RESIDUES AND TRACE ELEMENTS

Determination of PAHs, PCBs, Minerals, Trace Elements, and Fatty Acids in *Rapana thomasiana* as an Indication of Pollution

JACEK NAMIESNIK
Gdańsk University of Technology, Chemical Faculty, Department of Analytical Chemistry, 80 952 Gdańsk, Poland
PIOTR SZEFER
Medical University of Gdansk, Faculty of Pharmacy, Gdańsk, Poland
MAGDALENA SLIWKA-KASZYNSKA
Gdańsk University of Technology, Chemical Faculty, Department of Organic Chemistry, 80 952 Gdańsk, Poland
SNEJANA MONCHEVA
Institute of Oceanology, Bulgarian Academy of Sciences, 9000 Varna, Bulgaria
PATRICIA ARANCIBIA-AVILA and FERNANDO TOLEDO
Universidad del Bio-Bio, Department of Basic Sciences, Chillan, Chile
KYUNG-SIK HAM and SEONG-GOOK KANG
Mokpo National University, Department of Food Engineering, Muan, South Korea
SHELA GORINSTEIN[1]
The Hebrew University—Hadassah Medical School, School of Pharmacy, The Institute for Drug Research, Jerusalem, Israel

The aim of this investigation was to introduce several analytical methods for determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), minerals, trace elements, and fatty acids in *Rapana thomasiana* as a marine pollution indicator organism. The chemical differences of the gastropod *Ra. thomasiana* from polluted and nonpolluted sites of the Black Sea on the Bulgarian coast were investigated. Chromatography and high-resolution inductively coupled plasma/MS analyses were used for evaluation of PAHs, PCBs, fatty acids, minerals, and trace elements. These methods can be applied to other marine products.

Chemical components of different animals have been used as bioindicators of marine pollution (1–3). Therefore, new analytical methods for their detection and characterization are important.

There have been many investigations concerning application of chromatography and different types of extraction concerning the mussels *Mytilus galloprovincialis* (4–7). Direct extraction was used for determination of fatty acids (8) with derivatization (9) in shellfish. Evaluation of metals in fish, vegetables, and grains was based on ultrasonic-assisted acid digestion (10, 11), and in mussels (12) it was done using inductively coupled plasma (ICP)-atomic emission spectrometry. New approaches (13) for extraction, isolation, identification, and quantification of individual congeners/isomers of polychlorinated biphenyls (PCBs) in general and in food matrixes were reported (14). Evaluation was made of microwave-assisted extraction for the analysis of PCBs (15) in fish and 16 polycyclic aromatic hydrocarbons (PAHs) in water, sediment, and mussels (16). Matrix solid-phase microextraction (SPME) for measuring freely dissolved concentrations and chemical activities of PAHs in sediment cores from the western Baltic Sea (17) in food products (18) and the value of standards (19) were discussed. However, *Rapana thomasiana* has been investigated less often (20–23). Therefore, in this research, the chemical differences of *Ra. thomasiana* from polluted (RTP) and nonpolluted (RTN) sites of the Bulgarian Black Sea were studied by chromatography and high-resolution (HR) ICP/MS analyses for evaluation of PAHs, PCBs, minerals, trace elements, and fatty acids. Several analytical methods were introduced.

As far as we know, no results of such investigations have been published.

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[1] Corresponding author's e-mail: gorin@cc.huji.ac.il

Experimental

Chemicals and Standards

Enanthic acid, capric acid, myristic acid, linoleic acid, palmitic acid, oleic acid, stearic acid, arachidic acid (>98% pure), -bromoacetophenone (>99% pure), and triethylamine (>99% pure) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (ACN) and tetrahydrofuran (THF)—used in the mobile phase (HPLC grade)—and dichloromethane, methanol, and cyclohexane were purchased from Merck (Darmstadt, Germany). All solvents used as

reaction media were of analytical grade and were obtained from POCh (Gliwice, Poland).

Silica gel (40 m) was from Mallinckrodt Baker (Phillipsburg, NJ). A mixture of 16 compounds from the PAH group, with a concentration of each compound of 2000 g/mL, and standard solutions of seven PCBs in isooctane, each with a concentration 100 g/mL, were from Restek Corp. (Bellefonte, PA). Certified solutions of naphthalene-d8 and benz(a)anthracene in dichloromethane with concentrations of 2000 g/mL, and PCB 209 certified standard in acetone with a concentration of 200 g/mL, were obtained from Supelco (Bellefonte, PA).

