Effects of pollution on marine crustaceans in Qatari waters: a baseline survey and a case study on genotoxicity indicators in an endemic shrimp

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The endemic shrimp *Palaemon khori* of Al-Khor Qatar (photo: Environmental Science Center (ESC) 2017)

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To Mustafa (R.I.P), Ekhlas, Lala, De and Moe.

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Glossary of Terms

- **Accuracy** -- a term used in survey research to refer to the match between the target population and the sample.
- **Aneuploidy** -- the presence of an abnormal number of chromosomes in a cell.
- **Baseline** -- Analysis of current concentration of pollutants to identify the starting points.
- **Bioaccumulate --** The accumulation of substances, such as pesticides, or other chemicals in an organism.
- **Bioindicators** -- A bioindicator is any species (an "indicator species") or group of species whose function, population, or status can reveal the qualitative status of the environment.
- **Biotransfer** – The transfer of material by living organisms
- **Biomagnify --** The increasing concentration of a substance, such as a toxic chemical, in the tissues of organisms at successively higher levels in a food chain.
- **Biomonitors** -- defined as an organism that provides quantitative information on the quality of the environment around it. Therefore, a good biomonitor will indicate the presence of the pollutant and also attempt to provide additional information about the amount and intensity of the exposure.
- **Carcinogenic --** Can lead to causation of cancer.
- **Cytogenic –** analysis of the number and structure of chromosomes.
- **Case Study** -- the collection and presentation of detailed information about a particular participant or small group, frequently including data derived from the subjects themselves.
- **CRM** – Certified reference material
- **Chronic --** persisting for a long time
- **Chi-square Analysis** -- a common non-parametric statistical test which compares an expected proportion or ratio to an actual proportion or ratio.
- **Controlled Experiment** -- an experimental design with two or more randomly selected groups [an experimental group and control group] in which the researcher controls or introduces the independent variable and measures the dependent variable at least two times [pre- and post-test measurements].
- **Correlation** -- a common statistical analysis, usually abbreviated as r that measures the degree of relationship between pairs of interval variables in a sample. The range of correlation is from -1.00 to zero to +1.00. Also, a non-cause and effect relationship between two variables.
- **Cytogenetic endpoints --** Cytogenic indicators such as Milotic index and aneuploidy
- **DBT** -- Dibutyltin.
- **Data** -- factual information [as measurements or statistics] used as a basis for reasoning, discussion, or calculation.
- **Data Mining** -- the process of analyzing data from different perspectives and summarizing it into useful information, often to discover patterns and/or systematic relationships among variables.
- **Diploid** -- describes a cell that contains two copies of each chromosome.
- **EDI** – Estimated daily intake.

- **ESC** -- Environmental Science Center, Qatar University.
- **Genotoxic** -- destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. **Genotoxicity** describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. While **genotoxicity** is often confused with **mutagenicity**, all **mutagens** are **genotoxic**, whereas not all **genotoxic** substances are **mutagenic**.
- **LOD –** Below the limit of detection of the analytical instrument.
- **Mangroves** -- a shrub or small tree that grows in coastal saline or brackish water.
- **Measurement** -- process of obtaining a numerical description of the extent to which persons, organizations, or things possess specified characteristics.
- **Metaphase** -- the second stage of cell division, during which the chromosomes become attached to the spindle fibers.
- **Methods** -- systematic approaches to the conduct of an operation or process. It includes steps of procedure, application of techniques, systems of reasoning or analysis, and the modes of inquiry employed by a discipline.
- **Mutagenic** -- a chemical or physical agent's capacity to cause mutations (genetic alterations). Agents that damage DNA causing lesions that result in cell death or mutations are genotoxins.
- **PCBs** -- polychlorinated biphenyl (**PCB**) is an organic chlorine compound with the formula $C_{12}H_{10-x}Cl_x$.
- **QA/QC –** Quality assurance and Quality Control.
- **Sample** -- the population researched in a particular study. Usually, attempts are made to select a "sample population" that is considered representative of groups of people to whom results will be generalized or transferred. In studies that use inferential statistics to analyze results or which are designed to be generalizable, sample size is critical, generally the larger the number in the sample, the higher the likelihood of a representative distribution of the population.
- **Standard Deviation** -- a measure of variation that indicates the typical distance between the scores of a distribution and the mean; it is determined by taking the square root of the average of the squared deviations in a given distribution. It can be used to indicate the proportion of data within certain ranges of scale values when the distribution conforms closely to the normal curve.
- **Statistical Analysis** -- application of statistical processes and theory to the compilation, presentation, discussion, and interpretation of numerical data.
- **Statistical Significance** -- the probability that the difference between the outcomes of the control and experimental group are great enough that it is unlikely due solely to chance. The probability that the null hypothesis can be rejected at a predetermined significance level [0.05 or 0.01].
- **Statistical Tests** -- researchers use statistical tests to make quantitative decisions about whether a study's data indicate a significant effect from the intervention and allow the researcher to reject the null hypothesis. That is, statistical tests show whether the differences between the outcomes of the control and experimental groups are great enough to be statistically significant. If differences are found to be statistically significant, it means that the probability [likelihood] that these differences occurred solely due to chance is relatively low. Most researchers agree that a significance value of

.05 or less [i.e., there is a 95% probability that the differences are real] sufficiently determines significance.

- **Trace metals** -- Trace metals are important elements in the biogeochemistry of aquatic ecosystems. They are normally present in small but measurable amounts.
- **Unit of Analysis** -- the basic observable entity or phenomenon being analyzed by a study and for which data are collected in the form of variables.
- **Weighted Scores** -- scores in which the components are modified by different multipliers to reflect their relative importance.

Abstract

In this thesis the baseline and seasonal variations of the trace metals (TM) (Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb), Vanadium (V) and Zinc (Zn).

Methylmercury (CH_3Hg), organotins (OT) and polychlorinated biphenyls (PCBs) concentrations were investigated within the tissue of three selected marine organisms together with sediment and water. The first round of sampling was carried out in September, November and December 2014. Samples were then collected in May and June 2015, and finally in July 2016. The species used for the research are all indigenous to Qatari waters and included *Portunus pelagicus,* the blue swimming crab, a commercially important edible species, *Balanus amphitrite*, the striped barnacle, an invasive species that arrived in Qatar on the hulls of merchant ships, and now part of the local fauna. This species is often used as bio-indicator of coastal pollutants. The final selected species is the endemic caridean shrimp, *Palaemon khori* which inhabits the *Avicenna marina* mangrove forest at Al-Khor, Qatar.

The order of the heavy metal concentrations for the three species was Zn > Cu > As > Fe > Mn > Pb > Cr > Cd > Co > Ni > V for *P. pelagicus*, Zn > Fe > Cu > As > Cd > Mn > Ni > Cr > V > Pb > Co for *B. amphitrite* and Zn > Cu > Fe > Mn > Ni > Pb > As > Cr > Cd > Co > V for *P. khori*. Methylmercury was measured in all of the three species averaging 1.25µg/kg throughout. The three organotin monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT) were detected in all three species. TBT levels were observed at 0.58ngSng^{-1}, 0.78ngSng^{-1} and 1.91ngSng^{-1} for *P. segnis*, *B. amphitrite* and *P. khori* respectively. The results also revealed that concentrations of the contaminants within water samples were negligible, the majority being below detection limits. However, PCB congeners 2,2,3,5-Tetrachlorobiphenyl and 2,2,4,5,5-Pentachlorobiphenyl were detected in the tissue of *P. pelagicus* averaging 1.70 µg/kg for the former and 4.56 µg/kg for the latter. Results from the tissue of *P. khori* ranged from 1.5µg/kg - 2µg/kg and 4.28µg/kg – 5.21µg/kg respectively.

Seasonal variation studies showed fluctuating degrees of variability among pollutants depending on the target species and the pollutant. The results showed an increase in some pollutant concentrations from the winter months through to the summer, while subsequently other pollutants revealed a decrease in concentrations.

The direct effect of varying levels and combinations of pollutants (the maximum concentrations of trace metals, OT and MeHg found in the environment according to the literature, and a tenfold increase in those concentrations in an individual setting and in a combined setting) under laboratory controlled conditions on the endemic *P. khori* was assessed using classical (mortality) and genotoxicological (aneuploidy) endpoints, after 4 and 8 weeks of exposure. Our results showed that with regards to mortality on average the highest mortality was observed in shrimps exposed to TM at x10 of the maximum environmentally observed levels or TM in combination with other pollutants. The pollutant inducing the highest aneuploidy levels were trace metals and CH_3Hg in combination with OT (both at x10 concentration).

The data presented in this study represents the first ever estimated baseline for seasonal variations of contaminants, in both the marine environment and associated animal tissues from the coastal waters of Qatar. The data obtained from the genotoxic investigations are a fundamental part in establishing the first ever record for the karyotype of *P. khori*, while also providing a genotoxicological overview of the effect of these pollutants on the species at a genetic level.

Chapter I: Introduction

1.1 General introduction

The Arabian Gulf is a Mediterranean sea in western Asia. This water body is an extension of the Indian Ocean through the Strait of Hormuz and lies between Iran to the northeast and the Arabian Peninsula to the southwest. It is enclosed by eight countries: Iran, Iraq, Kuwait, Saudi Arabia (K.S.A), Bahrain, Qatar, United Arab Emirates (UAE), and Oman (Figure 1.1).

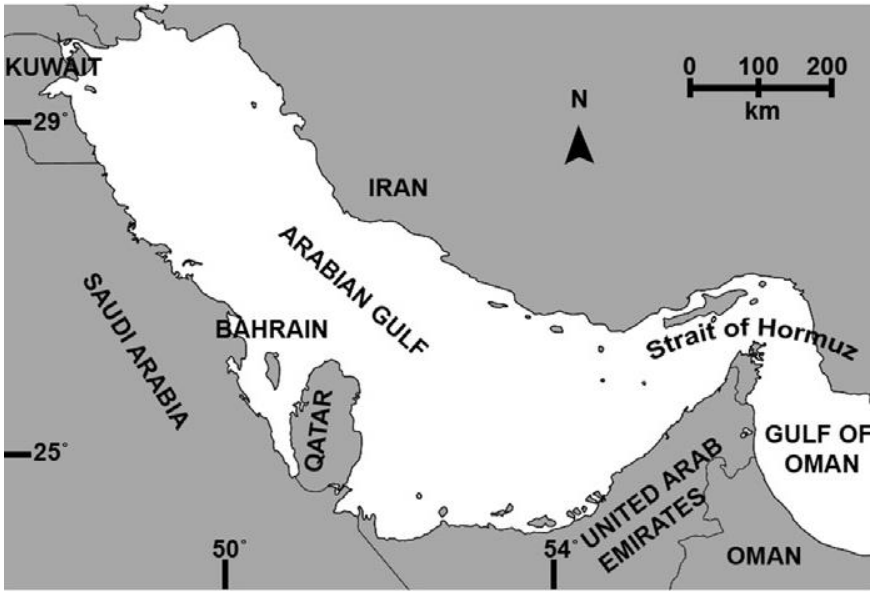

Figure 1. 1: The Arabian Gulf and surrounding countries

Being the location for one of the richest oil reserves in the world, the Arabian Gulf marine ecosystem continuously faces the risk of pollution. The Arabian Gulf is also a major shipping line and oil transportation, and thus accidental spilling is unavoidable. It is estimated that over 24 thousand oil tankers use these lanes annually (Al-Saad & Salman, 2012). The oil industry activities' such as exploration, drilling and transportation have taken their toll and resulted in a variety of adverse effects, such as damage to coral reefs, algal mats and mangroves (Elshorbagy, 2005). The Arabian Gulf is also characterized by shallow waters, the deepest point being about 100 m with an average depth of 35 m, and the majority of areas near the coast are less than 10 m deep. This lack of depth influences other ecological factors, such as water temperature (Sheppard et al., 2010). Due to the small amount of rain within the

region, the Arabian Gulf has also a higher salinity than most seas (38 - 44 psu, as compared to 35 psu). Finally the Arabian gulf is semi isolated from the world sea waters, the only connection being through the Strait of Hormuz a narrow connection that is not enough to dampen the effect of the high salinity and wide temperature swings (Al-Saad & Salman, 2012).

Historically, estuaries and coastal areas were chosen as location of early settlements due to the ease of marine transportation of goods and materials. Consequently Until this day most of the population centers are located either on the sea or in locations where ready access to the sea is available, with increasing population numbers (Kummu et al., 2011). Concomitant with the evolution and growth of these coastal populations, and due to similar reasons, there has been an increase in the number of industries along the coastline. This has placed a long-lasting overwhelming stress on the marine environment (Van Lavieren et al., 2011).

Anthropogenic activities have modified the marine environment globally, with very few coastal areas still unaffected by human activities. Marinas, seashore residential compounds and harbor developments have required extensive dredging (Erftemeijer et al., 2012) and the creation of other structures to safeguard shipping lanes and stabilize construction processes. The demand for coastal land and docking space has led to the filling of large areas of the seashore with waste, produced from dense populations and from industries, discharged into coastal waters with little understanding or appreciation of the possible effects (Van Lavieren et al., 2011).

Initially, this issue of impact was not of concern, as the receiving capacity of the body of water was not exceeded, enabling the ecosystem to recover from these activities, seemingly without permanent damage (UK Technical Advisory Group, 2007). Unfortunately nowadays, in some of the most densely populated parts of the world, the stress pressure from anthropogenic activities has increased past the threshold level of the marine ecosystem (Akpor, 2011).

Marine ecosystems provide animal proteins that play a very important role in maintaining vital nutrition to our dense population (Titilade & Olalekan, 2015). The aquatic environment also fulfills the growing needs of recreational opportunities supporting a long time favorite and growing industry within the developed world, and specifically within the Middle East countries, which have limited fresh water sources and thus need to rely heavily on seawater for the provision of fresh water from desalination plants. All these emphasize the need for desirable healthy marine ecosystem conditions.

Qatar is a peninsula almost completely surrounded by the Arabian Gulf; one of the world's most impacted regions due to anthropogenic activities (Naser, 2013). Approximately two thirds of the world's reserve oil deposits are situated within the Gulf region with the Gulf countries accounting for a quarter of the world's oil production (Khan, 2002). Qatar's main revenue is from oil and gas production and other petrochemical industries. Moreover, Qatar has no natural fresh water resources and thus there is a heavy dependence on desalination plants (Saif, 2012). With this in mind, the view of most scientists working in the region is that the most substantial threat to the marine ecosystems within the Gulf region comes from the large coastal modification through dredging and the conversion of natural areas into land fit for urban housing and industrial facilities (Khan, 2007; Munawar et al., 2002). All these factors contribute to the degradation of the marine environmental health, with the overall effects of accumulated contaminants in the terrestrial and aquatic environments rapidly on the rise (Elshorbaghy, 2005).

On the intertidal coastline of Qatar, the grey or white mangrove *Avicennia marina* occurs (Figure 1.2). There are eight mangrove forests situated in the eastern coast of the state, the oldest and richest in biodiversity is located at Al-Thakira and Al-Khor. Recent research has shown that *A. marina*' stands have the capability to acclimatize to the varying weather cycles that occur along the Qatar coastline (Yasseen & Abu-Al-Basal, 2008).

Figure 1. 2: Al-Khor mangroves at low tide.

Mangroves are ecologically and environmentally beneficial: they provide shelter and breeding grounds for many species of birds and other animals, they also stabilize the coastline acting as greenbelts and buffer zones against harsh arid conditions and most

importantly they combat the negative effects of greenhouse gases (Pernot et al., 2015). Their complex root systems act as anchor for the plants thus slowing the incoming tides and settling the sediment (organic and inorganic matter). Mangrove trees in general have limited oxygen due to the low oxygen availability in the anoxic conditions of the waterlogged mud, thus slowing the rate of decay, and increasing the amount of carbon that is accumulating in the sediment (Marchio et al., 2016)

Given the anthropogenic activities in the Arabian Gulf, a variety of pollutants, such as trace metals, methylmercury, organotins, and polychlorinated biphenyls have been widely reported in the area (Agah et al., 2006; de Mora et al., 2004; De Mora et al, 2003; Freije, 2015; Lyons et al., 2015).

1.2 Trace metal

Trace metal contamination of the marine ecosystem has lately gained notoriety and is classified as a worldwide problem due to trace elements toxicity and their bioaccumulation within marine environments even at low concentrations(Censi et al., 2006; DeForest, Brix, & Adams, 2007; Herber, 2004). Their toxicity arises not only from the level of contamination but also from the biochemical role they play in the metabolic processes as well as the extent to which they are absorbed and excreted by marine organisms (Jakimska et al., 2011) Although it is recognized that metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are essential nutrients that are required for various biochemical and physiological functions (World Health Organization , 1996), others such as aluminum (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) have no recognized biological functions and are classified as non-essential metals (Chang et al., 1996). Metals form one of the major contaminants resulting from anthropogenic activities on coastal and marine environments (Yu et al., 2008). These are a serious risk to humans' health, marine organisms and the natural environments. Some of them are also potentially carcinogenic (Zeng et al., 2015). They may enter the marine ecosystem as nontoxic metals in their elemental form, via various sources such as natural input, domestic and industrial sewage effluent (Goldberg, 1995; Islam & Tanaka, 2004), and then they can be incorporated in the sediment or in the seawater.

Most coastal areas and seas contain a limited amount of naturally occurring trace metals. Nevertheless, their cationic forms are dangerous to living organisms because of their capacity to bind with short carbon chains. These forms bioaccumulate in protein-rich tissues of marine organisms and may eventually end up in humans. Exposure to certain heavy metals can affect cellular components such as cell membrane, mitochondria, etc. (Wang & Shi, 2001) and they can interfere with cellular metabolic functions causing harmful side effects (Davis, 1979).

The effect of the Gulf War in 1991 and the ensuing oil spills on the concentration levels of several heavy metals in the marine environment and in aquatic organisms was the focus of several studies (e.g. (Al-Sayed et al., 1996; Madany et al., 1996). Levels of Cd, Pb, Zn, Ni, Cu, Mn, and Fe were studied in the tissues of fish from various parts of the Gulf such as United Arab Emirates (UAE), Oman, Kuwait, and Bahrain from 1991 to 1996 (Al-Sayed et al., 1996; Fowler et al., 1993; Madany et al., 1996) and during this timeframe trace metal concentration in the fish tissue were within international acceptable limits (Table 1.1), apart from Pb and Zn which were both considered to be in the upper limits. Similar results were found in Qatar (Al-Sayed et al.,1996; Kureishy, 1993) where no significant increase in the levels of Cd, Co, Cu, Hg, Ni and Pb were detected in the muscle tissue of various benthic and semi pelagic organisms analyzed.

Among mollusks, pearl oysters, *Pincatada radiate*, from the state of Bahrain contained higher levels of Cu, Zn, Pb, Mn, Ni, Cd, and Fe than those levels found in the seawater. Nevertheless, the levels were within the limits set by the World Health Organization (WHO; Table 1.1), with the exception of Pb and Cd (Al-Sayed et al., 1994). Heavy metals (As, Cd, Cu, Fe, Mn, Ni, Pb, V, Zn, and Hg) from the regional waters of Bahrain were assessed from over 23 locations, chosen as they were considered good fishing grounds (Juma & Al-Madany, 2008). The levels of the studied metals were found to be within the United Kingdom Quality Standards, except for Cu (4.53 μg/l –119 μg/l) in all sites and Hg (0.38 μg/l) in one site, confirming that the Bahraini marine waters are of good quality. Sadiq & McCain, (1993) studied the levels of Cr, Pb, Co, Mo, Cd, Zn, V, and Ni within clams, *Meretrix meretrix*, seawater, and sediments obtained from the Saudi coast during April–May 1999. The investigators compared their levels to levels of the same metals from 1985, all collected from the same area. They concluded that the levels varied at some stations showing an increase while other showed a decrease. It was noted however, that the scale of the increase was far larger in the clams collected in 1991 from locations toward the north (i.e., toward Kuwait), suggesting that the 1991 spill had an effect on the heavy metal

concentration levels. However, samples of fish from the Kingdom of Saudi Arabia and analysed for trace metals (Cd, Pb, Ni, V, and As) showed that these trace metals were below the maximum fish human consumption permissible level allowed by international legislations, indicating a return to normal expected levels, and decrease in the effect of the Gulf war (Iman Al-Saleh & Shinwari, 2002). A study performed in the Gulf of Oman in 2000-2001 detected high concentrations of Cd within the tissue of the orange spotted grouper *Epinephelus coioides* and the spangled emperor *Lethrinus nebulosus*, and this was later attributed to food-chain bioaccumulation due to upwelling in the region (de Mora et al., 2004). Moreover, it was noted that certain bivalve species (*Pinus radiata* and *Saccostrea cucullata*) exhibited very high levels of As but these high levels were attributed to natural origins rather than anthropogenic contamination (de Mora et al., 2004). Another study conducted in the cuttlefish *Sepia pharaonis* from the same country and investigating the levels of Cr, Cu, Zn, Ni, Cd, and Pb showed that the levels were within the safe limits for human consumption, although these samples were collected from areas within Saudi Arabia that were well known for anthropogenic and industrial pollution, and receiving urban effluents (Almasoud, Usman, & Al-Farraj, 2014). In Qatar, the edible portions including skin of 20 popular fish species were examined for heavy metal pollution (Cu, Zn, Pb and Hg), and were found to be safe for human consumption (Al-Jedah & Robinson, 2001). Al-Abdali, Massoud, & Al-Ghadban, (1996) examined core sediment samples from the Arabian Gulf in the western offshore area off Bahrain, Qatar, and the UAE for trace metals contents (Fe, V, Ni, Pb, and Cu), used as indicators of pollution levels in relation to the Kuwait oil slick that occurred in 1991. The results indicated that the levels of the metals studied were within permissible natural background levels. High levels of Pb (111 mg/kg) were reported in samples taken from coastal stations within Bahrain (Akhter & Al-Jowder, 1997). These levels were attributed to pollution from land-based industrial and urban sources, in particular automobiles. Analyzing the contents of these pollutants in aquatic organisms which can have the ability to bioaccumulate them (Sures, 2004), is a good estimate of the biological availability of environmental pollutants (Ali & Fishar, 2005).

Table 1. 1: International set limits for some trace metals, and selected pollutants in the tissue of aquatic organisms.

Analyte	International Limits (mg/kg)	PTWI* (µg/kg b wt.)	Reference	Comment
As	0.10 – 0.50		GB2762-2012	No legislation for As in UK or USA except for rice
Cd	0.05	7.00	EU/Reg.1881/2006/EU	
Cu	30.00	3500.00	FAO/WHO 2011	
Co	-	-	-	
Cr	-	-	-	
Fe	100.00	5600.00.	FAO/WHO 1983	
Hg	0.50	7.00	EU/Reg.1881/2006/EU	
Mn	1.00	980.00	FAO/WHO 1989	
Methylmercury	1.00	7.00	Codex, WHO, 2011	
Ni	70.00	35.00	USFDA	
Pb	0.30	25.00	EU/Reg.1881/2006/EU	
PCBs	2.00	-	FDA	
TBT	0.12	*250.00	OSPAR	*EFSA 2004
Zn	100.00	7000.00	FAO/WHO 1989	

Codex / WHO, (2011) committee on contaminants in foods
FAO/WHO,(2011)Evaluation of certain food additives and the contaminants mercury, lead and cadmium (1989) WHO Technical Report Series No. 505
EU/Reg.1881/2006/EU Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
OSPAR Commission, (2007) JAMP Guidelines for monitoring contaminants in biota and sediments
PTWI Provisional Table Weekly Intake (µg/ kg of body weight) WHO
National Standards (PRC), (1988) National Food Safety Standard. Maximum Levels of Contaminants in Food.
USFDA the Food and Drug Administration (FDA)
EFSA European Food Safety Authority

1.3 Methylmercury

Methylmercury (CH_3Hg) is one of the organic forms of mercury (Hg) and it is a known contaminant that can be present in fish and other seafood that are important for local consumption (Jacob, 2013), often exceeding international guidelines (Table 1.1; Chen et al., 2012). Indeed, human exposure to this toxic element is associated to its chemical speciation in marine ecosystems. Coal burning, medical waste, mining of gold are just of the few pathways by which Hg can enter the environment, although atmospheric deposition is the major source. Within the marine environment, the inorganic form of Hg is transformed into the stable organic form MeHg (World Health Organization (WHO), 2003), which is the principal organic form of Hg in seafood (U.S. Environmental Protection Agency (USEPA), 1991). Its toxicity comes from the fact that it is difficult to eliminate and it bioaccumulates in

the food chain with potentially high concentrations in certain fish and invertebrate species, which may be consumed by carnivorous predators or humans.

The apprehension about mercury contamination in the aquatic environment, began with the infamous Japanese Minamata Bay incident in the 1950s, in which a number of people were poisoned and some later died as a result of consuming seafood with high levels of MeHg (McCurry, 2006). Some large long-lived predatory fish may contain high levels of methyl mercury, small fish absorb CH_3Hg from water as they consume other smaller aquatic organisms. The longer the fish lives the more methylmercury the fish accumulates in its body. Larger fish that feed on other fish (high in the food chain) accumulate the highest levels of methylmercury (FDA) and over 90% of total Hg concentrations are attributed to MeHg (WHO, 2003). The biotransformation process may occur either within the water column or in the sediment (US EPA, 2011), this takes place through several pathways mainly facilitated by specific microorganisms, such as sulphur-reducing anaerobic bacteria (Gilmour & Henry, 1991; Jensen & Jernelöv, 1969; Regnell & Tunlid, 1991; WHO, 1993). These bacteria process the sulphate within the ecosystem by taking up Hg in its elemental form and converting it to MeHg (Figure 1.3) through metabolic processes.

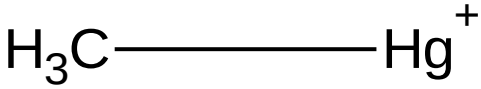

Figure 1. 3:Structure of methylmercury

Methylmercury then gets quickly adsorbed by plankton, which is in turn is consumed by the next level in the food chain (US Geological Society (USGS), 2000). Even at very low rates of atmospheric deposition, in locations remote from point sources, Hg biomagnification can result in toxic effects to marine organisms at the top of the aquatic food chains ((Braune et al., 2006; Singh, 2005).

Methylmercury is known to affect the nervous system (Horvat, 2001; WHO, 1990) and both organic and inorganic forms af mercury affect enzymatic activities leading to possible cell damage (Zhao et al., 2010). Methylmercury has a high attraction to lipids, thus allowing movement through the cell membranes, and therefore affects cell metabolism (de Pinho et al., 2002). Methylmercury might also interfere with the way cells divide, leading to unequal distribution of chromosomes to the daughter cells (Laws, 1993).

The transformation of mercury to methylmercury facilitated by microorganisms and under anaerobic conditions(Benoit, Gilmour, Heyes, Mason, & Miller, 2002; Compeau & Bartha, 1985), is the main pathway by which CH_3Hg is produced. Nevertheless,

environmental factors that govern such a transformations are poorly understood (Morel, Kraepiel, & Amyot, 1998). One of the most critical factors is the coupled reactions between Hg redox transformation and complexation with natural dissolved organic matter (DOM), this governs the speciation, biological uptake, and methylation of aqueous Hg in aquatic environments (Mason et al., 2006). Although this is not in the scope of this thesis it is noted that the research into the matter is still ongoing (Delongchamp et al., 2009; Li et al., 2009; Merritt & Amirbahman, 2009).

There has not been a lot of research done on the levels of methylmercy in the species selected for this thesis, within the Arabian Gulf region in general and Qatar in particular. This represents a fundamental gap in the knowledge regarding these important species. This research aims to add to the limited data available to better understand the levels of this hazardous pollutant.

1.4 Organotins

Organotins comprise a group of very important industrially used compounds. They are often used as stabilizers in process related to additives in plastics and silicone products, and have been extensively used as an active ingredient in wood protecting paints and disinfectants (CAS, 1998). These compounds show variable degrees of toxicity to a wide-ranging field of marine organisms and thus have been used as biocides (Blunden et al., 1990) often as anti-fouling agents in paints (Omae, 2003), as is the case of Tributyltin (TBT) , which now can be found worldwide in the marine ecosystem, from the coastal zones to the open seas. Other widely used organotin compounds are monobutyltin (MBT) and dibutyltin (DBT) (Rüdel, 2003), which are a versatile group with a variety of uses, from their role as stabilizers in process related to additives in plastics, silicone products, to their integration in wood protecting paints and disinfectants (CAS, 1998).

Organotin compounds mainly enter the marine environment via leaching from the soil, degradation of plastics, release from boat paints, and wastewater (Hussein K Okoro et al., 2011). They breakdown at a slow rate and thus persist and bioaccumulate in the marine environment (Iwata et al., 1997; Michel & Averty, 1999; Stewart & Mora, 1990).

In marine organisms these compounds may initiate the acquisition of male characteristics by female organisms, produce physical deformations and even induce larval mortality (Alzieu, 1998). Contamination from TBT has also resulted in the deformation of Pacific oyster's shells (Alzieu, 1996) and the increase of imposex characteristics in marine gastropods (Gibbs, P., 1996). The ban on antifouling organotin containing paints applied to

ships started in France in the 1980s and was later adopted in other western countries as well as Japan (89/677/CEE) (Díez, Ábalos, & Bayona, 2002). Moreover, research in Taiwan showed that TBT levels in oysters construed a risk to the health for fishermen in coastal marine communities (Chien et al., 2002). One of the studies looking into the impact of organotins on marine organisms was carried out using the marine microalga Nannochloropsis oculata, within the South Korean coastal waters (Sidharthan et al., 2002) the investigation showed that there is an adverse effect from TBT also on these organisms, particularly on the photosynthetic pigments By the beginning of 2008 there was a global ban in the use of organotin based antifouling paints (Champ, 2003).

Sediments and marine organisms' tissue provide a valuable resource in assessing organotin contamination. For example, the levels of TBT within clams (*Mya arenaria* and *Tresus nuttallii*) collected from Oregon's Coos Bay estuary, USA triggered a health advisory warning in 1995 when they reached 460µg/kg (Elgethun et al., 2000). Organotin compounds levels were analyzed in marine sediment from Suez Gulf: the levels of TBT averaged 1370µg/kg dry weight while the levels of DBT averaged 580µg/kg dry weight (Shreadah, 2011). The study also established a relationship between TBT and DBT, and postulated that DBT was mostly obtained from the degradation of TBT, and the high levels reported attributed to shipping activity within the harbor. Following the ban of antifouling paint that contains organotins species, various studies were performed on the strait between Denmark and Sweden, using the clam *Nuculana pernula* (commonly known as the pointed nut clam). The data gathered showed that TBT was bioaccumulating within the sample species, as a result of sediment pollution. The research also showed that levels of TBT concentrations in *N. pernula* were gradually reducing with time may be due to degradation (Strand & Asmund, 2003). TBT and its degradation products were also investigated in the west coast of Greenland using the bivalve *Mytilus edulis*. TBT levels were observed to be elevated in the research species collected from harbors (Strand & Asmund, 2003). In 2002, an evaluation of organotin pollution in Poland was undertaken within the tissue of the mussel, *M. edulis* and the flounder, *Platichthys flesus* both collected along the coast of Baltic Sea. The study concluded that the maximum values were recorded in specimens from the Gdansk Gulf, and that butyltins were mostly observed in the fish liver (Albalat et al 2002). In another investigation accumulation of BT was studied in rabbitfish (*Siganus vermiculatus*) living along the west coast of Sri Lanka and also here the highest concentration levels of BT were within the liver (Guruge & Tanabe, 2001). Organotin concentrations have been extensively

studied in developed countries (Champ & Seligman, 1996; de Mora, 1996), but data pertaining to organotin concentration within the aquatic ecosystems of the Arabian Peninsula are sparse. Hasan & Juma, (1992) analyzed TBT in sediment from Bahrain, while other organotin species were investigated in fish from the Arabian Gulf (Watanabe et al., 1998). Data was also reported for the Regional Organisation for Protection of the Marine Enviroment (ROPME) Sea Area, which included sampling locations of Umm Said, Dukhan, Doha, Ras Laffan and Ras Al-Nouf, all location in Qatar, from which sediment and grouper fish were analyzed (De Mora et al., 2003). The authors reported that the concentration of organotins in sediment ranged from < 0.23 – 3.1 for MBT, < 0.06 – 1.4 for DBT and < 0.06 – 1.7 for TBT ngSng^{-1} dry weight. There are no guidelines for MBT and DBT, however, the Oil Spill Prevention, Administration and Response (OSPAR) has a 120 ng Sn g^{-1} recommendation of TBT. The highest concentration of MBT and DBT were found in Umm Said, while the highest concentration of TBT was reported in Dukhan, both heavy industrialized sites for petrochemical and desalination of water, respectively. Data from fish (grouper and spangled emperor) collected form Al-Dakhira, an area very close to the Al-Khor mangroves, was of particular interest as it represent the only available organotin biota data that can be used for a comparison with our findings. The reported concentrations in the fish muscle was < 4.6 for MBT, < 4.7 for DBT and < 4.0 for TBT ng Sn g-^{1} dry weight respectively (De Mora et al. 2003).

Studies carried on organotins (Dong, Chen, & Liu, 2004) have offered compelling evidence demonstrating that the buildups of butyltins exhibited periodic variations with respect to their makeup and levels within some selected marine organisms, with concentrations being considerably higher in winter. For example, MBT, DBT and TBT levels varied according the season, with TBT prevailing in winter and spring seasons, and DBT and MBT in summer and autumn, respectively.

