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Assessing Genotoxic and Cytotoxic Effects in Bivalves Influenced by Marine Pollution in Bahrain, Arabian Gulf

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Abstract: The Arabian Gulf is increasingly subjected to habitat alteration due to dredging, reclamation activities, and pollution from various anthropogenic sources, including industrial and domestic effluents and oil spills. These activities can affect the naturally stressed marine ecosystems. Genotoxicity and cytotoxicity biomarkers are widely recognized as an important tool to assess the biological response of organisms exposed to a variety of contaminants. This study investigated the genotoxic and cytotoxic effects in bivalves influenced by marine pollution in Bahrain. The results revealed that high percentage of abnormal cells (45.57%) was associated with a shallow bay influenced by elevated levels of nutrients from a major sewage station. Characterization of genotoxic and cytotoxic biomarkers can be adopted to monitor coastal and marine areas influenced by anthropogenic land-based activities in the Arabian Gulf.

Key words: Genotoxicity, cytotoxicity, marine pollution, Arabian Gulf.

Introduction

Marine pollution is the harmful impact on living resources, marine life and human health that is caused by the direct or indirect introduction of a complex mixture of pollutants into the ocean (Tobias, 2008). Anthropogenic activities pose a great hazard on the marine life and affect the quality of seawater (Barsiene et al., 2012). The discharge of industrial wastes, population growth, development of industries, expansion of harbours, and tourism activities produce potentially toxic materials that adhere and accumulate to tiny deposited particles and filter feeders, causing physiological problems and genetic alterations in their tissues (Barsiene et al., 2012; Wright, 2013; Wadrop et al., 2016).

Persistent organic pollutants, nutrients, oils, radionuclides, heavy metals, and pathogens can cause severe impacts on the marine environment (Shanmugam et al., 2006; Wardrop et al., 2016). Even though these contaminants can occur in low concentrations, they are capable of modifying the genetic material of living organisms and act as genotoxic compounds (Hylland et al., 2008).

Biomarkers are generally defined as the measurable alternation in the cellular or biochemical components, processes, structures or functions in a biological system or a sample of tissues and fluids. They predict, determine and assess the incidence of a disease, or monitor the consequences after the exposure of organisms to a source of contamination (Ladeira et al., 2012).

Genotoxicity biomarkers are the alterations of the genetic material in the tissues of a living organism such as cancer, mutations and defects. They are caused by genotoxic agents, including radiation and chemical substances (Shah, 2012).

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One of the most reliable assays used in environmental genotoxicity assessment is the micronuclei (MN) test. This assay is fast and highly sensitive in detecting the chromosomal abnormalities caused by genotoxic agents using the differential staining by Geimsa stain. Such chromosomal abnormalities are mostly formed from DNA fragments that have been improperly condensed, amplified, or formed after failed replication. They include cells with micronuclei, cells with nuclear buds, fragmented-apoptotic cells, and bi-nucleated cells (Barsiene et al., 2012).

Additionally, micronuclei are small nuclei-shaped cytoplasmic chromatin masses. These micronuclei arise at the anaphase of cell division from lagging chromosomes or centric chromosome fragments. When the cell divides, these abnormal chromosomal forms lag behind and get excluded from the main nuclei in the daughter cells (Sabharwal et al., 2015).

Cytotoxicity is defined as the cell-killing property that is monitored by a chemical agent or a mediator cell that is independent from the accidental and the normal cell death mechanisms (i.e. necrosis and apoptosis) (Rode, 2008). Cytotoxicity is another biomarker that is used to monitor the genomic stability of a cell (Ladeira et al., 2012). The damage of the plasma membrane of a cell causing the release of enzymes to the outer of the cell is one example of cytotoxicity that can be detected by measuring the quantity of the leaked enzyme by several enzymatic reactions (Rode, 2008; Holmes and Goldberg, 2009).

Arabian Gulf is characterized by extreme environmental conditions represented by high aridity and low precipitation. High levels of salinity and surface temperature are the main natural stresses in marine environment of the Arabian Gulf (Naser, 2013). It is increasingly subjected to habitat alteration due to dredging and reclamation activities, and pollution from various anthropogenic sources, including industrial and domestic effluents and oil spills, which can affect the naturally stressed marine ecosystems (Vaughan et al., 2018). Likewise, Bahrain coastal and marine environments are intensively subjected to several anthropogenic activities that can alter the seawater quality and pose series hazard on the marine organisms and ecosystems. For instance, the shallow subtidal areas in Bahrain are receiving effluents containing ammonia, hydrocarbons, phosphorus and heavy metals from several land-based activities including, oil refining facilities, aluminium and petrochemical industries, sewage treatment plants and desalination plants (Naser, 2011).