Sample Collection

Samples of *Ra. thomasiana* were collected from an ecologically nonpolluted site (Galata Station, 3 miles offshore Varna Bay) and from a polluted site (Varna Bay) at a sea depth of 3–4 m. The sampling was carried out in late July to early August 2006. The samples were collected as previously described (5, 7). The collected *Rapana* and mussels from both polluted and nonpolluted sites were characterized by a similar maximum length and size of analyzed organisms: 75–85% of the maximum size reached within each population. This approach guaranteed that compared *Rapana* had similar metabolic conditions, and the influence of physiological differences between the two populations was less pronounced (24).

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 (GEA Process Engineering, Inc., Columbia, MD) for 36 h.

Determination of the Metal and Mineral Contents of the Studied Bioactive Compounds

Trace metals and minerals were determined as follows: around 0.5 g lyophilized sample was weighed and transferred to PTFE-Teflon vials (18 mL). Subsequently, 3.25 mL ultrapure water (ELGA Labwater, Veolia Water Systems, Ltd, Marlow, UK) and 3.25 mL concentrated HNO_3 (Scanpure, Elverum, Norway) were added to the vessels. Digestion of these portions was carried out in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany) according to a temperature profile that increases gradually from room temperature up to 250 C within 1 h. In addition, there was a cooling step, which allowed temperature to return to the initial value within approximately 1 h. After cooling to room temperature, the digested samples were diluted with ultrapure water to achieve a final acid concentration of 0.6 M. HR-ICP/MS analyses were performed using a Thermo Finnigan model Element 2 instrument (Bremen, Germany). The radio frequency power was set to 1400 W. The samples were introduced using a SC-FAST flow injection analysis system (ESI, Omaha, NE) with a peristaltic pump (1 mL/min). The instrument was equipped with a concentric Meinhart nebulizer connected to a Scott PFA spray chamber, platinum skimmer and interface cones, and a quartz burner with a guard electrode (Thermo Finnigan Ltd, Cambridge, UK). The nebulizer argon gas flow rate was adjusted to give a stable signal with maximum intensity for the nuclides ^{7}Li, ^{115}In, and ^{238}U. Methane gas was used in the analysis to minimize interferences from carbon and to provide enhanced sensitivity, especially for Se and As. The instrument was calibrated using 0.6 M HNO_3 solutions of matrix-matched multielement standards. A calibration curve consisting of five different concentrations was made from these standards. To check for the instrument drift, one of these multielement standards was analyzed for every 10 samples. The accuracy of the method was verified by analyzing the standard reference material Oyster Tissue NIST 1566b (Table 1; National Institute of Standards and Technology, Gaithersburg, MD). To assess possible contamination during sample preparation,

Table 1. Concentrations of chemical elements (certified and obtained) in reference material Oyster Tissue (NIST 1566b)

Element	Declared value, g/g DW	Obtained value, g/g DW	Recovery, %	RSD, %[a]
Se	2.06	2.228	109	6
Zn	1424	1644	115	6
Ca	838	884	106	4
Cd	2.48	2.72	107	5
Sn	0.031	0.031	100	17
Hg	0.0371	0.0367	99	5
Pb	0.308	0.319	104	4
Th	0.0367	0.037	100	7
U	0.255	0.265	104	3
B	4.5	5.6	123	6
Na	3297	2454	74	64
Mg	1085	1157	107	5
Al	197.2	190	96	7
S	6887	7447	108	6
V	0.577	0.605	105	5
Mn	18.5	19.8	107	5.4
Fe	205.8	218	106	5
Co	0.371	0.39	106	6
Ni	1.04	1.08	104	7
Cu	71.6	75.97	106	5
Rb	3.262	3.4	105	5
Sr	6.8	7.1	105	6
Ag	0.666	0.674	101	4
Sb	0.011	0.009	86	15
Ba	8.6	8.9	104	3
K	6520	6769	104	5
As	7.65	8.121	106	9

[a] n = 11.

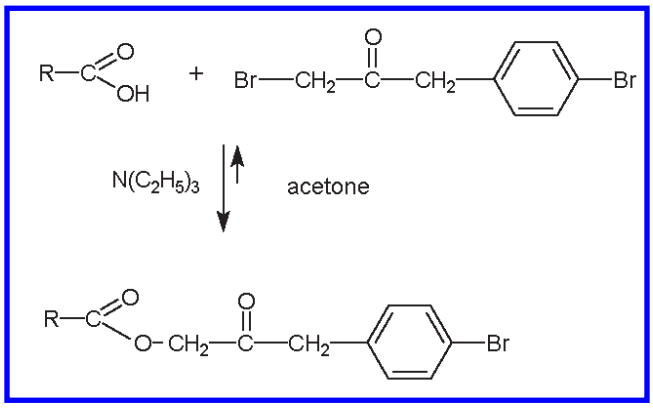

Figure 1. Scheme of the free fatty acid derivatization reaction.

blank samples of HNO_3 and ultrapure water were prepared using the same procedure as for the samples. Because most of the blanks were negligible, instrument detection limit was preferably used for calibration of LOD.

