Characterization of *Rapana thomasiana* as an indicator of environmental quality of the Black Sea coast of Bulgaria


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The aim of this investigation was to determine the contents of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), minerals, trace elements and bioactivity in the gastropod *Rapana thomasiana*, which can be used as an environmental bioindicator organism. The chemical differences between *Rapana thomasiana* from polluted (RapaPol) and non-polluted (RapaNPol) sites of the Black Sea coast in Bulgarian were investigated. Chromatography and high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) were used for evaluation of PAHs, PCBs, minerals and trace elements. Methanol extracts from RapaPol and RapaNPol (to a lesser degree) contained relatively high amounts of free phenolics (2.50 ± 0.3 and 1.57 ± 0.18 mg GAE/g DW, respectively) and exhibited the following respective levels of antioxidant activities determined by two radical-scavenging assays (μMTE/g DW): 1.8 ± 0.2 and 0.98 ± 0.08 by 1-diphenyl-2-picrylhydrazyl method (DPPH); 1.74 ± 0.17 and 1.04 ± 0.12 by cupric reducing antioxidant capacity (CUPRAC). The total amounts of elements, PAHs and PCBs were higher in RapaPol than in RapaNPol. The obtained indices of *Rapana thomasiana* can serve as a bioindicator of the environmental ecological quality.

**Keywords:** *Rapana thomasiana*; polluted and non-polluted areas, chemical indices

1. Introduction

*Rapana thomasiana* (*Rapana venosa*) is a predatory marine snail that may affect both natural and cultivated populations of oysters, mussels and other molluscs. In areas where it has been introduced, it has caused significant changes to the ecosystem. *Rapana thomasiana* is considered as one of the most unwelcome invaders worldwide and an active predator of epifaunal bivalves. Its proliferation is a serious threat to cultivated and natural populations of oysters, mussels and other molluscs. *Rapana* are very voracious predators and are blamed in the Black Sea for the decline of the native, edible bivalve fauna [1,2]. It has a high ecological fitness as evidenced by its high fertility, fast growth rate and tolerance to low salinity, high and low temperatures, water pollution and oxygen deficiency. Long-distance dispersal is facilitated by ship ballast water, in which the larvae of the snail are found in its plankton phase [3].

Different animals and changes in their chemical composition have been used as bioindicators of marine pollution; therefore it is important to introduce new methods for characterization of their properties in polluted and non-polluted areas [4–7]. The application of bioindicator organisms is very important from an environmental biochemical standpoint, as there are very few organisms that regularly produce certain molecular signals in response to changes in their environmental conditions. Two green algae (*Ulva rigida* and *Cladophora coelothrix*), marine animals such as mussel (*Mytilus galloprovincialis*), and a snail (*Rapana thomasiana*) were investigated and compared in order to find the most suitable as biomonitor of seawater pollution [2]. However, *Rapana thomasiana* has been less investigated [1–3,8,9]. An environmentally relevant bioindicator organism should experience stress, which normally indicates an elevation of metals, antioxidant activity, PAHs and PCBs in the polluted environment. Evaluation of heavy metals in mussels and gastropods has been based on ultrasonic-assisted acid digestion and inductively coupled plasma atomic emission spectrometry [10–13].

Therefore, in this research, the bioactivity differences between *Rapana thomasiana* from polluted (RapaPol) and non-polluted (RapaNPol) sites of the Black Sea were studied by chromatography, high-resolution inductively cou-
pled plasma mass spectrometry (HR-ICP-MS) analyses, and three-dimensional fluorometry (3D-FL) for evaluation of PAHs, PCBs, minerals, trace elements and bioactive compounds. Several analytical methods such as radical-scavenging assays (cupric reducing antioxidant capacity [CUPRAC] and 1-diphenyl-2-picrylhydrazyl method [DPPH]) were introduced in this regard [14–16]. As far as we know, no results of such investigations have been published.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN) and tetrahydrofuran (THF) used as mobile phase were of HPLC grade; dichloromethane, methanol and cyclohexane were purchased from Merck (Darmstadt, Germany). All solvents used as reaction media were of analytical grade and were obtained from POCb (Gliwice, Poland). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent (FCR), CuCl2xH2O and 2,9-dimethyl-1,10-phenanthroline (neocuproine) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Silica gel (40 μm, J.T. Baker BV Deventer, The Netherlands). A mixture of 16 compounds from the PAH group, each compound at a concentration of 2000 μg/mL; PCB standard solutions of seven polychlorinated biphenyls in isooctane, each with a concentration 100 μg/mL were purchased from Restek Corporation (Bellefonte, Pennsylvania, USA). Certificated solutions of naphthalene-d8 and benz(a)anthracene in dichloromethane, with concentrations of 2000 μg/mL, and PCB 209 certificated standard in aceton, with a concentration of 200 μg/mL, were obtained from Supelco (Bellefonte, PA, USA).

