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EFFECTS OF TRACE METALS, HARMFUL ALGAL BLOOMS, NUTRIENTS AND HYDROLOGICAL VARIABLES TO MULLET (*LIZA KLUNZINGERI)* IN KUWAIT BAY

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ABSTRACT

A catastrophic 'fish kill' took place in Kuwait Bay between 1999 and 2000, specifically to mullet, *Liza klunzingeri*. Its recurrence in the consecutive years in low profile only in the Bay region and not elsewhere along the Kuwait Coast stimulated us to investigate the causative factors in five important sites of Kuwait Bay. Among various Harmful Algal Blooms (HABs), *Dinophysis caudata* was found predominant in the five sites of the Bay. Trace metal levels in seawater, HABs and *L. klunzingeri* were higher (8.11 ìg l^{-1}, 45.14 ìg $g^{-1}$and 67.81 ìg g^{-1}, respectively) during HABs enrichment than during their absence (6.11ìg l^{-1} 40.29ìg g^{-1} 67.41ìg g^{-1}, respectively). Observations revealed high mortality of this species due to high trace metals with low nutrient levels (Phosphate, Nitrate and Silicate), and high ammonia levels during high HABs enrichment. Further, high temperature, turbidity and low dissolved oxygen were found to enhance the HABs in the Bay. These variations can be attributed to pollution from wastewater discharges, anthropogenic sources, rapid industrialization and human activities in the Bay. Furthermore, such 'fish kills' may not only recur in the near future but are anticipated to cause (1) direct and indirect harmful effects to fish consumers, in man and (2) economical loss from fishing industry, and hence the investigation.

Key words*:* Trace metals; HABs; *L. klunzingeri*; Kuwait Bay.

INTRODUCTION

The first massive outbreak of a HAB that led to "Fish Kills', particularly of the mullet, *Liza klunzingeri* ('Maid fish'), was reported in Kuwait Bay, during the year 1999[1]. Lists on few phytoplankton species (inclusive of oil and trace metal indicator species)[2] were found to support evidences to the species listed in five coastal areas of Kuwait Bay[3]. However, during the year 1999, most of the phytoplankton was found dominated by HABs species *such as Dinophysis caudata, D. norvegica (a diorrhetic shell fish poisoning-DSP), Protoperidinium spp., Peridinium spp., and Gymnodinium spp.,* in the Kuwait Bay. Earlier studies revealed increased trace metal levels in seawater due to anthropogenic inputs, seasonal influences and low photosynthetic activity[4-5]. The relationship between nutrient depletion during summer and the enrichment of HABs was observed[6]. The hydrological variables that influence the blooms of six species of red tide flagellates in Osaka Bay was described earlier[7]. Along with these studies, high temperature and pH leading to the formation of free ammonium in seawater were also reported[8]. The HABs are anticipated to recur in the future, during favorable conditions in the Kuwait marine ecosystem. Hence, a baseline study was made to determine (a) the effects of trace metals levels in seawater, HABs, phytoplankton and *L. klunzingeri*, (b) nutrients levels and hydrological variables from five sites of Kuwait Bay that exacerbate marine pollution problems and (c) if dominating HABs such as *D. caudata* are specifically linked to mass mortality of *L. klunzingeri*.

MATERIALS AND METHODS

Five sites, namely Subiyah, Umm-Al-Nammil Is. near Doha, Khadma, Kuwait Tower and Salmiya in Kuwait Bay (Sites I-V in Fig.1), affected by 'maid fish kill' were selected for the present study. A mechanized boat with a towing speed maintained at 0.3m s^{-1} was employed to collect samples by (a) using phytoplankton net of 5mm mesh size, (b) Vandorn water sampler and (c) trawl net for mullet fish. Based on the densities of HABs, samples were labeled "HABs" and "non- HABs" respectively.