Genotoxicity and cytotoxicity biomarkers are widely recognized as an important tool to assess the biological response of organisms exposed to a variety of contaminants (Leitao et al., 2017). It is hypothesized that genotoxicity and cytotoxicity studies could be used as effective indicators of pollution in the naturally and anthropogenically stressed marine environment of the Arabian Gulf. Consequently, the main aim of the present study was to investigate the genotoxic and cytotoxic effects in bivalves influenced by marine pollution in Bahrain. The objectives of this study include: (1) characterizing micronuclei of white clams *Diplodonta globosa* collected from three sites that are subjected to different levels of pollutants, (2) applying Lactate Dehydrogenase enzyme assay to detect environmental pollution using the white clams *Diplodonta globosa*, and (3) investigating the levels of selected physical parameters and nutrients in the marine environment of Bahrain during the period 2009-2012 and linking them to the tested biomarkers.

Material and Methods

Sampling Sites

The white clams *Diplodonta globosa* was selected as the target species to examine the genotoxic and cytotoxic effects due to marine pollution. This clam is rounded bulbous shell up to 35 mm with buff to white colour, which can be found buried in sand (Jones, 1986). Because clams are feeding by filtering the water column, they are widely used as a tool for monitoring ecological quality of marine environments (Simboura and Zenetos, 2002). The clam samples were collected from three sites, namely Tubli bay, Al-Hid coast and Jaradah in the period July-August 2012. Generally, the clams of 14 to 16 years old were chosen according to their age identified by counting the number of the rings on the outer shell, as each year a clam produces one ring on its shell (Hernandez et al., 2014). Therefore, this marine organism can be a good representative record for cumulative biological changes occurred during its life span which is exposed to environmental alteration.

The sites are characterized by a variety of human-induced pressures. Tubli Bay is located in the east-northern part of the main island of Bahrain. This bay is receiving around 160,000 m^3 per day of sewage discharges from the main water treatment plant in Bahrain that contain high amount of nutrients such as ammonia, nitrate and phosphate (Naser, 2011). Subtidal areas near the Hid area are influenced by discharges from a major ship repair yard, desalination plant and

other industrial activities. The last site, Jaradah, is located in the territorial waters of Bahrain at a distance of around 17 km from Hid area. Jaradah is a small sandy island located at proximity from main routes of shipping. Approximate locations of sampling sites are shown in Figure 1.

Micronuclei Test

The micronuclei test was conducted based on the protocol developed by Barsiene et al. (2008). Twenty clam samples from each site (i.e. 20 × 3 = 60 clams) were dissected and gills were isolated and placed in a drop of 3:1 ethanol acetic acid solution on clean microscopic slide. The gill arches were nipped gently with tweezers for 2-3 min until cells spread within the drop of solution. The cell suspension produced was carefully smeared on the surface of the slide and air-dried, then were fixed subsequently in methanol for 10 min and stained with 4% Giemsa solution in phosphate buffer (pH 6.8). The stained slides were analyzed under a light microscope at final magnification of ×1000.

Micronuclei were characterized as round and ovoid-shaped non-refractory particles in the cytoplasm, colour and structure similar to chromatin, and particles are completely separated from the main nucleus. Nuclear buds (NB) were characterized by extruded nuclear material that appears like micronucleus with a narrow or definite nucleoplasmic bridge to the main nucleus. Fragmented apoptotic cells (FA) in early stages were characterized by the presence of chromatin condensation within the nucleus and intact cytoplasmic and nuclear boundaries; late apoptotic cells exhibit nuclear fragmentation. The two nuclei of a bi-nucleated (BN) cell were approximately equal in size; the staining pattern and staining intensity have intact nuclear membranes and are situated within the same cytoplasmic boundary. The two nuclei may touch, but ideally should not overlap each other.

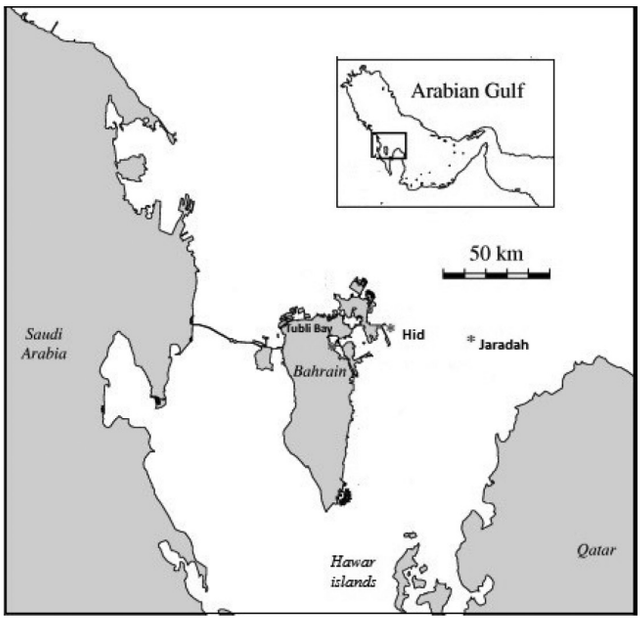

Figure 1: Maps showing the location of Bahrain in the Arabian Gulf and the approximate locations of sampling in Bahrain.

