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Trace elements risk assessment for consumption of wild mussels along South Africa coastline

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ABSTRACT

The study aimed to contribute to the scarce data on concentrations in the soft tissue of wild mussels growing in coastal cities of South Africa. The intake of 26 micro and macroelements was estimated. The mass fractions of sample sets from 8 sites along the South African coast from the West (Port Nolloth) to the East (Durban) were studied by neutron activation analysis at the Joint Institute for Nuclear Research (Russia). The following elements were identified as potentially hazardous due to high consumption risks: Al, Cr, Co, Zn, As, and I at stations in the Cape Town area (Waterfront, Hout Bay) and Durban. The mean concentrations of these elements among all individuals were 208, 0.8, 0.46, 60, 2.6, and 11 ppm of wet weight, respectively. In the studied mussels, the concentrations of Cr, Zn, As, and Se (ranging between 0.2−2.8, 14–290, 1.6−4.6, and 0.31−2.4 ppm of wet weight, respectively) exceeded maximum permissible levels for fish products. The weekly consumption of more than 250 g of fresh mussels per person could increase the risk for human health by potential intake of such elements as Al, As, and I.

1. Introduction

1.1. Significance of mussels in South Africa

Coastal resources in South Africa are vital for local coastal communities that depend on them, as many live in abject poverty. Mussel meat is the most accessible source of natural food besides fish. However, mussels, as organisms that filtrate the water, could accumulate high concentrations of trace or other elements originating from coastal sources of pollution. It was estimated that in South Africa, 58 % of the coastal and inshore ecosystem types, 41 % of the offshore ecosystem types, and 43 % of the estuary ecosystem types are threatened by pollution (Van Niekerk and Turpie, 2012). Mussels are benthic organisms that act as ecosystem engineers in the coastal zones and as attached molluscs, they improve the quality of water, food, and substrate basis of the ecosystem.

Mussels can reflect levels of trace elements present in seawater and seston due to natural inputs and anthropogenic activities. Wastewater discharges are the main source of local pollution in coastal municipalities. They contain high levels of toxic elements that could increase in the number of harmful events associated with bioaccumulation in marine organisms, resulting in the fall of populations and pose risk to human health. It is generally acknowledged that since 2004, the increasing human population in coastal regions has certainly resulted in further significant increases in the volumes of domestic sewage and municipal wastewater discharge in the sea (Sink et al., 2012). In South

Abbreviations: BW, body weight (kg); CF, conversion factor; CR, consumption rate (g/week or g/day); DW, dry weight; EDI, estimated daily intake (μg/kg bw/day); EPA'sIRIS, Environmental Protection Agency's Integrated Risk Information System; EWI, estimated weekly intake (μg/kg bw/week); HI, total hazardous index; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LOAEL, lowest-observed-adverse-effect level; MPCR, maximal permissible consumption rate (calculated according to study) (kg); MPL, maximum permissible level (μg/g w.w.); NOAEL, no-observed-adverse-effect level; PTWI, provisional tolerable weekly intake (mg/kg b.w./week); PMTDI, provisional maximum tolerable daily intake (in this study in μg/kg b.w./ day); RfD, oral reference dose (μg/kg bw/day); RQ, risk quotient; TDI, tolerable daily intake (in this study in μg/kg b.w./day); THQ, target hazardous quotient; WW, wet weight.

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Africa preliminarily treated sewage, secondary treated effluent discharges in the surf zone and estuaries, and untreated sewage from informal settlements occurring in stormwater runoff are discharged into the marine environment. Stormwater runoff from urban areas is difficult to control or predict since it is heavily dependent on rainfall which is collected and channeled from polluted surfaces into outlets and eventually to beaches or rocks, where the mussels can catch different types of particles and bioconcentrate the ions of metals directly from the water.

Historical data about the concentration of elements in mussels along South Africa are extremely limited. The fragmented results of local studies could not be merged into one dataset. Nowadays, there is no national monitoring program focusing on the assessment of industrial and urban pollution influence on mussels and human health via consumption of local mussels at South African coastline.

The use of high amounts of mussel meat for food in coastal municipalities is justified by the high protein content. However, the amounts of microelements can vary widely between polluted and pristine local areas. Analysis of the total content of a wide range of elements in mussels could be a useful approach to assessing differences in the levels of toxic and other trace elements at a regional scale.

1.2. Consumption of mussels

Consumption of mussels provides proteins, essential minerals, and vitamins, and thus, some protection from certain diseases related to nutritional deficiencies the risks, and benefits of their consumption are still hard to assess because of the level of metals bioaccumulated from the marine environment, with their reviewed toxicity (Gupta and Singh, 2011). It is important to know the diet-related hazard for the local population, which uses natural indigenous mussel meat as a main dish.

There are several approaches to evaluate the amounts of weekly or daily consumption per person around the world. In statistical reports in different countries, the average consumption of seafood (shellfish group) per capita usually is used. In South Africa, approximately 312,000 tonnes of seafood is eaten annually, with per capita seafood consumption at 6.25 kg in 2010 (Hofherr et al., 2016). But this approach does not take into account the local population in coastal cities, in which the citizens can eat mussels in huge quantities as a main dish. In regional studies, the consumption of mussels varies greatly. Ferrara et al., 2001 set the consumption of molluscs for the general population in the range of 20−40 g/person/day and strong social seafood consumers in the range of 150−270 g/day/person. Whyte et al., 2009 reported that the values of recommended and real consumption of mussels by New Zealand local populations range between 3.2 g (for average citizens) and 160 g per day (for Maori). Storelli and Marcotrigiano (2001) proposed that the consumption levels of bivalve molluscs per capita differ among geographical regions of Italy and range from 0.8 to 3.6 g/d. This corresponds to less than 2 individuals of mussels per week and that is the low-level consumption group. Stankovic et al., 2012, set the consumption of mussels at 28.6 g (17.8–35.7) per person per day to calculate TDI for the low consumption group. Ho and Leung (2014) considered the consumption of molluscs per capita for Hong Kong at the level of 52 g/d (Ho and Leung, 2014) that is considerably higher than the average values in the country according to reports of FAO. The official consumption of molluscs for a country is typically underestimated. For example, for South Africa from 2014 to 2017, it was 0.22—0.11 kg per year (that means 0.6—0.3 g per day) with a minimum value of 0.07 kg/person/yr in 2015 (FAOSTAT, 2017). Average consumption in European countries is higher and ranges from 1.17 to 3.94 kg per inhabitant per year. All these values included the entire population with its continental part, but locals in the coastal regions consumed more seafood (and bivalve molluscs). Although the average consumption throughout a person's life (per capita) is on average extremely low (average molluscs consumption per capita in South Africa from 2014 to 2017 ranged from 0.07 to 0.22 kg/person/year – FAOSTAT, 2017), mussel consumption was not regulated in coastal areas. Consequently, the daily dose can be increased to one hundred grams per person per day, contained in one dish (which corresponds to one cup of mussels and consists of 7–15 individuals, depending on their size).

