Validating Ctenophore *Pleurobrachia pileus* as an Indicator to Harmful Algal Blooms (HABs) and Trace Metal Pollution in Kuwait Bay

Abdul Hadi Bu-Olayan[1,]*, Verghese Thomas Bivin[1]
[1] Chair & Research Associate Department of Chemistry, POB 5969, Kuwait University, Safat-13060, Kuwait.

* Corresponding Author: Tel.: +965-4987075; Fax: -
E-mail: buolayan@yahoo.com

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Abstract

Trace metal levels were high in five Kuwait Bay sites (I-V) during harmful algal blooms (HABs) enrichment following an abundance of Ctenophore, *Pleurobrachia pileus*. Observations during HABs revealed higher trace metal levels in *P. pileus* and in seawater than during the non-existence of HABs. High trace metal levels in *P. pileus* were observed during winter (November-March) rather than in summer (April-September) validating the non-usage of trace metals by the primary producers as a result of low photosynthetic activity during winter. Trace metal levels in seawater and in *P. pileus* were observed high in Site-III followed by Site-IV > Site-V > Site-II > Site-I irrespective of the two seasons signifying the degree of trace metal pollution in Kuwait Bay sites. Furthermore, high abundance of Ctenophore and HABs on one hand significantly evidenced altercation of phytoplankton species diversity recorded earlier in Kuwait Bay sites; and on the other hand it evidenced a temporary depletion of nutrient levels. Therefore, it is evident that the abundance of *P. pileus* could label HABs enrichment, trace metals and nutrient levels in Kuwait Bay which is an outcome of anthropogenic inputs discharges, local industrialization and altered hydrological variables.

Keywords: Trace metals, Ctenophore, HABs, Kuwait.

Introduction

Recently, HABs have increasingly been found all over the world. Some investigators suggested that the causes for such enrichment are: eutrophication of coastal waters which is partially caused by sewage discharge (Smayda and Shimizu, 1991), climatic changes, grazing pressure or changed physical stability of the water column favoring dinoflagellates to meet nutrient demands by vertical migration, and also through trace metal pollution (Edna *et al*., 1986). Rao *et al.* (1993) reported specific HABs enrichment like *Gonyaulax digitale* and *Dinophysis norvegica* during peak summer and before the onset of winter. Experimentally, HABs such as *Gymnodinium nelsoni*, *Heterosigma akashiwo* and *Chatotonella antiqua* were found inhibited by trace metal levels like Cu, Fe and Mn (Fuse, 1987). However, in seawater, trace metals along with nutrients and organic compounds were found to exceed the threshold limits of HABs enrichment (Pan and Rao, 1997).

In Kuwait Bay, the first massive adversities of HABs that led to "Fish Kills' were reported during the year 1999 (Arab Times, 1999). Phytoplankton species listed by Palmer (1980) and observed by Bu-Olayan *et al.* (2001) were found temporarily displaced by HABs. Furthermore, zooplankton comprising mainly of crustaceans and echinoderms larvae were found displaced by abundant Ctenophore, *Pleurobrachia pileus* in five Kuwait Bay sites in the aftermath of the 'Fish Kill' phenomenon. Apart from the grazing behavior of *P. pileus* on phytoplankton and zooplankton (Deason and Smayda, 1982), one could suspect the major impact of (a) HABs, (b) accidental spills of trace metals from the Shatt al-Arab river located at the north of Kuwait, (c) domestic sewage outfalls, (d) the loss of predation pressure due to 'fish kills', (e) new introduction of invasive Ctenophore, (f) increase in prey due to loss of fish, and (g) oil pollution, for such displacement in Kuwait Bay over the recent years. Thus, investigations were carried out to determine Ctenophore as a possible indicator to HABs and trace metal pollution in Kuwait Bay.

Materials and Methods

Five sites (Figure 1) from Kuwait Bay, which were observed for the multidisciplinary activities, were chosen for the present investigation. A mechanized boat with a towing speed maintained at 0.3 m s^{-1} was employed to collect samples using (a) phytoplankton and zooplankton nets of 10 μm and 100 μm mesh size, respectively, and (b) a Vandorn water sampler (2 L). HABs and zooplankton samples were collected at 0.1 m depth from the surface.

