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Combined toxicity of mercury and plastic wastes to crustacean and gastropod inhabiting the waters in Kuwait

AH Bu-Olayan and BV Thomas*
Department of Chemistry, Kuwait University, Khaldiya Campus Safat 13060, Kuwait
*Corresponding Author's E-mail : bivint@yahoo.com

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Abstract

The present study determined total mercury (T-Hg) in crustacean *Portunus pelagicus* (blue crab) and mollusc *Tapes sulcarius* (Furrowed Venus: Cockle) following suspected rise in beach plastic wastes and their effect on marine organisms. Live samples were collected from beaches representing six Kuwait Governorate areas and exposed to toxicity (96hr) and bio accumulation tests for 180 d with inclusion of plastic wastes and environmental conditions simulated in laboratory. Results revealed high T-Hg concentrations in *T. sulcarius*(1.44ng l^{-1}) compared to *P.pelagicus* (1.03ng l^{-1}) during winter than summer, with bio accumulation factor (BAF)>1 labelled these species as hyper-accumulators. Significantly, combination of T-Hg concentrations from plastic wastes and in seawater validated the possibilities of detrimental effects of other marine lives besides deteriorating the aesthetic values of scenic beaches and likelihood of invasive species in such coastal areas.

Key words:

Bioaccumulation, Mercury, Plastic wastes, Toxicity

Introduction

Plastic materials pose a serious threat to marine lives and Coasts as they are found to leach toxic materials into their surrounding waters. Pelagic plastic pieces in the ocean have outnumbered live plankton and have been found to enter the food chain in marine life (Moore *et al.,* 2001). Environmental, health and aesthetic problems are the outcome of plastic materials littered along the beaches. Plastic waste spoils the serene coastal areas besides affecting water quality and physical damage to the ecosystems (UNEP 2005; Ribic *et al.,* 2012). Plastic wastes transport invasive species across many seas. Dispersal of aggressive invasive species endangers sensitivity and risk in both marine and coastal ecosystems from their native habitats (Murray, 2009). Many species accidentally ingest trash or get entangled in abandoned fishing gears (Sheavly, 2007). Ocean current patterns, climate, tides, proximity to urban, industrial and recreational areas and fishing grounds influence the types and amount of plastic wastes that are found in the open ocean or along the beaches (Duruibe *et al.,* 2007). Heavy metals are often released in water when metals rust, leaches from plastic manufacturing units and, when floating plastic wastes act as 'transport vector' dispersing metals along the coastal areas by ocean current and wind action (Nakashima *et al.,* 2012). One such metal that is detrimental to marine lives is total mercury (T-Hg) that is available in water bodies and in air-water interface (Hammerschmidt and Bowman, 2012). Researchers (Mackay and Fraser, 2000; Lewis and Chancy, 2008; Ryan *et al.,* 2009) observed traces of Hg in marine debris released into the beach sand sustained for long period, became toxic and accumulated in marine organisms and secondary consumers including man. Various investigators have observed the effect of Hg toxicity and bioaccumulation in mollusc and crustacean species (Callil and Junk, 2001; Costa *et al.,* 2011; Bordon *et al.,* 2012; Elahi *et al.,* 2012; Ramakritinam *et al.,* 2012). However, least evidences have been observed in the light of Hg toxicity from plastic materials affecting marine organisms. Cardoso *et al.* (2009) described transfer of total mercury (T-Hg) concentration from their surrounding environment as a result of Hg uptake mechanisms at the organism level leading to bioaccumulation that is defined as

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ratio of contaminant in an organism to the concentration in the ambient environment in steady state. They also validated the bioaccumulation factor (BAF) using the standard ASTM (2000) formula.

Kuwait Coast has rich flora and fauna biodiversity besides mud to fine sandy beaches diverse topography. Over the years, the increasing trend of wastes could be attributed to the activities of beach visitors, diverse ethnicity, occupation and negligence to environmental awareness. In Kuwait, beach wastes is mainly collected by private firms. Short awareness programme by these agencies reported a collection of over three tons of wastes from selected areas on the Kuwait Coast (En.v., 2012). Despite various environmental policies, evidences showed no stringent action against plastic waste pollution by beach visitors, or control of dispersion of Hg pollution from the Kuwait Coasts. The nature of plastic materials evinced interest to focus the seasonal effects of toxic effect of T-Hg concentration from plastic wastes, bioaccumulation factor (BAF) and, impact of plastic wastes in molluscs and Crustacean, *Tapes sulcarius* and *Portunus pelagicus* species, respectively.

