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Heavy Metals and PAH Assessment Based on Mussel Caging in the North Coast of Tunisia (Mediterranean Sea)

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ABSTRACT: In the framework of the Mytilos project (Interreg IIIB Medocc), international research cruises were carried out in 2006 in order to evaluate the level of Heavy metals and polycyclic aromatic hydrocarbons (PAH) along the coast of the western basin of the Mediterranean Sea. Caged mussels *(Mytilus galloprovincialis)* were placed in situ at six sites in the North coast of Tunisia. Results showed total PAH levels were in the range 45.6-241.6 ng/g dry weight (dw). The ranges of trace metals concentrations expressed in µg/gdw are: Hg (0.1-0.2), Pb (0.4-0.7), Cd (0.9-2.9), Cu (2.9-3.9), Fe (117-248) and Zn (250-426). Higher concentrations were observed at Rades, La Galite and Tabarka which can be attributed to the industrial activities implanted in the coast of Algeria and in the city of Rades. Concentrations of PAH, Cd, Pb, Fe and Zn are generally different from initial concentrations (before caging), depending on the adaptability of transplanted mussels to be used as bio-indicator of contaminants. Yet bio-monitoring using mussels give information on compound bioavailability which depends on their ability to accumulate contaminants in its tissue.

Key words: PAHs, Heavy metals, Mediterranean Sea, North coast of Tunisia, Mussel caging

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are compounds containing two or more fused aromatic rings in linear, angular or clustered arrangements. PAH have high molecular weight and low volatility and are known to be highly persistent in the environment (Jawad Al–Wadae and Raveendran, 1991; Meire *et al.,* 2008; Perugini *et al.,* 2007). Hydrocarbons may arise from petroleum hydrocarbons pollution and incomplete combustion of organic materials such as wood, coal and oils. The health hazard posed by these compounds has been extensively studied by several authors (Agbozu and Opuene, 2009; Villeneuve *et al.,* 2002). Several polycylic aromatic hydrocarbons were studied, including sixteen that are reported in the priority list of pollutants of the US Environmental Protection Agency (EPA). PAHs are bioavailable to fish and other marine organisms through the food chain, as waterborne compounds and from contaminated sediments. PAHs uptake always depends on their bioavailability as well as the physiology of the organisms (Meador *et al.,* 1995). Such compounds have adverse effects on health (carcinogenic and/or mutagenic activity) and ecosystem (Long *et al.,* 1995). Numerous studies indicate that one-, two- and three-ring compounds are acutely toxic, while higher molecular weight are considered to be genotoxic (Mersch-Sundermann *et al.,* 1992; Nylund*et al.,* 1992). Heavy metals, as defined by (Neiboer and Richardson, 1980) are normal constituents of marine environment. At least 11 are known to be essential to marine organisms: iron (Fe), copper (Cu), zinc (Zn) cobalt (Co), manganese (Mn), chromium (Cr), molybdenum (Mo), vanadium (V), selenium (Se), Magnesium (Mg) and Nickel (Ni).Metals occur normally at low concentrations yet are capable of exerting considerable biological effects even at such levels (BU-Olayan *et al.,* 2008). All metals are toxic at levels that are higher than the respective threshold limit. Silver (Ag), mercury (Hg), copper (Cu), cadmium (Cd) and lead (Pb) are particularly toxic. Metal pollution has a significant negative effect on marine ecosystems and humans.

The use of sentinel organisms particularly bivalve molluscs such as mussels (*Mytilus galloprovincialis*) to measure the levels of bioavailable contaminants has been established by various international (MED POL) and national (RECNO) pollution monitoring programs. The advantage of using these sentinel organisms (biomonitors) is the ability to concentrate