Even though the direct input of TBT to the aquatic ecosystems has been banned, non-pesticidal use of TBT continues in some countries and therefore continuous monitoring of TBT concentrations in the marine environment is warranted.

1.5 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a wide range of organic aromatic chlorinated synthetic compounds, which consist of the biphenyl structure with two linked benzene rings in which some or all of the hydrogen atoms have been substituted by chlorine atoms (Fig 1.4).

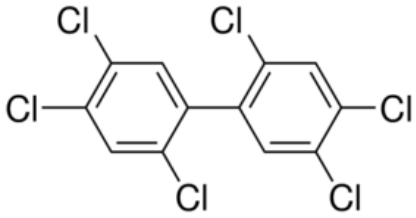

Figure 1. 4: Example of the structure of a polychlorinated biphenyl.

Polychlorinated biphenyls do not occur naturally, but rather are created solely through industrial processes and have been available commercially since the 1930s. They are used for various products, including fluorescent light fittings, coolants, internal electronic parts and also used in the construction of cutting and lubricating oils (Boyle & Highland, 1979). Polychlorinated biphenyls are prevalent and persistent in all environments (Combi et al., 2016; Risebrough et al., 1968) since they are chemically stable at high temperatures and possess a high resistivity to acids and microbial attack (Freitag et al., 1982). They have been found in seawater (Song et al., 2012), aquatic sediments (Filipkowska, 2013) and in various marine species throughout the world (Lavandier et al., 2013). The main concern over PCBs is their high bioaccumulation capacity. McGovern, (2006) reported that these compounds can cause liver impairment in humans, or in some cases affect the reproductive development of children born to exposed mothers. Exposure to some type of PCB congeners may elicit toxic responses ranging from neurochemical (Isaacson & Jensen, 1992; Seegal, 1990) to cardiovascular disease (Lind, et al., 2004) and endocrinological deficits (Brouwer, 1991). Due to health concerns and their environmental impact, their manufacture in Europe and North America ceased in the late 1970s and in Russia by 1990. Studies on PCBs have revealed that some have low toxicity to aquatic organisms such as fish, shrimp and oysters (Duke et al., 1970; Hansen et al., 1974). However, it was also suggested that these compounds inhibit the development in some algal species and may change the species conformation of algae within a laboratory setting and in open waters (Mosser et al., 1972; O'Connors et al., 1978) These toxic pollutants can still enter the marine environment via the destruction and disposal of industrial plants and equipment, or from emissions of construction materials (Kohler et al., 2005) as well as from landfill sites (leaching from old electrical equipment). Polychlorinated biphenyls were included in the Stockholm Convention (UNEP, 2009) due to their persistence, bioaccumulation, and toxicity. Marine mammals are particularly susceptible to the accumulation of PCBs as they accumulate through the fish that

they eat (which have high levels of PCB) and it gets stored in their blubber (Mull et al., 2013).

Data regarding PCBs concentrations from the Arabian Gulf are few. Freije, (2015) reported that the levels of the total PCBs in samples collected from Doha and southern Oman were within the limits of international guideline (set at 33 mg/kg by the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health). Several studies have also documented PCBs in sediments from lakes/rivers in Europe (Monikh et al 2014; Sundqvist et al., 2009), the United States of America (Martinez et al., 2010) and Asia (Ilyas et al., 2011). They all concluded that the levels of PCBs were amplified with the stage of fish development, pointing to possible bioaccumulation. Also, since fish size is correlated to age, older fish were found to be more exposed to PCBs than younger fish.

Reports regarding the concentration of the PCBs within crustaceans are vertically none existent in Qatar. However, a recent article (Ghaeni, Pour, & Hosseini, 2015) reported the concentration of PCBs (16, 44, and 99) from various crustaceans species from the Arabian Gulf (Iran). The concentration of PCBs within *Portunus pelagicus* , *Penaeus semisulcatus*, *Metapenaeus affinis* and *Penaeus merguiensis* were reported values reported ranged from 27μg/kg – 421μg/kg , all values were below the 2000μg/kg FDA limit (Table 1.1).

1.6 Crustaceans as biomonitors

According to established research (Markert et al., 2003), organisms that can show the effect of various pollutants from their environment are termed bioindicators. A bioindicator is defined as "a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental change on a habitat, community, or ecosystem, or is indicative of the diversity of a subset of taxa, or of the wholesale diversity, within an area" (Mcgeoch, 1998). The utilization of invertebrates for evaluating environmental conditions in aquatic ecosystems has been recognized for a long time (Cairns & Pratt, 1993) and their use expanded to encompass various biomonitoring protocols (Hellawell, 1986). The methods being used are easily applied and documented and the response of the aquatic invertebrates (the bioindicators) easily noted. Marine invertebrates have continually shown their usefulness in evaluating aquatic resources and their use as bioindicators has grown to include a wide range of species. These species can show environmental trends, impacts, and change or the ecological value of sites (Dallinger, 1994; Paoletti, Bressan, & Edwards, 1996).

This research utilizes three invertebrate species (Figure 1.5). The blue crab, *Portunus pelagicus* renamed *Portunus segnis* in 2016 by ESC researchers in Qatar (Giraldes et al., 2016), is a bento-pelagic species, slow-growing and longevous as compared to other crustaceans (Williams, 1981). These crabs are a commercially important edible species within Qatar and are widely consumed (Khoramnejadian & Fatemi, 2015). The shrimp *Palaemon khori* is endemic in the mangrove forest at Al-Khor where it was originally discovered (De Grave & Al Maslamani, 2006). This species represents, with other crustaceans, a main component of the food chain. Qatar has a large local fishing fleet, and the barnacle *Balanus amphitrite* is a species of common occurrence on all types of vessels ((M. D. Naser, Son, & Yasser, 2011).

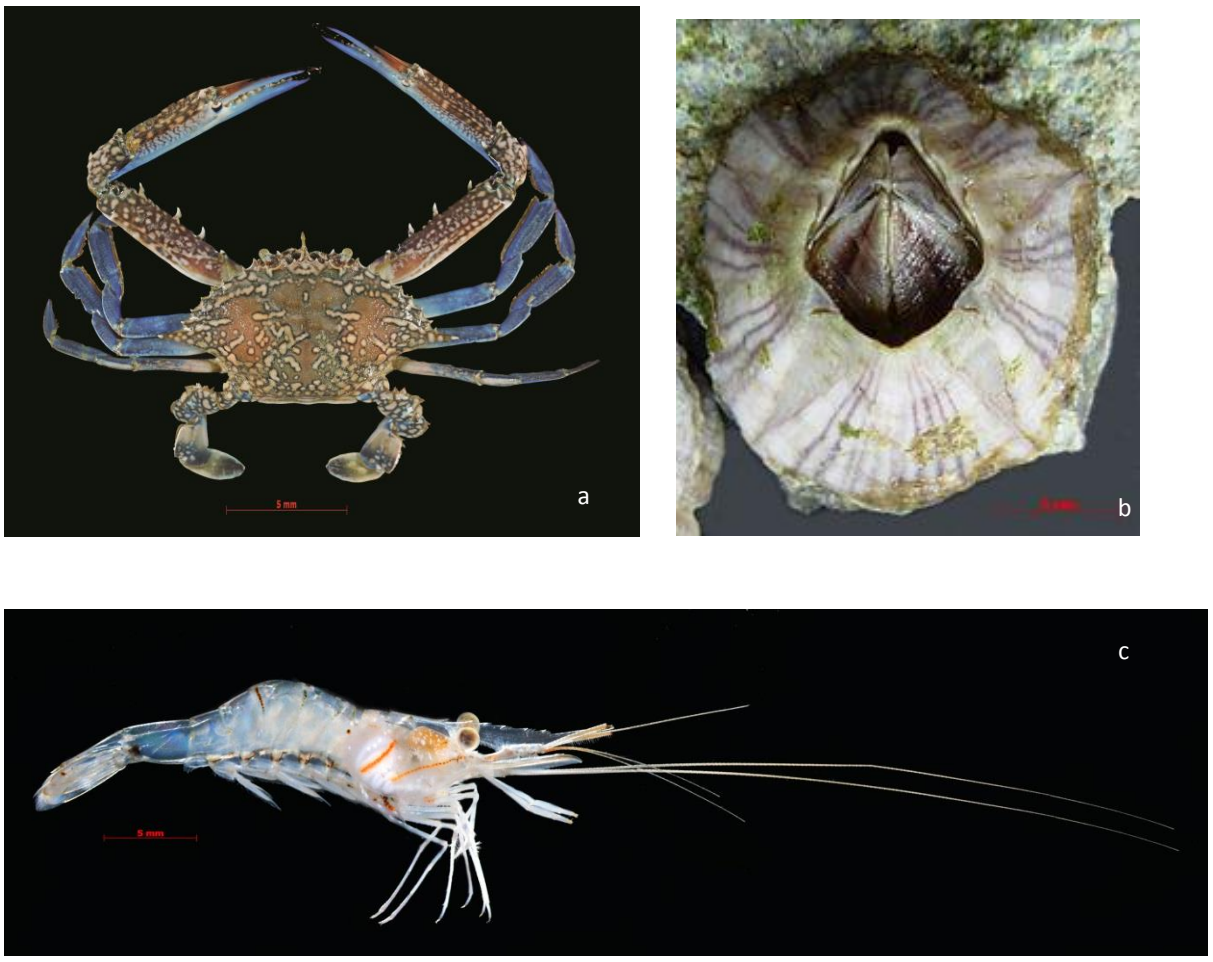

Figure 1. 5: The sentinel species used in this research: a) *Portunus segnis,* b) *Balanus amphitrite,* and c) *Palaemon khori.*

Barnacles are mobile during their planktonic life cycle, and attach themselves to hard surfaces at the latter stages of their life cycle. Their abundance and wide distribution make them good biomonitors of contaminants in coastal waters (Rainbow, 1995).

1.7 Genotoxicity

A major portion of contaminants introduced into the marine environment nowadays can be genotoxic, carcinogenic and mutagenic (J Baršienė, 1994). These compounds have the ability to influence genetic material at non-lethal and non-cytotoxic concentrations, and can lead to belated effects that are important from the individual to the population and community levels. It is now internationally agreed that the assessment of marine environmental health and the design and implementation of measures to improve environmental quality are best undertaken on the basis of an integrated approach using both chemical measurements and appropriate biological measurements in key sentinel species (Lyons et al., 2017).

Some marine species have the capability to absorb either through the skin or gills high levels of toxic substances in their tissues (Hagger et al., 2002). Some pollutants can manifest their impact via genotoxic and metabolic pathways, causing cancer, embryo toxicity and long-lasting damage in organisms (Jha et al., 2000). Genotoxins have particularly high ecotoxicological relevance in situations of chronic exposure to low doses and to multiple contaminants (e.g., as in the case of Polyromantic Hydrocarbon (PAH) rich tar balls arriving in the Qatari shorelines). This raises the need to establish genotoxicological profiles with endpoints on target study species for laboratory studies. Indeed several regulatory bodies stressed explicitly the need for the detection and assessment of potential carcinogenic and mutagenic toxicants using genotoxicity as an endpoint (Leitão et al., 2017). One of the most informative and intensively used test to determine the genotoxic properties of various chemical compounds is the analysis of chromosomal aberrations (Zhang et al., 1998). Owing to their biological significance, genotoxic effects are thought to be important endpoints in assessing pollution-related toxicity. Hose, (1994) and Mix, (1986) both demonstrated that there is a link between genotoxicity and chronic health effects at the population level, by using large scale pollution monitoring programs on fish and shell fish. The analysis of cytogenetic endpoints in organisms exposed to contaminants in their natural environment contributes significantly to the early detection of genotoxic damage. Numerous researchers have used aneuploidy (the presence of an abnormal number of chromosomes in a cell)as an environmental biomonitoring tool (Bouilly et al., 2004; Carrilho et al., 2008; Leitão et al., 2008). The main bulk of research focusing on environmental contaminates and their genotoxic effects have employed the use of invertebrate sentinel species, mainly crustaceans (Shaukat et al., 2014) and gastropods (Barðienë & Buèinskienë, 2002).

Furthermore, the impact of pollutants on the aneuploidy level, within and between generations, of the Pacific oyster, *Crassostrea gigas* has also been established (Barranger et al., 2014) as well as a negative link between aneuploidy and growth, on the same species (Leitao et al., 2001). In shrimps, however, data on the effect of environmental stressors such as genotoxic contaminants on the levels of genetic abnormalities such as aneuploidy is very sparse and non-existent in Qatar.

1.8 Aims and objectives

The aims of this research are to determine the presence and assess the effects of pollutants on selected marine organisms in Qatari waters (Fig 1.6). This was accomplished in three stages:

Stage 1 – Quantify the amounts of trace metals, MeHg, organotins and PCBs present in the environment (water and sediment) and the tissue of target species (blue crab, barnacles, and Khori shrimp).

Stage 2 – Investigate the seasonal variation of the above mentioned contaminants and their changes in the tissue of the target species.

Stage 3 – Determine if these chemicals under current concentration are affecting the Khori shrimp using a cytogenetic end point (aneuploidy) as an indicator of pollution.

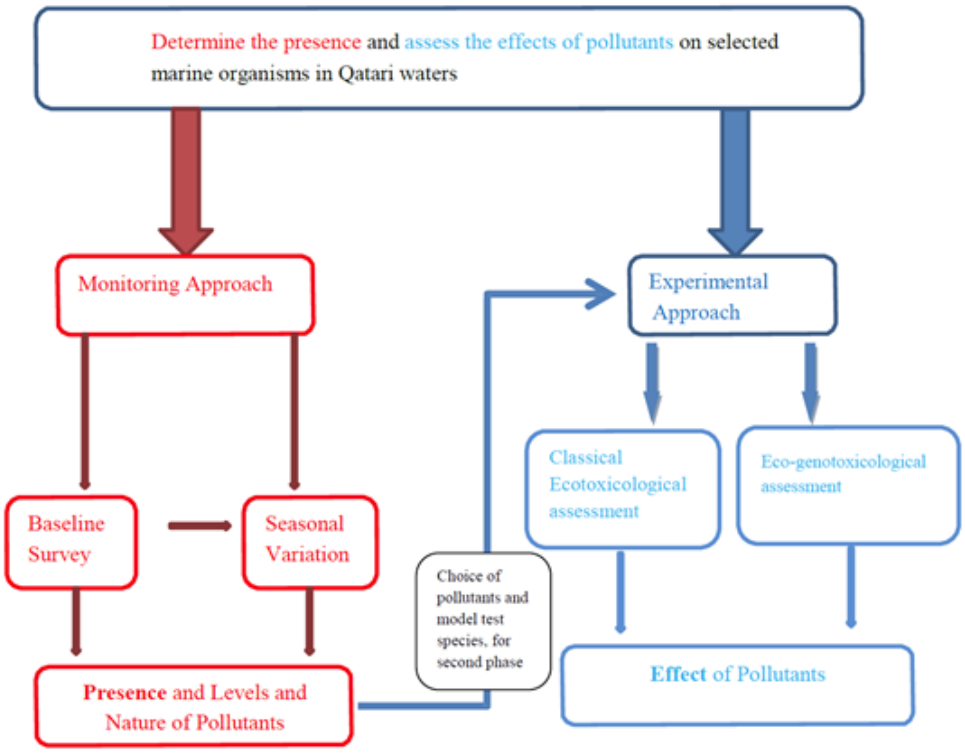

Figure 1. 6: Aims and objective pathway

This introductory chapter introduced the contaminants, the study area, and the target species. Chapter two describes the baseline concentrations and investigates the levels of the heavy metals As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn, and PCBs in the three selected test organisms *Portunus segnis*, *B. amphitrite* and *P. khori*. Chapter three describes the levels and distribution of organotins within the selected biota and sediment from the mangrove habitat in the Arabian Gulf (Qatar), while chapter four describes the determination of methylmercury levels from the same locale. Chapter five focuses on measuring the eco-toxicological effects of the trace metals (Cd, Cr, Mn, Pb, and V) as separate entities or in combination with methylmercury and organotins, on the Khori shrimp using genotoxicological tests (aneuploidy). Decapod crustaceans are considered to be ideal indicators for the study of marine pollution, as they respond well to biochemical stresses and these responses mimic other crustaceans and invertebrates reactions (Vijayavel & Balasubramanian, 2009).

Chapter II: Sampling Location and methodology

2.1 Sampling Area

Al-Khor is a city located in the north of the state of Qatar, 50 kilometers from the capital, Doha. It name came from the Persian word, meaning creek. Huddled along its coastline is a vast mangrove forest. The area supports a lot of wildlife (Al-Thani 2013).

Figure 2. 1: Sampling location and study area

Blue crabs (*Portunus segnis*) n= 30 were purchased from local fishermen caught within the Al Khor mangrove waterway and were brought alive, in ice boxes at 9°C, to ESC.

Barnacles (*Balanus amphitrite*) were collected from planks tethered to a buoy chain at subsurface level (1.2 m deep). Planks were positioned on site and removed after 35 days. They were brought in an icebox to ESC where the barnacles were scraped from the surface.

Palaemon khori specimens were collected at low tide from Al-Khor mangroves using fine nets. Samples were collected for shrimp (300), blue crab (20) and barnacles (75). Sediment and water samples were collected from Al-Khor mangroves (25.322629°N, 51.579759°E) following ESC guideline (adapting the protocol of Sampling and Preservation of Marine Environmental samples). Briefly, sediment samples were collected in 250 ml amber glass jars, taken to ESC and stored in the freezer until analyzed. Seawater samples were collected from the estuary in 2 l amber glass bottles, transported in an ice box to ESC and kept in the refrigerator at 2° – 8°C till analysed.

Sampling was performed at different times of the year to address seasonal variation. Winter sampling took place on 24th September, 20th November and 4th December 2014 while summer sample collection took place on13th May and 9th June 2015 and 29th July 2016. The inclusion of the July 2016 sampling served two purposes, one supporting an equal number of sampling points (N=3) both for winter and summer and it will help us determine any temporal variation.

The samples collected were used for the trace metal analysis, organotin, and methylmercury.

2.2 Analysis Methodology:

In this thesis concentration level of Trace metals, PCB, Organotins and Methylmercury were assessed using various techniques. Three species were used; the blue crab *P. segnis*, the striped barnacle *B. amphitrite*, and the endemic caridean shrimp, *P. khori*.

2.2.1. Trace Metals

All analysis was performed using the PerkinElmer® Optima™ 5300 DV ICP-OES instrument (Perkin Elmer, USA) fitted with an S10 autosampler and equipped with WinLab32™ for ICP Version 4.0 software for simultaneous measurement of all analyte wavelengths. Table 1 shows the optimized instrumental conditions for trace metals measurements.

Table 2. 1: Optimized instrumental conditions for Optima 5300 DV.

Nebulizer Gas Flow	0.6 L/min
Auxiliary Gas Flow	0.2 L/min
Plasma Gas Flow	15 L/min
Sample Pump Rate	2 mL/min
Processing Mode	Peak area
Auto Integration (min-max)	1-5 sec
Read Delay	60 sec
Replicates	3
Background Correction	1 or 2-point, manual

The wavelength selected followed U.S. EPA method 200.7 (USEPA 1994)and ISO regulation and took into consideration, spectral interferences and sensitivities against the expected concentration in the samples. A selection of the used analytical wavelengths are shown in Table 2.2.

Table 2. 2: A selection of the wavelength used.

Analyte	**Wavelength (nm)**
Al	308.212
Ba	233.520
Cd	226.499
Ca	315.881
Cr	205.557
Cu	324.756
Fe	259.934
K	766.457
Mg	279.079
Mn	257.605
Ni	231.602
Pb	220.350
V	292.400

The validity of the calibration was monitored by the Quality Control Check. This quality control check were run at selected intervals in an unattended automatic analysis run, thus ensuring ensure that the instrument performance remained consistent over the length of analysis.

2.2.2.PCB

The method used for the analysis of PCB , Table 2.3 displays the Aroclors investigated, and the method detection limit.

Table 2. 3: Method detection Limt for all analysed Aroclors

Compound	**MDL**
2, Chlorobiphenyl	1.47ppb
2,3 Dichlorobiphenyl	1.48
2,2,5 Trichlorobiphenyl	1.45
2,4,5 Trichlorobiphenyl	1.54
2,2,3,5 Tetrachlorobiphenyl	1.20
2,2,5,5 Tetrachlorobiphenyl	1.35
2,3,4,4 Tetrachlorobiphenyl	1.16
2,3,4,5 Pentaachlorobiphenyl	1.21
2,2,4,5,5 Pentaachlorobiphenyl	1.13
2,3,3,4,6 Pentaachlorobiphenyl	1.20
2,2,3,4,4,5 Hexachlorobiphenyl	1.32
2,2,3,4,5,5 Hexachlorobiphenyl	1.16
2,2,3,5,5,6 Hexachlorobiphenyl	1.15
2,2,4,4,5,5 Hexachlorobiphenyl	1.16
2,2,3,3,4,4,5 Heptachlorobiphenyl	1.45
2,2,3,4,5,5 Heptachlorobiphenyl	1.52
2,2,3,4,5,6 Heptachlorobiphenyl	1.20
2,2,3,4,5,5,6 Heptachlorobiphenyl	1.19
2,2,3,3,4,4,5,5,6 Nonachlorobiphenyl	1.13

Analysis of PCB was performed using a Gas Chromatography coupled to an Electron Capture Detector (GC/ECD). As per EPA method 8082A (U.S. EPA 2007).

Sample analyses are performed using a Agilent 6890 GC/ECD, equipped with dual injector, column, and electron capture detector capabilities. Table 2.4 displays the GC conditions used for the PCB analysis.

Table 2. 4: GC/ECD condition used in analysis.

Injector Temperature	250°C
Oven Temperature Program	120°C hold for 1 minute (min)
	9°C/min to 285°C, 10 min at 285°C
Detector Temperature	300°C
Carrier Gas	Helium
Make-up Gas	Nitrogen
Column Flow Rate RTX-XLB	3.0 milliliters/minute (mL/min);
Amount Injected	1 microliter (μL)
Data System	Chem Station

2.2.3. Organotins

The three species of organotins investigated were; Monobutyltin, dibutyltin and tributyltin. Some of the chemicals used for the derivatization of the organotins are very hazardous e.g. Sodium tertraethylborate ($NaBEt_4$) can ignite in air, and can produce toxic fumes when in contact with water. Thus care and attention was a must when using these compounds. All work was carried out in the fume cupboards, with strict chemical waste disposal protocols in effect.

Glassware was cleaned using Environmental Protection Agency (EPA, 2016) protocols, where it was cleaned with at the appropriate cleaning solution and after rinsing soaked overnight in a dilute solution of HCl, the glassware was then dried in an oven set to 60°C.

Samples were analyzed using an Agilent 7890B gas chromatograph equipped with a 7693 auto-sampler coupled to a 5975 inert MSD triple Axis detector mass spectrometer (Agilent Technologies, USA). The samples (c. 1 μL) were injected into a 30 m × 0.32 mm capillary column (film thickness 0.25 μm). The helium gas flow velocity was controlled at 10 cm/s. The instrumental temperature regime was as follows: 2 mins at 40 °C, to 150 °C at 10 °C/min. The GC-MSD interface temperature was set to 250 °C. Detection was performed in the electron impact ionization mode and single-ion monitoring (SIM). Correct identification and quantification of a given analyte was assured by using two compound-specific ions and a mass

ratio similar to the one determined with calibration. Table 2.5 shows the GC/MS instrumental condition

Table 2. 5: GC/MS condition used in analysis

GC/MS conditions	
Oven:	40°C to 100°C hold for 2 min @25°C/min, to 350°C hold for 13.4min @5°C
Flow:	1.1ml/min
Injector:	300°C/ 8.13psi
Column:	Restek HP5-MS (30m x 0.25mm x 0.25mm film thickness)

2.2.4. Methylmercury

Methylmercury analysis was performed by using The MERX-M instrument (brook Rands) according to the EPA draft method 1630. This is a closed analytical system comprised of four modules an autosampler, a purge and trap module, a GC/Pyrolytic module, and a detector. Extracts were diluted and then buffered using sodium acetate to a pH of 5.0. The extract was then ethylated with Sodium Tetraethylborate, resulting in oxidized mercury species. These volatile species were then stripped from the liquid phase with Argon gas, and retained on Tenex traps. The mercuric species were then desorbed back into the sample stream, and separated with a gas chromatography column. Figure 2.1 shows the instrument setup as per the manufacturer

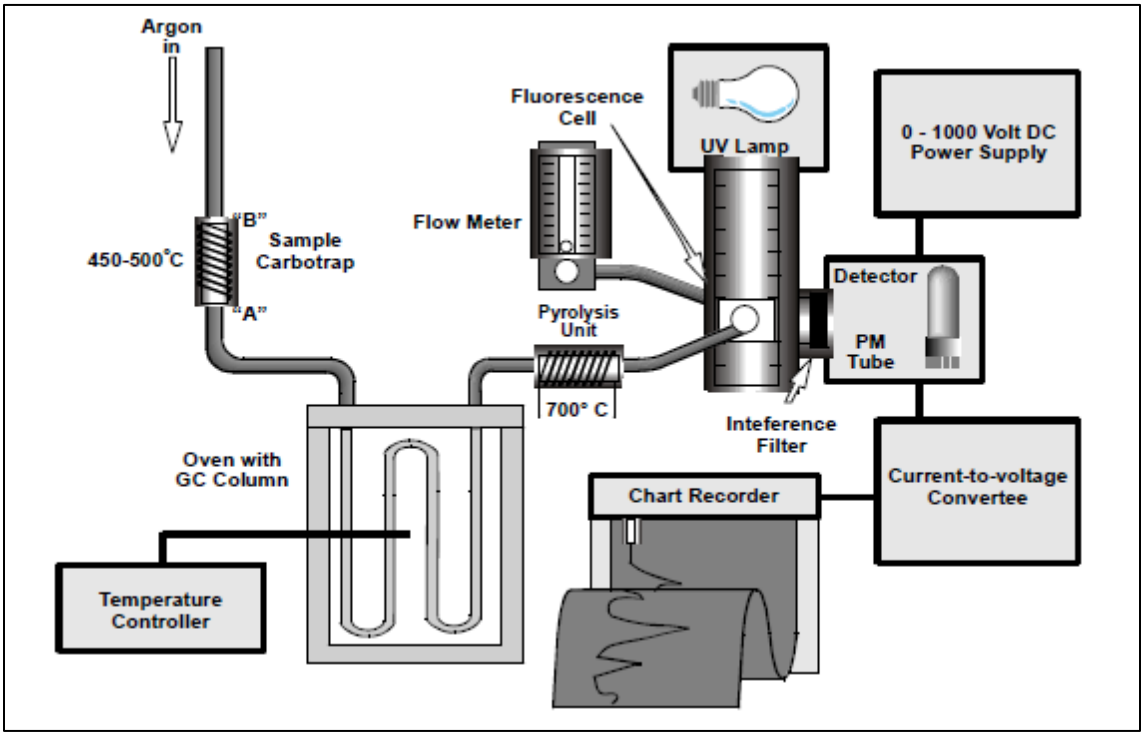

Figure 2. 2: Schematic Diagram of the Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) - *Brook Rands*

2.3. Sentinel Species

2.3.1. The blue Crab - *P. segnis*

This species of crab was renamed recently within Qatar by ESC researchers from *P. Pelagius*. They are one of Qatar's most valuable commercial fishing products and are a key component of the ecosystem, serving as both prey and predator for various marine species. Blue crabs are also good indicators of marine ecosystem health, because they inhabit many different parts of the ecosystem during their life cycle and can thus give an indication the ecosystem health within these areas

Figure 2. 3: *P segnis* – Picture courtesy of Giraldes et al., 2017 with permission

2.3.2 The striped barnacle – *B. amphitrite*

This barnacle species was an invasive organism and now part of the local fauna and is often used as bio-indicator of coastal pollution. Studies have shown that for some species of barnacles the accumulation of pollutants such as heavy metals is linear with time and the accumulation ratio is proportional to the concentration of the metal within the water.

Figure 2. 4: *B.amphirite*- Picture courtesy of ESC

2.3.3 The endemic shrimp – *P. khori*

The final selected species is the endemic caridean shrimp, *Palaemon khori* which inhabits the mangrove forest at Al-Khor, Qatar.

Figure 2. 5: *P. khori*- Picture courtesy of ESC

2.4 Quality Control and Quality Assurance

Most results from analyses performed on environmental samples are used to determine if contamination exists and if remediation is needed. Due to the different type's environmental matrices, the limits of investigative methods, the character of the analytes, and inherent error accompanying sampling and analysis procedure. Furthermore, the results of analysis may contain an component of uncertainty or bias, and as such may not be representative of the actual concentrations of the analytes in the environmental matrices. It is for these reasons there is a need for the evaluation of the quality of the analytical data in relation to the intended use , allowing the investigator to make decisions which are supported by data of known and sufficient quality.

Laboratory data should be produced under a quality system that integrates planning, implementing, and internal assessment of the work performed by the laboratory, including QC. The ESC laboratories used in these analyses are ISO17025-2005 accredited from A2LA (USA). The analysis of all samples used

As mentioned the E.S.Center implements QA/QC guidelines, and these include field & laboratory replicates, blanks, spiked samples and certified reference materials. These are applied

to all surveys. Instrumental calibration is equally checked according to the manufacturer's instructions.

The collection and analysis of blind field duplicate samples is an excellent means by which to evaluate sampling procedures as well as analytical precision (extraction procedures and analytical systems). A field duplicate sample set consists of a thoroughly homogenized sample collected from one desired location that has been split between two sets of bottle ware and labeled as representing two separate sample locations.

A common misconception is that field duplicates provide an estimate of the laboratory's ability to analyze field samples in a consistent manner. While the laboratory's ability is a factor in producing comparable results from the analysis of field duplicates, the homogeneity of the duplicate samples submitted from the field is a major factor. If field duplicates are significantly different due to either the collection procedure or the homogeneity of the area, comparable results between the two samples may not be achieved regardless of the laboratory's expertise.

Laboratory duplicates on the other hand are sample aliquots taken from either the same or identical sample containers containing a field sample. The aliquots are treated in the same manner through the entire analytical procedure from preparation through instrumental measurement. In many cases, samples for laboratory duplicate analyses are provided in separate containers from the field. In effect, these are no different than field duplicates. When the laboratory takes aliquots from the same container, the aliquots should be collected in a manner to ensure that the aliquots are comparable to each other and representative of the total container contents. Collected appropriately, laboratory duplicates can provide a measure of the laboratory's precision in performing the analytical method.

In addition, all methods employed utilized the use of Certified Reference Material (CRM) analysis. CRMs were from the National Institute of Standard Technology (NIST), while PACS-3 was from National Research Council Canada.

Samples were analysed in triplicate as per ESC protocols to quantify uncertainty in the estimate of the mean concentration within the sample, At the ESC labs batch replicates are used for determining lab analysis precision to enhance the quality control of the analysis. All analyses were carried out in the ESC labs which are ISO17025-2005 accredited from A2LA (USA).

Certified Reference materials (CRMs) NIST 2977 for heavy metals, NIST 1974c for PCBs, PACS 2 (Marine Sediment Reference Materials for Trace Metals and other Constituents) for

organotins, and IAEA 433, Trace Elements and Methylmercury in Marine Sediment and NIST2976, Mussel Tissue: Trace Elements & Methylmercury Freeze-dried) used for methylmercury. We had a good recovery (within 85-110%) for the selected analytes. Table 2.6 shows the recoveries from the various CRM used throughout the analysis.

Table 2. 6: Recoveries of selected analytes in the various CRM used.

Parameter / Analyte		**Analysis1 µg/kg**	**Analysis 2 (µg/kg)**	**Average (µg/kg)**	**Certified value (µg/kg)**	**Percentage Recovery**
Heavy Metals (CRM - NIST 2977)	As	8.239	7.860	8.049	8.830	91.16
	Cd	0.182	0.177	0.180	0.179	100.32
	Cr	3.831	4.029	3.930	3.910	100.50
	Cu	9.344	8.743	9.043	9.420	96.00
	Ni	6.090	7.310	6.700	6.060	110.56
	Pb	2.206	2.433	2.319	2.270	102.18
PCBs (CRM used NIST 1974c)	PCB 8	0.166	0.167	0.166	0.190	89.5
	PCB 28	13.104	12.960	13.032	14.400	90.5
	PCB 31	9.944	13.248	11.596	11.596	102.6
	PCB 49	16.072	16.126	16.099	17.100	94.1
	PCB 63	1.237	1.112	1.176	1.340	87.65
Organotin	DBT	570	580	575	610	94.3
	TBT	430	350	390	410	95.1
Methylmercury		*168.52	*169.01	168.765	170.00	99.27
		**28.960	**28.930	28.950	28.090	103.06

* IAEA 433** NIST2976

Chapter III: Occurrence and persistence of trace metals and polychlorinated biphenyls in marine organisms inhabiting Qatari waters

3.1 Abstract

The coastal marine environment within Qatar has been subject to anthropogenic pressures including increasing industrial development leading to the introduction of pollutants to the coastal environment via different pathways. Baseline and seasonal variations of heavy metals and polychlorinated biphenyls (PCB) concentrations were investigated within the tissue of three selected marine organisms, together with water and sediment sampled in September, November and December of 2014, May and June of 2015 and July of 2016. The species used for the research are all indigenous within Qatari waters and include the blue swimming crab (*Portunus segnis*), which is a commercially important edible species, the striped barnacle (*Balanus amphitrite*), which is often used as bio-indicator of coastal pollutants and the endemic caridean shrimp (*Palaemon khori*) which inhabits the *Avicenna marina* mangrove forest at Al-Khor.

