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Concentration of bioactive compounds in mussels *Mytilus galloprovincialis* as an indicator of pollution

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ABSTRACT

The polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organotins were analyzed in mussels *Mytilus galloprovincialis* from polluted and unpolluted sites from Mokpo Bay, Korea. The total PAH's concentrations (10^{-3} mg kg^{-1}) measured by GC–MS were in the range from 31 ± 23 to 1 ± 1. Among the eight PAHs the predominant ones were fluoranthene, phenanthrene and pyrene and accounted approximately 63% of the total PAHs. Among the four detected PCBs the highest content was of PCB 153, which accounted about 47% of the total PCBs. The main organotin compounds were dibutyltindichloride (DBT) and tributyltinchloride (TBT) and their composition was approximately 33% and 24%. PAHs, PCBs and organotins were found only in the mussels from polluted site. The antioxidant activity by ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] test was higher in mussels from polluted than from unpolluted sites ($P < 0.05$). It was found a correlation between the determined compounds (PAHs, PCBs and organotins) and the antioxidant activity of the mussel tissue from polluted site and the correlation coefficients were 0.96, 0.92 and 0.80, respectively. Such correlation can be explained by the properties of mussels. Since the mussel cell wall and tissues are hydrophobic, they can concentrate a number of hydrophobic pollutants like PAHs and PCBs from the marine environment by solubility rules. On the other hand, proteins are lipophilic compounds having antioxidant properties. Certain amino acid residues and thiol (-SH) groups, contained in proteins, respond to the ABTS antioxidant activity assay. Thus there may be a correlation between the total antioxidant activity of the organism and the PAH–PCB pollutants which were concentrated from its environment. The studied properties of mussels from polluted site can be used as an additional indicator of pollution.

1. Introduction

The subject of industry and pollution has been monitored for several decades. Bivalves (*Mitylus galloprovincialis*) and other marine organisms coming from the Central Adriatic Sea (Perez-Cadahia et al., 2004; Wang et al., 2005; Bihari et al., 2007; Perugini et al., 2007), the western coast of Alexandria, Egypt (Said, 2007), the west coast of Scotland (Webster et al., 2006), the Arcachon Bay, France (Devier et al., 2005), the Galician coast (Nieto et al., 2006), the Kastela Bay (Milun et al., 2004), the Mediterranean sea (Andral et al., 2004), coastal lagoon of Italy (Fabbri et al., 2006) were chosen for determination of the sea pollution. These marine organisms were selected because of their multitude, wide distribution and common use as sea food. Minier et al. (2006) also show that chemical and biological measurements bring different but complementary results that can help diagnose environmental health. In their study mussels were used to monitor and assess areas suspected of oil contamination by transplanting animals from unimpacted to impacted sites and vice versa.

PAHs were measured in different varieties of mussels: *Mytilus trossulus* (Page et al., 2005); *Mytilus californianus* (Oros and Ross, 2005); in blue mussels (*Mytilus* spp.) from Nordic coastal areas (Skarpheoinsdottir et al., 2007); in mussel *Perna perna* (Francioni et al., 2007), in marine bivalves *Perumytilus purpuratus* along the Chilean coast (Mendoza et al., 2006), in seven taxa of intertidal plants and animals at seventeen shoreline sites in Prince William Sound, USA (Neff et al., 2006). Seasonal variations of six mussel (*M. galloprovincialis*) biomarkers at two sites in the Mediterranean Sea were compared with physiological indices (condition, growth

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and gonad maturation), environmental parameters (temperature, salinity and turbidity), and chemical contamination levels of other organisms (Fernández-Reiriz et al., 1989; Bodin et al., 2004; Burlando et al., 2006).

Marine organisms (mussels *Mytilus edulis* and fish) have been shown as target species of peroxisome proliferators when these animals were exposed to North Sea crude oil, a mixture of oil, alkylphenols and extra PAHs (Cajaraville and Ortiz-Zarragoitia, 2006).

Trophic transfer of PCB congeners in zebra mussels (*Dreissena polymorpha*) and other animals were reported by Kwon et al. (2006).

Fang (2004) has described concentrations and patterns of organochlorine pesticide residues, and PCBs were analyzed in mussel (*Perna viridis*) samples from 10 coastal sites along the Pearl River Delta, southern China. Contaminant levels (organotins, trace metals, PCBs and PAHs) were measured in tissues of *Mytilus* sp. mussels.

PCB levels in cultivated mussels *M. galloprovincialis* from the Galician coast have been studied as biomarkers of pollution (Carro et al., 2005).

Organotin compounds such as butyl- and phenyltin used mainly as biocides for protection of vessels and agricultural crops, respectively, but this application has been limited or even forbidden due to their detrimental impact on the aquatic environment (Giergielewicz-Możajska et al., 2001; Wasik and Ciesielski, 2004).

Telli-Karakoc et al. (2002) have investigated PAHs and PCBs along the coast of Izmit Bay. Also Ruus et al. (2006) reported about the measurements of PCBs in blue mussels *M. edulis* from Western Norway, but the specific mechanisms and effects of such PCB accumulation in marine organisms were not investigated till now.

