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Seaweed and Chlorophyll as Biomarkers of Metals in the Persian Gulf, Iran

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Abstract Algal chlorophyll content and chlorophyll type ratios, as biomarkers of stress, were investigated. *Ulva intestinalis* and *Sargassum angustifolium* were sampled at low tide, in the intertidal zone of Bushehr Province in January and May, 2010. The mean concentrations of metals in the algae were in the following order: Pb > Ni > Cu > Cd. High negative correlations between chlorophyll a content ($r = -0.84$, $p < 0.01$), chlorophyll c content ($r = -0.82$, $p < 0.01$), and ratio of chlorophyll c/a in *S. angustifolium* ($r = -0.93$, $p < 0.001$) and Ni concentration in this algae shows that both the content and ratio of chlorophyll may clearly reflect a negative effect of high metal concentrations in this algae.

Keywords Seaweed · Metal · Chlorophyll · Biomarker · Persian Gulf · Iran

Conservation and restoration of impacted littoral ecosystems depend on early intervention. Appropriate intervention strategies must be based on bio-monitoring studies that can be implemented easily. Early ecological warning signs of stress may be used to detect, intervene, and protect vulnerable ecosystems. Use of aquatic plants is an important tool for ecotoxicological research and plant bio-monitoring can indicate if an ecosystem has been adversely affected and to what degree.

Chlorophyll is a key molecule in photosynthesis. Environmental stress that interferes with photosynthesis can induce responses in plants that may be detected as biomarkers of stress. Cadmium, lead, copper and mercury have been shown to cause obstructions in chlorophyll biosynthesis. It has been suggested that chlorophyll concentration in plants can be used as a biomarker in ecotoxicological studies. Excess metal can obstruct chlorophyll production causing adverse effects on the photosynthesis which ultimately impairs plant growth and development (Tarpley et al. 2005). Soil, contaminated with crude oil, has been shown to result in reduced chlorophyll levels in *Paspalum conjugatum* (a type of sourgrass) indirectly through metal effects (Ibemesim 2010). Studies in aquatic plants and blue-green algae have confirmed decreased chlorophyll levels and reduced photosynthesis as a result of metal exposure. Green algae or seaweed also respond to metal pollution by decreases in biomass and chlorophyll concentration (Giloni-Lima et al. 2010). This experimentally derived knowledge is the basis for using seaweed as a biomonitoring tool. *Enteromorpha* and *Ulva* have been used as biomonitors of trace metals across the world. Other studies suggest that brown seaweed (like *Sargassum*) may offer a better biomonitoring tool in coastal tropical environments (Jothinayagi and Anbazhagan 2009).

Increasing environmental degradation of the Persian Gulf (due to war related and industrial contamination) threatens the coastal ecosystems that are normally high in productivity and which serve as vital nursery areas for fish and shellfish. The aims of this study were to: 1) measure Ni, Cd, Cu, and Pb accumulation in two species of

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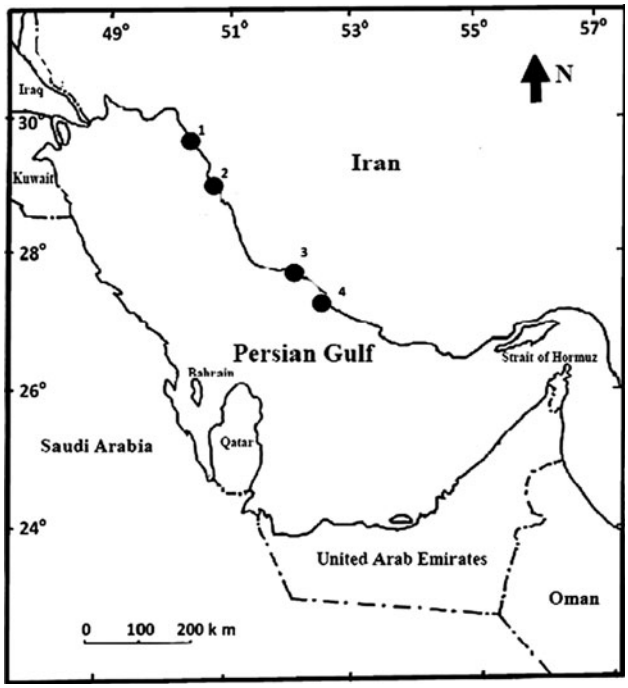

Fig. 1 Sampling sites in the Bushehr Province, Persian Gulf. (*1*) Oil facilities, (*2*) Nuclear power facilities, (*3*) Village of Olee, and (*4*) Gas Facilities

marine green and brown macroalgae (*Ulva intestinalis*, and *Sargassum angustifolium*), and 2) investigate potential effects of metal accumulation on in vivo chlorophyll content. We selected Ni, Cd, Cu, and Pb for our investigation because they are listed in the Dangerous Substances Directive (76/464/EEC) and its annex (a list of substances originally published by the European Economic Community in 1976), as substances that pose particular concern in aquatic environments, due to their high production volume, environmental persistence, and bioaccumulation and toxic properties.