Among the heavy metals, zinc (Zn) was recorded to have the highest concentration in all the studied organisms. Arsenic was detected in all of them, with highest concentrations in the crab muscle (33.78 ± 5.88 mg/kg) and barnacle tissue (31.08 ± 4.71 mg/kg). Barnacle showed the highest concentration of Zn, copper (Cu), and iron (Fe) in all three species, with significant seasonal variation in the levels of these elements and also arsenic (As). The shrimp showed no seasonal variation in most elements apart from Cr and Ni, while the crab muscle showed seasonal variation in As, Fe and Zn. Sediment analysis showed that Fe is the most abundant element.

Two PCB congeners, 2,2,3,5-Tetrachlorobiphenyl and 2,2,4,5,5-Pentachlorobiphenyl, were detected in the muscle of the crab (Mean ± SD: 2.01 ± 0.43µg/kg and 4.77± 0.50µg/kg, respectively) and the shrimp (1.96± 0.92µg/kg and 4.19 ± 0.61µg/kg). The data presented in this study represent the first baseline and seasonal variations these contaminants, in animal tissues, water and sediment from the coastal waters of Qatar.

3.2 **Introduction**

During the early nineties, the State of Qatar began to export oil and natural gas from its vast reservoirs, which resulted in an increased economic, industrial and social development. Power supply and desalination plants were the first to be commissioned as Qatar has no natural resource of freshwater apart from some small wells scattered throughout its territory ("The Nu'aija Wells"., 2017). Most of these plants, together with the numerous oil and gas processing units that were built, are situated in the Arabian Gulf itself or in its coastal areas. In the past, it was believed that the sea could act as a sink with an unlimited capability to permanently assimilate pollutants (Martin & Windom, 1991; Zwolsman, Eck, & Burger, 1996). Thus, these industries have discharged a variety of contaminants into the surrounding marine environment (Al-Ghadban et al., 2002; Readman et al., 1992). Moreover, the Gulf War have resulted in further detrimental effects on coastal and marine habitats (Gevao et al., 2016). As a result, there has been a major concern among the Gulf countries, including Qatar, about the real risk and extent of pollution to the marine ecosystem (Al-Naimi et al., 2015).

Mangroves are facing a rapid decline in many parts of the world due to anthropogenic impacts (Bayen, 2012; M. Lewis, Pryor, & Wilking, 2011; Sandilyan & Kathiresan, 2014). The Arabian Gulf is characterized by fluctuating water temperature and high salinities (Sheppard et al., 2010) factors known to favor the accumulation of pollutants (Blackmore & Wang, 2002). In this challenging environment, with desert climate and limited vegetation, the Qatari mangrove forests of *Avicennia marina* still remain a hotspot of biodiversity (Al-Maslamani et al., 2013; Naser, 2014). Anthropogenic activities, combined with the ability of mangrove habitats to act as a sink for pollutants, are threatening this already naturally stressed ecosystem (Maiti & Chowdhury, 2013; Natesan, Madan Kumar, & Deepthi, 2014).

Most coastal areas and seas contain a limited amount of naturally occurring heavy metals. Nevertheless, their cationic forms are harmful to living organisms as they can readily bind to short carbon chains(A. M. Freije, 2015). These forms bioaccumulate in protein-rich tissues of marine organisms and eventually can affect humans consuming them in their diet. Metals may interfere with the cellular metabolic functions causing harmful side effects (Leitão et al., 2017). These pollutants may affect marine organisms even at low concentrations: their toxicity arises not only from their concentration levels but also from the biochemical role they play in the

metabolic processes as well as the extent to which they are absorbed and excreted by marine organisms (Jakimska et al., 2011). Exposure to certain heavy metals can affect cellular components such as cell membrane, mitochondria, etc. (Wang & Shi, 2001).
Polychlorinated biphenyls (PCBs) are a wide range of chlorinated organic compounds. Their production started commercially in the 1930s, and were used for various products, including fluorescent light fittings, coolants, internal electronic parts and also used in the formulation of cutting and lubricating oils (Boyle & Highland, 1979). These compounds are chemically stable at high temperatures and possess a high resistivity to acids and microbial attack (Freitag et al., 1982) thus, they are highly persistent in the environment (Combi et al., 2016).

PCB can through their accumulation can lead to detrimental side effects, and inductory endocrine disruption compounds (Jamieson, Malkocs, Piertney, Fujii, & Zhang, 2017). Exposure to some type of PCB congeners may elicit toxic responses ranging from neurochemical (Isaacson & Jensen, 1992; Seegal, 1990) to carcinogenic (Lind et al., 2004) and endocrinological deficits (Brouwer, 1991). Due to these concerns and their environmental impact, their manufactures in Europe and North America ceased their production in the late 1970s and in Russia by 1990. These toxic pollutants can still enter the marine environment via the destruction and disposal of industrial plants and equipment, or from emissions of construction materials (Kohler et al., 2005). They can also enter from landfill sites (leaching from old electrical equipment). Polychlorinated biphenyls were included in the Stockholm Convention (UNEP, 2009) due to their persistence, bioaccumulation, and toxicity. Marine mammals are particularly susceptible to the accumulation of PCBs as they are the top of the food chain and these compounds have a high binding affinity to lipids(Jamieson et al., 2017), thus tend to be stored in the blubber

PCB and trace metal pollution can affect the distribution, abundance, breeding cycles and migration of marine organisms, on which millions of people rely for food and income, all over the world. Pollutants can also bioaccumulate along the food web, resulting in contamination of sea food (Jan P. Boon et al., 2002). Often the availability of pollutants, such as heavy metals, is governed by physiochemical factors, including temperature and salinity of both water and sediment, which regulate the bioaccumulation by marine organisms (Blackmore & Wang, 2002;

Mohammad, Nabavi, Parsa, Hosseini, & Nabavi, 2013). Hence, concentrations of pollutants both in the environmental media and within the organisms, can vary depending on the season. Crustaceans are often utilized as bioindicators and biomonitors in different marine ecosystems (Hamza-Chaffai, 2014). They are a successful group of animals, inhabiting marine, terrestrial and freshwater environments. They are ideal candidates for comparative investigations, possessing useful features that can aid in interpretation of data from bio-physiological studies. In this study we employed three invertebrate species collected from the coastal areas of Qatar: the blue crab *Portunus segnis*, appreciated as a commercially important edible species and widely consumed, the barnacle *Balanus amphitrite*, found attached to natural substrates, ships, boats and used as a bioindicator of contamination in coastal waters (Rainbow, 1995) and the shrimp *Palaemon khori*, endemic to the Al-Khor mangroves (De Grave & Al Maslamani, 2006), where it constitutes a main component of the food chain in this important nursing ground.

We aim to determine the baseline data of contaminants, in marine water, sediment and selected organisms. This will provide a record of the current situation in terms of pollution by heavy metals and PCBs in Qatari coastal waters and mangrove forests, following decades of anthropogenic pollutions. This study's other target is to evaluate the effectiveness of the three groups of crustaceans selected as bioindicators of pollutions.

3.3 Material and Method**:**

3.3.1 Sample Collection and Preparation

For sample collection location please refer to chapter 2 section 2.1. All sample preparation and contaminant analyses were performed using established protocols at ESC. In the lab, the semi frozen crabs were washed with distilled water and the soft tissue was removed from the carapace using a pair of stainless steel tweezers and a blade. Barnacles were identified to the species level: the flesh of *B. amphitrite* was meticulously separated from the shell using a sterile knife. Crabs' muscle and carapace, barnacles' tissue and shell and the full shrimp were then placed in a drying oven at 60°C. After drying all samples were individually ground.

Dry ground animal samples and sediment were further kept at 50°C for 48 hours to ensure constant weight.

To calculate the PCB concentration in animal and sediment, samples were extracted using a Dionex Accelerated Sample Extraction machine (ASE350). Ten grams of each sample were mixed with 15g of diatomaceous earth and placed in an extraction cell. Dichloromethane was added as the extraction solvent at a temperature of 105°C and at a pressure of1500 psi. For the animal tissue, 10g of alumina was added to the extraction cell to remove lipids from the samples. After the extraction, the mixture was concentrated to 1ml using a gentle stream of nitrogen, and the samples were injected into the Gas Chromatography coupled to an Electron Capture Detector (GC-ECD) prior to analysis.

3.3.2 Sample Preparations and Analysis

Dry sediment and animal samples were further dried at 50 °C for 48 hours till constant weight and then ground to powder using a mortar and pestle. Muscle and shell from crab and barnacles were analyzed separately while the shrimp comprised of the whole body. Sample preparation including extraction of the contaminants was done using the established protocols at ESC.

Briefly, for PCB analysis of animal and sediment samples 10 g of ground samples were extracted using a Dionex Accelerated Sample Extraction (ASE350). The weighed sample was mixed with 15 g diatomaceous earth and placed in an extraction cell and dichloromethane was added as the extraction solvent at a temperature of 105°C and 1500 psi pressure. For the animal tissue 10g of alumina was added to the extraction cell to remove lipids from the samples. After the extraction, the mixture was concentrated to 1ml using a gentle stream of nitrogen, and the samples was injected into the Gas Chromatography coupled to an Electron Capture Detector (GC-ECD) for the analysis (Fig 3.1).

Figure 3. 1: Sample analysis using gas chromatography coupled to an electron capture detector.

For the heavy metal analysis of animal and sediment samples 9ml of concentrated nitric acid was added to 0.25g of finely ground dry samples. The digests were initially heated for 30 minutes at 95°C on a hot block and then were further heated for another 30 minutes after addition of 3ml of hydrofluoric acid . For animal tissues and shells, 5ml of nitric acid were used instead of hydrofluoric acid. The temperature was then increased to 130°C for 1 hour, and further increased to 150°C until the liquid part was completely evaporated. At this point, 2ml of nitric acid and 50ml of deionized water were added and left to boil for 5 minutes. The digests were then allowed to cool down and were diluted to100ml using deionized water. The samples were finally filtered and analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Fig 3.2).

Figure 3. 2: Heavy metal analysis using inductively coupled plasma coupled to optical emission spectrometry**.**

Water samples were filtered and directly nebulized into the ICP/OES for metal analysis. PCB analysis of the water was carried out after liquid/liquid extraction using hexane as solvent: one liter seawater was decanted into a separating funnels with 30ml of hexane and the mixture was mixed for 3 minutes using an electric shaker. After this cycle, the two liquids were allowed to settle and the bottom layer, run off into a glass bottle, while the organic layer run off into a separate stoppered glass bottle containing 5g of sodium sulphate (drying agent). The procedure was repeated three times, each time adding the organic layer to the stoppered glass. The sodium sulphate was then removed via filtration, the filtrate reduced to 1ml by evaporating under a gentle stream of nitrogen and sample was then injected into the GC/ECD for analysis.

3.3.3 Statistical analysis

Analysis of variance (ANOVA) and Welch test (unequal variances t-test) were used to compare the difference in concentration for each element and PCB congener between the two seasons (winter vs. summer). For samples below the limit of detection the LOD divided by the square root of 2, was used.

3.4 Results:

We analysed As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn and any detected PCB congeners in the tissue of the organisms (Table 3.1). In the crabs (n = 30) we found, overall, higher concentrations of As, Cd, Cu, and Pb in the muscle compared to the carapace; the concentration of all other elements analyzed were instead higher in the carapace. Analysis data also shows that statistically significant higher concentrations of As, Cr, Cu, Ni and Zn were found in the winter while Fe, Mn and V were found to be higher in the summer. The muscle had higher concentration of As, and Zn in the winter and Cd, Co, Fe, Mn, Pb and V in the summer.

Despite covering a wide range of congeners in the analysis of PCBs, we could not detect any PCB in the carapace. Only two congeners, 2,2,3,5-Tetrachlorobiphenyl (PCB44) and 2,2,4,5,5-Pentachlorobiphenyl (PCB101) were detected in the muscle with significant high concentrations in the summer compared to winter.

Barnacle muscle had higher concentration of all the heavy metals compared to the shell. The highest average annual concentration was found for Zn (5684.5 mg/kg) and the lowest for Co (0.702 mg/kg). The heavy metals present displayed a trend of Zn > Fe > Cu > As > Cd > Mn > Ni > Cr > V > Pb > Co. Seasonal variation of the concentration of trace metals within the soft tissue of the barnacles were seen in Co, Cu, Fe, Ni, Pb, and V, with Ni and Zn showing higher concentration in the winter with the rest having higher values during the summer season

In the Barnacles shell, statistically significant higher concentrations of Cd, Cr, Mn, Ni, and Zn were found in the winter while As, Cu, and Fe were higher in the summer. The muscle had higher concentration of Ni in the winter and Co, Cu, Fe and Pb in the summer. PCBs were not detected in either the shell or muscle of the barnacles.

Shrimp analysis (whole body of the shrimp) showed that Cr and Ni, had significantly higher concentrations in the winter while Pb and Zn were higher in the summer. Similar to the crab muscle, the two PCBs detected in the shrimp were PCB101 and PCB44. However no significant difference was observed between the two seasons for both the congeners.

The overall order of the average concentration of the heavy metals in the shrimp were Zn> Cu> Fe> Mn> Ni> Pb> As> Cr > Cd > Co> V.

The mangrove sediment analysis (Table 3.3) revealed that concentration of Fe was the highest (757.46 mg/kg dry wt.) and that of Pb (0.05 mg/kg dry wt.) was the lowest with the following order: Fe > Mn > Cu > As > Zn > V > Cr > Ni > Co > Cd > Pb. No significant difference was observed between the winter and summer concentrations.

Analysis of the seawater showed that the concentrations of As, Cd, Co, Fe, V and Zn were all below the quantification limit of the instrument. Cr was found to have a concentration range of 0.99 µg/kg – 1.62 µg/kg, Cu 3.89 µg/kg – 5.41 µg/kg, Mn 0.25µg/kg – 0.521 µg/kg, Ni 0.17 µg/kg – 0.468 µg/kg, and Pb 0.68 µg/kg – 0.987 µg/kg.

The seawater was also analysed for some selected physiochemical parameters at the time of collection using a handheld Mettler Toledo multi parameter meter: temperature ranged between 24.0°C and 44.2°C, conductivity of the seawater ranged from 78.2µS/cm to 80.32µS/cm, specific conductivity from 70.82µS/cm to 78.62µS/cm, salinity from 48.7ppt – 50.2ppt, with total dissolved solids (TDS) ranging from 55.9 ng/kg to 58.7 ng/kg.

Table 3. 1: Table of trace metal and PCB analysis

		POLLUTANTS														
				Heavy Metals (mg/kg dry weight)											PCBS (µg/kg dry wt.)	
		SEASON	MONTH	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	PCB1	PCB2
CRAB	Carapace	Winter	Sept	5.344	0.007*	0.032*	13.144	8.532	0.258*	8.915	68.213	0.052*	0.014*	411.333	ND	ND
			Nov	5.525	0.007*	0.032*	14.143	8.787	0.258*	9.166	70.855	0.052*	0.014*	397.427	ND	ND
			Dec	6.398	0.007*	0.032*	12.781	9.125	0.258*	8.985	68.521	0.052*	0.014*	402.855	ND	ND
			Average	5.756			13.356	8.815		9.022	69.197			403.871		
			Std.dev.	0.564			0.705	0.297		0.130	1.445			7.009		
		Summer	May	0.1979*	0.007*	0.111	1.051	0.790	15.555	13.933	6.450	0.052*	0.141	3.153	ND	ND
			Jun	0.1979*	0.007*	0.109	1.241	0.914	15.822	13.755	6.394	0.052*	0.121	4.050	ND	ND
			Jul	0.1979*	0.007*	0.009	1.190	0.901	14.988	13.241	6.111	0.052*	0.019	4.010	ND	ND
			Average			0.076	1.161	0.869	15.455	13.643	6.319		0.094	3.738		
			Std.dev.			0.058	0.098	0.068	0.426	0.359	0.182		0.065	0.507		
		Annual Average		**5.756****		**0.076**	**7.258****	**4.842****	**15.455****	**11.333****	**37.758****		**0.094****	**203.805****		
		Standard devation		**0.564**		**0.058**	**6.695**	**4.357**	**0.426**	**2.543**	**34.452**		**0.065**	**219.207**		
	Muscle	Winter	Sept	35.644	0.007*	0.032*	0.064*	66.575	0.258*	0.019*	0.041*	0.052*	0.014*	184.160	2.100	5.300
			Nov	41.184	0.007*	0.032*	0.064*	59.041	0.258*	0.019*	0.041*	0.052*	0.014*	165.964	1.600	4.500
			Dec	39.785	0.007*	0.032*	0.064*	58.963	0.258*	0.019*	0.041*	0.052*	0.014*	159.215	1.420	3.890
			Average	38.871				61.526						169.780	1.707	4.563
			Std.dev.	2.880				4.372						12.903	0.352	0.707
		Summer	May	29.333	0.111	0.050	0.064*	62.230	11.090	0.760	0.041*	0.170	0.050	143.860	2.010	4.980
			Jun	28.742	0.121	0.070	0.064*	61.870	12.019	0.761	0.041*	0.184	0.032	142.940	2.510	5.014
			Jul	27.990	0.118	0.050	0.064*	59.990	12.000	0.755	0.041*	0.177	0.028	141.880	2.401	4.895
			Average	28.688	0.117	0.057		61.363	11.703	0.759		0.177	0.037	142.893	2.260	4.997
			Std.dev.	0.673	0.005	0.011		1.203	0.531	0.003		0.007	0.012	0.991	0.263	0.061
		Annual Average		**33.780****	**0.117****	**0.05**7**		**61.445**	**11.703****	**0.759****		**0.177****	**0.037****	**156.337****	**1.928**	**4.737**
		Standard devation		**5.883**	**0.005**	**0.011**		**2.869**	**0.531**	**0.003**		**0.007**	**0.012**	**16.848**	**0.431**	**0.499**

		SEASON	MONTH	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	PCB1	PCB2
BARNACLE	Shell	Winter	Sept	2.333	5.362	0.032*	7.032	4.680	0.258*	15.980	26.385	0.365	0.014*	171.423	ND	ND
			Nov	1.722	0.007*	0.032*	0.064*	1.660	0.258*	16.821	0.041*	0.052*	0.014*	161.323	ND	ND
			Dec	2.100	0.007*	0.032*	0.064*	1.895	0.258*	19.580	0.041*	0.052*	0.014*	153.250	ND	ND
			Average	2.052	1.792	0.032*	7.032	2.745		17.461	26.385	0.365		161.999		
			Std.dev.	0.308		0.032*		1.680		1.883				9.105		
		Summer	May	30.070	0.110	0.041	0.064*	63.572	11.583	0.782	0.041*	0.100	0.061	147.324	ND	ND
			Jun	28.590	0.098	0.038	0.064*	60.944	12.180	0.834	0.041*	0.091	0.049	142.583	ND	ND
			Jul	27.652	0.087	0.0318*	0.001	58.278	12.011	0.795	0.041*	0.088	0.052	141.630	ND	ND
			Average	28.771	0.098	0.039	0.001	60.931	11.925	0.804		0.093	0.054	143.846		
			Std.dev.	1.219	0.012	0.003		2.647	0.308	0.027		0.006	0.006	3.050		
		Annual Average		**15.411****	**1.414****	**0.039**	**3.517****	**31.838****	**11.925****	**9.132****	**26.385**	**0.161**	**0.054**	**152.922**		
		Standard devation		**14.656**	**2.632**	**0.003**	**4.972**	**31.932**	**0.308**	**9.201**		**0.136**	**0.006**	**11.651**		
	Muscle	Winter	Sept	23.862	4.558	0.041	16.158	4.370	0.258*	16.821	9.911	0.446	0.363	5156.543	ND	ND
			Nov	37.073	16.917	0.070	3.213	4.257	0.258*	13.443	11.233	1.036	1.364	6647.481	ND	ND
			Dec	35.216	19.253	0.080	2.147	7.142	0.258*	14.214	11.852	0.987	0.885	6782.483	ND	ND
			Average	32.050	13.576	0.064	7.173	5.256		14.826	10.999	0.823	0.871	6195.503		
			Std.dev.	7.152	7.897	0.020	7.799	1.634		1.770	0.992	0.327	0.501	902.294		
		Summer	May	31.517	16.574	1.262	0.560	120.320	317.633	13.954	6.433	1.592	2.913	5086.430	ND	ND
			Jun	29.890	16.011	1.331	0.511	119.985	320.010	14.101	6.625	1.621	3.091	5221.211	ND	ND
			Jul	28.971	16.882	1.425	0.600	120.014	320.997	14.891	6.857	1.871	3.212	5212.630	ND	ND
			Average	30.126	16.489	1.340	0.557	120.106	319.547	14.316	6.638	1.695	3.072	5173.424		
			Std.dev.	1.289	0.442	0.082	0.045	0.186	1.729	0.504	0.212	0.153	0.150	75.461		
		Annual Average		**31.088**	**15.032**	**0.702****	**3.865**	**62.681****	**319.547**	**14.571**	**8.819****	**1.259****	**1.972****	**5684.463**		
		Standard devation		**4.715**	**5.251**	**0.701**	**6.121**	**62.915**	**1.729**	**1.197**	**2.473**	**0.529**	**1.250**	**800.828**		
SHRIMP		Winter	Sept	3.597	1.063	0.125	4.375	107.377	44.919	8.637	10.973	3.958	0.119	127.062	ND	ND
			Nov	4.124	1.085	0.121	3.432	108.251	44.854	7.854	9.854	3.365	0.101	117.063	2.000	5.210
			Dec	3.891	1.074	0.154	5.141	117.211	47.012	8.541	10.014	2.890	0.121	118.923	1.500	4.280
			Average	3.871	1.074	0.134	4.316	110.946	45.595	8.344	10.281	3.404	0.114	121.016	1.750	4.745
			Std.dev.	0.264	0.011	0.018	0.856	5.443	1.228	0.427	0.605	0.535	0.011	5.318	0.354	0.658

SEASON	MONTH	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn	PCB1	PCB2
Summer	May	3.720	1.040	0.073	0.800	111.361	46.882	7.747	0.041*	4.784	0.240	128.431	1.400	3.810
	Jun	3.689	1.102	0.791	0.841	115.241	46.971	7.854	0.041*	4.587	0.321	128.941	1.374	3.654
	Jul	4.331	1.092	0.215	0.785	108.520	46.320	8.011	0.041012	4.125	0.121	128.980	1.534	3.701
	Average	3.914	1.078	0.360	0.809	111.707	46.725	7.871	0.041	4.499	0.227	128.784	1.387	3.732
	Std.dev.	0.362	0.033	0.380	0.029	3.374	0.353	0.133		0.338	0.101	0.306	0.086	0.080
Annual Average		**3.892**	**1.076**	**0.247**	**2.562****	**111.327**	**46.160**	**8.107**	**7.721****	**3.952****	**0.171**	**124.900****	**1.569**	**4.239**
Standard devation		**0.284**	**0.022**	**0.271**	**1.996**	**4.072**	**1.017**	**0.384**	**5.144**	**0.721**	**0.089**	**5.427**	**0.254**	**0.652**

*LOD
ND = not detected

** Statistically calculated P values were used to indicate any statistically significant variation ($p<0.05$)

Table 3. 2: Trace metal concentration within sediment collected form the mangroves sampling location (Al-Khor).

	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	V	Zn
Winter	4.498	0.123	0.527	3.744	5.935	713.000	20.653	1.876	3.825	4.069
	5.140	0.214	0.685	3.965	6.140	752.000	17.632	2.800	4.010	4.140
	4.819	0.169	0.606	3.855	6.038	732.500	19.143	2.338	3.918	4.105
Summer	6.101	0.019	0.795	4.125	7.014	785.210	21.251	2.487	4.014	4.201
	6.151	0.021	0.856	4.215	7.860	789.630	20.150	3.100	4.800	4.620
	5.977	0.018	0.755	3.989	7.000	772.470	19.852	2.955	4.625	4.441
Annual Average	**5.756**	**0.094**	**0.704**	**3.982**	**6.664**	**757.468**	**19.780**	**2.593**	**4.199**	**4.263**
Standard deviation	**0.564**	**0.087**	**0.123**	**0.172**	**0.757**	**30.528**	**1.272**	**0.452**	**0.408**	**0.219**

Table 3. 3: Comparison of trace metals within shrimp tissue and sediment form the mangroves

Annual Average	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	V	Zn
Shrimp	3.89215	1.076083	0.246667	2.562367	111.3269	46.15993	8.107433	7.720653	0.170633	124.9
Sediment	5.755533	0.093833	0.704	3.982083	6.664417	757.4683	19.78008	2.592667	4.198583	4.262583

3.5 Discussion

3.5.1 Trace Metals

The Arabian Gulf is one of the world's most impacted regions due to anthropogenic activities. These activities strain the ecosystems and the populations that depend on this environment (Naser, Son, & Yasser, 2011). Qatar is a peninsula almost completely surrounded by the Arabian Gulf. Qatar's main revenue is from oil and gas production and other petrochemical industries. Moreover, Qatar has no natural fresh water resources and thus there is a heavy dependence on desalination plants (Saif, 2012).

Most scientists within the region agree that the most substantial threat to the marine ecosystems within the Gulf region comes from the large coastal modification through dredging and the conversion of established marine ecosystems into land fit for urban housing and industrial facilities (Nuzrat Yar Khan, 2007; Munawar, M., Price, A.R.G., Munwar, I.F., Carou, S., Niblock, H., Lorimer, 2002).

All these factors contribute to the degradation of the marine environmental health. Pollutants such as heavy metals, and polychlorinated biphenyls (PCBs) have been widely reported in the Arabian Gulf and surrounding areas (De Mora, Fowler, Wyse, & Azemard, 2004; A. M. Freije, 2015; Lyons et al., 2015).

One of these major contaminants resulting from anthropogenic activities on coastal and marine environments are heavy metals (Yu et al., 2008). These are toxic and are a serious risk to humans health, marine organisms and the natural environments due to their perseverance and bioaccumulation characteristics (DeForest et al., 2007). They are also believed to be potentially carcinogenic (Zeng et al., 2015).

The World Health Organization (WHO), has indicated that heavy metals must be monitored and controlled to guarantee public safety (Heidarieh et al., 2013), thus in few decades the determination and monitoring of trace elements in living organisms has become an important topic (Bat, 2012). Many species have been utilized to monitor these pollutants. Species such as the he blue crab, shrimp and other filter feeders can bioaccumulate trace metals from their surrounding marine environment and thus can be important biological monitor of trace pollutants within the ecosystem. Therefore this study was undertaken to determine the baseline data and

seasonal variations of trace metals and PCBs in marine water, sediment and the selected organism.

Regarding heavy metals within the crab carapace the highest was for Zn (average 203.8), followed by, Ni (37.75), Fe (15.45), Mn (11.33), Cr (7.25), As (5.75), Cu (4.84,)V (0.09), Co (0.07) all mg/kg dry wt. , with Cd and Pb both below the limit of detection of the instrument. Studies conducted on crabs from within the Arabian / Persian Gulf showed that the heavy metals in the carapace followed the trend Cu > Cd> Ni>Pb (Bastami et al., 2012). The same group also reported that the trend held true for the muscle tissue. Our studies produced a different order which follows the trend Zn> Ni>Mn>Cr>Fe>Cu>As. However Cd, Co, Pb and V were all below the limit of detection. Within the muscular tissue our studies showed the following trend: Zn> Cu>As> Fe>Mn>Pb>Cr>Cd>Co>Ni>V. Hosseini et al., (2012) postulated that the buildup of heavy metal contaminants in aquatic invertebrates is related to the relative permeability of their membranes and their enzyme systems, this function is species specific leading to different metallic contaminates accumulating at different orders (Hosseini et al., 2012). The Zinc average was the largest at 159.2 mg/kg. Notable was the presence of As within both the carapace and tissue samples, at annual average levels of 33.80 mg/kg combining the carapace and the muscle. These levels may indicate contamination, but there are different species of As in the sea-food and total As is not representative of toxic As (Srivastava, Srivastava, Suprasanna, & D'Souza, 2013). A great portion of studies on heavy metals is conducted in the different parts of the body, here the exoskeleton was removed and the rest was clumped together to represent the soft tissue, mainly composed of muscular mass (Al-Mohanna & Subrahmanyam, 2001). Arsenic concentration in the soft tissue of the blue crab from our analysis was much greater than previous research data, conducted in various marine organisms such as crabs, fishes and lobsters of the Arabian Gulf and reported high levels of As in bivalves collected from the Arabian Gulf, and attributed the findings to natural sources instead of man-made pollution (Al-Mohanna & Subrahmanyam, 2001; S. De Mora et al., 2004). High levels of As are also observed in the sediment (Table 3.2), and as the area is still undergoing industrialization and urban planning activities the dragging of the sediment may cause the As already sored in the sediment to be released into the water column..

As expected Zn (203.8mg/kg) represented the highest concentration, this element is vital for the growth and metabolic functions within the organism(Ayas & Ozogul, 2011; Coast, Pradesh, & Jyothirmayi, 2014; Ismahene & Hadi, 2012; Pourang, Dennis, & Ghourchian, 2005) . Iron and Cu are also considered as important elements for the biological function of these crustaceans. Although these elements are important trace metals, high concentrations of these can be harmful to the organisms (Phillips & Rainbow, 2013). Our data shows that within the crab tissue (carapace and muscle) the concentration of Fe were below the detection limit in the winter season while summer averages 11-15 mg/kg dry wt. Chromium levels were below the detection limit for the muscle tissue content , but the carapace analysis showed levels of up to 14.4 mg/kg dry wt. Although our data for the metal content within the carapace and muscle, seems to disagree with Bastami and Esmailian, (2012) who proposed that the concentration of heavy metal distribution within the crab were higher in the muscle than the carapace (Bastami & Esmailian, 2012). Sumpton et al., (1994) suggests that this species of crab mature rapidly during the summer and molt, while growth slows down in the winter (Sumpton, W., Potter, M. and Smith, 1994). Weather the trace metal content within the crab's carapace and muscle is related to the molting ,which might warrant further investigation. Apart from Zn all other elements present within the carb muscle were below the international limits.

Limited sediment analyses in the NE of Qatar where the crabs were caught have been reported (Basaham & Ai-lihaibi, 1993). These studies showed a trend of Mn>Zn>Ni>V>Cu>Co. The majority of reported data pertaining to barnacles refers to amount of contaminants found within the soft tissue of the organism;. Little work has been done on the monitoring of metals within the barnacle shells. (Royo-Gelabert & Yule, 1994; Watson, Foster, & Walker, 1995) These studies analysed levels of some selected trace metals throughout the development stages within the barnacle *Semibalanus balanoides* from the UK Anglesey coast, for over a period of twenty four months. Our data showed that the annual average of trace metals within the barnacles shell displayed the following trend Zn>Cu>As>Mn>Fe>Ni>Cr>Cd>Pb>Co>V, with Zn registering at 152.9 mg/kg and V at 0.03 mg/kg. The barnacle shell data also indicated that most trace metal concentrations apart for Co and Pb displayed a significant difference in concentrations between the winter and summer seasons. As barnacles are filter feeders, these

differences can be the result of changes in anthropogenic input of metals or to other physicochemical factors such as salinity, temperatures together with other factors that may affect the bioavailability of these metals (Farraj, Gendy, Kahtani, & Hedeny, 2011).

There has been a number of reports on the analysis of heavy metals in the barnacles' soft tissue (Barbaro et al., 1978; Barber & Trefry, 1981; Reis, Salgado, & Vasconcelos, 2011). Researchers employed barnacles as a biomonitoring tool in the lagoons of the North Adriatic; they reported that these organisms have a large ability to uptake and accumulate trace metals, from sea water which contained high levels of contaminants present (Barbaro et al., 1978). Making them a good candidate for a biomonitoring organisms , they also concluded that barnacles possess two essential properties that make them suitable for use as biomonitors: the strong susceptibility of uptaking and retaining metals, and secondly the accumulation of such metals to levels above the environmental levels (Dionísio, Costa, & Rodrigues, 2013). Reis et al (2011) reviewed the literature regarding concentration of trace metals within the barnacle *B. amphitrite* the trace metal concentrations ranges reported are shown in table 2.5.
The results from our analysis show most analysed metals lie within the reported ranges, apart from Cr which appears to higher. This elevation maybe attributed to the ongoing dredging within the Bay at the time of sampling (September) and the release of Cr stored in the sediment into the water column.

Table 3. 4: Comparison of trace metal concentration from our results with international findings

	Cd	Cr	Fe	Mn	Ni	Pb	Zn
Reis et al (2011)	**0.69 – 30.9**	**0.2 – 3.32**	**313 – 5929**	**96 – 469**	**1.3 – 98.9**	**0.4 – 39.2**	**919 – 23300**
Present study	**15.032**	**3.865**	**319.547**	**14.571**	**8.819**	**1.259**	**5684.463**

As the studied species of shrimp within report was only recently discovered (2006), data regarding heavy metal concentration within this species are practically nonexistent. However different authors reported data from other species of shrimp from various global locations. Some of these reported values are shown in table 6, the data shows that the khori shrimp levels for

trace metals are lower than the levels reported (Firat, Gök, Coğun, Yüzereroğlu, & Kargin, 2008; Heidarieh et al., 2013; Pourang & Dennis, 2005).