2.2. Sample collection

Samples of Rapana (Rapana thomisiana) were collected at a sea depth of 3–4 m from (a) an ecologically non-polluted site (Galata station, three miles offshore Varna Bay, A) and (b) a polluted site (Varna Bay, B) (Figure 1). The sampling was carried out from late July to early August 2006. The samples were collected as previously described [5,7]. The collected Rapana from both polluted and non-polluted sites were characterized by a similar maximum length and a similar size of analysed organisms, which was 75–85% of the maximum size reached within each population. This approach guaranteed that the Rapana from the two sites had similar metabolic conditions, and so the influence of physiological differences between the two populations was less pronounced [17]. Whole soft tissue from 30 specimens of each population, with a maximum shell size of about 70 mm, was rapidly frozen in liquid nitrogen and stored at −80°C. Then the samples were lyophilized in glass flasks on Finn-Aqua Lyovac GT-2 equipment for 36 hours.

Figure 1. Sampling sites of molluscs along the Black Sea. A: Galata station; B: Varna Bay.
2.3. Analytical methods

2.3.1. Determination of the metal and mineral contents of the studied bioactive compounds

Trace metals and minerals were determined in 0.5 g of lyophilized sample, which was transferred to PTFE-Teflon vials (18 mL). Subsequently 3.25 mL of ultrapure water (Elga) and 3.25 mL of concentrated HNO₃ (Scanpure) were added to the vessels. Digestion of these portions was carried out in a high-pressure microwave system (Milestone UltraCLAVE II; EMLS, Leut-kirch, Germany) using a temperature programme. After cooling to room temperature, the digested samples were diluted with ultrapure water to achieve a final acid concentration of 0.6 M. The 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; the system of integration: MSD ChemStation; the temperature programme: 40°C; column: Rtx-5MS (30 m × 0.25 mm × 0.25 μm); detector: Agilent Technologies 5975C with electron ionization (EI), working in the SIM mode; the interface temperature: 280°C; the interface temperature: 280°C; the temperature programme: MSD ChemStation; the temperature programme: 280°C, then held 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 [17].

2.3.2. PAH and PCB determinations

The freeze-dried samples (1 g) were hydrolysed 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 methanol–water (bottom) layer was transferred into a second funnel, and the extraction step was repeated with 10 mL of cyclohexane. The cyclohexane extracts were combined and washed with 10 mL of methanol–water (1:1) solution. 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. The extract introduced into the column was eluted with 8 mL of dichloromethane and then reduced to 0.3 mL under a stream of nitrogen. The extract was injected to a gas chromatography–mass spectroscopy (GC-MS) system.

2.3.3. Gas chromatographic analysis

An Agilent Technologies gas chromatograph equipped with a mass spectrometric detector and on-column injector was used. The conditions of the GC-MS (which was operated in SIM) analysis were as follows: the injection system: spilt/splitless injector with automatic sample introduction; volume of sample injected: 2 μL; inert gas: helium – 70 kPa; the interface temperature: 280°C; column: Rtx-5MS (30 m × 0.25 mm × 0.25 μm); detector: Agilent Technologies 5975C with electron ionization (EI), working in the SIM mode; the system of integration: MSD ChemStation; the temperature programme: 40°C to 120°C at 40°C/min, then 5°C/min until 280°C, then held for 12 min. The

2.3.4. Determination of the contents of polyphenols and antioxidant activity

Unconjugated (‘free’) polyphenols (FreeP) were extracted from dried defatted samples (50 mg) with 5 mL of 50% methanol–water and heated at 90°C for 3 h. The FCR was used for polyphenol determination, and the measurement was performed at 765 nm with gallic acid as the standard. Results were expressed as mg of gallic acid equivalent (GAE) [7,17].