(a) HABs and Ctenophore analysis:

Samples were collected in sterile plastic bottles (75 ml) and stored in an icebox. Sub-samples (25 ml) were subjected to 5 ml dilution and fixed in Lugol's solution for HABs identification and counted as described by Rao and Mohanchand (1988). Ctenophore, *P. pileus* were isolated from the

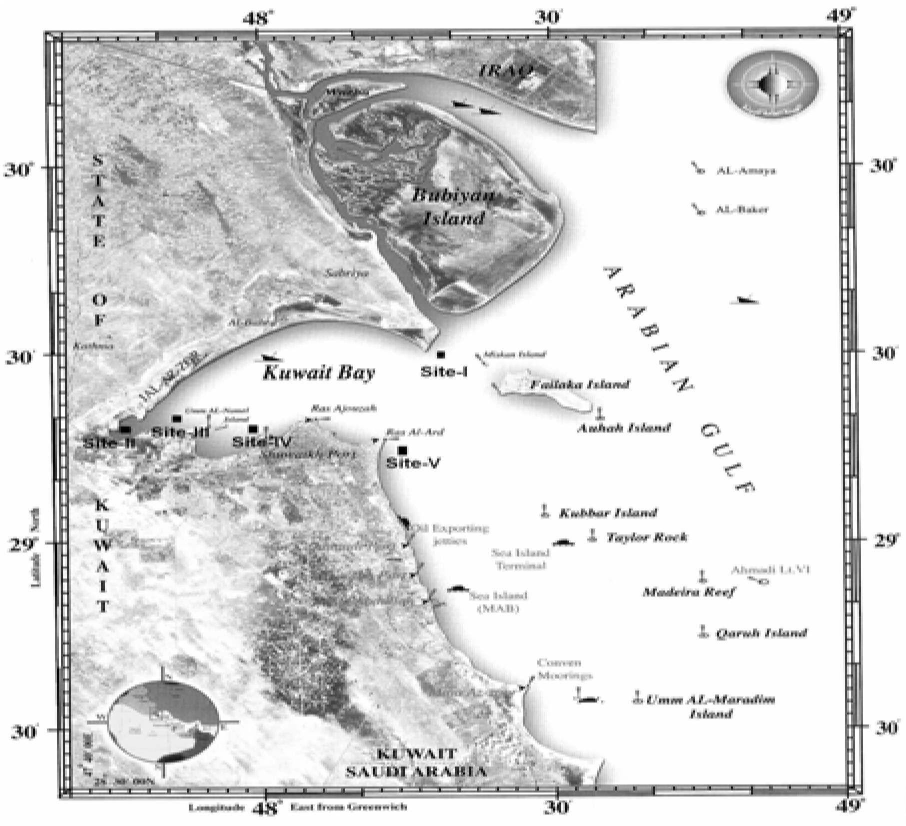

Figure 1. Map of Kuwait Bay indicating the five sites.

zooplankton collection, identified and counted from the total volume of net sample. HABs and *P. pileus* were dried separately at 40°C in an oven (GallenKamp) for 72 h. HABs (0.023 g) and *P. pileus* (0.62 g) were pre-digested in 6% Nitric acid (v/v) and 4% HCl (v/v) for 48 hrs in a 50ml Fischer brand disposable sterile centrifuge tube. The samples were diluted to 50ml in de-ionized water and digested in an automatic microwave digester (SpectroPrep-CEM). The accuracy of the method was verified using Peach leaves Standard Reference Material (SRM-1547) and Oyster Tissue (SRM 1566a) from the National Bureau of Standards, respectively. Recoveries above 96% were obtained (Table 1).

(b) Seawater analysis:

Seawater was collected by Vandorn water sampler in sterile plastic bottles (2 l) from 2 m depths from five Kuwait Bay sites. Seawater was filtered in a 0.45 μm membrane filter. One-liter seawater was added with 25 ml Ammonium-pyrrolidine-dithiocarbonate (2% v/v), 10 ml HCl and 35 ml methyl isobutyl ketone in a separatory funnel, shaken for 2 minutes and left undisturbed for 15-20 minutes. Two separate phases, namely, upper and lower solutions (A & B) were obtained. One liter of seawater was added with the upper solution (A) and the above-mentioned chemicals, and the process was repeated. The lower solution (B) was eluted in another separatory funnel. The upper solutions were collected in a 50 ml volumetric flask, and the lower solutions were discarded. The upper solutions were analyzed in Perkin Elmer 5100 Atomic Absorption Spectrophotometer (AAS) and the concentration of trace metals were measured (Table 2). Quality measures of the above procedure were carried out as described by Arnold *et al.* (1992).