In our study, we deemed that the average value of mussel soft tissue consumed as human food should be set at about 200 g weekly per person (28.6 g per day) based on the study by Stankovic et al., 2012. This is close to real consumption rates taking into account the size of average dishes in coastal cities, according to our experience. It corresponds to the amount of soft tissue (meat) of several mussels that one person can eat per day on average.

1.3. Application of mussels in risk assessment

The hot spots of coastal pollution associated with the status of local mussels, based on a balance of anthropogenic pressure and adaptation processes through self-purification, should be taken into consideration in coastal management and selection of the location for future farms. Key pollution areas and elemental groups which could affect the health of the local population can be estimated by different elemental ratios and calculation of risk assessment indices.

Mussels are a local self-caught seafood group as identified by the World health organization (WHO). This group is important because of insufficient data about intakes of elements through the group of typical seafood in human organisms of inhabitants of local cities. Thus, it is an unexplored potential health risk factor. Mussels have high bioaccumulation and a low biotransformation potential for both organic and inorganic contaminants. They are suitable long-term bioaccumulators (Smolders et al., 2003).

When mussels are exposed to pollutants, three distinct phases in stress response could be observed: alarm, compensation, and exhaustion, which correspond to alterations at the biochemical and physiological levels, and finally effects in the whole organism (Smolders et al., 2003). The last stage can be detected by changes in internal concentrations of a given element in the whole body. Mussels could be used as biomonitors with long-term exposure (years), which fits well for the purposes of prolonged risk assessment studies as they are used repeatedly as food.

The Mussel Watch Program, as part of the South African National Committee for Oceanographic Research (SANCOR) and the Marine Pollution Research Programme (MPRP), has been active since 1985, but the effects have not been reported regularly and in full (Sparks et al., 2014). It was based on the estimation of the concentration of 7 elements (Cu, Cd, Pb, Zn, Hg, Fe) in blue alien mussel *Mytilus galloprovincialis* on the West Coast (Saldanha Bay, Cape Town). The data about concentrations of hazardous metals and parameters of mussels at the West coast of South Africa were partially presented in Clark et al. (2017). Greenfield et al. (2011) reported data for brown mussel *Perna perna* – at the East coast (Richard Bay) for the period from 1976 to 2009.

(Wepener and Degger, 2020) showed results of mussel surveys conducted in 2008 and 2009, which were performed using brown mussels (*Perna perna*) at navigational buoys from the harbors along the South African coast (from Cape Town to Richard Bay). Their study showed that metal concentrations significantly decreased (by one or two orders of magnitude) from 2008 to 2009, which was explained by variabilities of non-point discharges of metals into the harbors and larger-scale oceanographic changes in upwelling events. (Erasmus et al., 2020) assessed the biogeographical differences of trace element accumulation in mussels (*Perna perna*) between the subtropical and warm temperate, at the beaches of the East Coast (Sheffield Beach and Tsitsikamma Marine Protected Area).

In general, fragmented data about concentrations of a small number of microelements in mussels as biomonitors were presented for different locations and regions without assessing the level of marine pollution and the influence of urban and industrial activity on human health along the whole South African coastline (Farrington et al., 2016). It is important to note that risk assessment studies were not conducted during large-scale mussel biomonitoring investigations.

Numerous studies on the concentration of trace elements in mussels assessed risks associated with their consumption collected in the world regions of modern large-scale producers of cultivated molluscs (for example, Montenegro, Turkey, China, USA, Italy, New Zeland, Malaysia: Stankovic et al., 2012; Bat et al., 2018; Fung et al., 2004; Ho and Leung, 2014; Goldhaber, 2003; Spada et al., 2013; Whyte et al., 2009; Yap et al., 2016). Usually, they focused on key pollutants, namely, on four trace elements (Cd, Pb, Hg, and As) and discussed their concentrations in mussels in comparison with guideline limits.

In South Africa, the mussels were earlier studied by direct comparison of the concentration of a small set of elements with maximum permissible levels. Other studies were focused on the species specificity (Firth et al., 2019) in the bioaccumulation of elements or intakes of other pollutants (microplastics, organic pollutants, etc.) (Li et al., 2016). However, studies on marine mussels' contamination with metals around the African coast, especially related to human health risks through consumption, are very limited, and further research is needed to ensure that mussels do not pose unknown risks to consumer health.

In our study, the impact on human health in this study was assessed by the most commonly used risk indexes (such as RQ, THQ, HI) based on the maximum concentration of elements that could be reached via consumption of mussels. The nutritional value of selected elements with a constant diet was not considered. The main task was to estimate the hazardous levels of consumption of elements according to international guidelines in various south African coastal cities based on their concentrations in one of the main food sources - mussels.

2. Material and methods

2.1. Sampling

The samples of mussels were collected by hand from 8 sites (stations) along the South African coastline (Fig. 1, Table 1) in 2017 out of the spawning season. The water temperatures during sampling were in the typical ranges for the studied regions (Table 1).

The stations were chosen as the most representative in terms of availability of mussels, natural environment, and typical ecological conditions among the coastal regions of South Africa.

Four closely located stations (2, 3, 4, 5) with known sources of pollution (grey wastewater discharge) in Saldanha Bay (2 – Bok river and 3 – Strandloper) and Cape Town region (4 - Waterfront, 5 - Hout Bay) were included in the study due to the known local pollution features for the purposes of comparison with the rest. From each site, mussels with a size of 40–80 cm were collected by hand from different local spots with 2 m distance. The samples were collected by taking into account the availability of mussels and their substrates (rocks), so as to be representative of the whole coastal population. 90 % of the mussels were *Mytilus galloprovincialis*. For comparison of concentrations on a wet weight basis, in our study for all samples, we have used the average conversion factor of batch mussels from Saldanha Bay, which corresponded to 0.32 dry/wet weight. The use of an average value simplifies the conversion and enables an easier comparison of the data with that by other researchers. After collection, the samples were frozen for transportation.

Description of stations:

St. 1. Port Nolloth: It is an isolated (very far from other cities) small town that was founded around the offshore diamond industry. Samples were collected under a jetty open to the ocean.