The numerous reviewed reports were focused on the determination of PAHs, PCBs, but not in the connection with other indices such as the antioxidant activity of the mussels, polyphenols composition and their use as biomarkers for pollution. We also found very limited data about the Korean cost. It is estimated that 90% of human exposure to persistent organic pollutants is through food. Fish and shellfish represent an important source of contamination, therefore the mussels from Mokpo Bay were characterized in this report by PAHs, PCBs, organotins and antioxidants.

As far as we know, no studies of the relationship of antioxidant capacity of mussels in connection with the main polluted components were conducted, and there are no published articles describing these properties of mussels as an indicator of pollution.

2. Materials and methods

2.1. Animals and sites of collection

Mussels were collected in two regions of Mokpo coast: an ecologically unpolluted (out of the port) and in polluted site (the Mokpo port), on April 10, 2004. Pollinated Mokpo sea is the bay of Halla Ship Construction Company which belong to Hyunday groups, while unpolluted sea is apart from 20 miles West-North from Mokpo bay.

The collected mussels (*M. galloprovincialis*) from both unpolluted and polluted sites were characterized by a similar maximum length and size of analyzed organisms (4.37 ± 0.5 cm): it was 75–85% of the maximum size reached within each population. This approach guaranteed that compared mussels had similar metabolic conditions and the influence of physiological differences between two populations was less pronounced (Regoli, 2000). The samples were designated as follows: MUP, for unpolluted site from Mokpo Coast, and MP, for polluted sites from Mokpo Port. Whole soft tissue from 30 specimens of each population were rapidly frozen in liquid nitrogen and stored at −80 °C. Then the samples were dried in glass flasks on Finn – Aqua, Lyovac GT-2 equipment for 36 h.

2.2. Reagents

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)], Folin-Ciocalteau reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The following chemicals were used for gas chromatographic analyses: dichloromethane, methanol, cyclohexane (Merck-Germany), silica gel (40 μm, J.T. Baker – Holland); the mixture of 16 compounds from the PAHs group with the concentration of each compound 2000 μg mL^{-1} (Restek Corpotation – USA); certificated solution of Naphthalene-d8 in dichloromethane with concentration 2000 μg mL^{-1} (Supelco – USA); certificated solution of benz(a)anthracene in dichloromethane with concentration 2000 μg mL^{-1} (Supelco – USA); PCB standard-solutions of seven polychlorinated biphenyls in isooctane, each with concentration 100 μg mL^{-1} (Restek Corpotation – USA); PCB 209 certified standard in acetone with concentration 200 μg mL^{-1} (Supelco – USA); all organotin compounds (OTC) were purchased from Sigma–Aldrich and Merck. All chemicals used in this investigation were high purity reagents (HPR).

2.3. Analytical methods and instrumentation

2.3.1. Gas chromatographic analysis

All experiments were performed using a TraceGC gas chromatograph (*ThermoQuest*) equipped with a mass spectrometric detector and on-column injector.

The analytes in each sample were identified by matching the retention time of each peak with the retention times of external standards.

2.3.2. Determination of PAHs and PCBs in mussels

The freeze-dried samples (1 g) were hydrolyzed in 15 mL of 4 N methanolic KOH solution at a slow rate for 4 h. The cooled digest was then transferred to a separatory funnel, and the reflux-flask was rinsed with 10 mL of a methanol–water (9:1) solution. The sample digest was extracted with 10 mL of cyclohexane. The extract was evaporated to approximately 0.5 mL under a stream of nitrogen. For sample clean-up, a short column packed with silica gel (0.5 g) was used and eluted with 8 mL dichloromethane. The eluate volume was reduced to 0.3 mL under a stream of nitrogen. The extract was injected to GC–MS system (2 μL).

2.3.3. Determination of organotin compounds

2.3.3.1. Accelerated solvent extraction (ASE). The mixture of 2 g (freeze-dried sample and 26 g quartz sand) was extracted with solution of 1 M acetic acid and 1 M sodium acetate and 0.3 g of tropolone in methanol–water mixture. Extracts were collected for analysis to calculate total mass of extract and masses of successive portions, respectively.

2.3.4. Derivatization and analysis

ASE extract was placed in a centrifuge vial with 10 mL acetic acid–sodium acetate buffer solution (pH 5), 2 mL of 0.1 μg mL^{-1} tetrabutyltin standard solution in hexane (IS). After addition of 2.5 mL of 2% solution of sodium tetraethylborate ($NaBEt_4$) the vial was tightly capped, then centrifuged at 4,400 rpm for 3 min. A portion (1.5 mL) of the hexane layer was cleaned by passage through a column with silanized glass wool and filled with 1 g Al_2O_3 (3% water) and a 1-mL layer of anhydrous Na_2SO_4 on the top. After elution of organotin compounds with 10 cm^3 hexane, the volume of extract was reduced to approximately 1 cm^3 under a stream of nitrogen. For analysis 2 μL of the extract was injected into gas chromatograph GC 8000 series, Thermo Quest Italia S.p.A., Carlo Ebra Instruments, Milano, Italy with a capillary column, Alltech Multi-Cap™, MC-5 HT (1000 × 1 m × 40 μm i.d. × 0.2 μm,