Materials and Methods

Sample sites: Beaches encompassing six Kuwait Governorates were fixed for plastic waste collection (Fig. 1). G-I Al-Jahra-the Northern Kuwait Coastal beach was observed for the angling activities by fishing hobbyists and stress from thermal pollution. G-II Al-Asimah, Kuwait City- wastes cast by beach visitors and from fishing boats in the Bay got deposited between the rocks because of intertidal activities. This site is subjected to stressed ecosystem due to dense inhabitation, discharge outfalls, power and desalination plants. G-III Al-Hawali- commercial activity site houses several recreation centers of Kuwait. G-IV Al-Farwaniya is in the central part of Kuwait. Typically, this region has no beaches. However, domestic wastes from this Governorate are let into drain outfalls that are discharged in the Kuwait Bay. G-V Mubarak Al- Kabeer has an evenly developed coast known for recreational activities. Occasional cleaning activities are executed in these beaches. G-VI Al-Ahmedi beaches in the northern area consist of industrial and oil wells and hence are contaminated with heavy plastic wastes. However, the southern area has less human inhabitation and low plastic waste dispersion.

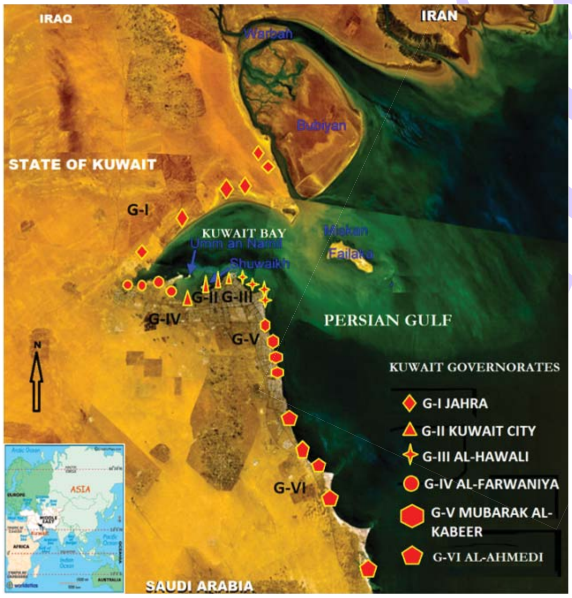

Fig.1.: Sampling sites GI to GVI of Kuwait Governorates

Sample collection : Plastic wastes were collected four times a month from the sampling sites, off the Kuwait beaches during the years 2012-2013, following the method of Sheavly (2007). These wastes were segregated by their polymer constituents as described by SPI (society of plastic industries Inc.) resin ID codes, washed in deionized water, dried and packed in labeled sterile bags before analysis. Dry weight of each plastic waste was recorded. Sub-samples (10mg), at random from each item, were cut into small pieces and stored in sterilized plastic containers for analysis.

Kuwait has rich intertidal fauna distribution all along the coast. Twenty replicates each, of mollusc: cockle (*Tapes sulcarius*) and crustacean: crab (*Portunus pelagicus*) species from six Kuwait Governorates beaches were also collected (Fig. 1).

Analysis of mercury: Mercury (T-Hg) in the wastes (0.1g) was analyzed direct by Mercury analyzer (DMA-80, Milestone, Italy). The DMA-80 in three combination principles (thermal decomposition, catalyses and amalgamation of samples) determined T-Hg concentrations with precision, low detection limits and standard error (0.0015-1000µgg^{-1} and ± 0.01 ngg^{-1}) as compared to EPA 7471 method (1-5 µgg^{-1}and ±5µgg^{-1}). Quality assurance was validated using standard reference materials, blanks, control charts and standard T-Hg. Sample recovery >97% to that of standard reference materials was considered to determine Hg concentrations in plastic wastes.

Tapes sulcarius (cockles: mollusc) and *Portunus pelagicus (crab: crustacean)* (n=10) were acclimated for 24 hr separately in an aquarium tanks in laboratory. Filtered seawater collected from the Kuwait coast (GI-GVI) of importance was added six different tanks. Stock solution (1 µg l^{-1}) of Hg (standard 1000µgml^{-1} Hg in 10% HNO_3 ICP-MS grade) was added to filtered seawater in these tanks containing *T. sulcarius* and *P. pelagicus* replicates to produce the required LC_{50} test concentrations (0.8-2.8 ng l^{-1}) and subjected to 96 hr toxicity tests respectively (USEPA, 1993). Hg solution was renewed every 24 hrs to prevent lowering of toxicant levels. 96 hrs toxicity tests were conducted to determine lethal Hg concentration for a given population which included three concentrations of Hg at LC_5, LC_{15}, and LC_{50} values for each species.