St. 2. Bok River: This site is located in Saldanha Bay near the greywater outlet of the city of Saldanha.

St. 3. Strandloper: This site is located in Saldanha Bay at the greywater outlet of the neighboring town of Langebaan.

St. 4.Waterfront: This site is located on a breakwater on the Cape Town waterfront that is exposed to the ocean.

St. 5. Hout Bay: This site is located in Hoot Bay, at the greywater outlet of the city.

St. 6. Plettenberg Bay: The exact sampling site was on the rocks in the middle of the city's main beach. The area is not densely populated and does not appear polluted.

St. 7. Port Elizabeth: This site is on the dock in the middle of Port Elizabeth's main beach. The area is densely populated and appears affected by pollution, as it is located near a large industrial port.

St. 8. Durban: The sampling site is on the mouth of South Africa's busiest industrial port and, according to numerous studies, the area is "heavily polluted".

Fig. 1. Sampling sites along the South African coast in 2018.

Table 1
Number of mussels and characteristics of sampling sites.

№	Station (West-East)	Number of individuals in sets	Population of the city [a] (2011)	Ocean	Temperature [b], °C	Salinity [b], PSU
1	Port Nolloth	12	6092	Atlantic	11−18	34.7−35.4
2	Bok River	10	99000*	Atlantic	11−18	34.7−37
3	Strandloper	10	99000*	Atlantic	11−18	34.7−37
4	Waterfront	12	433688**	Atlantic	11−18	34.7−37
5	Hout Bay	12	17900	Atlantic	11−18	34.7−37
6	Plettenberg Bay	12	31804	Indian	13.5−23	35.4
7	Port Elizabeth	12	312392	Indian	13.5−23	35.4
8	Durban	12	595061	Indian	16−27	>35.4

* Saldanha Bay Local Municipality.
** Population of the Cape Town city.
[a] Population data from Statistics South Africa, 2012.
[b] Average regional data from RSA-DEA, 2018, particularly Smit et al., 2013.

2.2. Elemental analysis

The concentrations of 26 elements were determined by instrumental neutron activation analysis at the REGATA facility of the IBR-2 reactor, JINR (Dubna, Russia).

From each local batch of samples, 10−12 mussels of one size group were chosen. The number of mussels was determined based on the minimum number of individuals per sampling site, necessary, on the one hand, for the representativeness of the subsample, and on the other hand, to minimize the deviations within the group. Also, the removal of such a small amount of mussels (corresponding to about one average daily serving) does not harm the natural population.

Each individual was defrosted and dissected to shells and soft tissues. After that, the soft tissues were rinsed with deionized water to remove mud and sediments and other particles, lyophilized to constant weight, pulverized to powder using a planetary mono mill with agate milling balls (PULVERISETTE 6, Fritsch Laboratory Instruments GmbH, Germany) at 400 rpm. The homogenized mussel soft tissues (0.3 g) were packed into plastic bags for the determination of short-lived isotopes and aluminum cups for long-lived isotopes of elements.

The instrumental neutron activation analysis was performed differently for 3 groups of elements according to the determination types: short-lived isotopes (Mg, Al, S, Cl, Ca, Ti, V, Mn, I) – subsamples were irradiated with full spectra of neutrons in the channel at a neutron flux of $1.6{\cdot}10^{12}$ n cm^{-2} s^{-1} during 3 min, after 3 min decay they were measured for 15 min; long-lived isotopes – samples were irradiated with epithermal neutrons in another irradiation channel with a cadmium shield at a neutron flux of $3.31{\cdot}10^{11}$ cm^{-2} s^{-1} for 3 days, after 3 and 20 days of decay they were measured for 30 min (for such elements as Na, K, As, Br, and U) and 90 min (for Sc, Cr, Fe, Co, Ni, Zn, Se, Rb, Sr, Sb, Cs, and Th), respectively.

The spectra of induced gamma activity were measured with HPGe detectors (Canberra) with a resolution of 1.9 keV for the total-absorption peak of 1332 keV of ^{60}Co. The detector is calibrated using standard spectrometric and certified reference materials (Pavlov et al., 2016). The detailed procedure of neutron activation analysis, performed at the REGATA facility, was described by Frontasyeva, 2005.

For quality control assurance (Table 2), standard reference materials of different origin provided by the National Institute of Standards and Technology (NIST) and Institute for Reference Materials and Measurements (IRMM) were used: NIST1633c (coal fly ash), NIST1547 (peach leaves), NIST1632c (trace elements in coal), IRMM690cc (calcareous soil), NIST2710, NIST2710a (Montana soil), NIST2709 (trace elements in soil), NIST1572 (citrus leaves), and NIST1566b (oyster tissue). The use of standards of different matrixes allowed to expand the number of elements with certified values, which can be determined in mussel samples, since the standard material for oyster tissue (1566b) contains only a limited number of certified values. Chemical matrix effects, known to be significant sources of error in some other types of instrumental chemical analysis, are insignificant in NAA. The use of reference materials with the matrix different from the analyzed samples in NAA is explained by insignificant matrix effect in case of small samples (size and weight) (Greenberg et al., 2011).

Specialized software developed in FLNP JINR (Pavlov et al., 2016) was used to create a Group Standard Sample (GSS) from all above-mentioned reference materials to calculate the content of 26 elements in the analyzed samples with maximum accuracy. The GSS was used to check the quality of the analysis by determining the content of the same elements. This procedure, applied to SRMs, allowed to compare the obtained values with the certified and provided quality control of the analysis (Zinicovscaia et al., 2018).

The majority of elements were in the range of 10 % of deviation between determined and certified values. Selenium was determined near detection limit levels and their mean concentrations among sample sets were with high standard deviations (71 % of mean recovery). Several elements were excluded from consideration due to the lack of reference data on concentrations in mussels and their tolerable intakes (PTWI and RfD).

2.3. Statistics

Element concentrations were determined on a dry weight basis for each individual of mussels, and then by using the conversion factor the minimum, maximum, mean values, and standard deviations were calculated on a wet weight basis for each batch.

All data sets were tested for normality and homogeneity of variance by using Shapiro-Wilk and Levene's tests respectively in STATISTICA software. The majority of elements were not normally distributed ($p < 0.05$ except Cl and K), thus the nonparametric Kruskal-Wallis test (Zar, 1984) was applied to examine the significance of differences of the determined concentrations of elements in mussel tissues among stations. For the calculation of key ratios, the maximum values in each set were used with attention to outliers.

Principal component analysis (PCA) was applied for the differentiation of groups of elements and identification of their contribution at the stations to demonstrate suggested sources.

