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Total antioxidant capacity in the black mussel (*Mytilus galloprovincialis*) from Black Sea coasts

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Abstract

The aim of this investigation was to evaluate the total antioxidant radical scavenging capacity (TARSC) in tissue extracts for understanding biochemical adaptations involving the antioxidant defense system of a bivalve mussel, *Mytilus galloprovincialis*, sampled in polluted (Pol) and non-polluted (Npol) sites from Black Sea coasts. Antioxidant-rich polyphenol fractions were extracted from whole dry mussel tissue with methanol and water in different proportions. The extracts were screened for polyphenol content and their potential as antioxidants using various in vitro models, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), β-carotene-linoleate (β-carotene) and nitric oxide (NO$^{\bullet}$). The total polyphenol extract of the whole mussel tissue from polluted site (TPMEPol) showed the highest antioxidant capacity among all of the extracts with the tested methods and was comparable with the antioxidant capacity of butylated hydroxyanisole (BHA). The antioxidant capacities of this extract determined by DPPH$^{\bullet}$, β-carotene and NO$^{\bullet}$ methods and polyphenol content showed the highest correlation coefficients (R^2) such as 0.9985, 0.9915 and 0.9895, respectively.

The free polyphenol extracts (FPMEPol and FPMENPol) had lower antioxidant capacity than the two others of total polyphenols (TPMEPol and TPMENPol) with three scavenging methods. Responses were linear in all tested methods and the antioxidant capacity values of soluble antioxidants showed the following relative order: Trolox > BHA > TPMEPol > TPMENPol > FPMEPol > FPMENPol.

Nutritional antioxidants, such as polyphenols, were probably the main antioxidant contribution to mussel antioxidants. The mussel extracts from the polluted site exhibited higher antioxidant capacity than from the non-polluted one. The results presented in this report indicate that the antioxidant capacity of the whole tissue of the mussel extract could possibly be a useful biomarker for aquatic environments.

Keywords: mussels; polyphenols; antioxidant capacity; pollution

1. Introduction

It is well known that monitoring marine pollution is very important (Galloway et al., 2002; Regoli et al., 2002; Romeo et al., 2003; Sudaryanto et al., 2002) and various biomarkers have been proposed for this purpose (Bebianno and Serafim, 2003; Nasci et al., 2002; Porte et al., 2001; Riveros et al., 2002).

Several classes of environmental pollutants are known to enhance the intracellular formation of reactive oxygen species in marine invertebrates with different consequences on their antioxidant system. The overall efficiency of antioxidant system is not evaluated from

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such data despite the fact that variations in the endogenous levels of antioxidants may reveal biological effects induced by pollutants. The analysis of individual antioxidants can be useful for their sensitivity and for understanding the mode of action of a stressor. Superoxide dismutase, catalase, glutathione peroxidases and glutathione reductase (specially adapted enzymes), and vitamin E and β-carotene (free radical scavengers in membranes), ascorbic acid, uric acid and reduced glutathione (for the aqueous phase) were approached for an analysis of the efficiency of antioxidant system. It is possible to observe the contemporary depletion of some antioxidants together with the induction of others because antioxidants can greatly vary in their biosynthetic pathway, intracellular localization, chemical nature and mode of action and their response to stressors can be very different.

It was suggested some years ago that the role of polyphenols was minimal and only connected to the colour of plants. Antioxidants act as a cooperative network, employing a series of redox reactions. Interactions between ascorbic acid and glutathione, and ascorbic acid and phenolic compounds have been well described (Blokhina et al., 2003).

A large body of the literature supports the fact that dietary antioxidants such as phenols are useful in the prevention of oxidation (Fang et al., 2002; Gorinstein et al., 2002; Singh et al., 2002; Velioglu et al., 1998; Vinson et al., 2001). Most phenolic compounds exhibit interesting antioxidant properties. The formation of reactive oxygen species (ROS) is prevented by an antioxidant system: low molecular mass antioxidants (glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A, and polyphenols), enzymes regenerating the reduced forms of antioxidants, and ROS-interacting enzymes such as superoxide dismutase (SOD) enzymes, peroxidases and catalases. In plant tissues, many phenolic compounds (in addition to tocopherols) are potential antioxidants: flavonoids, tannins and lignin precursors may work as ROS-scavenging compounds.

Some work on this topic has been done in bivalve mussels. Digestive glands of the mangrove mussel *Mytella guyanensis*, collected at one non-polluted and two polluted sites, had different antioxidant defenses (Torres et al., 2002). Cheung et al. (2002) showed that the apparent lack of correlation between trace organic pollutants and some of the enzymatic antioxidants may be due to the inhibitory effects caused by the chemicals. Antioxidative defenses have also been studied in blue mussels *Mytilus edulis* L. (Sukhotin et al., 2002; Walker et al., 2000). Adult mussels of the species *Perna perna* (Wilhelm Filho et al., 2001) were analysed for antioxidant and detoxifying enzymes. Partial characterization of *Mytilus galloprovincialis* was done by Cavallo and Stabili (2002) and Orbea et al. (2002).