SE-54); carrier gas (H_2: 50 mL min^{-1} at 85 °C); autosampler AS 2000; injection mode-splitless; detector – FPD-800, 610 nm interference filter; detector gases: H_2 (45 cm^3 min^{-1}), air (160 cm^3 min^{-1}). The temperature program was the following: isothermal 1: 90 °C for 3 min; rate 1: 15 °C min^{-1}; isothermal 2: 170 °C for 0 min; rate 2: 25 °C min^{-1}; isothermal 3: 240 °C for 3 min. Injector temperature was 250 °C and detector temperatures of base: 250 °C, body: 200 °C. The detection limit of organotin compounds was 17–0.03 mg kg^{-1} dry weight (DW) (Giergielewicz-Możajska et al., 2001; Wasik and Ciesielski, 2004).

2.3.5. Extraction of polyphenols

Defatted lyophilized mussel samples were extracted from a 50-mg aliquot with 5 mL of 1.2 M HCl in 60% methanol/water for total polyphenols (TPH) with some modifications with heating at 90 °C for 3 h. The samples were cooled, diluted to 10 mL with methanol and centrifuged for 5 min at 4000*g* with a benchtop centrifuge to remove solids (Vinson et al., 2001).

2.3.6. Polyphenol determination

The Folin-Ciocalteu method was used (Singleton et al., 1999), and the measurements were performed at 765 nm with gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents (GAE) kg^{-1} DW.

2.3.7. Antioxidant activity

The ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). The absorbance was monitored exactly 1 and 6 min at 734 nm after the addition of 990 μL of ABTS solution to 10 μL of mussel extracts or Trolox standards in methanol or phosphate buffered saline (pH 7.4). Trolox equivalent antioxidant capacity (TEAC) was expressed as molar trolox equivalents (TE) kg^{-1} DW (Ozgen et al., 2006).

2.4. Statistical analyses

The results of this investigation *in vitro* are means ± SD of three measurements. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The p values of <0.05 were considered significant.

3. Results

The results of the determination of the PAHs concentrations in mussels are shown in Fig. 1 and Table 1. From the 16 polycyclic aromatic hydrocarbons only eight were determined and the total sum of the eight PAHs was 96 × 10^{-3} mg kg^{-1} DW. The individual PAHs (10^{-3} × mg kg^{-1} DW) ranged from 31 ± 23 to 1 ± 1 (Table 1). Among the detected eight PAHs the predominant were fluoranthene, phenanthrene and pyrene which accounted approximately 63% of the total PAHs. The retention times (min) of the individual PAHs were similar to the reviewed reports, such as 17.62 (phenanthrene, anthracene); 22.89 (fluoranthene); 23.87 (pyrene); 30.47 (benzo(a)anthracene, chrysene); 35.65 [benzo(b)fluoranthene]; 37.52 (benzo(a)pyrene) (Fig. 1).

From four detected PCBs the highest one was PCB153 which was about 47% of the total sum (Table 2).

The following organotin compounds were detected in mussels from polluted area (Table 3, Fig. 2): monobuthyltin (MBT); dibuthyltin (DBT); tributhyltin (TBT); monophenyltin (MPhT); diphenyltin (DPhT); triphenyltin (TPhT). The main organotin compounds were DBT and TBT and compose approximately 33% and 24%.

