ISSN: 2642-9020

Research Article

Journal of Marine Science Research and Oceanography

Potential Disease Hazards of Petrolium Hydrocarbons Contamination in *Gafrarium Pectinatum* (Linnaeus, 1758) From Egyptian Lake Temsah with Emphasis On *Gafrarium* DNA Parcoding

Iman M K Abumourad*, Taghreed B Ibrahim, Soliman W S and Elgendy M Y

Hydrobiology Department, Veterinary Research Division, National Research Centre, 33 El Bohouth St Egypt

***Corresponding author**
Iman M K Abumourad. Abumourad, Hydrobiology Department, Veterinary Research Division, National Research Centre, 33 El Bohouth St Egypt

Submitted: 18 Nov 2020; **Accepted**: 30 Nov 2020; **Published**: 09 Jan 2021

Citation: *Iman M K Abumourad, Taghreed B Ibrahim, Soliman W S and Elgendy M Y (2021) Potential Disease Hazards of Petrolium Hydrocarbons Contamination in Gafrarium Pectinatum (Linnaeus, 1758) From Egyptian Lake Temsah with Emphasis On Gafrarium DNA Parcoding. J Mari Scie Res Ocean,4(1):140-144.*

Abstract

Assessment of petroleum hydrocarbon (PHC) contamination was carried out in water, sediments and the bivalve Gafrarium pictinatum as a part of monitoring program work. DNA barcoding was applied on G. pictinatum using Cytochrome c oxidase subunit 1 (COX1); accession no. HQ703080.1. assessment of disease risk probability due to PHC contamination using comet assay in G. pectinatum gills was studied. Results revealed that the value of dissolved petroleum hydrocarbons was 44 mg/l in water of the investigated area, 365.5 mg/kg in sediment and 135.2 mg/kg and in G. pictinatum tissue. Phylogeny of the collected bivalves was confirmed as Gafrarium pictinatum using COX1 representative primers, PCR, sequencing and blast tool on the NCBI. TPH caused DNA damage in G. pictinatum, the value of the damage differed from location to another which may be attributed to the level of hydrocarbons pollution. This study provides a basis for studying hydrocarbon contamination in marine environment.

Keywords: Petrolium Hydrocarbons, Pollution, Marine Ecosystem, *Gafrarium Pictinatum* and Disease Hazards

Introduction

The Suez Canal represents an important position worldwide as a World Commercial Shipping Line. Biologically it binds the Mediterranean Sea and the Red Sea with two different environments. Both seas receive oil pollution from anthropogenic sources including chronic discharges from oil refineries and loading/unloading operations in addition to oil spills and pipeline leakage.

Lake El Timsah is also known as Crocodile Lake, is a lake in Egypt on the Nile delta. It lies in a basin developed along a fault extending from the Mediterranean Sea to the Gulf of Suez through the Bitter Lakes region [1]. The surface area of Lake Timsah covers 5.4 square miles, it is a small and shallow lake and one of four lakes connected to the Suez Canal, it lies on the Suez Canal at mid way between Port Said and Suez, it receives brackish water from the western lagoon overtopping its high saline water. El Temsah lake is the main source of fish for the area, also its bottom is mainly muddy or sandy and is the habitat of a wide range of ecological and economical important bivalve's taxa. The lake is the end-point of several wastewater effluents, it lies 76 km from the northern part of the Suez Canal (Port Said), it is impacted by different pollution sources like agriculture, domestic wastes, and shipping activities.

Petroleum hydrocarbons are quantitatively the most important constituents of petroleum, they enter the marine environment from various sources such like but not only sporadic oil spills, hydrocarbons are important contaminants in the marine environment because of both human activities and natural processes [2]. Petroleum products are discharged into the marine environment through surface runoff, industrial effluents, storm water banks, and shipping and spill activities. Oil pollution is a severe global environmental problem causing a number of adverse negative impacts on aquatic organisms and mariculture, human health, tourism, ecosystem and consequently the national income. Water pollution by hydrocarbons has been and continues to be of increasing interest in public and research areas.

Bivalves are extensively used in monitoring programs in the marine environment due to their ability to concentrate pollutants to several orders of magnitude above ambient levels in sea water.

Gafrarium pectinatum of the family Veneridae (Linnaeus 1758) which is the most popular edible clams and represents an important bivalve fishery in Lake Timsah where it consumed locally in Egypt and had been exported to Europe.

