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Research Article

Validating mercury levels in catfish *Netuma thalassina* (Rüppell, 1837) during and aftermath 'fish kill' in Kuwait Bay

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Abstract

Mass mortality of Catfish *Netuma thalassina* during the peak summer in Kuwait Bay prompted the study of mercury concentrations using direct mercury analyzer (DMA-80) during the 'fish kill' otherwise, found below the detectable limits. The mean mercury (Hg) concentrations in seawater, sediment and body parts of *N. thalassina* (0.27 ng L^{-1}, 0.35 ng g^{-1}, 0.23 ng g^{-1}) was high during 'fish kill' on 29th April, 2017 when compared aftermath the 'fish kill' (0.20 ng L^{-1}, 0.29 ng g^{-1} and 0.15 ng g^{-1}) respectively. Hg concentrations in the collected samples were observed in the sequence of Site-II>Site-I>Site-III. Fish stress was validated by hepatosomatic index (HSI). During 'fish kill' a decrease in liver weight due to liver shrinkage against their body weight was observed in contrast aftermath the 'fish kill'. Mass mortality of *N. thalassina* was also suspected because of underwater explosion-a plan that is adopted when complex construction activities were made across the Kuwait Bay sites. Since, fish with swim bladder is susceptible to explosion, environmental variations and hazardous effluents, such factors are validated, and futuristic research delved in this line.

Keywords: Fish kill, Hepatosomatic index, Marine environment, Mercury, Pollution

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Introduction

Kuwait Bay showed similar attributes of disturbed ecosystem like some Bays elsewhere the globe (Hu *et al*., 2013; Yongmin *et al*., 2013; Al-Mutairi *et al*., 2014; El-Moselhy *et al*., 2014). Diverse reasons for fish mortality due to their sensitivity include: high nutrient load, significant rise in temperature and salinity, bacterial contamination, upwelling of water current and high concentrations of toxic pollutants from domestic and industrial discharges from drain outfalls into the Kuwait Bay (Vaselali and Vaselali 2009; Panigrahi and Tripathy 2011). Meanwhile, few evidences revealed certain fish were in a state of sheer physical exhaustion ultimately leading to their deaths (Gulf News, 2017; Kuwait Times, 2017). Ever since, the Gulf war-I in 1991, the Kuwait Bay region has been subjected to stressed ecosystem by oil spills, 'red tide, influx of freshwater, and environmental changes because of the rapid industrialization and population outgrowth (Heath, 1995; Al-Yamani, 2008; Pottinger, 2008.). The Salt Chlor-alkali plant (SCP) that used mercury (Hg) electrolytes in the past, underwater explosions undertaken during construction activities of massive bridges causing stress, habitat dislocation of marine lives and suspected discharges of toxic pollutants into the marine waters cannot be excluded for this massive 'fish kill'. Earlier findings showed comparatively, high trace metals levels in the gills, liver and muscle tissues of commercial fish inhabiting the Bay than the metals levels in deep-sea fishes during the cataclysmic effects (Costa *et al*., 2008; Govoni *et al*., 2008; Frias *et al*., 2011; Ghedira *et al*., 2010; Yongmin *et al*., 2013; Sarayut *et al*., 2010). Among the non-essential trace metals, Hg showed uncertainty of results obtained from atomic absorption spectrophotometry since their sensitivity to Hg detection was limited to ±0.01ppm mercury (Ghedira *et al*., 2010; Sarayut *et al*., 2010; Frias *et al*., 2011; Yuan *et al*., 2014). Additionally, the precision of results was also limited as they were interfered by the organic constituents. This shortfall in instrumental detection limits were overcome by using Tri-cell Direct Mercury Analyzer (DMA-80, Milestone, Italy) that detects Hg concentrations of 0.0015 ng g^{-1} onwards and with least errors for any given type of samples.