2.4. Human health risk assessment

The information on hazard, exposure, and dose-response was combined to provide an estimate of the likelihood that any of the identified adverse effects will occur in exposed people. The environmental health risk was assessed by comparing the environmental status, as represented by the concentrations of the metals in mussels, and threshold values likely to cause adverse effects in human consumers. In this context, a risk quotient can be calculated as follows (based on Jovic et al., 2012; Yap et al., 2016; Korkmaz et al., 2017):

Table 2
Quality control of Neutron Activation Analysis used in the present study.

	SRMs	Determined concentrations, mg/kg	Certified concentrations, mg/kg	Recoveries, %
Na	1633c (coal fly ash)	1580 ± 150	1700 ± 60	92
Mg	1547 (peach leaves)	4870 ± 330	4320 ± 80	111
Al	1547 (peach leaves)	260 ± 12	250 ± 8	106
S	1632c (trace elements in coal)	14900 ± 970	14620 ± 600	102
Cl	1547 (peach leaves)	320 ± 30	360 ± 20	88
K	1632c (trace elements in coal)	1060 ± 200	1100 ± 30	96
Ca	1547 (peach leaves)	16400 ± 1820	15600 ± 200	105
Sc	690cc (calcareous soil)	8.4 ± 0.2	7.9 ± 0.9	106
Ti	2710 (Montana soil)	2920 ± 370	2830 ± 100	103
V	2710 (Montana soil)	70 ± 4	80 ± 2	94
Cr	2709 (trace elements in soil)	128 ± 7	130 ± 4	99
Mn	1572 (citrus leaves)	24 ± 2	23 ± 2	102
Fe	2709 (trace elements in soil)	34000 ± 1600	35000 ± 1100	97
Co	1632c (trace elements in coal)	3.4 ± 0.1	3.5 ± 0.2	99
Ni	2709 (trace elements in soil)	80 ± 5	88 ± 5	91
Zn	2709 (trace elements in soil)	101 ± 4	106 ± 3	96
As	1632c (trace elements in coal)	6.5 ± 0.4	6.2 ± 0.3	105
Se	2710a (Montana soil)	0.76 ± 0.18	1.0 ± 0.3	76
Br	1632c (trace elements in coal)	7.9 ± 3.3	8.2 ± 0.4	97
Rb	1632c (trace elements in coal)	7.9 ± 1.5	7.5 ± 0.3	106
Sr	1632c (trace elements in coal)	64 ± 5	64 ± 1	100
Sb	2709 (trace elements in soil)	7.7 ± 0.3	7.9 ± 0.6	98
I	1547 (peach leaves)	0.29 ± 0.09	0.3 ± 0.09	98
Cs	1632c (trace elements in coal)	0.58 ± 0.03	0.59 ± 0.01	98
Th	2709 (trace elements in soil)	11.5 ± 0.4	11 ± 3.3	93
U	2709 (trace elements in soil)	2.7 ± 0.2	3 ± 0.9	110

1 is the direct comparison of concentrations in soft tissues (wet weight basis) with seafood safety guidelines based on established maximum permissible limits (**MPLs**);
2 is the characterization of the amount of mussel meat (maximal provisional consumption rate MPCR, kg/week) that would need to be consumed per week by a 70-kg average adult to reach the provisional tolerable weekly intake (PTWI) established by the JECFA or related reference limits;
3 is the assessment of differences between estimated daily intakes (**EDI**) with **RfD** and calculation of risk quotient (**RQ**), which corresponded to the ratio between estimated weekly intakes (**EWI**) and prescribed PTWI values of the element;
4 is the estimation of target hazardous quotient (**THQ**) and total hazardous index (**HI**) which corresponded to the sum of all quotients from elements as its combinations for each station for the local coastal population

The key ratios and limits were used in the following specification:

The maximal permissible levels (**MPL**) were used for the determination of limits for food and were established by organizations such as the European Commission or United States Food and Drugs Administration. The MPLs for seafood were set for Cr, Mn, Zn, As, Se; expressed in μg/g of wet weight and characterized by upper levels of element-specific concentrations in an object of the environment, which, during a year of human consumption, would not cause any adverse effects.

PTWI represents the permissible weekly exposure in humans as a result of the natural occurrence of a substance in food and drinking water. PTWI is used for food contaminants such as heavy metals that are characterized by cumulative properties. On any particular day, consumption of seafood containing above-average levels of the contaminant may exceed the proportionate share of its weekly tolerable intake. The assessment takes into account such daily variations, the primary concern being prolonged exposure to the contaminant because of its ability to accumulate within the body over a period of time. PTWI is a more representative ratio than TDI because the daily fluctuations in elemental contents are considered and accounted for.

Reference dose (**RfD**) is a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects over a lifetime. The RfD can be derived from NOAEL or LOAEL, benchmark dose with uncertainty factors generally applied to reflect limitations of the data used.

MPCR (kg) is the maximal permissible consumption rate, which can lead to exceeding the PTWI limit. This value was calculated as an average of all maximal individual concentrations obtained from all stations (n = 92).

EWI (μg/kg bw/week) is the estimated weekly intake, which was calculated using the equation:

$$EWI = \frac{C \cdot CR_W}{BW},$$

Where C – concentration of element (μg/g); CR_W – weekly consumption rate (200 g/week in our study); BW – body weight.

Average **BW** (body weight, kg). For South Africa the average BW was calculated from the mean body mass index and height of men and women according to a national survey conducted in 2016 (NDoH, 2019). The mean weight of women aged 25+ was 75.6 kg and for men aged 25+, it was 71.8 kg. Therefore, we used a nominal 70 kg as a representative mean value of weight among the whole adult population, including the 18–25 age group.

EDI (μg/kg bw/day) calculated similarly to EWI, by using daily consumption rate (28.6 g/day).

Risk quotient (**RQ**) was assessed as a ratio between estimated weekly intake (EWI) and provisional tolerable weekly intake (PTWI), which was presented by JECFA, 2019 (for several elements it was calculated on PMTDI). Risk quotients were assessed based on the adopted ratio from

Stankovic et al., 2012. RQ > 1 corresponded to a potential human health risk scenario in the case of consumption of mussels with average and maximum concentrations of the given element.