Determination of Fatty Acids

Chromatographic analyses were performed using a Series 1200 HPLC system (Agilent Technology Inc., Wilmington, DE) equipped with quaternary pump, autosampler, thermostated column compartment, and diode-array detector (DAD). Analytes were dissolved in ACN–acetone (1 + 1, v/v), and 5 L of this solution was injected into the chromatographic column. A gradient of A: water and B: ACN–THF (99 + 1, v/v) was used as the mobile phase at a flow rate of 2.0 mL/min. The gradient of %B at 5 min was 80%; at 8 min, 85%; and at 20 min, 98%. The total run was 22 min. The UV detector was operated at 258 nm (DAD in the single-wavelength mode). All analyses were thermostated at 40 C.

The concentrations of fatty acids in the studied samples were calculated using fatty acid standards and an internal standard (hexadecanoic acid) as a measure of extraction yield. The LOD was calculated as an S/N of 3, while the LOQ was defined as 10 times the noise level.

(**a**) *Sample preparation.*—Lyophilized *Rapana* samples (approximately 150 mg) were hydrolyzed with 1 mL 2 M KOH in methanol (MeOH)–H_2O (1 + 1, v/v) at 80–85 C for 1.5 h in PTFE-lined, screw-capped 5 mL amber tubes. After cooling, the hydrolyzates were acidified with 4 M HCl (approximately 0.5 mL) to pH of approximately 2, and the free fatty acids were extracted twice with 1 mL *n*-heptane. The upper organic layer was separated and dried with Na_2SO_4, then heptane was removed under a gentle stream of nitrogen at 40 C. The residue was used for derivatization as described below.

(**b**) *Derivatization procedure.*—Fatty acid standards (0.5–100 g/mL) and fatty acids released by saponification from biological lipid extract were converted to fatty acid *p*-bromophenacyl ester derivatives (Figure 1). To the residue in a PTFE-lined, screw-capped 1.5 mL amber tube, 200 L -bromoacetophenone solution (10 mg/mL in acetone) and 200 L triethylamine solution (10 mg/mL in acetone) were added. The contents were ultrasonicated and heated for 30 min at 50 C in an ultrasonic bath. The resulting solutions were evaporated to dryness under a gentle stream of nitrogen at 40 C. A 250 L volume of ACN–acetone (1 + 1, v/v) was added to the tube. The resulting solution was filtered and injected onto the column.

PAH and PCB Determination

(**a**) *Sample preparation.*—The freeze-dried samples (1 g) were hydrolyzed in 15 mL 4 M 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 MeOH–water (9 + 1, v/v). The rinsings were added to the separatory funnel. The sample digest was extracted with 10 mL cyclohexane by hand shaking for 5 min, and the two layers were allowed to separate. The MeOH–water (bottom) layer was transferred into a second funnel, and the extraction step was repeated with 10 mL cyclohexane. After discarding the MeOH–water layer, the cyclohexane extracts were combined and washed with 10 mL MeOH–water (1 + 1, v/v). The extract was evaporated down to approximately 0.5 mL under a stream of nitrogen. For sample cleanup, a short column packed with silica gel (0.5 g) was used. The extract introduced into the column was eluted with 8 mL dichloromethane. The eluate volume was reduced to 0.3 mL under a stream of nitrogen. The extract prepared in this way was injected into the GC/MS system.

(**b**) *Gas chromatographic analysis.*—All experiments were performed using an Agilent Technologies Model 5975C gas chromatograph equipped with a mass spectrometric detector and on-column injector. The conditions of GC/MS, which was performed in the selected-ion monitoring (SIM) mode, were the following: injection system, spilt–splitless with automatic sample introduction; volume of sample injected, 2 L; inert gas, helium–70 kPa; the interface temperature, 280 C; column, Rtx–5MS (30 m 0.25 mm id, 0.25 m film thickness); detector, Agilent Technologies 5975C with the electron ionization, working in the SIM mode; integration software, MSD ChemStation; and temperature program, 40 to 120 C (40 C/min), to 280 C (5 C/min), held at 280 C for 12 min. The analytes in each sample were identified by matching the retention time of each peak with the retention times of external standards.

Statistical Analyses

The results of this investigation are reported as means SD of three measurements. Differences between groups were tested by two ways analysis of variance. Linear regressions were also calculated. P values of <0.05 were considered significant.