Table 3. 5: Comparison of trace metal concentration from shrimp samples with international findings, all results are in mg/kg dry wt.

	As	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Literature(Firat et al., 2008; Heidarieh et al., 2013; Pourang & Dennis, 2005)	8.28	0.4 – 4.5			18.69-288.0	25.4			27.75 – 148.89
Present study	0.28	0.27	1.99	4.07	1.02	0.38	5.14	0.72	5.43

A wider range of heavy metals were analysed in this study and thus comparative data will be limited to the previously reported heavy metal concentrations. The heavy metal trend within *P. khori* shrimp follows Zn > Cu >Fe > Mn >Ni > Pb > As > Cr > Cd > V >Co. With Zn the highest registering at 124mg/kg and Co the lowest at 0.1057mg/kg.. Seasonal statistical variation was recorded in Cr, Ni, Pb and Zn, with Cr and Ni higher in the winter with Pb and Zn were highest during the summer periods.

3.5.2 PCBs:

PCBs are hydrophobic compounds, that have a high affinity for fine particulate matter, and thus they tend to portion themselves into the sediment and organic matter (Heidarieh et al., 2013) rather than in the aqueous phase. This may also be associated with the amount of chlorine within the PCB (Bat, 2012).

The results of the PCB analysis within the carb tissue shows that they were not detected in the in the carapace but they were present in the muscle.

As expected the average levels of PCB101 (4.78µg/kg) detected in the crab muscle was greater than that of PCB44 (average 1.99 µg/kg) detected within the same tissue. This supports Voorspoels et al (2004) suggestions that the levels of heavy PCB 101 should be greater than lighter PCB, due to their chlorine content and position, as compared with light-chlorinated congeners which are readily metabolized and eliminated (Voorspoels, Covaci, Maervoet, De Meester, & Schepens, 2004a). The data can also be rationalized by the crabs' feeding characteristics. These organisms feed on other benthic animals as well as crustaceans, fish,

bivalves and some aquatic plants but are also foragers that feed on decaying marine organic material, and these sediments may in certain instances have high levels of pollutants (Fazel Abdolahpur Monikh et al., 2013). Even though there are not a lot of data pertaining to PCB levels for organisms within the Arabian Gulf, the limited data available report values of 10.2 µg/kg – 1448 µg/kg as a total of several congeners (Al-Mohanna & Subrahmanyam, 2001). In South Carolina, USA researchers reported ∑PCBs of 860 µg/kg in some crabs close to a waste treatment plant, although this concentration dropped to 80 µg/kg a hundred meters away (Coast et al., 2014) . Maximum concentration of 130 µg/kg were also reported(Pourang & Dennis, 2005). Values obtained from our studies are well below these values. There were no statically significant differences in the values of the PCBs within the two seasons. PCBs 44 and PCB101 were both detected in the tissue of the shrimp but there were no statistical seasonal variation in their concentration. PCB 101 and PCB 44 were detected in our specimens. The level of PCBs within other shrimp species inhabiting the Arabian Gulf was reported to be in the range of 0.5µg/kg - 30µg/kg (Fowler, 1987; Nhan et al., 1998). Moreover, it was suggested that PCB levels in shrimps, crabs and lobsters, that inhabit moderately unpolluted waters, should be expected to be less than 10µg/kg (Monod & Arnaud, 1995).

In a recent publication on the levels of PCBs (16, 44, and 99) in crustaceans from various locations in Iran (Arabian Gulf), PCB 44 from the study ranged from 101 – 254µg/kg for the *P. pelagicus*, and 27µg/kg – 211µg/kg for five species of shrimp. These levels are considerably higher in magnitude in comparisons to our studies. These differences can be to various factors that govern the crab and shrimp diets. One vital element that has a significant role in pollutants accumulation in aquatic organisms is the metabolic activity (Voorspoels et al. 2004). Contaminants' accumulation in organisms is controlled by uptake, detoxification, and elimination mechanisms (Morsi et al. 2003). The diet of swimming crab varies with age, and seasonality as it captures other smaller live water as fish (Paoki et al. 2012). Crabs are also classified as bottom-feeding carnivores that prey on a wide variety of slow- moving organisms. Baseline data for PCBs in mangrove shrimp from similar environments as our study area are currently unavailable.

The highest concentration was Fe 754.47 ppm, and the least was Cd (0.109 µg/kg). Comparative analysis of the limited data on the shrimp and the sediment from the mangroves seem to mirror the levels of As, Cr, Fes and Mn within the shrimp, with others such as Cu Ni and Zn showing higher concentration within the shrimp compared to the sediment. Moreover, the levels trace metals within the shrimp is not only a reflection of the sediment levels but other aspects such as the scavenging nature of these shrimps, and their feeding habits.

Sediment results showed a wide range of heavy metal levels, occurring in the following descending trend Fe > Mn > Cu > As > Zn > V > Cr > Li > Ni >Co > Cd. Pb was below the LOD. All levels were within the acceptable limits (See appendix) set by the M.o.E Qatar. De Mora et al. in 2000 surveyed the "ROPEME" Sea Area (Qatar UAE, Oman Bahrain and the KSA), although the survey did not sample from the Qatari mangroves, data comparison showed that the concentration of heavy metals from Doha and Ras Laffan were much higher in all trace metals compared to our data.

3.9 Conclusion

In this study we evaluated the levels of 11 trace elements, and PCB concentration in crab (*Portunus segnis*), barnacle (*Balanus amphitrite*) and shrimp (*Palaemon khori*) collected from mangroves of Al Khor, Qatar. Within the crab higher concentrations of As, Cd, Cu, and Pb were found in the muscle compared to the carapace. Statistical significance within the levels of As, Cr, Cu, Ni and Zn was detected between the seasons. High levels of As were detected in the crab muscle, though to be from natural sources. Significant higher concentrations of PCB44 and PCB101 were detected in the summer compared to winter.

Analysis data also showed seasonal variation of the concentration of trace metals within the soft tissue of the barnacles were seen in Co, Cu, Fe, Ni, Pb, V and Zn, with Ni and Zn showing higher concentration in the winter with the rest having higher values during the summer season. As was also present within the barnacle tissue, and is thought to originate from natural sources. The study also shows that the barnacle muscle had higher concentration of all the heavy metals compared to the shell, but no PCB detected in shell and muscle.

Data from the shrimp tissue revealed that Zn was the highest registering at 124mg/kg and Co the lowest at 0.1057mg/kg. The heavy metal trend within P. khori shrimp follows Zn > Cu

>Fe > Mn >Ni > Pb > As > Cr > Cd > V >Co. Seasonal statistical variation was recorded in Cr, Ni, Pb and Zn, with Cr and Ni higher in the winter and Pb and Zn in the summer. PCBs detected in the shrimp had no significant difference observed between the two seasons for both the congeners (PCB44 and PCB101).

Chapter IV: Assessment of Organotin Contamination in Selected Marine Biota and Sediment from the Mangrove Habitat in the Arabian Gulf.

4.1 Abstract

Throughout the last century, organotin compounds have found use in a wide number of applications including stabilizers, coatings, preservatives, biocides, and as antifouling coatings. The latter has led to high concentrations of organotins prevailing in the marine environment, mostly in proximity to marinas and harbors. In the marine environment, organotin compounds: monobutyltin (MBT) and dibutyltin (DBT) are mainly derived from tributyltin (TBT) degradation, rather than from direct sources. These compounds can lead to a number of adverse effects on the organisms inhabiting the marine ecosystem, even at trace concentrations.

To ascertain the concentrations of these pollutants within the Qatari marine environment, levels of TBT, and its degradation products MBT and DBT, were measured within the sediment (top layer 10cm) and tissue of three selected marine organisms, all indigenous to Qatar's coastal marine mangroves. Our study organisms were *Portunus segnis*: the blue swimming crab, a commercially important edible species; *Balanus amphitrite*, the striped barnacle, which is often used as bio-indicators of coastal pollutants; and *P. Khori*, the endemic caridean shrimp, which inhabits the *Avicenna marina* mangrove forest at Al-Khor, Qatar.

Data revealed that concentrations of the organotins within sediment samples ranged from 1.99 nano grams of tin per gram ($ngSng^{-1}$) – $3.24ngSng^{-1}$, and averaged $2.77ngSng^{-1}$ for all three analysed tin species. Monobutyltin, DBT and TBT were detected in all three species. TBT levels were $0.58ngSng^{-1}$, $0.78ngSng^{-1}$ and $1.91ngSng^{-1}$ for *Portunus segnis* muscle (both muscle and carapace were analysed separately), *Balanus amphitrite* tissue and *Palaemon khori* respectively.

This study show that levels of organotin compounds (TBT DBT and MBT) in selected marine biota and sediments sampled from the mangroves in Qatar are generally lower than levels reported in the literature from elsewhere, although it must be noted that similar data for other mangrove habitat within the region of the Arabian Gulf is extremely limited.

The data presented in this study represent the first-ever estimated levels of organotin contaminants in animal tissues and sediments in the mangroves ecosystems of Qatar.

4.2 Introduction:

The term organotins generally refers to a tin (tetravalent atom, Fig 3.1) that is connected to one carbon atom at a minimum. The common formula for these organotin complexes is $R(4\text{-}n)\ SnX_n$ with n = 0 – 3 and R signifies an alkyl or aryl groups while X represents the anion (Ronald et al., 2002).

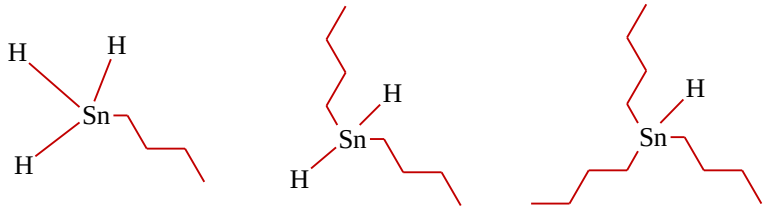

Figure 4. 1Organotin compounds ((MBT), dibutyltin (DBT), and tributyltin (TBT)) structures.

These comprise a large group of compounds that have a wide range of properties, their use has been wide spread due to their versatility for applications. The most widely used was a tri-substituted organotin compound TBT, due to its powerful biocidal activity towards marine organisms. Thus, it was widely used in antifouling paints, wood preservatives, and as antifungals (Hoch, 2001). This extensive use has led to the prevalence of these compounds in the marine ecosystem. Studies have shown that organotin compounds are released into the marine environment via various sources. They thus, represent a potential hazard for marine environment (Díez, Lacorte, Viana, Barceló, & Bayona, 2005)

Research into these compounds has mainly focused on their presence in waters and marine organisms, stemming from their use as antifouling agents (Antizar-Ladislao, 2008; Rantakokko et al., 2008; Rüdel et al., 2007) .

Antifouling paint were introduced to curtail the build-up of barnacles and to improve on speed as well as economic efficiency of maritime vessels. It works by the formation of a thin film released from the paint that envelopes the vessel, thus preventing fouling organism attachment (Universit & Universit, 2016).

The accumulation of these compounds within the marine organisms depends on the individual organisms' life style e.g. Some organotins, such as phenyltins have been reported at higher levels in benthic marine organisms while other such as butyltin levels were higher in pelagic marine organisms(Stäb et al., 1996). Other reports have postulated that some butyltins are more concentrated in some migratory fish species (Takahashi et al., 1999). Moreover, some reports indicate that aquatic organism inhabiting shallow water may contain more butyltin compounds when compared to deep water dwellers.

Reports on organotin accumulation have mainly focused on seafood, from aquatic invertebrates such molluscs (bivalves) and crustaceans (decapods) (Furdek et al., 2012)., to fish as these are ecologically important and dominant in many habitats. Ashraf et al., (2017) published a report accessing the levels of organotins within common commercial fish from the Arabian Gulf. The study indicated that MBT ranged from 12.7 $ngSng^{-1}$ – 85.9 $ngSng^{-1}$, DBT ranged from 34.7$ngSng^{-1}$ – 77.3$ngSng^{-1}$, while TBT within the analysed fish ranged from 43.7$ngSng^{-1}$ - 98.5$ngSng^{-1}$. All the reported values are much higher than the one detailed in this thesis, noting that our data refers to crab, barnacles and shrimp, and not fish. The only record for organotins within marine biota from Qatar was reported by de Mora in which he reported organotin concentrations ($ngSng^{-1}$) in fish, The Author reports levels of MBT ranging from 3.1 $ngSng^{-1}$ – 5.5 $ngSng^{-1}$, a DBT range of 3.2 $ngSng^{-1}$ – 5.1 $ngSng^{-1}$ and TBT ranging from 2.8 $ngSng^{-1}$ – 6.4 $ngSng^{-1}$(Stephen J. De Mora et al., 2003) . All data reported from our study lie with the range of these findings. No other records for organotin concentrations within marine organisms from Qatar were found.

Kannan & Falandysz, (1999) reported quantifiable BT concentrations in human liver obtained by autopsy from samples collected from the of the Baltic Sea's polish coast, probably a result of seafood consumption. Barnes and Stoner reviewed the pharmacological effects of organotins compounds, and reported that some of these compounds seemingly affect the cardiovascular and respiratory system in humans (Barnes & Stoner, 1959). For the majority of countries, no data on organotin levels in seafood were available (Belfroid, Purperhart, & Ariese, 2000). Noted, however, that the amount of seafood consumed varies from country to country, thus in order to assess the risk in consuming seafood tainted with organotin compounds it is important to take into account the over- all fish consumption habits. This can be done as proposed by Belfroid et

al. (2000) who calculated the tolerable average residue levels(TARL). Tolerable average residue levels are the basis for the concerned authorities to develop a maximum residue limit (MRL) of TBT in seafood.

It can be a tool to ensure the health of the population by comparing the TARL values directly with measured residue levels of TBT in seafood. TARL calculated for people living in the Gulf area were 13.7ug/g for TBT .

The mangrove present in the north of Qatar is a unique area that warrants special attention for various reasons. The Arabian Gulf's marine environment is fragile as a whole, arid condition and high variations of temperature (7°C in winter while 52°C in summer) inland and up 38°C within the water column make life for the local marine organisms very difficult, making them at the extreme limits to any additional stress, such as contamination of the habitat by pollutants like organotin compounds.

The closeness of the mangroves to the Al-Khor marina makes the occurrence of organotins likely due to the continued presence of the fishing port and the large volume of marine traffic within the area. In this chapter the aim is to determine the presence of any of the three organotin species in the sediment and biota (the crab *Portunus segnis*, the barnacle *Balanus amphitrite*, and the shrimp *P. Khori*) within this important habitat, as it harbors a unique species of shrimp endemic to Al-Khor mangroves, and acts as a nursery for many fish and crab species.

4.3 Material and Methods

4.3.1 The Study Area

Along the coast near Al Khor (Arabic word, meaning creek), is a vast mangrove forest. The area supports a lot of wildlife, and subjected to tidal activities. (Fig 4.2 & 4.3) (Al-Thani, 2013). For sampling location points refer to chapter two.

Figure 4. 2: Al-Khor mangroves at high tide

Figure 4. 3: Al-Khor mangroves at low tide

4.3.2 Sample Collection:

The shrimp *Palaemon khori* were sampled at low tide from Al-Khor mangroves (25.690020, 51.55572), by the aid of nets (Figure 4.4).

Figure 4. 4: Collecting *Palaemon khori* samples.

The collected samples (Figure 3.6) were then transferred to the Environmental Science Center (ESC) in cool boxes containing aerated fresh sea water.

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Figure 4. 5: Caught palaemon shrimps from the Al-Khor mangroves.

4.3.3 Sample preparations and analysis

After sample as per chapter 2, the samples were taken to the ESC. The sample were then allocated to the different parameters to be analysed, For organotin analysis both biota and sediment samples were dried in the drying oven set at 60°C for 48 hours, after which they were ground using a mortar and pestle. Tissue and shell (carapace) from crab and barnacles were separated while the shrimp comprised of the whole animal. Barnacle shells were ground using an electric grinder.

For sample analysis, c. 2.5 g of dried sample was weighed into 25 ml beaker, to which 500 µl organotin (OT) standard solutions was added giving a 100 ng/g organotins concentration. The samples were then dispersed in diatomaceous earth, relocated to a 34 ml accelerated solvent extractor cell. Using a Dionex 350 Accelerated Sample Extractor (ASE) (Fig 4.6), the samples were then extracted using the following parameters: Extraction Solvent: 1 M Sodium acetate, 1 M acetic acid in methanol (1:1); Temperature: 100 °C; Pressure: 1500 psi; Heat-up Time: 5 min; Static Time: 5 min; Flush Volume: 60% of collection volume; Purge Time: 100 s; Static Cycles: 3–5; (Dionex application note #339).

The extracts were then placed in a 250 ml volumetric flask containing 7.3 g of sodium chloride in and 50 ml distilled water, the final solution was then pH adjusted to 5.0 using 1 M NaOH. Organotins were ethylated using 1 ml of 5% w/v $NaBEt_4$ aqueous solution, and the final volume was made up to 250 mL using distilled water. 2 ml of hexane (Pesticide Grade, Sigma

Aldrich) was added and the samples were then shaken electrically for c.12 hours. After the shaking time had elapsed, 500 µl aliquot of the organic layer were pipetted into a 2 ml vial and 10 µl tetrabutyltin equivalent to 50 ng butyltins was added as a surrogate standard. After sample preparation the samples were stored at -20°C, till injection into the GC/MS. Ethylated samples were then injected into the Gas Chromatography coupled to Mass Spectrometry (GC-MS) for analysis. (Fig 4.7).

Figure 4. 6: Sample extraction using Dionex Accelerated solvent extractor.

Figure 4. 7: Sample analysis using Agilent GC/MS

Samples were analysed by the GC/MS in triplicate (i.e. the analytical instrument analyses the sample three times) and gives the average The samples were run once through the instruments while the CRMs run twice. All statistical analysis was carried out using SPSS v23.

4.4 Results

Analytical results from both the biota and sediment samples are shown in Table 3.7. In summary all three organotin species were found in the tissue of the three selected organisms and analysed sediment.

Within the tissue of the crab the highest concentrations of the three organotin species were detected in the carapace registering 9.2ngSng^{-1} for MBT, 12.66ngSng^{-1} for DBT and 10.52ngSng^{-1} for TBT (Figures 4.8). Similar analysis results were obtained for the barnacle (Figure 4.9), shows that the barnacle shell has higher levels of the three organotin species.

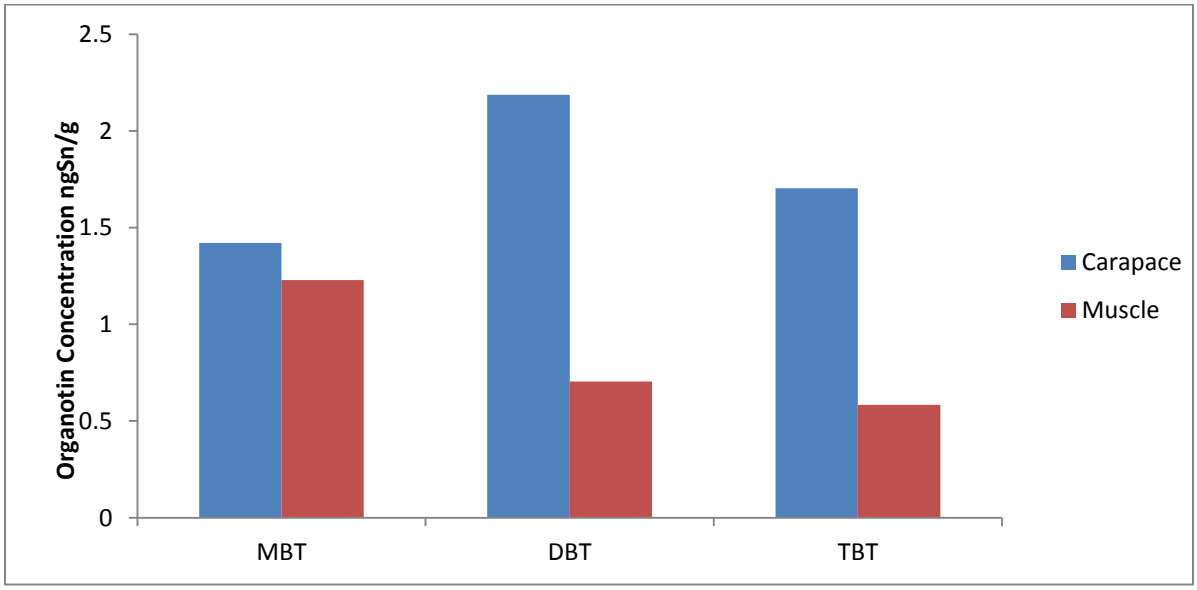

Figure 4. 8: Overall total organotin in blue crab *Portunus segnis* carapace and muscle.

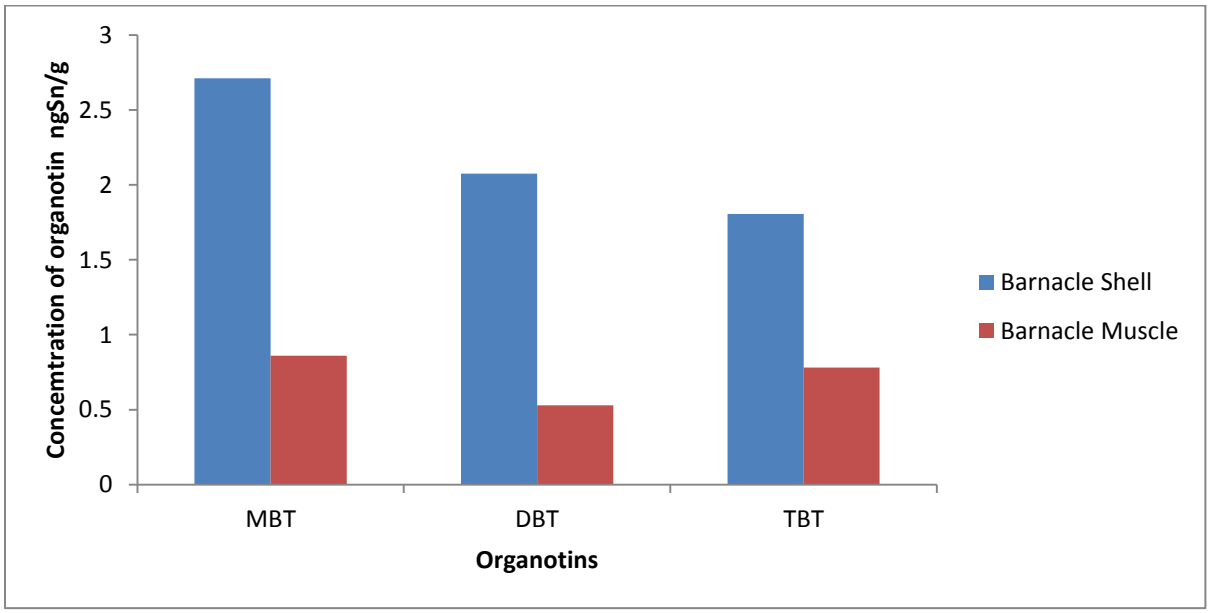

Figure 4. 9: Overall average concentrations of MBT, DBT and TBT in barnacle shell vs muscle.

With respect to the total of the three organotin species (∑OTC), the highest concentration was recorder in the barnacles (52.9 ngSng-^{1}), followed by the crab (46.7 ngSng-^{1}) and shrimp (41.06 ngSng-^{1}). Pearson correlation (2 tailed) statistics was applied to the result from each species. The results from the crabs analysis showed that there is no correlation between MBT and both DBT and TBT. There is however a significant (p=0.12) positive correlation between DBT and TBT in the carb (Table 4.1)

Table 4. 1: Pearson Correlation for organotin species with the carb

		MBT in crab	DBT in crab	TBT in crab
MBT in crab	Pearson Correlation	1	.182	.324
	Sig. (2-tailed)		.572	.305
	N		12	12
DBT in crab	Pearson Correlation		1	.695*
	Sig. (2-tailed)			.012
	N			12
TBT in crab	Pearson Correlation			1
	Sig. (2-tailed)			
	N			

*. Correlation is significant at the 0.05 level (2-tailed).

Barnacle results statistics show that there is a strong positive correlation between MBT and both DBT (p= 0.000) and TBT (p=0.000). A strong correlation also exists between DBT and TBT (p=0.000) (Table 4.2).

Table 4. 2: Pearson Correlation for organotin species with the shrimp.

		MBT in Barnacle	DBT in Barnacle	TBT in Barnacle
MBT in Barnacle	Pearson Correlation	1	.884**	.849**
	Sig. (2-tailed)		.000	.000
	N		12	12
DBT in Barnacle	Pearson Correlation		1	.946**
	Sig. (2-tailed)			.000
	N			12
TBT in Barnacle	Pearson Correlation			1
	Sig. (2-tailed)			
	N			

Shrimp tissue analysis display a strong correlation between MBT, and both DBT and TBT, similarly a correlation exist between DBT and TBT (Table 4.3)

Table 4. 3: Pearson Correlation for organotin species with the barnacle

		MBT in Shrimp	DBT in Shrimp	TBT in Shrimp
MBT in Shrimp	Pearson Correlation	1	.967**	.919**
	Sig. (2-tailed)		.002	.010
	N		6	6
DBT in Shrimp	Pearson Correlation		1	.848*
	Sig. (2-tailed)			.033
	N			6
TBT in Shrimp	Pearson Correlation		.	1
	Sig. (2-tailed)			
	N			

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

To access the correlation of the concentration levels between the shrimp and sediment a Durbin – Watson regression test was applied to the data. The results showed that there is no correlation

between the levels of MBT in the sediment and shrimp. The data however shows that there is a positive significant correlation (R^2= 0.541) between DBT in the sediment and BDT in shrimp. Similarly TBT in shrimp and TBT in sediment display a positive significant correlation (R^2= 0.552) (Table 4.4).

Table 4. 4: Durbin –Watson regression test analysis

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.735[a]	.541	.426	.251852	2.135

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	1.811	.476		3.802	.019		
	DBT in SEDIMENT	.304	.140	.735	2.171	.096	1.000	1.000

a. Dependent Variable: DBT in SHRIMP

TBT Analysis

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.743[a]	.552	.440	.288740	1.906

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	.420	.653		.642	.556		
	TBT in SEDIMENT	.647	.291	.743	2.221	.091	1.000	1.000

a. dependent variable: TBT in shrimp

The mean concentration of all three organotin species was plotted for all three sentinel species and sediment (Figure 4.10) , the result show that concentrations within the sediment is

"mirrored" within the shrimp and the crab , the fact that they are both sediment dwellers can be a factor.

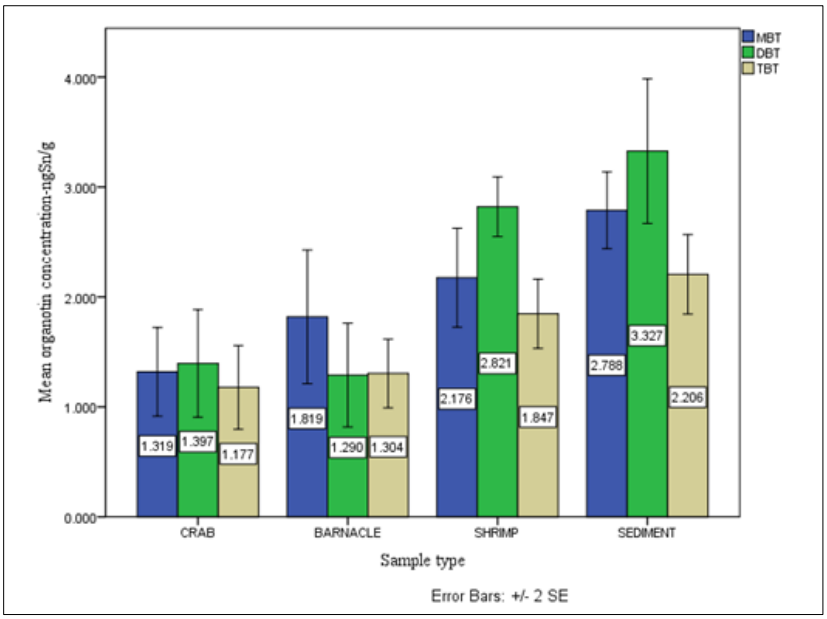

Figure 4. 10: Mean concentration of all three organotin species.

Table 4. 5: Analysis result for MBT, DBT and TBT from biota (blue crab, barnacles, shrimp) and sediment.

		POLLUTANTS					POLLUTANTS					P)LLUTANTS			
		Organotins (ngSng-^{1})					Organotins (ngSng-^{1})					Organotins (ngSng-^{1})			
		MONTH	MBT	DBT	TBT		MONTH	MBT	DBT	TBT		MONTH	MBT	DBT	TBT
CRAB	Carapace	Sept	1.20	2.30	1.10	BARNACLE	Sept	2.40	2.50	2.10	SHRIMP	Sept	2.80	3.10	2.50
		Nov	0.90	3.00	1.50	Shell	Nov	2.80	2.20	1.90		Nov	2.90	3.30	2.10
		Dec	0.70	1.80	2.00		Dec	2.00	1.90	1.70		Dec	2.10	2.90	1.80
		May	2.10	2.00	1.90		May	3.10	1.80	1.60		May	1.90	2.60	1.60
		Jun	2.20	1.83	2.02		Jun	3.25	1.97	1.73		Jun	1.79	2.53	1.58
		Jul	2.10	1.73	2.00		Jul	3.10	1.89	1.64		Jul	1.56	2.50	1.50
		average	**1.42**	**2.19**	**1.70**		**average**	**2.71**	**2.07**	**1.81**		**average**	**2.30**	**2.89**	**1.92**
		STD	**0.68**	**0.48**	**0.37**		**STD**	**0.49**	**0.26**	**0.19**		**STD**	**0.55**	**0.33**	**0.39**
	Muscle	Sept	1.90	0.40	0.60	Muscle	Sept	1.10	0.30	0.80		Total	**13.06**	**16.93**	**11.08**
		Nov	1.60	1.40	0.52		Nov	0.80	0.85	0.70	SEDIMENT	Sept	3.24	4.13	2.74
		Dec	1.75	0.51	0.36		Dec	0.68	0.36	0.40		Nov	2.85	3.79	2.31
		May	0.44	0.62	0.71		May	0.90	0.52	0.90		Dec	3.01	4.01	2.60
		Jun	0.45	0.59	0.73		Jun	0.82	0.61	1.10		May	1.99	2.02	1.52
		Jul	0.49	0.58	0.69		Jul	0.88	0.57	1.08		Jun	2.75	3.00	2.07
		average	**1.23**	**0.70**	**0.58**		**average**	**0.86**	**0.53**	**0.78**		Jul	2.90	3.02	2.00
		STD	**0.71**	**0.36**	**0.14**		**STD**	**0.14**	**0.20**	**0.26**		**average**	**2.77**	**3.39**	**2.25**
		Total	**15.83**	**16.76**	**14.12**		**Total**	**21.83**	**15.48**	**15.65**		**STD**	**0.43**	**0.81**	**0.44**
												Total	**16.73**	**19.96**	**13.24**

*MBT = Monobutyltin, DBT = Dibutyltin, TBT = Tributyltin. SD = Standard deviation

4.5 Discussion

Data pertaining to the concentration of organotin species in the blue crab from the Arabian Gulf are virtually non-existent; where most of the available data is based on the amount of organotins within the sediment, or the tissue of various fish species (De Mora et al., 2003). Nuhu & Al-Shatri. (2015) studied the amount of monophenyltin (MPhT), a phenyl derivative of organotin, in various marine biota from Saudi Arabia, and reported values of 1420 $ngSng^{-1}$ within crab tissue and 4050 $ngSng^{-1}$in shrimp (Nuhu & Al-Shatri, 2015). Internationally, organotin concentrations within three species of crab *Scylla serrata* (giant mud crab), *Portunus sanguinolentus* (three spot crab) and *Portunus pelagicus* (blue swimming crab) sampled from Mumbai market, India were assessed by Jadhav et al., (2011). They reported values of butyltin in *Scylla serrata* of 112 ngSng-^{1}; *Portunus sanguinolentus* of 111 ngSng-^{1}; and *Portunus pelagicus* of 42 ngSng-^{1} (Jadhav et al., 2011). They also postulated that the differences observed may be due to the crab's different habitats and resultant organotin exposure, and that seasonal variability was more important than the stress induced by the exposure to different levels of the pollutants. In France Guérin et al. (2007) reported organotin levels in crab tissues at 8.7ngSng-^{1}, although the authors did not elaborate on the species of the crab. The concentration observed in both studies is higher than organotin concentrations recorded in our study.