Determination of trace metals in seawater

Trace metals, Cu, Zn, Fe, Pb and Ni, in seawater that were found (i) significant to marine pollution in the Kuwait Bay[3] and (ii) within the

Table 1 : Metal-wise statistical analysis in seawater, HABs, phytoplankton and 'Maid fish' collected during HABs and non-HABs enrichment in Kuwait Bay

Metals	Mean	S.D.	Range	Tests description	Pearson's correlation (r)	t-value/* significance
1. Seawater – during HABs						
Cu	2.40	±0.26	2.02-2.84	1. Vs 2.	0.99	2.67*
Zn	3.98	±1.15	2.31-5.72	1. Vs 3.	0.92	
Fe	5.47	±1.58	3.71-8.11	1. Vs 4.	0.93	
Ni	0.15	±0.08	0.05-0.31	1. Vs 5.	0.90	
Pb	2.40	±0.42	1.95-3.14	1. Vs 6.	0.90	
2. Seawater –during non-HABs						
Cu	2.11	± 0.08	1.98-2.25	2. Vs 2.	-	
Zn	3.35	± 1.05	2.15-4.62	2. Vs 3.	0.91	
Fe	4.12	± 1.07	2.89-6.11	2. Vs 4.	0.92	
Ni	0.07	± 0.06	0.01-0.19	2. Vs 5.	0.86	
Pb	1.87	± 0.63	1.12-3.02	2. Vs 6.	0.86	
3. HABs						
Cu	19.92	±16.36	0.54-36.87	3. Vs 2.	0.91	
Zn	22.42	±11.33	5.10-32.84	3. Vs 3.	-	
Fe	30.77	±14.83	9.41-45.14	3. Vs 4.	0.99	14.87*
Ni	11.25	± 6.92	2.54-19.75	3. Vs 5.	0.94	
Pb	12.93	± 9.96	0.85-29.87	3. Vs 6.	0.94	
4. Non-HABs –(Phytoplankton)						
Cu	16.01	±13.22	0.45-31.12	4. Vs 2.	0.92	
Zn	18.77	±11.66	2.59-31.11	4. Vs 3.	0.99	
Fe	27.16	±13.68	8.15-40.29	4. Vs 4.	-	
Ni	8.15	± 5.42	1.56-15.46	4. Vs 5.	0.95	
Pb	9.84	± 6.66	0.41-18.91	4. Vs 6.	0.95	
5. Maid fish- during HABs						
Cu	7.90	±0.10	7.77-8.02	5. Vs 2.	0.86	
Zn	32.93	±0.86	31.91-33.79	5. Vs 3.	0.94	
Fe	66.64	±1.00	65.48-67.81	5. Vs 4.	0.95	
Ni	1.44	±0.13	1.32-1.62	5. Vs 5.	-	
Pb	2.74	±0.16	2.54-2.93	5. Vs 6.	1.00	2.47*
6. Maid fish- during non-HABs						
Cu	7.74	±0.21	7.48-7.95	6. Vs 2.	0.86	
Zn	32.18	±1.17	30.98-33.68	6. Vs 3.	0.94	
Fe	66.12	±1.04	65.12-67.41	6. Vs 4.	0.95	
Ni	1.32	±0.14	1.21-1.54	6. Vs 5.	1.00	
Pb	2.66	±0.18	2.49-2.86	6. Vs 6.	-	

Mean & Range in (ìg l^{-1}) for seawater and (ìg g^{-1}) for other samples; S.D.: Standard Deviation; HABs: dominated by Dinophysis sp., Protoperidium sp., and Peridinum sp., non- HABs: phytoplankton (diatoms, harmless dinoflagellates); *significant at $P < 0.05$.

detectable limits of Atomic Absorption Spectrophotometer (AAS) were chosen for the present study. Two-liter seawater samples collected from Kuwait Bay sites (I-V) were filtered in a 0.45mm membrane filter. One-liter were amended with 25 ml ammonium-pyrrolidine-dithiocarbonate (APDC-2% v/v), 10mL HCl (0.5 M) and 35mL methyl isobutyl ketone (MIBK-99.5%) 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. To one liter of fresh seawater, the upper solution (A) was added with APDC, HCl and MIBK and the process was repeated. In another separatory funnel, the lower solution (B) was amended with the above chemicals and eluted. The upper solutions from both A and B were collected in a 50mL volumetric flask and the lower

