Environmental Science and Pollution Research (2022) 29:61352–61369
https://doi.org/10.1007/s11356-022-20186-1

RESEARCH ARTICLE

# Trace elements in the detoxifying and accumulating body parts of *Mytilus galloprovincialis* Lamark, 1819 (Crimea, Black Sea): human health risks and effect of the sampling site location

**Natalya S. Chelyadina[1] · Sergey V. Kapranov[1] · Mark A. Popov[1] · Lyudmila L. Smirnova[2] · Nikolay I. Bobko[1]**

Received: 15 November 2021 / Accepted: 6 April 2022 / Published online: 20 April 2022


## Abstract

The mussel *M. galloprovincialis* is one of the most popular species in world's mariculture and environment pollution bioindicators. Although this mollusk was in a focus of numerous publications, the detoxifying and accumulating role of some of its body parts was insufficiently studied. The goals of the present work were as follows: (a) to study the distribution of potentially toxic elements (Cr, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) in soft tissues, byssus, and shell liquor of this bivalve as a function of sampling location in the Black Sea near the southwestern coast of Crimea and (b) to assess human health risks from consuming soft tissues of mussels cultivated on a mollusk farm. Multivariate analysis showed significant differences in the overall distribution of the elements among the body parts and sampling sites under consideration. The trace element contents in soft tissues of *M. galloprovincialis* decreased in the following order: Zn > Cu > As > Ni > Pb > Cd > Cr > Co > Hg. The noncarcinogenic hazard index from the cultivated mussel consumption was found to be well below one and the carcinogenic risk index was found within the tolerable limits, which indicate the safety of consuming these mussels for humans. Byssus of *M. galloprovincialis* was characterized as a perfect indicator of marine environment pollution with Ni, Cu, Pb, Co, and Cr. For the first time, the concentrations of trace elements were determined in the shell liquor and the function of byssus and shell liquor as the systems of trace element excretion from soft tissues was demonstrated.

**Keywords** Black Sea · Mussels · Trace elements · ICP-MS · Bioaccumulation · Soft tissues · Shell liquor · Byssus

## Introduction

The Mediterranean mussel *M. galloprovincialis* is an important source of animal protein with high nutritional value and one of the most popular target species in bivalve mariculture (Venugopal and Kumarapanicker 2017; Voultsiadou et al. 2010). This mollusk thrives in the coastal waters of the Mediterranean, Black Sea and the Atlantic of the Northern Hemisphere. It is also cultivated in countries of the southern Mediterranean, in South Africa, and China (Atasaral et al. 2015; Gosling 2003; Kholodov et al. 2017; Lutz et al. 1991). In the Black Sea, *M. galloprovincialis* is extensively cultured and is one of the dominant indigenous mollusk species (Ivanov et al. 1989; Kholodov et al. 2017; Massa et al. 2017).

*M. galloprovincialis* is also widely used as an environmental biomonitor. A substantial progress in this application of the mollusk has been achieved through the implementation of the "Mussel Watch" program (Cantillo 1998; Rainbow and Phillips 1993; Stankovic and Jovic 2012). Mollusks are good bioindicators of trace element pollution of environment (Casas et al. 2008; Gupta and Singh 2011; Stankovic and Jovic 2012) because of the ability to accumulate trace elements in their body parts to levels several orders of magnitude higher than in the marine environment (Adams and Rowland 2003; Casas et al. 2008; Rainbow and Phillips 1993; Stankovic and Jovic 2012). Trace element contents in marine organisms depend not only on environmental factors, such as the trace

Responsible Editor: Lotfi Aleya

✉ Natalya S. Chelyadina
chelydina2007@mail.ru

[1] A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, Nakhimov ave. 2, Sevastopol, Russian Federation 299011

[2] Institute of Natural and Technical Systems, Russian Academy of Sciences, Lenin str. 28, Sevastopol, Russian Federation 299011

element concentrations in seawater, temperature, salinity, dissolved oxygen concentration, and pH value, but also on biological characteristics, such as aquatic species identity, sex and sexual ripening stage, type of tissue, organ, and diet (Boening 1999; Sunlu 2006).

Many trace elements, including heavy metals, are biologically active and capable of disrupting human metabolic processes (Korish and Attia 2020). Estimation of heavy metal contents in edible aquatic mollusk tissues is important for the assessment of human health risks from the mollusk consumption (Gupta and Singh 2011). The most hazardous elements for human health are cadmium, copper, arsenic, nickel, mercury, lead, zinc, chromium, and cobalt (Briffa et al. 2020; Gupta and Singh 2011; Stankovic and Jovic 2012).

There exist a number of publications on the trace element levels in soft tissues (Abderrahmani et al. 2020; Esposito et al. 2021; Kapranov et al. 2021a; Kapranov et al. 2021b; Turanlı and Gedik 2021) and byssus of mytilids (Nicholson and Szefer 2003; Szefer et al. 2002, 1999; Yap et al. 2003a; Yap et al. 2003b). Mussel byssus is known to be a better bioindicator of coastal heavy metal pollution than other tissues (Szefer et al. 2002, 1999). In *M. galloprovincialis* shell liquor, trace element levels were studied very poorly, and to our knowledge, there is only one publication on the content of Zn and Cu in this fluid (Chelyadina and Smirnova 2018). At the same time, the need for the study of the mussel shell liquor element composition is quite obvious as shell liquor is an important mediator in the metabolism that occurs between tissue cells and the open circulatory system of the mussel and affects the shell liquor composition (Chelyadina et al. 2015).

The goals of this work are as follows: to study the distribution of the above-mentioned potentially toxic elements (Cd, Cu, As, Ni, Hg, Pb, Zn, Cr, Co) in soft tissues, byssus, and shell liquor of the mussel *M. galloprovincialis* and its dependence on the sampling location in coastal waters of the southwestern Crimea (Black Sea); to reappraise the ability of byssus to serve as a trace element accumulator and bioindicator of water pollution with the trace elements under study; to assess the role of the shell liquor as the heavy metal detoxification medium; and to assess human health risks from the consumption of the cultivated mussels.

The novelty of the present study lies in the following. In this work, the contents of potentially toxic trace elements in byssus of *M. galloprovincialis* from the Black Sea are measured for the first time. We make also the first appraisal of the potential of Black Sea mussel byssus as a trace element contamination bioindicator. Furthermore, novel data on trace element levels in shell liquor are reported, and first indications of shell liquor as a detoxifying medium in mussel organism are obtained. Finally, trace element-related carcinogenic risks due to consuming soft tissues of *M. galloprovincialis* from the northwestern Black Sea are determined for the first time in this study.

## Materials and methods

### Object of research

The mollusk under study is the bivalve *Mytilus galloprovincialis*, as it follows from its appearance. The shell of *M. galloprovincialis* is rectangular-wedge-shaped with narrow umbo curved forward; moderately convex; black-violet, with blue or blue-violet nacre. The shell length is up to 140 mm, height up to 75 mm, and width up to 52 mm (Kholodov et al. 2017; Morduchai-Boltovsky 1972).

The object of this study was *M. galloprovincialis* with the shell size of 54.01 ± 2.9 mm. The mussel *M. galloprovincialis* is a common species in the Black Sea shelf zone and in the fouling of various hydraulic structures. The animals form independent biocenoses and are part of other communities. *M. galloprovincialis* has a significant potential for acclimatization, which allows them to adapt to different living conditions and occur in nearly all biotopes of the region. At present, this species has spread throughout temperate shelf waters of almost all oceans. It can exist in wide ranges of salinity (8–40 $g{\cdot}L^{-1}$) and water temperature (1–28 °C). The optimum temperature is 12–20 °C and salinity 12–25 $g{\cdot}L^{-1}$ (Ivanov et al. 1989; Kapranov et al. 2021b). The mussel *M. galloprovincialis*, being an active filter feeder, filters water at a rate of 0.5 to 7.5 $L{\cdot}h^{-1}$ and higher and, as a result, accumulates trace elements from the incoming water and food, phytoplankton, and detritus (Coombs and Keller 1981; Temerdashev et al. 2017). Soft tissues of mussels accumulate trace elements and partly remove them into shell liquor, which is involved in the metabolism between tissue cells and circulating blood (hemolymph). Shell liquor, byssus, feces, pseudo-feces, and mussel gametes during the spawning make up the mollusk's excretion system (Chelyadina et al. 2015; Kapranov et al. 2021b; Pospelova 2008).

*M. galloprovincialis* is a sedentary organism that, after the larval stage, settles and attaches to the substratum (rock, sediments, rope collectors, etc.) with byssal threads, which are secreted from a byssal gland in the mussel foot and provide tight attachment. Byssal threads consist of collagen fibrils incorporated into matrix protein core. Byssus plays not only the role of an attachment organ, but also performs the function of extracting heavy metals from the mussel body (Nicholson and Szefer 2003; Suhre et al. 2014, 2006).

### Characteristics of the sampling sites

In this study, mussels were sampled in June 2017, and the temperature of the seawater environment was measured

at the sampling time using a meteorological thermometer TM-10 (*Termopribor*, Klin, Russia).

The sampling sites were selected so as to encompass environments with different physicochemical properties and different expected levels of trace element exposure. The first sampling site was the mollusk farm (Station 1) located at the outer roadstead of Sevastopol (44°37′13.4″ N; 33°30′13.6″ E, Fig. 1). The animals at Station 1 were harvested from rope collectors at a depth of 2–3 m, where the water temperature was 21.4 °C. The second sampling site was the seafloor under the farm (Station 2). The depth at Station 2 was 16 m and the water temperature at the seafloor was 11.0 °C. The third place for the mussel sampling (44°36′42.6″ N; 33°35′12.2″ E, Fig. 1) was the innermost part of Sevastopol Bay (Station 3). The animals at this station were collected from the quay wall at a depth of 2–3 m at the water temperature of 21.8 °C.

The water in the mollusk farm area (Station 1) is ranked as mesotrophic; the trophic index (TRIX) value was 2.73. Hidden upwellings are typical in this area. The farm has sufficient water exchange and nutrient supply due to local currents even in the small wind-wave mixing regime (Kuftarkova et al. 2006). Food resources in this marine farm are favorable for the growth and development of mollusks as microalgae included in the diet of the cultivated mussels are permanently present in the phytoplankton (Ryabushko et al. 2017). The seasonal variation of salinity in the surface water is weak. The amplitude of the seasonal fluctuations of the long-term mean values did not exceed 0.4‰ over the last two decades, and interannual salinity fluctuations in any season were below 1‰, being in the range of 17.26–18.39‰. The pH values in the surface water on the farm varied in the range of 8.14–8.88, with the median being 8.35 (Kapranov et al. 2021b; Kuftarkova et al. 2006).

The seabed under the farm (Station 2) consists of highly silted sand with rare boulders. The salinity in the farm water gradually increases from the surface to the bottom. The median difference between the bottom and surface salinities is 0.1‰, and the median difference between the respective pH values is nearly 0.

The innermost part of Sevastopol Bay (Station 3) is a typical estuary, with the Chernaya River, a major source of heavy metals (Gruzinov et al. 2019), flowing into this area. Close to this sampling site, there are a shipbreaking plant and a city power plant, which are the additional sources of heavy metal pollution. In the inner part of the bay, the salinity of the upper layer varied in the range from 11.16 to 17.74‰. This area is characterized by an increase in the water salinity from the surface to the bottom and seawards, with a pronounced wedge of salt water penetrating relatively far upstream from the river mouth. The pH value at the sampling site was 8.42 (Boltachev et al. 2010), and the mean TRIX value in Sevastopol Bay was 4.01 (Slepchuk et al. 2017), which corresponds to the moderate trophic level.