Lactate Dehydrogenase (LDH) Enzyme Assay

The assay was conducted by using of gill's tissue homogenate prepared in 0.1 M phosphate buffer (PBS, pH 7.0). A sample of 25 mg of freshly detached gills tissue was weighed and washed with 0.85% saline solution followed by homogenization in 10 ml PBS using a glass homogenizer. The homogenate was centrifuged for five minutes at 5000 rpm followed by supernatant collection and storage at 4°C until use. This tissue homogenate was used for protein quantification and LDH enzyme assay.

Enzyme assay procedure was adopted from Aanand et al. (2010). Total 3 ml mixture of 2.7 ml of 0.1 M phosphate buffer, 0.1 ml of NADH solution, and 0.1 ml of tissue homogenate was prepared and the reaction was started after the addition of the substrate sodium pyruvate. The optical density (OD OD_{340nm}) was recorded every 15/30 seconds using the UV spectrophotometer. For each site, five replicate samples were examined (i.e. 3 × 5 = 15 clams).

The OD reading was decreased with time as the NADH was oxidized by the LDH enzyme into NAD^+ as an indicator of more leakage of the enzyme from the homogenized tissue resulting in the death of cells. The quantity of protein was calculated using the formula:

$$\frac{\Delta A \,.\, V \,.\, D}{6.3 \,.\, d \,.\, \nu} = UI/\text{ml}$$

where ΔA is the change in absorbance at 340 nm/min., V = total volume of reaction mixture (3 ml), D = enzyme dilution factor (10), 6.3 = mM extinction coefficient of NADH, d = light path length (1 cm), and v is volume of enzyme sample; i.e. tissue homogenate (0.1 ml).

Protein Estimation

Total protein quantification in gill's tissue was done using Lowry's method (Lowry et al. 1951). Tissue homogenate (0.1 ml) was mixed with 1 ml of 10% tricarboxylic acid (TCA), vortexed and incubated in water bath at 95°C for 15 minutes. The sample was

cooled to room temperature followed by centrifugation at 14000 rpm for 20 minutes. The residue was dissolved in 0.5 ml of 0.1 N NaOH from which 0.1 ml was used for protein estimation.

Environmental Parameters

Measurements for chemical and physical parameters of the selected locations were provided by the Supreme Council for Environment in Bahrain. These parameters include ammonia, nitrate, phosphate, chlorophyll (a), dissolved oxygen, hydrocarbons, and total suspended solids (TSS). These parameters span over a period of four years (2009-2012) in order to detect their cumulative impacts on the indicator species. Parameters were statistically analyzed using PASW statistics 18 to distinguish the temporal and spatial differences between the selected sites.

Results

Micronuclei (MN) test revealed that the percentage of the normal cells were in their highest at Jaradah site, with a percentage of 98.44% and in their lowest at Tubli bay with a percentage of 54.09%. Hid area showed a relatively high amount of normal gill cells of 88.99% (Table 1).

Table 1. Percentage of MN examined cells as compared with normal examined cells of clam gills tissue imported from target sites

	Jaradah	*Hid*	*Tubli bay*
% normal cells	98.44	88.99	54.09
% MN cells	01.56	11.27	45.57

The MN cells obtained from Tubli bay and Hid areas showed several nuclear abnormalities, including micronuclei, fragmented-apoptotic, and bi-nucleated cells (Figures 2 and 3). Conversely, most of the cells obtained from Jaradah area exhibited normal characteristics (Figure 4).

The LDH test showed that the enzyme activity is more in tissue homogenates of clam samples obtained from Tubli bay. Conversely, trace amounts of the enzyme activity was detected in the samples obtained from Jaradah and Hid areas (Table 2).

Measurements for chemical and physical parameters of the targeted sites from 2009 to 2012 are shown in Table 3. The measurements showed that the availability of ammonia was increasing with time in all sites.