Materials and Methods

Four sampling sites along the coast in the Bushehr Province were selected (Fig. 1). Locations of sampling sites were recorded using a Global Positioning System (GPS): (1) Oil facilities site (Ganaveh Port) (29°39′N, 50°24′E), (2) Bushehr Nuclear Power Facilities site (28°50′N, 50°52′E), (3) control site (Village of Olee) (27°49′N, 51°55′E), and (4) Gas facilities site (Haleh village) (27°24′N, 52°38′E). The sampling sites in this study represent areas of importance in seaweed harvest (Gas and Oil facilities and Village of Olee) and areas close to sources of anthropogenic pollution (Gas and Oil facilities, and Nuclear power facilities). Sample collection months (January and May 2010) subscribe to cold and warm seasons. Seasonal physicochemical parameters of the seawater (temperature, salinity and pH) are reported in (Table 1). *Ulva intestinalis* and *Sargassum angustifolium* samples (0.5 to 1 kg fresh weight) were collected at low tide in the intertidal zone at a depth of ≤1 m. Five cm from the tip of the algae was removed. To obtain a more representative sample within each collection site, an area of approximately 15 m in diameter was sampled at five different points. Seaweeds were cleaned in the field by rinsing with sea water and then with double-distilled water (DDW). They were kept in pre-cleaned glass jars with seawater and stored in a cooler on ice before transferring to the laboratory.

Dogfish muscle (DORM-3, National Research Council of Canada), and GBW07313 (Marine sediment, China) were used as reference material for all analyses. Analytical blanks were run with each batch of samples (ten samples in each batch). Instrument wavelength accuracy was tested by the use of SRM 930e standard (National Institute of Standards and Technology, Gaithersburg, MD). Absorbance of an acetone solution blank (90 %) at 750 nm and at 630, 647 and 664 nm were measured to correct for primary pigment absorbance (Table 2). Chlorophyll was extracted from seaweed (10 mL acetone: 90 mL H_2O for each 0.5 g sample) the next day as described in De Jong et al. (1994). Extracts were homogenized using a B-Brawn type homogenizer (B. Braun Biotech International, model type 853023/8, Germany) at 1,000 rpm for one minute. The homogenate was filtered through two layers of cheese cloth and centrifuged using a Nuve Fuj 650 model centrifuge (Nüve, model 650, Turkey) at 2,000 rpm for 10 min. The

Table 1 Physiochemical parameters of the seawater including temperature, pH, and salinity (mean of 3 samples ± SEM) for four collection sites along the coast of Bushehr Province, in the Persian Gulf

Factor	Oil facilities		Nuclear facilities		Village of Olee		Gas facilities	
	Jan 2010	May 2010	Jan 2010	May 2010	Jan 2010	May 2010	Jan 2010	May 2010
Temperature (°C)	15 ± 0.1	24 ± 0.2	19 ± 0.2	28 ± 0.1	21 ± 0.2	28 ± 0.1	18 ± 0.1	28 ± 0.1
PH	7.4 ± 0.1	7.3 ± 0.1	8.5 ± 0.1	7.8 ± 0.1	8.1 ± 0.2	7.9 ± 0.1	8.3 ± 0.1	7.8 ± 0.2
Salinity (ppt)	38 ± 0.1	39 ± 0.1	38 ± 0.1	42 ± 0.2	40 ± 0.2	42 ± 0.1	39 ± 0.1	39 ± 0.1