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In another set of experiments, two representative species (10 replicates each) were exposed for 180 d and Hg concentration at LC_{15} after measuring the initial T-Hg concentrations in seawater, plastic wastes and in whole tissues of the two species. *T. sulcarius* and *P. pelagicus* were fed with brine shrimp (*Artemia franciscana*) nauplii, free from trace T-Hg concentrations to prevent possible food contamination in the tank. At random, two species were separately sacrificed prior to ethical permission from the local statutory bodies. Seawater and the species tissues from the control and the 180d exposed samples were analyzed for T-Hg concentration by direct in mercury analyzer (DMA-80) and the results were incorporated. Bioaccumulation factor (BAF) of total mercury (T-Hg) for 180d exposed species, *T. sulcarius* and *P. pelagicus* was calculated. Furthermore, bioaccumulation test resulting BAF=1, BAF<1 and BAF >1 for a given species were characterized as accumulators , indicator and hyper accumulators', respectively (ASTM, 2000).

Results and Discussion

Irrespective of both summer and winter seasons, the number of wastes per year was high in Governorate-II beaches when compared to the wastes collected from other five Kuwait Governorates beaches. Reasons attributed were of fishing and recreational activities, innumerable breakwater structures on the beaches, wastes washed through the storm water outfalls, and slow water current in the Bay causing immobility of plastic wastes. These attributes were in line with the earlier observations (UNEP, 2005; Murray, 2009). In contrast to other beaches elsewhere on the globe, beach visitors were less in Kuwait beaches during peak summer (June-August). High temperature and emigration of large number of population to other countries is attributed to such decrease of plastic wastes during the summer season. This is in confirmation with the previous report of (Ribic *et al.,* 2012). Evidences showed two to three tons of plastic wastes/13,500sq.m, over a period of three months assessment in a year, in selected Kuwait beaches of pollution interest (En.v., 2012). Physical collection during this study(2012-2013) revealed 7-8 tons of plastic wastes from the Kuwait coastline (Fig.2).

High T-Hg concentration in plastic wastes was observed during winter in comparison to summer samples. Governorate-wise analysis showed T-Hg concentrations in the sequence GII>GI>GIV>GV>GIII>GVI (Fig.3). High T-Hg concentration in GII beaches attributed to the large quantity of wastes deposited over the shoreline as a result of low water current in the Kuwait Bay and also due to Hg containing sources that adhered with the plastic wastes. Beach cleansing programme by non-governmental organization and less number of visitors attribute to low quantity and T-Hg concentrations of plastic wastes in GV-GVI beaches. This was similar to earlier observations of Ryan *et al.* (2009); Nakashima *et al.* (2012). The major plastic wastes comprised of oil cans, syringes and plastic trash bags (Fig. 4).High T-Hg concentration was found in empty

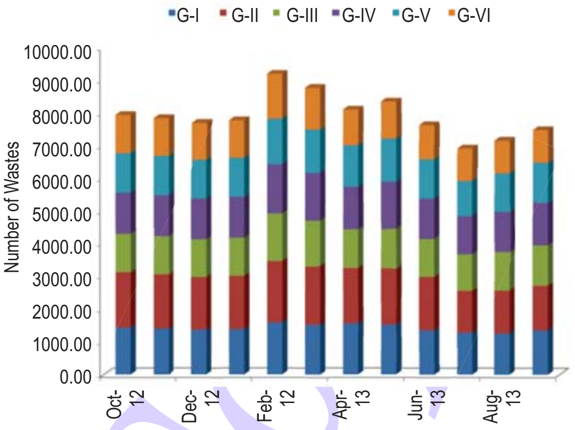

Fig.2.: Number of plastic wastes in six Kuwait beaches during 2012-2013 GI-GVI: Kuwait Governorate

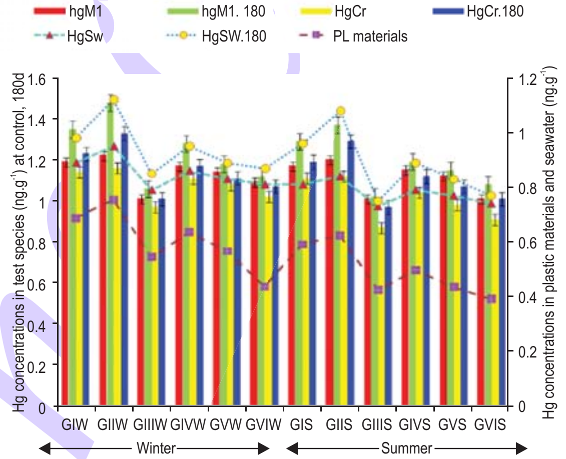

Fig. 3 Toxicity test exposure (control, 180d) of Hg concentrations in seawater, test species and plastic inclusions. GI-GVI: Six Kuwait Governorates, W: winter, S: summer, hgMI: mollusc, HgCr: crustacean, SW: seawater, PL: mean of plastic wastes from each Governorate beaches