The total oxyradical scavenging capacity assay (TOSCA) in mussels measures the biological resistance to various kinds of oxyradicals, thus providing useful indications to predict oxyradical-medicated adverse effects on the physiological condition of the organisms (Regoli et al., 1998; Regoli, 2000; Regoli et al., 2002; Winston et al., 1998). The integration with the analysis of the total antioxidant capacity provided a more holistic assessment of the overall biological significance of some variations (Regoli et al., 2002).

In the cited literature, most of the proposed methods for antioxidant determination of mussels as potential biomarkers are based on the water-soluble enzymes. The approach of extracted polyphenols as the main antioxidants has not been used for these purposes. In our recent investigations, the antioxidant scavenging capacity of different products was determined using the total radical-trapping antioxidative potential (TRAP) test (Gorinstein et al., 2002). However, TRAP is a relatively unspecific marker of free radical scavenging capacity in alcohol solutions, because only water extracts could be preferably used for measurements in this assay.

It can be interesting to isolate the polyphenols from whole tissue of the investigated mussel samples and to test the extracts for their 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging capacity (Cos et al., 2002). The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) solution-based chemical assay has been widely used in plant extracts as well for the determination of antioxidant radical scavenging capacity in pure marine natural products (Singh et al., 2002; Takamatsu et al., 2003).

Polyphenols also have a protective function against oxidative damage, and singlet oxygen was very powerfully quenched by β-carotene. β-Carotene, analysed spectrophotometrically, has been widely used in model systems to investigate the radical scavenging capacities of several natural compounds such as phenolic compounds, anthocyanins, or crude mixtures such as methanol extracts of plants (Singh et al., 2002; Velioglu et al., 1998). Nitric oxide (NO$^{\bullet}$) interacts with oxygen to produce stable products, nitrite, and nitrate. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous or alcohol solutions have been assayed spectrophotometrically (Marcocci et al., 1994).

In the present investigation for the determination of the total antioxidant radical scavenging capacities (TARSC) in polyphenol extracts at the same concentration as the samples from whole mussel tissue, more specific methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), β-carotene-linoleate (β-carotene) and nitric oxide (NO$^{\bullet}$) were used for comparison purposes. As far as is known such investigations have not already been done in mussels.

The aim of the present report was to evaluate TARSC in polyphenol extracts from whole tissue of

mussels for understanding biochemical adaptations involving the antioxidant defense system of *Mytilus galloprovincialis* in polluted and non-polluted sites. Polyphenols as the main antioxidant contribution to mussels were determined and the correlation was found between these compounds and the antioxidant capacity in mussels from polluted and non-polluted sites.

2. Materials and methods

2.1. Collection of mussel samples

Samples of mussels (*Mytilus galloprovincialis*) were collected in two regions of the Black Sea Coast: an ecologically non-polluted area (Cape Galata) and a polluted site (the area of Port Varna) characterized by high environmental pollution. Monitoring data were collected for plant nutrients (nitrate + nitrate, orthophosphate and silicate), dissolved oxygen, chlorophyll and surface sediments (Shtereva et al., 1998). Total and individual aliphatic and polycyclic aromatic hydrocarbons (T-PAHs), polychlorinated biphenyls (PCBs), phenols in water and sediments, and pesticides from two locations in Port Varna and Cape Galata have been analysed (Moore et al., 1998; Shtereva, 2001; Report on environmental assessment impact, 1999). Cape Galata showed increased retention times in conjunction with relatively low inputs of biochemical oxygen demand (BOD) and oil (Shtereva et al., 1998). Port Varna received the highest loading of BOD, oil, total suspended sediment (TSS) and metals (Balashov et al., 1998; Moore et al., 1998). Port Varna was extremely highly polluted because it was a closed area while other ports were open to the sea, therefore the concentrations of total PAHs were much lower in other places than in Port Varna (Balashov et al., 1998). The most important indices of polluted (Port Varna) and non-polluted (Cape Galata) sites are shown in Table 1.

Results collected in the Varna region were in correspondence with other regions (Porte et al., 2001; Okay et al., 2001; Orbea et al., 2002). The data proved the existence of a pollution gradient between sampling sites and a chronic exposure to petrogenic and pyrolitic hydrocarbons, especially in Port Varna. Benzo (a) pyrene hydroxylase (BPH) activity did not show any difference among sampling sites (Moore et al., 1998).

Mussels from both regions were characterized by a similar maximum length and the size of analysed organisms (4.37 ± 0.5 cm) was 75–85% of the maximum size reached within each population. This approach guaranteed that mussels compared had similar metabolic conditions and the influence of physiological differences between two populations was less pronounced (Regoli, 2000). The samples were designated as follows: NPol = non-polluted site and Pol = polluted site.