Table 2. Concentration of minerals and trace elements in *Rapana* from two sampling sites

Elements	RTN[a] Concentration, g/g DW	RTN[a] RSD, %	RTP[b] Concentration, g/g DW	RTP[b] RSD, %
Se	2.56	3.1	2.90	1.7
Zn	117.3	2.7	156.8	2.5
Ca	12910	2.1	5890	3.2
Cd	13.36	3.8	16.74	2.9
Mo	0.19	8.7	0.34	1.7
Sn	0.18	3.7	0.18	2.0
Cs	0.014	2.4	0.016	2.3
Au	0.0069	2.1	0.0021	11.4
W	0.009	7.5	0.017	5.1
Hg	0.199	0.5	0.251	1.5
Tl	0.003	5.4	0.003	2.9
Pb	0.71	0.5	0.83	2.0
Bi	0.022	0.2	0.022	1.0
Th	0.021	1.2	0.030	0.7
U	0.063	2.7	0.084	1.8
Li	0.21	20.9	0.45	3.0
Be	0.0065	22.9	0.0055	35.3
B	3.36	3.6	4.29	4.0
Mg	8478	5.6	10670	6.6
Al	95.2	4.9	119.2	5.7
Si	213	2.6	293	4.7
P	6867	6.1	7947	2.5
S	9868	9.2	10829	9.1
Cl	6740	5.9	9050	1.5
Sc	0.022	10.7	0.028	9.4
Ti	3.42	8.4	3.98	12.2
V	0.34	5.1	0.43	8.2
Cr	1.12	1.3	0.71	4.0
Mn	9.52	4.9	9.99	1.2
Fe	303	3.0	438	3.5
Co	0.32	3.7	0.46	2.9
Ni	1.10	1.1	0.77	2.8
Cu	32.80	1.8	32.49	1.9
Ga	0.023	12.0	0.030	13.8
Rb	3.50	1.2	3.67	3.8
Sr	57.67	1.4	39.76	3.4
Ag	0.56	2.9	0.90	5.7
Sb	0.014	9.8	0.013	15.0
Ba	0.90	1.8	1.28	4.3
K	5223	4.3	5470	2.0
As	19.55	3.2	22.24	1.0
Br	130	2.0	223	3.4
Nb	0.008	49.5	0.009	34.2
Y	0.096	3.3	0.138	1.7
Zr	0.042	14.0	0.045	3.3
La	0.16	2.3	0.19	2.8
Ce	0.25	2.3	0.31	2.5
Pr	0.029	4.2	0.037	2.6
Sm	0.026	5.1	0.033	1.6
Tb	0.004	3.5	0.005	3.3
Dy	0.011	7.9	0.014	1.0
Er	0.008	1.7	0.011	1.6
Tm	0.001	5.3	0.001	6.4
Yb	0.005	3.9	0.008	6.6
Hf	0.0012	3.0	0.0013	5.8

[a] RTP = *Ra. thomasiana* from polluted site.
[b] RTN = *Ra. thomasiana* from nonpolluted site.

Results and Discussion

Minerals and Trace Elements

In this investigation, the chemical differences of RTP and RTN sites of the Bulgarian Black Sea were studied. The results of the determination of minerals and trace elements are shown in Table 2. Sixty elements were quantified for each *Rapana* sample. All elements were detected in the samples, except Ir and Ru. Six elements were very abundant (Ca, Mg, P, S, K, and Cl); eight were not abundant (Al, Zn, Fe, Cu, Sr, As, Se, and Cd); and 23 were trace elements (Cs, Pt, Au, W, Tl, Bi, Th, U, Be, Sc, Ga, Nb, Ta, Zr, Pr, Sm, Tb, Dy, Er, Tm, Yb, Lu, and Hf). *Rapana* from polluted sampling sites contained higher levels, than nonpolluted, of the following trace elements: Li (2 times higher); W (1.9 times higher); Mo (1.8 times higher); Ag (1.6 times higher); Ba (1.4 times higher); Zn (1.5 times higher); Ni (1.3 times higher); Cd (1.3 times higher); and Hg (1.3 times higher). Pb, Cd, Hg (MeHg), organic Sn, As (inorganic compounds of As), Tl, Ni, and Cr(VI) are the main elements for pollution characterization. Some essential elements, for instance, Zn and Cu, can be anthropogenic in origin, and their elevated levels are considered to be toxic for biota. Such high levels of the elements mentioned above, especially Li, W, and Mo (at a polluted compared to a nonpolluted station), can be attributed to external environmental parameters (e.g., anthropogenic factors) and/or internal biological parameters (gender and size of *Rapana* specimens). The influence of these internal factors can be eliminated as a source of variations between both sampling sites when these two populations studied