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many organic contaminants by a factor of 10 above the ambient sea water levels and even higher than sediments, providing a direct representation of pollutants bioavailability (Nasci and Fossato, 1982). Besides, they are resistant to a wide range of contaminant concentration (Vinodhini and Narayanan, 2008). In addition, mussels have only a limited ability to metabolize organic pollutants especially PAHs and are therefore often used to monitor PAHs contamination in the marine environment (Farrington *et al.,* 1983). These sessile organisms are easy to collect and handle for active biomonitoring (caging) experiments (Andral *et al.,* 2004). The Mediterranean Sea is a semi-closed, system with high anthropic pressures from the South and the industrialized North European countries. Although accounting for 1% of the world's ocean surface, it receives about 25% of worldwide petroleum inputs to the ocean, with a quantity of 750×10^3 ton yr^{-1} (Burns, 1986). In the Mediterranean Sea, many scientists have studied the biological effects of chemical contaminants on wild and farmed the mussel (*Mytilus galloprovincialis* (Porte *et al.,* 2001). However monitoring programs can only be developed if sentinel organisms were available in the area to be studied. If not, caging technology, where organisms are put in cages deposited in various locations, has to be used. Monitoring contamination with caged organisms is a developing technology (Harries *et al.,* 1997; Nasci and Fossato, 1982). This technique has been highly developed in recent years (Roméo *et al.,* 2003) and has the advantage of being able to place caged mussels at sites where they are not usually present (e.g. sites close to the coast). In this study, caged mussels were placed in various sites at the North coast of Tunisia. Three months later, the caged mussels were collected and analysed for their PAH and heavy metals content. The main objective of this investigation is to assess the level and the sources of contamination by PAH and heavy metals along the North coast of Tunisia (Mediterranean Sea) using mussel caging.

MATERIALS & METHODS

Oceanographic cruises in the South-Western Mediterranean Sea were conducted between April and May 2006 along the Tunisian coast using the research vessel "R/V L Europe". Transplantation experiment was carried out in six sites situated in the North coast of Tunisia (Fig. 1). The used Mussels *(Mytilus galloprovincialis)* were collected by IFREMER from an aquaculture farm in Languedoc-Roussillon which harvests contamination-free mussels from the open sea. Batches were composed of mussels with an average shell size of 50 ± 5 mm. The 3-kg samples were stored in polyethylene bags and then mounted on PVC tubing and re-immersed for 10 days so they can re-cluster prior to transplantation (Galgani *et al.,* 2010). The experiment was carried for a three months period. Cages were deployed in six stations between April-May 2006 and were recovered in August 2006. Caged mussels were sampled at t = 0 (the beginning of transplantation) and t= 3 months (the end of the transplantation). A number of 30–40 mussels were dissected and the whole flesh was then lyophilized.

Fig. 1. Map of the stations of caged mussels in the North coast of Tunisia

(LG: Languedoc-Roussillon, T: Tabarka, B: Bizerte, SA: Sidi Ali, R: Rades and K: Korbous)

ll chemicals, reagents and acids were of analytical grade and of highest purity. Nitric acid, chloridric acid, fluorhydric acid and standards of Cu, Fe, Zn, Cd, Pb and Hg were provided by Merck. Methanol, hexane and dichloromethane that were used for the analysis of hydrocarbons were obtained from Scharlau. Potassium hydroxide that was used during saponification and the silica gel and alumina that weer used for cleaning were supplied by Fluka. A PAH standard mixture was provided by International Atomic Energy Agency (IAEA) containing: naphthalene (Naph), 1-methylnaphtalene (1MNaph), ethylnaphtalene (ENaph), acenaphthylene (Ace), acenaphthene (Acty), fluorene (Fl), phenanthrene (Phe), anthracene (An), 2-methylphenanthrene (2MPhe), 1-methylphenanthrene (1MPhe), 3,6-Dimethylphenanthrene (3,6DMPhe), fluoranthene (Flt), pyrene (Py), 1-methylpyrene (1MPy), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo (k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (Per), indeno(1,2,3-cd)pyrene) (IPy), dibenzo(a,h) anthracene (DBA) and

benzo(ghi)perylene (BPer) for chromatography analysis.

Analysis of mussels was performed according to the technique proposed by Villeneuve, 1995. Appropriate blanks were analysed with each set of analyses. Sample comparisons were made with reference material (IAEA 406) for quality control purposes. Recoveries ranged from 71% to 89% and the detection limits ranged from 0.05 to 0.25 ng/g.