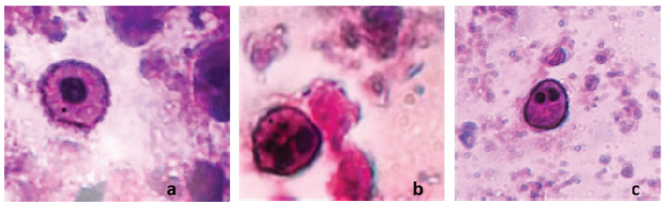

Figure 2: Nuclear abnormalities in gills of clam samples obtained from Tubli bay: (a) cell with micronuclei, (b) fragmented-apoptotic cell and (c) bi-nucleated cell.

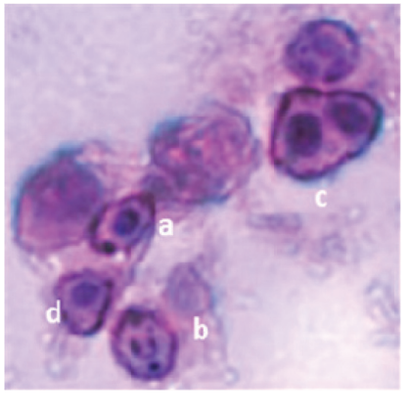

Figure 3: Nuclear abnormalities in gills of clam samples obtained from Al-Hid: (a) cell with micronuclei; (b) fragmented-apoptotic cell, (c) bi-nucleated cell and (d) normal cell.

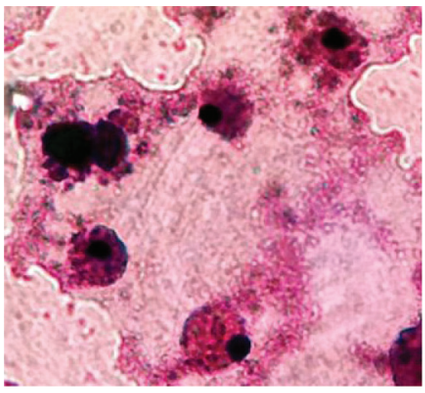

Figure 4: Normal cells of clam gills sample obtained from Jaradah area.

Table 2: Concentration (UI/ml) of lactate dehydrogenase enzyme (LDH) in examined homogenate of clam gills tissue collected from target sites

	Jaradah	*Hid*	*Tubli bay*
Protein Concentration (UI/ml)	0.10	0.30	1.70

Enzymatic activity is expressed as units/mg protein/min 25°C

However, small amounts ranging from 7-14 µg/L were found in Jaradah and Hid areas, while a large

concentrations were obtained from Tubli bay ranging from 230-375 µg/L.

Nitrate concentrations were at their highest in Tubli bay ranging from 37.5-97.5 µg/L, while small amounts of 5-18 µg/L were measured in Jaradah and Hid areas. Phosphate concentrations were found in Jaradah and Hid ranging from 0.6-13 µg/L while dramatically higher concentrations were observed in Tubli bay as they range from 177-303 µg/L.

Higher concentrations of chlorophyll(a) were measured in Tubli bay (7-19 µg/L), while much less concentrations were obtained from Jaradah and Hid areas (0 to 2 µg/L). The concentrations of dissolved oxygen measured in Tubli bay were lower than those from Jaradah and Hid sites (6-7 mg/L).

Hydrocarbons were almost 0 mg/L in Jaradah and Hid areas in years 2009 and 2010 but they were elevating to around 1 mg/L in 2011, to 6.5 mg/L in Jaradah and to 2 mg/L in Hid. Elevated levels of hydrocarbons were observed in Tubli bay ranging from 23-40 µg/L. Total suspended solids (TSS) were found in high concentrations in Tubli bay (12-20 mg/L) compared to lower concentrations measured in Jaradah (3-4 mg/L) and Hid (4-9 mg/L) areas.

Most of the physical and chemical parameters were significantly different between the selected sites (P value ≤ 0.05). However, some parameters showed no significant differences in certain years, including nitrate (2009), chlorophyll-*a* (2009, 2011, 2012), dissolved oxygen (2011), and total suspended solids (2011, 2012) (Table 4).

Principal Components Analysis (PCA) for the physical and chemical parameters, percentage of MN cells, and concentration of LDH indicated that all of them (apart from dissolved oxygen) are significantly correlated with Tubli bay (Figure 5).