Results and Discussion

Seawater, HABs and Ctenophore were analyzed for major trace metals. Five metals indicated significance to marine pollution in Kuwait Bay during the catastrophic ‘Fish Kill’ and hence the study. Trace metals were in the magnitude of Fe>Zn>Cu>Pb and Ni, in seawater and Ctenophore collected from the five sites of the Kuwait Bay. Higher trace metals were found in samples collected from Site-III (Doha) than the trace metal levels from other sites (Table 3). This could be attributed mainly to: (1) the discharge of huge amount of domestic sewage outfall from Shuwaikh, an adjoining industrial area, (2) the influence of chemical effluent discharge from wastewater treatment plant from Shuwaikh as well as from the nearby desalination plant, and (3) the non-

Table 1. Recovery of trace metals in standard reference materials, peach leaves (SRM 1547) and Oyster tissue (SRM 1566a) against HABs and Ctenophore samples

Metals	Certified values (µg/g)		Present study (µg/g)		Recovery (%)	
	SRM-1547	SRM-1566a	SRM-1547	SRM-1566a	SRM-1547	SRM-1566a
Cu	3.70	66.30	3.65	65.74	98.64	99.15
Zn	17.90	830.00	17.84	825.69	99.66	99.48
Fe	218.00	539.00	217.00	520.51	99.54	96.56
Ni	0.69	2.25	0.67	2.23	97.10	99.11
Pb	0.87	0.37	0.86	0.36	98.85	97.29

Table 2. Operating conditions for AAS (Perkin Elmer 5100) in determining the trace metal levels in samples

Metals	Gas	Wavelength (nm)	Slit-Width (nm)	Lamp Current (mA)	Conc. Range (µg/L)	D.L. Spoiler (µg/L)	Sensitivity (µg/L)
Cu*	a.ac	324.8	0.7	3	500.0	0.002	0.09
Fe*	a.ac	248.3	0.2	5	500.0	0.004	1.00
Zn*	a.ac	213.9	0.7	5	100.0	0.002	0.12
Ni**	Argon	232.0	0.2	4	37.5	1.0	0.15
Pb**	Argon	283.3	0.5	5	37.5	1.0	0.50

*: Flame, **: Graphite furnace, a.ac: air acetylene gas, D.L.: Detectable limits.

Table 3. Trace metal levels in seawater (µg/L) and Ctenophore (µg/g) from five sites of Kuwait Bay

Sites	During HABs					During Non-HABs				
	Cu	Zn	Fe	Ni	Pb	Cu	Zn	Fe	Ni	Pb
Seawater-Summer										
Site-I	2.02	2.31	3.71	1.85	0.05	1.58	2.11	2.89	1.52	0.01
Site-II	2.12	3.41	3.98	1.67	0.06	1.61	2.18	3.12	1.53	0.02
Site-III	2.76	5.55	7.56	3.01	0.29	2.17	4.59	5.46	2.19	0.16
Site-IV	2.51	4.39	5.99	2.29	0.16	2.15	4.01	4.18	1.75	0.11
Site-V	2.41	4.22	5.33	2.23	0.14	2.03	3.68	4.15	1.62	0.09
Seawater-Winter										
Site-I	2.45	2.45	3.94	2.09	0.12	1.84	2.35	3.85	2.07	0.09
Site-II	2.46	3.45	4.13	2.18	0.14	1.98	3.38	3.52	2.12	0.11
Site-III	2.84	5.82	7.45	3.14	0.61	3.05	5.62	6.11	2.45	0.33
Site-IV	2.69	4.56	6.59	2.65	0.55	2.58	4.44	4.58	2.38	0.21
Site-V	2.81	4.12	5.48	2.58	0.49	2.27	3.79	4.26	2.26	0.18
Ctenophore-Summer										
Site-I	3.55	4.19	4.62	2.54	1.84	2.56	3.01	4.15	2.49	1.25
Site-II	4.02	5.82	5.88	2.61	3.26	3.18	3.55	5.61	2.59	2.52
Site-III	4.88	6.75	11.02	4.45	4.64	4.12	6.22	9.18	3.71	3.12
Site-IV	4.61	6.48	10.12	3.69	3.71	3.54	6.19	9.07	3.25	2.98
Site-V	9.92	5.68	9.62	4.51	3.61	3.76	5.14	8.93	3.57	2.92
Ctenophore-Winter										
Site-I	4.51	5.21	5.78	2.65	1.98	3.61	4.24	5.19	2.61	1.96
Site-II	4.54	5.95	6.86	2.91	2.54	3.73	5.59	6.61	2.75	2.14
Site-III	6.87	6.84	7.81	4.77	3.75	5.15	6.11	7.29	5.01	3.46
Site-IV	6.12	6.51	7.74	4.65	2.64	4.45	5.63	7.18	4.37	2.94
Site-V	4.59	6.26	6.98	4.49	2.63	4.19	5.44	6.92	4.16	2.42