The only data available for comparison or organotin levels within barnacles from within the Arabian Gulf, is for barnacles from Oman, where MBT concentrations ranged from 6.6 $ngSng^{-1}$-31 $ngSng^{-1}$; DBT from 24 $ngSng^{-1}$ - 57 $ngSng^{-1}$; and TBT concentrations from 56 $ngSng^{-1}$ – 85 $ngSng^{-1}$ dry weight (De Mora et al., 2003). These values are also higher than our results. TBT concentrations were measured in barnacles collected from the Aegean Sea, Turkey. The authors report values for TBT of 635ngSn g^{-1} in barnacles (Kucuksezgin et al., 2011). Both sets of data show higher concentrations of organotin compared to our findings. Nuhu et al. (2015) measured three phenyl derivatives (i.e. MPhT, DPhT, and TPhT) in shrimps from Saudia Arabia, and reported values of 4.05, 0.140, and 0.221mg/kg respectively (Nuhu & Al-Shatri, 2015). In France, organotins within shrimp tissue were measured at 0.3$ngSng^{-1}$for MBT, and DBT , while TBT was observed at 0.7$ngSng^{-1}$, the authors also reported levels of 1.1$ngSng^{-1}$ fort MBT, 1.4$ngSng^{-1}$ DBT and 2.8$ngSng^{-1}$TBT in crab. (Guérin et al., 2007). These levels are lower than the observed levels in our study

Organotin data from the carapace and the muscle of the blue crab (*Portunus pelagicus*) present some interesting observations. Based on average concentration data for the carapace and muscle, no significant differences in the concentration of MBT are apparent, with noticeable difference in the levels of DBT and TBT (see Figure 3.8). However, seasonal comparison (winter vs summer) of organotin levels within the carapace showed that there is a significant difference ($p= 0.01$) in the levels of only MBT, with no significant differences in the levels of DBT and TBT.

The monthly average data for MBT in crab muscle show that MBT levels within the carapace decreased between September and December, while that of the muscle was constant. In contrast, MBT data from May to July show that the carapace concentrations were considerably higher than those of the muscle tissue (see Figure 3.9). Noted however, that although not related to a seasonal trait some species of crabs are known to shed contaminants during molting (Bergey & Weis, 2007; Raessler, Rothe, & Hilke, 2005), although this phenomenon is not apparent in all species (Keteles & Fleeger, 2001). The variation of the levels can also be attributed to other factor such abiotic factors (temperature pH, etc.), that play an important role in the distribution of contaminants within water and sediment(Al-Naimi et al., 2015). These localized conditions can influence the life cycle of the crab in several ways: size maturity, growth, fecundity, spawning, recruitment and mortality(Giraldes et al., 2016).

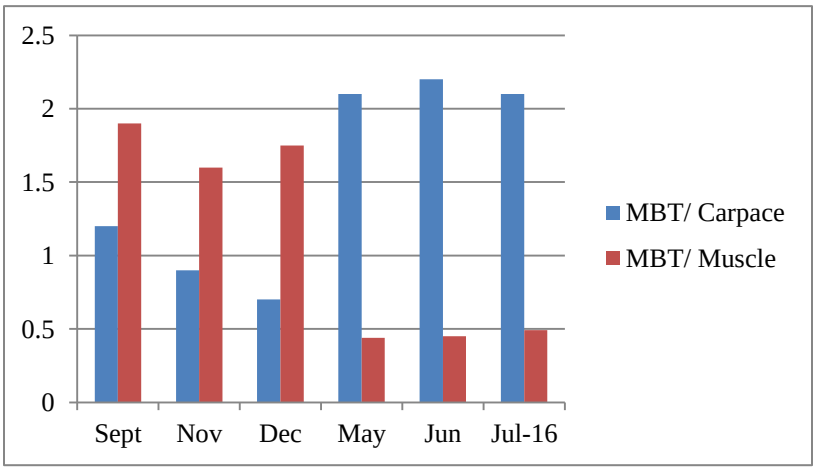

Figure 4. 11: Monthly trends (Sept 14 – Jul 16) in MBT concentration in blue crab *Portunus segnis* (carapace and muscle).

In contrast, within barnacle, the levels of MBT, DBT and TBT remain significantly different ($p > 0.05$) between the shell and tissue over the measurement period. Although MBT levels occur at a slightly higher concentration overall, the difference between total organotin levels in the shell and the muscle remained the same, where organotins were present at higher concentrations in the shell barnacles do not shed their shell during their life cycle, and store some contaminants within them (Figure 3.11).

The levels of OTs with the tissue of the shrimp are comparable to those found in the sediment. Statistically, correlation coefficients (r): were calculated for DBT, and TBT with values of 0.54, and 0.55 showing a positive correlation between the two values. This is to be expected as this species of shrimp resides in the top sediment, and consume or scavenge food in this niche. Table 3.6 shows the correlation in the levels of MBT, DBT and TBT within the shrimp relative to that in the sediment. The data shows that there is a positive significant correlation ($R2= 0.541$) between DBT in the sediment and DBT in shrimp. Similarly TBT in shrimp and TBT in sediment display a positive significant correlation ($R2= 0.552$).

The fate of organotins within the marine environment has been examined by a number of reports; where some have suggested that bacteria play an important role in the degradation of these compounds, while other research has indicated phytoplankton play the dominant role (Hoch, 2001; Lee et al., 2012; Sampath et al., 2011). Moreover, the conditions that govern the growth of these microorganisms, such as pH, temperature, oxygen, turbidity, and light also govern the fate of organotins. The parent compound, TBT, within the sediment, is thought to have a half-life that of several years (Dowson et al., 1993; Takeuchi et al., 2004), where degradation of organotins in sediment is dependent on the type and concentration of organotins present and their propensity to inhibit decomposition at high concentrations (Dowson et al., 1993; Hoch, 2001a; Stewart & Mora, 1990). It is thus difficult to ascertain the precise time of the occurrence of organotin sediment contamination.

The content of butyltins within a particular matrix can however be analysed by the butyltin degradation index (BDI) first suggested by (Díez et al., 2002). BDI assess the extent of degradation of butyltins and is calculated by the formula;

$$BDI = \frac{(MBT+DBT)}{TBT}$$

a BDI value of less than 1 indicates recent TBT contamination, whereas higher values are considered more historic contamination (Filipkowska et al., 2014). Based on our study data, BDI values ranged from 3.31 to 4.75, which indicate historic TBT contamination. Tolerable average residue levels (TARL) were calculated using the method proposed by Belfroid et al., (2002). Within Qatar seafood is one of the main staple diets, with crabs a favorite of the populace, given a TDI (tolerable daily intake) of 0.25 µg/kg BW/day (Santos et al., 2009), an average body weight of 70kg and an average daily seafood consumption of 10.7g/day (Ashraf et al., 2017) TARL values can be calculated as follows.

$$\text{TARL} = \frac{\text{TDI} \times 70 \text{ kg BW}}{\text{Average daily seafood consumption}}$$

Obtained values for Qatar was calculated at 13.7µg/g. The TARL values levels from the edible blue crab in this study pose no immediate risk to public health

4.6 Conclusions

The data presented in this study show that levels of organotin compounds (TBT, DBT and MBT) in selected marine biota and sediments sampled from the mangroves in Qatar are generally lower than levels reported in the literature from elsewhere, although it must be noted that similar data for other mangrove habitat within the region of the Arabian Gulf is extremely limited. It is notable, however, that there is a correlation between the levels of organotins in the mangrove sediment and the levels found in shrimp, which is in contrast to findings from other studies that show a lower concentration of all three organotin species within shrimp tissue (Benson, 1997; Penninks). This study has, however, raised some interesting questions in the context of the life cycle and ecology of the biota studied, with respect to: i) the correlation between organotins in mangrove sediment and the shrimp *P. Khori*, and: ii) whether the Blue crab *Portunus segnis* can actively remove organotins via the carapace molting cycle.

Chapter V: Determination of methylmercury, in selected fauna and sediments from the mangroves of Qatar.

5.1 Abstract:

The concentration of methylmercury (CH_3Hg) in the blue crab (*Portunus segnis*), the barnacle (*Balanus amphitrite*), the shrimp (*Palaemon khori*), and sediments from Al-Khor mangroves were determined. CH_3Hg concentrations in all samples were observed form September 2014 – July 2016, and concentrations ranged from1.0 µg/kg – 2.0 µg/kg in the crab carapace, and 0.9 µg/kg – 1.3 µg/kg in the muscle, from 0.66 µg/kg – 1.20µg/kg for the barnacle shell, 0.58 µg/kg – 2.2µg/kg in the barnacle soft tissue, 1.32 µg/kg – 2.2 µg/kg for the shrimp and 1.6 µg/kg – 2.01 µg/kg for the sediment. Sediment concentrations do not correlate with that of the shrimp *P. khori* although the concentration levels in the sediment are higher. Comparison of levels found in the biota and sediment from the mangroves with some international levels indicate that the levels of CH_3Hg within the mangrove sediment are, below the Interim Sediment Quality Guidelines (ISQGs) limits for mercury in soil is 130µg/kg, far below the WHO limit (300µg/kg).

5.2 Introduction:

Mercury (Hg) is one of the most potent marine environmental pollutants, due to its toxicity and its accumulation in aquatic organisms (Heyes et al., 2006). It is an element of special concern as its elemental inorganic form is transformed biologically to the lipophilic organic compound methylmercury (CH_3Hg) which bioaccumulates and biomagnifies as it propagates up the marine food chain (Monikh et al., 2014). Methylmercury within the sediment is thought to be created by microorganisms within the aquatic system,. This compound is then taken up by filter feeder such as zooplankton, which in turn are eaten by small fish. The pollutant is then accumulated in the tissue of the fish as they grow bigger and mature. The now grown fish are consumed by bigger fish, increasing the level of methylmercury with each step of the food chain (Liang et al., 2007). Methylmercury is at its peak in predatory fish, some of which are consumed by human and thus are the main threat to human health.. Over 90% of human health risks associated with seafood consumption (fish, shrimp and crabs) is related to Hg-contaminated organisms (Raymond & Ralston, 2004; Risher, 2003). The exposure of marine

organisms to pollutants such a methylmercury is governed by levels of this pollutant in the surrounding ecosystem, such a seawater and sediment. Water chemistry (Monikh et al., 2013) , and the dynamic of suspended particulate matter due to natural or manmade input are important factors governing some pollutant concentrations. General human activities have resulted in the pollution of most marine ecosystems by this potent contaminant, while discarded mercury from the various industries can find its way into aquatic systems and thus accumulate in the bottom sediment (US EPA. 1997).

Methylmercury and total Hg levels in the tissue of fish and other marine crustaceans have been investigated by numerous environmental analysts since 1980s (Al-Hashimi & Al-Zorba, 1991; Al-Majed & Preston, 2000; S. de Mora, Fowler, Wyse, & Azemard, 2004; A. Freije & Awadh, 2009; Khordagui & Al-Ajmi, 1991). In Kuwait in 1991 the level of Hg in fish and shrimp was below the set by EU/Reg.1881/2006/EU as an action level (Al-Hashimi & Al-Zorba, 1991), but later studies (Al-Majed & Preston, 2000) showed that the levels of both MeHg and Hg in 20.6% of the samples (zooplankton and fish) from Kuwait territorial waters exceeded the WHO limit, even though they were below the USFDA limit. Similar values were found from Oman (de Mora, et al., 2004), where concentrations of total Hg in fish were < 500µg/kg and also from Bahrain (Madany, Wahab, & Al-Alawi, 1996) where the mean of total mercury (Hg + MeHg) in fish was 84µg/kg, again below the USFDA limit. Another study on Hg and MeHg in fish from Bahrain reported levels of Hg in the range of 22 µg/kg –117µg/kg wet weight, and levels of MeHg in the range of 28 µg/kg –123 µg/kg (A. Freije & Awadh, 2009a). Studies from Saudi Arabia also showed that the levels of total Hg were less than the limit proposed by the USFDA, and the Japanese and Brazilian legislation (Al-Saleh & Al-Doush, 2002). Similarly, investigation on the levels of Hg and MeHg in the tissue of fish collected from the water of Iran (off the Arabian Gulf) showed values ranging from 12µg/kg to 87µg/kg much lower than the WHO guideline of 500 µg/kg (Agah et al., 2006). Seasonal changes in CH_3Hg levels production was reported with values reaching a maximum in the summer and late spring (Croston, Bubb, & Lester, 1996). Temperature was observed as one of the most significant constraints found to drive MeHg formation (Benoit et al., 2002; Fitzgerald, 2007).

Although recent articles have been published describing the levels of methylmercury in the Blue Crab *Portunus segnis* from the Arabian Gulf (Ghaeni et al., 2015) data are nevertheless sparse within the region. The reported methylmercury concentration of 112mg/kg was for

samples collected from four locations in Iran(Ghaeni et al., 2015). Other recent studies in the blue crab focus mainly in the total mercury within the species (Hosseini et al., 2015)

The main objectives of the present work are: (1) to investigate the levels of methylmercury in the biota and sediments within the mangrove stands in Al Khor; (2) to assess the relationship between, methylmercury within the sediment and biota; (3) to compare methylmercury levels in the sediments with literature data.

5.3 Material and methods

5.3.1 Sample Collection

The blue crab (*Portunus segnus*) has been renamed recently in 2016 by the ESC to replace *P. Pelagius* (Giraldes et al., 2016). For all sample collection refer to chapter two.

Sediment samples were collected in accordance with the ESC protocol of Sampling and Preservation of Marine Environmental samples (Figure 5.1).

The sediment samples were collected by scooping using a 250 ml glass jar (Figure 5.2), and samples stored in the freezer until analysed.

Figure 5. 1:Collecting sediment samples

5.3.2 Sample Preparation

Sample preparation and analysis was based on the method supplied by Brooke Rands. In essence approximately 0.5 g of a sediment sample was digested in 5 ml 18% KBr + 5% $CuSO_4$ and 1 ml of 1M H_2SO_4 for 1 hour, to which 10 ml dichloromethane (DCM) was then added and the mixture shaken every 5 min for 1 hour before being centrifuged at 3000 rpm. A small aliquot (2

ml) of the DCM layer was pipetted into a teflon tube and 20 ml of deionized water (DI) added, this was heated at 70°C for 1 hour. A 2 ml aliquot was then removed and added to 38 ml of deionized water, the pH was then adjusted between 4.5 - 5 using acetate buffers, maximizing the potential of ethylation. The resulting solution was then ethylated with 1% sodium tetraethylborate ($NaBEt_4$) for 15 minutes after which, nitrogen gas was bubbled into the mixture for 20 minutes. The reacted mercury was then adsorbed on a Carbotrap. These mercuric compounds were then desorbed from the trap using elevated temperatures, segregated using a gas chromatographic (GC) column, reduced by passing them through a pyrolytic column and finally detected using a Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) Figure 5.2.

Figure 5. 2: Sample analysis using Cold Vapor Atomic Fluorescence Spectrometry

Samples were analysed using Brooks Rand MERX III system equipped with an autosampler and MODELIII Atomic Fluorescence detector. Method validation for biota was carried out as per The European International Conference on Harmonisation (ICH) guidelines, in which accuracy repeatability, quantification / detection limit, linearity and range were all examined.

5.4 Results and Discussion

The analysis results from all our samples are presented in table 5.1. The results of CH_3Hg concentrations are shown as averages, minimum concentration, maximum observed concentration with standard deviation on dry weight basis (µg/kg). The Environmental Studies Board, Panel on mercury, reports that the natural expected levels of methylmercury in marine organisms are in the range of 0.4µg/kg –2.0 µg/kg.

Table 5. 1: Analysis results for methylmercury in the sediment, *Portunus segnis*, *Balanus amphitrite*, and *Palaemon khori* from the mangrove study area

CH_3Hg (µg/kg dry wt.)										
		Month / Year				Month / Year			Month / Year	
Portunus segnis	Carapace	Sept /2014	1.97	*Balanus amphitrite*	Shell	Sept /2014	1.20	*P. Khori*	Sept /2014	1.48
		Nov / 2014	2.00			Nov / 2014	1.00		Nov / 2014	1.90
		Dec / 2014	1.00			Dec / 2014	0.98		Dec / 2014	2.20
		May /2015	1.60			May /2015	0.80		May /2015	1.42
		Jun/ 2015	1.70			Jun/ 2015	0.70		Jun/ 2015	1.40
		Jul / 2016	1.72			Jul / 2016	0.66		Jul / 2016	1.32
		Average	**1.67**			**Average**	**0.89**		**Average**	**1.62**
		Min	**1.00**			**Min**	**0.66**		**Min**	**1.32**
		Max	**2.00**			**Max**	**1.20**		**Max**	**2.20**
		STD	**0.36**			**STD**	**0.21**		**STD**	**0.35**
	Muscle	Sept /2014	1.09		Muscle	Sept /2014	1.50	Sediment	Sept /2014	1.96
		Nov / 2014	1.10			Nov / 2014	1.60		Nov / 2014	1.87
		Dec / 2014	0.90			Dec / 2014	2.20		Dec / 2014	2.01
		May /2015	1.10			May /2015	0.80		May /2015	1.60
		Jun/ 2015	1.25			Jun/ 2015	0.69		Jun/ 2015	1.77
		Jul / 2016	1.32			Jul / 2016	0.60		Jul / 2016	2.04
		Average	**1.13**			**Average**	**1.23**		**Average**	**1.86**
		Min	**0.90**			**Min**	**0.60**		**Min**	**1.60**
		Max	**1.32**			**Max**	**2.20**		**Max**	**2.04**
		STD	**0.15**			**STD**	**0.64**		**STD**	**0.17**

The analysis of crab tissue produced results in the range of 1.0µg/kg – 2.0µg/kg in the carapace, and 0.9µg/kg – 1.3µg/kg in the muscle. Studies on the concentration of methylmercury within

the blue crab are rare; Sarasiab et al., (2014) reported a range of 108 µg/kg - 541 µg/kg from Khuzestan Shore, Iran. Another report lists a value of 2.8µg/kg (Hardy & Jones, 1997) for samples from Thailand. Adams & Engel also reported ranges of 63 – 493 µg/kg in blue crabs, *Callinectes sapidus* (Adams & Engel, 2014). It seems that the concentrations of methylmercury are sporadic among this species of crab depending on the location where it was harvested. In comparison to published data our result show that the CH_3Hg within the muscular tissue of the blue crab collected during this study, are lower than data published recently regarding the blue crab.

Metabolic activity may influence pollutant levels in aquatic animals is their (Voorspoels et al., 2004). It is also postulated that the bioaccumulation of such pollutants depends on the organisms and may be controlled by the organisms' uptake, and subsequent elimination of these contaminats (Monikh et al., 2014) (Monikh et al., 2014). The feeding habits of the *P. segnus* are understood to change with seasonality, abiotic factors such as temperature and salinity will undoubtedly influence the animals biology (Kamrani et al., 2010). Statistical analysis of the data showed that there is not significant difference in the levels of CH_3Hg recorded for both the crab's carapace and the muscle tissue during the hot and cold times of the year (Figure 5.3).

The methylmercury levels recorded in the blue crab from this study are much lower than recorded data from various location within the Arabian Gulf, and lower than the action levels of the WHO and the UK standard. Levels in the sediment remain higher than the levels recorded for both the carapace and muscular tissue.

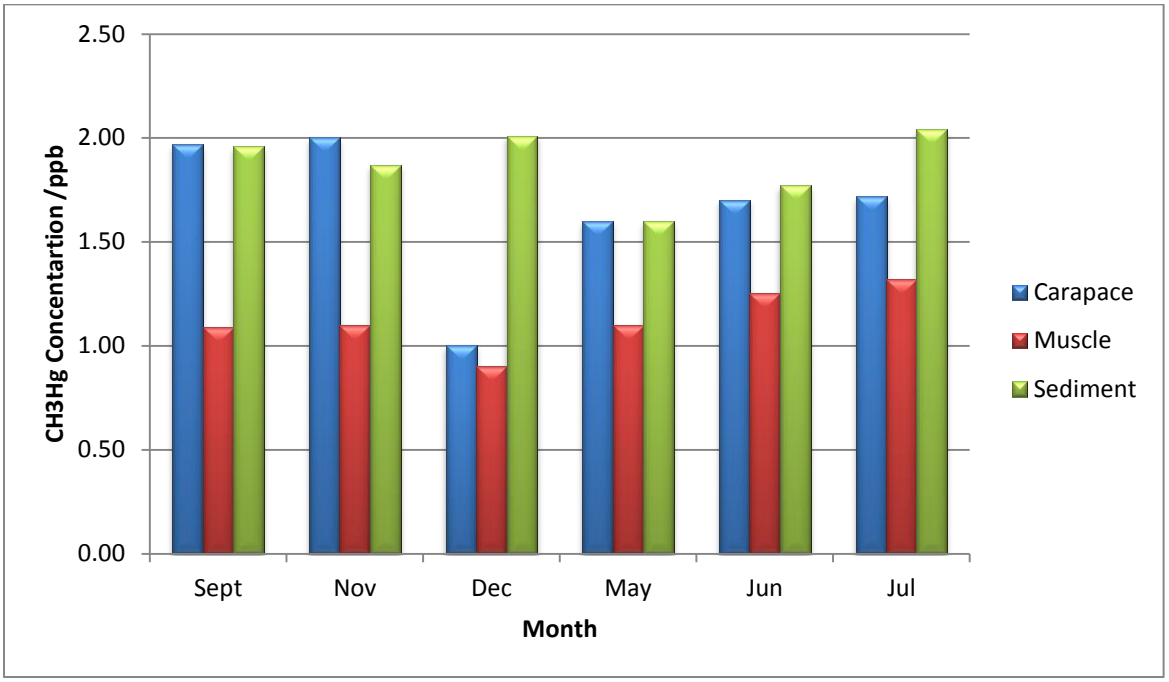

Figure 5. 3: Methylmercury concentrations in the crab's (*P. segnus*) carapace and muscle tissue in comparisons with levels found in the sediment.

Table 5. 2: Comparative values from methylmercury levels within the blue swimming crab from the Arabian Gulf

Species	**Location**	**Conc. µg/kg**	**Reference**
Portunus pelagicus	Khuzestan	850	Ghaeni et al., 2015
Portunus pelagicus	Khuzestan	112	
Portunus segnis	Iran	550 - 930	
Portunus segnis	**Qatar**	**1.3 – 1.7**	**This Study**

Methylmercury detected in the shell and in the muscular tissue of the barnacle ranged from 0.66 µg/kg – 1.20µg/kg and averaged 0.895µg/kg for the shell, with a range of 0.58 µg/kg – 2.2µg/kg in the soft tissue averaging 1.23µg/kg. In a report by the U.S National Research Council, there seem to be no significant difference between our reported data for CH_3Hg within barnacle and these limits. Extensive search for levels of methylmercury within barnacles yielded several reports that deal with other aspects of this pollutant but no reported levels.

Multifaceted relations among chemical, physical, and some biological aspects in the marine coastal ecosystems may play a part in the variation in CH_3Hg transformation from elemental Hg.

Dissolved Organic matter (DOM) level and composition changes may explain some seasonal variation in CH_3Hg levels that are available to phytoplankton. DOM may originate from autochthonous (Fenchel et al., 1998), and such differences are more noticeable in coastal waters than in open waters (Cauwet G., 2002; Thurman EM., 1985), thus showing the more varied biological production in coastal ecosystems plus the input from land sources. Complexed metals attached to the DOM are in general less available to aquatic microorganisms than free elemental metal (Campbell, 1995). Mercury ions if bound to DOM may be reduced by photochemical pathways to elemental Hg, thus influencing bioavailability (Ravichandran, 2004). As the composition and levels of DOM changes with the temperature differential within the Gulf region (Porcal et al., 2015), (High temperature in the summer with low temperatures in the winter/wet season) its effect on the bioavailability of CH_3Hg also varies. Noted however that in low levels of DOM this relationship appears to be variable (Gorski et al., 2008).

Data regarding the yearly variation in the levels of CH_3Hg within barnacles is rare and virtually nonexistent in the Arabian Gulf. It is evident from our data that there is a small difference in the concentration throughout the year, there seem to be a decrease in the recorded concentrations, in the colder month (Sept to Dec) as compared to the summer values (May – Jul) figure 5.5 . As these are filter feeders the CH_3Hg may be due to their feed or levels of CH_3Hg in the water column.

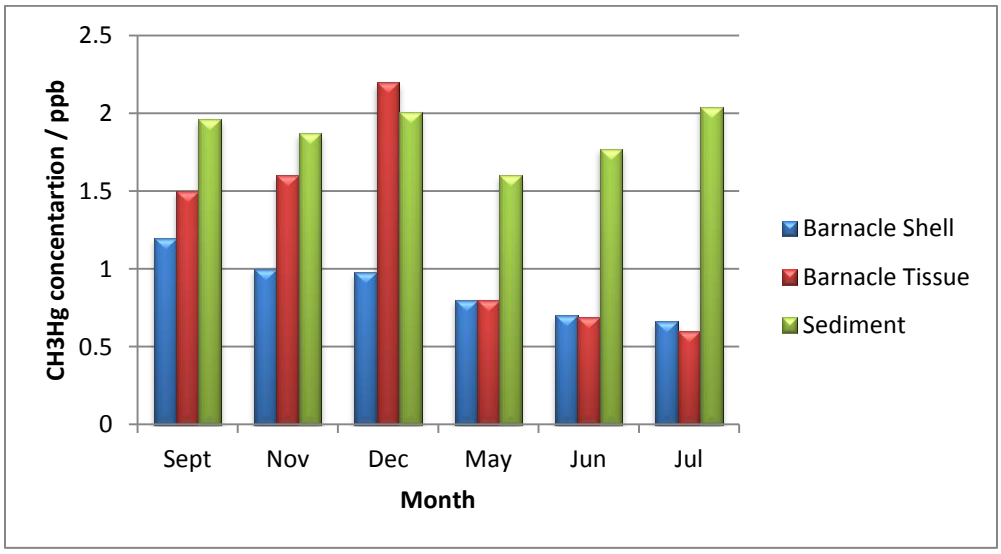

Figure 5. 4: Methylmercury concentrations in the barnacle shell and muscle tissue in comparisons with levels found in the sediment

Shrimp samples had a concentration range of 1.32µg/kg – 2.2µg/kg, and averaged 1.62 µg/kg. In an FDA report entitled "*National Marine Fisheries Service Survey of Trace Elements in the Fishery Resource*" levels of methylmercury were reported in Shrimp at 9µg/kg. Data regarding the amount of methylmercury from the Arabian Gulf is virtually non-existent. Table (4.4) shows some of the data for methylmercy in various species of shrimp from several international locations. Data from our study are much lower than these reported values.

Table 5. 3: Methylmercy concentration range in various species of shrimp from several international locations.

Species	**Location**	**Range µg/kg**	**Reference**
Penaeus monodon(Black tiger shrimp)	Vietnam	6.5 – 14.0	Hoang et al., 2017
‘’	India	4.3 – 21.7	
‘’	Australia	35.0 – 83.8	
Litopenaeus vannamei(Vannameri Shrimp)	India	4.3-5.8	
‘’	Ecuador	25.1 – 39.8	
‘’	Malaysia	10.0 – 12.1	
Marsupenaeus japonicus	Japan	30.4 – 140.8	
Metapenaeus joyneri	Japan	10.9 – 17.7	
Pleoticus muelleri	Argentina	5.9 – 38.5	
Penaeus semisulcatus	Indonesia	23.1 – 117.2	
Palaemon khori	**Qatar**	**1.3 – 2.2**	**This Study**

Sediment data showed that the levels of methylmercury in the range of 1.60 – 2.04µg/kg with an average of 1.86µg/kg. In studies conducted by Luoma (I977), levels of mercury contamination within the shrimp (*Palaemon debilis*), a deposit-feeding organism, are linked to the bioavailability of the contaminant from the seawater instead of the sediment in which it resides (Samuel N. Luoma, 1977). The results of the variation of CH_3Hg throughout the year showed that there a very slight increase in the levels of CH_3Hg during the cold month but these does not reflect any significant difference when compared to the hot summer month.

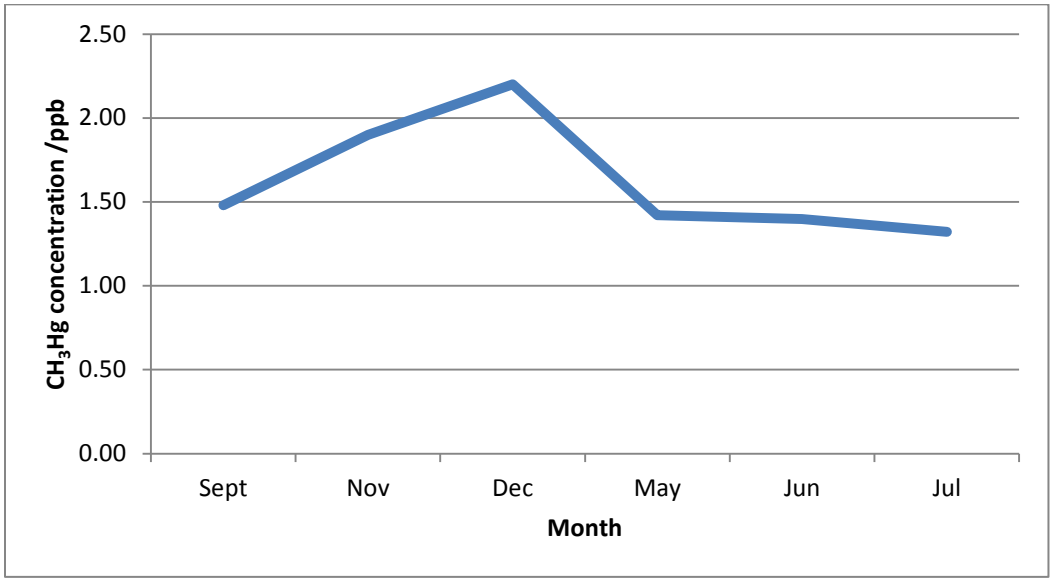

Figure 5. 5: Methylmercury trend within the tissue of the shrimp *P Khori.*

Table 5. 4: Comparison of CH_3Hg levels (µg/kg.) in sediments from the present study as compared to values from USA, Europe and Russia

Location	**Average**	**Year**	**Reference**
Estuarine–coastal system	0.51	2002	(Kwokal et al.,2002)
North Sea, (Belgium)	0.24	2001	(Leermakers et al., 2001)
Scheldt Estuary	0.25	2001	(Leermakers et al., 2001)
Mainstem, Chesapeake Bay (USA)	0.37	2010	(Fazeli, 2010)
Lake Balaton, (Hungry)	0.49	2005	(Nguyen et al., 2005)
Ore (Sweden)	0.71	2002	(Kwokal et al.,,2002)
Lot-Garonne (France)	0.90	2006	(Schäfer et al., 2006)
Annapolis Harbor, Maryland (USA)	1.75	1999	(Mason et al., 1999)
Tagus (Portugal)	2.10	1986	(Craig & Moreton, 1986)
Seine–Vasière Nord (France)	2.17	2008	(Ouddane et al., 2008)
Mangrove , Al-Khor Qatar, Arabian Gulf	**1.86**	**2016**	**This study**
Seine (France)	3.10	2008	(Ouddane et al., 2008)
Adour (France)	3.10	2004	(Mikac et al., 1999)
Belgium	12.50	1998	(Baeyens & Leermakers, 1998)
Nerbion-Ibaizabal Estuary, Bilbo Basque, (Spain)	48.00	2004	(Sanz Landaluze et al., 2014)

Methylmercury levels found in the mangrove sediment, ranged from 1.6 µg/kg – 2.01 µg/kg with an average of 1.86 µg/kg. Table 4 shows a comparative list of the amount of methylmercury reported in sediment from various international reports. The table seems to indicate that the

levels of CH_3Hg within the mangrove sediment are on the upper end of the scale, however there is no significant differences between data collected in this project and those of Tagus (Portugal) and Seine–Vasière Nord (France) both lower in concentration that our samples, this insignificance can also be applied to the Seine and Adour (France), both higher in concentrations to our results. Data collected by Baeyens & Leermakers (1998) and Sanz Landaluze et al. (2004) show much higher concentration in Belgium and Spain. The Interim sediment quality guidelines (ISQGs) limits for mercury in soil 130µg/kg, far above any of the values obtained in this survey.

5.5 Conclusions

This work was done to ascertain the amount of methylmercury concentration within selected biota and sediment from the mangroves of Qatar. In general the amounts of methylmercury found in the biota are lower than most action levels WHO and UK standards; although on average they exceed the levels set by the EPA (0.94µg/kg). Barnacles were also analysed for methylmercy levels, but comparative data pertaining to these organisms were sparse. Sediment data trend do not correlate to value within the crab or the shrimp *P Khori* although the concentration levels in the sediment are higher. Comparison of levels found in the sediment and with the international levels seems to indicate that the levels of CH_3Hg within the mangrove sediment are on the upper end of the scale, although the Interim sediment quality guidelines (ISQGs) limits for mercury in soil is 130µg/kg, far above any of the values obtained in this survey. Data on the levels of methylmercury with the shrimp have shown that these levels are much lower than internationally reported values for methylmercury in shrimp.

This study was the first to report on the levels of methylmercury within the mangrove ecosystems of Qatar, its results providing useful information for future studies related to the levels, and effect of methylmercury on a regional scale.

Chapter VI: Characterization of the genotoxic effects of trace metals, organotins and methylmercury on the endemic Qatari mangrove shrimp *P. Khori*

6.1 Abstract

The local shrimp *Palaemon khori* is endemic to the Qatari mangroves of Al-Khor, where it was originally discovered in 2006. The area has recently seen major development and is in close proximity to the local harbor, exposing the shrimp to varying anthropogenic pollutants such as spilt fuels and debris from boat traffic. Although the assessment of the toxicity of heavy metals, methylmercury and organotins on aquatic organisms has been widely performed, few studies have focused on the assessment of their genotoxic impact and until now no study has been performed on the endemic mangrove species within Qatar.