Extraction of mussel tissue was performed by a Soxhlet extractor during 8 h with Methanol. Internal standards 9,10-dihydroanthracene, n-octadecene (C18:1) and n-dotriacontane (n-C32) were added to mussel samples before Soxhlet extraction in order to determine the recoveries during the analytical procedure. Extracts were concentrated with a rotavapor. To eliminate fatty acids, a saponification was realised with addition of potassium hydroxide in a Soxhlet extractor for two hours. The mixture of KOH/MeOH was first extracted twice with hexane into a glass separating funnel with a Teflon stopcock and then was concentrated.

Glass column was filled with silica gel and alumina (pre-baked at 300 °C for 4 h) with the bottoms end plugged with cleaned glass wool. The column was rinsed with hexane. The hexane extract (1 mL) was added to the top of the column and PAH was eluted with 30 mL of a mixture of hexane and dichloromethane (90:10 v/v). Hewlett Packard model 5890 II series with 30 m, 0.25 i.d. fused silica capillary column (Ultra 2: 5% diphenyl-dimethyl-polysiloxane) connected with a flame ionisation detector (FID) was used for analysis of individual alkanes and PAH. A splitless injection mode was used. Nitrogen was used as the carrier gas with a flow rate of 1 mL/min. The injector was maintained at 290 °C. All injection volumes were 1 μL in the splitless mode. The column temperature was initially held at 60 °C for 2 min, ramped to 290 °C at a rate of 3 °C min^{-1}, and then the temperature was held constant at 300 °C for 10 min.

Identification and quantification was performed by using external and internal standard, respectively. Twenty-four typical PAH were used as external standards for the identification of each PAH peak in the samples. A mixture of external standards was injected in the GC-FID and the corresponding response factor for each individual compound was estimated. Internal standards (9,10-dihydroanthracene, n-octadecene (C18:1) and n-dotriacontane (C32) were used to calculate the concentration of each individual compound in the sample. A confirmation analysis was performed by HRGC-MS Varian 4000 equipped with a CP-8400 autosampler, CP-8410 Autoinjector and a 30 m, 0.25 i.d. DB-5ms fused silica capillary column. Helium was used as the carrier gas with a flow rate of 1 mL/min. The injector and transfer line were maintained at 290 °C and 250 °C, respectively. Injection volumes were 1 μL in the splitless mode. We maintained the same temperature settings as with the GC-FID. The mass spectrometer was used in electron ionization mode and all spectra were acquired using a mass range of *m/z* 50-400.

Digestion of Cd, Pb Cu, Zn and Fe was carried out in a Teflon bombs with 5 mL HNO_3 using a microwave (Millestone type.Ethos) digestion at 100% power with pressure set at 120 psi for 20 min, the overall digestion time for the one cycle was 40 min. Blank acid mixtures were digested in the same way. Mercury was treated separately using 300 mg of mussel tissue samples in 5 mL HNO_3 using a water bath at a temperature of 90°C to dryness. The samples were then diluted to 50 mL with milli Q water acidified with 2 ‰ of HNO_3 (Loring *et al.*, 1992). Mussel tissue samples were analysed by AAS (type Varian) equipped with Zeeman correction. Analysis of Zn and Fe were performed by flame AAS, whereas Cu, Pb and Cd were analysed by electrothermal AAS with a graphite furnace. For Hg, cold vapour was used with a VGA-AAS (Varian AA10) system. The instrument was calibrated with diluted solution prepared from a known stock solution of each element. The accuracy and precision of the overall procedure have been determined and are estimated to be around 5 % for most elements. The quality assurance of the analytical results was controlled with the use of certified reference marine organism IAEA-407 provided by International Atomic Energy Agency (Monaco).

RESULTS & DISCUSSION

Common fish and mollusc from Mediterranean Sea are widely employed as bio-indicators of marine pollution by micropollutants. Most of them are used in the framework of the MED POL Programme Mediterranean Pollution co-ordinated by UNEP (United Nations Environmental Programme). The programme aims to: (a) monitoring the levels of organic compounds around the Tunisian coasts; (b) controlling the contaminant content in different tissues for public health purposes; and (c) investigating the effectiveness of the selected tissues as bio-indicators. Molluscs were used also for national pollution survey in the framework of the Network of harmful contaminants (RECNO) along the coast of Tunisia.