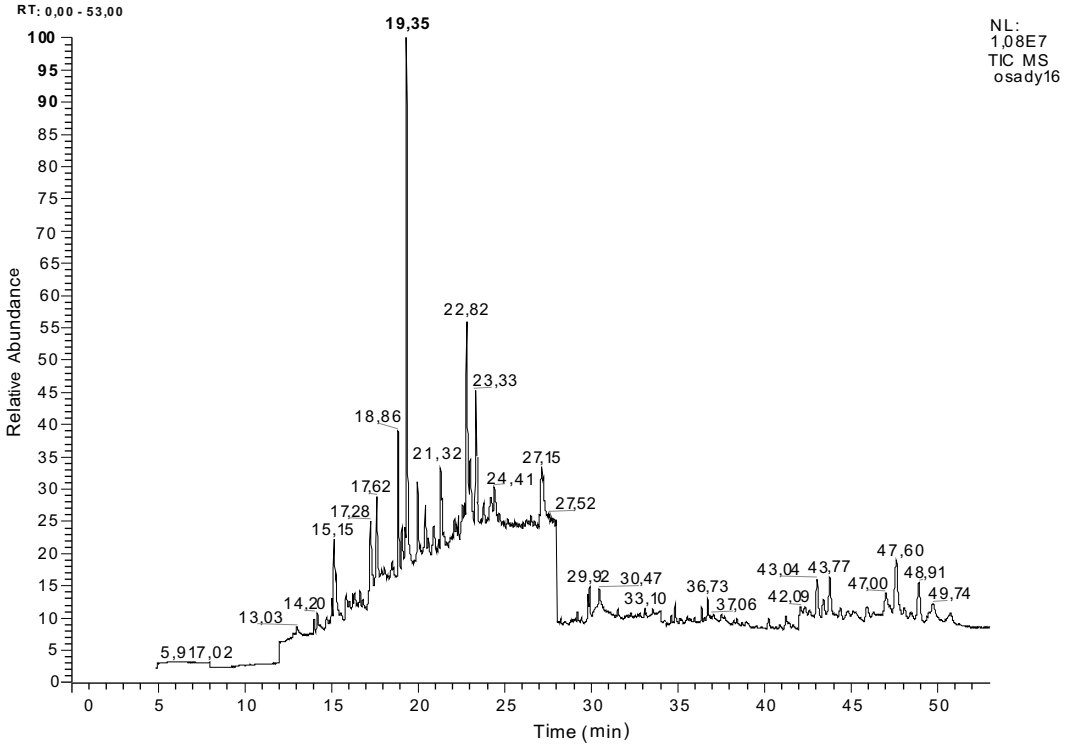

Fig. 1. The GC–MS chromatogram of solvent extract from mussels collected from polluted site (in TIC mode), showing the PAHs: naphthalene; benz(a)anthracene; chrysene; benzo(b)fluoranthene; benzo(k)fluoranthene; benzo(a) pyrene; indeno(1,2,3cd) pyrene; dibenzo(a,h)anthracene; benzo(g,h,i) perylene; acenaphthylene; acenaphthene; fluorene; phenanthrene; anthracene; fluoranthene; pyrene. Retention time (min) 17.62 (phenanthrene, anthracene); 22.89 (fluoranthene); 23.87 (pyrene); 30.47 (benzo(a)anthracene, chrysene); 35.65 [benzo(b)fluoranthene]; 37.52 (benzo(a)pyrene).

Table 1
Determination of PAHs in mussels ($10^{-3} \times$ mg kg^{-1} DW)

	Unpolluted	Polluted	LOQ	LOD
Naphthalene	–	–	1	0.3
Acenaphthylene	–	–	1	0.3
Acenaphthene	–	–	1	0.3
Fluorene	–	–	1	0.3
Phenanthrene	–	19 ± 14	2	0.6
Anthracene	–	9 ± 6	2	0.6
Fluoranthene	–	31 ± 23	2	0.6
Pyrene	–	17 ± 12	2	0.6
Benz(a)anthracene	–	13 ± 9	3	1
Chrysene	–	5 ± 4	3	1
Benzo(b)fluoranthene	–	[1 ± 1]	3	1
Benzo(k)fluoranthene	–	–	3	1
Benzo(a)pyrene	–	[1 ± 1]	3	1
Indeno(1,2,3cd)pyrene	–	–	4	1.3
Dibenzo(a,h)anthracene	–	–	4	1.3
Benzo(g,h,i)perylene	–	–	4	1.3

(–) – below limit of quantification (LOQ).
[···] – between limit of quantification and limit of detection.
LOD – limit of detection.

Table 2
Determination of PCBs in mussels ($10^{-3} \times$ mg kg^{-1} DW)

	Unpolluted	Polluted	LOQ	LOD
PCB 28	–	–	1	0.3
PCB 52	–	–	1	0.3
PCB 101	–	2 ± 2	1	0.3
PCB 118	–	2 ± 2	1	0.3
PCB 138	–	4 ± 3	1	0.3
PCB 153	–	7 ± 5	1	0.3
PCB 180	–	–	1	0.3

(–) – below limit of quantification (LOQ).
LOD – limit of detection.
PCB 28 – 2,4,4′-trichlorobiphenyl; PCB 52 – 2,2′,5,5′-tetrachlorobiphenyl; PCB 101 – 2,2′,4,5,5′-pentachlorobiphenyl; PCB 118 – 2,3′,4,4′,5′-pentachlorobiphenyl; PCB 138 – 2,2′,3,4,4′,5′-hexachlorobiphenyl; PCB 153 – 2,2′,4,4′,5,5′-hexachlorobiphenyl and PCB 180 – 2,2′,3,4,4′,5,5′-heptachlorobiphenyl.

Table 3
Determination of organotin compounds in mussels ($10^{-3} \times$ mg kg^{-1} DW)

ANALYTE	MBT	DBT	TBT	MPhT	DPhT	TPhT
Limit of detection [LOD]	6	13	17	5	3	0.03
Limit of quantification [LOQ]	18	40	51	14	9	0.1
Unpolluted	n.d	n.d	n.d	n.d	n.d	n.d
Polluted	26 ± 5	54 ± 3	40 ± 1.5	16 ± 3.5	29 ± 3.0	<LOQ

Abbreviations: monobutyltintrichloride (MBT), dibutyltindichloride (DBT), tributyltinchloride (TBT), monophenyltintrichloride (MPhT), diphenyltindichloride (DPhT) and triphenyltinchloride (TPhT).