The following two radical-scavenging tests were used:

1. Cupric reducing antioxidant capacity (CUPRAC). This assay is based on utilizing the copper(II)-neocuproine (Cu (II)-Nc) reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank.
2. 1,1-Diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable [7,15,16].

2.3.5. Three-dimensional (3D-FL) spectra

Fluorescence spectra of methanol extracts of Rapana thomasi ana at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells and a thermostat bath. The widths of the excitation and the emission slits were set to 10.0 and 5.0 nm, respectively. The three-dimensional spectra were collected with subsequent scanning emission spectra from 270 to 400 nm at 1.0 nm increments by varying the excitation wavelength from 250 to 320 nm at 10 nm increments. The scanning speed was set at 1000 nm/min for all measurements [18,19].

2.4. Statistical analyses

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

3. Results and discussion

3.1. Three-dimensional fluorescence (3D-FL) spectra and bioactive substances

In this investigation the chemical differences of Rapana thomasi ana from polluted (RapaPol) and non-polluted (RapaNPol) sites of the Bulgarian Black Sea were studied.
As was mentioned above, the presence of bioactive compounds was studied by 3D-FL spectra, which are shown in Figure 2. The y-axis represents the excitation spectra from 250 to 320 nm, while the x-axis is the emission spectra from 270 to 400 nm of RapaPol (A,B) and RapaNPol (C,D) in methanol extract. In 3D-FL spectra the excitation and the emission wavelengths and the fluorescence intensity were used in order to investigate the information of the extracted bioactive compounds in the samples. The contour and cross maps of methanol extracts showed one peak for RapaPol at a location of λ Em/Ex 305/270 nm and one for RapaNPol at λ Em/Ex 300/270 nm, with the fluorescence intensity for RapaPol being about twice as much as that for RapaNPol. Our obtained results can be compared with other reports, where the excitation and emission wavelengths derived from the top ring of each contour line for dyes from plant *Rubia cordifolia* L. var. *munjista* Miq. root, sea invertebrate *Rapana thomasiana* hypobranchial gland, from plant *Polygonum tinctorium* Lour. leaf [18]. Our data are similar to those of [19], where seven types of fluorescence peaks were detected from samples of dissolved organic matter. There were protein-like fluorescence peaks with Ex max/Em max = 275/300 nm [19], which are equal to the peaks determined in the *Rapana* samples (Figure 2). The results of 3D-FL can be used as an additional tool to study the changes in *R. thomasiana*.

### 3.2. Polyphenols and antioxidant activity

Methanol (50% methanol/water) was used for unconjugated (‘free’) polyphenol extraction, and the results showed variation in the amounts of the bioactive compounds, depending on the animals used and the site collection. Polyphenols (mg GAE/g DW) in *Rapana thomasiana* from the polluted site were significantly higher than from the non-polluted site (Figure 3, *P* < 0.05).