Table 2 Results of our quality assurance/quality control work

Chlorophyll	Certified values	This study values	Recovery (%)
a	4.86	4.9	101
b	1.02	1	98
c	0.37	0.35	95
Metal	Certified values	This study values	Recovery (%)
Ni	1.28	1.13	88
Cd	0.23	0.29	79
Cu	15.5	14	90
Pb	0.4	0.36	90

supernatant was separated and the absorbance was recorded with a UV/VIS spectrophotometer (LKB Ultraspec II UV/VIS, model 4050, England. spectral resolution 1.00 nm). Wavelengths chosen for analysis were 647 and 664 nm for chlorophyll b and a of *U. intestinalis*, and 630 and 664 nm for chlorophyll c and a of *S. angustifolium*. Pigment contents were calculated in mg g^{-1} weight by applying the absorption coefficient equations described by Jeffrey and Humphrey (1975). Seaweeds were washed, oven dried to constant weigh, crushed, homogenized, labeled, and stored in sterile polyethylene bottles. Samples (1–2 g) were put in sealed flasks (10 mL HNO3/HCl (1:3 v/v) and digested for 12 h on a hot plate at 140°C until the solution was clear. The digests were then filtered through acid-cleaned 0.45 mm membrane filters, diluted to 25 mL with DDW and stored at 4°C for soluble metal analysis. The digests were then filtered through acid-cleaned 0.45 mm membrane filters, diluted to 25 mL with DDW and stored at 4°C for soluble metal analysis.

A flame atomic absorption spectrometer (model Spectr AA 220FS, M ulgrave, Victoria, Australia) with deuterium background corrector was used to measure Ni, Cd, Cu, and Pb in seaweed samples. The following wavelengths (in parentheses) were used for the detection of Ni (232 nm), Cd (228.8 nm), Cu (324.8 nm) and Pb (283.3 nm). The flame composition was acetylene 2.0 and air 13.5 (liter/min). The nebulizer aspiration flow rate was kept between 5.5 and 6.0 mL/min. To each standard, sample, blank and certified reference material the following matrix modifiers were added; ammonium nitrate for Cd and Cu and magnesium nitrate for Ni and Pb. Measurements for each standard, sample, blank and reference material were replicated three times. The detection limits were calculated as IUPAC recommendation (IUPAC-Analytical Chemistry Division, 1978). Detection Limits in the sample tests were: Ni (0.05 µg g^{-1}), Cd (0.02 µg g^{-1}), Cu (1 µg g^{-1}) and Pb (2 µg g^{-1}). Reference materials were included for all analyses. The instrument was calibrated based on a linear six-point calibration curve for Ni and Pb (0.5, 1, 10, 50 and 100 mgl^{-1}); and for Cu and Cd (0.1, 0.5, 1, 10 and 50 mgl^{-1}). Standard calibration curves for Ni ($r^2 = 0.9989$), Cd ($r^2 = 0.9787$), Cu ($r^2 = 0.9899$), and Pb ($r^2 = 0.9824$) were generated. Analytical blanks were run with each batch of samples (ten samples in each batch) and run similarly.

All statistical analyses were performed by SPSS for Window (version 12, Texas Instruments, IL, USA). Analytical precision gave a mean error of 5 %. The means of