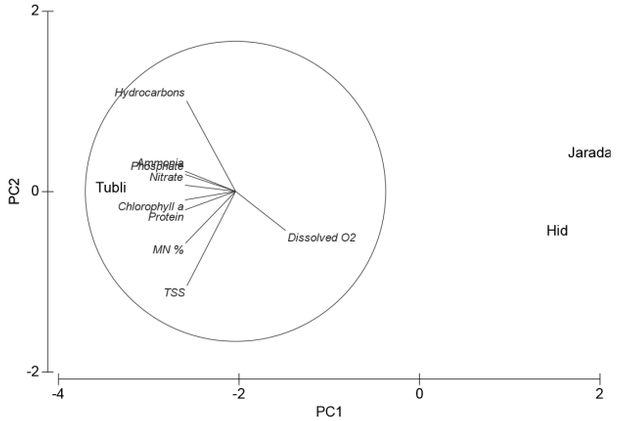

Figure 5: A plot of PCA for physical and chemical parameters, percentage of MN cells, and concentration of LDH.

Table 3: Measurements for chemical and physical parameters of the sampling sites from 2009 to 2012

	Jaradah				*Hid*				*Tubli*			
	2009	*2010*	*2011*	*2012*	*2009*	*2010*	*2011*	*2012*	*2009*	*2010*	*2011*	*2012*
Ammonia	07.33	18.36	06.51	13.07	08.75	12.92	10.90	14.40	231.28	247.08	327.27	375.77
Nitrate	11.40	05.17	05.20	05.62	17.86	08.75	04.94	10.17	38.71	56.10	37.52	97.51
Phosphate	06.52	01.02	00.87	00.70	13.54	04.32	05.55	05.12	186.17	234.22	177.13	302.90
Chl (a)	00.36	00.31	00.37	00.63	01.05	00.76	01.07	02.43	13.04	11.15	07.38	19.54
DO	06.00	06.30	06.60	06.65	06.25	06.20	07.15	06.25	01.95	02.30	06.20	02.85
Hydrocarbons	00.60	00.51	01.81	06.50	00.62	00.72	00.80	02.00	31.30	30.37	38.95	23.22
TSS	4.10	4.05	4.15	3.25	9.20	7.95	4.00	6.30	20.40	13.20	18.80	12.30

Table 4: Variance of physical and chemical parameters over the studied period (2009-2012)

	2009		*2010*		*2011*		*2012*	
Ammonia	$F_{2,9}$= 37.2	P≤ **0.001**	$F_{2,9}$=21.5	P≤ **0.001**	$F_{2,9}$= 98.2	P≤ **0.001**	$F_{2,9}$=111.2	P≤ **0.001**
Nitrate	$F_{2,9}$=1.5	P=0.267	$F_{2,9}$=25.7	P≤ **0.001**	$F_{2,9}$=777	P=**0.011**	$F_{2,9}$=10.9	P=**0.003**
Phosphate	$F_{2,9}$=8.9	P=**0.007**	$F_{2,9}$=5.6	P=**0.026**	$F_{2,9}$=13.7	P=**0.002**	$F_{2,9}$=7.0	P=**0.014**
Chl (*a*)	$F_{2,9}$=3.5	P=**0.073**	$F_{2,9}$=15.4	P=**0.001**	$F_{2,9}$=2.6	P=0.127	$F_{2,9}$=2.1	P=0.182
DO	$F_{2,9}$=15.3	P=**0.001**	$F_{2,9}$=17.2	P≤ **0.001**	$F_{2,9}$=0.33	P=0.720	$F_{2,9}$=6.4	P=**0.019**
Hydrocarbons	$F_{2,9}$=12.9	P=**0.002**	$F_{2,9}$=27.9	P≤ **0.001**	$F_{2,9}$=38.7	P≤ **0.001**	$F_{2,9}$=20.3	P≤ **0.001**
TSS	$F_{2,9}$=5.11	P=**0.032**	$F_{2,9}$=9.18	P=**0.007**	$F_{2,9}$=4.1	P=0.053	$F_{2,9}$=2.3	P=0.158

Discussion

Anthropogenic activities such as the discharge of industrial, municipal, and agriculture wastes cause the introduction of contaminants, including hydrocarbons and other organic and inorganic compounds that induce biological alternations and lead to genotoxic and cytotoxic effects (Barsiene et al., 2012). These effects can be used as an indicator for environmental pollution that can affect the health of marine organisms, population, and community (Leitao et al., 2017). The present study provides an initial characterization for the genotoxic and cytotoxic effects due to increased levels of pollutants. Generally, high percentages of abnormal cells in the form of micronuclei, fragmented-apoptotic cells, nuclear buds, and bi-nucleated cells were associated with Tubli bay area. Bouilly et al. (2007) studied the aneuploidy and hemocyte parameters in Pacific Oyster, *Crassostrea giga* and proposed a classification for aneuploidy based on the percentages of abnormal cells. For instance, a percentage between 10-14 is classified as 'Normal Weak', while a percentage over 30 is considered 'Very High'.