Amongst the fishes in Kuwaiti waters, the catfish *Netuma thalassina* and their body parts are mostly used as bait and not relished by the residents. They are often found in estuaries. They feed on crabs, shrimps and small fishes and molluscs. Evidences showed the use of their skin in healing diabetic wound ulcers (Al-Banaw *et al*., 2010). However, their high rate of mortality posed suspicious accountability of the stressed Bay waters when compared with the less stressed fish (Sabullah *et al*., 2015; Shafei, 2015). Several possibilities such as the untreated effluent discharges from the water treatment plants and domestic outfalls, beach wastes, rapid urbanization and use of some chemicals in constructional activities were suspected to cause such magnitude of Hg pollution and specific 'fish kills'. The dead *N. thalassina* fish that were caught from the Bay showed varying Hg concentrations ~~of~~ in their body tissues during the outbreak of 'fish kill'. Based on the need-of-the-hour, studies were conducted both during and aftermath

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the 'fish kill' by: (a) direct Hg determination in the *N. thalassina* body parts, (b) validating the possible influence of pollution using hepatosomatic index (HSI), (c) correlating the environmental variables (temperature, salinity, dissolved oxygen) to label this species as a possible bio-indicator to Hg pollution and, (d) investigating the significant changes in the marine environment and (e) recommend the safe consumption of seafood.

Materials and methods

Kuwait Bay is located between latitudes 29.3576 N, and longitudes 48.0977 E, and borders Iraq to the North and to the South of Saudi Arabia. Kuwait Bay is characterized by mudflat to fluviomarine and sandy sediment. This study categorized moderate, heavy and comparatively less polluted, three sites encompassing the Northern, Central and Southern regions of the Kuwait Bay respectively (Fig. 1). Environmental parameters such as temperature, salinity, dissolved oxygen and pH were recorded using a multi-checker water monitoring system (Horiba -U235, USA).

Seawater samples (108 Nos.) were collected by using Vandorn's water sampler (2L) from three sites of Kuwait Bay (4 transect with an equidistance of ~2m) /site x triplicates x 3 locations x 3 months). Seawater was filtered using a 0.45µm membrane filter. Mercury concentrations of the samples were measured by using the Direct Mercury Analyzer (DMA-80, Milestone, Italy) that had detection limits from 0.0015 ng g^{-1} onwards. Hg in the seawater samples were also validated by following the standard methods APHA (2017).

Sediment samples (108 Nos.) collected by Vaan-Veen grab from three Kuwait sites were analyzed for mercury in the DMA-80 following the standard method (APHA 2017).

Netuma thalassina, catfish (10 Nos. each) was collected randomly from the three Kuwait Bay sites. Most of them were males, found either dead and floating on the water surface or reaching the mortality state especially in the central region (Site-II) of the Kuwait Bay (Fig. 1). Fish samples (10 Nos.) from each site were collected using ranger nets. The dead fish were segregated from the live fish, stored in cool box and, transported to the laboratory for analysis. The length, weight and sex of the fish were recorded. Twenty body-parts (~1-10 g) from each fish collected during the 'fish kill' (April-May, 2017) and aftermath the 'fish kill' (June-July, 2017) were dissected, segregated and directly measured for total mercury in the DMA-80.

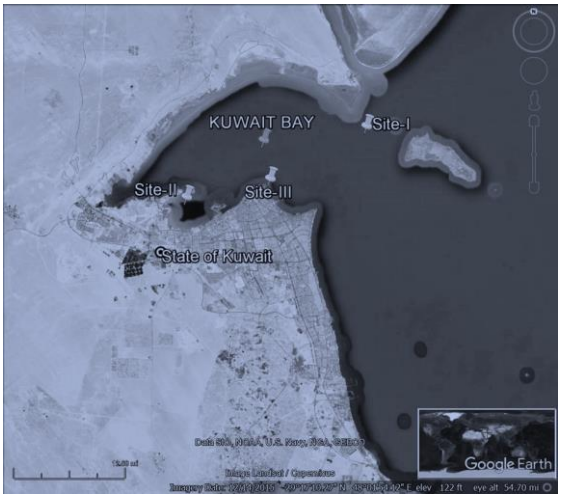


Figure 1: Sampling sites of Kuwait Bay Sites I-III: Subiyah, Doha, Kuwait Towers

The accuracy of the analytical procedure was validated by attaining >95% recovery of the appropriate reference materials

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(CRM-403: marine water, SRM-2702: marine sediment and DORM-3: dogfish muscle) to that of the samples other than the use of mercury standard (ICP grade) and blanks for quality assurance (APHA, 2017). Furthermore, the significance of the test results was validated using the hepatosomatic index and statistical tests using Minitab-17, respectively. Hepatosomatic Index (HSI: Goede and Barton 1990; Cristina *et al*., 2011) = Liver weight/Body weight of fish x 100 (Eq.1).