Table 1
Main indices of pollution in Port Varna and Cape Galata[a]

Indices	Port Varna	Cape Galata
T-PAHs, ng g^{-1}	253.2	94.4
PH_{sed}, mg kg^{-1}	12.59	1.10
PH_w, mg l^{-1}	0.40	0.11
PCBs, ng g^{-1}	1565.0	243.1
Ph_w, mg l^{-1}	0.030	0.016
Ph_{sed}, mg kg^{-1}	0.12	0.08
Pes, μg kg^{-1}	0.619	0.301
BOD, ml l^{-1}	6.1	2.7
Cu_w, μg l^{-1}	7.1	4.2
Fe_w, μg l^{-1}	70.1	43.2
As_w, μg l^{-1}	5.0	1.3
Zn_w, μg l^{-1}	13.1	5.0
Pb_w, μg l^{-1}	13.2	4.1

[a] Data taken from Balashov et al. (1998), Moore et al. (1998), Shtereva (2001), Shtereva et al. (1998), and Report on environmental assessment impact (1999).

T-PAHs, total polycyclic aromatic hydrocarbon; PH_{sed}, petroleum hydrocarbons in sediment; PH_w, petroleum hydrocarbons in water; PCBs, polychlorinated biphenyl; Ph_w, phenols in water; Ph_{sed}, phenols in sediment; Pes, pesticides; BOD, biochemical oxygen demand.

2.2. Reagents

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid) was purchased from Aldrich Chemical Co. (Milwaukee, WI). 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), β-carotene, Greiss reagent (modified, G4410), sodium nitroprusside and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

2.3. Preparation of tissue extracts

Whole tissue from 30 specimens of each population were rapidly frozen in liquid nitrogen and stored at −80 °C and then freeze-dried in glass flasks on Finn-Aqua, Lyovac GT-2 equipment for 36 h. Following the approach of the present study that polyphenols were the main components which affected the total antioxidant capacity in mussels, polyphenols were extracted from the whole dry tissue, using two solvents: 50% methanol/50% water and 50% methanol/50% water/1.2 M HCl at 90 °C (Vinson et al., 2001). Dry matter of whole tissue from mussel were defatted with acetone (1:1) for 24 h at −20 °C for further extraction of polyphenols and then air-dried. Defatted samples were extracted from a 50 mg aliquot with 5 ml of 50% methanol/50% water and the sample was vortexed for 1 min and heated at 90 °C for 3 h with vortexing every 30 min. After the samples were cooled, they were diluted to 10 ml with methanol and centrifuged for 5 min at 4000 g with a bench top centrifuge to remove solids (Free Phenol Mussel Extract—FPME). The second extract was obtained with 5 ml of 1.2 M HCl in 50% methanol/50% water (Total Phenol

Mussel Extract—TPME) and treated as described above (Vinson et al., 2001). Aliquots of extracts were stored in liquid nitrogen and examined for each assay of polyphenol content and antioxidant capacities.

2.4. Determination of total polyphenol content and spectrophotometric measurements

Aliquots from two extractions were used for determination of total polyphenols by the Folin–Ciocalteu method and the values were read at 675 nm (Singleton and Rossi, 1965). TPMEPol, TPMENPol, FPMEPol and FPMENPol mussel samples in concentration of 2 mg ml^{-1} in 0.01 mM phosphate buffer were scanned in the range (nm) of 200–500. The antioxidant capacities of the extracts were determined by three scavenging radical methods. In all methods the same concentration of mussel extract was measured in order to compare the results obtained.

2.5. Total antioxidant radical scavenging capacity (TARSC) using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) method

Five millilitres of a 0.1 mM DPPH methanol solution was added to 2 mg ml^{-1} of the two methanol extracts (TPME and FPME) from mussels of polluted and non-polluted regions. The tubes were allowed to stand at 27 °C for 20 min to complete the reaction. The control was prepared as described above without any extract, and MeOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. For comparison, BHA standard was used. The total antioxidant radical scavenging activity was expressed as the inhibition percentage: % TARSC = (control OD − sample OD/control OD)100 (Singh et al., 2002).

2.6. Antioxidant assay using β-carotene-linoleate model system (β-carotene)

β-Carotene (0.2 mg) in 0.2 ml of chloroform, linoleum acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed (Singh et al., 2002; Velioglu et al., 1998). Chloroform was removed at 40 °C under vacuum, and the resulting mixture was diluted with 10 ml of water and mixed well. Forty millilitres of oxygenated water was added to this emulsion. Four millilitre aliquots of the emulsion was pipetted into different test tubes containing 2 mg ml^{-1} of mussel extracts and BHA in methanol. A control containing 0.2 ml of methanol and 4 ml of the above emulsion was prepared. The tubes were placed at 50 °C in a water bath, and the absorbance at 470 nm was taken at zero time ($t = 0$). Measurement of absorbance was continued until the colour of β-carotene disappeared in the control tubes ($t = 135$ min) at an interval of 15 min. A mixture prepared as described above without β-carotene served as blank. The total antioxidant radical scavenging capacity of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula: $\%\ \mathrm{TARSC} = 100[1 - (A_0 - A_t)/(A^{\circ}_0 - A^{\circ}_t)]$, where A_0 and A°_0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and A_t and A°_t are the absorbance measured in the test sample and control, respectively, after incubation, and kinetics was monitored during 135 min. Trolox and BHA were used as standards.