The present study recorded percentages of 11.27 and 45.57 of abnormal cells in Hid and Tubli bay, respectively. Therefore, the genotoxic and cytotoxic effects in Tubli bay is considered very high. This bay is influenced by several environmental stressors, including sewage discharges and reclamation activities (Naser, 2011). On the other hand, abnormal cells were limited in samples obtained from Jaradah area. This could be attributed to the relatively remote location of the area from the main land-based environmental stressors.

LDH is considered among the useful biomarkers of water pollution. Lactate dehydrogenase is an important glycolytic enzyme that may be induced when organisms are exposed to low levels of oxygen or contaminants (Wu and Lam, 1997; Nunes et al., 2015). LDH activity can function as a marker to assess the respiratory status of organisms (Antunes et al., 2010). In several studies, LDH activity variation indicates the response to the stressful environmental conditions and pollution caused by chemical and physical parameters (Aanand et al., 2009; Osman et al., 2010). Likewise, cytotoxicity results obtained from applying the LDH assay showed more enzyme activity in gills tissue homogenate obtained from clam samples collected from Tubli bay compared with the samples collected from Hid and Jaradah areas.

The higher activity of LDH enzyme is likely due to more stressful environment in Tubli bay because of high pollutant presence. The enzyme activity of LDH in gills is highly affected by anoxic environment (Lee and Lee, 2011). Similarly, data from the present study represented by Tables 3 and 4 show that Tubli bay is under low dissolved oxygen values which may explain the higher LDH activity in this site.

Several studies adopted the genotoxicity and cytotoxicity as biomarkers to assess the impacts of a variety of environmental pollution on marine organisms. For instance, Janiva et al. (2012) conducted a study to assess the genotoxicity and cytotoxicity levels in mussels caused by an oil spill in a marine oil terminal in the Baltic Sea using MN test. That study indicated that the gills of the collected samples were severely impacted on the genetic level in that the frequencies of abnormal gill cells were higher after the oil spill than the ones used as a reference before the oil accident (Janiva et al., 2012).

Elevated levels of nutrients and other pollutants such as hydrocarbons are considered major threats to marine ecosystems in the Arabian Gulf (Vaughan et al., 2018). Generally, most of measured physical and chemical parameters in the present study showed a progressive increase in the marine environment of Bahrain in the areas influenced by land-based activities.

Nitrogen and phosphorus are essential elements for the marine life and are important for the growth and development of aquatic organisms in marine environment when provided at their optimum and suitable concentrations (Li et al., 2015). However, anthropogenic activities have globally increased the levels of available essential elements in the environment over the past century (Chen et al., 2012).

Ammonia, nitrate and nitrite are natural components of the nitrogen cycle. Ammonia is produced in trace concentrations as a by-product from the metabolism of proteins and decomposition of organic materials in the coastal waters (Sraj et al., 2014). Generally, ammonia levels were significantly increasing through the years 2009 to 2012 in Tubli bay compared to the concentrations determined in Jaradah and Hid areas.

It is stated that ammonia levels found to be greater than approximately 100 µg/L indicate for polluted waters (Knepp and Arkin, 1973). Therefore, waters nearby the main outlet of the main sewage and treatment plant in Bahrain in Tubli bay could be considered polluted with ammonia. Typically, ammonia damages the gills of fish, filter feeders and other aquatic organisms when reaches the concentration of 60 µg/L, and it is lethal to fish at the level of 200 µg/L (Knepp and Arkin, 1973). Although the concentrations of nitrate obtained

from Tubli bay were relatively high compared to those in Jaradah and Hid areas, these levels did not exceed the toxicity limits in marine environment proposed by Knepp and Arkin (1973).

Phosphates enter the marine environment through the organic wastes of humans and animals. They tend to cause harmful effects on the aquatic organisms when exceed the normal ranges reaching the levels above 100 µg/L of phosphates as a result of industrial effluents and fertilizers runoff (Sulaiman et al., 2014). The fluctuations of phosphate levels noticed in Tubli bay through the years from 2009 to 2012 around the range of 100 to 300 µg/L indicate that the waters of Tubli bay are considered to be relatively polluted with phosphates.

Harmful amounts of phosphate in marine waters lead to direct effects on the algae causing them to grow rapidly and therefore decreasing the levels of available dissolved oxygen causing many aquatic organisms to die eventually (Bussel et al., 2013). This explains the presence of higher concentrations of chlorophyll content where phosphates is concentrated more in Tubli bay site than other sites, exceeding the standard concentration of 15 mg/L or less in 2012 (Shanmugam et al., 2006).