Because of their lipophylic nature, PAH tend to accumulate more in marine organisms than in other matrices such as sediment. However, marine organisms can rapidly convert up to 99% of the PAHs to metabolites within 24 h of uptake. In addition, the half

life of PAHs is generally very short. It ranges from six to nine days for fluorene, phenanthrene, anthracene and fluoranthene which is contrary to other persistent organics pollutants class, as polychlorinated biphenyls (Meador *et al.,* 1995).

For all investigated samples, the lowest concentration of total PAH is obtained for the Languedoc-Roussillon sample at t=0 (45.6 ng/g) (Table 1). At t= 3 months total PAH varied from one station to another. The lowest one was obtained in Korbous station (51.8 ng/g) and the highest was obtained in Rades station (241.6 ng/g). The difference between the two stations may be explained by the trophic position and food regime. It could also be derived from direct PAH inputs, such as shipping, oil spill, dry and wet atmospheric deposition, air-water exchange etc. In our study area, industrial and vehicle atmospheric emissions, a great deal of sewage and waste water from the city of Rades, and diesel oil leakage/contamination from frequent cargo and fishing ships contributed to PAH inputs.

The PAH levels at Rades station were similar to that found at Tabarka and La Galite stations and twice as high as levels found at Bizerte and Sidi Ali stations (Table 1). The contamination of Tabarka and La Galite may originate from industries implanted in the coast of Algeria and that were transported by the currents.

Concentrations of individual compound of PAH able varied from less than detection limits to 122.7 ng/g. This maximum value was obtained for fluoranthene in the La Galite station. For the other compounds, the majority of PAH are below the detection limits in the different analysed samples (Table 1).

The average background values for biological tissue range from 0.01 to 1 µg/kg for individual PAH (EUH, 2002). Benzo(a)pyrene was selected as a general indicator of total PAH in a given sample. The joint FAO/WHO expert committee on food additives has adopted a specification, which requires that the concentration of benzo(a)pyrene should not exceed a limit of 10 µg/kg (OJEU, 2005). Benzo(a)pyrene was not detected in mussel tissue in all of the stations.

Mussels that were sampled in different environments showed an increased accumulation of PAHs for the most contaminated sites, while those exposed to the least contaminated sites showed an apparent constant PAH concentrations compared to Languedoc-Roussillon site at t=0.

Contamination in mussel tissue results from equilibrium between uptake, accumulation and depuration (excretion and/or biotransformation). It is therefore obvious to find that the most contaminated mussels are located in the most contaminated sites. Mussels are not exposed directly to the contamination adsorbed on sediment grains, but they are exposed to the contamination present in the water column. However, because of tide currents in the studied area, sediment particles are regularly re-suspended and are therefore made available for filter-feeding organisms. When bivalves are exposed to a low level of pollution, they can depurate the contaminants at the same rate at which they accumulate them. However, this will not be the case if the pollution level is high.

The concentrations of PAH in tissue were proportional to the quantity of lipids (Table 1) for all of the collected samples. It is well known that tissue lipid content is influenced by several factors, such as age, species, feeding and spawning status (Perugini *et al.,* 2007). In this study such lipid variation would influence PAH tissue concentrations. The lowest level of PAH was found with low tissue lipid concentration. This variation could be attributed to the environmental conditions and probably to feeding because this period corresponds to the post-spawning phase for mussels in all of the stations. Moreover, it was reported that low levels in post-spawned species is due to the elimination of hydrocarbons through gamete emissions (Perugini *et al.,* 2007). Therefore Spawning periods can be disturbed or even shifted by natural factors such as temperature, richness of the diet and chemical contamination (Bayne *et al.,* 1976). Bodin *et al.,* 2004 showed that two spawning periods per year occurred in the mussel *Mytillus galloprovincialis* that were maintained in cages, i.e. preponderant spawning in January/February and partial spawning in April/June: a period of gamete emission between January and June and a period of gonad restoration between August and January.

PAH levels obtained for caged mussel in Tunisian coast are different to those obtained for other coastal regions in the world (Table 2).

The composition of PAH was variable in mussel samples. The PAH with 2,3-rings, 4 rings and 5,6 rings ranged from 32.5 to 68.3 % (average 42.9 %), 5.7 to 25.2 % (average 17 %) and 24.6 to 56.6 % (average 40 %), respectively, in mussel tissues sampled from all of the stations.

