing of *Ulva* can be attributed to the high nitrogen content especially in the form of NH_4^+ resulting from sewage discharge from the nearby sewage treatment plant (Table 1).

5. Conclusions

Overall, it can be said that the algae in these three different sites are affected to some extent by their environment and the pollution within their environment. The most striking observation is that the high levels of NH_4^+ influenced algal growth and photosynthesis, perhaps masking the effects of other pollutants in Laucala Beach and Nasese, while in Lami in the presence of relatively low levels of NH_4^+, other pollutants appeared to have an adverse effect on the growth of these algae.

This investigation illustrates that *Ulva* can be used as a bioindicator for marine pollution in Fiji with respect to high nitrogen content (especially for high NH_4^+ levels). *Ulva* presence in any intertidal zone itself indicates high level of nitrogen. Since *Ulva* is an opportunist alga, it prefers and is well adapted to grow in coastal areas where there are moderate levels of pollution. Hence, logically it would only exhibit stress responses when the pollution level is very high and beyond its tolerance range as shown in the Lami area.

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