## Analytical sample preparation

The mussel shells were cleaned of epifauna, algae, and mineral residue with a knife and wire brush and washed with seawater. Then, each mussel was dissected by cutting the adductor muscle using a plastic scalpel to isolate individual body parts (soft tissues, byssus, and shell liquor), which were further analyzed as separate samples. From the open shell, after cutting the mantle in the anterior part, the turbid shell liquor was poured in dry test tubes pre-cleaned with nitric acid and deionized water, and then, byssus and soft tissues were excised with the scalpel. The weight of shell liquor in the mussels under study was up to 30% of their

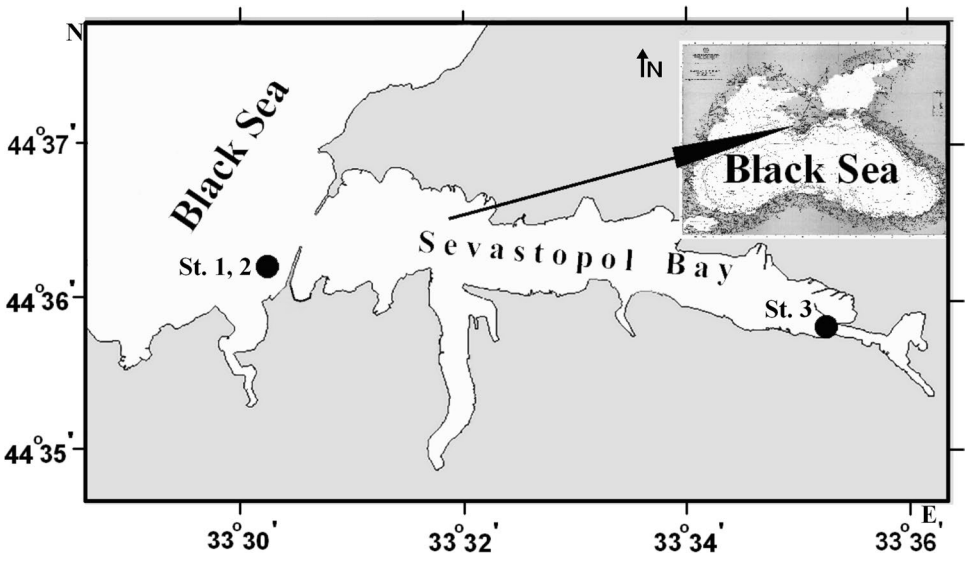


**Fig. 1** Location of the sampling stations (St. 1–3)

total weight. The soft tissues and byssus were blotted with filter paper and weighed. The dry weight of the soft tissues was determined after oven-drying at 105 °C. Weights of all body parts were measured on an analytical balance AXIS ANG200C (Gdańsk, Poland). Byssus and tissue samples were placed in digestion tubes made of PTFE using a plastic spatula. The following samples (mean ± SD) were weighed for the analysis: 72 ± 17 mg soft tissue, 44 ± 26 mg byssus, and 100 ± 2 mg shell liquor. For the digestion, concentrated nitric acid of analytic grade was additionally purified by sub-boiling distillation in an acid purification system DST-1000 (*Savillex*, USA) and added into the digestion tubes in a proportion of 4 mL per 100 mg sample. The PTFE-capped digestion tubes with the samples and nitric acid aliquots were kept in an autoclave at 120 °C for around 1.5 h. Deionized water was used to dilute the digested samples to about 1000 mL·g$^{-1}$ dry weight (d.w.).

## Trace element analysis

The concentrations of trace elements (Cr, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) in the diluted samples were measured using inductively coupled plasma mass spectrometry (ICP-MS) on an instrument PlasmaQuant® MS Elite (*Analytik Jena*, Germany). The plasma flow was 9.0 L·min$^{-1}$, the sampling depth was 8 mm, and the RF power was 1.25 kW. The dwell time for each element was 10 ms, one point per peak in the peak-hopping mode. To make sure there are no significant polyatomic interferences, the measurements were carried out with the collision reaction interface (CRI) switched off and on. In the CRI mode "on," gaseous hydrogen with the flow rate 40 mL·min$^{-1}$ was used as the skimmer gas. No internal standard was used since the undesirable matrix effects were not expected due to the high dilution of the samples. The signal drift was taken into account by measuring the element concentrations in the diluted standard IV-ICPMS-71A after every fifth sample and using an interpolating polynomial relationship to correct the apparent concentrations in time (Kapranov et al. 2021a).

The calibration curves were obtained using a multielement standard IV-ICPMS-71A (*Inorganic Ventures*, USA) and a standard solution of mercury (II) nitrate (*Supelco*, USA) diluted with deionized water. The $R^2$ coefficients for all linear calibration fits were greater than 0.999. The detection limits in this analysis range from 0.03 (Pb) to 10 (Ni) ng·L$^{-1}$ (Chemnitzer 2019). The accuracy and precision of the ICP-MS analysis was verified by the measurement of the element concentrations in the certified European Reference Material ERM®-CE278k (tissue of the mussel *Mytilus edulis* Linnaeus, 1758). Samples of the reference material (0.1 g) were digested in the extra pure nitric acid and diluted with deionized water as described above. The certified and observed values are given in Table S1 (Supplementary Material).

The seawater sampling was performed in three replications at the mussel sampling sites at Stations 1 and 3. The seawater was filtered through a membrane filter with 0.45 µm pore size (Sartorius) and acidified with the extra pure nitric acid in a proportion of 0.1 mL per 100 mL seawater. For the ICP-MS analysis, the samples were diluted 10-fold, and the dwell time was increased to 1 s.

## Human health risk assessment

It is common to assess the human health risk from the estimated daily intake of a pollutant, i.e., the rate of daily consumption of the pollutant per body weight unit (Zhelyazkov et al. 2018):

$$\mathrm{EDI} = \mathrm{CR} \times C/\mathrm{BW_a} \quad (1)$$

where CR is the food consumption rate, i.e., the average weight of daily consumed food (in kg·day$^{-1}$·capita$^{-1}$), $C$ is the pollutant content in the food (in mg·kg$^{-1}$), and $\mathrm{BW_a}$ is the average human body weight (b.w.), which is typically assumed to be 70 kg. For the Russian population, CR extrapolated to the year 2017 was 2.132 g·day$^{-1}$·capita$^{-1}$ (Ryabushko et al. 2022).

The calculated EDI values are compared with the reference data: provisional tolerable daily intake (PTDI) established by FAO/WHO (Bat and Öztekin 2016; FAO/WHO 2011; Filippini et al. 2020), tolerable upper daily intake (UDI) set by European Food Safety Authority (EFSA 2006; Filippini et al. 2020), or oral reference dose ($\mathrm{RfD_o}$) introduced by the United States Environmental Protection Agency (USEPA 2021).

Target hazard quotient (THQ) set by USEPA is frequently used as the measure of human health risk from ingesting pollutants with various foodstuffs (Bat et al. 2018a, 2021; Chijioke et al. 2020; Filippini et al. 2020; Kapranov et al. 2021a; Khandaker et al. 2021; Rakib et al. 2021; Ryabushko et al. 2022; Zhelyazkov et al. 2018). It is the ratio of the estimated daily intake of the pollutant to its upper oral reference dose:

$$\mathrm{THQ} = \mathrm{EDI}/\mathrm{RfD_o} \quad (2)$$

If THQ < 1, there are no likely toxic risks for a consumer in the long-term dietary exposure.

To assess the overall noncarcinogenic risks from the consumption of multiple contaminants, hazard index (HI) is used, which is the sum of the THQ values for each contaminant (USEPA 1989):

$$\mathrm{HI} = \sum\nolimits_i \mathrm{THQ}_i \quad (3)$$

The values of HI < 1 indicate no likely toxic risks to human health from the prolonged consumption of contaminated food.

The long-term carcinogenic risk from the oral pollutant intake is assessed by means of cancer risk index (CRI):

$$\mathrm{CRI} = \mathrm{EDI} \times \mathrm{SF} \quad (4)$$

where SF is oral slope factor in $(\mathrm{mg \cdot kg^{-1} \cdot day^{-1}})^{-1}$ for individual pollutants (USEPA 2021). Carcinogenic risks are considered insignificant if $\mathrm{CRI} < 10^{-6}$, tolerable if $10^{-6} < \mathrm{CRI} < 10^{-4}$, and significant if $\mathrm{CRI} > 10^{-4}$ (Bat et al. 2021).

The total cancer risk index ($\mathrm{CRI_t}$) is found as the sum of cancer risk indices for individual pollutants:

$$\mathrm{CRI_t} = \sum\nolimits_i \mathrm{CRI}_i \quad (5)$$

## Statistical analysis

All results are presented as mean ± 95% confidence interval. Statistical comparisons were performed using two-way permutational multivariate analysis of variance (PERMANOVA) and permutational multivariate analysis of dispersion homogeneity (PERMDISP). The effects of the group factors (body part type and sampling location) on the overall accumulation of trace elements were analyzed and visualized by means of principal coordinate analysis (PCO) with the Euclidean distance as the similarity measure. All statistical procedures were realized in PRIMER 6.1.16 & PERMANOVA+ 1.0.6 (Clarke et al. 2014). The differences were considered significant at $P < 0.05$.

# Results

## Trace element contents in M. galloprovincialis and seawater

Table 1 shows the trace element contents in soft tissues, byssus, and shell liquor of *M. galloprovincialis* sampled at the three stations as well as the concentrations in seawater from Stations 1 and 3. The highest concentrations (above 10 $\mu g \cdot L^{-1}$) at Station 1 are registered for As, Zn, and Cu and at Station 3 for Zn, As, Cu, Ni, Cd, and Cr. There are differences in the trace element contents in soft tissues, byssus, and shell liquor that are associated with the overall mean levels of each element at the sampling sites. In all samples, the zinc content is the highest. The trace element contents in the animals from different biotopes decrease in the following order: Station 2 > Station 3 > Station 1.

The largest shares of the trace element contents in soft tissues among the three body parts are observed for Zn, As, and Cd (Fig. 2). The contents of many elements under consideration are highest in byssus. The greatest shares of the trace element contents in mussel byssus are found for Ni (81–90%), Cu (61–79%), Pb (57–79%), and Co (54–69%).

**Table 1** Trace metal concentrations in three body parts of *M. galloprovincialis* from three biotopes ($\mu g \cdot g^{-1}$ d.w.) and in the seawater environment ($\mu g \cdot L^{-1}$)

| | Station 1 | | | | Station 2 | | | Station 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soft tissues | Byssus | Shell liquor | Seawater | Soft tissues | Byssus | Shell liquor | Soft tissues | Byssus | Shell liquor | Seawater |
| Zn | 88.7 ± 11.9 | 98.32 ± 18.85 | 6.91 ± 2.3 | 27.98 ± 5.4 | 131.43 ± 20.2 | 145.86 ± 9.59 | 5.31 ± 1.32 | 111.62 ± 14.84 | 121.1 ± 18.59 | 19.7 ± 5.3 | 107.64 ± 12.6 |
| Cu | 7.8 ± 3.6 | 37.36 ± 8.54 | 2.19 ± 1.7 | 15.27 ± 3.5 | 12.47 ± 3.4 | 45.22 ± 6.77 | 3.1 ± 0.51 | 10.59 ± 3.56 | 25.22 ± 6.77 | 5.42 ± 1.7 | 20.25 ± 3.8 |
| As | 6.0 ± 0.88 | 5.37 ± 1.87 | 1.96 ± 0.69 | 47.84 ± 8.9 | 7.79 ± 2.42 | 4.97 ± 0.86 | 1.56 ± 0.45 | 5.5 ± 0.44 | 4.1 ± 1.1 | 2.1 ± 0.9 | 49.27 ± 8.9 |
| Ni | 3.94 ± 1.69 | 39.38 ± 10.72 | 1.24 ± 1.66 | 7.37 ± 2.5 | 4.2 ± 1.37 | 44.49 ± 7.69 | 0.43 ± 0.26 | 3.43 ± 2.45 | 24.49 ± 7.69 | 2.4 ± 0.9 | 10.95 ± 3.2 |
| Pb | 1.7 ± 0.26 | 3.4 ± 0.8 | 0.92 ± 0.35 | 1.28 ± 0.38 | 2.89 ± 1.01 | 5.36 ± 1.61 | 0.66 ± 0.18 | 2.95 ± 1.32 | 12.64 ± 3.3 | 0.49 ± 0.15 | 1.53 ± 0.31 |
| Cd | 1.1 ± 0.16 | 3.27 ± 0.94 | 1.28 ± 0.5 | 2.05 ± 0.42 | 1.5 ± 0.4 | 2.1 ± 0.71 | 0.45 ± 0.22 | 1.5 ± 0.3 | 0.73 ± 0.12 | 0.2 ± 0.08 | 10.31 ± 2.4 |
| Cr | 1.1 ± 0.16 | 1.52 ± 0.50 | 0.18 ± 0.21 | 4.39 ± 1.1 | 1.49 ± 0.43 | 1.73 ± 0.22 | 1.2 ± 0.94 | 1.2 ± 0.4 | 2.24 ± 0.4 | 0.8 ± 0.2 | 10.70 ± 2.6 |
| Co | 0.35 ± 0.07 | 0.98 ± 0.39 | 0.22 ± 0.17 | 2.20 ± 0.46 | 0.45 ± 0.07 | 1.39 ± 0.2 | 0.15 ± 0.01 | 0.23 ± 0.16 | 0.39 ± 0.1 | 0.1 ± 0.008 | 2.29 ± 0.64 |
| Hg | 0.09 ± 0.01 | 0.1 ± 0.04 | 0.18 ± 0.04 | 0.04 ± 0.02 | 0.19 ± 0.07 | 0.11 ± 0.02 | 0.22 ± 0.06 | 0.05 ± 0.009 | 0.06 ± 0.02 | 0.27 ± 0.08 | 0.08 ± 0.04 |