The aim of this study is to describe the distribution of petroleum hydrocarbons pollution and determination of total petrolium hydrocarbones in water, sediment and *Gafrarium pectinatum* bivalve tissues as bio-indicator and to assess the its impact on the clam using detection of DNA alterations or breaks by comet assay with emphasis on the DNA barcoding of this mussele.

Materials and Methods

Study Area: Lake Temsah (Figure 1).

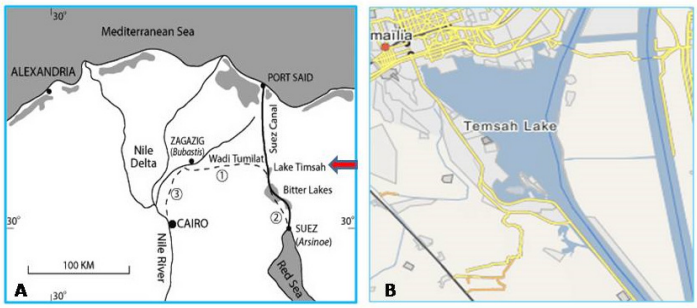

Figure 1: Lake Temsah; A: Sampling of Water, Sediment and Bivalve: [3]

Six surface water samples (1m depth) were collected from six stations in Lake Temsah by local motorboat using a Nisken bottle during 2018. Surface sediments (0-3cm) samples were collected from the same sites of water sampling using a stainless steel grab sampler. All the collected water and sediment samples were stored in refrigerator at 4 C until analysis. In addition, 84 *Gafrarium pictinatum* samples (Figure 2) were collected; 30 for analysis of total poly hydrocarbons (TPH). Another 30 *Gafrarium pictinatum* samples were kept in liquid nitrogen till further processing of DNA barcoding and 24 were kept in aerated seawater tank for further investigation of DNA breaks and damage using single cell gel electrophoresis assay.

Figure 2: *Gafrarium pictinatum* collected from Lake Temsah

Water Samples Extraction

Water samples were extracted according to Moustafa et al. , 1997. Two times with 60 ml of dichloromethane in a separating funnel. Sample extracts were combined and concentrated by rotary evaporation to 5 ml [4]. Finally, samples were concentrated under a gentle stream of pure nitrogen to a final volume of 1 ml.

Sediment Samples Analysis

Determination analysis of TPH in sediment samples were done according to Bernard et al., (1996).

Bivalve Samples Analysis for Determination of Tph

Samples were frozen-dried, pooled (5 individuals per station), then 50 g were Soxhlet-extracted with 260 ml of n-hexane for 8 h and then re-extracted for 8 h into 260 ml of dichloromethane [5]. These extracts were combined and concentrated down using a rotary evaporator at 30 °C followed by concentration with a nitrogen gas stream down to a volume of 1 ml for measurement of TPH.

Spectrofluorometric (Uvf) Analysis

Total petroleum hydrocarbons were measured using the UVF-spectrofluorometer (Sequoia-Turner Model 450) at 360 nm excitation and 415 nm emission according to Parsons et al. [6].

Phylogeny/ DNA barcoding of *Gafrarium Pictinatum* DNA Extraction

Small portions of foot muscles were excised and flash frozen in liquid nitrogen before stored in a -80 C til further DNA and RNA analysis. genomic. DNA was isolated and purified from 30-35mg of foot muscles using Wizard Genomic DNA Purification Kit (Promega), according to the manufacturer's instructions. Whole genomic DNA was then quantified using a spectrophotometer and diluted to a standard concentration of 30 ng/µl and then the DNA was stored at -20◦c.

PCR for Amplification of Cox1

A small region (~ 600- 700 bps) of the COX1 gene was amplified in the thermo cycler (BioRad, USA) using the COX1 primers: Left: 5'-TGATTGTTGCCGGGGTCTAT-3'. and Right: 5'-CCCCAC-GTGTAAAGCCAAAA-3' (GenBank accession no. HQ703080.1). PCR reaction was performed in volume of 25 µl containing 12.5 µl Master Mix, 0.5 µl of each primer (10 pmol), 3 µl of template DNA (about 90 ng template DNA) and sterile distilled water to final volume of 25 µl. To optimize PCR products, annealing temperature and times were varied. PCR conditions were as follows: an initial denaturation for 3 min at 94◦C, followed by 45 sec at 94◦C, 1 min at annealing temperature 55◦C and 2 min at 72◦c for 35 cycles, and a final extension of 10 min at 72◦C.