Target hazardous quotient (**THQ**), developed by USEPA (1989) fits well for the purposes of human health risk assessment for non-carcinogenic elements in the local human population over a lifetime in comparison with the reference oral dose (RfD). THQ was calculated using the equation (Yap et al., 2016):

$$THQ = \frac{EF \cdot ED \cdot CR \cdot C \cdot 10^{-3}}{RfD \cdot BW \cdot AT}$$

Where EF is the exposure frequency (365 days); ED is exposure duration (average expectancy is 70 years); CR is consumption rate (28.6 g/day/person), C is the concentration of element (wet weight basis), RfD is reference dose (μg/kg bw/day); BW is body weight (for South Africa the average weight was established as 70 kg); AT is 30 years, an average exposure time for non-carcinogens (USEPA, 2011). THQ > 1 reflects the high potential risk at human health via the consumption of mussels in constant dietary conditions over a lifetime.

HI (total hazard index) – represents as summarize values of THQs of n elements for each station:

$HI = \sum_{i=1}^{n} THQ_i$. Total hazard index HI > 1 designates the hazard risk on human health for the local population based on multiple elements in mussel meat.

Risks were assessed for all determined elements to identify the potentially hazardous elements among those analyzed. Depending on the different approaches to assess the potential health risk of mussels' consumption for local coastal populations, different conclusions were suggested.

3. Results

3.1. Group of microelements in mussels

The ranges of concentrations and means of the determined elements in all stations are presented in Table 3. The PCA showed 4 basic groups of elements, which differ by their sources, way of entering the organisms, and suggested origin: terrigenous (Al, S, Cl, K, Ca, Sc, V, Cr, Mn, Fe, Co, Ni, Rb, Sb, Cs, Th), anthropogenic (Zn, As, Br, I, U), hydrogenous (Na, Mg, Cl), and biogenic (Ca, Sr). It was obtained that the mussels with high concentrations of the terrigenous group were at st. 8 (Durban), and anthropogenic group at st. 2 (Strandloper), st. 4 (Waterfront), and st. 5 (Hout Bay) with local differences.

Significant spatial differences between concentrations in mussels were found. The highest concentrations of Al, Sc, Ti, V, Cr, Mn, Fe, Co, Th, U were obtained at st. 8 (Durban), while Zn, As, and Br – at st. 4 (Hout Bay).

In studied mussels, the elements such as Cr, Zn, As and Se (which ranged 0.2−2.8, 14−290, 1.6−4.6, and 0.31−2.4 ppm of wet weight, respectively) exceeded the average maximum permissible levels for fish products in different countries, established by Nauen (1983); USFDA (1993, 2007), and DOH (2004).

3.2. Comparison of the concentrations in soft tissues with maximum permissible levels for seafood products

Compared to the reference data (based on raw mussels and in diet, in wet weight of mussels, high levels (by a factor of 2–4) of concentrations of Al, Cr, Mn, Fe, Ni, Zn, Se, Sb were observed (Table 3), as well as equal levels of V and Co, and low levels of As. For all considered elements (mean values), the majority of individuals were characterized by element concentrations lower than existing MPLs for Cr, Zn, As, and Se (regarding shellfish and other marine food, according to Nauen, 1983 and USFDA, 2007) (Fig. 2). The maximum content of Cr (2.8 ppm), As (4.6 ppm) and Se (2.4 ppm) in mussel batches exceeded the average

Table 3
Ranges of individual concentrations among all stations in mussels and reference data (all data are presented on a wet weight basis).

	Ranges of individual concentrations (μg/g), $n = 92$		Reference data (μg/g)		MPL
			raw mussels	table shellfish	
	min-max	mean	Richir and Gobert, 2014 (a)	Leblanc et al., 2005 (b)	(μg/g) (c)
Na	6140−18900	10,510		5422	
Mg	1020−4320	2200		509	
Al	8−1090	208	13−280	17	
S	2440−26000	9500			
Cl	10100−30400	18220			
K	930−4060	2500			
Ca	970−14100	3550		753	
Sc	0.002−0.189	0.035			
V	0.1−4.2	0.8	0.7−4.0		
Cr	0.2−2.8	0.8	0.06−0.69	0.09	1
Mn	0.3−29.9	4.8	1.1−6.6	2.7	
Fe	16−570	124	21−210		
Co	0.04−0.46	0.13	0.12−0.44	0.05	
Ni	0.06−1.69	0.58	0.23−1.08	0.23	(80)
Zn	14−290	60	11−72	66	100 (300)
As	1.6−4.6	2.6	5.4−14.7	1.9	1−5 (86)
Se	0.31−2.4	0.74	0.48−1.38	0.01	0.3−2
Br	44−220	103			
Rb	0.74−2.52	1.17			
Sr	9.2−86.4	24.8			
Sb	0.002−0.031	0.009	0.002−0.009	0.002	
I	2−36	11			
Cs	0.003−0.082	0.015			
Th	0.002−0.450	0.045			
U	0.03−0.22	0.09			

For comparison, the concentrations from the present study and reference from Richir and Gobert, 2014 were converted from dry weight to wet weight by using our CF = 0.32. (a) Richir and Gobert, 2014. All mussels (*Mytilus galloprovincialis*) were collected from 1 sampling site at the east Corsica before spawning season; (b) Leblanc et al., 2005 – concentrations in table shellfish group (French Total Diet Study); (c) average data from Nauen, 1983; in brackets – data from USFDA, 1993, 2007; DOH, 2004.

MPLs established by different countries (from minimums to maximums of MPL), and amounted to 1, 3, and 1.2 ppm, respectively. The individuals with maximum concentrations at the st. 4, 5 (Waterfront, Hout Bay) reached the MPL for Cr and the whole local set from st. 8 (Durban) was above the limit. The limit for Zn in South Africa is set at the level of 300 μg/g ww (DOH, 1994) that is higher than the maximum value obtained in mussels (290 μg/g ww). Zn was at the highest level in individuals from st. 3 (Strandloper), 4 (Waterfront), and st. 5 (Hout Bay). Arsenic was below the maximum MPL of 5 μg/g at all stations, but higher than average MPL (3 μg/g) at st. 3, 4, 5, and 8. The concentrations of arsenic in the majority of individuals from st. 5 were above the average limit. The maximum concentrations of selenium were higher than the limit (2 μg/g) at stations 4 and 5, but the means in these sets were lower than 1.5 μg/g ww.

3.3. Estimated intakes of mussels and maximal permissible consumption

The estimated consumption of mussels per week and per day was compared with the corresponding existing regulatory limits. For a convenient representation of the maximum potential intake of elements, we used the amount of mussel meat that must be eaten to exceed the reference limits.

The EWI for all studied elements were below the recommended PTWI (Table 4).