There were various contents of phenolic compounds in the extracts, depending on the extraction solvent. Polyphenols had maximum absorptions of their UV spectra in a narrow range between 217 and 264 nm and the methanolic extract of the samples had spectral similarities with catechin solution (standard), which indicated that flavonoids predominated in the phenolic compounds. The absorption units on the spectra were higher in the extract from polluted site than in the methanol one from unpolluted.

The amounts of total polyphenols TPH ($10^3 \times$ mg GAE kg^{-1} DW) for mussels from contaminated (MC) and mussels uncontaminated (MUC) sites ranged from 29.35 ± 2.65 to 24.87 ± 2.34, when the extraction was done using 60% methanol/water (Fig. 3) in comparison with 28.48 ± 2.74 to 23.56 ± 2.23, where the extraction was done using 50% methanol/water.

The related antioxidant activities (mM TE kg^{-1} DW) in total polyphenol extracts ranged from 71 ± 6.4 to 59 ± 5.1 in comparison with 66 ± 6.1 to 56 ± 4.9 (Fig. 3), as determined by ABTS assay, were significantly higher in MC than in MUC ones ($P < 0.05$). Accordingly, the antioxidant activity was increased in mussels from the polluted site.

The antioxidant activity by ABTS test was higher in mussels from polluted than from unpolluted sites. It was found a correlation between the determined compounds PAHs, PCBs and organotins and the antioxidant activity of the mussel tissue from polluted site and the correlation coefficients were 0.96, 0.92 and 0.80 (Fig. 4A and B).

4. Discussion

The aim of our study was to determine the composition of PAHs, PCB, their bioavailability, the content of organotins and their influence on antioxidant activity of mussels (*M. galloprovincialis*) as pollution biomarkers.

Different conditions of pollution were compared in mussels and a significant increase of peroxisome proliferation was found when the animals were exposed to crude oil, alkylphenols and extra PAHs (Burlando et al., 2006; Cajaraville and Ortiz-Zarragoitia, 2006). Our results were in accordance with others, confirming that a special influence was in oil mixture, alkyl phenols and PAHs. In our report we have used only one fixed condition. Okay et al. (2001) found that higher PAH concentrations in mussels were detected around the refinery area (110–170 mg kg^{-1} DW), which are higher than our data. Mean tissue total PAH (TPAH) concentrations in intertidal clams, mussels, and worms from oiled sites range (from 24 to 36) $\times 10^{-3}$ mg kg^{-1} DW, according to Neff et al. (2006), which is twice lower than the obtained data. Total polycyclic aromatic hydrocarbons (TPAH) have been obtained in mussel tissue (16.99 vs. 17.03 mg kg^{-1} DW). Mussels feed on particulate matter and therefore concentrate particle-associated PAHs. The reported data (Perez-Cadahia et al., 2004) were higher than our obtained data. TPAH obtained for mussels from unoiled sites were in the range of (3–355) $\times 10^{-3}$ mg kg^{-1} DW) (Page et al., 2005). In the reported data by us TPAHs were also in this range, even lower, therefore the mussels were taken also from unoiled site. The range of sum PAH detected in mussels *M. californianus* was 0.021–1.093 mg kg^{-1} DW (mean 0.175 mg kg^{-1} DW). PAH isomer pair ratios applied as diagnostic indicators suggested that the bioaccumulated PAH were derived primarily from petroleum combustion, with lesser amounts derived from biomass and coal combustion, and unburned petroleum (Oros and Ross, 2005). Our data can be compared also with the data of Telli-Karakoc et al. (2002), who measured the total PAH concentrations in the Bay of Marmara Sea by spectrofluorometry, which was used also in our study. The total 16 PAHs were in the range from 5.67 to 14.81 mg kg^{-1} wet weight (WW) in edible part of mussel. Total PAH (2- to 6-ring parent and alkylated) concentrations ranged from 0.013 to 0.151 mg kg^{-1} WW. Seasonal trends were evident with concentrations being significantly higher for samples collected between November and March compared to those collected between April and October: for April to October: 0.031 mg kg^{-1} WW and for November to March: 0.063 mg kg^{-1} WW. Individual PAH concentrations exceeded only for the heavier 4- and 5-ring PAHs (fluoranthene, pyrene, benz[a] anthracene and benzo[a] pyrene) in samples collected between November and March. Our results were similar, except only benzo[a] pyrene. Differences were also seen in the PAH profiles with season. Mussels collected between November and March had a higher proportion of the heavier PAHs compared to mussels collected in the summer and autumn (Webster et al., 2006). In this report mussels were collected only