The use of "fingerprint" ratios of certain isomeric pairs of PAH concentrations provides a less subjective means to identify PAH sources: petrogenic and/or pyrogenic (Table 1). Generally petrogenic PAH are characterized by predominance of alkylated compounds over their parent homologues and by the dominance of two to three aromatic ring compounds, whereas in the pyrolytic PAH mixtures, the parent compounds with four or more aromatic rings are the

Table 1. Concentration of individual compounds of PAH, total PAH in ng/g dw, lipid content (mg/g) and different ratios characterising the origin of hydrocarbons

Compounds	Languedoc-Roussillon	Tabarka	La Galite	Bizerte Lagoon	Sidi Ali	Rades	Korbous
Naph	3.3	12.5	1.4	2.3	2.1	8.2	0.6
1MNaph	0.3	0.8	0.3	0.7	1	0.8	0.2
ENaph	0.2	0.5	1.9	0.3	0.4	2.4	0.3
Ace	0.2	1.8	1.5	1	0.9	0.9	0.1
Acty	0.4	nd	3.1	0.2	1.2	2.5	0.6
Fl	0.9	nd	5.9	0.4	1.5	5.6	0.5
Phe	2.1	11.8	17.9	6.2	6.1	15.2	0.7
An	1.4	1.8	6.6	0.6	0.7	6.9	0.6
2MPhe	2	1	0.7	1	1.2	0.6	0.6
1MPhe	0.2	nd	1.2	nd	nd	1.4	0.04
3,6DMPhe	nd	0.1	1.6	1	1	1.8	0.2
Flt	3.8	42.8	122.7	42.1	38.7	104.5	13.2
Py	1.9	1.2	5	2.5	2.1	4.9	1.5
1MPy	0.08	7.8	0.7	2.3	6.6	0.7	0.4
BaA	nd	nd	nd	nd	nd	nd	nd
Chry	0.7	2.4	1.8	1.1	1.3	1.7	1.2
BbF	0.9	3.9	nd	2	nd	0.7	nd
BkF	1.3	54.1	9.5	26.4	22.3	6.8	5.1
BeP	3.8	65.3	8.5	nd	nd	21.7	8.2
BaP	8.2	nd	nd	nd	nd	nd	nd
Per	7.9	nd	35.6	27.6	27.1	34.1	9.5
IPy	0.8	nd	7.9	4.9	5.4	5.3	2.5
DBA	3.6	nd	3.9	17.4	16.9	nd	3.5
BPer	1.5	14.4	3.5	6.6	7	34.3	2.2
ΣHAP	45.6	222.6	241.6	146.9	143.5	205.1	51.7
CPAH	14.8	58.1	21.3	50.8	49.6	12.8	11.2
Total TEQ	12	0.9	4.8	18.3	17.4	0.7	3.8
Phe/An	1.5	6.4	2.7	9.9	9.9	2.2	1.3
Flt/Py	2	33.7	24.3	16.8	16.3	21.2	8.7
An/An+Phe	0.4	0.1	0.3	0.09	0.09	0.3	0.4
Flt/ Flt+Py	0.7	1	1	0.9	0.9	1	0.9
LMW/HMW	0.5	0.5	2.2	0.6	0.6	1.4	0.5
Lipid content (mg/g)	17.1	26.6	27.1	21.3	20.5	23.4	18.2

naphthalene (Naph), 1-methylnaphtalene (1MNaph), ethylnaphtalene (ENaph), acenaphthylene (Ace), acenaphthene (Acty), fluorene (Fl), phenanthrene (Phe), anthracene (An), 2-methylphenanthrene (2MPhe), 1-methylphenanthrene (1MPhe), 3,6-Dimethylphenanthrene (3,6DMPhe), fluoranthene (Flt), pyrene (Py), 1-methylpyrene (1MPy), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (Per), indeno(1,2,3-cd)pyrene) (IPy), dibenzo(a,h) anthracene (DBA) and benzo(ghi)perylene (BPer).

phenanthrene / anthracene : Phe/An, fluoranthene / pyrene: Flt/Py, anthracene/anthracene + phenanthrene: An/An+Phe, fluoranthene to fluoranthene + pyrene: Flt/ Flt+Py, low molecular weight PAH/high molecular weight PAH: LMW/HMW, Total concentration of potentially carcinogenic PAH: CPAH, Total toxic benzo(a)pyrene equivalent: Total TEQ.