Results

Replicate of *N. thalassina* (10 Nos. each) ranged between 23.0cm-45.68cm and 412.0 g–569.19 g length and weight, respectively caught from the three sites from Kuwait Bay (Fig.1). Fish other than these dimensions and females which were found scarce were not considered for this study.

The male fish body weight was correspondingly found to increase the liver (9.12 g -9.84 g) weights in the absence of 'fish kill'. The calculated HSI (1.87-1.99) values were high with corresponding liver weight (Table 1). In the presence of 'fish kill" (FK) the liver weight ranged 8.37 g - 8.73 g as against the HSI 1.74-1.81. Statistical test by ANOVA showed significant differences between the length, weight and HSI of *N. thalassina* and their analysis during and after the 'fish kill' effect (Table 2).

Table 1: Biometrics of *Netuma thalassina* and hepatosomatic index (HSI).

No.	Description	Total Length (cm)	Body Weight (g)	Liver Weight (g)	HSI
1.	SI-AFK	37.87±3.45	491.10±48.90	9.63±0.46	1.96
2.	SII-AFK	34.74±5.91	488.97±59.74	9.12±0.62	1.87
3.	SIII-AFK	40.25±3.26	493.70±48.55	9.84±0.10	1.99
4.	SI-FK	32.98±7.05	479.06±56.57	8.45±0.38	1.76
5.	SII-FK	29.40±5.27	480.30±54.66	8.37±0.57	1.74
6.	SIII-FK	36.10±3.71	481.81±56.27	8.73±0.53	1.81

HSI= weight of liver/body weight of fish; FK: during fish kill; AFK aftermath fish kill

Table 2: ANOVA tests between *Netuma thalassina* body parts, growth parameters, HSI and hydrological variables.

Source of Variation	SS	df	F	*p*-value	F crit
A. Hg in body parts during and aftermath 'Fish-kill'					
Parts-wise	1.4609	19	159.621	0.0002	1.697
†Event-wise	0.2951	5	122.532	0.0007	2.310
Error	0.0458	95			
Total	1.8018	119			
B. Hg in samples Vs during and aftermath the 'Fish-Kill'					
†Event-wise	0.035	5.0	52.343	0.001	3.326
Sample-wise	0.054	4.0	199.779	0.003	4.103
Error	0.001	10.0			
Total	0.091	19.0			
C. Significance of *N. thalassina* biometrics to HSI					
†Event-wise	133.91	5	3.035	0.043	2.901
††Biometric-wise	999678.83	3	37764.886	0.0001	3.287
Error	132.36	15			

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Table 2 continued:					
Total	999945.09	23			
D. Hydrological variables Vs 'during' and aftermath the 'Fish kill'					
†Event-wise	40.419	5	1.3629*	0.2797	2.710
Variables-wise	5971.578	4	251.6992	0.0008	2.866
Error	118.625	20			
Total	6130.623	29			

† Event-wise: during and aftermath "Fishkill", * non-significant, SS: sum of squares, df: degree of freedom, F: calculated value, F Crit: critical table value

The Hg concentrations in all the samples have done by DMA-80 showed a consistent pattern of results. The mean Hg concentrations were high in seawater (0.32 ng L^{-1}), sediment (0.35 ng g^{-1}) and *N. thalassina* body parts (0.27 ng g^{-1}) samples collected from Site-II followed by Sites-I and Site-III (Figs. 2,3). Among the 20 body parts of *N. thalassina*, high Hg concentrations was observed in the liver (0.39 ng g^{-1}) followed by gills (0.36 ng g^{-1}) and other parts. The least Hg concentrations were observed in the otolith (0.04 ng g^{-1}) (Fig. 3). Statistical test by ANOVA showed significant differences between the body parts of *N. thalassina* and their analysis during and after the 'fish kill' effect (Table 2).