Table - 2 : Station-wise statistical analyses in seawater, HABs, phytoplankton and Maid fish during HABs and non-HABs enrichment in Kuwait Bay

Stations	Mean	S.D.	Range	Tests description	Pearson's correlation (r)	t-value/* significance
1. Seawater – during HABs						
Subiyah	2.09	±1.24	0.05-3.84	1. Vs 2.	0.99	6.63*
Khadma	2.40	±1.44	0.05-4.13	1. Vs 3.	0.93	
Doha	3.92	±2.72	0.29-8.11	1. Vs 4.	0.93	
Tower	2.83	±1.89	0.09-5.48	1. Vs 5.	0.95	
Salmiya	3.15	±2.19	0.15-6.59	1. Vs 6.	0.95	
2. Seawater –during non-HABs						
Subiyah	1.79	±1.04	0.01-3.15	2. Vs 2.	-	
Khadma	1.86	±1.07	0.02-3.22	2. Vs 3.	0.92	
Doha	3.07	±2.07	0.16-6.11	2. Vs 4.	0.92	
Tower	2.22	±1.64	0.02-4.26	2. Vs 5.	0.96	
Salmiya	2.51	±1.67	0.11-4.68	2. Vs 6.	0.97	
3. HABs						
Subiyah	3.74	±3.49	0.54-9.62	3. Vs 2.	0.92	
Khadma	9.50	±6.97	2.48-19.62	3. Vs 3.	-	
Doha	32.30	±8.84	18.6-44.81	3. Vs 4.	1.00	3.76*
Tower	23.38	±8.54	13.9-35.18	3. Vs 5.	0.82	
Salmiya	28.35	±12.35	14.1-40.29	3. Vs 6.	0.82	
4. Non-HAB's –(Phytoplankton)						
Subiyah	2.94	±3.08	0.41-8.19	4. Vs 2.	0.92	
Khadma	7.68	±5.78	2.12-16.10	4. Vs 3.	1.00	
Doha	26.86	±9.81	14.1-40.29	4. Vs 4.	-	
Tower	19.81	±8.65	9.86-32.12	4. Vs 5.	0.81	
Salmiya	23.64	±10.8	11.1-40.28	4. Vs 6.	0.82	
5. Maid fish- during HABs						
Umm-al				5. Vs 2.	0.96	
Nammil				5. Vs 3.	0.82	
Is. (site				5. Vs 4.	0.81	
near				5. Vs 5.	-	
Doha)	11.43	±4.53	6.73-18.21	5. Vs 6.	1.00	2.91*
6. Maid fish- during non-HABs						
Umm-al				6. Vs 2.	0.97	
Nammil				6. Vs 3.	0.82	
Is. (site				6. Vs 4.	0.82	
near				6. Vs 5.	1.00	
Doha)	10.59	±4.35	5.55-16.32	6. Vs 6.	-	

Mean & Range in (ìg l^{-1}) for seawater and (ìg g^{-1}) for other samples; S.D.: Standard Deviation; HABs: dominated by *Dinophysis sp., Protoperidium sp.*, and *Peridinum sp.*; Phytoplankton: dominated by diatoms, non-HABs dinoflagellates, *significant at P <0.05.

solutions discarded. The upper solutions were analyzed in AAS and the concentration of trace metals measured. Quality measures were followed[9].

Phytoplankton analysis

Phytoplankton samples (75ml) were collected at a depth of 0.1m from the surface in sterile plastic bottles. Sub-samples (25ml) were fixed in Lugol's solution for identification of species. Sub samples were further diluted to 5ml based on plankton density. HABs causing phytoplankton were identified and counted as described[10]. The remaining phytoplankton (50ml) filtered by 0.5ìm Whatman filter paper was placed in a sterile petridish and dried in an oven at 40^{o}C for 12hrs. Dried phytoplankton weighing 0.023g (constant), was pre-digested separately in 6% Nitric acid (v/v)

Table - 3 : Mean hydrological parameters in seawater during HABs and non-HAB off the Kuwait Bay