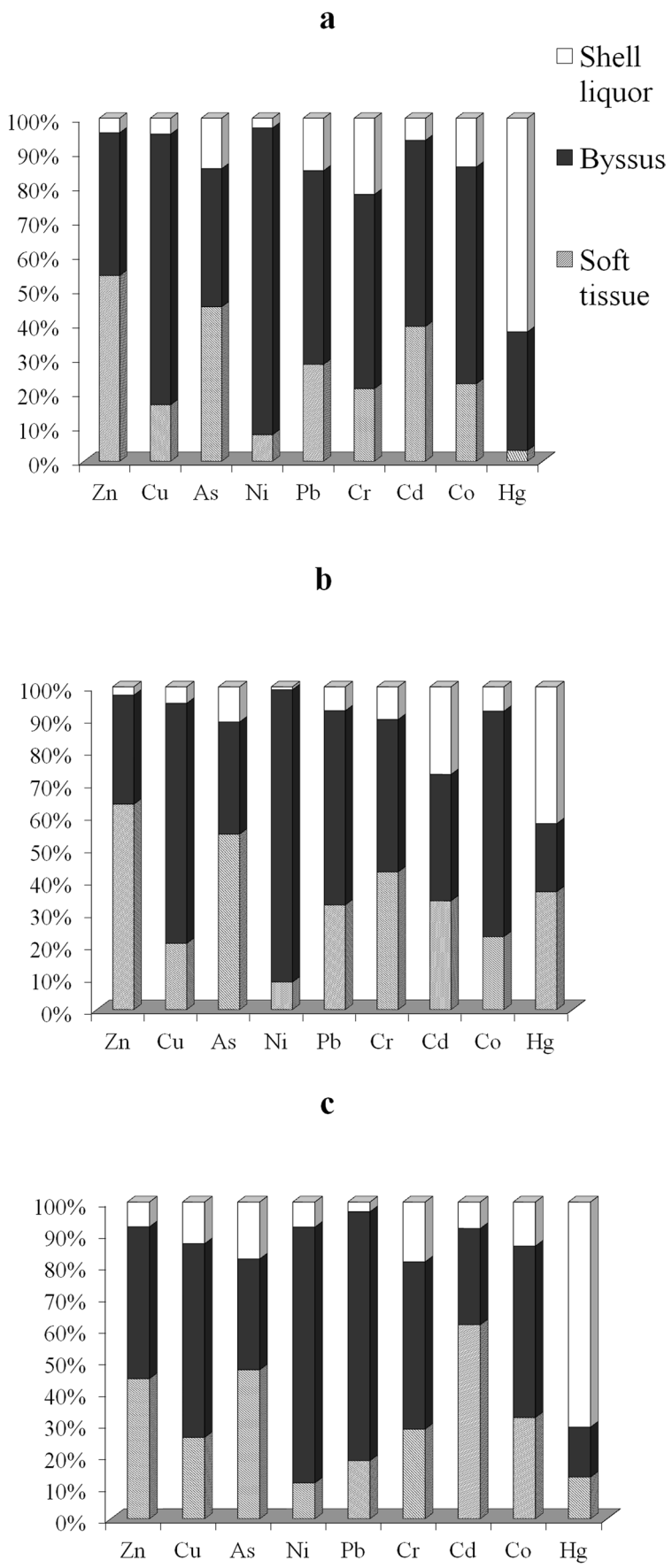


**Fig. 2** Proportions of the trace element contents in soft tissues, byssus, and shell liquor of *M. galloprovincialis* sampled at **a** Station 1, **b** Station 2, and **c** Station 3

The shares of the trace element contents in byssus among the three body parts (Fig. 2) decrease as follows:

(Station 1) Ni > Cu > Co > Pb > Cr > Cd > As > Zn > Hg;
(Station 2) Ni > Cu > Co > Pb > Cr > Cd > As > Zn > Hg;
(Station 3) Ni > Pb > Cu > Cr > Co > Zn > As > Cd > Hg.

The shell liquor has low trace element concentrations at all stations (Table 1). However, the level of Hg in it is higher than in the other body parts (up to 71% among the three ones), which indicates that the liquor makes a considerable contribution to the mercury excretion for the organism detoxification.

## Trace element content correlations

To examine interdependence of the element contents in different parts of mussels from the three stations, we analyzed the corresponding correlations of the power-law type (Table 2). The strongest ones ($R^2 = 0.93$–$0.97$) were found between the contents in soft tissues and in byssi from the three stations as well as between the concentrations in seawater. The weaker correlations were detected between the contents in soft tissues and in other parts as well as between the shell liquors of attached mussels ($R^2 = 0.72$–$0.88$). The weakest correlations with $R^2 = 0.58$–$0.78$ were among the other body parts and seawater.

## Multivariate analysis of element concentrations

The overall trace element distributions in all the three body parts of the animals are significantly different ($P < 0.05$, PERMANOVA) at all the three stations (Table S2, Supplementary Material). There are no significant differences in the element content dispersions when sampling location is used as group factor ($P = 0.5641$, PERMDISP), and it can be asserted that the overall element levels are significantly different at the three stations. However, the dispersions with the body part type as group factor (except for the byssus–shell liquor pair) are significantly heterogeneous ($P = 0.0102$, PERMDISP), and it cannot be unequivocally stated whether the differences in the distributions are due to the mean levels or dispersions.

In soft tissues and byssus of *M. galloprovincialis*, there are significant differences in the overall element contents among the stations ($P = 0.0037$ and $P = 0.0003$, respectively) with no significant differences in the dispersions (Tables S3 and S4). The pairwise PERMANOVA for the soft tissues shows significant differences between Stations 1 and 2 and between Stations 1 and 3 (Supplementary Table S3). The similar test for byssus indicates significant differences between Stations 1 and 2 and between Stations 2 and 3 (Supplementary Table S4). There are no significant differences in the shell liquor trace element levels among the stations ($P = 0.2092$, Table S5).

Principal component analysis (Fig. 3) applied to the square-root-transformed standardized element contents in soft tissues and byssus shows that the vectors of all elements are oriented in the positive direction of principal component 1 (PCO1), which characterizes the overall accumulation of the elements in the mussel parts. For both soft tissues and byssus, PCO1 accounts for > 40% of the dispersion. Principal component 2 (PCO2) explains 16–22% of the total variation. The negative direction of PCO2 is associated with Zn, As, Cd, Pb, and Hg, and in the positive direction of PCO2, there are vectors of Co, Ni, and Cr for soft tissues and Co, Ni, and Cu for byssus. It appears that the PCO2 is related to the station location: its positive half-plane is dominated mostly by the observations from Station 1, and in its negative half-plane are mainly the Station-2 points. The data for

**Table 2** Parameters of the most meaningful correlations of the form $Y=aX^b$ between the element contents in soft tissues (T), byssus (B), shell liquor (L), and seawater (W) at the three stations (St.1–3): *a*|*b* above the diagonal and $R^2$ below the diagonal

| X\Y | | T | | | B | | | L | | | W | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | St.1 | St.2 | St.3 | St.1 | St.2 | St.3 | St.1 | St.2 | St.3 | St.1 | St.3 |
| T | St.1 | - | 0.98\|1.08 | 0.75\|1.07 | 2.80\|1.02 | | | 0.71\|0.62 | | | 1.84\|0.91 | |
| | St.2 | 0.96 | - | 0.77\|0.99 | | 2.22\|0.92 | | | 0.62\|0.51 | | | |
| | St.3 | 0.93 | 0.95 | - | | | 4.11\|1.01 | | | 2.42\|0.55 | | 3.83\|0.81 |
| B | St.1 | 0.88 | | | - | 0.78\|0.99 | 0.93\|1.11 | 0.41\|0.56 | | | 1.03\|0.84 | |
| | St.2 | | 0.86 | | 0.97 | - | 1.29\|1.10 | | 0.46\|0.47 | | | |
| | St.3 | | | 0.82 | 0.96 | 0.94 | - | | | 1.28\|0.48 | | 2.35\|0.69 |
| L | St.1 | 0.79 | | | 0.76 | | | - | 0.85\|0.82 | 2.88\|0.92 | 4.43\|1.29 | |
| | St.2 | | 0.73 | | | 0.60 | | 0.76 | - | 3.44\|0.86 | | |
| | St.3 | | | 0.72 | | | 0.69 | 0.81 | 0.62 | - | | 1.52\|1.25 |
| W | St.1 | 0.73 | | | 0.69 | | | 0.60 | | | - | 1.90\|0.97 |
| | St.3 | | | 0.72 | | | 0.58 | | | 0.78 | 0.93 | - |

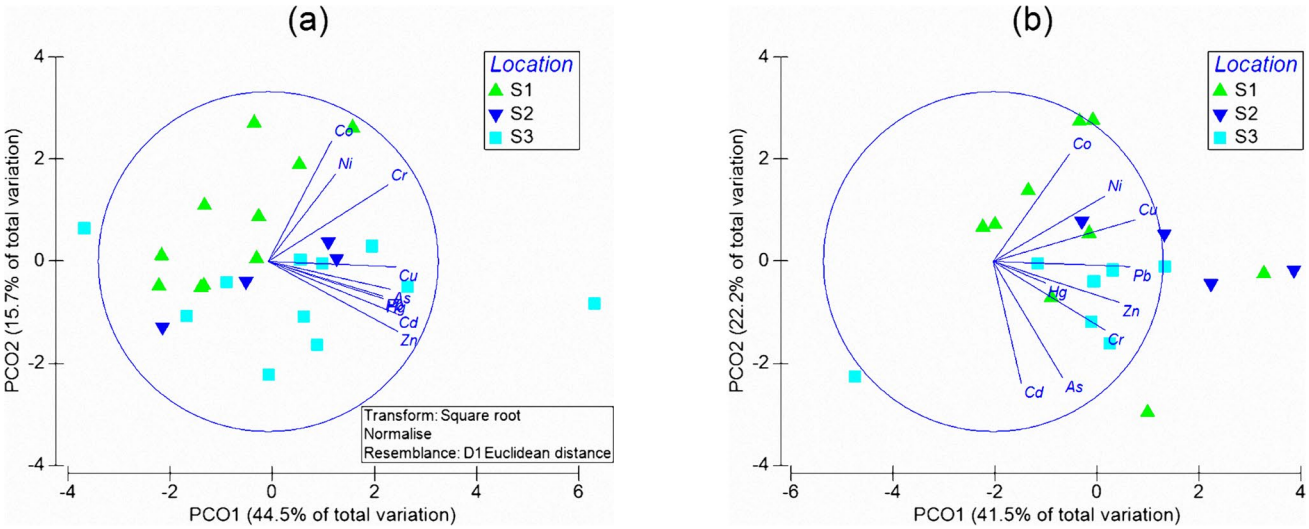


**Fig. 3** Principal component analysis of trace element contents by location factor in (**a**) soft tissues and (**b**) byssus of *M. galloprovincialis*. S1, Station 1; S2, Station 2; S3, Station 3. Vectors: projections of the Pearson correlation of variables onto the PCO1–PCO2 plane

Station 3 are in between and largely close to PCO2 = 0. This illustrates the overall element accumulation in mussel tissues increasing from Station 1 through Station 3 to Station 2. Interestingly, sampling location, and thus, different element levels in the environment, was not the main factor determining the dispersion of the points.