In this study, we performed the first genotoxicity assessment of trace metals (TM), methylmercury (CH_3Hg) and organotins (OT) present in the Qatari mangroves, through the evaluation of the aneuploidy levels on the endemic shrimp *P. khori*. In the lab, two different concentrations were used for each contaminant (individually or in combination), one environment concentration equivalent to the peak value found in the environment through literature research [Env], and the other 10 times higher [10Env], to predict effects under a more extreme pollution scenario. Aneuploidy rate, as well as mortality rates, were assessed at the start of the study, four weeks (T_4) into the study and finally at the end of eight weeks (T_8) exposure period.

Concerning aneuploidy, the control group revealed significantly lower levels of aneuploidy when compared to all treatments. It was surprising to observe that there was no significant difference between levels of aneuploidy with exposure time from T_4 to T_8. Trace metals were the only contaminants which resulted in a significant increase in aneuploidy levels between [Env] and [10Env]. When comparing single contaminant treatment with combination treatments, significant differences were only observed in the combination of OT and CH_3Hg in which an increase in the levels of aneuploidy was noted, and in the combination treatment of TM and CH_3Hg in which decrease in the aneuploidy levels was found. Higher mortality rates, after eight weeks of

exposure were observed in all treatments that involved TM, either alone or in combination with other pollutants. Our results highlighted that the combinations, inducing the highest aneuploidy levels, were [TMx10], [OTx10+CH_3Hgx10] the followed by [TMx10+CH_3Hgx10]. The evaluation of aneuploidy in endemic species helps in identifying the effects of pollution from genotoxic chemicals released from various anthropological activities.

6.2 Introduction

Anthropogenic activities constitute a constant source of pollution to water and sediment in coastal areas worldwide (Halpern et al., 2015; Nogales et al., 2011) and can cause a multitude of effects on aquatic organisms, contributing to the degradation of the marine environmental health. The Arabian Gulf is a particularly impacted area (Halpern et al., 2008; Halpern et al., 2015), with Qatar facing substantial threats to marine ecosystems from oil pollution and large coastal modifications to accommodate industrial facilities and urban housing (Cole et al., 2015; H. A. Naser, 2013; Rushdi et al., 2017; Sheppard et al., 2010). A significant percentage of contaminants introduced into the marine environment nowadays can be genotoxic, carcinogenic and mutagenic (J Baršienė, 1994; Bolognesi & Cirillo, 2014a; C. Lewis & Galloway, 2009). These compounds have the ability to influence genetic material at non-lethal and non-cytotoxic concentrations, and can lead to delayed effects that are important at the individual level and can also be reflected at the population level (Leitão et al., 2017; Pampanin et al., 2017). Genotoxic contaminants, such as methylmercury, trace metals and organotins, have been widely reported in the Arabian Gulf (De Mora et al., 2003; Freije, 2015) and surrounding countries, including Kuwait (Lyons et al., 2015), Iran (Agah et al., 2006) and Qatar (Al-Naimi et al., 2015).

Methylmercury (CH_3Hg) enters the marine environment through various pathways, but mainly through atmospheric deposition (Sundseth et al., 2017). Its toxicity stems from its persistence and its tendency to bioaccumulate and biotransfer in the food chain with potentially high concentrations in carnivorous fish and invertebrates species (National Research Council, 2000). For example, the levels of CH_3Hg in a selection of fish from Bahrain range from 0.028 ppm to 0.123 ppm (A. Freije & Awadh, 2009b) and the levels of mercury reported within the shrimp *Penaeus semisulcatus* sampled from Saudi Arabia ranged from 0.012 ppm to 0.039ppm (I. Al-Saleh & Al-Doush, 2002), both below the UK regulation recommended limit of 0.3ppm. However, data regarding the levels of CH_3Hg in invertebrates from the Arabian region are rare.

Most coastal areas and seas contain a limited amount of naturally occurring heavy metals, especially in the sediments. Nevertheless, even in low concentrations their cationic forms are dangerous to living organisms because of their capacity to bind with short carbon chains (Davis, 1979; Freije, 2015). Depending on the metal, these pollutants might not exert a strong impact individually, but when combined their effect may be magnified, as in the case of cadmium (Cd) and arsenic (As) (Ghiani et al., 2014; Marking & Mauck, 1975; Waalkes et al., 1992), even though sometimes the combined effect might amend the negative impact of the most impactful, as in the case of the antagonistic effects of silver (Ag) and copper (Cu) on jellyfish (Lucas & Horton, 2014). Trace metals (TM) bioaccumulate in protein-rich tissues of marine organisms and may interfere with cellular metabolic functions causing harmful side effects (Bolognesi et al., 1999; Dallas et l., 2013). They can affect marine organisms even at low concentrations: their toxicity arises not only from the level of contamination but also from the biochemical role they play in the metabolic processes as well as the extent to which they are absorbed and excreted (Jakimska et al., 2011). The genotoxic effect of TM on marine invertebrates such as molluscs has been previously shown [*e.g.,* in the bivalve *Mytilus edulis* (Bolognesi & Cirillo, 2014), the Manila clam *Ruditapes philippinarum* (Piló et al., 2017) the pearl oyster *Pinctada radiata* (Leitão et al., 2017) and the barnacle *Balanus improvisus* (Baršiene, 2002).

Maritime activities have in the past lead to the introduction of organotins (OT) into the marine environment until their ban in the 1980s (89/677/CEE). Organotins were used as wood protecting paints, disinfectants and biocides, especially in marine anti-fouling paints (Blunden, SJ, Evans, 1990; Omae, 2003), thus spreading worldwide to most ecosystems, from coastal zones to the open seas. This group of pollutants has a slow breakdown rate and as CH_3Hg and TM also persists in the marine environment and bioaccumulates (Michel & Averty, 1999; Stewart & Mora, 1990). Organotin compounds can cause DNA damage, double-strand breaks, base damage and intra-strand crosslinks (Anderson et al., 1997; Cookson et al., 1998). Organotins genotoxic effects have been reported for marine invertebrates, *e.g.* for the marine worm, *Platynereis dumerilii* using cytogenetic biomarkers and have been found to be dose-dependent on exposure (Hagger et al., 2002).

Cytogenetic parameters and atypical cytogenetic features, such as numerical chromosomal abnormalities, *i.e.*, aneuploidy, have shown their importance as endpoint indicators for assessing marine environmental genotoxicity (Baršiene et al., 2012; Leitão et al., 2017; Piló et al., 2017)

The impact of pollutants on the aneuploidy level of the Pacific oyster, *Crassostrea gigas* has been established ((Barranger et al., 2014; Bouilly et al., 2004), as well as the negative link between aneuploidy and growth (Leitao et al., 2001).

Mangroves are one of the coastal ecosystems more at risk by anthropogenic pollutions (Maiti & Chowdhury, 2013). The grey or white mangrove *Avicennia marina* is the most established coastal tree found in Qatar (Al-Khayat & Balakrishnan, 2014). Large areas of these ecologically important mangroves have recently been up-rooted as part of a port development scheme (http://www.npp.com.qa) and work is currently underway to restore this damage (http://www.npp.com.qa/EnvironmentalProtection.). The range of mangrove assemblages is being extended along the intertidal zones of the country through the planting of samplings and propagules. The habitat enhancement and ecosystem services provided by *A. marina* are extensive and include habitat provision for wildlife, sediment stabilization and carbon capture. These mangroves provide one of the most productive areas of vegetation in a region where the extreme environmental conditions constrain most vegetation growth. An endemic species of palaemonid shrimp, *Palaemon khori* (De Grave & Al Maslamani, 2006) has been recently discovered in the *A. marina* mangrove forest at Al-Khor, Qatar. This species represents an important component of the trophic chain within this mangrove-associated faunal community.

A wide variety of marine organisms have been used as biomarkers to assess the state of the environment in which they reside (Hook et al., 2014). The utilization of endemic species as biomarkers for local environments can offer a regional specific indicator of environmental stressors exclusive to that site (Baršienė et al., 2008; Leitão et al., 2017). Moreover, analyzing the impact of various pollutants that can biologically affect endemic species can give a better understanding and a more effective means of monitoring and maintaining the integrity of local mangroves (Slingenberg et al., 2009).

There is now a scientific consensus that the assessment of marine ecosystems' well-being, and the measures to enhance environmental quality, should be accomplished using an approach that utilizes both chemical measurements and appropriate biological measurements in key sentinel species (Leitão et al., 2017; World Health Organization (WHO), 1996).

The objective of this study was to evaluate, for the first time, the genotoxic effect of CH_3Hg, the TM chromium (Cr), manganese (Mn), cadmium (Cd), vanadium (V) and lead (Pb), and OT on the endemic shrimp *P. khori* using aneuploidy as the cytogenetic endpoint.

6.3 Materials and Methods

6.3.1. Sample Collection

Live specimens (n = 500) of the shrimp *P. khori* (average size: 300 mm total length; Fig. 5.1) were collected at low tide from Al-Khor mangroves (25.690020°N, 51.55572°E; refer to chapter 2 for sampling location map), using fine nets (Fig. 5.3). Specimens were stored in ice boxes containing aerated fresh sea water collected from the same location and brought to the Environmental Science Center (ESC), Qatar University, where they were transferred into a large tank containing aerated seawater.

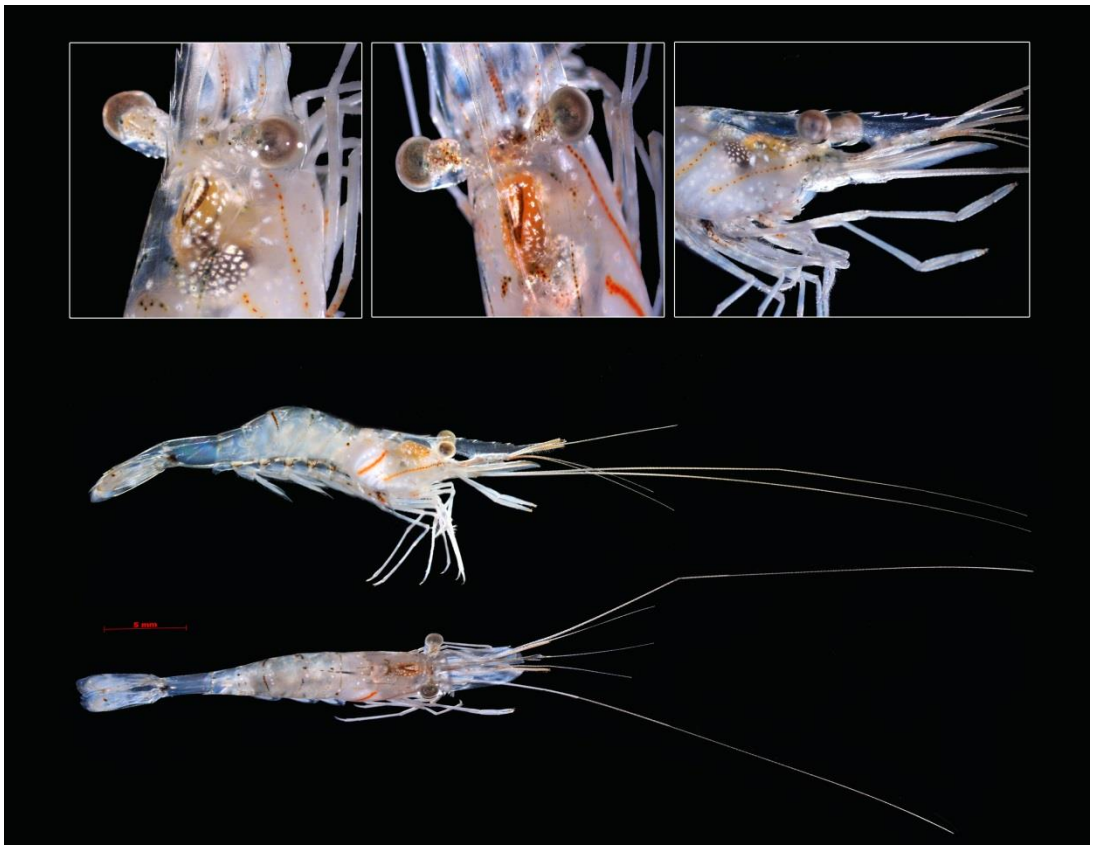

Figure 6. 1: *Palaemon khori* (Picture courtesy of ESC photographic section).

Figure 6. 2: *Palaemon khori* field sample collection.

6.3.2. Experimental design

In order to assess the genotoxic effect of CH_3Hg, TM and OT on the endemic mangrove shrimp *P. khori*, the following experimental design was set up under strict laboratory conditions. Twenty-five small tanks (5L) were prepared, each filled with 3L of seawater, gently aerated. Each tank was then dosed with the selected pollutant (or combination of pollutants; ©Sigma Aldrich) at a set concentration (Table 6.1): the highest concentration found in seawater obtained from the literature was used in some tanks [Env]; in others, these concentration were increased ten times [10Env] (Table 1), with a replicate tank for each treatment and a control tank (natural seawater). The TM Cr, Mn, Cd, V and Pb were chosen following Leitão et al. (2017), as these metals are toxic and have been shown to produce genotoxicological effects. The term organotins refers to a large group of compounds but here the three major ones, Monobutyltin (MBT), Dibutyltin (DBT) and Tributyltin (TBT) were targeted, following literature (Dopp et al., 2007; Kuballa et al., 1995). Thirty shrimps were housed in each tank at the beginning of the experiment. The water within each tank was checked every three days for volume (3L), temperature (22°C) and pH (8.01). Shrimp mortality within the tanks was assessed daily. Water within the experimental tanks was continually cleaned by constant carbon filtration. The shrimps

were fed with gold fish flakes (c.500 mg) every three days. Sampling, for aneuploidy evaluation, was carried out over three time periods: ten specimens were sampled at the beginning of the study (T_0), 10 were sampled four weeks after initial dosing (T_4), and finally the last sampling was done at week 8 (T_8).

Figure 6. 3: Experimental Dosing Tanks set up.

Table 6. 1: Concentration of pollutants added to each tank. CH_3Hg = Methylmercury; OT = Organotins TM =Trace metals; MBT = Monobutyltin; DBT = Dibutyltin; TBT =Tributyltin

Tank	Description	Polluant [Concentration in µg/kg]
1	Control	--
2 & 14	[CH_3Hg]	CH_3Hg [5.55][1]
3 & 15	[10CH_3Hg]	CH_3Hg [55.50]
4 & 16	[OT]	MBT, DBT and TBT [2.75][2]
5 & 17	[10OT]	MBT, DBT and TBT [27.50]
6 & 18	[TM]	(Cr [333] + Mn [316] + Cd [170] + V [8] + Pb [10])[3,4,5,6.]
7 &19	[10TM]	Cr [3330] + Mn [3160] + Cd [1700] + V [80] + Pb [100]
8 & 20	[TM+CH_3Hg]	Cr [333] + Mn [316] + Cd [170] + V [8] + Pb [10] +CH_3Hg [5.55]
9 & 21	[10TM+10CH_3Hg]	Cr [3330] + Mn [3160] + Cd [1700] + V [80] + Pb [100] +CH_3Hg [55.50]
10 & 22	[TM+OT]	Cr [333] + Mn [316] + Cd [170] + V [8] + Pb [10] + OT [2.75]
11 & 23	[10TM+10OT]	Cr [3330] + Mn [3160] + Cd [1700] + V [80] + Pb [100] + OT [27.5]
12 & 24	[CH_3Hg+OT]	CH_3Hg [5.55] + OT [2.75]
13 & 25	[10CH_3Hg+10OT]	CH_3Hg [55.5] + OT [27.5]

[1]Shi et al., 2005, [2] Shreadah, 2011, [3]Kiatananchai, 1984, [4]Heidarieh et al., 2013; [5]Nelson et al., 1999, [6]de Mora et al., 2004.

6.3.3. Aneuploidy scoring

Specimens were first submerged for 4 h in an aerated 0.09% solution of colchicine in seawater and kept at room temperature (23°C - 26°C). The cephalothorax was then dissected to isolate the muscular tissue (Hassan & Leitão, 2015), which was subjected to a hypotonic treatment for 45 min in 0.9% sodium citrate and fixed in a freshly prepared mixture of absolute ethanol and glacial acetic acid (3:1). The muscular tissues were left in the fixative for 48 h after which they were removed and dried with a tissue and slides were prepared from each individual muscular sample, following the air drying technique of Thiriot-Quieveux & Ayraud, (1982): each sample was placed in a small holder and agitated with a small amount of solvent (diluted glacial acetic acid) and then a droplet was poured on a slide from a vertical distance of 30 cm to allow good dispersion of the metaphases. Slides were then stained with Giemsa (4%, pH 6.8) for 15 min (Leitão et al., 2017).

Chromosome counts were made directly, by microscope observation (Nikon Eclipse E400 with incorporated Nikon DS-Fi1 image acquisition camera; Fig. 6.4), on apparently intact metaphases (induced by the colchicine treatment).

Figure 6. 4: Slide observation for aneuploidy.

Palaemon khori normally presents 48 chromosome pairs (2*n* = 96), 26 metacentric, 7 submetacentric, 12 subtelocentric and 3 telocentric (Hassan & Leitão, 2015). The level of

aneuploidy was estimated by counting the total number of aneuploid metaphases over the total number of metaphases per individual/treatment (n = 30 per treatment, 15 per tank). The pooled average (two tanks pretreatment) gave us the final percentage of aneuploidy for each group of individuals. The counting of chromosomes of all individuals was performed by the same observer to reduce the subjectivity associated with different observers in recording of the results.

6.3.4 Mortality

Mortality was checked daily in all tanks, and any dead shrimp was removed. The number of dead shrimps was recorded to ascertain the total mortality after the eight-week treatment period; this number was then expressed as a percentage of the total number of shrimps at the start of the experiment.

6.3.5 Statistical analysis

Measurements of abiotic factors (temperature, pH, salinity) were carried out in triplicate. MEDCALC® statistical software was used to quantify any significant difference between the two calculated percentages for both mortality and aneuploidy.

6.4. Results

The mortality levels of the shrimp in the control tank within the 8 weeks of study were 6.7% at T_4 and 11.1% at T_8 (Table 6.2).

Table 6. 2: Mortality (%) and Aneuploidy (%) 4 and 8 weeks after exposure (T_4 and T_8)

Treatment	% Mortality T_4	% Mortality T_8	% Aneuploidy T_4	% Aneuploidy T_8
Control	6.7	11.1	5.0	7.0
CH_3Hg	10.0	11.8	17.0	16.0
CH_3Hg x 10	16.7	20.0	19.0	19.0
OT	10.0	23.5	13.0	12.0
OT x 10	16.7	40.0	15.0	19.0
TM	20.0	21.4	12.0	12.0
TM x 10	13.3	56.3	18.0	21.0
TM + CH_3Hg	26.7	33.3	11.0	16.0
TMx10 + CH_3Hg x 10	30.0	100.0	18.0	-
TM + OT	26.7	58.3	13.0	14.0
TMx10 + OT x 10	26.7	100.0	15.0	-
OT + CH_3Hg	30.0	54.4	19.0	18.0
OTx10 + CH_3Hg x 10	33.3	90.0	18.0	21.0

In the treatment tanks, the mortality rate ranged from 10.0% to 100.0%, with the highest values registered with the trace metal exposure mixes, in combination with other pollutants (CH_3Hg and OT, Figure 6.5). Using MEDCALC®, to calculate Chi squared test, statistical analyses (χ^2 = 0.279, df = 1 p = 0.597) show that there was a significant difference between the mortalities at T_4 and T_8 for all treatments.

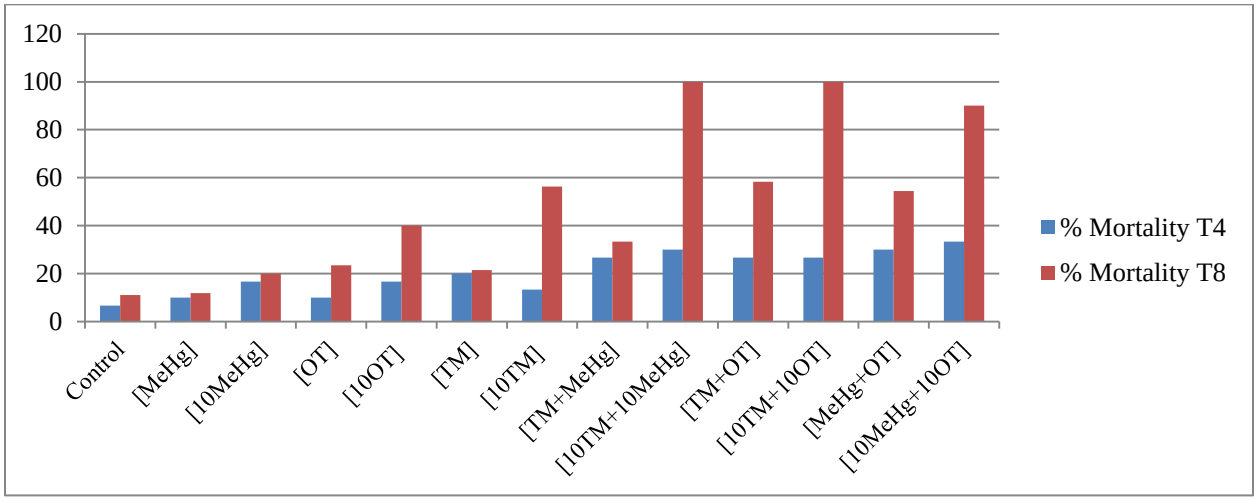

Figure 6. 5: Mortality of *Palaemon khori* subjected to varying levels of pollutants or combination of pollutants at T_4 and T_8.

Aneuploid cells were observed in all treatments (Figure 6.6), ranging from 5% – 19% at T_4 and 7% - 21% at T_8, with the lower levels always observed in the control.

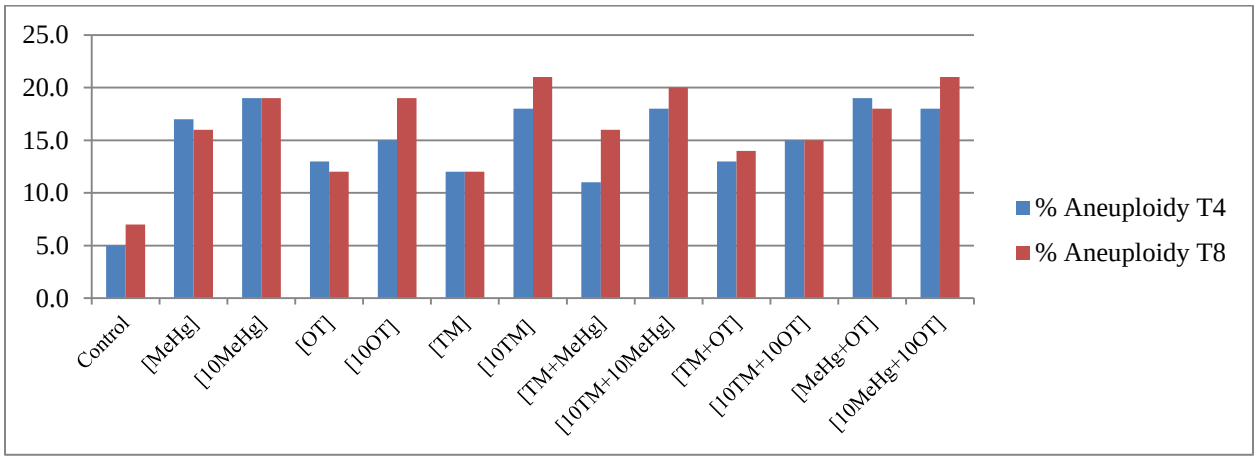

Figure 6. 6: Aneuploidy in *P. khori* after 4 and 8 weeks of exposure to the varying levels and pollutants types.

The levels of aneuploidy observed for the T_4 and T_8 at [Env] levels show the same trend, apart from the difference observed for [TM+CH_3Hg] (c.5% difference, Figure 6.6). The [10Env] graph also shows a similar trend but there is a difference in the levels between T_4 and T_8 for [OT], [TM], [TM+CH3Hg], and [OT+CH_3Hg] (Figure 5.9).

There was no statistically significant difference ($p > 0.05$) in the aneuploidy levels between T_4 and T_8 for the same pollutant/pollutant combination, but rather a difference between the aneuploidy levels of the same pollutant/pollutant combination between the two conditions; [Env]

and [10Env]. At T_4, the highest observed aneuploidy was for [CH_3Hg x10], and [OT+CH_3Hg], [TM], [TM+ CH_3Hg], and [OTx10+ CH_3Hgx10]. The lowest levels of aneuploidy besides the control were observed in the [TM] and [TM+CH_3Hg]. Aneuploidy observed at T_8 showed that the highest levels were recorded in tanks that contained [TM x10] and in tanks with the [TMx10+CH_3Hgx10], and, [OTx10+CH_3Hgx10]. The lowest aneuploidy recorded at T_8 apart from the control was observed in the [OT] and [TM].

Although *P. khori* has a normal diploid number of 2n =96 (Figure 6.7; Hassan *et al* 2015), aneuploid cells were observed in all treatments including the control.

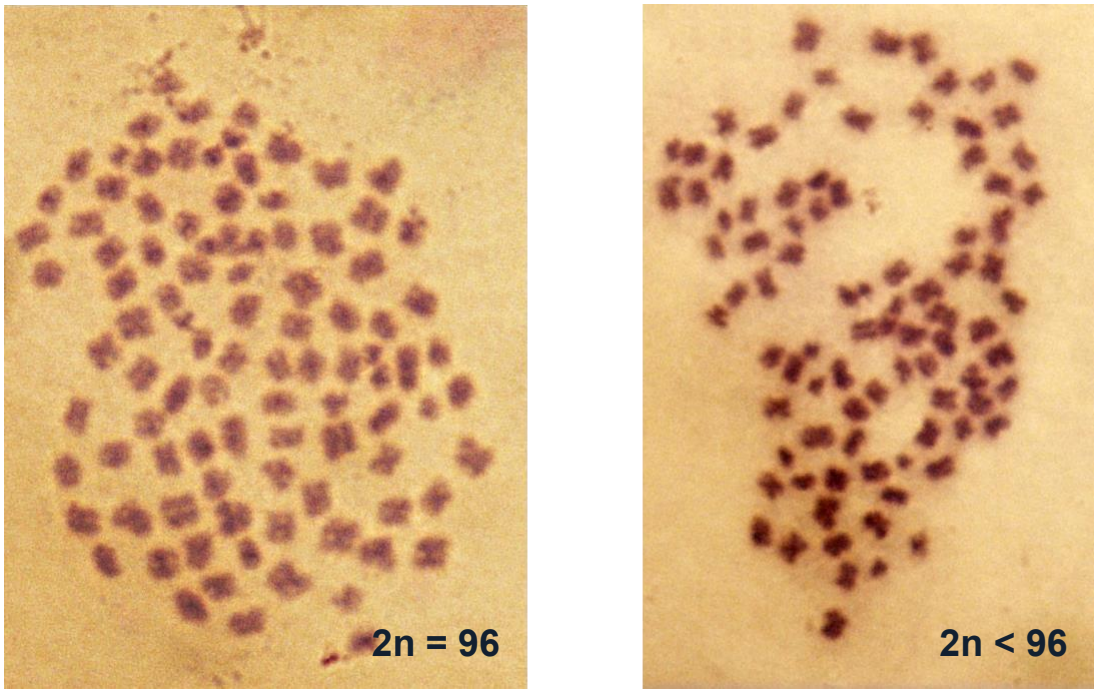

Figure 6. 7: Examples of a) diploid (2n = 96) and b) aneuploidy (2n < 96) metaphases of *Palaemon khori.*

The aneuploidy levels observed ranged from 5% – 19% at T_4 and 7% - 21% at T_8, with the lower levels always observed in the control (5% and 7% at T_4 and T_8 respectively).

Graphically the levels of aneuploidy observed for the T_4 and T_8 [Env.] levels show the same trend, apart from the difference observed for the combination of TM and CH_3Hg (c.5% difference) (Figure 6.8).

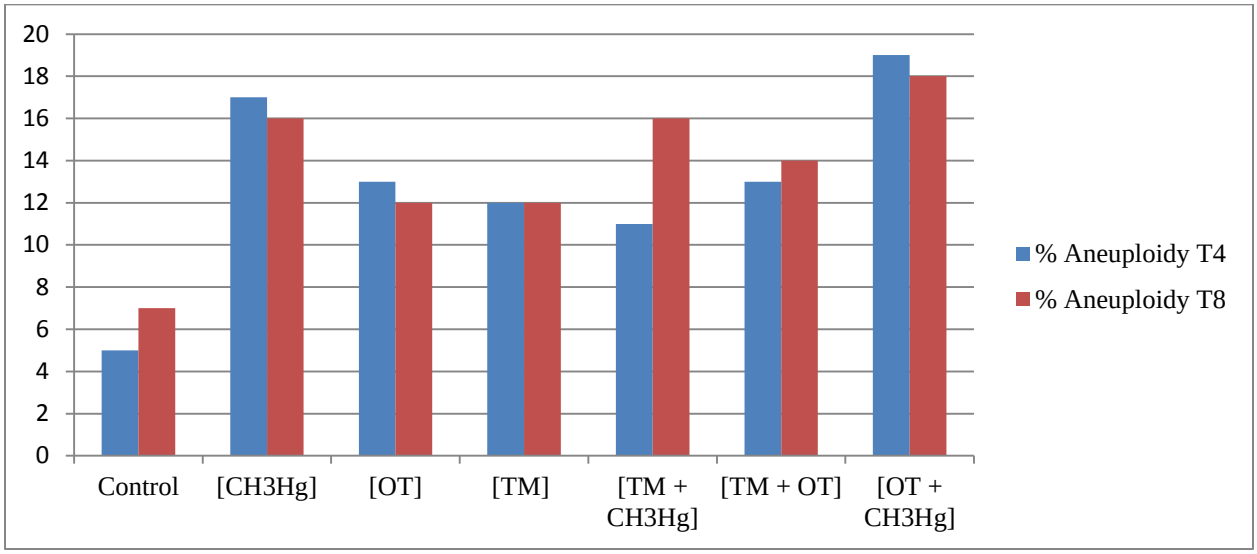

Figure 6. 8: Percentage of aneuploidy at [Env.]

The [10Env.] graph also shows a similar trend but there is a difference in the levels between T_4 and T_8 for [OT], [TM]. However no aneuploidy was calculated for [TM+CH_3Hg], and [OT+CH_3Hg] as both tanks had 100mortality. (Figure 6.9).

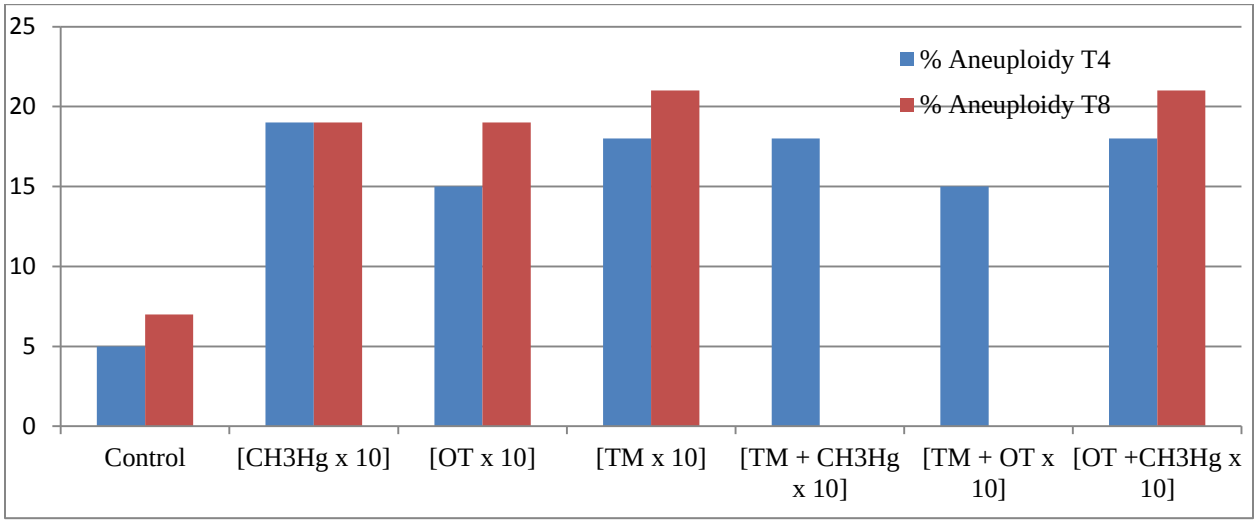

Figure 6. 9: Percentage of Aneuploidy at [10Env.]

There was no statistically significant difference ($p > 0.05$) in the aneuploidy levels between T_4 and T_8 in the tanks with viable number of survivors, for the same pollutant/pollutant combination, but rather a difference between the aneuploidy levels of the same

pollutant/pollutant combination between the two conditions, [Env.] and [10Env.]. The % aneuploidy observed at T_4 and T_8 for all pollutants / combination of pollutants at both concentration levels, show that at T_4, the highest observed aneuploidy was for [CH_3Hgx10], and [OT+CH_3Hg], [TM], [TMx10+CH_3Hgx10], and [OTx10+CH_3Hgx10]. The lowest levels of aneuploidy besides the control were observed in the [TM] and [TM+CH_3Hg]. Aneuploidy observed at T_8 showed that the highest levels were recorded in tanks that contained [TMx10] and in tanks with the combination of [OTx10+CH_3Hgx10] , [CH_3Hgx10], , and [OTx10]. The lowest aneuploidy recorded at T_8 apart from the control was observed in the [OT] and [TM].