The antioxidant activity, determined by two antioxidant-scavenging methods, in methanol extract was significantly higher in *Rapana thomasiana* from the polluted area than from the non-polluted area. The DPPH and CUPRAC antioxidant values were in approximately the same relationship as for polyphenols (Figure 3, *P* < 0.05). A direct relationship was obtained between the polyphenols and the antioxidant activities, with correlation coefficients between the polyphenols and the antioxidant activities, determined by DPPH and CUPRAC, ranging from 0.87 to 0.96 in
Rapana thomisiana. The antioxidant values of Rapana in the polluted site were significantly higher than in the non-polluted site, and this was found in a number of previous reports [1, 2, 14]. The same data were obtained in three mollusc species from the Black Sea: Mytilus galloprovincialis, Mya arenaria and Rapana besoar. The studies found the highest catalase activity in the mid-gland of M. arenaria, probably because of its evolution in a very polluted environment [14]. Rapana thomisiana samples reacted slightly differently: the antioxidant characteristics in polluted samples (polyphenols and overall antioxidant activities by CUPRAC and DPPH assays) were significantly higher than in non-polluted samples (\( P < 0.05 \)). These results are in agreement with others [2], where two green algae (Ulva rigida and Cladophora coelothrix), a mussel (Mytilus galloprovincialis) and a snail (Rapana thomisiana) from the Bulgarian Black Sea shore were treated with diesel fuel. The changes appeared to be larger in the evolutionarily less-advanced species from both groups of marine organisms, algae and invertebrates (Ulva rigida and Mytilus galloprovincialis, respectively), than in Rapana thomisiana. These results can be also explained by the differences in the feeding process of the two types of marine animals (the feeding of R. thomisiana is slightly different from other marine animals).

3.3. Determination of metal contents

The results of the determination of minerals and trace elements are shown in Figure 4. Six elements were very abundant (Ca, Mg, P, S, K, Cl, Figure 4A), others were not abundant or were present in trace amounts (Figure 4B and 4C, respectively). Lanthanides are natural elements and terrigenic in origin; hence, they are used as terrigenic normalizers in geochemistry. So we can expect that no anthropogenic sources are responsible for their presence in the molluscs. However, some lanthanides can be pollutants. For instance, La sometimes occurs in atmospheric particulate matter as a result of refinery emissions, because it is used as a catalyst in oil refineries. The transition metals belong to d-block elements, except Sc, Y, La, Ac as well as Zn, Cd, Hg and Cn. Potentially anthropogenic in origin could be the chemical elements Hg, Cd, Sn, As, Tl, Zn, Cu, Ag, Cr, Co, Ni, Fe and V. Some authors determined the optimum values of the contents of As, Cd, and Pb in muscle tissue of five fish species, used as bioindicators for Lake Manchar (Sindh, Pakistan), to investigate whether consumption of these fishes threatened human health [13]. It seems that Rapana from a polluted sampling site contained higher levels of the following trace elements: Li (2.0 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), Hg (1.3 times higher. The main elements for pollution characterization are Pb, Cd, Hg (MeHg), organic Sn (TBT), As (inorganic compounds of As), Tl, Ni and Cr(VI). Some essential elements, for instance Zn and Cu, can be anthropogenic in origin, and their elevated levels are considered to be toxic for biota. This difference (between polluted and non-polluted sites) can be due to variations in metal concentrations associated with natural internal parameters (physiological), e.g. age, sex, or external ones (ecological), e.g. salinity of water; food resources, including their structure and availability; geochemical composition of the bottom sediments that are habitats of the bivalvia [4].

The influence of these internal factors can be eliminated as a source of variation between the two sampling sites because a comparable number of specimens belonging to the same sex and size class were collected from the two sites. If this were not the case, then only chemometric analysis can give an explanation for this variation [8, 9].

If the soft tissue of the molluscs is edible, then the risk associated with consumption should be assessed. Rapana is a sea fruit, therefore the safety levels of metals are important. RDAs are acceptable from the view of these requirements. The concentration data, in terms of US FDA or EPA levels, are more reasonable: permissible concentrations of toxic metals (Hg, Pb, Hg) have not exceeded the levels relative to FDA and EPA. Safety Levels in Regulations and Guidance [20] even for Cd with its high tissue levels, were below permissible levels (4.0 mg Cd/kg clams, oysters and mussels). However, according to the Polish Regulations of the Ministry of Health [21], referring to the edible kidney of mammals, and in EU document No. 1881/2006 [22], the concentration of Cd in Rapana has been somewhat exceeded. These Regulations [21] concern maximum concentrations of chemical and biological pollutants which may be present in foods, food components and food additives. According to the above Regulations [21], maximum concentration of Cd in kidney of mammals amounts to 1.00 mg/kg. Moreover, the maximum concentrations of Cd in mollusks and cephalopods were also 1.00 mg/kg [21].
Based on the FAO RDAs [23,24] for essential elements (Ca, Mg, K, Se, Cu, Zn, Mn, Fe), 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 [8], where one gastropod (Rapana thomasiana) and one mussel (Mytilus galloprovincialis), collected from two sites along the coastline of the Bulgarian Black Sea, were investigated for heavy metal contamination (Cd, Co, Cu, Ni, Pb and Zn). One certified reference material of oyster tissue (NIST 1566b) was used to validate the methods and the obtained results proved to be in good agreement with the certified values.