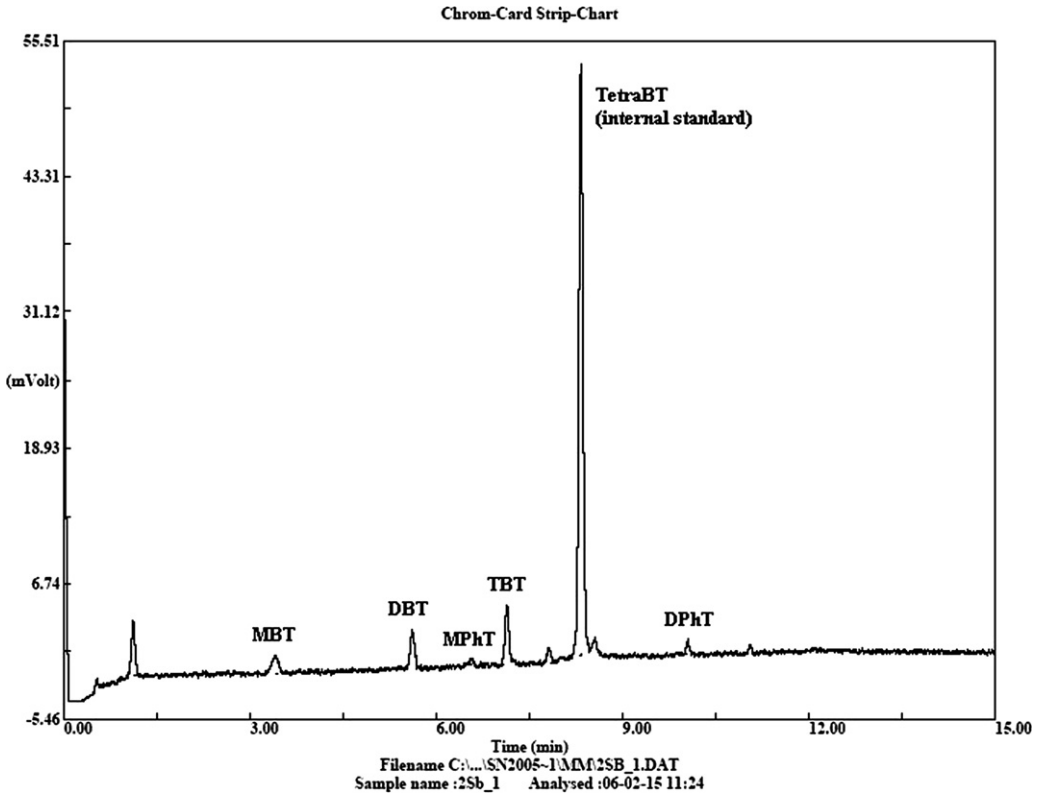

Fig. 2. The GC–MS chromatogram of solvent extract from mussels collected from polluted site, showing the organotin compounds. Abbreviations: Monobutyltintrichloride (MBT), dibutyltindichloride (DBT), monophenyltintrichloride (MPhT), tributyltinchloride (TBT), diphenyltindichloride (DPhT).

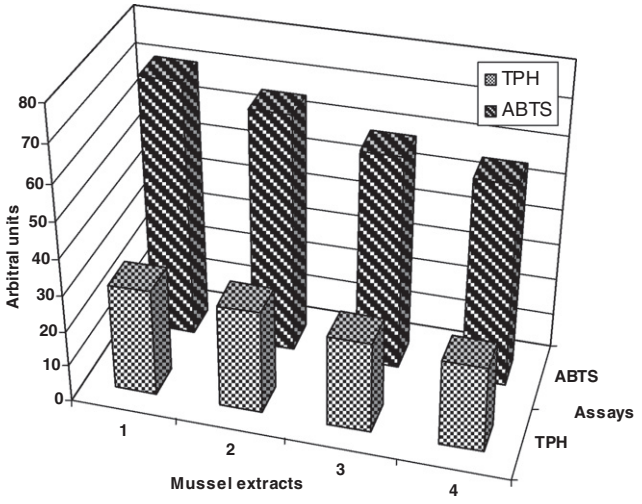

Fig. 3. Radical scavenging activity by [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS, mM TE kg^{-1} DW) and total polyphenols (TPH, $10^3 \times$ mg GAE kg^{-1} DW) in mussel extracts (1) from polluted site, extracted with 60% methanol/water in 1.2 M HCl; (2) from polluted site, extracted with 50% methanol/water in 1.2 M HCl; (3) from unpolluted site, extracted with 60% methanol/water in 1.2 M HCl and (4) from unpolluted site, extracted with 50% methanol/water in 1.2 M HCl.

once. Chrysene was detected only in mussels with very low values (average 0.74×10^{-3} mg kg^{-1} WW). In our report the concentration of chrysene was higher than in cited article. Sixteen polycyclic aromatic hydrocarbons in green mussels (*P. viridis*) were detected. The results showed that some of PAHs in green mussels significantly affected on the growth rate (mussels size) and type of tissues: small mussels > middle mussels > big mussels; mantle > visceral mass, while the content of PAHs in gill varied widely. Mussels easier concentrate the low molecular weight (MW) of PAHs. With mussels growing, the contents of the MW of PAHs were decreased, while the contents of the high MW of PAHs were increased slightly (Wang et al., 2005). Similar conclusions and numbers of accumulation of molecular weights were described by Bihari et al. (2007). The total concentrations of ten PAHs vary from below detection limit (from 49.2 to 134) $\times 10^{-3}$ mg kg^{-1} WW in mussel tissues. Mussels from majority of sampling sites tend to accumulate PAHs of lower MW. The PAH dynamic between different matrices is complex and site specific. The relationship between the total sum of PAHs and their contents in different marine matrices and their ability to affect mussels revealed specific interactions between an organism and complex mixture of toxic contaminants present in the marine environment (Bihari et al., 2007).