Figure 4 demonstrates the results of principal component analysis of square-root-transformed standardized trace element data assorted according to the body part type, with both the element contents (Fig. 4a) and sampling location (Fig. 4b) used as variables. In both cases, most of observations related to a certain body part type are bunched in clusters separated from each other. This feature allows deciding which body part a particular suite of element contents originates from. By analogy with Fig. 3, PCO1 in Fig. 4a explaining 44.3% of the total variation is determined by the overall element accumulation (mainly associated with Pb, Cr, Cu, Ni, Co, whose vectors are close to the positive direction of PCO1) in mussel body parts, from shell liquor to byssus. The greatest contribution to PCO2 explaining 23.4% of the total variation is made by the chalcophilic elements Cd, As, Zn, and Hg, which tend to be more concentrated in shell liquor and/or soft tissues. If the variable is sampling location (Fig. 4b), PCO1 explains 58.4% of the total variation

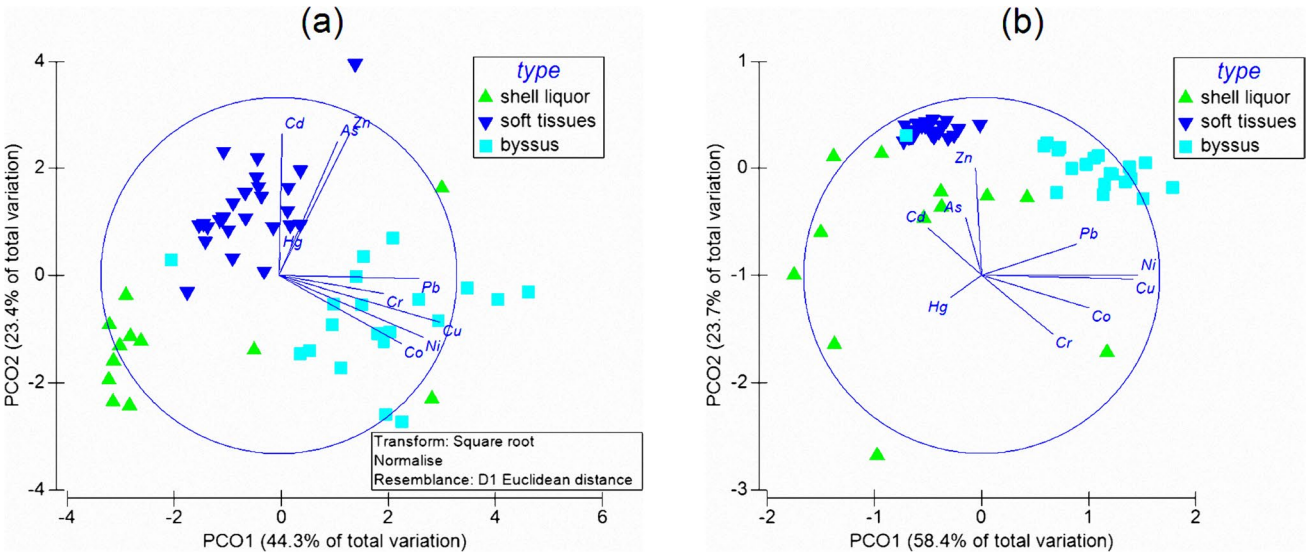


**Fig. 4** Principal component analysis of trace element contents in mussel body parts with **a** element contents and **b** locations as variables. Vectors: projections of the Pearson correlation of square-root-transformed standardized element contents onto the PCO1–PCO2 plane

and is related to all the three body parts, with shell liquor and byssus making the largest contribution, whereas PCO2 explaining 23.7% of the total variation is associated mainly with the element content dispersion in shell liquor. The vectors in Fig. 4b shifted to the origin point to the body parts in which the highest contents of the respective elements are observed: Cr, Co, Ni, Cu, and Pb in byssus; Zn, As, and Cd in soft tissues; and Hg in shell liquor.

### Human health risk assessment

Given the potential hazards to human health from the trace element intake, trace elements in cultivated mussels were compared with maximum permissible trace element levels in edible mollusks from the European, Turkish, and Russian regulations (EC (Commission of the European Communities) 2006; Official Gazette of Republic of Turkey 1995, 2002, 2009, 2011 SanPiN 42-123-4089-86 1992). The measured contents of Cu, As, Hg, Pb, and Zn in the mussels' soft tissues did not exceed the maximum permissible levels (Table 3).

It was found that EDI (Eq. (1)) for all elements under consideration were lower than the corresponding reference values ($RfD_o$, PTDI and UDI). THQ and HI calculated from EDI according to Eqs. (1)–(3) were well below one, which indicate no likely noncarcinogenic health risk from consuming soft tissues of *M. galloprovincialis* harvested from the mollusk farm.

Carcinogenic risks are assessed by calculating CRI and $CRI_t$ from Eqs. (4) and (5) using slope factors given in (USEPA, 2021). Among the elements in question, they were reported for As, Cr, and Pb. The highest CRI ($5.4 \cdot 10^{-6}$) is found for As, and the total $CRI_t$ is $9.5 \cdot 10^{-6}$, which indicates tolerable carcinogenic risks from consuming cultivated mussels' soft tissue.

## Discussion

*M. galloprovincialis* can absorb trace elements from water, as well as ingest with phytoplankton and other suspended particles entering the digestive system (Haryono et al. 2017; Mikac et al. 1996). Trace element entering the food chain can be accumulated in the mollusk tissues to hazardous levels and be harmful to mollusk consumers' health (Manahan 2000). Cd, Pb, Hg, and inorganic As have a negative impact on organisms and are harmful even in trace amounts. They cannot be metabolized into harmless forms and are accumulated in the human body over time causing chronic illness and other health problems (Ati-Hellal and Hellal 2021; Stankovic and Jovic 2012). In the present study, the content of the most toxic elements in soft tissues of *M. galloprovincialis* from the Black Sea decreased in the following order: As > Pb > Cd > Hg. The same order was observed for *M. galloprovincialis* from the southeastern Adriatic, Montenegro (Stankovic et al. 2011).

As emphasized in (Horne 1969), seawater from different oceans and seas contains all trace elements. Their concentrations increase in coastal waters, which are under impact of many anthropogenic factors. The mussel sampling sites under study (Fig. 1) are affected by numerous discharges, including untreated domestic wastewater, shipyard and dock effluents, storm water outlets, urban and industrial emissions, farmland runoff (containing fertilizers and pesticides), and sewage of fleet stationed in city bays (Gruzinov et al. 2019; Smirnova and Riabinin 2013). The selected sampling sites differ also in the chemical composition of seawater and mussel food supply. Station 1 (mollusk farm area) is characterized by unhindered water exchange with the open sea and abundant food supply for mussels (cf. *Characteristics of the sampling sites*). At the seafloor under the mollusk farm (Station 2), trace elements are extracted from sediments and the proportion of trace element-enriched suspended matter increases in the mussel diet (Ergül et al. 2008; Tankéré et al. 2001). Station 3 is located in the area where trace element-contaminated waters of the Chernaya River flow into Sevastopol Bay (Gruzinov et al. 2019). In this estuarine area, the concentrations of free heavy metal forms (Zn, Cd, Cu, Pb) progressively increase with the salinity rise (Egorov 2021; Lapin and Krasyukov 1986).

As mentioned, due to the sufficient water exchange with the open sea areas and the lack of river discharge in the vicinity, seawater at Station 1 is cleaner than at Station 3 and the trace element concentrations at Station 1 are lower (Table 1). This makes the mollusk farm environment suitable enough for the mussel cultivation because (a) the hazard index of the trace element intake is well below one, which implies no likely noncarcinogenic health risks, in line with other estimates for the Black Sea mussels (Bat et al. 2018b), and (b) the total cancer risk index is in the order of $10^{-5}$, which indicates tolerable (allowable) carcinogenic risks. These risk estimates are somewhat higher than those found for mussels in the Sinop area (Turkey) in the southern Black Sea in summer 2019 (Bat et al. 2021). This spatial difference is in overall agreement with the trace element accumulation pattern in the gastropod *Rapana venosa* collected at the same sites (Ryabushko et al. 2022). A probable reason for this difference is that waters in the Sinop nearshore area may be cleaner, in terms of the trace element levels, than those near the southwestern coast of Crimea.

In benthic mussels collected from the seafloor under the farm (Station 2), the trace element contents in soft tissues were highest. It was noted that seafloor sediments are the main repository of trace elements, including potentially hazardous metals, in marine environment (Okoro et al. 2012; Sakai et al. 1986). Consequently, sediments can be

**Table 3** Measured versus maximum allowable (max) contents of trace elements and their estimated daily intakes (EDI, Eq. (1)) with soft tissues of *M. galloprovincialis* (collected at Station 1 = mollusk farm) versus upper tolerable levels established in different regulations for noncarcinogenic ($RfD_o$, PTDI, and UDI) and carcinogenic ($SF^{-1}$) risks. The resulting target hazard quotients (THQ, Eq. (2)) and hazard index (HI, Eq. (3)) are the measures of the long-term noncarcinogenic risk, and the cancer risk indices (CRI, Eq. (4); $CRI_t$, Eq. (5)) are the measures of the long-term carcinogenic risk

| | Content, $\mu g \cdot g^{-1}$ w.w.[a] | European max, $\mu g \cdot g^{-1}$ w.w. (EC (Commission of the European Communities) 2006) | Russian max, $\mu g \cdot g^{-1}$ w.w. (SanPiN 42-123-4089-86 1992) | Turkish max, $\mu g \cdot g^{-1}$ w.w. (Official Gazette of Republic of Turkey 1995, 2002, 2009, 2011) | EDI[b], $mg \cdot kg^{-1}$ b.w.$\cdot day^{-1}$ | $RfD_o$, $mg \cdot kg^{-1}$ b.w.$\cdot day^{-1}$ | PTDI, $mg \cdot kg^{-1}$ b.w.$\cdot day^{-1}$ | UDI, $mg \cdot kg^{-1}$ b.w.$\cdot day^{-1}$ | THQ | SF, $(mg \cdot kg^{-1}$ b.w.$\cdot day^{-1})^{-1}$ | CRI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Zn | 19.29 | - | 200 | 50[c] | $5.9 \cdot 10^{-4}$ | 0.3 | 1 | 0.36 | $2.0 \cdot 10^{-3}$ | - | |
| Cu | 1.70 | - | 30 | 20[c] | $5.2 \cdot 10^{-5}$ | 0.04 | 0.5 | 0.07 | $1.3 \cdot 10^{-3}$ | - | |
| As | 1.30 | - | 2 | - | $4.0 \cdot 10^{-5}$ | 0.0003 | 0.002 | - | $1.2 \cdot 10^{-2}$[d] | 1.5 | $5.4 \cdot 10^{-6}$ |
| Ni | 0.74 | - | - | - | $2.6 \cdot 10^{-5}$ | 0.02 | 0.003 | 0.0004 | $1.3 \cdot 10^{-3}$ | - | |
| Pb | 0.37 | 1.5 | 10 | 1.5 | $1.1 \cdot 10^{-5}$ | - | 0.004 | - | - | 0.0085 | $9.5 \cdot 10^{-8}$ |
| Cr | 0.27 | - | - | - | $8.1 \cdot 10^{-6}$ | - | - | - | - | 0.5 | $4.0 \cdot 10^{-6}$[e] |
| Cd | 0.24 | 1.0 | 2 | 1.0 | $7.5 \cdot 10^{-6}$ | 0.0001 | 0.001 | 0.0004 | $7.5 \cdot 10^{-2}$ | - | |
| Co | 0.08 | - | - | - | $2.3 \cdot 10^{-6}$ | 0.0003 | - | 0.0002 | $7.6 \cdot 10^{-3}$ | - | |
| Hg | 0.02 | 0.5 | - | 0.5 | $5.7 \cdot 10^{-7}$ | 0.0003 | 0.0006 | 0.0006 | $1.9 \cdot 10^{-3}$ | - | |
| HI | | | | | | | | | 0.10 | | |
| $CRI_t$ | | | | | | | | | | | $9.5 \cdot 10^{-6}$ |

[a]factor of conversion from dry to wet weight (w.w.) is 4.6

[b]factor of conversion from dry to cooked weight is 3.82 (Biandolino et al. 2021)

[c]withdrawn from Turkish Food Codex

[d]converted from total to inorganic As using the factor 0.09 (Neff 2002)

[e]assumed to be Cr(VI) only

considered indicators of long-term trace element pollution of aquatic environment. Although many trace elements, being micronutrients, are necessary for the normal physiological activity of aquatic organisms, many of them can be concentrated in excess of physiological requirements in soft tissues and become toxic (Briffa et al. 2020; Rouane-Hacene et al. 2015; Stankovic and Jovic 2012).

Growing mussels assimilate essential elements, which can be divided into two groups based on their levels in tissues (Horne 1969). The first group is the structural elements that are most abundant in mussel tissues and form organic and inorganic compounds in the mollusk tissues (shells, soft tissues, and byssal threads). The second group includes catalytic trace elements (micronutrients) that are present in small amounts, mainly as components of compounds that catalyze biochemical processes. Most of the trace elements under study (Cr, Co, Ni, Cu, Zn, As) have low atomic numbers (from 24 to 33) and may be involved in the functioning of marine organism cells as minor components of proteins, carbohydrates, and lipids. In particular, ions of catalytic elements Cu, Zn, Co, and Ni are known as enzyme activators (cofactors) (Horne 1969).

The contents of trace elements in different mussel body parts, including byssus, soft tissues and shell liquor, depend on many factors, such as concentrations of these elements in water and food (microalgae, detritus and various kinds of suspended matter), chemical speciation of the elements (free or bound in organic and inorganic compounds), their affinity and strength of interaction with tissue structures, food composition, filtration rate, and physicochemical characteristics of the marine environment in biotopes under consideration.