The results obtained by the optimized method of ultrasound-assisted pseudodigestion for toxic metals followed by electrothermal atomic absorption spectrophotometry showed good agreement with the certified values and sufficient high recovery [12]. Relative standard deviation values were 1.21%, 5.52% and 5.32% for As, Cd, and Pb, respectively, which is in agreement with our data. Our results are comparable with the data of others, where mostly Cd, Cr, Ni and Pb were determined [10,11], and where other trace elements such as As, Al, Cr, Cu, Fe, Mn and Zn were estimated as well [13]. The results of the present study showed that Rapana thomasiana possessed a much greater ability for bioaccumulation of Cu and Zn than did the species in the other studies. Rapana thomasiana manifested the most bioaccumulation capacity for Cd (Figure 4B). Among the five freshwater fish species, Ruditapes philippinarum possessed the highest content of Ni [13]. Furthermore, Cd, Cu and Zn contents in some gastropod and oyster samples exceeded the maximum permissible levels established by WHO [25]. Because of their special bioaccumulation capacity for Cd and Ni, Rapana venosa and Ruditapes philippinarum have the potential of being used as biomonitors to assess aquatic contamination with heavy metals. It was found that the contents of minerals and trace elements in Rapana thomasiana from RapaPol were higher than from RapaNPol. The highest differences were for Ca and Cd. Higher levels of Cd in Rapana soft tissue could be associated with chemical plants located near
the polluted area, and the high Ca levels could have been provided from fertilizers (anthropogenic source) or from calcareous bottom sediments (natural source). Other investigators also found that the contents of Cd, Pb, Cu and Zn in shells of *Mytilus galloprovincialis* and *Rapana thomasiana*, collected from polluted sea sites of the Romanian Black Sea coast, are high [8]. A high concentration of Zn (16.82 μg/g) in *Rapana* samples was observed, compared with the other samples [8]. The *Rapana* samples also contained a lower concentration of Cu (5.88 μg/g) and the highest concentration of Cd (0.94 μg/g) [8]. Our results were similar to the ones cited in [10]: the metal concentrations (μg/g DW) in the total soft tissues of *Anadora granosa* were 1.30–9.44 (mean: 4.69) for Cd, 91.9–203.5 (mean: 130.2) for Zn, 0.80–16.15 (mean: 7.67) for Ni, 455.91–1125.50 (mean: 715.30) for Fe and 5.41–7.39 (mean: 6.14) for Cu. The study by Yap *et al.* [8] revealed that *A.granosa* was a potential bioindicator for Cu and Zn as observed by the comparison with those metals in the sediment. Our results are also in agreement with other reports where As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn and other heavy metal elements were determined, by ICP-MS, in the body of *Rapana venosa* from the shallow sea of northern Liaotong Bay. The distribution characteristics of the heavy metal elements and the influence of the growing environment were detected and the results were in the range found in our studies. Trace element content in marine algae species were 1.70–17.1 μg/g for Cu, 3.64–64.8 μg/g for Zn, 9.98–285.0 μg/g for Mn, 99–3949 μg/g for Fe, 0.50–11.6 μg/g for Cr, 0.27–36.2 μg/g for Ni, 11–694 μg/kg for Se, 0.50–44.6 μg/g/kg for Cd, 1.54–3969.00 μg/kg for Pb and 1.56–81.90 μg/kg for Co. Iron was the highest trace element concentration and cadmium was the lowest in samples [26–28]. The pollution is characterized by heavy metals and sulphates as toxic constituents and other organic compounds [29–32].