Stations	(HAB) pH	Tem. (°C)	Sal. (‰)	DO (mg l^{-1})	Tb.	(Non-HAB) pH	Tem. (°C)	Sal. (‰)	DO (mg l^{-1})	Tb.
Subiyah-I	8.2	30	35	3.3	5	8.1	27	34	4.1	6
Khadma-II	8.1	29	34	3.5	2	8.1	26	36	3.8	2
Doha-III & UmmAl-Nammil Is.	8.2	30	36	3.4	2	8.2	28	38	4.4	3
K.Tower-IV	8.2	31	36	3.5	1	8.2	29	35	4.4	3
Salmiya-V	8.1	31	37	3.6	2	8.2	29	36	4.3	3

I-V: Sites in Fig.1, HAB: Harmful Algal Blooms, Tem: Temperature,
Sal: Salinity, DO: Dissolved Oxygen, Tb: Turbidity.

Table - 4 : Comparative analysis on the trace metals levels in seawater, HABs, phytoplankton, fish and nutrients in other parts of the globe

Description	Seawater concentration (µg l^{-1})	HABs concentration (µg g^{-1})	Phytoplankton concentration (µg g^{-1})	'Maid' Fish concentration (µg g^{-1})	References
Trace metals					
Cu	0.45-3.86	2.80-38.73	5-59.17	6.40-7.80	1,2,5
	2.02-2.84	**0.54-36.87**	**0.45-31.12**	**7.48-8.02**	**Present Study**
Fe	4.12-9.90	5.60-42.55	4.90-126.8	49.50-66.90	2,3,5
	3.71-8.11	**9.41-44.81**	**8.15-40.29**	**30.98-33.79**	**Present Study**
Zn	0.54-9.90	2.84-40.76	10.40-210	7.60-32.20	1,2,5
	2.31-5.72	**5.10-32.84**	**4.01-31.11**	**65.12-67.81**	**Present Study**
Ni	0.18-2.80	ND	2.45-41.01	1.50-1.60	1,2,5
	0.05-0.31	**2.54-19.75**	**1.56-15.46**	**1.21-1.62**	**Present Study**
Pb	0.03-12.2	0.05-32.50	4.73-209	0.20-2.80	1,2,5
	1.95-3.14	**0.85-29.87**	**0.41-18.91**	**2.49-2.93**	**Present Study**
Nutrients					
Phosphate	0.01-0.30	ND	ND	ND	4,5
	0.01-0.04	**ND**	**ND**	**ND**	**Present Study**
Silicate	0.15-0.85	ND	ND	ND	4,5
	0.12-0.81	**ND**	**ND**	**ND**	**Present Study**
Nitrate	0.02-0.55	ND	ND	ND	4,5
	0.02-0.69	**ND**	**ND**	**ND**	**Present Study**

ND: Not detected; References 1:[15]; 2:[16]; 3: [10]; 4: [17]; 5: [3].

and 4% HCl (v/v) for 48 hrs in a 50ml Fisher brand disposable sterile centrifuge tube[9]. The samples were diluted to 50ml in de-ionized distilled water and digested in an automatic microwave digester (SpectroPrep-CEM) and the metal content (ìg/g) read in the AAS. The accuracy of the method was verified using a Standard Reference Material (SRM-1547-Peach leaves) from the National Bureau of Standards. The average recoveries (%) of all the trace metal levels were 94.20 ±0.07.

Trace metals in *L. klunzingeri*

L. klunzingeri was collected from the Umm-al Nammil Island located near Doha (site III) in Kuwait Bay. This island was found (1) easily accessible to fish collection from traditional fish trap called 'Hadrah', (2) prone to varied hydrological fluctuations and (3) the center point among the five