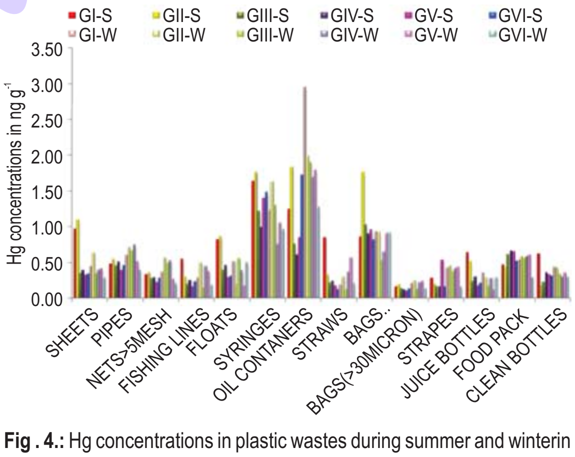

Fig . 4.: Hg concentrations in plastic wastes during summer and winterin the Kuwait beaches

Table 1. : Acute mercury toxicity test on mollusc, *Tapes sulcarius* and Crustacean, *Portunus pelagicus* (10 replicates each)

Kuwait Governorates/ Type of Conc.	Test Conc. (ng l^{-1})	†Est. Conc. (ng l^{-1})	LC Point	95 % C.I. limits		X^2 calculated	Mortality number
				lower	upper		
GI-ML: 1	1.2	1.10	05	0.65	1.15	0.363*	-
2	1.6	1.36	15	0.95	1.59		3
3	2.0	1.93	50	1.66	2.25		5
GI-CR:1	0.9	0.83	05	0.58	0.97	0.413*	-
2	1.1	0.97	15	0.76	1.09		3
3	1.3	1.26	50	1.12	1.40		6
GII-ML:1	1.2	1.10	05	0.68	1.35	0.097*	-
2	1.6	1.33	15	0.95	1.56		3
3	2.0	1.85	50	1.59	2.12		6
GII-CR:1	0.9	0.85	05	0.61	0.98	0.309*	-
2	1.1	0.98	15	0.77	1.10		3
3	1.3	1.25	50	1.13	1.39		5
GIII-ML:1	1.2	1.05	05	0.55	1.34	0.316*	-
2	1.6	1.34	15	0.88	1.60		3
3	2.0	2.04	50	1.73	2.47		5
GIII-CR:1	0.9	0.81	05	0.52	0.97	0.417*	-
2	1.1	0.97	15	0.73	1.11		3
3	1.3	1.33	50	1.18	1.53		5
GIV-ML:1	1.2	1.15	05	0.69	1.40	0.813*	-
2	1.6	1.39	15	0.99	1.62		2
3	2.0	1.94	50	1.67	2.23		6
GIV-CR:1	0.9	0.87	05	0.62	1.01	0.510*	-
2	1.1	1.01	15	0.80	1.13		2
3	1.3	1.29	50	1.17	1.45		4
GV-ML:1	1.2	1.17	05	0.70	1.42	0.644*	-
2	1.6	1.42	15	1.00	1.65		2
3	2.0	1.98	50	1.71	2.29		5
GV-CR:1	0.9	0.89	05	0.62	1.03	0.853*	1
2	1.1	1.03	15	0.81	1.15		2
3	1.3	1.32	50	1.18	1.47		4
GVI-ML:1	1.2	1.14	05	0.61	1.43	0.426*	-
2	1.6	1.44	15	0.96	1.70		2
3	2.0	2.13	50	1.83	2.60		4
GVI-CR:1	0.9	0.84	05	0.51	1.00	0.276*	-
2	1.1	1.02	15	0.74	1.16		2
3	1.3	1.41	50	1.25	1.69		4