2.7. Scavenging capacity against nitric oxide ($NO^{\bullet}$) test

A 0.5 ml portion of a mixture (2 mg ml^{-1} of extract and 0.1 ml of sodium nitroprusside solution) was diluted with 0.3 ml of Greiss reagent for one step analysis of nitrite (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotination of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with the Greiss reagent (Marcocci et al., 1994).

2.8. Statistical analysis

Data are reported as mean ± standard deviation. The analysis of variance and least significant difference tests were conducted to identify differences between means, while a Pearson correlation test was conducted to determine the correlations between means. Statistical significance was declared at $p < 0.05$.

3. Results

3.1. Determination of polyphenol content

The maximum peak in polyphenol extracts TPMEPol, TPMENPol and FPMEPol was near 280 nm, and the UV spectra were typical of polyphenols (Fig. 1, curves 1–3). The maximum peak slightly shifted to the right side only in FPMENPol (Fig. 1, curve 4). The amount of polyphenols in the polluted site (Fig. 1, curves 1 and 3) was higher than in the non-polluted one (Fig. 1, curves 2 and 4).

The highest values of polyphenols (μg gallic acid/100 g DW) were observed in polluted samples: TPMEPol (615.3 ± 50.5) and FPMEPol (301.8 ± 26.1) in comparison with the non-polluted: TPMENPol (360.2 ± 30.2) and FPMENPol (254.2 ± 23.4) and decreased in the following order: TPMEPol > TPMENPol > FPMEPol > FPMENPol (Figs. 1 and 2).

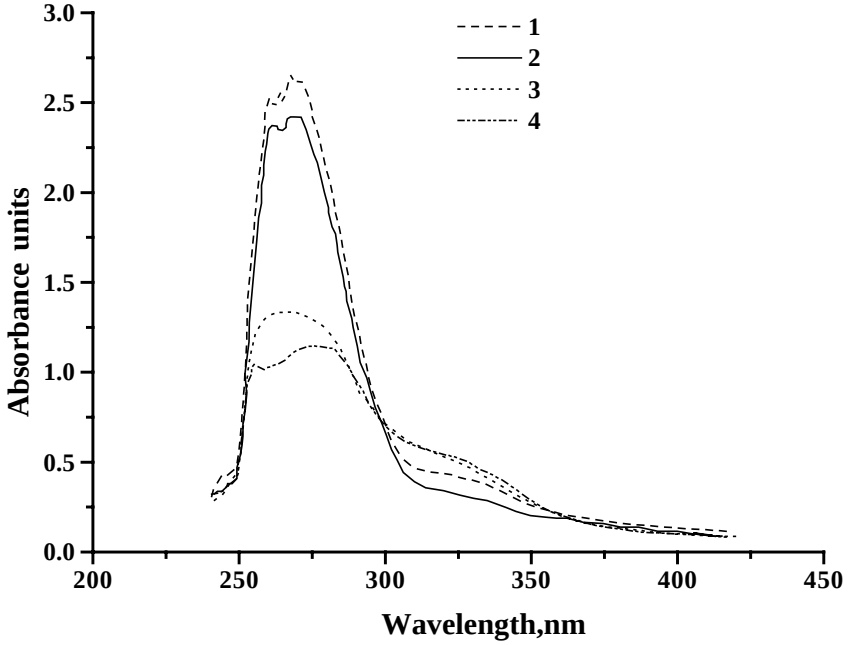

Fig. 1. Spectrophotometric measurements of TPMEPol (1), TPMENPol (2), FPMEPol (3) and FPMENPol (4). TPME, total polyphenol mussel extract from polluted region with 50% methanol/50% water/1.2 M HCl; FPME, free polyphenol mussel extract with 50% methanol/50% water; NPol = mussels from non-polluted region and Pol = polluted region.

3.2. Total antioxidant radical scavenging capacities (TARSC)

The relative amounts of polyphenols and the antioxidant capacities are shown in Fig. 2. The highest antioxidant capacity was found in TPMEPol with DPPH•. The relative proportions of radical quenching activities of NPol group with the maximum of DPPH• were 1:0.95:0.66 against β-carotene and NO•, while the Pol groups showed 1:0.98:0.66 against β-carotene and NO•.