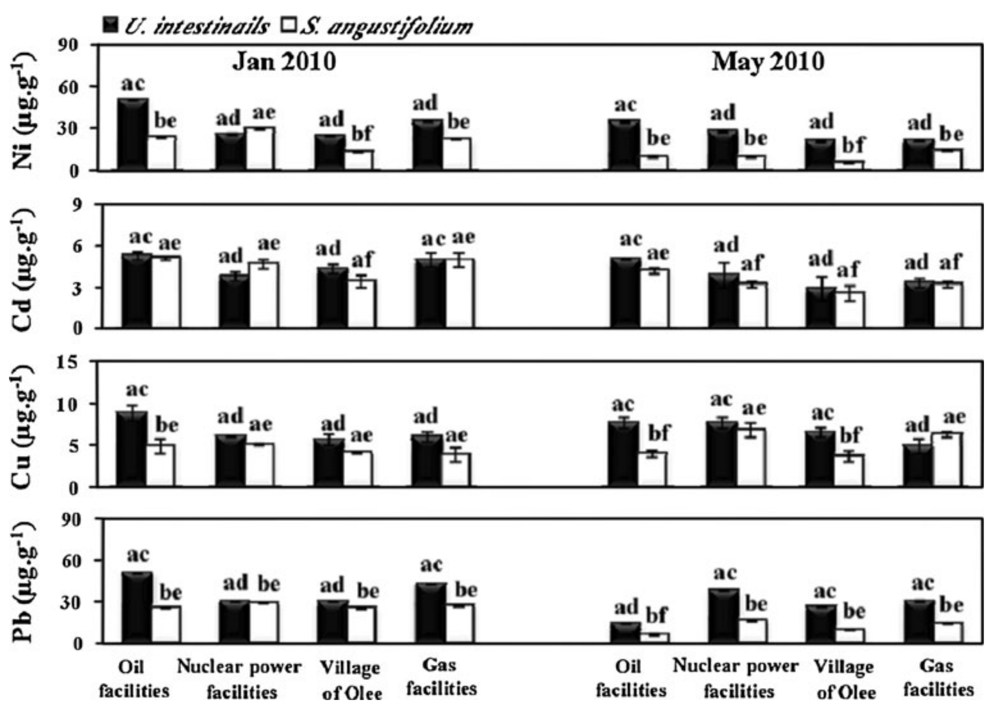

Fig. 2 Mean (±SEM) concentrations (µg g^{-1}) of Ni, Cd, Cu and Pb in *U. intestinalis* and *S. angustifolium* tips from four sites on the Iranian coast of Bushehr Province. Notations: **a** and **b** show significant metal differences between species; **c** and **d** show significant differences between stations for *U. intestinalis* within each collection period; **e** and **f** show significant differences between stations for *S. angustifolium* within each collection period

three replicates were used. All data were tested for normality of distribution and homogeneity of variance before the parametric statistical analysis. Variability between sampling sites was analyzed for each metal by one-way ANOVA. To detect differences between individual means, we used Tukey's Multiple Comparison test. The relationships between chlorophyll content and heavy metal concentration in the macroalgal species were evaluated by simple correlation analysis (p was set at ≤0.05).

Results and Discussion

Metal levels decreased in the order: Pb > Ni > Cu > Cd (Fig. 2). Overall, there were higher metal levels in seaweed in January than in May. In *Ulva,* Ni, Cu and Pb levels were higher than in *Sargassum.* This may be attributed to several factors. First, increased precipitation in the colder season may have facilitated the transport of terrestrial pollutants into the aquatic environment, making pollutants more readily available for uptake by algae. In January, there is also increased oil-tanker traffic in the Persian Gulf, which is accompanied by more incidents of oil-leaks, and an overall increased use of fossil fuels that adds to metal pollution. In addition, metabolic changes in algae can affect metal levels. Algal growth is accompanied by decreased metal concentrations. Riget et al. (1995) have suggested that reduced metabolic activity of algae in winter leads to increased metal accumulation. Increased algal metabolism in the spring has a "diluting" effect of metal levels in the algae.

Chlorophyll results are shown in Fig. 3. Table 3 tabulates significant correlations between metal levels and chlorophyll content. In *U. intestinalis*, we detected a negative correlation between chlorophyll b content and increasing Ni ($r = -0.64$, $p < 0.01$); and between the ratio of chlorophyll b/a and increasing Ni ($r = -0.67$, $p < 0.01$). A negative correlation also existed between chlorophyll c/a and increasing Ni in *S. angustifolium*. Existing high negative correlations between chlorophyll a content ($r = -0.84$, $p < 0.01$), chlorophyll c content ($r = -0.82$, $p < 0.01$), and ratio of chlorophyll c/a in *S. angustifolium* ($r = -0.93$, $p < 0.001$) and Ni concentration in this algae shows the content ratio of chlorophyll reflects the negative effect of high concentrations of metals. Negative correlations existed between chlorophyll b and increasing Pb concentration ($r = -0.52$) and between chlorophyll b to a (b/a) and increasing Pb concentration ($r = -0.55$) in *U. intestinalis*; and between chlorophyll c to a (c/a) and increasing Ni ($r = -0.62$), and between chlorophyll a and increasing Pb concentration ($r = -0.51$) in *S. angustifolium*. The plants are likely responding to both of these stressors simultaneously. Both Ni and Pb had high levels in both species when compared to levels in