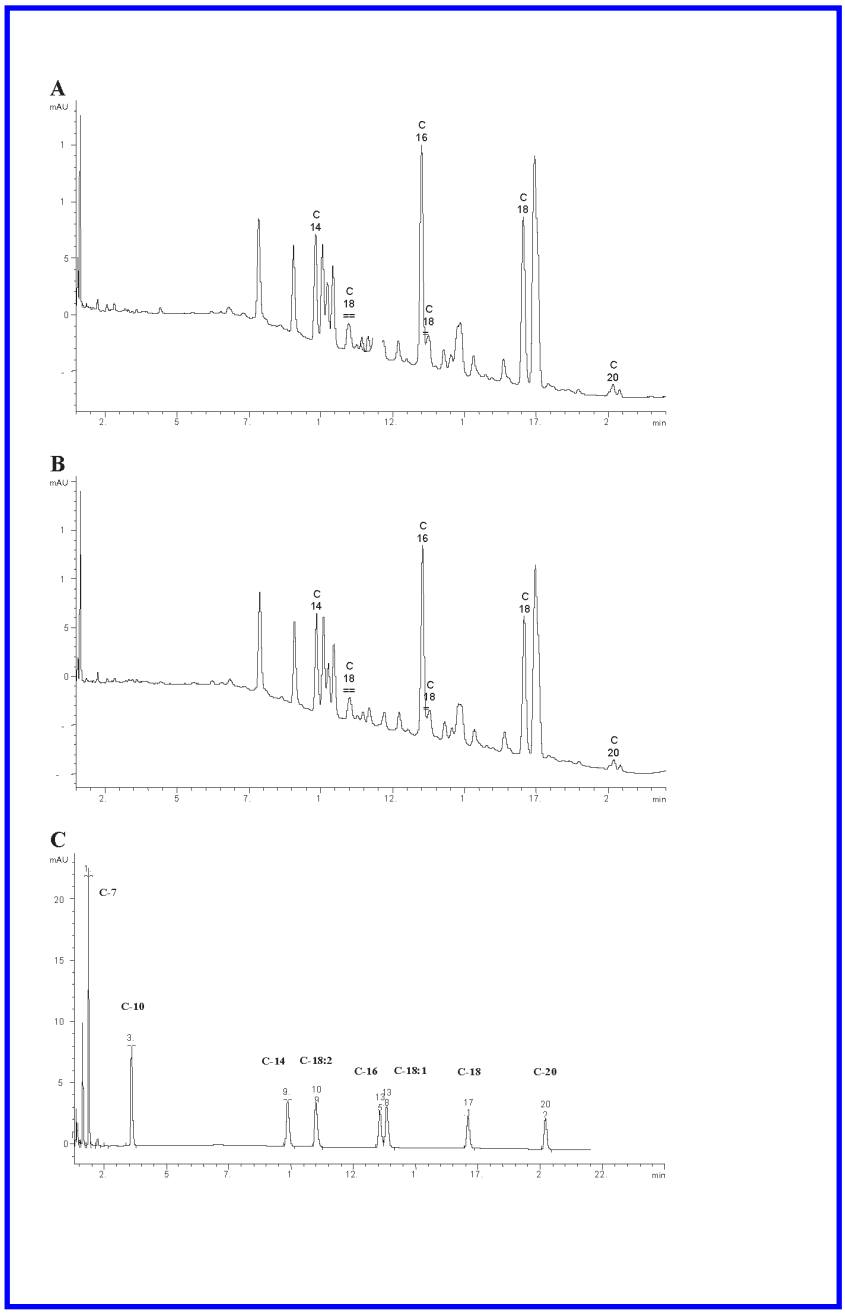

Figure 2. Chromatograms of *Rapana* samples: (A) RTP; (B) RTN; (C) typical chromatogram of fatty acids standard solution: C-7 enanthic acid, C-10 capric acid, C-14 myristic acid, C18:2 linoleic acid, C-16 palmitic acid, C-18:1 oleic acid, C-18 stearic acid, and C-20 arachidic acid.

(originating from nonpolluted and polluted sampling sites) consist of a comparable number of specimens belonging to the same sex and size class. If this is not the case, then only chemometric analysis can give response to this question (21, 22).

Since *Rapana* is edible, it is possible to compare the data obtained for chosen nutritive and toxic elements with recommended daily allowance (RDA) and provisional tolerance weekly intake values, respectively. The macroelements Ca, Mg, K, Na, P, Cl, and S and the microelements Zn, Cu, Mn, Fe, Cr (III), Co, Se, Mo, I, F, and B are important for nutrition.