Fig. 3 presents the % of total remaining scavenging radical DPPH• of total (TPME) and free (FPME) phenol mussel extracts. The DPPH• scavenging effect of TPMEPol at a concentration of 2 mg ml^{-1} was higher than those of the other extracts, but showed significantly lower radical scavenging effect than that of Trolox at the same concentration ($p<0.05$). The scavenging effect of TPMEPol was nearly equal to that of BHA at the end point of 20 min. Trolox showed higher antioxidant capacity than BHA of about only 0.1% of remaining DPPH. TPMEPol, Trolox and BHA were similar especially at 20 min, showing that TPMEPol had the same activity as BHA and Trolox. FPMEPol was lower than TPMENPol of about 13%, showing the highest position on the graph and the lowest antioxidant capacity (Fig. 3).

The antioxidant TPMEPol sample and BHA had similar reaction kinetics curves against β-carotene (Fig. 4) and were very close to each other. The difference in the antioxidant activity of these two samples was about 7.9%. The mussel extracts of TPMEPol and TPMENPol at 2 mg ml^{-1} with β-carotene showed 86.5% and 58.7% antioxidant capacities, respectively. TPMENPol was less effective in quenching free radicals in this system in comparison to TPMEPol and the difference in their antioxidant activities was about 27.8. The average kinetic curve of Pol group (Fig. 4) showed higher scavenging effect than the kinetic curves of NPol samples. BHA (94.4%) compared to other samples was close to Pol sample (Fig. 4). The results of this kinetic study showed that the antioxidant activities of the group of samples from the polluted area were much higher (1.5–2 times) than in the non-polluted one.

The NO• (Fig. 2) scavenging effect of TPMEPol sample (58.3%) was higher than those of the other extracts, but was significantly lower than that of Trolox (66%) at the same concentration ($p<0.05$). The NO• scavenging effects of TPMENPol and FPMEPol were nearly equal to that of BHA (40%). The best correlation found (Fig. 5A) was between polyphenols in TPME and DPPH• ($R^2 = 0.999$) and (Fig. 5C) β-carotene ($R^2 = 0.992$), followed by the NO• (Fig. 5E) method ($R^2 = 0.989$). Relatively high correlations were found (Fig. 5B) also between polyphenols in FPME and DPPH• ($R^2 = 0.985$) and (Fig. 5D) β-carotene ($R^2 = 0.981$), followed by NO• (Fig. 5F) method ($R^2 = 0.980$).

4. Discussion

This study was conducted to evaluate the total antioxidant radical scavenging capacities of mussel

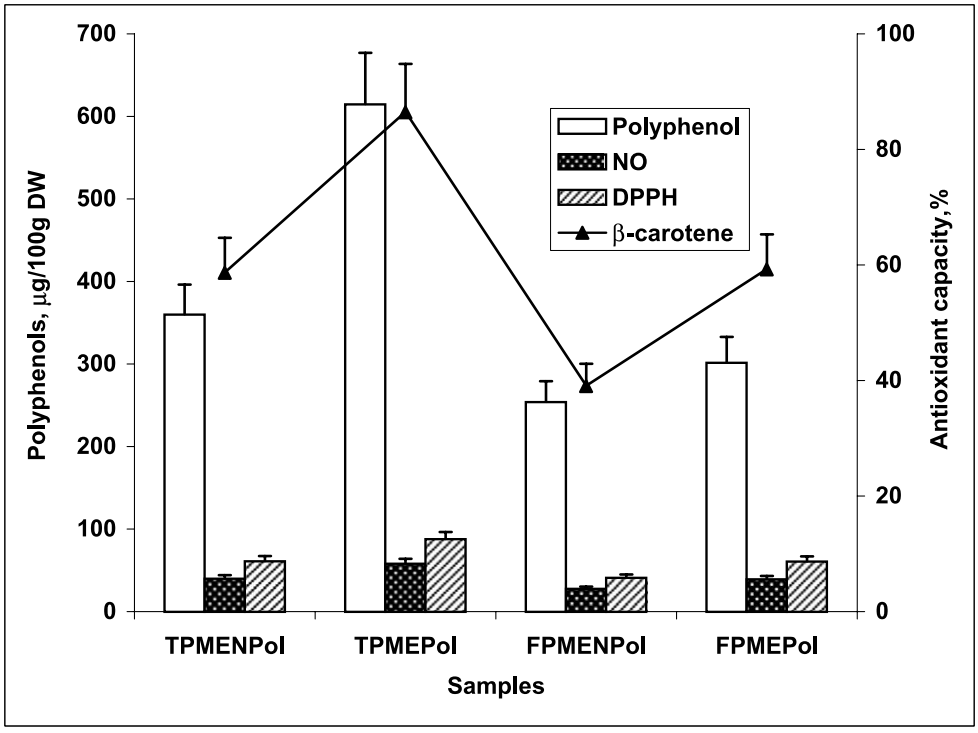

Fig. 2. Quantitative comparison of total radical scavenging capacities and the polyphenols in the mussel extracts. DPPH• = 1,1-diphenyl-2-picrylhydrazyl, β-carotene = β-carotene-linoleate system; NO• = nitric oxide.

extracts from whole tissue and their use as a marker for pollution. All tested samples from the polluted area showed significantly higher scavenging capacities against DPPH•, β-carotene and NO•, whilst samples from non-polluted area had the lowest capacities against those radicals.