### 3.4. Determination of PAHs and PCBs

The results of the determination of the PAH and PCB concentrations in *Rapana thomasiana* are shown in Figure 5. In polluted and non-polluted areas in Rapana, seven PAHs were detected, in comparison with the 16 PAHs which

![Figure 5. Concentrations of (A) PAHs and (B) PCBs in *Rapana* samples. Napht: naphthalene (ng/g × 10); Acena: acenaphthene; Phenan: phenanthrene (ng/g × 10); Anthr: anthracene; Fluoran: fluoranthene. PCB52: 2,2',5,5'-tetrachlorobiphenyl; PCB101: 2,2',4,5,5'-pentachlorobiphenyl; PCB118: 2,3',4,4',5'-pentachlorobiphenyl; PCB138: 2,2',3,4,4',5'-hexachlorobiphenyl; PCB153: 2,2',4,4',5,5'-heptachlorobiphenyl; PCB180: 2,2',3,4,4',5,5'-octachlorobiphenyl.](image-url)
were tested in the standard run. As can be seen from Figure 5A, the amount of PAHs in the polluted area was higher than in the non-polluted area, and the total sum of the seven PAHs for the samples from the non-polluted site was 401 \(\pm 50\) mg/kg DW, whereas for the polluted site it was 572 \(\pm 50\) mg/kg DW. The individual PAHs \((\times 10^{-3}\) mg/kg DW) ranged from 130 \(\pm 36\) to 5 \(\pm 3\) for the non-polluted area and 329 \(\pm 50\) to 4 \(\pm 3\) for the non-polluted area (Figure 5A). Among the detected seven PAHs, the predominant were naphthalene, phenanthrene and fluoranthene, which accounted for 80.3% and 86.2% of the total PAHs for the non-polluted area and polluted area, respectively (Figure 5A). The amount of naphthalene in the polluted site was about 2.5 times higher than in the non-polluted site. The other PAHs in the polluted samples were at similar levels to those in the non-polluted sample. In other studies [33,34], analysis was performed by gas chromatography coupled to mass spectrometry and internal standard quantification was performed using five deuterated PAHs, where 18 PAHs in phytoplankton/seston, zooplankton, five invertebrates, five fish, and one seabird species collected were from Bohai Bay [33]. The PAH concentrations (2.0–64.5 ng/g WW) in these samples were moderate compared with those in other marine organisms.

From six detected PCBs, the highest one was PCB 153, which was about 47% of the total sum (Figure 5B). PCB 153 had the highest content among the six detected in non-polluted animals and accounted for about 33%. In polluted samples the content of PCB 153 was higher in comparison with the non-polluted samples and was about 45%. Our results corresponded with those of [35], where it was shown that indicator PCBs (PCBs 28, 52, 101, 138, 153 and 180) represented 58.9% of \(\Sigma\)PCB. Our results are in agreement with other reports, showing that for solid samples three extraction methods can be used for PCBs: supercritical fluid extraction, microwave-assisted extraction and accelerated solvent extraction. Our determination of PCBs differed from the other methods [29,30,35,36] as we used accelerated solvent extraction. Our determination of PCBs differed from the other methods [29,30,35,36] as we used accelerated solvent extraction. Our determination of PCBs differed from the other methods [29,30,35,36] as we used accelerated solvent extraction. Our determination of PCBs differed from the other methods [29,30,35,36] as we used accelerated solvent extraction.

In conclusion, \textit{Rapana} can be used as a bioindicator to evaluate the toxic effects of chemical pollutants, especially heavy metals, in marine organisms, and represents an important tool for biomonitoring environmental pollution in coastal areas. Trace metal contents in \textit{Rapana} collected at polluted sites were 3–4-fold higher than the non-polluted site. Our results are in agreement with others where algae, mussel and \textit{Rapana thomasiiana} were compared under pollution conditions, and it was determined that the changes appeared to be larger in the evolutionarily less advanced species from both groups of marine organisms, algae and invertebrates [2]. The changes in fluorimetric measurements, bioactive substances and organic matter for the polluted area showed that the selected \textit{Rapana thomasiiana} species met the standards set for environmental bioindicator organisms.

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