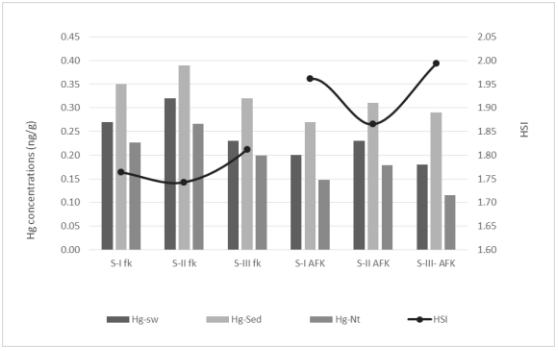


Figure 2: Mercury concentrations in seawater, sediment, *Netuma thalassina* and HSI
Sw: seawater, Sed: sediment, Nt: *Netuma thalassina* fish, HSI: hepatosomatic index, S-I to SIII: Kuwait Bay sites, fk: fish kill,
AFK: aftermath fish kill

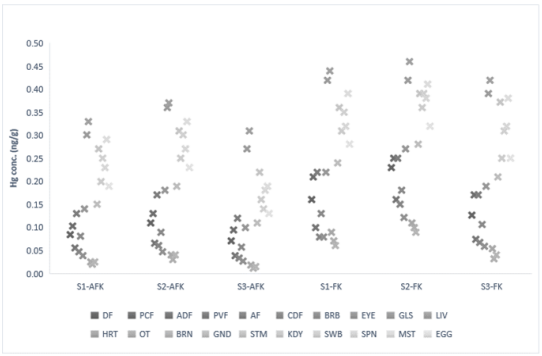


Figure 3: Mercury concentrations in the body parts of *Netuma thalassina.*
DF: dorsal fin, PCF: pectoral fin, ADF: adipose fin, PVF: pelvic fin, AF: anal fin, CDF: caudal fin, BRB: barbels, GLS: gills, LIV: liver, HRT: heart, OT: otolith, BRN: brain, GND: gonad, STM: stomach, KDY: kidney, SWB: swim bladder, SPN: spleen, MST: muscle tissue, SI-SII: sites, AFK: aftermath fish kill, FK: fish kill

Hydrological parameters such as temperature (34.2 ^{o}C) and salinity (40.9 ‰) were found high during the summer. Site-wise analysis showed low temperature and salinity in Site-I compared to Sites-II and Site-III and irrespective of the 'fish kill' (Table 1). Statistical tests validated the correlation between hydrological parameters, Hg in seawater, sediment, and *N. thalassina*, site-wise variations, hepatosomatic index (HSI) and, during and aftermath the 'fish kill' (Fig. 4). A negative correlation was found between HSI (hepatosomatic index) and Hg in seawater, sediment and *N. thalassina* (Fig. 4). The HSI was found to increase with increasing temperature, salinity, pH but the

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reverse with dissolved oxygen (DO). The dissolved oxygen was low during the summer. However, during the 'fish kill' the DO was found low below the threshold limits (<4-5 mg L^{-1}). A decrease in HSI for N. *thalassina* from the three sampled sites was observed during 'fish kill' compared to aftermath the 'fish kill'. Statistical test by ANOVA showed significant differences between the hydrological variables but showed no significance difference between during and after the 'fish kill' effect the analysis (Table 2).

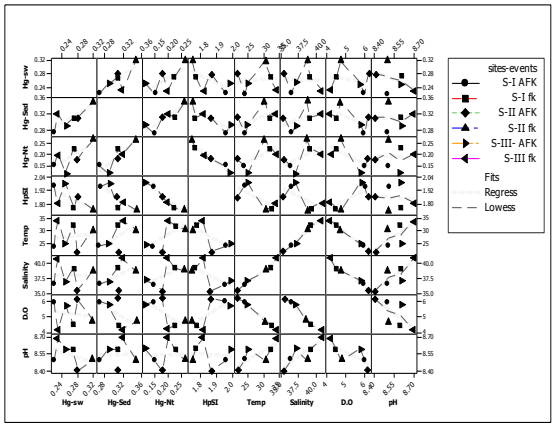


Figure 4: Correlation coefficient matrix on environmental variables
Hg-sw: mercury in seawater, sed: sediment, Nt: *N. thalasinna*, HSI: hepatosomatic index; Temp: temperature; DO: dissolved oxygen; AFK: aftermath fish kill; fk: fish kill

Discussion

This study revealed a catastrophic effect of 'fish kill' specifically to catfish *Netuma thalassina*. These fish were washed ashore during the onset of summer indicating the diverse stress on the Kuwait Bay's marine ecosystem (Fig. 1) by statutory bodies' reports (Kuwait Times 2017; Gulf News 2017). However, the distribution pattern of Hg pollutant that substantiated the cause of 'fish kill' in the Kuwait Bay waters was not evidenced and hence, 'the need-of-the hour' studied.