HABs: Harmful algal blooms: dominated by *Dinophysis* sp., *Protoperidium* sp., and *Peridinum* sp., non-HABS: Dinoflagellates, Desmids and diatoms, Sites I-V: Subiyah, Doha, Khadma, Towers, Salmiya.

removal of sediment deposition due to the typical nature of low water current action in the Bay. This provides evidences to the findings of Smyada and Shimizu (1991) and Bu-Olayan *et al.* (2001). Seasonal analysis revealed higher metal levels in seawater and Ctenophore during winter than in summer, irrespective of the sites sampled in Kuwait Bay. The views of Ahner and Morel (1995), and Benderliev and Ivanova (1996), describing the non-utility of trace metals by the primary producers due to low photosynthetic activity, could support such seasonal changes in trace metal levels.

Comparatively, considerable increases of Fe and Pb levels were observed in the aftermath of the ‘fish kill' in 1999-2000 in Kuwait Bay (Table 3). Fe and Pb levels were found to be higher than the levels of seawater described by Michael (1994) and Bu-Olayan *et al.* (2001). Thus, observations showed a significant displacement of HABs over beneficial phytoplankton along with an increase in trace metal levels in the Kuwait Bay sites.

The occurrence of HABs and 'fish kill' could be related to the abundance of Ctenophore *P. pileus* in Kuwait Bay. The Ctenophore mainly fed on diatoms and crustacean zooplankton (Frank, 1986). *P. pileus* was observed highly and temporarily (10^3 species/L) in Site-I (Subiyah), and they migrated within few days towards Site II-V (Khadma, Doha, Kuwait Towers and Salmiya). This could be attributed to (a) the unidirectional flow of water current from the north to the south (Subiyah to Salmiya) and (b) the low nutrient levels (0.01, 0.09 and 0.64 µg/L of phosphate, nitrate and silicate) in Subiyah when compared to (0.02, 0.72 and 0.92 µg/L of phosphate, nitrate and silicate) other Kuwait Bay sites respectively (Bu-Olayan *et al.*, 2001). Thus, we could also correlate to the temporary displacement and low nutrient levels due to the enrichment of Ctenophore population.

P. pileus abundance were in the sequence of $10^3>10^4>10^2>10^1$ species/L in Site II-V, respectively. Comparatively, *P. pileus* were noted during the onset of winter (November) and summer (March) rather than the other periods of the year and found to coincide with HABs enrichment. They were rarely observed in the absence of HABs, thus supporting the views of Deason and Smayda (1982) and Rao *et al.* (1993).

Analysis revealed higher trace metal levels in seawater during HABs than during their absence (Table 4). Low trace metal levels were observed during the absence of *P. pileus*. Thus, one cannot ignore the possibilities of trace metal accumulation in Ctenophore which could also alter the seawater quality to a certain extent. ANOVA tests revealed high significant differences between trace metal levels in seawater and Ctenophore with that of the HABs enrichment irrespective of seasons (Table 5). Furthermore, studies are recommended to establish: (1) Ctenophore abundance as an indicator to HABs enrichment, (2) the remedial measures in the Bay to prevent Ctenophore population and (3) to control industrial pollutants from domestic sewage outfalls, chemical treatment and power plants in Kuwait Bay region.