Gel Electrophoresis, Purification& Sequencing

Electrophoresis was carried out, PCR products were run on a 1.5 % agarose gel stained with ethidium bromide. PCR product was visualized and photographed in UV gel documentation system. Purification was carried out using QIAquick PCR Purification Kit (QIAGEN). The purified PCR product was sequenced in Macrogen Ltd (Korea). By using Blast from the NCBI Gen Bank data base, COX1 sequence of the *G. pictinatum* was screened.

Comet Assay (For DNA Damage Assessment)

To examine the DNA damage in clam *Gafrarium pictinatum* caused by petrolium hydrocarbons, a method of Slobodskova et al. (2010)., was used, where the gill cells of the mussels were used [7]. Slime from the gills was removed by triple washing with a cold (4°C) Ca2+ and Mg2+ free isotonic solution (500 mM NaCl, 12.5 mM KCl, 5 mM EDTA_Na2, and 20 mM Tris_HCl, pH 7.4). The gills were then cut into small pieces with scissors and placed in 4–5 ml of isotonic solution. After a 30–40 min incubation, the gill cells were removed from the gill fragments by filtering through

a 40_µm sieve. Cells in the filtrate were precipitated by centrifugation and resuspended in isotonic solution to a concentration of 10^5 cells/ml. 50 µl of the cell suspension was added to 100 µl of 1% low melting point agarose (LKB, Sweden) in 0.04 M phosphate buffer (pH 7.4) at 37°C, thoroughly mixed, placed on a glass slide coated with 1% agarose for better adhesion, and covered with a cover glass. The sample was placed for 3 min in a fridge for gel to form. The cover glass was carefully removed, each slide was placed for 1 h in a lysing solution (2.5 M NaCl; 0.1 M EDTA_Na2, 1% Triton X_100; 10% DMSO; 0.02 M Tris, pH 10) and placed in darkness. After washing with cold distilled water, the slides were transferred to an electrophoresis buffer (300 mM NaOH and 1 mM EDTA Na2) and maintained for 40 min. Electrophoresis was carried out at 2 V/cm for 15 min. After neutralization (0.4 M tris HCl, pH 7.4), the slides were stained with ethidium bromide (2 µg/ml).

The DNA comets were visualized and registered using a scanning fluorescence microscope equipped with a digital camera. Digital images were processed using CometScore Freeware v.1.5 (http://www.auto_ comet.com/products_cometscore.php)

Results

Gravimetric determination of oil content for water samples (Figure 3) indicates that the total concentration of dissolved petroleum hydrocarbons (DDPH) in water of the investigated area ranged from 24 mg/l to 68 mg/l with average value of 44 mg/l. In sediment the TPH concentration ranged between 171 mg/kg and 623 with average value of 365.5 mg/kg and in *G. pictinatum* tissue ranged between 99.5 mg/kg to 214 mg/kg with average value of 135.2 mg/kg.

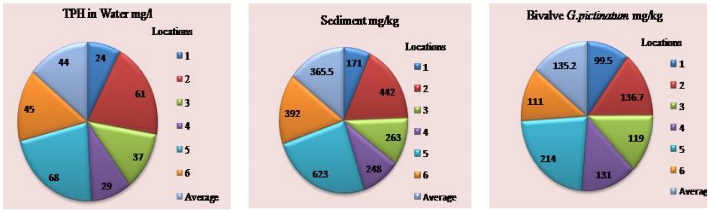

Figure 3: Total dissolved petroleum hydrocarbons (TPH) in water, sediment and bivalve

PCR technique and direct sequencing of COX1 gene were selected to assess the phylogenitic taxonomy of the collected bivalves. PCR product yielded 633 bp, by using blast tool of the NCBI the taxonomy of the collected bivalve was indicated as *Gafrariym pictinatum.*

Comet Assay (Single Cell Gel Electrophoresis)

Location 1, 3 & 4 had no comet cells and appear as a control normal cells (Figure 4, A), while comet cells were appeared clearly in number and level of DNA damage in the cell in other locations and in certain order; location 5 followed by location 2 and finally location 6 (Figure 4; B, C & D). Concerning percent cells of damaged DNA; location 5 showed the highest rate of comet cells and DNA breaks (15.13%) followed by location 2 (7.86 %) then finally location 6 (3.13%). The same order of DNA damage measured was noticed in concern to tail length and percent DNA in tail (Table 1).