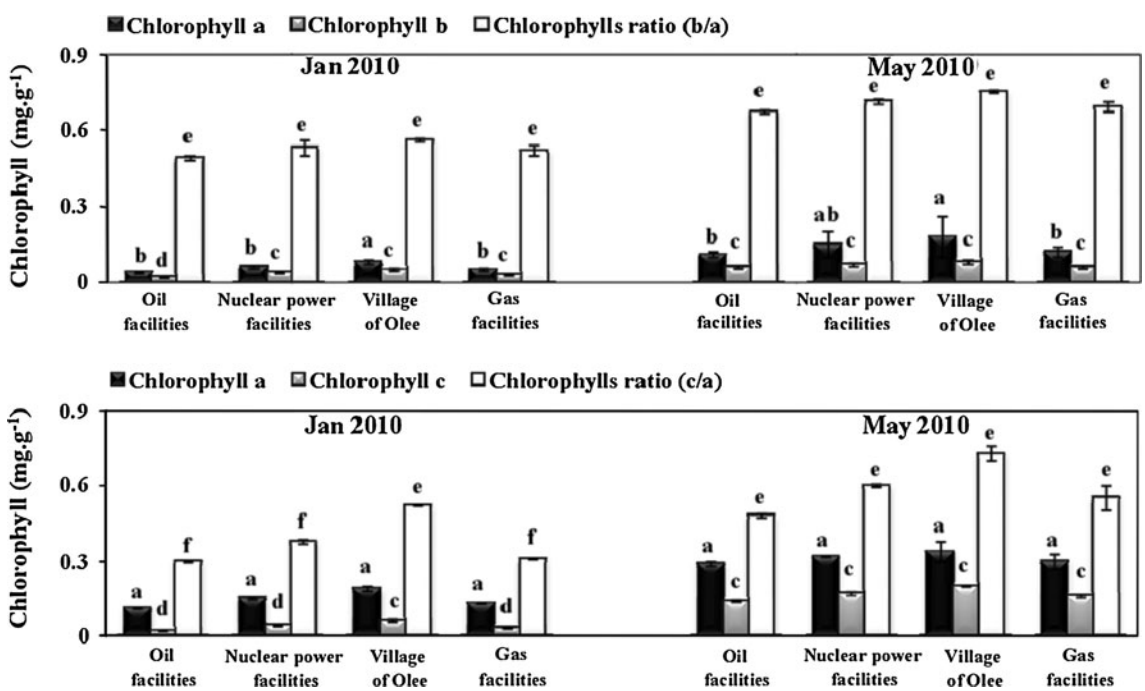

Fig. 3 Mean (±SEM) chlorophyll content (mg g^{-1}) in *U. intestinalis* and *S. angustifolium* tips from four sites on the Iranian coast of Bushehr Province. Notations: **a** and **b** show significant differences between stations in chlorophyll a concentrations; **c** and **d** show significant differences between stations in concentrations of chlorophyll b in *U. intestinalis* and chlorophyll c in *S. angustifolium,* respectively; **e** and **f** show differences between stations in chlorophyll ratios

Table 3 Significant correlations between metal concentrations and chlorophyll content in two seaweed species from Bushehr Province on the coast of the Persian Gulf, Iran

Species	Chlorophyll type	Ni	Cd	Cu	Pb
U. intestinalis	Chlorophyll a	−0.30[ns]	0.36[ns]	0.14[ns]	0.11[ns]
	Chlorophyll b	−0.64**	0.23[ns]	0.34[ns]	−0.52*
	Chlorophylls ratio (b/a)	−0.67**	−0.43[ns]	0.58*	−0.55*
S. angustifolium	Chlorophyll a	−0.84**	−0.40[ns]	0.17[ns]	−0.51*
	Chlorophyll c	−0.82**	0.24[ns]	−0.05[ns]	0.40[ns]
	Chlorophylls ratio (c/a)	−0.93***	−0.61*	0.11[ns]	−0.62**

ns = not significant at the level of 0.05

* significant at 0.05; ** significant at 0.01; *** significant at 0.001

Table 4 *Ulva* (green seaweed), and *Sargassum* (brown seaweed), metal concentrations (μg g^{-1} dry weight) from various geographical locations

Species, region	Metal levels (μg g^{-1} dry weight)			
	Ni	Cd	Cu	Pb
U. intestinalis, Kuwait coast[a]	6–9	ND	60–120	2–9
Ulva sp., Moreton Bay (Australia)[b]	ND	<1	15	6
U. intestinalis, Delmarva Peninsula (USA)[c]	<1–2	ND	<1–17	3–5
Ulva sp., San Jorge Gulf (Argentina)[d]	<5	<4	<4	<2
U. lactuca, Saudi coast of the Persian Gulf[e]	26–33	<1	8–19	13–18
U. intestinalis present study	16–50	2–5	6–9	26–51
S. subrepandum, Red Sea coast (Saudi Arabia)[f]	0.5	0.1	ND	0.4
S. angustifolium, Kuwait coast[a]	3.5	ND	85	7
Sargassum sp., West coast of Aegean Sea[g]	14	0.1	2	0.02
S. angustifolium, Saudi coast of the Persian Gulf[e]	ND	1.3	4–7	12–17
S. binderi, Gulf of Aden (Yemen)[h]	3	1	10	2
S. angustifolium, present study	2–30	2–5	2–7	9–32