Table 2. Comparison of data on metal analysis (µg/ g) and PAH (ng/g) in caged mussels transplanted in Tunisian coast with those of other coastal regions

Areas	Cage duration	Hg	Cd	Pb	Cu	Fe	Zn	HAP	References
French coast	3 months	0.04-0.34	0.3-5.5	0.4-5.3	3-9.2	-	80-240	2-17	Andral et al., 2004
French coast	3 months	-	-	-	-	-	-	21.9-105.5	Galgani et al., 2010
Italian coast	3 months	-	-	-	-	-	-	25.6-80.4	Galgani et al., 2010
Spanish coast	3 months	-	-	-	-	-	-	29.8-99.9	Galgani et al., 2010
Algerian coast	3 months	-	-	-	-	-	-	25-72.5	Galgani et al., 2010
Moroccan coast	3 months	-	-	-	-	-	-	29.8-75.8	Galgani et al., 2010
La Fourcade	2 years	0.1-0.14	0.9-1.2	2.1-3	5.4-6.5	-	223.6-292.4	49.8-81.3	Bodin et al., 2004
Carteau	2 years	0.1-0.15	0.6-0.9	1.2-1.7	4.8-5.9	-	173.5-238.8	101.5-161.4	Bodin et al., 2004
Arcachon Bay	3 months	-	-	-	-	-	-	302-2420	Baumard et al., 1998
Arcachon Bay	1 Year	-	0.8-1	1.4-1.6	720	-	-	100-1600	Dévier et al., 2005
Bay of Cannes	Spring (1month)	-	0.9-1.2	-	8.6-9.3	-	124-137	-	Stien et al., 1998
Bay of Cannes	Autumn (1 month)	-	1.19-1.31	-	9.7-11	-	151-176	-	Stien et al., 1998
Tunisian coast	3 months	0.1-0.2	0.9-2.9	0.4-0.7	2.9-3.9	117-248	251-426	45.6-261.4	This study

most abundant components. Biogenic PAH come from natural terrestrial or marine sources and are equally produced during pyrolytic processes (Baumard *et al.,* 1998; Stangroom *et al.,* 1998). Ratios of phenanthrene to anthracene (Phe/An) and fluoranthene to pyrene (Flt/Py) have been widely used to distinguish petrogenic and pyrogenic sources of PAHs (Sicre *et al.,* 1987). PAHs of petrogenic origin are generally characterized by Phe/An values >10, whereas combustion processes often result in low Phe/An ratios (<10) (Benner *et al.,* 1990). For the Flt/Py ratios, values greater than 1 have been used to indicate pyrolytic origins, and values less than 1 are attributed to petrogenic source (Sicre *et al.,* 1987). In the present study, Phe/An and Flt/Py ratios in mussel samples (Table 1) were less than 10 and more than 1, respectively for all stations, indicating pyrolytic source of PAHs. A ratio of Anthracene to anthracene + phenanthrene (An/ An+Phe) lower than 0.1 indicates a petrogenic source while a ratio higher than 0.1 reflects a combustion source (Budzinski *et al.,* 1997). A fluoranthene to fluoranthene + pyrene (Flt/ Flt+Py) ratio <0.4 is generally characteristic of petrogenic sources (oil, diesel, coal), a ration between 0.4 and 0.5 indicates liquid fossil fuel (crude oil and vehicle) combustion, whereas a ratio greater than 0.5 is generally found in kerosene, grass, coal and wood combustion samples and creosote (Budzinski *et al.,* 1997; Yunker *et al.,* 2002). For gasoline, diesel, fuel oil, crude oil combustions and emissions from cars and diesel trucks Flt/ Flt+Py ratio is less than 0.5 (Yunker *et al.,* 2002). The plot Flt/ Flt+Py ratio against An/An+Phe ratio (Fig. 2) indicates that PAH found at all stations are clearly from pyrolytic source.