PTWI for total arsenic was determined by using the ratio of 42 % inorganic/total for mussels based on the study by Chiesa et al. (2018) from the equation: 15 / 0.42 = 35.7 μg/kg bw/week, calculated using

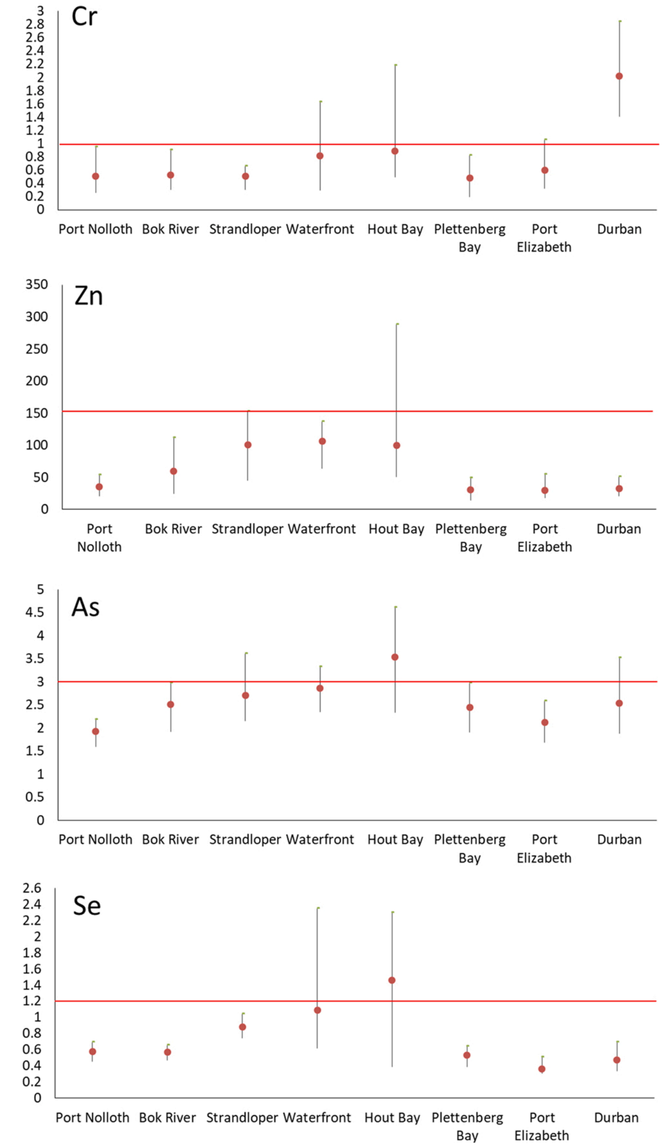

Fig. 2. Means, maximum and minimum concentrations (vertical bars) of Cr, Zn, and As (wet weight basis) in soft tissues of mussels along South African coastline. Red line corresponds to average MPLs.

the most current estimated PTWI (15 μg/kg bw/week, JECFA, 2011).

MPCR (Table 4) reflected the maximum intake of mussel meat, which a person could eat per week without any health risk (below or at the level of prescribed or calculated PTWI). The MPCR in kg/week could be used for the regulation of potentially safe daily dishes in local coastal regions. For each determined element, the total consumption of mussel meat was normalized using the minimal values (in bold), and the maximum recommended weekly dietary intake was calculated as lower than 250−770 g (according to the MPCR for Al, Cr, As, and I at the maximal levels in all coastal stations) throughout life at worst. This corresponds to 36−111 g/day (10–20 individual mussels).

The MPCR for elements such as Cl and Br could not be assessed correctly without knowing the form of the element (chemical compound) in mussels. The reference limits for bromine were consistent with bromate, while the intakes were calculated for total bromine.

Our data revealed lower levels of MPCR (kg) for Zn, Fe, As. An exception was the high amount of Ni (2.7–3.5) in comparison with data by Jovic et al., 2012 (7.1–21.7, 11.5–14.8, 3.6–8.6 respectively) on the amounts of mussel meat in kg, which could be consumed by a 70 kg person per week, to exceed the limiting PTWI at the level of CR = 125 g mussels/person/week. The lower values can be explained by higher maximum concentrations of elements in our study and higher consumption rate (CR = 200 g/person/week). Also, Jovic et al. (2012) used PTWI 350 μg/kg bw/week for total arsenic referencing Whyte et al., (2009). In our case, the diminished MPCR was calculated using a lower level of PTWI (35.7 μg/kg bw/week) corresponding to total As.

Table 4
Regulation limits for maximum consumptions of elements (total amounts) estimated for weekly and daily intakes and maximal permissible consumption rate.

	PTWI μg/kg bw/week	RfD μg/kg bw/day	PMTDI μg/kg bw/day	EWI[d] (μg/kg bw/week)	EDI[d] (μg/kg bw/day)	MPCR (kg/week)
Na	24500000 [a]		3500000	39600	5660	123
Al	2000			1230	180	**0.3**
Cl	1050 [a]	100[e]	150	68000	9740	–
Cr	5−20 [b]	3 (Cr^{+6}) 1500 (Cr^{+3})	100	3.95	0.56	**0.25-1.0**
Mn	980 [a]	140		24.2	3.5	8.1
Fe	5600 [a]	700[f]	800	630	90	1.8
Co	700 [a]	300[f]	100	0.53	0.08	265
Ni	35	20	5	2.46	0.35	2.8
Zn	7000 [a]	300	300−1000	320	46	4.4
As	35.7 [a] 15 (inorganic)	0.71 0.3 (inorganic)		9.22	1.32	**0.77**
Se	35 [c]	5	0.3	3.18	0.45	2.2
Br	–	4 (bromate)		390	56	–
Sr	4200 [c]	600		140	20	6.1
Sb	2.8 [c]	0.4	0.36	0.042	0.006	13.2
I	119 [a]		17	49	7	**0.49**
U	21 [c]	3 (soluble salts)		0.42	0.06	10.1

[a] Calculated from PMTDI (data from 87th JECFA database – June 2019).
[b] Used in Tam and Mok (1991). Calculation based on recommended dietary allowances (0.05−0.2 mg/day) and the body weight 70 kg (Lin et al., 2004).
[c] Calculated from RfD (data from IRIS EPA, 2019).
[d] Average values of maximum intakes from each station (per week and per day).
[e] Chlorine RfD was not relevant as the total amount of chlorine and corresponded to the pure compound.
[f] For RfDs for Fe and Co were used subchronic and chronic LOAEL respectively.