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Table 4. Seasonal analysis of seawater (µg/L) and Ctenophore (µg/g) collected during the presence and absence of HABs off the Kuwait Bay.

Metals	During HABs		During Non-HABs	
	Mean±S.D.	Range	Mean±S.D.	Range
Seawater-Summer				
Cu	2.36±0.29	2.02- 2.76	1.90±0.08	1.58-2.17
Zn	3.97±1.19	2.31- 5.55	3.31±1.10	2.11-4.59
Fe	5.31±1.57	3.71- 7.56	3.96±1.02	2.89-5.46
Ni	2.21±0.09	1.67- 3.01	1.72±0.06	1.52-2.19
Pb	0.14±0.42	0.05- 0.29	0.07±0.65	0.01-0.16
Seawater-Winter				
Cu	2.65±0.25	2.45-2.84	2.34±0.09	1.84-3.05
Zn	4.08±1.25	2.45-5.82	3.91±1.13	2.35-5.62
Fe	5.51±1.77	3.94-7.45	4.46±1.21	3.52-6.11
Ni	2.52±0.08	2.09-3.14	2.25±0.07	2.07-2.45
Pb	0.38±0.45	0.12-0.61	0.18±0.68	0.09-0.33
Ctenophore-Summer				
Cu	5.39±1.20	3.55-4.88	3.43±0.90	2.56-4.12
Zn	5.78±1.30	4.19-6.75	4.82±1.04	3.01-6.22
Fe	8.25±1.61	4.62-11.02	7.38±1.42	4.15-9.18
Ni	3.56±1.15	2.54-4.45	3.12±0.51	2.49-3.71
Pb	3.41±1.08	1.84-4.64	2.55±0.63	1.25-3.12
Ctenophore-Winter				
Cu	5.32±1.50	4.51-6.87	4.22±1.69	3.60-5.15
Zn	6.15±1.70	5.21-6.84	5.40±1.99	4.24-6.11
Fe	7.03±2.30	5.78-7.81	6.63±2.19	5.19-7.29
Ni	3.89±1.02	2.65-4.77	3.78±1.07	2.61-5.01
Pb	2.70±1.00	1.98-3.75	2.58±0.81	1.96-3.46

S.D.: Standard Deviation; HABs: dominated by *Dinophysis* sp., *Protoperidium* sp. and *Peridinum* sp.;
non-HABS: Dinoflagellates, Desmids and diatoms

Table 5. Statistical test by ANOVA between seawater, and Ctenophore of the Kuwait Bay, Kuwait

Variables	*DF*	*SS*	*F*	*P*	Test of Significance
A. During HABs enrichment:					
Between seawater & Ctenophore (S)	24	59.35	39.74	0.003	*
Between seawater & Ctenophore (W)	24	72.61	95.05	0.002	*
Between seawater (S) & seawater (W)	24	92.98	2953.19	0.001	*
Between Ctenophore (S) & Ctenophore (W)	24	114.18	77.68	0.002	*
B. During Non-HABs enrichment:					
Between seawater & Ctenophore (S)	24	42.54	74.95	0.003	*
Between seawater &Ctenophore (W)	24	55.00	104.08	0.002	*
Between seawater (S) & seawater (W)	24	53.86	710.01	0.001	*
Between Ctenophore (S) & Ctenophore (W)	24	87.84	88.07	0.002	*
C. HABs Vs Non-HABs enrichment:					
Between seawater (H) Vs seawater (NH)--(S)	24	91.26	860.85	0.002	*
Between Ctenophore (H) Vs Ctenophore (NH)--(S)	24	114.18	77.68	0.001	*
Between seawater (H) Vs seawater (NH)--(W)	24	85.48	418.20	0.002	*
Between Ctenophore (H) Vs Ctenophore (NH)--(W)	24	69.62	287.22	0.001	*

DF: Degree of freedom, *SS*: Sum of squares, *P*: Probability, *significant;
S: summer, W: winter, H: HABs, NH: Non-HABs

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