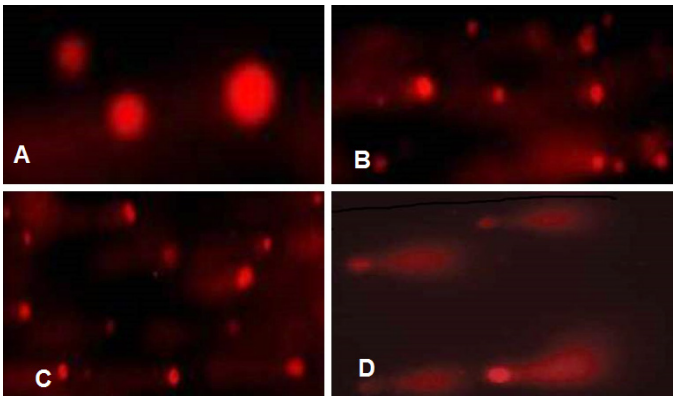

Figure (4): comet assay in *G. pictinatum* cells showing large numbers of damaged DNA. A: represent location 1,3&4, B: Location 6, C: Location2 and D: Location 5.

Table 1: DNA damage estimated by comet assay in *G. pictinatum* (Mean±S.D).

Location	Tail Length (px)	% DNA in Tail	Tail Moment	Tail Intensity	% cells with comets
L2	19.2±0.13	39.85±0.41	8.23±0.77	5317.52±0.65	7.86±0.42
L5	46.8±0.22	56.99±0.14	31.9±0.97	12219.04±0.17	15.13±0.21
L6	12.8±0.36	16.39±0.15	6.34±1.21	3219.04±0.85	3.13±0.19

Discussion

Pollution with petrolium hydrocarbons is attributed to the disposed oil originated from shipping activities, maintenance and maritime activities in several docks around Temsah Lake. One of the serious threats to marine water ecosystems is the hydrocarbons contamination originated from oil. Lake Temsah located North of Suez Canal is a land engulfed embayment, it has been subjected to significant environmental changes caused by various anthropogenic activities; it receives several kinds of pollutants like raw sewage from the city network, industrial pollution from shipping activities and maintenance and agricultural drainage water and possibly marine pollution [8].

Bivalve molluscs such as mussels and oysters have the ability to concentrate relatively high concentrations of anthropogenically derived chemicals so, they commonly used in the environmental monitoring programs [8]. The Venus clam *G. pectinatum* of the family Veneridae is one of the most popular edible clams which

represent an important bivalve fishery in Lake Timsah, it is consumed locally in Egypt and also exported to Europe.

Authors used molecular method in many purposes like providing information on the evolutionary history of the organism and biodiversity conservation issues [9-10]. The present phylogenetic analysis revealed that using of DNA barcoding was successful and provide clear results were enough to identify and taxonomy of *G. pictinatum.*

Figure (3) illustrates the concentrations of TPH measured in water, sediment and *G. pictinatum* using the UVF spectrofluorometer; TPH measured in *G. pictinatum* tissues ranged between 99.5 mg/kg to 214 mg/kg with average value of 135.2 mg/kg.

Results also revealed that TPH content in water samples ranged from 24 mg/l to 68 mg/l with average value of 44 mg/l. Concerning the lethal hazards effects that might follow the petroleum pollution; Egaas et al., 1982 reported that disease hazards and death of living organisms may occur in the range of 1 to 10mg/L, and the sub-lethal effects range is up to 1mg/L. From this work' obtained results, the studied areas have values high enough to cause lethal toxicity in the aquatic organisms [11]. The components of the oil, especially aromatics may interfere with cellular or sub-cellular processes in the living organisms and causing hazardous diseases like carcinogenesis and death in some cases [12].