Permissible concentrations of toxic metals (Hg, Pb, and Cd) have not been exceeded relative to U.S. Food and Drug Administration and U.S. Environmental Protection Agency safety levels in regulations and guidance (http://www.cfsan.fda.gov/~comm/haccp4x5.html), even for Cd with its high tissue levels, but are below permissible levels (4.0 mg Cd/kg clams, oysters, and mussels). However, according to Polish Regulations of the Ministry of Health (referred to edible kidney of mammals), the concentration

Table 3. Fatty acid composition of the biological samples

Rapana samples	Fatty acid, g/g DW[a]													
	Enanthic acid C 7:0	Capric acid C 10:0	Myristic acid C 14:0		Palmitic acid C 16:0		Stearic acid C 18:0		Oleic acid C 18:1		Linoleic acid C 18:2		Arachidic acid C 20:0	
RTN	ND[b]	ND	47.82	0.40	146.31	19.09	114.22	4.05	9.68	0.16	18.45	1.80	13.56	1.61
RTP	ND	ND	50.76	1.62	138.41	3.77	106.49	3.21	9.63	0.19	17.09	0.51	13.88	0.49

[a] Mean SD (n = 3).
[b] ND = Not detected.

of Cd in *Rapana* has been somewhat exceeded. Based on RDAs for essential elements (Ca, Mg, K, Se, Cu, Zn, Mn, and Fe), according to the Food and Agriculture Organization (http://www.fao.org/docrep/T7799E/t7799e00.htm #Contents), *Rapana* tissue is potentially a good source of nutritive elements such as Zn, Mg, and especially Se and Cu. Our results were in accordance with other reports (22).

One gastropod (*Ra. thomasiana*) and one mussel (*M. galloprovincialis*) were collected from two sites along the coastline of the Bulgarian Black Sea for the investigation of heavy metal contaminations. Cd, Co, Cu, Ni, Pb, and Zn were analyzed by using pressure nebulization-ICP/MS.

Ultrasound-assisted pseudodigestion for toxic metals followed by electrothermal atomic absorption spectrophotometry was developed for As, Cd, and Pb. The result obtained by the optimized method showed good agreement with the certified values of Table 1 and sufficiently high recovery. RSD values were 1.21, 5.52, and 5.32% for As, Cd, and Pb, respectively, which agreed with our data (11). Our results are comparable with others (10, 11), where mostly Cd, Cr, Ni, and Pb were determined; other trace elements, such as As, Al, Cr, Cu, Fe, Mn, and Zn, were estimated, as well (12). Value assignment of nutrient concentrations in five standard reference materials and six reference materials was discussed (19), showing the values of their elemental composition (major, minor, and trace elements), but no additional nutritional information was provided, mostly based on SRM 1566b Oyster Tissue, which we have used in our determination of the metals. One standard reference material, Oyster Tissue NIST 1566b, was used to validate the methods; the obtained results proved to be in good agreement with the certified values. The results of the present study showed that *Ra. thomasiana* possessed a much greater ability for bioaccumulation of Cu and Zn than did other species. Since Cu(II) is the strongest complex-forming metal cation among the first-row transition metals according to the Irwing-Williams order, it probably formed strong complexes with the carboxylate and amine groups of biological ligands and showed a high affinity for binding.

Ra. thomasiana manifested the most bioaccumulation capacity of Cd. Among the five species, *Ruditapes philippinarum* possessed the highest content of Ni. Furthermore, Cd, Cu, and Zn contents in some gastropod and oyster samples exceeded the maximum permissible levels established by the World Health Organization (WHO). Due to their special bioaccumulation capacity of Cd and Ni, *Ra. venosa* and *Ru. philippinarum* had the potential of being used as biomonitors to control the aquatic contaminations of heavy metals. It was found that the contents of minerals and trace elements in *Ra. thomasiana* from RTP are higher than from RTN.

The highest differences were of Ca and Cd. Other investigators also found that the contents of Cd, Pb, Cu, and Zn in shells of *M. galloprovincialis* and *Ra. thomasiana* collected from polluted sites of the Romanian Black Sea coast are higher (21). A high concentration of Zn in the *Mytilus* (22.99 g/g) and *Rapana* (16.82 g/g) samples compared to the other samples was observed. In the *Rapana* samples, a lower concentration of Cu (5.88 g/g) and the highest concentration of Cd (0.94 g/g) were detected.

The distribution characteristics of the heavy metal elements and their relation to its growing environment were studied in *Ra. venosa* from the shallow sea of northern Liaotong Bay (23, 25). Our results were in correspondence with these studies in the determination of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn, and other heavy metal elements by ICP/MS. The results show that the *Ra. venosa* tends to absorb and reserve heavy metal elements, as does *Ra. thomasiana*.