A novel approach for determination of total oxy-radical scavenging capacity in phenol extracts of whole dry tissue of mussel *Mytilus galloprovincialis*, as a new index of biological resistance to oxidative stress, is shown in the present study, compared to other investigations (Regoli et al., 1998; Gaspic et al., 2002; Lionetto et al., 2003; Monirith et al., 2003; Romeo et al., 2003).

All polyphenol extracts examined showed strong antioxidant capacity in the DPPH• assay, but were

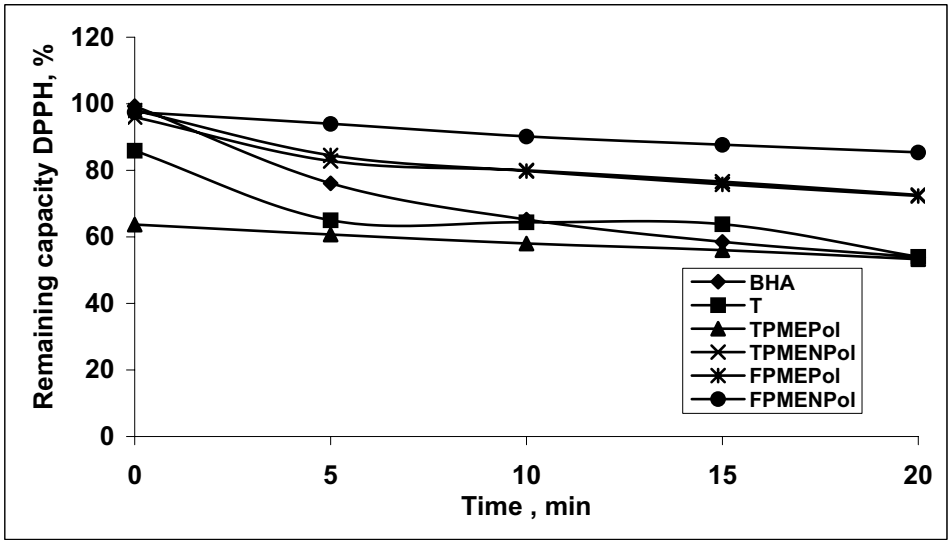

Fig. 3. Kinetics of DPPH• scavenging effects of extracts from: TPMEPol, TPMENPol, FPMEPol, FPMENPol, BHA and T. BHA, butylated hydroxyanisole; T, Trolox, 6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid.

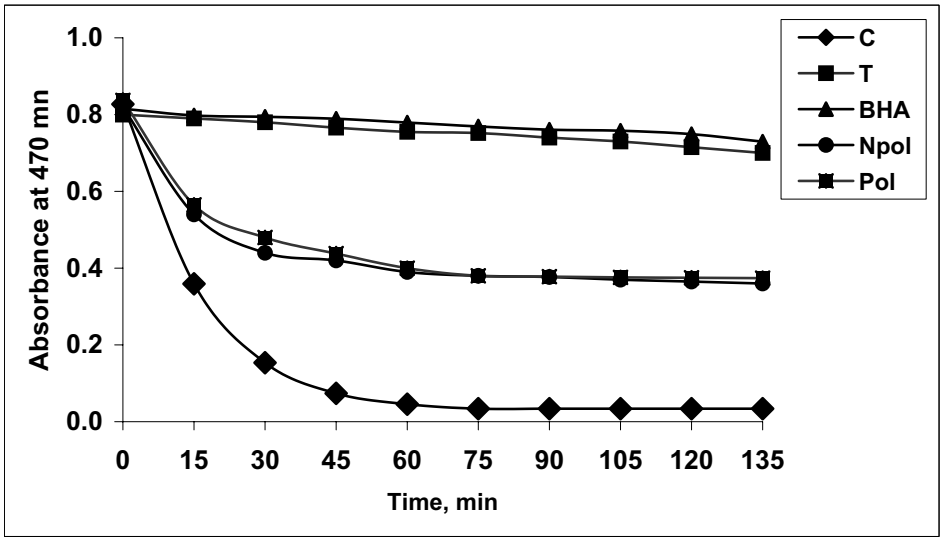

Fig. 4. Reaction kinetics of TPME with β-carotene: TPMEPol, TPMENPol, BHA and C, control.

relatively less active in NO• assay. These results were in accordance with others that marine natural products were more active to different radicals (Takamatsu et al., 2003). The different relative radical scavenging capacity of individual mussel extract against different testing radicals may be explained by the different mechanisms involved in the radical–antioxidant reactions. Other factors, such as stereoselectivity of the radicals or the solubility of the mussel extracts in different testing systems, may also affect the capacity of the mussel extract to react and quench different radicals. Mussels were exposed to high environmental levels of chemical pollutants and were subjected to increased intracellular flux of oxyradicals mainly produced by the Fenton reaction in the presence of transition metals or by the redox cycle of several organic compounds. The mechanisms involved in the beneficial actions of antioxidants in biological systems included directly quenching of free radicals to terminate the radical chain reaction, chelating transition metals to suppress the initiation of radical formation, acting as reducing agents, or stimulating the antioxidative defense enzyme activities.