Sediments are common used assessment techniques of petroleum pollution in coastal aquatic systems, the concept here is that sediments act as pollutant sinks and provide an integrated picture of the events taking place in the water column. Many studies have shown that hydrocarbons from spilled petroleum persist in the sediments for long time years [13]. Metwalley et al., (1997) reported that there are three groups in marine sediments according to the TPH levels: unpolluted sediments which have TPH range from 1 to 4mg/Kg, moderately polluted sediments which have <100mg/Kg and highly polluted sediments which have up to 2000mg/Kg.

Based on this classification, the resulted values listed in figure (3) for TPH concentration in sediments ranged between 171 mg/kg and 623 with average value of 365.5 mg/kg dry weight, indicate highly polluted sediments, same results were reported by El gendy and Moustafa (2007), as they found that the total petroleum hydrocarbon (TPH) content in water samples reached 103mg/l and in sediment samples reached 635mg/kg. This alarming concentration levels have potential threats to marine living organisms consequently their consumers [14]. From result data it was noticed that values of TPH in case of sediment samples was higher than that of the water, this may be attributed to the accumulation of oil on sediments and the ability of sediments to accumulate and retain oils while water is mobile and tends to migrate down through sediments where adsorption of pollutants may take place.

Ali et al., (2006) reported that, the qualitative and quantitative assessment of PAHs in water, sediments and fish samples of Lake Temsah indicated that they are highly affected by PAHs contamination, its threats may be due to their propensity to initiate carcinogenic and or mutagenic effects in terrestrial and aquatic biota. Elgendy and Moustafa, (2007) found that the total petroleum hydrocarbon (TPH) content in fish samples reached 139mg/kg. The main components of such pollutants were polyaromatic hydrocarbons (PAHs)

Detection of DNA alterations in aquatic organisms is highly suitable biomarker for evaluating the genotoxic effects of different contaminants like petrolium hydrocarbones. The comet assay proved to be a simple and sensitive genotoxic approach for evaluating DNA damage in aquatic organisms exposed to various contaminates in their environment [15].

In this study, comet assay showed greater DNA damage in some locations compared to others. Location 1, 3 & 4 had no comet cells and appear as a control normal cells, this may be due to lower levels of TPH in these locations where location 5 showed the highest rate of comet cells and DNA breaks (15.13%) followed by location 2 (7.86%) then finally location 6 (3.13%)in terms of percent cells with comets, also the tail length, and percent DNA in tail which may be attributed to higher levels of TPH detected in this species. Martins and Costa in their review reported that, in a similar study of Rank et al. they had an example with field-collected bivalves and found that comet assay, lysosomal stability (measured through the Neutral Red Retention Time assay) with acetylcholine esterase activity yielded results consistent with the effects of pollution [16-17].

The formation of PAH metabolite–DNA adducts has long been reported to occur in mussels as well as the presence of CYP isoforms similar to those in vertebrates [18-20]. Dallas et al. found that mussels and cockles collected from several sites within same estuarine area in England sea water yield different levels of comet-measured DNA strand breakage (determined in haemocytes) and distinct sensitivity to distinct metals, although both were apparently sensitive to environmental pollution [21].

From this study, it could be indicated that pollution by petroleum hydrocarbons was clear in lake Temsah water, sediments and the bivalve *G. pictinatum* tissues and had been found to affect *G. pictinatum* causing DNA damage.

G. pictinatum was proved to be a good model to be used in the monitoring programs [22-23].

Reference

1. Gmirkin Russell E (2006) Berossus and Genesis, Manetho and Exodus. Continuum International Publishing Group 2006: 231.
2. Moustafa YM (2004) Environmental assessment of petroleum contamination of Gamasa- Damiette beaches." Orient J Chem 20: 219-226.
3. Stiros S (2009) Misconceptions following Suez Canal Excavation. http://mediterranee.revues.org/162
4. Moustafa YM (2004) Contamination by polycyclic aromatic hydrocarbons in some Egyptian Mediterranean Coasts. Biosciences Biotech Research Asia 2:15-24.
5. Villeneuve J P, Carvalho F P, Fowler S W, Cattini C (1999) Levels and trends of PCBs, chlorinated pesticides and petroleum hydrocarbons in mussels from the NW Mediterranean coast: comparison of concentrations in 1973/1974 and