Fatty Acids

The HPLC profiles (Figure 2 and Table 3) revealed that the major unsaturated acid was linoleic, while the main saturated

Table 4. Determination of PAHs (ng/g DW) in *Rapana* samples

	RTN		RTP		LOQ	LOD
Naphthalene	130	36	329	50	1	0.3
Acenaphthene	20	10	34	10	1	0.3
Fluorene	29	34	21	5	1	0.3
Phenanthrene	131	33	119	14	2	0.6
Anthracene	5	3	4	3	2	0.6
Fluoranthene	61	26	45	25	2	0
Pyrene	25	18	20	14	2	0.6

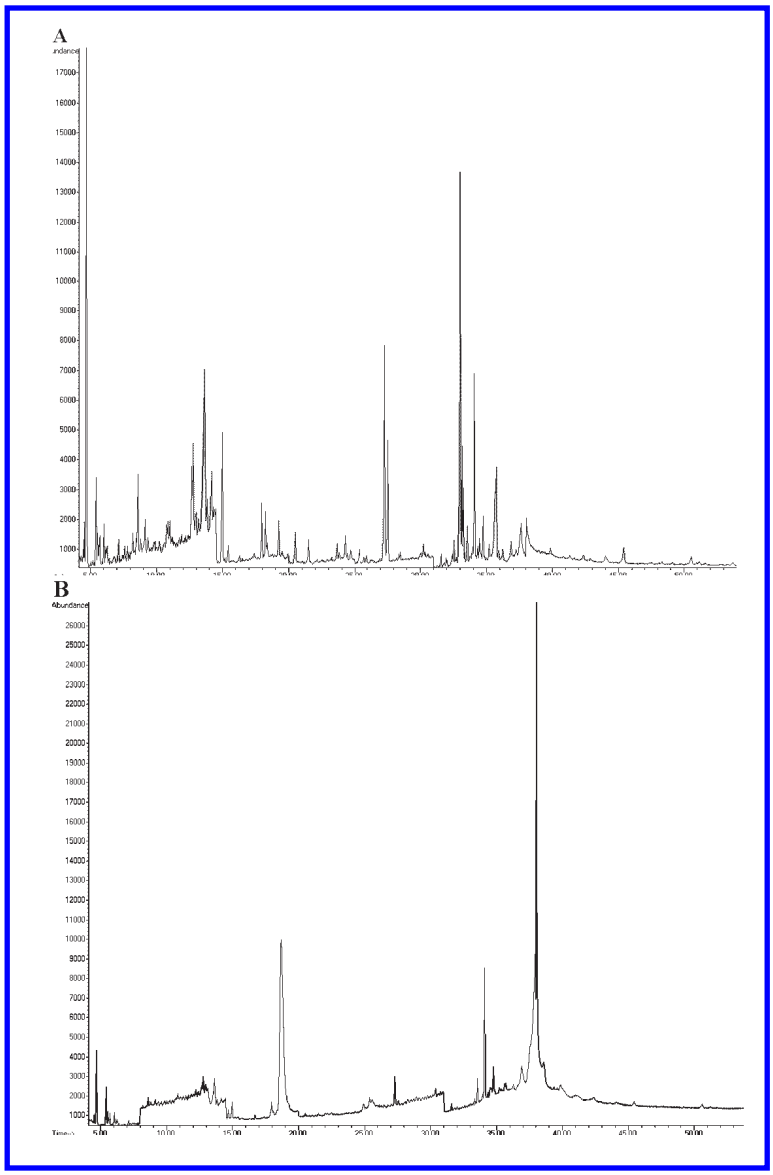

Figure 3. GC/MS chromatogram of PAHs from a solvent extract of *Rapana* samples: (A) RTN; (B) RTP; naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene (peaks left to right).

acid was palmitic. Myristic and stearic acids were found, as well. Palmitic (16:0), stearic (18:0), and linoleic (18:2) acids were among the major fatty acids throughout the *Rapana* samples. The levels of palmitic, stearic, and linoleic fatty acids were significantly different ($P < 0.05$) among the *Rapana* samples.

As was reported in other research, a simplification of the determination of fatty acids was based on extraction/methylation with methanolic hydrogen chloride (8). Our results are not in correspondence with other results (9), where derivatization in electrospray ionization and HPLC/MS was not applied. The LOQs of the method ranged from 60 to 560 g/g wet weight and were similar to our results. Our results correspond with other results (26), where in the mollusk *M. galloprovincialis* L. and the gastropod *Ra. venosa* were determined fatty acids as sources of biologically active substances that can have different applications, including as food additives. Various saturated, mono-, di-, and polyunsaturated fatty acids were present in the extracts (Table 3). Saturated fatty acids, including 14:0, 16:0, and 18:0, were found in different quantities in the lipid extracts but accounting for a similar saturated fatty acid content from two sites of *Rapana* samples. These saturated fatty acids were higher than 35% of the total fatty acid content in comparison with the cited literature (26). Similarly, monounsaturated acid (16:1n-7, 18:1n-9, and –10, 20:1n-9) content was also analogous (around 30% of the total fatty acid content) for both marine species (26).