As shown in Fig. 2, considerable levels of Zn As, and Cd were observed in soft tissues and byssus of mussels from the biotopes under consideration, which suggest that these elements are most tightly bound to organic components of soft tissues. Byssus has been described as an organ that not only adsorbs trace elements from seawater, but also participates in the extraction of some elements from soft tissues (Koide et al. 1982; Szefer et al. 1999; Yap et al. 2003b). High abundances of some elements (Ni, Cu, Pb, Co) in byssus are associated with histidine and lysine residues, which are the components of byssal proteins. These elements form strong coordination bonds with histidine and lysine (Leberman and Rabin 1959; Naik et al. 2012; Yamauchi and Odani 1996), which strengthen byssus (Babarro and Reiriz 2010; Lucas et al. 2002; Reinecke et al. 2017).

The analysis revealed strong power-law-type correlations ($R^2 \geq 0.58$) of the trace element contents in the media under study. In the strongest correlations, which were observed between the element contents in soft tissues and byssi from the three stations (Table 2), the exponent was close to 1, which implies the similar element accumulation mechanisms in these body parts at all the three stations. Strong correlations between the element contents in byssus and soft tissues ($R^2 = 0.82–0.88$) with the exponents around 1 and much weaker correlations between the contents in byssus and seawater ($R^2 = 0.58–0.69$) suggest that the elements in byssus are extracted mainly from soft tissues rather than from seawater, and thus, its metal pollution bioindication function is mediated by the element accumulation in mussel tissues. Yap et al. (Yap et al. 2005) noticed that if large amounts of heavy metals are accumulated in soft tissues of *M. edulis*, then the metals are transferred to byssus in almost equal proportions, and the same pattern was noted in the present research (Table 1 and 2). However, because the complexes of many trace elements with amino acids in byssus are strong enough, we do not expect these elements to be intensely purged into seawater and their transport through byssus to be the main mechanism of trace element detoxification in mussels. Szefer et al. (Szefer et al. 2006) found that byssus of mytilids can indicate environment pollution with two groups of trace elements associated either with metal refineries (Cu, Pb, Zn, and Cd) or with other industrial activities (Co, Fe, Cr and Ni). However, in the present work, mussel byssus was found to efficiently accumulate elements (Ni, Cu, Pb, Co, and Cr) from both groups, but none of these elements has been identified as a manifest pollutant in the mollusk farm area (Kapranov et al. 2021a). This suggests that the tendency of accumulation of trace elements in mussel byssus is not always directly related to their abundance in the environment. It is likely that some element forms (e.g., suspended or chelated forms), although strongly contributing to the environment pollution, cannot be deposited in byssus from soft tissues or seawater.

For shell liquor, the correlations with the soft tissue and seawater concentrations are not very strong, which indicate that some of the elements in shell liquor are accumulated or discarded more selectively than in soft tissues and water. The exponents in the correlations with the soft tissue contents are close to 0.5, suggesting diffusion to be the main mechanism of the element transport between soft tissues and shell liquor. The exponents above 1 in the correlations with the concentrations in seawater indicate that seawater is not fully mixed with shell liquor and only partly affects trace element composition in it. Expectedly, the effect of the seawater more contaminated with trace elements (at Station 3) on the shell liquor composition is more pronounced ($R^2 = 0.78$) than that at Station 1 ($R^2 = 0.60$).

The most convenient means to depict many objects of similar type (such as samples of different tissues or samples from different locations) and linkages among them based on multiple factors (such as multiple element contents) is the use of multivariate analysis. The first results on arranging mussel samples from different locations in multidimensional space according to the element contents date back to the 1990s (Struck et al. 1997). Similar to our approach (Fig. 3),

principal component analysis was applied by Besada et al. (2011) to separate groups of locations with the highest contents of individual heavy metals. However, in contrast to our results with all the element vectors pointing in the positive direction of PC1, there was no apparent correlation between element contents in mussels from different sites, and it was the scatter among stations that made the greatest contribution to the maximum dispersion along PC1. In our case, sampling location is not the main determinant of the sample dispersion (this could rather be attributed to PC2), and it is likely that the biological characteristics such as sexual differentiation and gonadal ripening (Kapranov et al. 2021b) play a major role in the different accumulation of elements in mussels collected at sampling sites not too far away from each other (about 7 km between Station 1 and Station 3).

The principal component analysis shows also the possibility of determining which body part was used for the sampling (Fig. 4). In the work of Richir and Gobert (Richir and Gobert 2014), the similar discrimination was observed among different parts of soft tissues before and after spawning. In our study, byssus and especially shell liquor make the largest contribution to the overall dispersion, and if sampling location is used as variable, the shell liquor dispersion outreaches the other ones. This indicates that it is the trace element contents in shell liquor that are most strongly affected by the station location.

It is worthwhile to discuss in detail the role and distribution of each element under consideration. The content of *Zn* in soft tissues or byssus was in the order of 100 $\mu g \cdot g^{-1}$ d.w. and reached 40–45% of the total Zn accumulated in the body parts under study (Table 1). The observed high levels of Zn in soft tissue are related to the essentiality of this trace element for mussels. Zn serves as a catalyst in many physiological processes; it regulates growth, development, reproduction, and metabolic processes in mollusks (França et al. 2005; Korish and Attia 2020; Viarengo et al. 1990). As mentioned above, the content of Zn in byssus depends on its concentration in seawater and in soft tissues.

*Cu* was concentrated mostly in soft tissues and byssus. The low proportions of Cu in shell liquor (5–13%) indicated little or no copper excretion through this fluid. The results showed that the Cu content in byssus reached 61–79% (Fig. 2). It is likely that this mussel body part can both concentrate copper from seawater and withdraw this metal from the mollusk tissues. Previously, *Mytilus edulis* was also shown to contain higher copper content in byssus than in soft tissue (Szefer et al. 1997). It is known that Cu ions, like Zn, act in mollusks as cofactors of some enzymes, playing a key role in their functioning (Horne 1969; Khristoforova et al. 1994). In particular, they stimulate polyphenol oxidase, ascorbate oxidase, and other enzymatic systems. The combined effect of excess zinc and copper leads to the destruction of mollusk mitochondria and suppresses sperm motility (Earnshaw et al. 1986; Lyngby and Brix 1987). The major sources of Cu in the water body in question are wastewater, transport, copper-containing fertilizers and pesticides, welding and galvanization processes, and combustion of hydrocarbon fuels.

*As* is a metalloid that is rarely found in nature as a free element, but its compounds are found in air, water, soil and all living tissues (Kaur et al. 2011; Mayer et al. 1993). In aquatic organisms, arsenic occurs in organic and inorganic forms. Inorganic As compounds are highly toxic, whereas natural organoarsenicals are non-toxic (Neff 2002). The most important anthropogenic source of arsenic pollution off the southwestern coast of Crimea is chemical warfare burial sites with the warfare containing lewisite (Smirnova et al. 2005). Inorganic As is strongly carcinogenic. Depending on oxidation state, cell type, concentration, and exposure, it can induce excessive apoptosis (Chiarelli and Roccheri 2014). In our study, the arsenic content in mussels from the mollusk farm did not exceed 1.3 $\mu g \cdot g^{-1}$ w.w., which is below the maximum permissible level according to the Russian regulations (Table 3). In soft tissues of mussels, organic arsenic accounts for 91% of the total arsenic (Neff 2002). There is evidence that in the mollusk farm area, the As concentration in seawater exceeded the maximum permissible level; however, *M. galloprovincialis* did not accumulate this element in soft tissues in large amounts (Ryabushko et al. 2017), in line with our results.

In our studies, the highest As content was in soft tissues of benthic mussels (Station 2), which fact was noted also by Wu et al. (Wu et al. 2014) for species sampled in the East China Sea: the closer the organism's habitat to the sediments, the higher the arsenic content in it. Despite the fact that the As content share in byssus reached as much as 40%, its content in soft tissues was significantly higher than in byssus and shell liquor (Table S6, Supplementary Material). Ünlü and Fowler (Ünlü and Fowler 1979) noted that active secretion of arsenic in byssus of the mussel *M. galloprovincialis* contributes to the elimination of arsenic from the mussel body.

*Ni* is discharged into the aquatic environment mainly with municipal and industrial wastewaters. For some invertebrates, it is a trace element with essential biological role since they produce enzymes that contain Ni in active sites. However, in high concentrations, Ni is toxic (Chalkiadakis et al. 2013). It is known that nickel, along with cadmium and arsenic, inhibits DNA repair mechanisms (Lucas et al. 2002). In byssus, the Ni content percentage was highest among all the elements under study (Fig. 2), and it was 8–10 times higher than in soft tissues (Table 1). Earlier, Szefer et al. (Szefer et al. 2002) noted that among all metals (Hg, Cd, Pb, Ag, Cu, Zn, Cr, Ni, Co, Mn и Fe), Ni was deposited

in greatest proportions in byssus of *Mytilus edulis trossulus* (southern Baltic) as compared to its soft tissues.

*Pb* is a heavy metal that is toxic to animals and humans. It damages nervous system and causes various disorders (Nava-Ruiz et al. 2012). The main source of lead in the environment is anthropogenic activity (in particular, this heavy metal is released from anti-fouling paints and acid batteries). Lead toxicity in marine invertebrates depends on the species identity and its life stage (Chiarelli and Roccheri 2014). In the present research, at all stations, the Pb content in byssus of *M. galloprovincialis* was significantly higher than in its soft tissues (Table S7), and we can suggest that this element is actively excreted through byssus. The highest percentage of Pb among the body parts, up to 79%, was in byssus of *M. galloprovincialis* from Station 3 (Fig. 2c). The high Pb concentration in this water area was apparently due to the shipbreaking plant activity and the inflow of the Chernaya River that can transport large amounts of Pb (Gruzinov et al. 2019). Thus, the results obtained support the idea that *M. galloprovincialis* byssus is an excellent indicator of the seawater pollution with lead. The Pb contents in soft tissues were comparable with other researchers' results obtained using mussels from the southwestern Black Sea (Bat et al. 2018a; Belivermiş et al. 2016; Çulha et al. 2017).

*Cd* is a very toxic environmental pollutant and cell poison that causes different types of damage including cell death. A high percentage of its content was observed in soft tissues, up to 62% (Fig. 2c). Significant differences were found for the Cd content between soft tissues and byssus (Table S8). Also, significant differences in the Cd content were registered between soft tissues and shell liquor, which suggest that shell liquor is not used for the removal of this toxicant. Li et al. (Li et al. 2006) noted that bivalves do not regulate Cd levels and usually accumulate this element. Cd is accumulated in cells by interacting with cellular components and molecular targets (Chiarelli and Roccheri 2014; Kingsley and Frazier 1979). In invertebrates, it stimulates the expression of antioxidant enzymes, metallothioneins, and heat shock proteins. It inhibits the expression of digestive enzymes, esterases and phospholipases. Cd also affects tissue organization, immune responses, and cell cycles by inducing apoptosis (Sokolova et al. 2004). The relatively low levels of Cd as compared to higher levels of Pb in byssus may be partly a result of the more efficient transfer of Pb from soft tissues to byssus, as opposed to Cd, which is strongly accumulated in soft tissues in hepatopancreas (Szefer et al. 2006). The high content of cadmium in soft tissues of mussels appears to be related to its high levels in sediments in the adjacent bays (Gubanov et al. 2010).

Sources of *Cr* in the environment can be both anthropogenic and natural. Natural chromium occurs mainly in trivalent state, whereas hexavalent Cr (VI) in the environment originates almost entirely from human activities (Liang et al. 2021; Sacchi et al. 2021). This metal, especially in the hexavalent state, is a very toxic trace element posing certain threats to coastal ecosystems. Coastal chromium pollution is mainly due to the discharge of untreated or poorly treated industrial wastes. Cr (VI) is 30 times as toxic as Cr (III), and it is both mutagenic and carcinogenic (Natale et al. 2000). In the southwestern Crimea, the anthropogenic source of chromium can be shipyards and the shipbreaking plant, and the natural source of chromium is volcanic rocks of the Crimean peninsula.