The ratio of the concentration of the lowest molecular weight PAHs (LMW: naphthalene to Fluoranthene) to the concentration of the other PAHs studied (HMW: pyrene to benzo(ghi)perylene) was calculated for mussels samples. All mussel samples except two stations (Rades and La Galite) were found to be apparently enriched in the highest molecular weight compounds (Table 1) which is in accord with what has been reported in the litterature (Hellou *et al.,* 1993; Mzoughi *et al.,* 2010). Water of the North coast of Tunisia is characterized by high turbidity due to the large amount of suspended particles. These particles are the main source of pollution for filter-feeding bivalves. Mussel tissue residues may be expected to reflect the particle PAH content. Mussel PAH residues reflect the distribution of the PAHs in the source i.e. in the particles, which could explain the apparent

enrichment of their tissues with the highest molecular weight compounds.

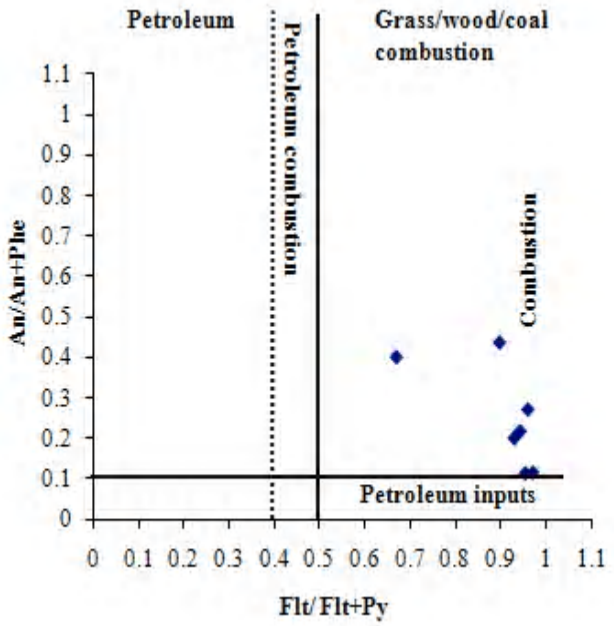

Fig. 2. Plot of anthracene/anthracene + phenanthrene (An/An+Phe) vs fluoranthene/ fluoranthene + pyrene (Flt/ Flt+Py)

The presence of low condensate ring structures may result from contributions from both petrogenic and pyrolytic PAH sources (Sericano, *et al.,* 2001; Perugini *et al.,* 2007). Thus the presence of low-molecular weight PAH in tissue was attributed to the high dispersion capacity of these pollutants associated with metrological conditions, such as precipitation rates, winds and tide currents (Meire *et al.,* 2008) and to the fact that less soluble PAHs are adsorbed on particulate matter, stored in bottom sediments and are preferentially adsorbed by the digestive system of mussels, while the assimilation of the lower PAHs, characterized by a reasonable water-solubility, could be due to the direct absorption by water and interstitial water. PAH bioavailability may depend on the degree of pollution. Thus, contaminated sediments could be characterized by a saturation of the adsorption sites for hydrophobic organic compounds and interactions between sediment particles and contaminants could then be weakened, increasing the availability of the compounds. It may also depend on the origin of the compound. For example, petrogenic hydrocarbons were found to be accumulated at higher levels than pyrolytic compounds by various organisms (McElroy *et al.,* 1989). The low availability of pyrolytic PAHs may be linked to their enhanced binding to particulate matter, especially their trapping into soot particles formed during pyrolysis at high temperature of the organic matter (McGroddy and Farrington, 1995).

Several PAH, and especially their metabolic products, are known to be carcinogenic (Connel *et al.,* 1997). Total concentration of potentially carcinogenic PAH (CPAH) (sum of benzo (a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo (a)pyrene, indeno (1,2,3-cd)pyrene and dibenzo (a,h)anthracene) varied from 11.1 to 58 ng/g, which accounts for 4.9 to 34.5 % of total PAH in mussel tissues.