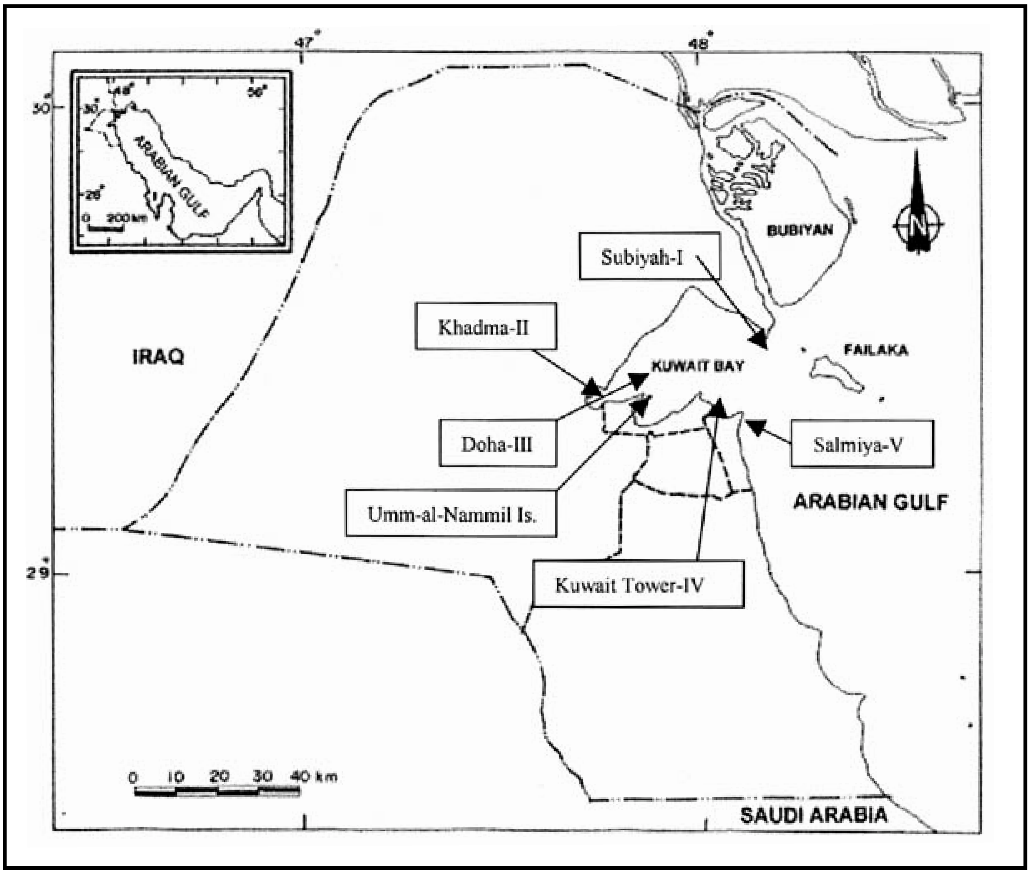

Fig. 1 : Kuwait Bay sites of the Arabian Gulf

sites of the Bay. Using non-contaminating laboratory measures, their body tissues (10g) were dried in a hot air oven (Gallen Camp II) at 50°C for 48 hrs. They were ground to <250mm size and packed in sterile polyethylene containers. Sample analysis on ground powder (2g) was carried out as described for phytoplankton. The accuracy of the method was verified by Certified Reference material: Dogfish muscle (DORM-2) from the National Bureau of Standards. The average recoveries (%) of all the trace metal levels were 95.61 ±0.06.

Determination of nutrients in seawater

The samples for nutrients analyses such as phosphate and silicate in seawater were pretreated for chloride interferences as described[9] and estimated by spectroscopy method with absorbencies measured at 880 and 810nm, respectively[11-12]. In the case of nitrate, the potential measurement of NO_3-N concentration standards and samples were recorded against a semi-logarithmic graph with a slope of +57 ± 3 mV/decade at 25°C and concentrations measured from the calibration curve[13].

Hydrological variables

Hydrological variables like pH, salinity, dissolved-oxygen, temperature and turbidity were checked using a multi-sensor array, Horiba (U-10), Int. Inc., USA. The methodologies of [9,14] were adopted to analyze ammonium level in the samples.