Naturally, hydrocarbons in the marine environment are derived from the combustion of organic matter and are available in 33 µg/L concentrations or less (Samarco et al., 2013). However, it is present in the marine waters from petrogenic sources, such as crude and refined petroleum and from diagenitic origins like short-term degradation of biogenic precursors (Li et al., 2015). Hydrocarbons levels measured in Tubli bay were indicating for signs of pollution in year 2011 (40 µg/L). However, in year 2012 the concentration dropped to 23 µg/L, which is considered below that of the contamination levels.

Higher concentrations of total suspended solids (TSS) measured in Tubli bay compared to other targeted sites were observed in year 2011. However, these concentrations did not exceed the standard TSS concentrations (25 mg/L or less) (Shanmugam et al., 2006). Suspended solids in the seawater are resulting from the discharge of wastes into the water leading to severe damages to the aquatic life (Tsuda, 1997).

Uncontrolled disposal of wastewater and other pollutants is generally associated with environmental degradation. For instance, Shanmugam et al. (2006) assessed the levels of coastal marine pollution in Chennai city, southern India and found that concentrations of dissolved oxygen, nitrate, phosphate, and chlorophyll-*a* reached levels that exceeded the permissible limits of international standards.

In conclusion, the findings of present study revealed that the effluent disposal in the waters of Tubli bay is reducing the quality of coastal water and therefore impacting the marine life and adding to the risk of marine pollution. Additionally, genotoxicity and cytotoxicity studies can be considered as an important tool for long-term monitoring in the marine environment of the Arabian Gulf.

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References

Aanand, S., Purushothaman, C., Pal, A. and K. Rajendran (2010). Toxicological studies on the effect of copper, lead and zinc on selected enzymes in the adductor muscle and intestinal diverticula of green mussel *Perna viridis*. *Indian Journal of Marine Sciences*, **39(2):** 299-302.

Barsiene, J., Andreikenaite, L., Garnaga, G. and A. Rybakovas (2008). Genotoxic and cytotoxic effects in the bivalve mollusks *Macoma balthica* and *Mytilus edulis* from Baltic Sea. *Ekologia*, **54(1):** 44-45.

Barsiene, J., Rybakovas, A., Garhaga, G. and L. Andreikenaite (2012). Environmental genotoxicity and cytotoxicity studies in mussels before and after an oil spill at the marine oil terminal in the Baltic Sea. *Environmental Monitoring and Assessment*, **184:** 2067-2078.

Bouilly, K., Bonnard, M., Gagnaire, B., Renault, T. and S. Lapegue (2007). Impact of diuron on aneuploidy and hemocyte parameters in Pacific Oyster, *Crassostrea gigas*. *Archives of Environmental Contamination and Toxicology*, **22:** 219-223.

Bussel, C., Mahlmann, L., Kroeckel, S., Schroeder, J. and C. Schulz (2013). The effect of high ortho-phosphate water levels on growth, feed intake, nutrient utilization and health status of juvenile turbot (Psetta maxima) reared in intensive recirculating aquaculture systems (RAS). *Aquacultural Engineering*, **57:** 63-70.

Chen, W., Tong, H. and H. Lin (2012). Effects of nitrate on nitrite toxicity to *Microcystis aeruginosa*. *Marine Pollution Bulletin*, **64:** 1106-1111.

Hernandez, A., Gasper, M., Macho, G. and E. Vazquez (2014). Age and growth of the sword razor clams Ensis arcuatus in the Ria de Pontevedra (NW Spain): Influence of the environmental parameters. *Journal of Sea Research*, **85:** 59-72.

Holmes, R. and B. Goldberg (2009). Computational analysis of mammalian lactate dehydrogenase: Human, mouse, opossum and platypus LDHs. *Computational Biology & Chemistry*, **33(5):** 379-385.

Hylland, K., Tollefsen, K., Ruus, A., Jonsson, G., Sundt, R., Sanni, S., Utvik, T., Johnsen, S., Nilssen, I., Pinturier, L., Balk, L., Barisiene, J., Marigonmez, I., Feist, S. and F. Borseth (2008). Water column monitoring near oil installation in the North Sea. *Marine Pollution Bulletin*, **56:** 414-429.

Janiva, B., Alexandras, R., Galina, G. and A. Laura (2012). Environmental genotoxicity and cytotoxicity studies in mussels before and after an oil spill at the marine oil terminal in Baltic Sea. *Environmental Monitoring and Assessment*, **184:** 2067-2078.

Jones, D. (1986). A field guide to the sea shores of Kuwait and the Arabian Gulf. University of Kuwait, Kuwait.

Knepp, G. and G. Arkin (1973). Ammonia toxicity levels and nitrate tolerance of channel catfish. *The Progressive Fish-Culturist*, **35(4):** 221-224.