3.4. Risk quotients based on estimated intakes

The EDIs (μg/kg bw/day) of all elements were lower than RfDs except for Br and As (Table 5), which were presented as informative. Instead of the RfD for the total bromine was presented the existing RfD for bromate. In the case of arsenic, we could use the RfD for the inorganic form that corresponded for total As to 0.3/0.42 = 0.71 μg/kg bw/day (based on 42 % inorganic/total As according to Chiesa et al., 2018), which is higher than each of our EDI levels at the stations. These elements are included in anthropogenic factors and cause increasing potential risks in mussel consumption.

3.5. Risk

Quotients for the majority of elements, which were calculated for ranges from minimum and maximum concentrations in mussels from each station, were less than 1.0 (Fig. 3). The exceptions were the maximums of Cr at st. 4 (Waterfront), st. 5 (Hout Bay), and the major part of the batch from st. 8 (Durban) revealed high values of RQ for Cr. Also, the maximum values of RQ for Al at the Durban station (8) were higher than

Table 5
Estimated daily intakes of selected elements (EDI, µg/kg bw/day) for stations along the South African coastline (from west to east). The highest levels among means and maxima are given in bold.

		Cr	Mn	Fe	Co	Ni	Zn	As	Se	Br	Sr	Sb	U
1	mean	0.2	0.9	36	0.06	0.2	15	0.8	0.24	41	8	0.003	0.04
	max	0.4	1.5	51	0.09	0.3	22	0.9	0.28	48	12	0.005	0.06
2	mean	0.2	0.3	24	0.02	0.1	24	1.0	0.23	37	11	0.001	**0.05**
	max	0.4	0.7	35	0.03	0.1	46	1.2	0.27	45	17	0.002	0.08
3	mean	0.2	0.5	24	0.03	0.2	41	1.1	0.36	46	**13**	0.002	**0.05**
	max	0.3	0.7	34	0.03	0.2	63	1.5	0.43	61	30	0.004	0.06
4	mean	0.3	1.3	55	0.05	0.3	**43**	1.2	0.44	41	9	**0.008**	0.03
	max	0.7	2.2	173	0.09	0.4	56	1.4	**0.96**	59	13	**0.013**	0.05
5	mean	0.4	0.9	25	0.03	0.2	41	**1.4**	**0.6**	**63**	8	0.005	**0.05**
	max	0.9	1.8	52	0.05	0.3	**118**	**1.9**	0.94	**90**	13	0.009	**0.09**
6	mean	0.2	1.3	19	0.03	0.1	12	1.0	0.22	30	8	0.002	0.02
	max	0.3	2.1	62	0.04	0.1	20	1.2	0.26	39	21	0.004	0.04
7	mean	0.2	3.5	50	0.06	0.4	12	0.9	0.15	38	11	0.003	0.03
	max	0.4	6.3	82	0.08	0.7	22	1.1	0.21	51	15	0.004	0.04
8	mean	**0.8**	**6.7**	**162**	**0.13**	**0.4**	13	1.0	0.19	42	**13**	0.006	0.03
	max	**1.2**	**12.2**	**233**	**0.19**	**0.7**	21	1.4	0.28	56	**35**	0.008	0.05
Oral RfD (µg/kg bw/day)		30[a]	140	700[b]	0.3[b]	20[c]	300	0.7[d]	5	4[e]	600	0.4	3[f]

[a] Calculated from RfD for Cr^{+6} - 3 µg/kg bw/day.
[b] Calculated from subchronic LOAEL (Fe) and chronic LOAEL (Co).
[c] For soluble salts.
[d] RfD for total As, calculated as 0.7 = 0.3/0.42 from RfD for inorganic As.
[e] RfD for bromate.
[f] For soluble salts.

1 and close at the Waterfront station (4).

The low-risk levels are attributed to the low intake of elements through the mussel meat in accordance with the prescribed PTWI and the established average intake of 200 g/week per person.

Aluminium reached the highest level of RQ = 1.2 at the Durban (st. 8) (higher at st. 8 than at st. 2, 3, 5, 6; Kruskal-Wallis test, $p < 0.0002$), while arsenic RQ amounted to 0.9 at the Hout Bay (higher at st. 5 than in mussels from st. 1, 7, 8; Kruskal–Wallis test, $p < 0.03$). Other elements had the RQ levels less than 0.5. In general, this corresponds to an unlikely potential risk for human health via consumption of local mussels.

3.6. Target hazardous quotients and total hazard index

The maximum values (among maxima, Table 6) of HI were found for mussels from Hout Bay (Cape Town region) and Durban (in bold), which correlated well with the other approaches of risk evaluation (e.g. RQs for Fe). Low values (among all data) for HI were found at the Bok river (Saldanha Bay), Plettenberg Bay, and Port Elizabeth.

The elements As and Br were excluded from the contribution to HI (Table 6) due to the specificity of RfD, but their calculated values were in the range of 0.9–2.2 and 7.8–19.2, respectively. Iodine, with a PMTDI value of 17 µg/kg day was adopted for the calculation of RfD, made maximum contributions in total HI for all stations, except st. 6 (Plettenberg Bay) and st. 7 (Port Elizabeth).

The elements Mn, Fe, Co, Ni, Se, Sr, Sb, and U did not exceed 0.2 of THQ at any site. Levels of Cr, Co, Zn, I were in some cases higher than 0.2 and could contribute to the total hazardous index. In the case of iodine, the high THQs were provided by low RfD, which were calculated on PMTDI due to the absence of an official level of RfD by EPA. It is interesting to note the high level of THQ for iodine at the West Coast stations (1−5), which could be explained by the high hydrochemical levels in the surrounding waters, should be investigated in the future.

4. Discussion

4.1. Elements with high risks in the studied sites

All elements could be classified as potentially *safe* and *hazardous* based on the risk assessment by ratios of RQ, THQ, and HI and by accounting for their MPL and EWI.

According to the PCA results, the elements Al, Cr, and Ni corresponded to a terrigenous component present in the soft tissue of mussels. According to our data, Fe was associated with this group as well. Such elements could be accumulated in mussels via suspended mineral particles and could be ingested by humans. Al, considered as a non-volatile element, could be used as a marker for a terrigenous origin. The highest levels of Al intake (calculated on a wet weight basis) were found in the samples from Durban, Port Elizabeth, Waterfront, and Port Nolloth. Zn and As could be markers of an anthropogenic component in the mussel tissues and the maximum concentrations were determined in samples from the Hout Bay and Strandloper. The mean concentrations of Al, Cr, Ni, Zn, As, and I among all individuals were 208, 0.8, 0.58, 60, 2.6, and 11 ppm of wet weight, respectively.