The Cr content in our studies in soft tissues and byssus of *M. galloprovincialis* was more than twice as low as in *Mytilus edulis trossulus* from polluted waters of the Gulf of Gdańsk, Poland (Szefer et al. 2002). Our results showed that the content of Cr in byssus was significantly higher than that in soft tissues (Table S9). Earlier, the same pattern was noted for *M. edulis trossulus* from the Gulf of Gdańsk, and byssus was recommended for use in chromium pollution biomonitoring (Szefer et al. 2002). It is known that Cr (VI) significantly affects the functional and structural parameters of mussel gills, and this indicates that this tissue is the main target of the Cr (VI) exposure (Ciacci et al. 2012). There is evidence of the effect of Cr (VI) in vitro on immune system of *M. galloprovincialis* (Barmo et al. 2011). In *M. edulis*, the DNA chain breaks in the gill cells under the influence of Cr (VI) (Emmanouil et al. 2007). An increase in the total content of Cr (VI) in tissues and destabilization of lysosomal membranes were observed in digestive gland of *M. galloprovincialis* treated with a high concentration of this heavy metal (100 $\mu g \cdot L^{-1}$), and oxidative stress occurred in this organ (Barmo et al. 2011).

*Co* is an important component of vitamin $B_{12}$ and a cofactor for several enzymes (Lehninger 1976; Nolan and Dahlgaard 1991). In all the water areas under study, cobalt was slightly more accumulated in soft tissues (up to 32% among the three body parts). In relatively low concentrations, this trace element becomes toxic. In mammals, it can induce apoptosis, necrosis, or inflammatory response in the body and is genotoxic (Briffa et al. 2020).

Co was accumulated and extracted mainly by byssus and, to a slight extent, by shell liquor (Table 1, Fig. 2). Significant differences in the Co content were found between soft tissues and byssus (Table S10). As shown by Szefer et al. (Szefer et al. 2006), mytilids from industrialized regions also showed higher concentrations of Co in byssus than in soft tissues. Byssus of *M. edulis* was proposed to be used in Co pollution biomonitoring (Szefer et al. 1999).

There are three chemical forms of *Hg* in the environment: elemental, organic, and inorganic. These forms are mutually transformable into each other, and they all can cause systemic toxicity (Graeme and Pollack Jr 1998). Hg can occur naturally in the environment or come from anthropogenic sources. A significant fraction of Hg is volatilized and

returns to the atmosphere, but much of this metal entering the coastal areas precipitates due to very low solubility of its compounds (Stankovic and Jovic 2012). Anthropogenic sources of Hg in the water areas under consideration can be ballast discharge from submarines and domestic wastewater. Natural sources of mercury include numerous methane seeps off coasts of southwestern Crimea and in bays of Sevastopol (Egorov et al. 2011). This element is accumulated in sediments, which are its main sink. It was noted that most of the inorganic and organic Hg compounds in the aquatic environment are adsorbed on suspended matter and settle down to the bottom (Schiff 2000). For this reason, the highest content of mercury in soft tissue was found in mussels sampled from the seafloor under the farm. In sediments, bacteria can convert inorganic mercury to methylmercury, the most toxic Hg compound (Harada et al. 1998).

In our study, the mercury contents in soft tissues and byssus were below 0.2 $\mu g \cdot g^{-1}$ (Table 1). The same pattern was observed for *M. galloprovincialis* from the Cantabrian coast (Bartolomé et al. 2010). It should be noted that in *M. galloprovincialis*, Hg was removed from soft tissues mainly through shell liquor (up to 71%). In the body of marine animals, methylmercury cloride is quantitatively absorbed into blood stream (Wood 1975). As a result, shell liquor, being in contact with blood, contains high concentrations of mercury (Table 1). Our results show that the function of shell liquor and byssus in *M. galloprovincialis*, among others, is to protect its vitals by extracting and excreting toxic trace elements.

## Conclusions

In the present study, the distribution of potentially toxic trace elements (Cd, Cu, As, Ni, Hg, Pb, Zn, Cr, Co) in soft tissues, byssus, and shell liquor of the mussel *M. galloprovincialis* living in the coastal waters of the Black Sea off southwestern Crimea has been studied. For the first time, these elements have been quantified in shell liquor and the role of shell liquor as a detoxifying system has been demonstrated by an example of mercury.

At all the sampling stations, the trace element contents in soft tissues of *M. galloprovincialis* have been found to decrease in the following order: Zn > Cu > As > Ni > Pb > Cd > Cr > Co > Hg. The contents of Cd, Cu, As, Hg, Pb, and Zn in soft tissues of the cultured mussels did not exceed the permissible levels according to European, Turkish, and Russian regulations. Estimated daily intakes (EDI) of these elements have been found below the tolerable values set by international authorities (WHO, European Commission and USEPA), indicating no likely noncarcinogenic risks of consuming the mussels cultivated on the mollusk farm. The carcinogenic risk indices have been found to be in the tolerable range.

The mollusks collected from the seaflooor under the farm have shown the maximum content of potentially toxic metals in all body parts under study. Significant differences have been registered in the overall element contents in *M. galloprovincialis* from the three stations under study. Moreover, the contents of individual elements have been found to vary in different mussel body parts. Zn, Cd, and As have the largest shares in soft tissues: 44–3%, 34–62%, and 45–54%, respectively. Principal component analysis has shown a tendency of the data points to clustering with sample location as the grouping factor. A similar clustering tendency has been observed if the grouping factor is body part type.

Byssus of mussels from the three biotopes under consideration has been identified as a tissue with the highest percentage of contents of many potentially toxic metals: Ni (81–91%), Cu (61–79%), Pb (57–79%), and Co (54–69%). This body part performs the function of not only attachment to the substrate, but it also extracts heavy metals from soft tissues, apparently to a greater extent than from water. It is a better bioindicator of the environment pollution with Ni, Cu, Pb, Co, and Cr than mussels' soft tissues. The data obtained have shown the need for the further study of the role of byssus in translocation of trace elements in mussel tissues. In particular, the question about the ratio of the accumulating and excreting functions of byssus remains unresolved.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-20186-1.

**Acknowledgements** The authors are grateful to Dr. Nelya P. Kovrigina for providing the data on the trophic index TRIX.

**Author contribution** NSC and MAP took part in collecting the material, setting up and conducting the experiment, processing the data obtained, and discussing the materials. SVK edited the final version of the manuscript and contributed to the ICP-MS and data analysis. LLS contributed to the data analysis and editing the final version of the manuscript. NIB prepared analytical samples and contributed to the ICP-MS analysis.

**Funding** This work was supported by the Ministry of Science and Higher Education of the Russian Federation within the governmental research assignments (grant numbers 121030300149-0 and AAAA-A19-119031490078-9).

**Data availability** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** Ethical standards were met during the experiment. All samplings were conducted in accordance with the Russian Federation law on harvesting biological resources in natural waters.

**Consent to participate** All authors consented to participate.

**Consent for publication** All authors consented to the publication.

**Competing interests** The authors declare no competing interests.

## References


Abderrahmani K, Boulahdid M, Bendou N, Aissani A (2020) Seasonal distribution of cadmium, lead, nickel, and magnesium in several tissues of mussels from the Algerian coasts. Environ Sci Pollut Res 27:22547–22567. https://doi.org/10.1007/s11356-020-08682-8

Adams WJ, Rowland CD (2003) Aquatic toxicology test methods. In: Hoffman DJ, Rattner BA, Burton GA, Cairns J (eds) Handbook of Ecotoxicology. Lewis Publishers, Boca Raton, pp 19–43. https://doi.org/10.1201/9781420032505

Atasaral ŞŞ, Romero MR, Cueto R, González-Lavín N, Marcos M, Diz AP (2015) Subtle tissue and sex-dependent proteome variation in mussel (*Mytilus galloprovincialis*) populations of the Galician coast (NW Spain) raised in a common environment. Proteomics 15(23-24):3993–4006. https://doi.org/10.1002/pmic.201500241

Ati-Hellal ME, Hellal F (2021) Heavy metals in the environment and health impact. In: Otsuki T (ed) Environmental Health. IntechOpen. https://doi.org/10.5772/intechopen.97204

Babarro JMF, Reiriz FMJ (2010) Secretion of byssal threads in *Mytilus galloprovincialis*: quantitative and qualitative values after spawning stress. J Comp Physiol B 180(1):95–104. https://doi.org/10.1007/s00360-009-0392-y

Barmo C, Ciacci C, Fabbri R, Olivieri S, Bianchi N, Gallo G, Canesi L (2011) Pleiotropic effects of hexavalent chromium (Cr VI) in *Mytilus galloprovincialis* digestive gland. Chemosphere 83(8):1087–1095. https://doi.org/10.1016/j.chemosphere.2011.01.037

Bartolomé L, Navarro P, Raposo JC, Arana G, Zuloaga O, Etxebarria N, Soto M (2010) Occurrence and distribution of metals in mussels from the Cantabrian coast. Arch Environ Contam Toxicol 59:235–243. https://doi.org/10.1007/s00244-010-9476-7

Bat L, Öztekin HC (2016) Heavy metals in *Mytilus galloprovincialis*, *Rapana venosa* and *Eriphia verrucosa* from the Black Sea coasts of Turkey as bioindicators of pollution. Walailak J Sci Technol 13(9):715–728

Bat L, Arıcı E, Öztekin A (2018a) Human health risk assessment of heavy metals in the Black Sea: evaluating mussels. Current World. Environment 13(1):15–31. https://doi.org/10.12944/CWE.13.1.03

Bat L, Sahin F, Öztekin A (2018b) Toxic elements in edible mollusks from Igneada coasts of the Black Sea, Turkey. Korean J Food Health Converg 4:22–31. https://doi.org/10.13106/kjfhc.2018.vol4.no3.22

Bat L, Öztekin A, Arıcı E, Şahin F (2021) *Mytilus galloprovincialis* and metal contaminants: health risk assessment from Sinop coasts. Korean J Food Health Converg 7(4):13–21. https://doi.org/10.13106/kjfhc.2021

Belivermiş M, Kılıç Ö, Çotuk Y (2016) Assessment of metal concentrations in indigenous and caged mussels (*Mytilus galloprovincialis*) on entire Turkish coastline. Chemosphere 144:1980–1987. https://doi.org/10.1016/j.chemosphere.2015.10.098

Besada V, Andrade JM, Schultze F, González JJ (2011) Monitoring of heavy metals in wild mussels (Mytilus galloprovincialis) from the Spanish North-Atlantic coast. Cont Shelf Res 31:457–465. https://doi.org/10.1016/j.csr.2010.04.011

Biandolino F, Parlapiano I, Denti G, Di Nardo V, Prato E (2021) Effect of different cooking methods on lipid content and fatty acid profiles of *Mytilus galloprovincialis*. Foods 10(2):416. https://doi.org/10.3390/foods10020416

Boening DW (1999) An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environ Monit Assess 55:459–470. https://doi.org/10.1023/A:1005995217901

Boltachev AR, Karpova EP, Danilyuk ON (2010) Peculiarities of thermohaline parameters and ichthyocenosis of the Chernaya river estuary (the Sevastopol Bay). Mar Ecol J 2(9):23–36 (in Russian)

Briffa J, Sinagra E, Blundell R (2020) Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 6(9):2–26. https://doi.org/10.1016/j.heliyon.2020.e04691

Cantillo AY (1998) Comparison of results of mussel watch programs of the United States and France with worldwide mussel watch studies. Mar Pollut Bull 36(9):712–717. https://doi.org/10.1016/S0025-326X(98)00049-6

Casas S, Gonzalez JL, Andral B, Cossa D (2008) Relation between metal concentration in water and metal content of marine mussels (*Mytilus galloprovincialis*): impact of physiology. Environ Toxicol Chem 27:1543–1552. https://doi.org/10.1897/07-418.1

Chalkiadakis O, Paraskevopoulou V, Roussos I, Dassenakis M, Simantiris LN (2013) Comparative study of the accumulation of Ni in different tissues of mussels and soft clams. E3S Web Conf 1:11003. https://doi.org/10.1051/e3sconf/20130111003

Chelyadina NS, Smirnova LL (2018) Content of ascorbic acid, calcium, copper, and zinc in the interstitial fluid of cultivated mussel *Mytilus galloprovincialis* Lam. in waters of the south-west coast of Crimea. Scientific Notes of V.I. Vernadsky Crimean Federal University. Biol Chem 70(4):237–246 (in Russian)

Chelyadina NS, Pospelova N, Kopytov YP (2015) Distribution of copper in the tissues of males and females of *Mytilus galloprovincialis*. Hydrobiol J 51(4):74–79. https://doi.org/10.1615/HydrobJ.v51.i4.90

Chemnitzer R (2019) Strategies for achieving the lowest possible detection limits in ICP-MS. Spectroscopy 34(10):12–16

Chiarelli R, Roccheri MC (2014) Marine invertebrates as bioindicators of heavy metal pollution. Open J Metal 4:93–106. https://doi.org/10.4236/ojmetal.2014.44011

Chijioke NO, Khandaker MU, Tikpangi KM, Bradley DA (2020) Metal uptake in chicken giblets and human health implications. J Food Compos Anal 85:103332. https://doi.org/10.1016/j.jfca.2019.103332