The relationship between the total sum of PAHs and their molecular weights was shown as well by Nieto et al. (2006). The concentrations of the sum of the 16 PAHs determined in the mussel samples collected at the sampling points were between 2.5 and 5.9 mg kg^{-1} DW.

A relation between parent PAHs accumulated in the mussels and their MW has been found to provide an indication of hydrocarbon pollution. The concentration of cyclopenta [cd]pyrene and benzo[ghi]fluoranthene increased from undetectable levels in reference mussels withdrawn from the Adriatic sea to 10–30×10^{-3} mg kg^{-1} DW in transplanted mussels. Other contaminants bioaccumulated by caged mussels included pyrene, fluoranthene and mercury (Fabbri et al., 2006). In the mussels of polluted sites pyrene and fluoranthene were detected (Table 1). PAHs composition pattern was dominated by the presence of PAHs with 3-rings (62%) followed from those with 4-rings (37%) and 5-rings (1%). Mediterranean mussels that did not present very high levels of contamination expressed as sum of PAHs showed one of

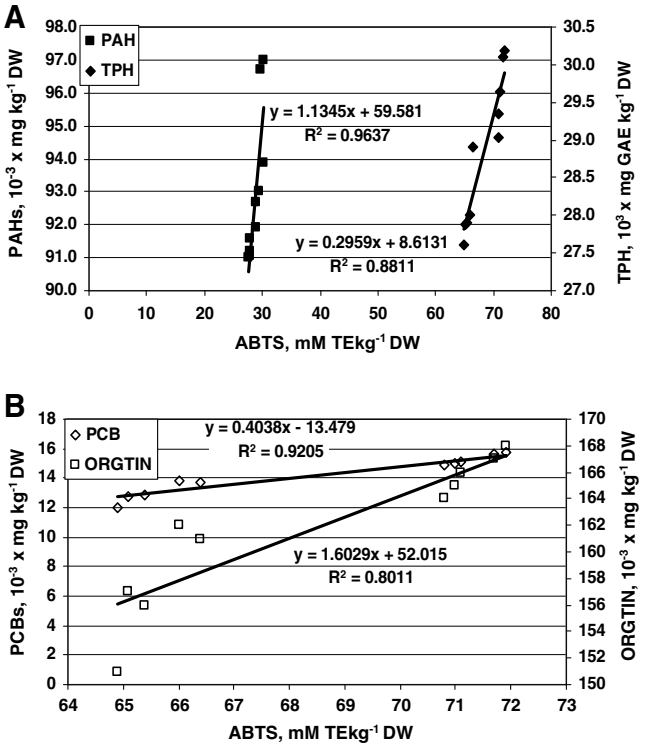

Fig. 4. Relationship, calculated by a linear regression analysis for mussel from polluted and unpolluted sites between polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organotins and antioxidants. A, (■) ABTS (mM TE kg^{-1} DW, X) and PAHs ($10^{-3} \times$ mg kg^{-1} DW, Y_1); (♦) ABTS (mM TE kg^{-1} DW, X); total polyphenols (TPH, $10^3 \times$ mg GAE kg^{-1} DW, Y_2). B, (□) ABTS (mM TE kg^{-1} DW, X) and PCBs ($10^{-3} \times$ mg kg^{-1} DW, Y_1); (◇) ABTS (mM TE kg^{-1} DW, X) and organotins (ORGTIN, $10^{-3} \times$ mg kg^{-1} DW, Y_2).

the highest values of benzo(a)pyrene equivalents (BaPEs) (Perugini et al., 2007). The conditions in the Mokpo Bay are different from the ones described by Perugini et al. (2007). Skarpheoinsdottir et al. (2007) found that the total PAH tissue levels in the mussels ranged between 40 and 11.67 $\times$ 10^{-3} mg kg^{-1} DW. PAH ratio values indicated that the PAHs were in most cases of pyrolytic origin, but with petrogenic input near harbours and an oil refinery.

Our results were similar to Kwon et al. (2006), who investigated the trophic transfer of PCB in zebra mussels *D. polymorpha* and found that total PCB levels were about 29–97 $\times$ 10^{-3} mg kg^{-1} WW. Zebra mussels were dominated by penta- and hexachlorine homologues and the average degree of chlorination of PCBs was 56.1%. Regression analysis indicated that PCB concentrations in mussels were significantly correlated with sediment concentrations, however, concentrations in mussels were several times higher than in surrounding sediment (Fang, 2004), but we have connected these concentrations with the antioxidative stress.