6.5 Discussion

One of the major concerns regarding the well-being of marine organisms is the introduction of pollutants into their ecosystems. These pollutants can be either chemical or biological in nature, and their effect may prompt genetic changes in those marine organism (Osman et al., 2011). In general, pollutant bioaccumulate with time, this accumulation within the tissue of the organism may lead to a multitude of effects which may affect the ecosystem a whole by affecting the individual organism within it (Wani et al., 2007). Since anthropogenic activities continue to increase, further affecting the marine environment, analysis of the genotoxic potential of introduced pollutants has become increasingly important (Osman et al., 2012). These analyses may help in providing an early warning system to the eventual hazardous long term effects of these pollutants (Rybakovas et al., 2009). Genotoxins may have particularly high ecotoxicological relevance in situations of chronic exposure to low doses and to multiple contaminants (Abdel-Shafy & Mansour, 2016).

The results obtained in our study after exposure of the endemic mangrove shrimp *P. khori* to various levels of CH_3Hg, OT and the TM (Cr, Cd, Mn, Pb and V) alone or in combination showed that the highest observed mortalities (100%) were in the tanks that contained TM in combination with other pollutants. Generally, apart from [TMx10], the mortalities arising from one pollutant are lower when compared to a combined pollutant effect. The mortality results showed that a tenfold of the [Env.]. Methylmercury and OT had 50 - 60% increase, when compared to [Env.].. Surprisingly this increase *jumped* to 100% when one of the pollutants was trace metals. Tchounwou et al., (2012) have postulated that the trace metals are pollutants that are known to cause multiple organ damage, even at lower levels of exposure. Moreover, positive

correlations between mortality, increased concentrations of heavy metals and exposure time had also been previously observed (Rehman et al., 2017).

Our result show that there was no significant differences in the levels of aneuploidy when comparing T_4 and T_8 for [Env.] levels for trace metals, while [OT+CH_3Hg], [CH_3Hg], and [OT], displayed a non-significant decrease in the levels of aneuploidy. However, the combination of TM and CH_3Hg at [Env.] showed an increase of c.45% in the levels of aneuploidy between T_4 and T_8, this was followed by TM and OT at [Env.] producing an increase of c.8%. In the cases of the [10Env.], the highest increase (c.27%) was recorded in the tanks with OT, followed by TM and the combination of OT with CH_3Hg (c.17%). Trace metal combination with CH_3Hg had a 100% mortality so no results were possible, similarly [TM + OT].

One of the most prevalent pollutants that have previously shown to have genotoxicity effect in marine organisms are TM (Bolognesi & Hayashi, 2011), studied the effect of TM on the cell aneuploidy in the mussels *Anodonta cygnea*, *Unio tumidus* and the gastropod *Viviparus viviparous*, their results showed high levels of polyploid cells (42%) resulting from contamination by Cd, V, Cr, Ni, Cu, Mn, Zn, Fe, Sr and Hg. Cross & Rebordinos (2003) also studied the consequences of heavy metal contamination on the bivalve *Crassostrea angulata* using genotoxic endpoints (and concluded that marine contamination by Cd, Fe and Zn was linked to higher genetic abnormalities. More recently Piló et al. (2017) studied the genotoxic effect of trace metals on the Manila clam *Ruditapes philippinarum* and showed a strong relation between aneuploidy and sediment contaminations. Similarly, in a study conduicted in Qatar Leitão et al. (2017) also demonstrated the effect of trace metals on the pearl oyster *Pinctada radiata*.

Both CH_3Hg and OT also showed no significant differences in the levels of aneuploidy between T_4 and T_8, as single pollutants, however as mentioned earlier both OT and its combination with CH_3Hg (both at [10Env]) produced an increase of 17%.

Although the literature regarding methylmercury and aneuploidy or other genetic abnormalities is sparse, a study regarding the genotoxicological effect of this pollutant in killifish embryos reported by Perry et al., (1988) showed that methylmercury may reduce mitoses in killifish embryos. Al-Sabti (1994) studied the induction of micronuclei by low concentrations of methylmercury added as a separate pollutant and in combination with selenium, on the Prussian carp, and reported that the frequencies of micronuclei were elevated in a dose-dependent manner.

Organotins have a high selectivity regarding their mode of action and have been classified as having a neuro-, cyto- and genotoxic effect on several biological models such as the invertebrate (tunicate) *Styela plicata* (Dopp et al., 2004; Florea & Büsselberg, 2006), and their toxicity is well documented in several model invertebrate species (Claude Alzieu, 1998a; Grun & Blumberg, 2006). Specific reports on their genotoxicity are however rare compared to the reports on their other toxicities. Micaelet al., (2007) studied the effect of tributyltin (TBT) and triphenyltin (TPT) in the model fish *Danio rerio* and concluded that chronic exposure to low levels of TBT, TPT and binary mixtures of TBT were genotoxic to zebrafish.

Week four (T_4) aneuploidy data did not reflect the high mortality findings associated with TM, although at 18% was one of the highest. With the exception of TM, the higher levels were generally found in the specimens exposed to CH_3Hg. Methylmercury showed the highest levels of aneuploidy both, as an individual pollutant and in combination with others. Methylmercury may complex with other chemical groups within the cells leading to DNA damage through free radical formation (Ehrenstein et al., 2002), chromosomal aberrations and aneuploidy(Rania et al., 2011).

Analysis of the T_8 results show that the highest levels of aneuploidy were observed in the tanks containing the TM and the combination of OT and CH_3Hg (c. 21%). These were followed closely by the combination of CH_3Hg and OT all at [10Env.]. Similar to the mortality studies, TM was one of the pollutants inducing the highest levels of aneuploidy, this agrees with Pilo et al., 2017 in which the authors postulated that trace metals caused an increase in genetic abnormalities (DNA single-strand breaks and micronuclei frequency). Also noted, was the influence of CH_3Hg, four of the top six aneuploidy inducing pollutants/combination of pollutants. Methylmercury, the organic form of mercury has been reported as a as a genotoxic contaminant (Ochi, 2002). More recently, Leitão et al., (2017) assessed the effect of some selected contaminants on the pearl oyster *Pinctada radiata* and reported that Hg was highly positively correlated to aneuploidy.

The results of this study highlighted the high level of genotoxicity levied by [TMx10] and the combined effect of more than one pollutant [OTx10+CH_3Hgx10]. The data also shows that for some pollutants/combination of pollutants the levels of aneuploidy may have reached near maximum levels before eight week of exposure with the extent dependent on the amount and type of pollutant. Some pollutants or combination of pollutants have previously showed as

having a more profound effect than others (Oakes et al., 2014), and certain pollutants induce genotoxic effects either alone or they may enhance the effect of other chemical agents ((Snow, 1992). The identification of which chromosomal pairs are being affected by chromosomal loss/gain in the different treatments would allow a better clarification of the relationship between the different genotoxic agents studied and aneuploidy phenomenon (Torres et al., 2008).

6.6 Conclusion

This study evaluated the toxic effects of the maximum concentrations of TM, OT and CH_3Hg found in the environment according to the literature, and a tenfold increase in those concentrations in an individual setting and in a combined setting, on the model species *P. khori*, by classical (mortality) and genotoxicological (aneuploidy) endpoints, after 4 and 8 weeks of exposure. Our results showed that with regards to mortality on average the highest mortality was observed in shrimps exposed to [TMx10], or TM in combination with other pollutants. The pollutants inducing the highest aneuploidy levels were [TMx10] and [CH_3Hgx10+OTx10].

This study highlighted the importance of regular environmental biomonitoring of pollution that may arise for genotoxic contaminants within the marine ecosystem, given the fact that results are visible already after 4 weeks of exposure. The observation reported herein can provide useful insight towards better understanding of heavy metals, organotins and methylmercury as genotoxicants to endemic marine crustaceans such as *P. khori.* These results are the first (baseline) data evaluating the effect of several genotoxicants pollutants as individuals or in combinations within the Qatari mangroves region using *P. khori* as sentinel organisms.

Results tables

Table 6. 3: Mortality Observations

	Total at start	Mortality at T_4	% T_4 Mortality	Sampling at T_4	Number left in Tank after T_4	Mortality at T_8	% T_8 Mortality
Control	30.0	2.0	6.7	10.0	18.0	2.0	11.1
[CH_3Hg]	30.0	3.0	10.0	10.0	17.0	2.0	11.8
[CH_3Hg x 10]	30.0	5.0	16.7	10.0	15.0	3.0	20.0
[OT]	30.0	3.0	10.0	10.0	17.0	4.0	23.5
[OT x 10]	30.0	5.0	16.7	10.0	15.0	6.0	40.0
[TM]	30.0	6.0	20.0	10.0	14.0	3.0	21.4
[TM x 10]	30.0	4.0	13.3	10.0	16.0	9.0	56.3
[TM + CH_3Hg]	30.0	8.0	26.7	10.0	12.0	4.0	33.3
[TMx10 + CH_3Hg x 10]	30.0	9.0	30.0	10.0	11.0	11.0	100.0
[TM + OT]	30.0	8.0	26.7	10.0	12.0	7.0	58.3
[TM x10 + OT x 10]	30.0	8.0	26.7	10.0	12.0	12.0	100.0
[OT + CH_3Hg]	30.0	9.0	30.0	10.0	11.0	6.0	54.5
[OT x10 + CH_3Hg x 10]	30.0	10.0	33.3	10.0	10.0	9.0	90.0

Table 6. 4Aneuploidy Scoring

Treatment	Number of observed metaphases at T_4	Number of aneuploid metaphases T_4	Number of observed metaphases at T_8	Number of aneuploid metaphases T_8	% Aneuploidy T_4 (rounded to the nearest whole number)	% Aneuploidy T_8 (rounded to the nearest whole number)
Control	200	10	176	12	5	7
[CH_3Hg]	133	23	89	14	17	16
[CH_3Hg x 10]	111	21	98	19	19	19
[OT]	134	17	88	11	13	12
[OT x 10]	98	15	75	14	15	19
[TM]	91	11	90	11	12	12
[TM x 10]	87	16	56	12	18	21
[TM + CH_3Hg]	132	15	77	12	11	16
[TMx10 + CH_3Hg x 10]	87	16			18	-
[TM + OT]	121	16	102	14	13	14
[TMx10 + OT x 10]	119	18			15	-
[OT + CH_3Hg]	123	23	89	16	19	18
[OTx10 + CH_3Hg x 10]	141	25	78	16	18	21

Chapter VII: General discussion

7.1 Introduction

Throughout history, the world marine ecosystems have been used as major repositories of mankind's wastes. The problem is further magnified if these systems were shallow such as the Arabian Gulf. With significantly increasing impacts, sensitive habitats within these ecosystems are continuously been altered.

Prof. Elshorbaghy Keynote Address in 2005: *Overview of marine pollution in the Arabian Gulf with emphasis on pollutant transport modeling concentrations of Zn, Cd, Hg, and Pb in seawater*. He concluded that the levels of the measured heavy metals are similar in magnitude to those measured in many open ocean areas which suggest that the coastal water of the Gulf is not affected to any major extent (Hardy et al., 1993) . Similarly sediment analysis revealed that trace metals were in line with background levels and thus no evidence of anthropogenic contamination was found.

Data regarding the levels of heavy metals in the sediment with the Arabian Gulf countries have shown a general high background levels in the geographical north with low levels recorded in the south attributed to the grain size distribution In general, a positive correlation has been reported between increase of trace metal concentrations and decrease of grain size (Al-Abdal et al., 1996; Alam et al.,, I., 1998; Basaham & Ai-lihaibi, 1993). Some large size grains sediments show high heavy metals concentrations due to formation of large agglomerates from the smaller particles enriched by contaminations (El Tokhi, Mahmoud, & Alaabed, 2015).

Some historical results (Table 7.1). have shown extreme enhanced levels for the trace metals (Copper (Cu), Nickel (Ni), Zinc (Zn), and Vanadium (V).

Table 7. 1: Some historical levels of Trace metals within the sediment (μg/g dry weight) - (Elshorbagy, 2005).

Cu	Ni	Zn	V
3.2 – 44 (Kuwait 94) 19 - 45 (Iran 97) 11-77 (Bahrain 93 – 98)	22 - 130 (Kuwait 94) 6.7 - 121 (Kuwait 98) 28 -788 (UAE 94) 98 - 200 (Iran 97) 1035 - 1164 (Oman 96 -97) 4 – 667 (Oman 2001) 10 – 1167 (Oman 2002)	9 - 126 (Kuwait 1994) 66 - 130 (Iran 97) 21 - 117 (Bahrain 93 – 98)	12 - 63, (Kuwait 94) 7.1 - 88 (Kuwait 98) 66 – 118 (Iran 97)

Heavy metal data within Qatar is very rare. Most work has been carried out as part of projects and the data publication restricted; Table 7.2 shows some levels of selected heavy metals within sediment dating back to 1996. The table also lists some data regarding soil, and some selected biota.

Table 7. 2: Some selected historical Data from Qatar (µg/g dry weight) – Various sources

Sample Type	Year	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Barnacles	1997		0.142	1.73	1.77	0.142	13.66	7.4	23.12	11.39		46.59
Barnacles	1999	6.8			0.6				0.5	1.33		1.92
Biota	1999	0.68			0.6				0.5	0.133		1.92
Bivalves	1998		6.8	0.54	1.42	6.8	8.3	1.35	2.57	0.68	6.8	3.58
Crab	1994				9.84	4.26	99.8	3.81		8.35		3.39.5
Crab	1996		1.94	0.459	0.459	2.85	9	2.09	0.27	11.21		27
Crab	1998		6.8		1.42	6.8	8.6	1.35	2.57	0.68		35.8
Sediment	1996		0.56	1.71	9.38	0.42	146.5	311	7.04	1.21		56
Sediment	1997		0.49	0.76	1	2.25	731	127.2	7.75	4.1	4.15	64.25
Sediment	1998		0.72	0.76	8.01		101	36.26	6.21	0.94		0.4
Sediment	2002		0.31	5.4	21.03	2.7	635	72.9	12.1	2.8	0.008	28.8
Sediment	2005	15.6		4.7	46.9	19.6	886.5		37.3	2.39		14.8
Sediment	2007	1.71	0.13	1.45	7.8	3.8	578	17.5	11.8	1.6	5.12	9.97
Sediment	2009		0.06	2.31	29.09	10.14	519.3	80.5	15.24	6.51	33.65	28.31
Sediment	2009	0.92			1.3	0.7	397		2.2			1.59
Sediment	2011	4.53	0.23	2.22	10.9	14.66	1741	31.5	4.02		3.19	6.26
Sediment	2012	2.7			3.17	3.04	371.7	11.9	2.17	1.55	2.9	4.51
Soil	1998		0.72	0.76	8.01	1.5	101	36.2	6.21	0.94	4.01	11.42
Soil	1999	4.7	4.15		0.63	3.62			0.6	20.8	2.62	4.3
Soil	2001		0.021	7.5	14		38	72.8	5.3	10.3	0.01	0.08
Soil	2009	2.41	1.078	0.661	6.985	4.736	92.822	22.86	11.58		2.88	4.43
Soil	2010				1.47		307.6	10.58				8.61
Water	1998		0.05	0.1	0.45	0.05	0.8	0.25	0.3	0.3	0.05	0.3

Sample Type	Year	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Water	1999	0.087	0.75		1.3	0.1			1.27	0.955	0.049	0.57
Water	2007	0.65	0.15	1.7	0.75	3.97	0.05	0.63	0.13	0.29	0.27	1.56
Water	2009		0.44			2.34	26.5	0.9	1.94			10.6
Water	2009		0.17	3.07	7.5	9.46	106.5	2.59				
Water	2011						63.32	3.24	17.3	5.4		1.55
Water	2012			4.34	2	145.97	1282.7	8.33	4.02			103.49
Water	2012	1.27		0.13		0.13	2.19	0.36	0.98			164

Although bivalves are considered to be one of the best organisms used as bioindicators (Hamza-Chaffai, 2014), historical data on trace metals within bivalves from Qatar and the Arabian Gulf are rare. In 2004 de Mora, published a report containing some data regarding heavy metals in bivalves from Qatar, UAE, Abu Dhabi, Bahrain and Oman (de Mora et al., 2004).

Older studies regarding trace metals in bivalves have reported some elevated levels such as high Zn in Bahrain in 1994 (2400-3190 μg g^{-1} dry) and UAE in1994 (769 – 3110 μg g^{-1}dry). Another report in the same year showed high levels of mercury (Hg) from Bahrain (28-106 μg g-1dry) and UAE (15-152 μg g-1dry) all within the Pearl Oyster. Elevated levels of lead (Pd) were also found in Clamp from Kuwait 1994 (3.2 μg g-1dry) and, enhanced levels of V, Ni, and Sn were found in Pearl Oyster in Bahrain.

Pearl oyster analysis was also carried out by Leitão et al. (2017). The authors observed that metal concentration were lower than the one reported by de Mora et al. (2004) apart from Zn, which gave a higher concentration levels, that were similar in magnitude to the results reported from the ROPME area (ROPME, 2013).

Another important pollutants that is pertinent to shipping lane channels, fish farming and recreational sea going activities, is antifouling agents. Although no copper based antifouling compound analysis was carried out during this research, sediment and biota were analysed for organotins based compound.

The use of organotin compounds such as tributyltin (TBT) and triphenyltin (TPT) in the agricultural industry and as active ingredients in a wide field of applications has seen a vast increase. Tributyltin has been used since the 1970s when it gained notoriety as an excellent biocide used to prevent aquatic life from attaching to the haul of aquatic structures, and marine vessels (Claude Alzieu, 1998b). This allowed organotins compounds to leach into the coastal ecosystems in large quantities. They have so far been detected in various marine habitats and organisms such as fishes (Takahashi et al., 2000) seawater (Meena et al., 2009) and marine mammals (Tanabe, 1999). The use of TBT in marine antifouling paints is thought to be the main source for these compounds in marine waters.

Organotins, as discussed in chapter three are extremely toxic even at low levels (ng/l) ((Bhosle, 2007). Exposure time and concentration have been identified as the two main factors behind their toxicity (Hussein Kehinde Okoro & Kehinde, 2012). It has also been reported that organotins can

lead to imposex in gastropods and snails (Evans et al., 2000; Evans, 1992; Zhou et al., 2003). Other effect such as growth reduction has also been demonstrated in mussels (Salazar & Salazar, 1991).

From our study, results for the three organotin species show that, within the blue crab carapace, MBT averaged 1.42ngSng^{-1}; DBT averaged 2.19ngSng^{-1}; and TBT averaged 1.70 ngSng^{-1} , while within the muscle the concentration for the three organotin compounds averaged 1.23 ngSng^{-1}, 0.70ngSng^{-1} and 0.58ngSng^{-1} for MBT, DBT and TBT, respectively. Very sparse data was found for the levels of organotins in the Arabian Gulf; the only available historical data is from Oman and reported in 2003 by De Mora. In general the only regulation for organotins within aquatic tissue relates to the levels of TBT equivalent to120ngSng^{-1} set by OSPAR, concluding that the levels of TBT within the study area are below action levels.

Polychlorinated biphenyls (PCBs), formulation, such as Aroclors mixtures, were used in the electrical industrial, paints, and plastic industry. Normally these compounds exist as colorless oily liquids/waxy solids. They are regarded as hydrophobic, inert, non-flammable and resistant to heat. PCBs find their way into the marine ecosystem from sources of energy production, waste refills and combustion industries. In the 1970 the United States of America and Europe ceased PCBs production because of their environmental impact implications. Although these compounds can still enter the marine environment as a result of the disposal and dismantling of any equipment or site that might contain them. PCBs are included in the Stockholm Convention due to their persistence, bioaccumulation, and toxicity (Stockholm Convention, 2008).

Within the realm of toxic metals, mercury (Hg) is one of the most hazardous environmental pollutants (Nguyen et al., 2005), it is toxic in both the organic and inorganic form (Storelli et al., 1998). Mercury enters the marine environment as inorganic mercury, resulting from anthropogenic activities, (Manohar et al., 2002). It has been established that methylation which occurs in anoxic aquatic systems is the main route by which mercury is transformed to methylmercury (Compeau & Bartha, 1987; Hammerschmidt & Fitzgerald, 2006; Ullrich, Tanton, & Abdrashitova, 2001). The WHO (1991) suggests that the majority of studies on mercury suggest that on average 80% of the total mercury within the muscle tissue exists as the organic form methylmercury. This pollutant is extremely toxic and can lead to a multitude of adverse effect, even death (Goyer & Clarkson, 2001).

Qatar's marine ecosystem has seen many inputs from the growing population such as inputs of domestic sewage, industrial discharge, and the construction based dredging that leads to sediment resuspension and redepositing (Sheppard et al., 2010). All these anthropogenic activities s have led to the introduction of potentially (directly or indirectly) genotoxic, carcinogenic and mutagenic substances. Genotoxins are known to change the genetic material even a6t low level where the concentration is not lethal, and non-cytotoxic. However there are often belated effects which are far more harmful in the long run (Zuykov et al., 2013).

The exposure of marine organisms to high levels of pollutants may lead to high levels of mortality. The results for our genotoxic experiments showed high levels of mortality within the khori shrimp when exposed to high levels of trace metals and methylmercury. It can be surmised that there is a clear correlation between pollution and death in this instance. However exposure to lower levels of these pollutants may not lead directly to death, but may instead cause chronic damage, whose results may not be evident for a long time (Mayer et al., 1993). These low levels may weaken the organism leading to immunosuppression, and also lead to genetic abnormalities (Sövényi & Szakolczai, 1993).

As anthropogenic activities continue to increase, further affecting the marine environment, analysis of the concentration and genotoxic potential of induced pollutants into on marine ecosystem has become an important tool in the evaluation of marine environmental pollution (Osman et al., 2012). These analyses may help in providing an early warning system that can indicate hazardous long term effects of these pollutants (Rybakovas et al., 2009).

7.2 General findings

7.2.1 Trace metals

Our data for heavy metals within the blue crab the highest concentration within the carapace was for Zn (average 243.76), followed by Ni (44.08), Mn (10.95), Cr (8.47), Fe (6.43), Cu (5.62), As (3.53), V (0.061), Pb (0.052), Co (0.023) and Cd (0.007) mg/kg dry wt. respectively . Studies conducted on crabs from within the Arabian/Persian Gulf showed that the heavy metals in the carapace followed the trend Cu > Cd > Ni > Pb (Bastami & Esmailian, 2012). The same group

also reported that the trend held true for the muscle tissue. Our studies produced a different order which follows the trend Zn > Ni > Mn > Cr > Fe > Cu > As. However Cd, Co, Pb and V were all below the limit of quantification. The data for heavy metals within the crab's muscular tissue displayed the following trend: Zn > Cu > As > Fe > Mn > Pb > Cr > Cd > Co > Ni > V. The Zinc average was the largest at 159.2 ppm. Notable was the presence of As within both the carapace and tissue samples, at average levels of 17.808 ppm. These levels may indicate contamination, but there are different species of As in seafood and total As is not representative of toxic As (Srivastava et al., 2013). A great portion of studies on heavy metals is conducted in the different parts of the body, here the exoskeleton was removed and the rest was clumped together to represent the soft tissue, mainly composed of muscular mass (Al-Mohanna & Subrahmanyam, 2001). Arsenic concentration in the soft tissue of the blue crab from our analysis was much greater than previous research data, conducted in various marine organisms such as crabs, fishes and lobsters of the Arabian Gulf (Al-Mohanna & Subrahmanyam, 2001; De Mora et al., 2004). These high levels may be explained as the result of As contamination in the Arabian Gulf, but this warrants more investigation. As mentioned earlier in chapter two. Arsenic has many anthropogenic sources which could be present in the Gulf region but levels are on a steady decline.

The *P. segnis* carb species is a bottom-dwelling crab, in which lipophilic contaminants, such as heavy metal compounds, tend to accumulate in high concentrations because of its considerable fat content (Chaiyara, Ngoendee, & Kruatrachue, 2013). Also some studies have suggested that the high levels of heavy metal residues found in this species are due to biomagnification through the food chain. Studies of sediment heavy metal concentration from the area in which they were obtained showed a trend of Mn > Zn > Ni > V > Cu > Co (Basaham & Ai-lihaibi, 1993).

In our study, all metals were present in the soft tissue of the barnacles, the highest concentration was found was for Zn and the lowest for Co. This data somewhat agrees with the 1997 observation, which shows the highest concentration was Zn, although the lowest trace metal in 1997 was Cu and not Co. The heavy metal analysis within the soft tissue of the barnacles has been the subject of many reports (Barbaro et al., 1978; Barber & Trefry, 1981) although these reports show varying levels of concentrations, some describing much elevated levels than our data. Heavy metal analysis data regarding barnacles within the Gulf are rare; within Qatar historically the only data found was from 1997 and 1999 (Table 6.2). Most of the available data

describe the concentrations within the soft tissue, with little data on the barnacle shell (Royo-Gelabert & Yule, 1994; Watson et al., 1995), although a report by Hockett et al., (1995) describes the buildup of metals within the shell, describing several ways by which environmental levels can be assimilated into the shell.

The heavy metal trend within this shrimp follows Zn > Cu >Fe > Mn >Ni > Pb > As > Cr > Cd > Co >V. As this species of shrimp was only recently discovered (2006), data regarding heavy metal concentration within the *P khori* shrimp species are practically nonexistent. Heidarieh et al. (2013) reported a trend for another type of shrimp that shows a swap between the Zn and Fe (Fe > Zn > Mn > As > Co). No historical data regarding heavy metals in shrimp from Qatar were found. Recent data published in 2015 on a study of heavy metal in the giant red shrimp (*Aristaeomorpha foliacea*) showed that Zn and Cu were found in maximum levels, this agrees with our data, the reason for this is linked to the different seasonal food habits and may also be related to the shrimps environment (Olgunoglu et al., 2015). The high level of Zn can also be attributed to its essentialness for the development and metabolism of organisms in comparison with other metals (Meshram et al., 2014; Meshram et al., 2014; Pourang & Dennis, 2005).

Sediment and water concentration of heavy metals within study areas were also examined.

Sediment samples showed a wide range of heavy metal levels. The highest concentration was Fe, and the least was Cd, while Pb was below the LOD. All levels were within the acceptable limits set by the Ministry Of Environment, Qatar (M.o.E). Seawater was also analysed for the presence of heavy metals, most of which were below the limit of quantification. Those that were detected were in the range of 1.0µg/kg – 4.2µg/kg, with the exception of Li which was detected at a high level (42.7µg/kg). All detected values fall well below the limits set by the M.o.E (Appendix 1). As the *Palaemon khori* shrimp species is only endemic to Al-Khor mangroves, a comparative analysis of heavy metal present within the shrimp to those found in the water and sediment from the collection location show high levels of Li that is not evident within the shrimp, Cu, Cr, and Pb in the sediment follow the same trend as the shrimp but Mn and Ni are reversed in order. In the sediment, the metals Fe, As, Co and V follow the same trend as in the shrimp, but Mn and Ni are also reversed. From the present data it is difficult to form an option regarding the bioaccumulation factor; more analytical data is needed before a summarizing report can be formulated.

7.2.2 Organotins

Although there has been numerous reports pertaining to organotins from the rest of the world (Champ & Seligman, 1996; de Mora, 1996), data regarding organotins within the Arabian Gulf are also rare. Historical data are present for Bahrain (Hasan & Juma, 1992). In 1998 Watanabe and others measured the amount of organotins in various fish species from the gulf (Watanabe et al., 1998).

All three organotin species analysed were observed in the barnacle shell and muscle. Shrimp tissue analysis showed that MBT averaged 2.30ngSng^{-1}, DBT averaged 2.89ngSng-1, and TBT averaged 1.92ngSng-1. No historical data regarding organotins in shrimp from Qatar was found in the literature. Comparative analysis with other reports point to low levels of organotins within the specified biota. Sediment analysis data show that the MDT averaged 2.77ngSng^{-1}, DBT averaged 3.89ngSng^{-1}, and TBT averaged 2.49ngSng^{-1}. Results from the organotin compounds analysis within the three species and sediment in our study indicate that the levels present are generally lower than is reported elsewhere. It was also observed there is an indication of a similarity trend between levels of organotins within the sediment and the shrimp species dwelling on it.

7.2.3 Polychlorinated biphenyl

Our results from the PCB analysis show that no PCBs were detected in the crab carapace but they were present in the crab muscle and the shrimp tissue. Only two PCBs congeners were detected: PCB101 (4.78 µg/kg) and PCB44 (1.99 µg/kg) in the crab muscle. This is in line with the literature regarding the distribution of heavy and light PCBs which suggests that levels of heavy PCB are in general more than lighter ones (Voorspoels et al., 2004).

As these compounds are mostly found mainly within the sediment, their presence in the biota such as shrimp and crabs can be rationalized by the organisms feeding habits, as some prey items may contain high levels of pollutants (Monikh et al., 2013).

Observed historical data available shows that within the Arabian Gulf levels of 10.2µg/kg – 1448 µg/kg (a total of several congeners) were detected (Fowler, 1987). Values obtained from our studies are well below these values. Research suggests that levels less than 10µg/kg are considered as unpolluted(Monod & Arnaud, 1995). PCB analysis of the sediment and seawater from the mangrove forests showed no detectable PCBs levels.

7.2.4 Methylmercury (CH_3Hg)

Analysis results for our samples showed maximum CH_3Hg levels of 2µg/kg in the crab carapace and 1.3µg/kg in the muscle. Comparative studies (e.g., Hardy & Jones, 1997; Sarasiab & Hosseini, 2014) have reported values of 108-541µg/kg and 2.8µg/kg for samples from Iran and Thailand. The concentrations of methylmercury seem to have a wide range among this species of crab depending on it locale.

Within the barnacles this pollutant was detected in both the shell and muscle at 1.2µg/kg for the shell and 2.20µg/kg in the muscle. As mentioned in chapter four it seem apparent that differences between the data reported by the Panel on Mercury National Research Council Environmental Studies Board (NRCESB) and the levels found in this research.

The search for data pertaining to levels of methylmercury within barnacles only yielded data that dealt with other characteristics of this pollutant.

Methylmercury levels in the shrimp studied averaged 1.62µg/kg. Studies within the ROPME area displayed a range of 0.29µg/kg –1.30µg/kg (Al-Majed, & Rajab, 1998). This data seem to indicate that the levels found in the mangroves were on the high end, although the results can be rationalized by the nature of the mangroves in which the shrimp and sediment were collected. The Al-Khor mangroves are shallow and there is a lot of stagnant ponds of seawater, increasing the chance for anoxic sediment to develop, thus increasing the conversation of inorganic mercury to methylmercury (Gray et al., 2002). Moreover the NRCESB suggests that natural levels of methylmercury within marine organisms may range from 0.4µg/kg – 1.0µg/kg although level of 2µg/kg may be found. Analysis of the seawater and sediment from the sample collection location showed no detectable levels of methylmercury.

7.2.5 Genotoxic Study

The results obtained in our study after exposure of the shrimp *P. Khori* to various levels of MeHg, OT and TM alone or in combination showed that the highest recorded mortality were in the tanks containing trace metals, or trace metals in combination with other pollutants.

Moreover, mortality studies indicated that increased concentration of other pollutant (except trace metals) of up to ten folds produced half the percentage increase when compared to the inclusion of trace metals (60% and above). The data seem to agree with JunFeng et al., (2014) who postulated correlations between mortality, increased concentrations of heavy metals and exposure time.

As regards to aneuploidy, *P. khori* has a normal diploid number of 2n = 96 (Hassan & Leitão, 2015) and aneuploid cells were observed in all treatments including the control. Our study data showed that the aneuploidy levels observed ranged from 5% – 19% after four weeks of exposure (T_4) and 7% - 21% after eight weeks (T_8) of exposure, with the lower levels always observed in the control. The same trend for the levels of aneuploidy observed for the two concentration used (Max. environmentally observed concentration, and ten time that concentration) were seen for T4 and T8 for both concentration with slight differences. Noted however, the combination of TM and MeHg at maximum environmentally found levels showed an increase of c.45% in the levels of aneuploidy between T4 and T8, indicating that trace metals are one of the most prevalent pollutants that have a genotoxic effect in marine organisms, in agreement with (Bolognesi & Hayashi, 2011).

The main theme during the course of study presented in this thesis has been to investigate the presence and assess the effects of pollutants on selected marine organisms in Qatari waters. This was carried out by firstly quantifying the amounts of heavy metals, MeHg, organotins and PCBs present in the mangrove ecosystem of Al – Khor, ascertaining a baseline concentrations of these pollutants in the sediment, in the water and some selected biota (barnacles, shrimp, and the blue crabs) inhabiting this marine system. The baseline analysis and concentration for the heavy metals pollutant investigated: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr) , copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb),vanadium (V) and zinc (Zn), together with MeHg , OT and PCBs in the three selected test organisms: *P. khori*, *P. pelagicus* and *B. amphitrite*, sediment and water were reported in chapters two, three and four. The data presented in these chapters represents the first ever estimated baseline for seasonal variations of contaminants, in both the marine environment and associated animal tissues from the coastal waters of Qatar.