The composition and distribution of fatty acids in two mollusk species, *Ra. thomasiana* and *M. galloprovincialis*, was determined (20). The lipids were extracted from the meat of the mollusks. Then, the main classes of lipids from the

Table 5. Determination of PCBs (ng/g DW) in *Rapana* samples

Compound[a]	RTN	RTP	LOQ	LOD
PCB 28	—[b]	—	1	0.3
PCB 52	5 3	—	1	0.3
PCB 101	13 5	—	1	0.3
PCB 118	7 4	—	1	0.3
PCB 153	27 14	28 6	1	0.3
PCB 138	26 14	24 10	1	0.3
PCB 180	4 3	10 5	1	0.3

[a] PCB 28 = 2,4,4 -Trichlorobiphenyl; PCB 52 = 2,2 ,5,5 -Tetrachlorobiphenyl; PCB ,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; PCB 180 = 2,2 ,3,4,4 ,5,5 -Heptachlorobiphenyl.

[b] — = Below LOQ.

obtained extracts were separated and purified by column chromatography. The obtained lipid fractions were analyzed by GC after a specific treatment. The obtained results showed high content of essential fatty acids (especially in linoleic and arachidonic acids). The authors concluded that the two species of mollusks have a special therapeutic and nutritive potential. Our results were similar to the data of other authors (27) who investigated variation in the fatty acid content in five species of Korean shellfish. Relative percentages of the composition of fatty acids remained constant within the same species regardless of their geographical regions, which are shown as well in Table 3 and Figure 2.

PAHs

The results of the determination of the PAH concentrations in *Ra. thomasiana* are shown in Table 4 and Figure 3. Of the 16 PAHs, only seven were determined; the total sum of the seven PAHs [ng/kg; dry weight (DW)] were, for the samples from nonpolluted site, 401 ng/kg DW; and from the polluted site, 572. The individual PAHs (ng/kg DW) ranged from 130 36 to 5 3 for the nonpolluted site, and 329 50 to 4 3 for the polluted site (Table 4).

Among the seven detected PAHs, the predominant ones were naphthalene, phenanthrene, and fluoranthene, which accounted for 80.3% from the nonpolluted site and 86.2% from the polluted site.

PAHs were found in animals from polluted and nonpolluted sites; the amount of naphthalene in polluted site samples was about 2.5 times higher than in nonpolluted site samples. Other individual PAHs were comparable in these samples. Sixteen PAHs were determined in mussels. The analysis of mussels was undertaken using Soxhlet extraction, ultrasonic extraction, and pressurized liquid extraction, and the performances of several cleanup steps were compared. Alkaline digestion was necessary to remove coextracted compounds, and the method gave acceptable recoveries and LOD values of 0.5–7.7 g/kg dry mass, as for sediments. In all cases, analysis was performed by GC/MS, and internal standard quantification was performed using five deuterated PAHs (16).

Sediment-pore water partitioning of PAHs was studied in sediment cores of a dumping area in the western Baltic Sea and compared to a reference site. Freely dissolved concentrations of nine PAHs were measured in sediment samples using SPME, a cost- and time-efficient method with detection limits in the lower ng/L range (17).

PCBs

Among six detected PCBs was the highest one, PCB153, which was about 47% of the total sum (Table 5). In nonpolluted samples, only six PCBs were detected and the highest was PCB 153, which accounted for about 33% of the total amount of PCBs. In polluted samples, only three PCBs were determined, and PCB 153 content was higher than in nonpolluted samples and accounted for about 45%. These results are comparable with the mussels that were collected in the same area (7). Our results corresponded with earlier results for PCBs, where it was shown that indicator PCBs (PCBs 28, 52, 101, 138, 153, and 180) represented 58.9% of total PCBs (26).

Our results are in agreement with another report (14) showing that, for solid samples, three extraction methods can be used for determination of PCBs, i.e., supercritical fluid extraction, microwave-assisted extraction, and accelerated solvent extraction. All of these new procedures have the potential to replace classical extraction methodology. For liquid samples, SPE and SPME were evaluated.

Our determination of PCBs differs from another (15) using an isotope dilution method for quantification via analysis of the samples by GC/MS. The results were also compared to those of other extraction techniques (Soxhlet extraction, PLE, saponification, and homogenization).

Conclusions

Rapana can be used as a bioindicator to evaluate the toxic effects of chemical pollutants in marine organisms, especially heavy metals, representing an important tool for biomonitoring environmental pollution in coastal areas. Trace metal contents in *Rapana* collected at polluted sites were 3- to 4-fold higher, compared to the nonpolluted site. The overall conclusions are in agreement with others (28), introducing a number of bioindicators, including algae, macrophytes, zooplankton, insects, bivalves, mollusks, gastropods, fish, amphibians, and others in practical biomonitoring of aquatic metal pollution. The analytical methods applied in this study can be used for other marine products.

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