As we compare our obtained data with others (Mendoza et al., 2006) marine bivalves *P. purpuratus* turns out to be a good bioindicator of PCB levels in the coastal areas of Chile due to its wide distribution with total value of 298 $\times$ 10^{-3} mg kg^{-1} DW, which is 20 times higher than the obtained data. Future studies are needed to confirm our findings utilizing another environmental matrix such as collection of samples during different periods of time. Carro et al. (2005) reported that only PCB52 was correlated with depth, other ones such as 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180 were not correlated and only four (101, 118, 138 and 153) from this order were found in the present report. Milun et al. (2004) showed the amount of PCBs from 13.5 to 59.3 $\times$ 10^{-3} mg kg^{-1} DW. These data are in the same range as the obtained ones. Average contamination levels with organic compounds of PCBs in wild species are comparable to those measured in man-made cages and comparable to those shown by Andral et al. (2004). Maximum PCB (Neff et al. 2006) concentrations in mussels were measured in the SEKA (28.11 $\times$ 10^{-3} mg kg^{-1} DW) and the Dil Deresi River (25.68 $\times$ 10^{-3} mg kg^{-1} DW). Zebra mussel (*D. polymorpha*) accumulated PCBs and PAHs to a high degree with values reaching 800 $\times$ 10^{-3} mg kg^{-1} DW for PCBs (sum of 20 congeners), and 1,000 $\times$ 10^{-3} mg kg^{-1} DW of PAHs (sum of 14 compounds) in the whole body. These values are among the highest reported of PCBs and, to a lesser extent, of PAHs in other contaminated areas in the world (Minier et al., 2006). Concentrations of PCBs ranged from 0.79 to 64.9 $\times$ 10^{-3} mg kg^{-1} with an average 12.14 $\times$ 10^{-3} mg kg^{-1} WW.

The concentrations of organochlorines in fish species (*Euthynnus alleferatus*, *Scomberomorus commerson*, *Sphyraena Sphyraena*, *Diplodus vulgaris*, and *Alepes djedaba*) decreased in the following order: PCBs > DDTs > HCHs > total cyclodienes. Concentrations of DDTs in fish tissues ranged from 4.89 to 36.37 $\times$ 10^{-3} mg kg^{-1} WW with an average of 16.4 $\times$ 10^{-3} mg kg^{-1} WW. The concentrations of total HCHs ranged from 0.3 to 65.7 $\times$ 10^{-3} mg kg^{-1} WW with an average of 16.35 $\times$ 10^{-3} mg kg^{-1} WW pesticides and PCBs in all fishes were below the acceptable limit (Said, 2007). Contamination levels at Carteau are twice as high for PAHs (101.5 $\times$ 10^3 mg kg^{-1} DW) and PCBs (90.2 $\times$ 10^3 mg kg^{-1} DW) than La Fourcade. The seasonal contamination trend at Carteau showed six-fold higher levels of pyrolytic pollutants in winter (Bodin et al., 2004). Blue mussels (*M. edulis*) collected from 34 locations along the south and east coast of Korea was analyzed for PCBs and organochlorine (OC) pesticides (Khim et al., 2000). Maximum concentrations of PCBs and total OC pesticides were 98.5 and 20.5 $\times$ 10^{-3} mg kg^{-1} WW, respectively. The obtained data were in the accepted range.

There are many reports on PAHs, PCBs, but no data for comparison were found about the antioxidant activity, polyphenol content and the studied main pollution compounds. The influence of different concentrations of polyphenols on the interaction with pollutants in mussel *Unio tumidus* was reported only by Labieniec et al. (2003). Our previous and present results are in accordance with Labieniec et al (2003), based on the probability of interaction of pollutants with polyphenols. As it was mentioned the polyphenols are natural antioxidants and the antioxidant activity of the animals is based on their composition.

A good correlation was observed between the antioxidant activities determined by ABTS in total polyphenol extracts. Our results are corresponding with our data (Gorinstein et al., 2006) and Orbea and Cajaraville (2006), showing that the superoxide dismutase and glutathione peroxidase activities as well as the total antioxidant activity can be used as biomarker of pollution.

5. Conclusions

In the present report we studied the effects of seawater contaminants of Mokpo Sea oil on antioxidant levels in the whole tissue of the mussel, PAHs, PCBs and organotins.

The data presented in this report have shown that mussels to be used as biomarkers to establish physiological endpoints for chemical contaminant exposure. Bioindicator organisms are species used by environmental researchers to monitor the health of an environmental ecosystem. The biological species or group of species selected to serve this function should be able to influence the environmental integrity of the ecosystem in regard to its ecological role, population, or status. Bioindicator organisms are monitored for possible changes (chemical, physiological, or behavioral) within the ecosystem as a reflection of

environmental problems. The highly mobile organisms like fish may avoid pollution problems by escaping from the ecosystem of environmental concern. However, less mobile mussels basically stay in their environment, and may concentrate important ecosystem pollutants like PAHs and PCBs. Therefore they in a way reflect the environmental problems that the ecosystem faces. In the past, there have been cases where mussel species have been proposed as bioindicators (Goldberg, 1975), where the mussels have been regarded as a suitable bioindicator of marine health in marine biomonitoring programmes in regard to its ability of heavy metal bioaccumulation and other chemical contaminants. In the present study, other references are given to support this subject (Carro et al., 2005; Devier et al., 2005; Cajaraville and Ortiz-Zarragoitia, 2006).

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