Presently, there are no data in the literature about the antioxidants in polyphenol mussel extracts. Therefore, these results showing the reaction of different scavenging radicals with the polyphenol mussel extracts from polluted and non-polluted sites could only be compared with the observations of Torres et al. (2002), where mussels were analysed for antioxidant and detoxifying enzymes and their differences in polluted and non-polluted sites. With the exception of superoxide dismutase, the activities of catalase, glutathione reductase, glutathione S-transferase and glutathione peroxidase were higher at the polluted sites, where thiobarbituric acid-reactive substance (TBARS) and 8-oxo-7, 8-dihydro-2′-deoxyguanosine levels were enhanced at the polluted sites. The use of different relative activity of used radicals in the present report could be supported by other studies where selected oxidants (peroxyl radicals, hydroxyl radicals, peroxynitrite and others) and biomarkers of oxidative stress were used (Lionetto et al., 2003; Power and Sheehan, 1996; Wilhelm Filho et al., 2001). The present results can also be compared with other reports about this mussel (Cavallo and Stabili, 2002; Da Ros et al., 2000; Livingstone et al., 1995; Moore et al., 1998; Mourgaud et al., 2002; Nasci et al., 2002; Porte et al., 2001; Regoli et al., 1998), or with *Mytilus edulis* L. and some other species (Cheung et al., 2002; Gaspic et al., 2002; Livingstone et al., 2000; Riveros et al., 2002; Sukhotin et al., 2002; Walker et al., 2000). Compared to vitamin E (Trolox) and BHA, well-known synthetic antioxidants, mussel extracts showed greater DPPH• radical quenching capacity, although they differed from each other in their relative capacities. In the present report extracts from dry whole tissues of mussel were used to evaluate their overall antioxidant capacity. The present approach of the antioxidant capacity of mussels was based on the amount of polyphenols extracted from their whole tissue with methanol extraction. This did not mean that this approach was better than the others (Galloway et al., 2002; Lionetto et al., 2003; Livingstone et al., 2000; Mourgaud et al., 2002; Nasci et al., 2002), but it could be an additional evaluation of the polluted areas. The present results were in correspondence with Regoli et al. (1998, 2002), Regoli (2000) and Winston et al. (1998), showing that mussels from both polluted and non-polluted sites exhibited different relative efficiency in scavenging various forms of oxyradicals. In spite of the use of the water- and lipid-soluble antioxidants and extraction of separated parts of mussels such as glands and gills (Regoli et al., 1998, 2002; Regoli, 2000; Winston