Among all known potentially carcinogenic PAH, benzo(a)pyrene is the only PAH for which toxicological data are sufficient for derivation of a carcinogenic potency factor (Peters *et al.,* 1999). However, data is available to quantify the toxicities of other PAH relative to benzo(a)pyrene, expressed as toxic equivalency factors (TEFs). These are used to estimate benzo(a)pyrene-equivalent doses (BaP_{eq} dose). The application of benzo(a)pyrene (BaP)-toxic equivalent factor to polycyclic aromatic hydrocarbons (PAH) concentrations may provide a more accurate risk assessment for environmental exposure to PAH. According to the US Environmental Protection Agency (US EPA, 1993), TEFs for benzo(a)anthracene, benzo(a)pyrene benzo (b)fluoranthene, benzo (k)fluoranthene, indeno (1,2,3-cd)pyrene and dibenzo (a,h)anthracene are 0.1, 1, 0.1, 0.01, 0.1 and 1, respectively. According to IARC (1987) total toxic benzo(a)pyrene equivalent (TEQ) of all these PAH is:

$BaP_{eq}\ dose_i = TEF_i.dose_i$

$Total\ TEQ = \Sigma\ BaP_{eq}\ dose_i$

Total TEQ calculated for all of the analyzed samples varied from 0.7 to 18.3 ng $TEQg^{-1}$ (Table 1). The maximum value of Total TEQ was found at the station of Bizerte lagoon indicating the presence of local sources. Average values of relative contents BaP_{eq} doses in Total TEQ decreased in the order: DBA (49.7%) IND (16.6%), BaP (11.5%), BKF (11.4%) and BbF (9.2%).

Concentrations of all measured metals were higher than the initial mussel samples of Languedoc-Roussillon at t = 0. No difference was obtained for Cu in all stations at t = 0 and at t = 3 months. Some concentrations of bioaccumulated heavy metals (Cd, Pb, Cu, Fe and Zn) were higher at Tabarka, La Galite and Rades stations. Contaminant concentrations obtained during this study (Table 3) were compared with those obtained in the context of Network of harmful contaminants (RECNO) performed at various sites along the Tunisian Mediterranean coast between 1996 and 2010. They highlighted approximately the same levels.

Table 3. Concentration of Hg, Cd, Pb, Cu, Fe and Zn (µg/g dw) in caged mussels from different area of the North coast of Tunisia

Area	Hg	Cd	Pb	Cu	Fe	Zn
Languedoc-Roussillon	0.12	0.87	0.36	3.89	142	256
Tabarka	0.2	2.94	0.74	3.61	247	426
La Galite	0.15	2.18	0.54	3.61	117	314
Bizerte Lagoon	0.15	1.79	0.65	3.32	184	299
Sidi Ali	0.22	1.74	0.45	3.62	145	251
Rades	0.13	1.68	0.63	2.92	211	316
Korbous	0.21	1.79	0.54	3.54	248	274

Wang *et al.*, 1997 showed that shellfish tend to uptake metals in solute form and homogeneous levels in the water column seem to have little impact on variations in levels between marine stations. Under these conditions, levels of metals measured in natural populations sampled on the coast are nearly identical to those obtained from transplantation of caged mussels.

Heavy metal concentrations can be either natural due to geological substrates or man induced, due to direct or indirect input related to human activities. Consequently, the natural occurrence of metals complicates assessments of potentially contaminated sites because measurable metal bioavailability does not automatically infer contamination and some site specific high concentrations may represent entirely natural conditions.

Similarly to PAH content, higher concentrations of Cd, Pb, Fe and Zn are obtained for station of Rades, La Galite and Tabarka , it can be attributed to the industrial activities implanted in the coast of Algeria and in the city of Rades. Thus the absence of proper urban and/or industrial sewage treatment In Algeria have turned the coastal marine environment into a prime recipient of pollution (Taleb *et al.*, 2007).
Heavy metals concentrations were compared with caged mussel transplanted in different coastal regions in the world indicating similar behaviour for all metals studied (Table 2).

CONCLUSION

This paper provides important information on caged mussels allowing the assessment of the bioavailability and distribution of PAH and heavy metals (Hg, Pb, Cd, Cu, Fe and Zn). Results showed that bioavailability of the various contaminants varies and that low and constant mussel tissue concentrations constitute an apparent baseline level which is thought to result from an equilibrium between uptake and depuration of the absorbed PAH and heavy metals. Mussels are generally enriched with highest molecular weight reflecting the distribution of PAH in the sources. Fingerprint ratios allowed the distinction between pyrolytic and petrogenic sources depending on the activities in such areas. To identify the sources and history of pollution in these coastal areas we recommend the study of different core of sediment in a future work.

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