In the absence or aftermath, the 'fish kill' (AFK), increasing liver weight to that of their body weight indicated the Bay waters in normal condition whereas, during 'fish kill' the reverse took place, indicating the hypothesis of liver shrinkage related to cellular and organismal stress and starvation, a factor in line with the earlier findings (Goede and Barton, 1990; Pottinger, 2008; Vaselali and Vaselali, 2009; Sarayut *et al*., 2010; Panigrahi and Tripathy, 2011).

Samples analyzed in the DMA-80 revealed accurate results since the method adopted, instrumentation precision and minimizing manual errors reproduced consistent results compared to the earlier methods (Frias *et al*., 2011; Yuan *et al*., 2014). Observations revealed high Hg concentrations in seawater and sediment samples from Site-II because of high nutrient loading, low mobility of water current and enormous effluent discharges of pollutants from the drain outfalls. The high Hg levels in the liver are attributed to the metallothioneins associated essential metals displacement by the Hg in the hepatic tissues (Yongmin *et al*., 2013; El-Moselhy *et al*., 2014). Liver, gills and kidney of *N. thalassina* derive Hg concentrations from the seawater, sediment and food (Shafei, 2015). The influence of high temperature and salinity did attribute as one of the major factors for the 'fish kill' in the three sampled in Kuwait Bay sites. However, the low levels of salinity in Site-I may be attributed to the freshwater inflow of Shatt-Al Arab River from the North and dilution of seawater by thermal plant. This agreed with the earlier findings (Al-Yamani, 2008; Hu *et al*., 2013; Al-Mutairi *et al*., 2014). Although

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the environmental variables were moderate in Site-II compared to Sites-I and III, *N. thalassina* 'fish kill' was witnessed on a mass-scale in Site-II. Nutrient loading and effluent discharges from the drain outfalls are attributed for the occasional 'fish kill' in this site. Furthermore, the impact of sediment deposition, desalination plant in Site-II, besides, suspected underwater explosion undertaken for the recent construction of the lengthy Subiyah bridge (36 Km) connecting Sites: I-III could be attributed to the recent habitat displacement and stress that lead to the enigmatic 'fish kill'. Statistical test validating the positive correlation coefficient between temperature, salinity, and pH indicated their influence to the increase of Hg contamination in the studied samples. The negative correlation between the HSI and Hg in the seawater, sediment and catfish body parts samples validated the significant impact of Hg pollution in the Bay waters. Exceptionally, the DO values indicated attributed their independent correlation with HSI but, were dependent on temperature. The Low DO during the summer was associated with the degradation of organic matter and low mixing of water current. However, a steep decline of DO during the event of 'fish kill' showed noticeable effects on the behavioral and physiological process in the fish when compared to aftermath the 'fish kill'. This was in line with the earlier study (Costa *et al*., 2008; Al-Mutairi *et al*., 2014). The HSI decrease could be attributed to the stress that imposed an energy drain on the fish and often correlated with glycogen loss (Heath, 1995). Furthermore, the decline in liver mass could be related to low feeding, besides, the possible effect of some contaminants (Heath, 1995). In the present study, the decrease of HSI with increasing Hg concentration can be hypothesized to fish subjected to short duration stress. This contrasted with the earlier studies that hypothesized an increase in HSI due to prolonged exposure, increased metabolic xenobiotics association and adaptation to the presence of marine pollution (Heath, 1995; Ghedira *et al*., 2010; Frias *et al*., 2011; Al-Mutairi *et al*., 2014; Sabullah *et al*., 2015).

From the above facts, the present findings recommend (a) the use of HSI as an invaluable tool to label *N. thalassina* as an indicator species to Hg pollution, (b) Hg analysis at trace concentrations in any sample by direct mercury analyzer (DMA-80) that produces accurate, precise and reproducible results, (c) continuous monitoring and action plan to prevent dead fish being consumed by other fishes and spread the phenomenon in the marine sites especially during the catastrophic 'fish kill' events, besides (d) deducing the possibilities of health risks of few human consumers of contaminated fishes.

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