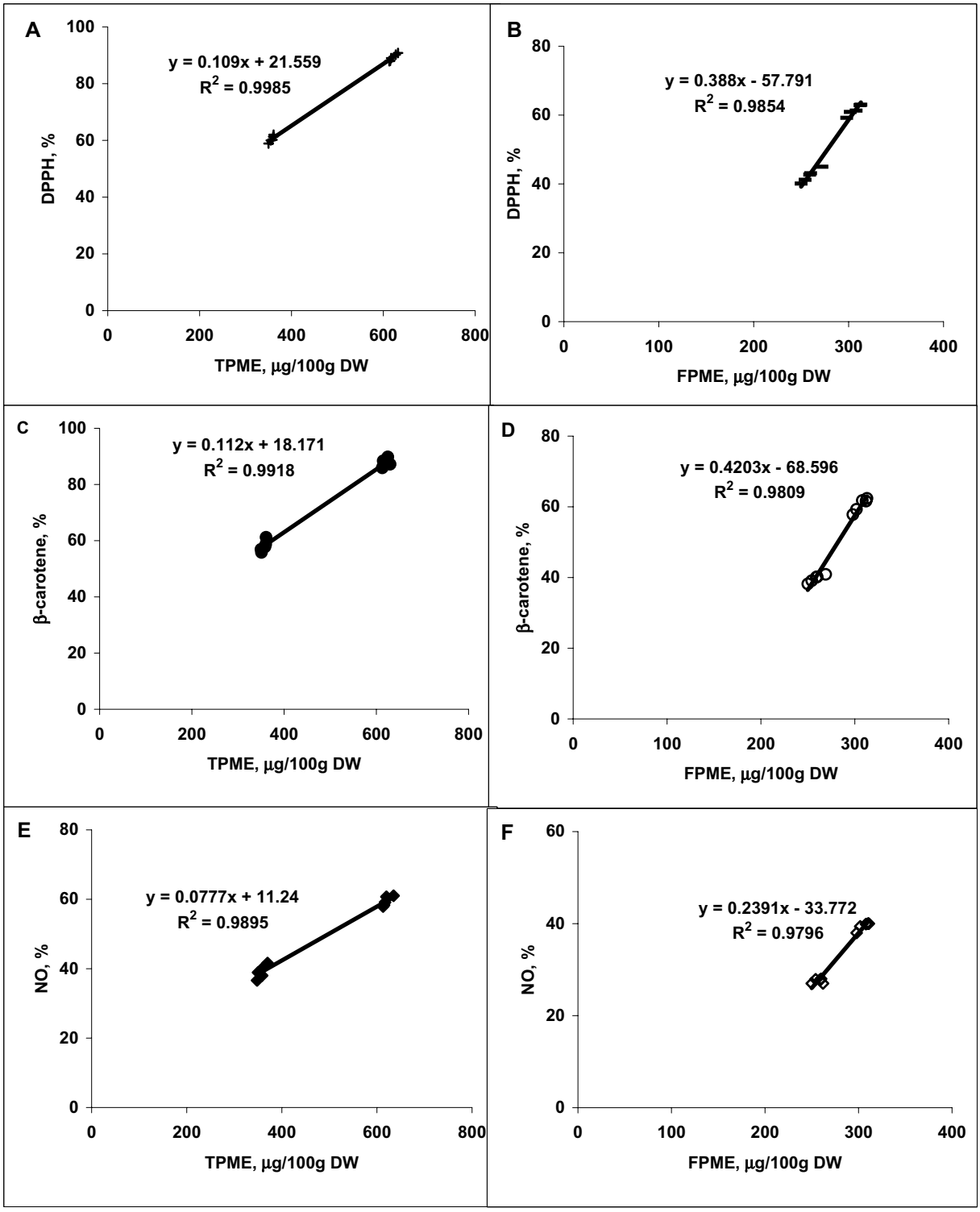

Fig. 5. Relationship, calculated by linear regression analysis for mussel extracts between: (A) (+) TPME (µg/100 g DW, *X*) to DPPH• scavenging effect (%, *Y*) and (B) (−) FPME (µg/100 g DW, *X*) to DPPH• scavenging effect (%, Y). (C) (●) TPME (µg/100 g DW, *X*) to β-carotene bleaching effect (%, *Y*) and (D) (○) FPME (µg/100 g DW, *X*) to β-carotene bleaching effect (%, *Y*). (E) (♦) TPME (µg/100 g DW, *X*) to NO• (%, *Y*) and (F) (◇) FPME (µg/100 g DW, *X*) to NO• (%, *Y*). Polyphenols, (µg gallic acid/100 g dry weight (DW).

et al., 1998), there was a conceptual similarity between the proposed methods in the present study with TOSCA that antioxidants determined in mussels could be used for the characterization of pollution. The difference between the two approaches is the following: in the present report the alcohol-soluble antioxidants were screened for their capacity. Responsiveness of antioxidants to pollutants is difficult to predict and a high degree of variability has

been reported as a function of class of chemicals, kind of exposure phase of the biological cycle. Variations in the levels or activities of antioxidants are potential biomarkers which may reveal a contaminant-mediated biological effect on the organisms (Porte et al., 2001). *Mytilus galloprovincialis* is routinely exposed to an oxidative stress condition at the polluted site, and considering xenobiotic bioaccumulation in bivalve molluscs, mussels represent excellent bioindicators for environmental monitoring studies.

The study of whole soft dried parts of mussels, implied that different tissues with different antioxidant capacities were mixed together, without distinguishing what is happening in a particular tissue, for instance, in the digestive gland or gills. Such evaluation of the overall antioxidant capacity gives a rapid estimation for the pollution contamination. Similar findings were reported by Torres et al. (2002) in digestive glands of the mangrove mussel *Mytella guyanensis*, collected at one non-polluted and two polluted sites, as well as by others (Da Ros et al., 2000; Power and Sheehan, 1996; Regoli, 2000; Winston et al., 1998).

In conclusion, nutritional antioxidants such as polyphenols were measured in this study in order to describe a whole and more reliable picture concerning antioxidant defenses in different animal species, especially in those filter-feeding with high bioaccumulation capacity. $DPPH^{\bullet}$, β-carotene and $NO^{\bullet}$ radical scavenging methods gave relatively similar results with high correlation between total polyphenols and antioxidant capacities and were suitable for the investigated samples. The total antioxidant capacity of mussel extracts as here determined for the first time by these methods, showed that polyphenols probably play a major role in comparison with other antioxidant components such as proteins, metals and the antioxidant capacities well correlated with the quantity of polyphenols. This confirms other studies (Regoli, 2000; Mourgaud et al., 2002) which showed that the total scavenging capacity acted towards different forms of oxyradicals and can be a useful biomarker for aquatic environments.

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