ND, not determined

[a] Buo-Olayan and Subrahmanyam (1996)

[b] Gosavi et al. (2004)

[c] Anish et al. (2007)

[d] Perez et al. (2007)

[e] Al-Homaidan (2008)

[f] El- Naggar and Al- Amoudi (1989)

[g] Sawidis et al. (2001)

[h] Al-Shwafi and Rushdi (2008)

same species from other studies (Table 4). In agreement with current knowledge, as water temperatures increased – followed by increased algae metabolism – we witnessed decreased metal in both algae types in this study. In *Ulva,* Ni, Cu and Pb levels were higher than *Sargassum.* This may be due to generally higher tolerance of *Ulva* to metals. Cadmium levels were higher in *Sargassum,* which may have been due to the presence of chelating compounds in the cell walls of this alga with a high affinity for adsorbing Cd. Based on the data in Table 3, it can be argued that *Sargassum* responds to changes in the environment more vividly than *Ulva.*

The village of Olee was the most undisturbed area we studied. This was reflected by significantly lower ($p < 0.05$) metal levels in the algae than at the other stations for both collecting seasons, with an exception for Pb in both seaweeds in May, 2010. This site, which is the furthest from any industrial facilities, exhibited the least amount of metal pollution. Samples collected from sites near Oil (#1) and Gas (#4) Facilities had the highest metal levels. These sites are near large petrochemical industries and support large urban populations. Domestic sewage runoffs may also contribute to high levels of metals in the sediment in these sites.

Levels of heavy metals in the same species of algae from other geographical locations (with emphasis on areas in the Persian Gulf) are presented in Table 4 Based on the data we present here and the data from other parts of the Persian Gulf, it is clear that the seaweed of the Persian Gulf may have been severely affected by metal contamination from oil industry. Metal variation in seaweed samples from different sampling sites may be related not only to different metal levels in the environment, but also to factors such as tidal range, temperature, and salinity. Table 5 provides information on the slope, bottom sediment and tidal area at each site. Our results showed that temperature and pH of water were significantly lower at the Oil Facilities site than at other locations ($p < 0.05$). Salinity did not differ between sites ($p > 0.05$). However, our sampling sites differed in tidal-area, slope, and bottom substrate (Table 5). Rock and gravel shores that experience wave actions allow sediment particles to remain suspended in the water column, increasing the availability of pollutants to

Table 5 Physical characteristics of sampling sites with associated pollution sources and highest and lowest metal levels in algae from the Bushehr Province of Iran in the Persian Gulf

Site		Algae metal	Tidal area (m)	Bottom substrate	Slope
1	Oil facilities	Highest algae levels of metals	400	Gravel and sandy	Relatively low
2	Nuclear power facilities		300	Rocky and some Sandy	Relatively steep
3	Village of Olee	Lowest algae levels of metals	200	Rocky with less Sandy	Relatively steep
4	Gas facilities		900	Gravel and some sandy	Low

algae. In steep slope shores, sediment particulates tend to settle in depths, making them less available to the algae. In shores with wider intertidal area, water covers the algae for longer periods, making pollutants available to the algae for extended periods. Wave action data is available for these stations. In general, wave action is considered high at the Nuclear Power Facilities site and at the Village of Olee site. The Gas Facilities site and Oil Facilities site are considered to have low and medium wave action, respectively. Despite high wave action, the Village of Olee site with a narrow tidal area and steep shores had the lowest sediment and algae levels of most metals. In contrast the Oil and Gas Facilities sites with low slopes and wide tidal areas had higher algae levels of metals than other sites. These findings corroborate with the current literature (Deheyn and Latz 2006).

The response of green algae to metal pollution by decreases in biomass and chlorophyll has been considered to be an important tool for biomonitoring and ecotoxicological research aimed at conservation and restoration of impacted littoral ecosystems. However, in this study, we generally documented stronger negative correlations between heavy metal concentrations and either chlorophyll concentrations or chlorophyll concentration ratios in the brown alga *S. angustifolium* than in the green alga *U. intestinalis*. Based on our data, *Sargassum* responds to changes in the environment more vividly than *Ulva* and appears to be a more sensitive bioindicator for stress caused by metal pollution.

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