Ciacci C, Barmo C, Gallo G, Maisano M, Cappello T, D'Agata A, Leonzio C, Mauceri A, Fasulo S, Canesi L (2012) Effects of sublethal, environmentally relevant concentrations of hexavalent chromium in the gills of *Mytilus galloprovincialis*. Aquat Toxicol 120:109–118. https://doi.org/10.1016/j.aquatox.2012.04.015

Clarke KR, Gorley RN, Somerfield PJ, Warwick RM (2014) Change in marine communities: an approach to statistical analysis and interpretation, 3rd edn. PRIMER–E, Plymouth

Coombs TL, Keller PJ (1981) Mytilus byssal threads as an environmental marker for metals. Aquat Toxicol 1(5–6):291–300. https://doi.org/10.1016/0166-445X(81)90023-0

Çulha ST, Çulha M, Karayücel İ, Çelik MY, Işler Y (2017) Heavy metals in *Mytilus galloprovincialis*, suspended particulate matter and sediment from offshore submerged longline system, Black Sea. Int J Environ Sci Technol 14:385–396. https://doi.org/10.1007/s13762-016-1158-1

Earnshaw MJ, Wilson S, Akberali HB, Butler RD, Marriott KRM (1986) The action of heavy metals on the gametes of the marine mussel, *Mytilus edulis* (L.)—III. The effect of applied copper and zinc on sperm motility in relation to ultrastructural damage and intracellular metal localization. Mar Environ Res 20(4):261–268. https://doi.org/10.1016/0141-1136(86)90052-8

EC (Commission of the European Communities) (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union L364:5–24

EFSA (2006) Tolerable upper intake levels for vitamins and minerals. European Food Safety Authority

Egorov VN (2021) Theory of Radioisotopic and chemical homeostasis of marine ecosystems. Springer, Cham. https://doi.org/10.1007/978-3-030-80579-1

Egorov VN, Artemov, Yu G, Gulin SB (2011) Methane seeps in the Black Sea – environmental and ecological role. EKOSI-Gidrofizika, Sevastopol (in Russian)

Emmanouil C, Sheehan TM, Chipman JK (2007) Macromolecule oxidation and DNA repair in mussel (*Mytilus edulis* L.) gill following exposure to Cd and Cr (VI). Aquat Toxicol 82(1):27–35. https://doi.org/10.1016/j.aquatox.2007.01.009

Ergül HA, Topcuoğlu S, Ölmez E, Kırbasoğlu Ç (2008) Heavy metals in sinking particles and bottom sediments from the eastern Turkish coast of the Black Sea. Estuar Coast Shelf Sci 78:396–402. https://doi.org/10.1016/j.ecss.2008.01.006)

Esposito G, Mudadu AG, Abete MC, Pederiva S, Griglione A, Stella C, Ortu S, Bazzoni AM, Meloni D, Squadrone S (2021) Seasonal accumulation of trace elements in native Mediterranean mussels (*Mytilus galloprovincialis* Lamarck, 1819) collected in the Calich Lagoon (Sardinia, Italy). Environ Sci Pollut Res 28:25770–25781. https://doi.org/10.1007/s11356-021-12380-4

FAO/WHO (2011) Evaluation of certain food additives and contaminants: seventy-second report of the joint FAO/WHO expert committee on food additives. World Health Organization, Geneva

Filippini T, Tancredi S, Malagoli C, Malavolti M, Bargellini A, Vescovi L, Nicolini F, Vinceti M (2020) Dietary estimated intake of trace elements: risk assessment in an Italian population. Exposure Health 12:641–655. https://doi.org/10.1007/s12403-019-00324-w

França S, Vinagre C, Caçador I, Cabral HN (2005) Heavy metal concentrations in sediment, benthic invertebrates and fish in three salt marsh areas subjected to different pollution loads in the Tagus Estuary (Portugal). Mar Pollut Bull 50(9):993–1018. https://doi.org/10.1016/j.marpolbul.2005.06.040

Gosling EM (2003) Bivalve molluscs: biology, ecology and culture. Blackwell, Oxford, UK

Graeme KA, Pollack CV Jr (1998) Heavy metal toxicity, part I: arsenic and mercury. J Emerg Med 16:45–56. https://doi.org/10.1016/S0736-4679(97)00241-2

Gruzinov VM, Dyakov NN, Mezenceva IV, Malchenko YA, Zhohova NV, Korshenko AN (2019) Sources of coastal water pollution near Sevastopol. Oceanology 59:523–532. https://doi.org/10.1134/S0001437019040076

Gubanov VI, Kopytov YP, Bobko NI (2010) Assessment of bottom sediments in Crimea coastal regions pollution by heavy metals. Mar Ecol J 9(4):38–47 (in Russian)

Gupta SK, Singh J (2011) Evaluation of mollusc as sensitive indicatior of heavy metal pollution in aquatic system: a review. IIOAB J 2(1):49–57

Harada M, Nakanishi J, Konuma S, Ohno K, Kimura T, Yamaguchi H, Tsuruta K, Kizaki T, Ookawara T, Ohno H (1998) The present mercury contents of scalp hair and clinical symptoms in inhabitants of the Minamata area. Environ Res 77(2):160–164. https://doi.org/10.1006/enrs.1998.3837

Haryono MG, Mulyanto, Kilawati Y (2017) Heavy metal Pb content in the seawater, sediment and green mussel tissue *Perna viridis*. Jurnal Ilmu dan Teknologi Kelautan Tropis 9(1):1–7. https://doi.org/10.29244/jitkt.v9i1.17864

Horne BA (1969) Marine Chemistry: The structure of water and the chemistry of the hydrosphere. Wiley, New York

Ivanov VN, Kholodov VI, Senicheva MI, Pirkova AV, Bulatov KV (1989) Biology of cultivated mussels. Naukova dumka, Kiev (in Russian)

Kapranov SV, Karavantseva NV, Bobko NI, Ryabushko VI, Kapranova LL (2021a) Element contents in three commercially important edible mollusks harvested off the southwestern coast of Crimea (Black Sea) and assessment of human health risks from their consumption. Foods 10(10):2313. https://doi.org/10.3390/foods10102313

Kapranov SV, Karavantseva NV, Bobko NI, Ryabushko VI, Kapranova LL (2021b) Sex- and sexual maturation-related aspects of the element accumulation in soft tissues of the bivalve *Mytilus galloprovincialis* Lam. collected off coasts of Sevastopol (southwestern Crimea, Black Sea). Environ Sci Pollut Res 28(17):21553–21576. https://doi.org/10.1007/s11356-020-12024-z

Kaur S, Kamli MR, Ali A (2011) Role of arsenic and its resistance in nature. Can J Microbiol 57:769–774. https://doi.org/10.1139/w11-062

Khandaker MU, Chijioke NO, Heffny NAB, Bradley DA, Alsubaie A, Sulieman A, Faruque MRI, Sayyed MI, Al-mugren KS (2021) Elevated concentrations of metal(loids) in seaweed and the concomitant exposure to humans. Foods 10:381. https://doi.org/10.3390/foods10020381

Kholodov VI, Pirkova AV, Ladygina LV (2017) Cultivation of mussels and oysters in the Black Sea. LLC Izdat-Print Publishing House, Voronezh (in Russian)

Khristoforova NK, Shulkin VM, Kavun VYA, Chernova EN (1994) heavy metals in commercial and cultivated mollusks in the Peter the Great Bay. Dal-nauka, Vladivostok (in Russian)

Kingsley BS, Frazier JM (1979) Cadmium transport in isolated perfused rat liver: zinc-cadmium competition. Am J Physiol 236:139–143. https://doi.org/10.1152/ajpcell.1979.236.3.C139

Koide M, Lee DS, Goldberg ED (1982) Metals and transuranic records in mussel shells, byssal threads and tissues. Estuar Coast Shelf Sci 15(6):679–695. https://doi.org/10.1016/0272-7714(82)90079-8

Korish MA, Attia YA (2020) Evaluation of heavy metal content in feed, litter, meat, meat products, liver, and table eggs of chickens. Animals 10:727. https://doi.org/10.3390/ani10040727

Kuftarkova EA, Gubanov VI, Kovrigina NP, Eremin IY, Senicheva MI (2006) Ecological assessment of modern state of waters in the region of interaction of the Sevastopol bay and part of the sea contiguous to it. Mar Ecol J 5(1):72–91 (in Russian)

Lapin IA, Krasyukov VN (1986) Effect of humic acids on the behavior of heavy metals in estuarine waters. Oceanology 26(4):621–627 (in Russian)

Leberman R, Rabin BR (1959) Metal complexes of histidine. Trans Faraday Soc 55:1660–1670. https://doi.org/10.1039/tf9595501660

Lehninger AE (1976) Biochemistry, 2nd edn. Worth Publishers Inc, New York

Li Y, Yu Z, Song X, Mu Q (2006) Trace metal concentrations in suspended particles, sediments and clams (*Ruditapes philippinarum*) from Jiaozhou bay of China. Environ Monit Assess 121:491–501. https://doi.org/10.1007/s10661-005-9149-6

Liang J, Huang X, Yan J, Li Y, Zhao Z, Liu Y, Ye J, Wei Y (2021) A review of the formation of Cr(VI) via Cr(III) oxidation in soils and groundwater. Sci Total Environ 774:145762. https://doi.org/10.1016/j.scitotenv.2021.145762

Lucas J, Vaccaro E, Waite J (2002) A molecular, morphometric and mechanical comparison of the structural elements of byssus from *Mytilus edulis* and *Mytilus galloprovincialis*. J Exp Biol 205(12):1807–1817. https://doi.org/10.1242/jeb.205.12.1807

Lutz R, Chalermwat K, Figueras AJ, Gustafson RG, Newell C (1991) Mussel aquaculture in marine and estuarine environments throughout the world. In: Menzel W (ed) Estuarine and Marine Bivalve Mollusk Culture. CRC Press, Boca Raton, pp 57–97

Lyngby JE, Brix H (1987) Monitoring of heavy metal contamination in the Limfiord, Denmark, using biological indicators and sediment. Sci Total Environ 64(3):239–252. https://doi.org/10.1016/0048-9697(87)90248-8

Manahan SE (2000) Environmental chemistry. Lewis Publishers, Boca Raton

Massa F, Onofri L, Fezzardi D (2017) Aquaculture in the Mediterranean and the Black Sea: a *Blue Growth* perspective. In: Nunes PALD, Svensson LE, Markandya A (eds) Handbook on the economics and management of sustainable oceans. Edward Elgar Publishing, Cheltenham, pp 93–123. https://doi.org/10.4337/9781786430724.00013

Mayer DR, Kosmus W, Pogglitsch H, Mayer D, Beyer W (1993) Essential trace elements in humans. Serum arsenic concentrations in hemodialysis patients in comparison to healthy controls. Biol Trace Elem Res 37:27–38. https://doi.org/10.1007/bf02789399

Mikac N, Kwokal Z, Martincic D, Branica M (1996) Uptake of mercury species by transplanted mussels *Mytilus galloprovincialis* under estuarine conditions (Krka river estuary). Sci Total Environ 184:173–182. https://doi.org/10.1016/0048-9697(96)05078-4

Morduchai-Boltovsky FD (1972) Identification book for the fauna of the Black and Azov Seas. Naukova Dumka, Kyiv

Naik KBK, Kumar BA, Raju S, Rao GN (2012) Speciation studies of L-histidine complexes of Pb(II), Cd(II), and Hg(II) in DMSO-water mixtures. Int J Inorg Chem 3(2):194–205. https://doi.org/10.1155/2012/265249

Natale G, Basso N, Ronco A (2000) Effect of Cr (VI) on early life stages of three species of hylid frogs (Amphibia, Anura) from South America. Environ Toxicol 15(5):509–512. https://doi.org/10.1002/1522-7278(2000)15:5<509::AID-TOX21>3.0.CO;2-S

Nava-Ruiz C, Méndez-Armenta M, Ríos C (2012) Lead neurotoxicity: effects on brain nitric oxide synthase. J Mol Histol 43:553–563. https://doi.org/10.1007/s10735-012-9414-2

Neff JM (2002) Bioaccumulation in marine organisms. Elsevier, Amsterdam

Nicholson S, Szefer P (2003) Accumulation of metals in the soft tissues, byssus and shell of the mytilid *Perna viridis* (Bivalvia: Mytilidae) from polluted and uncontaminated locations in Hong Kong coastal waters. Mar Pollut Bull 46(8):1039–1043. https://doi.org/10.1016/S0025-326X(03)00152-8

Nolan C, Dahlgaard H (1991) Accumulation of metal radiotracers by *Mytilus edulis*. Mar Ecol Prog Ser 70:165–174. https://doi.org/10.3354/meps070165

Official Gazette of Republic of Turkey (1995) Chemical, toxicological and microbiological acceptable values for live (fresh), chilled and frozen crustaceans and molluscs, 95/6533:22223

Official Gazette of Republic of Turkey (2002) Notifications about determination of the maximum levels for certain contaminants in foodstuffs of Turkish Food Codex. Turk J Agric-Food Sci Technol 63:24885 (in Turkish)

Official Gazette of Republic of Turkey (2009) Notifications changes to the maximum levels for certain contaminants in foodstuffs 22:27143 (in Turkish)

Official Gazette of Republic of Turkey (2011) Turkish Food Codex Contaminants Regulation, 28157

Okoro HK, Fatoki OS, Adekola FA, Ximba BJ, Snyman RG (2012) A review of sequential extraction procedures for heavy metals speciation in soil and sediments. Open Access Sci Rep 1(3):1–9. https://doi.org/10.4172/scientificreports.181

Pospelova NV (2008) Elements of budget of carotenoids, α-tocopherol and some metals in the *suspended matter – mussels – bio-deposits* system. Dissertation, A.O. Kovalevsky Institute of Biology of the Southern Seas, Sevastopol, Ukraine (in Russian).