RESULTS AND DISCUSSION

Trace metals were in the sequence of Fe>Zn>Cu>Pb and Ni, in *L. klunzingeri*, seawater, and phytoplankton (diatoms) collected from the five sites off the Kuwait Bay. L.klunzingeri was found to be susceptible during HABs outbreak among other fish in the Kuwait Bay. This may be attributed to its

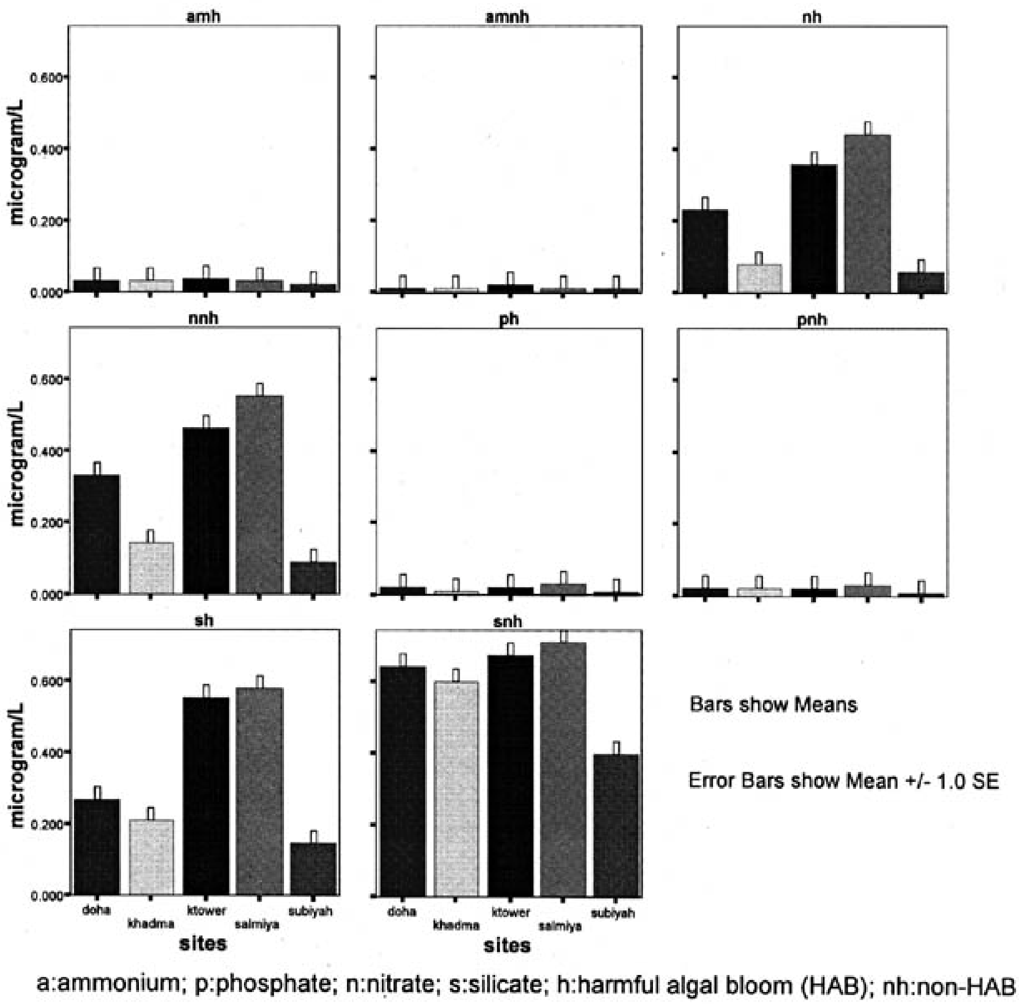

Fig. 2 : Station wise nutrient levels in seawater during HABs and Non-HABs

highly sensitivity to pollutants and varying hydrological variables. Trace metals levels was found relatively high in all the samples in Site-IV followed by Sites III>I>II>V in the presence of HABs. However, in their absence trace metal levels followed a sequence of Sites III>IV>V>II>I.

Trace metals in seawater

Trace metals were high in seawater during the occurrence of HABs enrichment than during its presence in very low densities irrespective of the sampling sites. This may be attributed to: (1) the discharge of domestic sewage outfall from Shuwaikh, an adjoining industrial area, (2) chemical effluent discharge from the nearby desalination plant and wastewater treatment plant from Shuwaikh area, (3) synergistic effects of density-stratified water column, low water current action, low utility of trace metals in seawater by the existing phytoplankton due to low photosynthetic activity or death of primary producers [4-5]. Further, t-tests revealed significant difference to trace metals in seawater analyzed during HABs than in their absence (Tables 1 and 2) and supports the earlier investigation[3].