Moreover there was a need to determine if the concentrations of TM, MeHg, and OT present are affecting the *P. khori* shrimp using a cytogenetic end point (aneuploidy) as indicators. In this stage we established the karyotype of *P. khori* for the first time (Hassan & Leitão, 2015). The species *P. khori,* was an ideal indicators for the study of marine pollution, as they respond well to biochemical stresses and these responses mimic other crustaceans and invertebrates reactions (Vijayavel & Balasubramanian, 2009).

The data obtained from the cytogenetic research are presented in the chapter five, in which the data emphasized the significance of environmental biomonitoring within the marine ecosystem. The study also provided a better understanding on the effects of these pollutants on an endemic crustacean. The data pertaining to genotoxicity in the first of its kind utilizing the mangrove shrimp, and will act as a guideline for future studies on other species in the marine ecosystem of Qatar Marine Zone.

Furthermore, the studies focused on the genetic effect of the studied pollutants on the *Palaemon khori* indicated that the shrimp proved ideal as it responded well to biochemical stress and could be a unique Qatari biomonitoring organism.

7.3 Recommendation

The results of the baseline analysis for most analytes studied are below the limits set by the Ministry of Municipality and Environment (MME) in Qatar. However, some important pollutants (e.g. OT, MeHg) have no set limits. Setting standards and limits based on research for the benefit of the MME is recommended. Furthermore, although the samples analysed were form the subsurface it is recommended that core samples be taken and further analysis performed to show the distribution, origin and contamination levels of these selected heavy metals and their relation to core depth.

As there is no set limits for methylmercury it is recommended that total mercury studies are performed and that the ratio of methylmercury to total mercury be examined to determine whether this ratio is influenced by the conditions of the environment with respect to mercury contamination.

The work on the genotoxic effect of pollutants on the endemic shrimp have shown that the levels of aneuploidy may be affected by certain pollutants , however is will be useful to see which chromosomal pairs are being affected by chromosomal loss/gain, this can be achieved by using differential staining techniques.

This thesis has focused in the secondary producers through the marine environment of Qatar; it also recommended that a similar study be performed on the primary producers within the mangroves such as the mangrove plant itself, the different algae within the mangroves, seaweeds and seagrasses.

7.4 Future direction

The study within this thesis focused on the secondary producers within the mangroves of Al-Khor, the next stage is to analyse the primary producers within the mangroves such seaweeds, seagrasses, phytoplankton and the mangrove plant. Together with the data from this thesis, we can begin to understand the bioaccumulation and biomagnification of pollutants within the mangrove ecosystem.

From an ecological stand point and as the shrimp *P. khori* is only local to the Al-Khor mangroves, the introduction of this species to another mangrove stand is one of the future plans. This relocation can insure the survival of the unique species in case of an ecological disaster in Al-Khor harbour. The choice of the new location will although depend on condition that it meets the same bio-physicochemical parameters present at the present location. These factors now recorded from this research can be used as guidelines for such an undertaking.

The present study focused on three species the swimming crab *Portunus segnis,* the barnacle *Balanus amphitrite,* and the shrimp *Palaemon khori,* within the same location. There are a number of mangrove crabs that would provide good comparative material for future studies. Some crabs are closely associated with the mangroves while others have a wider sphere of distribution, using both localized and un-localized crabs will no doubt produce good material for research focusing on these species as monitors of pollution. Moreover, there are other mangrove localized species such as juvenile fish of various species, gastropods and hermit crabs that can be used to ascertain the health of the mangroves.

The karyotypes of some marine species, within Qatar, have already studied, (*Palaemon khori*, *Pinctada radiata*). However, the characterization of the various small mangrove crabs need to be studied form all the mangrove locations to ascertain if these are unique population isolated and evolved to suit the extreme condition of salinity and aridity.

Sediment and water analysis from all the mangrove stands within Qatar is another avenue of research that needs to be investigated. A project to investigate the levels of methylmercy with surface and core sediment from the various mangrove stands is already in the planning stage.

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Appendix

Appendix. 1: Water Permissible parameters within Qatar , and Literature values review for heavy metals, methylmercury, organotin and PCBs for the GCC and international locations

Parameter	Maximum Permissible Limit	Unit
pH	6.5 – 8.3	
Salinity	33 – 45	
Dissolved Oxygen	More than 4	Parts per thousand (ppt)
Total Suspended Solids (TSS)	30	ppm
Phosphorus	30	ppm
Nitrates	100	ppm
Silica	900	ppm
Ammonia (Nitrogen)	15	ppm
Total Petroleum Hydrocarbons	5	ppm
Cadmium	0.7	ppm
Nickel	20	ppm
Mercury	Less than 0.4	ppm
Iron	90	ppm
Copper	15	ppm
Lead	12	ppm
Vanadium	10	ppm
Polychlorinated phenyl	Not permissible	ppm
Chlorophyll	1	ppm

Source Ministry of Environment Qatar

Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Arabian Gulf	Bottom sediments	Fe	353	32,150		µg /g dry wt	1996	Al-Abdali et al. (1996)	From Freije 2015
Australia	Barnacle	Cd	2.98	8.4		mg/kg	2005	Silva et all (2005)	From P.A. Reis et al. 2013
Azores	Barnacle	Zn			27.54	mg/kg	1995	Weeks et al (1995)	From M. Dionísio 2013
Azores	Barnacle	Cd			156	mg/kg	1995	Weeks et al (1995)	From M. Dionísio 2014
Azores	Barnacle	Cu			30.2	mg/kg	1995	Weeks et al (1995)	From M. Dionísio 2015
Bahrain	Pearl oyster	Pb	1.25	14		µg g−1 wet wt	1994	Al-Sayed et al. (1994)	From Freije 2015
Bahrain	Pearl oyster	Cd	0.25	3.8		µg g−1 wet wt	1994	Al-Sayed et al. (1994)	From Freije 2015
Bahrain	Fish	Pb	4.3	15.2		µg g−1wet wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Fish	Zn	223	1253		µg g−1wet wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Costal sediments	Pb			111	mg/kg dry wt	1997	Akhter and Al-Jowder (1997)	From Freije 2015
Bahrain	Pearl oyster	Zn			4290	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Pearl oyster	V			7.3	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Pearl oyster	Pb			3.92	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Bivalves	As	153	156		µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Sediments	Cu			48.3	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Sediments	Hg			0.22	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Sediments	Pb			99	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Sediments	Zn			52.2	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Sediments	Cd	109	195		µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
Bahrain	Water	Cu	4.53	119		µg l−1	2008	Juma and Al-Madany (2008)	From Freije 2015
Bahrain	Water	Hg			0.38	µg l−1	2008	Juma and Al-Madany (2008)	From Freije 2015
Bahrain	Sediments	Cd			19.14	mg kg−1	2010	Naser (2010)	From Freije 2015
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Bahrain	Sediments	V	4.9	36.6		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Mn	17.3	39.9		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013

Bahrain	Sediments	Fe	3233	4811.0		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Co	0.99	1.7		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Ni	9.4	19.6		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Cu	1.16	17.6		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Zn	2.34	3.8		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Cd	0.001	0.753		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain	Sediments	Pb	0.64	24.0		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	V	2.7	7.4		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	Mn	42.8	57.2		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	Fe	0.006	0.01		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	Co	1	1.6		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	Ni	0.2	12.8		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	Cu	3.8	4.0		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Bahrain Qatar Border	Sediments	Zn	20.4	32.2		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Baltic Sea	Sediments	V			130	mg/kg	1961	Manheim (1961)	From H Al Naimi - 2013
Baltic Sea	Sediments	Mn			4030	mg/kg	1962	Manheim (1961)	From H Al Naimi - 2013
Baltic Sea	Sediments	Fe			7.7	mg/kg	1963	Manheim (1961)	From H Al Naimi - 2013
Baltic Sea	Sediments	Co			22	mg/kg	1964	Manheim (1961)	From H Al Naimi - 2013
Baltic Sea	Sediments	Ni			43	mg/kg	1965	Manheim (1961)	From H Al Naimi - 2013
Baltic Sea	Sediments	Cu			78	mg/kg	1966	Manheim (1961)	From H Al Naimi - 2013
Baltic Sea	Sediments	Zn			110	mg/kg	1967	Manheim (1961)	From H Al Naimi - 2013
Brazil	Barnacle	Cd			5.13	mg/kg	2006	Silva et all (2006)	From P.A. Reis et al. 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Brazil	Barnacle	Cu			23.6	mg/kg	2006	Silva et all (2006)	From P.A. Reis et al. 2013
Brazil	Barnacle	Zn			1185	mg/kg	2006	Silva et all (2006)	From P.A. Reis et al. 2013
Brazil	Barnacle	Fe			466	mg/kg	2006	Silva et all (2006)	From P.A. Reis et al. 2013
Brazil	Barnacle	Mn			9.6	mg/kg	2006	Silva et all (2006)	From P.A. Reis et al. 2013

Brazil	Barnacle	Ni			9.1	mg/kg	2006	Silva et all (2006)	From P.A. Reis et al. 2013
China	Barnacle	Cd	2.1	10.1		mg/kg	1988, 1990	Philips And Rainbow (1988, 1990)	From P.A. Reis et al. 2013
China	Barnacle	Cu	59.3	3472		mg/kg	1988, 1990	Philips And Rainbow (1988, 1990)	From P.A. Reis et al. 2013
China	Barnacle	Zn	2726	11990		mg/kg	1988, 1990	Philips And Rainbow (1988, 1990)	From P.A. Reis et al. 2013
China	Barnacle	Cr	0.22	28		mg/kg	1988, 1990	Philips And Rainbow (1988, 1990)	From P.A. Reis et al. 2013
China	Barnacle	Pb	1.7	39.2		mg/kg	1988, 1990	Philips And Rainbow (1988, 1990)	From P.A. Reis et al. 2013
China	Barnacle	Cd	9.4	30.9		mg/kg	1992	Rainbow And Smith (1992)	From P.A. Reis et al. 2013
China	Barnacle	Cu	239	4865		mg/kg	1992	Rainbow And Smith (1992)	From P.A. Reis et al. 2013
China	Barnacle	Zn	5677	15940		mg/kg	1992	Rainbow And Smith (1992)	From P.A. Reis et al. 2013
China	Barnacle	Cd	5.02	11.9		mg/kg	1993	Rainbow et. al (1993)	From P.A. Reis et al. 2013
China	Barnacle	Cu	34.4	182		mg/kg	1993	Rainbow et. al (1993)	From P.A. Reis et al. 2013
China	Barnacle	Zn	1467	6965		mg/kg	1993	Rainbow et. al (1993)	From P.A. Reis et al. 2013
China	Barnacle	Cd	4.15	11.1		mg/kg	1996	Blackmore (1996)	From P.A. Reis et al. 2013
China	Barnacle	Cu	188	6317		mg/kg	1996	Blackmore (1996)	From P.A. Reis et al. 2013
China	Barnacle	Zn	3148	11298		mg/kg	1996	Blackmore (1996)	From P.A. Reis et al. 2013
China	Barnacle	Fe	1202	5929		mg/kg	1996	Blackmore (1996)	From P.A. Reis et al. 2013
China	Barnacle	Mn	17.8	223		mg/kg	1996	Blackmore (1996)	From P.A. Reis et al. 2013
China	Barnacle	Cd	0.87	5.5		mg/kg	1998	Blackmore et al (1998)	From P.A. Reis et al. 2013
China	Barnacle	Cu	29	2204		mg/kg	1998	Blackmore et al (1998)	From P.A. Reis et al. 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
China	Barnacle	Zn	1521	10		mg/kg	1998	Blackmore et al (1998)	From P.A. Reis et al. 2013
China	Barnacle	Fe	816	3126		mg/kg	1998	Blackmore et al (1998)	From P.A. Reis et al. 2013
China	Barnacle	Mn	38.8	277		mg/kg	1998	Blackmore et al (1998)	From P.A. Reis et al. 2013
China	Barnacle	Cu	52.4	1810		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013
China	Barnacle	Zn	2860	23300		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013

China	Barnacle	Cr	0.5	3.32		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013
China	Barnacle	Fe	313	1470		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013
China	Barnacle	Mn	14.5	95.4		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013
China	Barnacle	Ni	1.25	98.9		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013
China	Barnacle	Pb	0.4	9.08		mg/kg	2001	Rainbow and Blackmore (2001)	From P.A. Reis et al. 2013
Croaitia	Barnacle	Cu	41	109		mg/kg	1978	Barbaro et al (1978)	From P.A. Reis et al. 2011
Croaitia	Barnacle	Cr	2.1	3.9		mg/kg	1978	Barbaro et al (1978)	From P.A. Reis et al. 2011
Croaitia	Barnacle	Pb	7.1	11.7		mg/kg	1978	Barbaro et al (1978)	From P.A. Reis et al. 2011
Iran	Shrimp	Mn			25.43	mg/kg	2013	Heidarieh et al (2013)	
Iran	Shrimp	Fe			288	mg/kg	2013	Heidarieh et al (2013)	
Iran	Shrimp	Zn			68.73	mg/kg	2013	Heidarieh et al (2013)	
Iran	Shrimp	As			8.28	mg/kg	2013	Heidarieh et al (2013)	
Iran	Shrimp	Co			0.4	mg/kg	2013	Heidarieh et al (2013)	
Iran	Shrimp	Zn			41.76	mg/kg	2005	Pourang et al, (2005)	From Heidarieh et al (2013)
Iran	Sediments	V	76.5	145		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Iran	Sediments	Mn	470	1111		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Iran	Sediments	Fe	22231	44035		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Iran	Sediments	Co	6.91	24.2		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Iran	Sediments	Ni	29.4	67.8		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Iran	Sediments	Cu	13.2	50.9		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Iran	Sediments	Zn	55.9	149		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013

Iran	Sediments	Pb	11.3	24.6		mg/kg	2004	de Mora and Sheikholeslami. (2004)	From H Al Naimi - 2013
Iran	Crab	Mn			1.91	mg/kg	2013	Heidarieh et al (2013)	
Iran	Crab	Fe			62.87	mg/kg	2013	Heidarieh et al (2013)	
Iran	Crab	Zn			66.64	mg/kg	2013	Heidarieh et al (2013)	
Iran	Crab	As			21.38	mg/kg	2013	Heidarieh et al (2013)	
Iran	Crab	Co			0.15	mg/kg	2013	Heidarieh et al (2013)	
Kingdom of Saudia Arabia	Crab	Zn			165.7	mg/kg	1982	Sadiq et al (1982)	From Heidarieh et al (2013)
Kingdom of Saudia Arabia	Crab	Co			4.66	mg/kg	1982	Sadiq et al (1982)	From Heidarieh et al (2013)
Kingdom of Saudia Arabia	Shrimp	Zn			148.9	mg/kg	1982	Sadiq et al (1982)	From Heidarieh et al (2013)
Kingdom of Saudia Arabia	Shrimp	Co			4.56	mg/kg	1982	Sadiq et al (1982)	From Heidarieh et al (2013)
Kuwait	Fish	Total -Hg			⩾0.5	mg/kg dry wt	2000	Al-Majed and Preston (2000)	From Freije 2015
Kuwait	Crab	Mn			0.95	mg/kg	2001	Al-Mohanna& Surbrahmanyam(2001)	From Heidarieh et al (2013)
Kuwait	Crab	Zn			206	mg/kg	2001	Al-Mohanna& Surbrahmanyam(2001)	From Heidarieh et al (2013)
Kuwait	Crab	As			0.31	mg/kg	2001	Al-Mohanna& Surbrahmanyam(2001)	From Heidarieh et al (2013)
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Kuwait	Sediments	V	85.3	133.5		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Mn	551.0	941.2		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Fe	1.2	2.8		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Co	26.6	37.7		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Ni	149.5	209.1		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Cu	33.8	49.9		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Zn	91.4	126.7		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Kuwait	Sediments	Mn	167	500.0		mg/kg	1987	Anderlini et al (1987)	From H Al Naimi - 2013
Kuwait	Sediments	Fe	0.7	2.0		mg/kg	1987	Anderlini et al (1987)	From H Al Naimi - 2013

Kuwait	Sediments	Ni	55	120.0		mg/kg	1987	Anderlini et al (1987)	From H Al Naimi - 2013
Kuwait	Sediments	Cu	31	51.0		mg/kg	1987	Anderlini et al (1987)	From H Al Naimi - 2013
Kuwait	Sediments	Zn	24	89.0		mg/kg	1987	Anderlini et al (1987)	From H Al Naimi - 2013
Ligurain Coast	Sediments	Fe			4.1	mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Ligurain Coast	Sediments	Ni			130	mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Ligurain Coast	Sediments	Cu			39	mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Mediterranean	Crab	PCBs	10.2	90.5		µg /g	1993	Porte et al (1993)	From G.R.W. Denton 2006
Mediterranean	Crab	PCBs		1448		µg /g	1987	Fowler (1987)	From G.R.W. Denton 2006
Mediterranean	Shrimp	PCBs			<30	µg /g	1987	Fowler (1987)	From G.R.W. Denton 2006
Mexico	Sediments	Ni	5	10.9		mg/kg	1977	Roth & Hurnung (1977)	From H Al Naimi - 2013
Mexico	Sediments	Zn	14	28		mg/kg	1977	Roth & Hurnung (1977)	From H Al Naimi - 2013
Mexico	Sediments	Cd	0.4	1.1		mg/kg	1977	Roth & Hurnung (1977)	From H Al Naimi - 2013
Mexico	Sediments	Pb	3.4	9		mg/kg	1977	Roth & Hurnung (1977)	From H Al Naimi - 2013
North West of Arabian Gulf	Sediments	V	150	186.0		mg/kg	1986	Abayachi and DouAbul (1986)	From H Al Naimi - 2013
North West of Arabian Gulf	Sediments	Mn	915	1643.0		mg/kg	1987	Abayachi and DouAbul (1986)	From H Al Naimi - 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
North West of Arabian Gulf	Sediments	Fe	0.45	0.9		mg/kg	1988	Abayachi and DouAbul (1986)	From H Al Naimi - 2013
North West of Arabian Gulf	Sediments	Ni	386	637.0		mg/kg	1989	Abayachi and DouAbul (1986)	From H Al Naimi - 2013
North West of Arabian Gulf	Sediments	Cu	17.3	37.1		mg/kg	1990	Abayachi and DouAbul (1986)	From H Al Naimi - 2013
Arabian Gulf	Sediments	Zn	27	43.0		mg/kg	1991	Abayachi and DouAbul (1986)	From H Al Naimi - 2013
NorthSea	Sediments	Ni	6.5	22		mg/kg	2004	D. Secrieru and A. Secrieru. (2004)	From H Al Naimi - 2013
NorthSea	Sediments	Zn	19.7	197.5		mg/kg	2004	D. Secrieru and A. Secrieru. (2004)	From H Al Naimi - 2013
NorthSea	Sediments	Cd	0.1	0.8		mg/kg	2004	D. Secrieru and A. Secrieru. (2004)	From H Al Naimi - 2013
Northwestern Black Sea	Sediments	V	1	118		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013

Northwestern Black Sea	Sediments	Co	1	71.59		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Northwestern Black Sea	Sediments	Ni	1	117		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Northwestern Black Sea	Sediments	Cu	4.62	75.72		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Northwestern Black Sea	Sediments	Zn	1	174		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Northwestern Black Sea	Sediments	Cd	0.16	3.99		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Northwestern Black Sea	Sediments	Pb	2.1	43.5		mg/kg	1982	Cosama et al, (1982)	From H Al Naimi - 2013
Oman	Fish liver	As			9.6	μg g−1 dry wt	2007	Fowler et al. (2007)	From Freije 2015
Oman	Abalone	Cd	11	30		mg kg−1 dry wt	2007	Fowler et al. (2007)	From Freije 2015
Oman	Sediments	V	10.2	123.0		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Mn	89.1	310		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Fe	5051	#####		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Oman	Sediments	Co	1.98	22.1		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Ni	9.9	439.0		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Cu	1.61	13.9		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Zn	7.7	26.3		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Cd	0.03	0.093		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Oman	Sediments	Pb	0.37	25.9		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
Qatar	Sediments	V	1.04	18.7		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Mn	4.42	54.66		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Fe	123	2368		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Co	0.001	0.61		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Ni	0.001	8.55		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Cu	0.52	6.4		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Zn	0.001	14.83		mg/kg		From H Al Naimi - 2013	
Qatar	Sediments	Cd	0.11	0.48		mg/kg		From H Al Naimi - 2013	

Saudia Arabia	Sediments	V	2.0	48.8		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Mn	18.8	262.3		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Fe	0.002	2.0		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Co	0.001	16.6		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Ni	3.7	116.1		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Cu	1.5	27.4		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Zn	6.2	65.3		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Cd	0.089	0.3		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Saudia Arabia	Sediments	Pb	1.7	4.4		mg/kg	1993	Ali and Al-Lihaibi (1993)	From H Al Naimi - 2013
Spain	Barnacle	Cd			12	mg/kg	1975	Barbaro et al (1978)	From P.A. Reis et al. 2013
Spain	Barnacle	Cu	550	600		mg/kg	1975	Barbaro et al (1978)	From P.A. Reis et al. 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Spain	Barnacle	Zn	1780	3300		mg/kg	1975	Barbaro et al (1978)	From P.A. Reis et al. 2013
Taiwan	Sediments	Ni	42	285		mg/kg	1979	Shiber (1979)	From H Al Naimi - 2013
Taiwan	Sediments	Zn	103	3514		mg/kg	1979	Shiber (1979)	From H Al Naimi - 2013
Taiwan	Sediments	Cd	0.3	1.8		mg/kg	1979	Shiber (1979)	From H Al Naimi - 2013
Taiwan	Sediments	Pb	26	576		mg/kg	1979	Shiber (1979)	From H Al Naimi - 2013
Turkey	Shrimp muscle	Fe			18.69	mg/kg	2008	Firat et al (2008)	From Heidarieh et al (2013)
Turkey	Shrimp muscle	Zn			27.75	mg/kg	2008	Firat et al (2008)	From Heidarieh et al (2013)
Turkey	Crab muscle	Fe			18.93	mg/kg	2011	Ayas & Ozogul (2011)	From Heidarieh et al (2013)
Turkey	Crab muscle	Zn			104.8	mg/kg	2011	Ayas & Ozogul (2011)	From Heidarieh et al (2013)
UAE	Sediments	Co			45.2	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
UAE	Sediments	Cr			303	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
UAE	Sediments	Ni			1010	µg g−1 dry wt	2004	de Mora et al. (2004)	From Freije 2015
UAE	Sediments	V	7.3	70.1		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE	Sediments	Fe	3594	6055.0		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE	Sediments	Co	1.79	3.3		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013

UAE	Sediments	Ni	14.2	25.0		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE	Sediments	Cu	1.34	7.8		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE	Sediments	Zn	1.56	3.4		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE	Sediments	Cd	0.018	1.9		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE	Sediments	Pb	0.54	5.2		mg/kg	1993	Fowler et al (1993)	From H Al Naimi - 2013
UAE Coastal	Sediments	Mn	5.03	352.0		mg/kg	1999	M.A.Shriadah (1999)	From H Al Naimi - 2013
UAE Coastal	Sediments	Co	6.01	25.9		mg/kg	2000	M.A.Shriadah (1999)	From H Al Naimi - 2013
UAE Coastal	Sediments	Ni	8.01	214.5		mg/kg	2001	M.A.Shriadah (1999)	From H Al Naimi - 2013
UAE Coastal	Sediments	Cu	3.05	79.0		mg/kg	2002	M.A.Shriadah (1999)	From H Al Naimi - 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
UAE Coastal	Sediments	Zn	3.01	534		mg/kg	2003	M.A.Shriadah (1999)	From H Al Naimi - 2013
UAE Coastal	Sediments	Cd	4.32	9.6		mg/kg	2004	M.A.Shriadah (1999)	From H Al Naimi - 2013
UAE Coastal	Sediments	Pb	9.03	57.01		mg/kg	2005	M.A.Shriadah (1999)	From H Al Naimi - 2013
UAE	Sediments	Zn			10.15	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	Pb			2.82	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	Cd			0.105	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	Ni			16.92	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	Mn			119	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	Fe			3230	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	V			11.04	mg/kg	2015		El Tokhi et al - 2015
UAE	Sediments	Cu			5.05	mg/kg	2015		El Tokhi et al - 2015
Saudi Arabia (Intertidal)	Sediments	Fe	4100	530	1800	mg/kg	2014		Almasoud et al -2014
Saudi Arabia (Intertidal)	Sediments	Mn	120	9	36	mg/kg	2014		Almasoud et al -2014
Saudi Arabia (Intertidal)	Sediments	Zn	46	8	21	mg/kg	2014		Almasoud et al -2014
Saudi Arabia (Intertidal)	Sediments	Cu	18	1	5	mg/kg	2014		Almasoud et al -2014
Saudi Arabia (Intertidal)	Sediments	Cr	130	7.5	34	mg/kg	2014		Almasoud et al -2014

Saudi Arabia (Intertidal)	Sediments	Pb	12	1.1	4	mg/kg	2014		Almasoud et al -2014
United States	Barnacle	Cu	11	64		mg/kg	1981	Barber and Trefry (1981)	From P.A. Reis et al. 2013
United States	Barnacle	Zn	360	12000		mg/kg	1981	Barber and Trefry (1981)	From P.A. Reis et al. 2013
USA	Barnacle	Zn			910	mg/kg	1966	Alexander and Rowland (1966)	From P.A. Reis et al. 2013
USA	Barnacle	Cd	6	58		mg/kg	1998	Flalkowski And Newman (1998)	From P.A. Reis et al. 2013
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
USA	Barnacle	Cu	40	3750		mg/kg	1998	Flalkowski And Newman (1998)	From P.A. Reis et al. 2013
USA	Barnacle	Zn	620	37900		mg/kg	1998	Flalkowski And Newman (1998)	From P.A. Reis et al. 2013
USA	Barnacle	Fe	450	1150		mg/kg	1998	Flalkowski And Newman (1998)	From P.A. Reis et al. 2013
USA	Barnacle	Pb	5	15		mg/kg	1998	Flalkowski And Newman (1998)	From P.A. Reis et al. 2013
USA	Sediments	Zn	53	168		mg/kg	1977	Eisler et al. (1977)	From H Al Naimi - 2013
USA	Sediments	Cd	0.1	2.5		mg/kg	1977	Eisler et al. (1977)	From H Al Naimi - 2013
USA	Sediments	Pb	17	81		mg/kg	1977	Eisler et al. (1977)	From H Al Naimi - 2013
Vietnam	Shrimp	PCBs	0.5	1.1		µg /g	1998	Dang et al (1998)	From G.R.W. Denton 2006
Iran	Fish	THg	0.04	0.09		mg/kg			
Arabian Gulf	Fish Canned	THg			0.16	mg/kg	2005	Khansari et al. 2005	Mortazavi, and Sharifian, 2011
Bahrian	Fish	THg			0.084	mg/kg	1996	Madany et al 1996	Mortazavi, and Sharifian, 2011
Bahrian	Shellfish	THg			0.042	mg/kg	1996	Madany et al 1996	Mortazavi, and Sharifian, 2011
UAE	Fish	THg			0.107	mg/kg	1996	Ahmed And Al Ghais	Mortazavi, and Sharifian, 2011
Qatar	Fish	THg			0.02	mg/kg	1993	Kureishy	Mortazavi, and Sharifian, 2011
Qatar	Mussels	THg	0.02	0.46		mg/kg	1993	Kureishy	Mortazavi, and Sharifian, 2011
Qatar	Fish	THg	0.06	0.208		mg/kg	1999	ROPME	Mortazavi, and Sharifian, 2011

NorthSea	Fish	THg	0.039	0.61		mg/kg	2003	Baeyens	Mortazavi, and Sharifian, 2011
Belgium	Fish	THg			0.134	mg/kg	2003	Baeyens	Mortazavi, and Sharifian, 2011
UAE - Jebel Ali	Sediments	MBT			1.08	µg /g	2003		De Mora 2003
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
UAE - Jebel Ali	Sediments	DBT			0.1	µg /g	2003		De Mora 2003
UAE - Jebel Ali	Sediments	TBT			0.11	µg /g	2003		De Mora 2003
UAE - Jabu Dhabi	Sediments	MBT			0.17	µg /g	2003		De Mora 2003
UAE - Jabu Dhabi	Sediments	DBT			0.11	µg /g	2003		De Mora 2003
UAE - Jabu Dhabi	Sediments	TBT			0.12	µg /g	2003		De Mora 2003
UAE - Al Marfa	Sediments	MBT			0.14	µg /g	2003		De Mora 2003
UAE - Al Marfa	Sediments	DBT			0.08	µg /g	2003		De Mora 2003
UAE - Al Marfa	Sediments	TBT			0.09	µg /g	2003		De Mora 2003
UAE - Al Reweis	Sediments	MBT			0.12	µg /g	2003		De Mora 2003
UAE - Al Reweis	Sediments	DBT			0.07	µg /g	2003		De Mora 2003
UAE - Al Reweis	Sediments	TBT			0.08	µg /g	2003		De Mora 2003
UAE - ThreeRocks	Sediments	MBT			0.16	µg /g	2003		De Mora 2003
UAE - ThreeRocks	Sediments	DBT			0.1	µg /g	2003		De Mora 2003
UAE - ThreeRocks	Sediments	TBT			0.11	µg /g	2003		De Mora 2003
Qatar - Umm Said	Sediments	MBT			3.1	µg /g	2003		De Mora 2003
Qatar - Umm Said	Sediments	DBT			1.4	µg /g	2003		De Mora 2003
Qatar - Umm Said	Sediments	TBT			0.8	µg /g	2003		De Mora 2003
Qatar - Dukhan	Sediments	MBT			1.3	µg /g	2003		De Mora 2003
Qatar - Dukhan	Sediments	DBT			0.71	µg /g	2003		De Mora 2003
Qatar - Dukhan	Sediments	TBT			1.7	µg /g	2003		De Mora 2003
Qatar - Doha	Sediments	MBT			1.5	µg /g	2003		De Mora 2003
Qatar - Doha	Sediments	DBT			0.14	µg /g	2003		De Mora 2003
Qatar - Doha	Sediments	TBT			0.14	µg /g	2003		De Mora 2003

Qatar - Ras Laffan	Sediments	MBT			0.74	µg /g	2003		De Mora 2003
Qatar - Ras Laffan	Sediments	DBT			0.06	µg /g	2003		De Mora 2003
Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
Qatar - Ras Laffan	Sediments	TBT			0.06	µg /g	2003		De Mora 2003
Bahrain - Askar	Sediments	MBT			6.3	µg /g	2003		De Mora 2003
Bahrain - Askar	Sediments	DBT			2.4	µg /g	2003		De Mora 2003
Bahrain - Askar	Sediments	TBT			1.8	µg /g	2003		De Mora 2003
Oman - Hifi	Sediments	MBT			9.7	µg /g	2003		De Mora 2003
Oman - Hifi	Sediments	DBT			2	µg /g	2003		De Mora 2003
Oman - Hifi	Sediments	TBT			60	µg /g	2003		De Mora 2003
USA	Sediments	TBT	50	1400		µg /g	2006	Matthias et al., 1989	Bhosle et al., 2006
Chesapeake Bay	Sediments	TBT	37	1400		µg /g	2006	McGee et al., 1995	Bhosle et al., 2006
San Pedro Bay	Sediments	TBT			10	µg /g	2006	Venkatesan et al., 1998	Bhosle et al., 2006
UK	Sediments	TBT	50	5800		µg /g	2006	Thomas et al., 2000	Bhosle et al., 2006
Germany	Sediments	TBT	73	15130		µg /g	2006	Biselli et al., 2000	Bhosle et al., 2006
France	Sediments	TBT			8150	µg /g	2006	Amouroux et al., 2000	Bhosle et al., 2006
New Zealand	Sediments	TBT	5	3320		µg /g	2006	de Mora et al., 1995	Bhosle et al., 2006
Spain	Sediments	TBT			9260	µg /g	2006	Tolosa et al., 1992	Bhosle et al., 2006
Cadiz coast	Sediments	TBT	250	14150		µg /g	2006	Gomez-Ariza et al., 1995	Bhosle et al., 2006
Coast of Spain	Sediments	TBT	125	18722		µg /g	2006	Diez et al., 2002	Bhosle et al., 2006
Greenland	Sediments	TBT			417	µg /g	2006	Jacobsen and Asmund, 2000	Bhosle et al., 2006
Japan	Sediments	TBT	24	5125		µg /g	2006	Harino et al., 1998	Bhosle et al., 2006
Fiji	Sediments	TBT			10	µg /g	2006	Davis et al., 1999	Bhosle et al., 2006
Malaysia	Sediments	TBT	0.7	216		µg /g	2006	Tong et al., 1996	Bhosle et al., 2006
Malta	Sediments	TBT	82	1500		µg /g	2006	Axiak et al., 2000	Bhosle et al., 2006
East Gulf and Pacific coasts	Sediments	TBT	24	1800		µg /g	2006	Krone et al., 1996	Bhosle et al., 2006
Korea	Sediments	TBT	60269	3E+05		µg /g	2006	Hwang et al., 1999	Bhosle et al., 2006

Country	Media	Element	Min	Max	Mean	Unit of measure	Year	Source	Notes
USA	Sediments	TBT	60	30250		µg /g	2006	Page et al., 1996	Bhosle et al., 2006
USA	Sediments	TBT	9736	68613		µg /g	2006	Elgethun et al.<comma> 2000	Bhosle et al., 2006