Rainbow PS, Phillips DJH (1993) Cosmopolitan biomonitors of trace metals. Mar Pollut Bull 26:593–601. https://doi.org/10.1016/0025-326X(93)90497-8

Rakib MRJ, Jolly YN, Dioses-Salinas DC, Pizarro-Ortega CI, De-la-Torre GE, Khandaker MU, Alsubaie A, Almalki ASA, Bradley DA (2021) Macroalgae in biomonitoring of metal pollution in the Bay of Bengal coastal waters of Cox's Bazar and surrounding areas. Sci Rep 11:20999. https://doi.org/10.1038/s41598-021-99750-7

Reinecke A, Brezesinski G, Harrington MJ (2017) pH-responsive self-organization of metal-binding protein motifs from biomolecular junctions in mussel byssus. Adv Mater Interf 4(1):1–11. https://doi.org/10.1002/admi.201600416

Richir J, Gobert S (2014) The effect of size, weight, body compartment, sex and reproductive status on the bioaccumulation of 19 trace elements in rope-grown *Mytilus galloprovincialis*. Ecol Indic 36:33–47. https://doi.org/10.1016/j.ecolind.2013.06.021

Rouane-Hacene O, Boutiba Z, Belhaouari B, Guibbolini-Sabatier ME, Francour P, Risso-de-Faverney C (2015) Seasonal assessment of biological indices, bioaccumulation and bioavailability of heavy metals in mussels *Mytilus galloprovincialis* from Algerian west coast, applied to environmental monitoring. Oceanologia 57:362–374. https://doi.org/10.1016/j.oceano.2015.07.004

Ryabushko LI, Pospelova NV, Balycheva DS, Kovrigina NP, Troshchenko OA, Kapranov SV (2017) Epizoon microalgae of the cultivated mollusk *Mytilus galloprovincialis* Lam. 1819, phytoplankton, hydrological and hydrochemical characteristics in the mussel-and-oyster farm area (Sevastopol, Black Sea). Mar Biol J 2(4):67–83 (in Russian). https://doi.org/10.21072/mbj.2017.02.4.07

Ryabushko VI, Toichkin AM, Kapranov SV (2022) Heavy metals and arsenic in soft tissues of the gastropod *Rapana venosa* (Valenciennes, 1846) collected on a mollusk farm off Sevastopol (Southwestern Crimea, Black Sea): assessing human health risk and locating regional contamination areas. Bull Environ Contam Toxicol. https://doi.org/10.1007/s00128-021-03451-w

Sacchi E, Bergamini M, Lazzari E, Musacchio A, Mor J-R, Pugliaro E (2021) Natural background levels of potentially toxic elements in groundwater from a former asbestos mine in Serpentinite (Balangero, North Italy). Water 13(5):735. https://doi.org/10.3390/w13050735

Sakai H, Kojima Y, Saito K (1986) Distribution of heavy metals in water and sieved sediment in the Toyoher River. Water Res 20(5):559–567. https://doi.org/10.1016/0043-1354(86)90019-9

SanPiN 42-123-4089-86 (1992) Maximum permissible levels of heavy metals and arsenic in food raw materials and foodstuffs. In: Podolskiy VM (ed) Sanitary Rules and Regulations, Hygiene Standards, and a List of Guidelines and Recommendations for Food Hygiene, 5. Information and Publishing Center of the State Committee for Sanitary and Epidemiological Supervision of the Russian Federation, Moscow, pp 355–357 (in Russian)

Schiff KC (2000) Sediment chemistry on the mainland shelf of the southern California bight. Mar Pollut Bull 40(3):268–270. https://doi.org/10.1016/S0025-326X(99)00216-7

Slepchuk KA, Khmara TV, Man'kovskaya EV (2017) Comparative assessment of the trophic level of the Sevastopol and Yuzhnaya bays using E-TRIX index. Phys Oceanogr 24(5):60–70. https://doi.org/10.22449/1573-160X-2017-5-60-70

Smirnova LL, Riabinin AI (2013) Microbiological and elemental composition of aerosols falling on the Crimean coast of the Black Sea. Paleontol J 47(10):1198–1204. https://doi.org/10.1134/S0031030113100109

Smirnova LL, Andreeva NA, Antonova LS, Misyura AG, Gurik VV, Pichugin VM (2005) Microflora activity as an indicator of the toxicity of sea bottom sediments of the shelf zone of the Black Sea and the Kerch Strait. Ecol Saf Coast Shelf Zones Integr Use Shelf Resourc 12:533–539 (in Russian)

Sokolova IM, Evans S, Hughes FM (2004) Cadmium-induced apoptosis in oyster hemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. J Exp Biol 207(19):3369–3380. https://doi.org/10.1242/jeb.01152

Stankovic S, Jovic M (2012) Health risks of heavy metals in the mediterranean mussels as seafood. Environ Chem Lett 10:119–130. https://doi.org/10.1007/s10311-011-0343-1

Stankovic S, Jovic M, Milanov R, Joksimovic D (2011) Heavy metals concentrations (Zn, Cu, Pb, Cd, As and Hg) in the Mediterranean mussel (*Mytilus galloprovincialis*) and evaluation of mussel quality and possible human health risk from cultivated and wild sites of the southeastern Adriatic Sea, Montenegro. J Serbian Chem Soc 76(12):1725–1737. https://doi.org/10.2298/JSC110420095S

Struck BD, Pelzer R, Ostapczuk P, Emons H, Mohl C (1997) Statistical evaluation of ecosystem properties influencing the uptake of As, Cd, Co, Cu, Hg, Mn, Ni, Pb and Zn in seaweed (*Fucus vesiculosus*) and common mussel (*Mytilus edulis*). Sci Total Environ 207:29–42. https://doi.org/10.1016/s0048-9697(97)00246-5

Suhre MH, Gertz M, Steegborn C, Scheibel T (2014) Structural and functional features of a collagen-binding matrix protein from the mussel byssus. Nat Commun. https://doi.org/10.1038/ncomms4392

Sunlu U (2006) Trace metal levels in mussels (*Mytilus Galloprovincialis* L. 1758) from Turkish Aegean sea coast. Environ Monit Assess 114:273–286. https://doi.org/10.1007/s10661-006-4780-4

Szefer P, Ikuta K, Kushiyam S, Szefer K, Frelek K, Geldon J (1997) Distribution and association of trace metals in soft tissue and byssus of *Mytilus edulis* from the east coast of Kyushu Island, Japan. Arch Environ Contam Toxicol 32:184–190. https://doi.org/10.1007/s002449900173

Szefer P, Ikuta K, Frelek K, Zdrojewska I, Nabrzyski M (1999) Mercury and other trace metals (Ag, Cr, Co, and Ni) in soft tissue and byssus of *Mytilus edulis* from the east coast of Kyushu Island, Japan. Sci Total Environ 229(3):227–234. https://doi.org/10.1016/s0048-9697(99)00079-0

Szefer P, Frelek K, Szefer K, Lee CB, Kim B-S, Warzocha J, Zdrojewska I, Ciesielski T (2002) Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis trossulus* from the southern Baltic. Environ Pollut 120(2):423–444. https://doi.org/10.1016/S0269-7491(02)00111-2

Szefer P, Fowler SW, Ikuta K, Osuna PF, Ali AA, Kim BS, Fernandes HM, Belzunce MJ, Guterstam B, Kunzendorf H, Wolowicz M, Hummel H, Deslous-Paoli M (2006) A comparative assessment of heavy metal accumulation in soft parts and byssus of mussels from subarctic, temperate, subtropical and tropical marine environments. Environ Pollut 139(1):70–78. https://doi.org/10.1016/j.envpol.2005.04.031

Tankéré SP, Muller FL, Burton J, Statham P, Guieu C, Martin J-M (2001) Trace metal distributions in shelf waters of the northwestern Black Sea. Cont Shelf Res 21(13-14):1501–1532. https://doi.org/10.1016/s0278-4343(01)00013-9

Temerdashev ZA, Eletskii II, Kaunova AA, Korpakova IG (2017) Determination of heavy metals in *Mytilus galloprovincialis* Lamarck mussels using the ICP-AES method. Anal Control 21(2):116–124 (in Russian). https://doi.org/10.15826/analitika.2017.21.2.009

Turanlı N, Gedik K (2021) Spatial trace element bioaccumulation along with consumer risk simulations of Mediterranean mussels in coastal waters of Turkey. Environ Sci Pollut Res 28:41746–41759. https://doi.org/10.1007/s11356-021-13506-4

Ünlü MY, Fowler SW (1979) Factors affecting the flux of arsenic through the mussel *Mytilus galloprovincialis*. Mar Biol 51:209–219. https://doi.org/10.1007/BF00386800

USEPA (1989) Risk assessment guidance for superfund (RAGS). United States Environmental Protection Agency, Washington

USEPA (2021) Risk based concentration table. United States Environmental Protection Agency, Washington

Venugopal V, Kumarapanicker G (2017) Shellfish: nutritive value, health benefits, and consumer safety comprehensive reviews in food science and food safety. Compr Rev Food Sci Food Saf 16:1219–1242. https://doi.org/10.1111/1541-4337.12312

Viarengo A, Canesi L, Pertica M et al (1990) Heavy metal effects on lipid peroxidation in the tissues of *Mytilus galloprovincialis* Lam. Comp Biochem Physiol 97(1):37–42. https://doi.org/10.1016/0742-8413(90)90168-9

Voultsiadou E, Koutsoubas D, Achparaki M (2010) Bivalve mollusc exploitation in Mediterranean coastal communities: an historical approach. J Biol Res Thessaloniki 13:35–45

Wood JM (1975) Biological cycles for elements in the environment. Naturwissenschaften 62:357–364

Wu X, Gao M, Wang L, Luo Y, Bi R, Li L, Xie L (2014) The arsenic content in marketed seafood and associated health risks for the residents of Shandong, China. Ecotoxicol Environ Saf 102:168–173. https://doi.org/10.1016/j.ecoenv.2014.01.028

Yamauchi O, Odani A (1996) Stability constants of metal complexes of amino acids with charged side chains – part I: positively charged side chains. Pure Appl Chem 68(2):469–496. https://doi.org/10.1351/pac199668020469

Yap CK, Ismail A, Tan SG (2003a) Cd and Zn in the straits of Malacca and intertidal sediments of the west coast of Peninsular Malaysia. Mar Pollut Bull 46(10):1348–1353. https://doi.org/10.1016/S0025-326X(03)00193-0

Yap CK, Ismail A, Tan SG, Omar H (2003b) Can the byssus of green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a biomonitoring organ for Cd, Pb and Zn?. Field and laboratory studies. Environ Int 29(44):521–528. https://doi.org/10.1016/S0160-4120(03)00008-4

Yap A, Ismail S, Tan G (2005) Byssus of the green-lipped mussel *Perna viridis* (Linnaeus) as a biomonitoring material for Zn. Russ J Mar Biol 31(2):102–108 (in Russian). https://doi.org/10.1007/s11179-005-0050-5

Zhelyazkov G, Yankovska-Stefanova T, Mineva E, Stratev D, Vashin I, Dospatliev L, Valkova E, Popova T (2018) Risk assessment of some heavy metals in mussels (*Mytilus galloprovincialis*) and veined rapana whelks (*Rapana venosa*) for human health. Mar Pollut Bull 128:197–201. https://doi.org/10.1016/j.marpolbul.2018.01.024