†conc.: estimated exposure concentration; C.I: Confidence interval; χ2: Calculated Chi square for heterogeneity *: significant Chi square; ML: mollusc; CR: crustacean ; 1: LC5; 2:LC15; 3: LC50 LC: lethal concentration

plastic oil cans followed by other plastic items (Fig. 4). Such high concentration in empty plastic oil containers may be due to the contamination of plastic container, before it was emptied and littered. This supports the earlier views Duruibe *et al.* (2007); Lewis and Chancy (2008); Hammerschmidt and Bowman (2012). Furthermore, such containers with traces of lubricant could possibly leach coastal water and might cause detrimental effect to the marine organisms and secondary consumers. Sub-lethal toxicity tests in crustacean *P. pelagicus* and mollusc *T. sulcarius* revealed mercury sensitive ranging 0.97-1.03 ngl^{-1} and 1.33-1.44 ngl^{-1} (Table 1). High tolerance of mercury in *T. sulcarius* at LC_{15} is attributed to their filter feeding mechanism when compared to accumulation of pollutants in *P. pelagicus*. Similar Hg sensitivity to crustaceans was observed by Costa *et al.(*2011); Bordon *et al.*(2012); Elahi *et al.*(2012). Governorate-wise toxicity confirmed high T-Hg sensitivity to LC_{50} in *P. pelagicus* collected from GII beaches. This possibly leads to the assumption of inclusion of innumerable types of non-biodegradable materials from nature. (Nakashima *et al.,*2012). Comparatively, sensitivity of T-Hg at LC_{50} was found higher in *P. pelagicus* than the sensitivity of T-Hg in *T. sulcarius (Table 1).* The present study on Hg transfer from the environment was found supportive to both the test species exposed to T-Hg at LC_{15} and in agreement with the studies Cardoso *et al.* (2012).

Following the results of Table 1, experiments conducted for 180 d exposure to seawater collected from the seven Kuwait Governorate beaches, *T. sulcarius* and *P. pelagicus* species showed significant T-Hg bioaccumulation in the presence of plastic inclusion (Fig. 3), which indicated the possibility of T-Hg

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Table 2. : Mercury concentration in seawater, *Tapes sulcarius* and *Portunus pelagicus* for control and 180 day exposure

Governorates/	**T-Hg in ng.l^{-1}**		**T-Hg in ng.g^{-1}**			
Seasons	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**
GI-W	0.89	0.98	1.19	1.35	1.14	1.23
GII-W	0.95	1.12	1.22	1.48	1.16	1.33
GIII-W	0.79	0.85	1.01	1.06	0.97	1.01
GIV-W	0.86	0.95	1.17	1.28	1.11	1.17
GV-W	0.83	0.89	1.14	1.18	1.08	1.11
GVI-W	0.81	0.87	1.09	1.12	1.02	1.07
Mean-W	0.86	0.94	1.14	1.25	1.08	1.15
GI-S	0.81	0.96	1.17	1.29	1.11	1.19
GII-S	0.84	1.08	1.20	1.37	1.12	1.29
GIII-S	0.73	0.75	1.01	1.02	0.87	0.97
GIV-S	0.79	0.89	1.15	1.19	1.04	1.12
GV-S	0.77	0.83	1.12	1.15	0.98	1.07
GVI-S	0.74	0.77	1.01	1.08	0.91	1.01
Mean-S	0.78	0.88	1.11	1.18	1.01	1.11
Mean S and W	0.82	0.91	1.12	1.21	1.04	1.13

1:Seawater, 2: *T. sulcarius*, 3: *P. pelagicus*, a: control, b:180d exposure, GI-GVI: Kuwait Governorates beaches, W: winter, S: summer

accumulation from seawater as well from the plastic materials by leaching process in marine environment.Higher bioaccumulation of T-Hg and lower sensitivity to toxicity tests in *T. sulcarius* than *P. pelagicus* explains the possibility of lesser pollutant excretion in *P. pelagicus* than with *T. sulcarius*. However, bioaccumulation factor was >1 in all the analyses and high in *T. sulcarius* than in *P. pelagicus* (Table 2).This indicated that *T. sulcarius* could have accumulated by adsorption of T-Hg from the plastic materials or absorbed T-Hg from their surrounding medium as described earlier by Nakashima *et al.* (2012). Thus, two species could be labeled as 'hyper-accumulator' to mercury toxicity (Table 2) supporting the earlier findings Mackay and Fraser (2000); Callil and Junk (2001).

T. sulcarius and *P. pelagicus* exposed for 180 d revealed additive toxic and bioaccumulation effects of T-Hg and plastic wastes during winter than in summer, and labelled these species as hyperaccumulators. Governorate-wise assessment revealed high T-Hg in both seawater and in the two species inhabiting the beaches, where high recreational and fishing activities and low public awareness to waste management was observed besides the influence of wastewater discharges from drainoutfalls and industrialization.

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