HAB's abundance, phytoplankton and trace metal levels

Enrichment of HAB especially *D. caudata* displacing other phytoplankton was noted just before the onset of summer and winter in Kuwait Bay (sites: I-V). Site-I recorded the first observation of *D. caudata*. HABs spread to sites II-IV soon after their enrichment for a short period (within a week's time) from site-I. This may be attributed to the one way-directional flow of water current from the Shatt-al-Arab River, originating from Iraq to the North of Kuwait[3]. In the present study, trace metals were found high in *D. caudata* than in phytoplankton (harmless diatoms and dinoflagellates), attributing to: (1) favorable hydrological parameters like temperature, pH and salinity and (2) human activities in the five sites and (3) sewage and effluent disposals into the Bay. The significance of this observation was also confirmed statistically (Table 2).

Trace metals in *L. klunzingeri*

'Fish kill' to the tune of 2MT occurred first, in the year 1999 and reoccurred in the following year in an un-quantifiable measure, especially in Kuwait Bay (sites III-V)[1]. Relatively, high trace metals level was found significant during peak HABs enrichment (Table 2). Among the HABs, the most abundant species *Dinophysis caudata*, could be attributed to their indirect action to the 'fish kill', as their abundance was found to alter the dissolved oxygen at relatively high temperature in the Bay sites. Further, trace metals and site-wise Pearson's correlation revealed significant difference between seawater, HAB, phytoplankton and 'maid fish' (Tables 1 and 2). It is also interesting to observe that no other fish other than the 'maid fish', reported such high mortality in Kuwait Bay. Thus, the occurrence of HABs (*D. caudata*) could be used as an indicator species to 'fish kill'.

Nutrients in seawater

Nutrients were in the sequence of Silicate>Nitrate>Phosphate irrespective of seasons and sites. Mean nutrient levels were lower (Silicate: 0.15-0.58; Nitrate: 0.6-0.44; Phosphate: 0.01-0.03) during HABs enrichment than in the latter's absence (Silicate: 0.40-0.571; Nitrate: 0.9-0.55; Phosphate: 0.01-0.03), (Fig.2). This may be attributed to (1) the utility of nutrients from the marine environment by the HABs, (2) depletion of nutrient resources, a view that supports the earlier observation[6], (3) temperature stratification and (4) flow rate of water current in the Bay. However, in an overall view, the nutrient levels were observed lower than the earlier studies in Kuwaiti waters (Table 4).

Ammonia level was found relatively high during HABs enrichment than in their absence in this Bay when compared to the earlier studies (Fig.2) [8]. Acute ammonia toxicity with increasing temperature and low dissolved oxygen was reported by earlier workers[14]. This phenomenon was observed in the present findings during HAB enrichment and hence attributed to be one among the cause for 'fish kill' in this Bay.

Hydrological variables

Few environmental manipulations by human activities and HAB outbreak over recent years have been found to alter the hydrological features in the five sites of Kuwait Bay when compared to the earlier studies[3]. An interesting observation in the present study was that of the HABs abundance during high temperature (Table 3). This supported the other views [7]. This alteration of temperature in seawater could be attributed to (1) direct pumping of untreated sewage and effluents into the Bay and (2) warm water discharge from the power and thermal plants located in Subiyah region (Site I).

CONCLUSION

Both observations and statistical analysis supports the effects of (1) high trace metals pollution in the Bay that finds its pathway from various sources, (2) temporary replacement of *D. caudata* abundance (HABs) over other phytoplankton species (non-HABs) due to water current, biophysical and hydro-chemical factors, and the combination of marine pollutants, (3) hydrological alterations in the Bay through desalination, power and thermal plants causing high temperature and low dissolved oxygen, (4) altered nutrient levels and (5) rapid industrialization and recreational activities at the Kuwait Bay anchorage, leading to mass mortality of *L. klunzingeri* over recent years and label *D. caudata* as an indicator species to 'fish kill' in future studies.

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