1988/1989. Sci Total Environ 237/238: 57-65.
6. Parsons TR, Matia Y, Malli GM (1985) Determination of Petroleum Hydrocarbons. A Manual of Chemical and Biological Method for Seawater Analysis, Pergamon Press, Oxford.
7. Slobodskova VV, Solodova EE, Slinko EN, Chelomin VP, (2010) Evaluation of the genotoxicity of cadmium in gill cells of the clam Corbicula japonica using the comet assay. Russian Journal of Marine Biology 36: 311-315.
8. ELmoselhy Kh M, Yassien M H (2005) Accumulation patterns of heavy metals in venus Clams, Paphia undulata (born, 1780) and Gafrarium pectinatum (linnaeus, 1758), from Lake Timsah, Suez Canal, Egypt. Egypt. J Aquat Res 31:1-16.
9. Ahmed MI (2010) Morphological, Ecological and Molecular examination of the Seacucumber species along the Red Sea Coast of Egypt and Gulf of Aqaba, with the investigation of the possibility of using DNA barcoding technique as a standard method for Seacucumber ID. Thesis, Hull Univ 2010: 258.
10. Cunha RL, Blanc F, Bonhomme F, Arnaud-Haond S (2011) Evolutionary patterns in pearl oysters of the genus Pinctada (Bivalvia, Pteriidae). Mar Biotechnol 13: 181-192.
11. Egaas E, Varanis A (1982) Effects of polychlorinated biphenyls and environmental temperature on in vitro formation of benzo[a]pyrene metabolites by liver of trout Salmo gairdneri. Bio Chem Pharm 31: 561-566.
12. Ali HR, Elgendy NSH, Elezbewy S, Elgemaee G H, Moustafa YM, et al. (2006) Assessment of Polycyclic Aromatic Hydrocarbons Contamination in Water, Sediment and Fish of Temsah Lake, Suez Canal, Egypt. Current World Environment 1:11-22.
13. Wang XC, Sun S, Ma HQ, LiuY (2006) Sources and distribution of aliphatic &polyaromatic hydrocarbons in sediments of Jiaozhou Bay, Qingdao, China". Marine pollution Bulletin 52:129-138.
14. EL gendy N, Moustafa YM (2007) Environmental assessment of petroleum hydrocarbons contaminating Temsah Lake, Suez Canal, Egypt. Orient J of Chem 23:11-26.
15. Frenzilli G, Nigroa M, Lyons B (2009) The comet assay for the evaluation of genotoxic impact in aquatic environments. Celebrating the 20th Anniversary of the invention of the comet assay. Mutat Res Rev 681: 80-92.
16. Martins M, Costa P M (2015) The comet assay in Environmental Risk Assessment of marine pollutants: applications, assets and handicaps of surveying genotoxicity in non-model organisms. Mutagenesis 30: 89-106.
17. Rank J, Lehtonen KK, Strand J, Laursen M (2007) DNA damage, acetylcholinesterase activity and lysosomal stability in native and transplanted mussels (Mytilus edulis) in areas close to coastal chemical dumping sites in Denmark. Aquat Toxicol 84: 50-61.
18. Canova S, Degan P, Peters L D, Livingstone D R, Voltan R, et al. (1998) Tissue dose, DNA adducts, oxidative DNA damage and CYP1A-immunopositive proteins in mussels exposed to waterborne benzo[a] pyrene. Mutat Res 399: 17-30.
19. Akcha F, Izuel C, Venier P, Budzinski H, Burgeot T, et al. (2000) Enzymatic biomarker measurement and study of DNA adduct formation in benzo[a]pyrene-contaminated mussels, Mytilus galloprovincialis. Aquat Toxicol 49: 269-287.
20. Shaw J P, Large AT, Chipman J K, Livingstone DR, Peters L D (2000) Seasonal variation in mussel Mytilus edulis digestive gland cytochrome P4501A- and 2E-immunoidentified protein levels and DNA strand breaks (Comet assay). Mar Environ Res 50: 405-409.
21. Dallas L J, Cheung V, Fisher A S, Jha A N (2013) Relative sensitivity of two marine bivalves for detection of genotoxic and cytotoxic effects: a field assessment in the Tamar Estuary, South West England. Environ. Monit Assess 185: 3397-3412.
22. Anderson SL, Wild GC (1994) Linking genotoxic responses and reproductive success in ecotoxicology. Environ Health Perspect 102: 9-12.
23. Andral B, Stanisiere JY, Sauzade D, Damier E, Thebault H, et al. (2004) Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Marine Pollution Bulletin 49: 704-712.