Ladeira, C., Viegas, S., Carolino, E., Gomes, M. and M. Brito (2012). Genotoxicity Biomarkers: Application in Histopathology Laboratories. Biomarkers. Khan, T. INTEC Publications.

Lee, A. and K. Lee (2011). The enzyme activities of opine and lactate dehydrogenase in the gills, mantle, foot and adductor of the hard clam *Meretrix lusoria*. *Journal of Marine Science and Technology*, **19(4):** 361-367.

Leitao, A., Al-Shaikh, I., Hassan, H. and R. Hamadou (2017). First genotoxicity assessment of marine environment in Qatar using the local Pearl oyster *Pinctada radiata*. *Regional Studies in Marine Science*, **11:** 23-31.

Li, H., Zhang, C., Han, X. and X. Shi (2015). Changes in concentrations at oxygen, dissolved nitrogen, phosphate, and silicate in the southern Yellow Sea 1980-2012: Sources and seaward gradients. *Estuarine, Coastal and Shelf Science*, **163:** 44-55.

Li, P., Diao, X., Zhang, Y., Xie, Y., Yang, F., Zhou, H., Han, Q., Wang, F., Cheng, H. and H. Wang (2015). Polycyclic aromatic hydrocarbons in surface sediment from Yangpu Bay, China: Distribution, sources and risk assessment. *Marine Pollution Bulletin*, **99:** 312-319.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and R.J. Randall (1951). Protein measurement with the Folin phenol reagents. *Journal of Biological Chemistry*, **193:** 265-275.

Naser, H. (2011). Effects of reclamation on macrobenthic assemblages in the coastline of the Arabian Gulf: A microcosm experimental approach. *Marine Pollution Bulletin*, **62:** 520-524.

Naser, H. (2013). Assessment and management of heavy metal pollution in the marine environment of the Arabian Gulf: A review. *Marine Pollution Bulletin*, **72:** 6-13.

Nunes, B., Travasso, R., Goncalves, F. and B. Castro (2015). Biochemical and physiological modifications of *Sardina pilchardus*: Spatial and temporal patterns as a baseline for biomonitoring studies. *Frontiers in Environmental Science*, **3:** 1-14.

Rode, J. (2008). Apoptosis, Cytotoxicity and Cell Proliferation, Fourth Edition. Roche Diagnostics GmbH.

Sabharwal, R. (2015). Emergence of Micronuclei as a Genomic Biomarker: A Review. *Indian Journal of Medical & Pediatric Oncology*, **36(4):** 212-218.

Samarco, P., Lolian, S., Warby, R., Bouldin, J., Subra, W. and S. Porter (2013). Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater Horizon Oil spill, Gulf of Mexico. *Marine Pollution Bulletin*, **73(1):** 129-143.

Shah, S. (2012). Importance of Genotoxicity and S2A Guidelines for Genotoxicity Testing for Pharmaceuticals. *Journal of Pharmacy and Biological Sciences*, **1(2):** 43-54.

Shanmugam, P., Neelamani, S., Ahn, Y., Philip, L. and G. Hong (2006). Assessment of the levels of coastal marine pollution of Chennai city, Southern India. *Water Resources Management*, **21(7):** 1187-1206.

Simboura, N. and A. Zenetos (2002). Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. *Mediterranean Marine Science*, **3(2):** 77-111.

Sraj, L., Almeida, M., Swearer, S., Kolev, S. and I. McKelvie (2014). Analytical challenges and advantages of using flow-based methodologies for ammonia determination in estuarine and marine waters. *Trends in Analytical Chemistry*, **59:** 83-92.

Sulaiman, R., Ismail, Z., Othman, S., Ramli, A. and S. Shirazi (2014). A comparative study of trends of nitrate, chloride and phosphate concentration levels in selected urban rivers. *Measurements*, **55:** 74-81.

Tobias, N. (2008). Marine Pollution: New Research, First Edition. Nova Science Publishers, New York.

Tsuda, R. (1997). Turbidity and Suspended Solids Concentration in Coastal Areas. *Oceanographical Society of Japan*, **12:** 51-62.

Vaughan, G., Al-Mansoori, N. and J. Burt (2018). The Arabian Gulf. *In:* Sheppard, C. (ed.), World Seas: An Environmental Evaluation, second edition. Elsevier Science, Amsterdam, NL.

Wardrop, P., Shimeta, J., Nugegoda, D., Morrison, P., Miranda, A., Tang, M. and B. Clarke (2016). Chemical Pollutants Sorbed to Ingested Microbeads from Personal Care Products Accumulate in Fish. *Environmental Science & Technology*, **50(7):** 4037-4044.

Wright, S. (2013). The Physical Impacts of Microplastics on Marine Organisms: A Review. *Environmental Pollution*, **178:** 483-492.