Cantillo (1998) showed that Zn concentrations above 200 ppm are indicative of contamination. According to the literature data, the amount of inorganic As in mussels can vary from 1 to 50 % (Feldmann and Krupp, 2011). Sloth and Julshamn (2008) found that inorganic As amounts to 42 % of the total content. However, since 2011, the PTWI value of 15 has been rejected and a new one has not been introduced (Chiesa et al., 2018). Therefore, the MPCR, RQ, THQ, and HI calculated for As could be considered informative.

The EFSA Panel on Contaminants in the Food Chain decided to consider all reported analytical results in food as Cr^{+3} (EFSA, 2014). There is a lack of data on the presence of Cr^{+6} in food. Several studies have concluded that the percentage of Cr^{+6} relative to total Cr content in certain types of food is, on average, below 10 % (in the range of 1.31–12.9 %). However, it was also considered that all Cr^{+6} would turn into Cr^{+3} due to thermodynamic reactions varying from very fast (disappearance of Cr^{+6} in few minutes) to quite slow, with a partial survival of Cr^{+6} species for hours (EFSA, 2014).

In the case of 10 % of Cr^{+6} from the total content, the RfD for total chromium could be determined as 3/0.1 = 30 µg/kg bw/day (data by EPA's IRIS). This value is higher than EDI (0.56 µg/kg bw/day). The total RfD for chromium could be calculated on 90 % Cr^{+3} as 1500/90 = 1670 µg/kg bw/day, which is much higher than the total RfD calculated on Cr^{+6}.

4.1.1. Safe

The levels of Na, Mn, Fe, Ni, Sr, Sb, and U in mussels could be considered relatively safe for consumption according to the hazardous

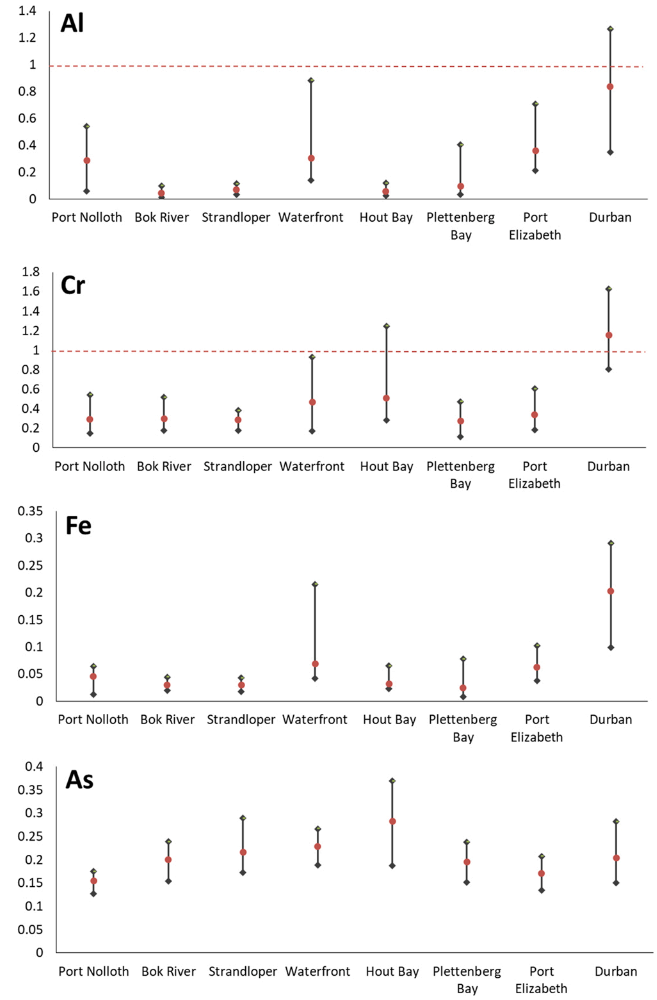

Fig. 3. Risk quotients for the best case, median case, and worst-case scenarios for Al, Cr, Fe, and As in consumption of mussel tissues (wet weight basis) based on the provisional tolerable weekly intake (PTWI). Red line corresponded to 1.0 of RQ.

quotients and indexes. To exceed the tolerable limits (PTWI) for such elements, the person would have to ingest more than 1.8 kg mussel meat every week. Elements such as Na are usually associated with marine water chemical composition. Mn, Fe, Ni, Sr, Sb, and U are usually associated with a terrigenous component (from weathering of rocks, f.e.) of suspended natural materials (sediments), but can increase due to anthropogenic pollution runoff. Differences in the concentrations of such elements between coastal zones are explained by local geological features and are reflected in the biochemistry of mussels.

It is important to note that in the coastal waters with intense runoff and sediment flows, the safe elements could be associated with the terrigenous group and create high risks due to pollution influence.

4.1.2. Hazardous

Al, Cr, Co, Zn, As, Se, and I can increase the health risk if mussel meat is consumed more than 300 g/week per person and in the case of higher than individual maximum concentrations in mussel meat in accordance with the following ratios:

MPL: the maximum concentrations of Cr, Zn, As, and Se can exceed permissible levels set for seafood products. The mussels were adapted to these levels in the environment, but it could be the result of local coastal pollution (e.g. at the st. 5), which was confirmed by other indexes (the highest RQ, and THQ of Zn at the same station).

MPCR: the high amounts of Al, Cr, As, and I in mussels revealed that maximum consumption of mussels should be lower than 250 g per person per week to avoid potential dietary risks.

THQs in the range of 0.3—0.7 for the elements Cr, Co, Zn, and I contributed maximums in local HI (st. 1, 4, 5, and 8).

Al, Cr, and Co are associated with the terrigenous component but can be accumulated by mussels due to human activities at the harbors (described further). Zn and I play an essential role in the organisms in optimal amounts, but at the high concentrations determined in mussels, they could cause risks for human health. Since total concentrations of Cr and As exceeded MPLs they were considered as hazardous elements.

4.2. Brief characteristics of hazardous elements and their input into the mussel environment

4.2.1. Aluminum

Aluminum is a major component of the earth's crust. It is released to the environment both by natural processes and from anthropogenic sources, whereby natural processes far outweigh the contribution of anthropogenic sources. Aluminum is highly concentrated in soil-derived dust from natural processes, coal combustion, and activities as mining and agriculture that could be transported to marine sediments by coastal runoff. Usually, this element is connected with the terrigenous component and it is important to assess the possible risks for human health in the consumption of indigenous mussels from selected populated coastal

Table 6
Target hazardous quotients of several elements and hazard indexes among studied stations.

		Mn	Fe	Co	Ni	Zn	Se	Sr	Sb	I	U	HI
1	mean	0.005	0.04	0.17	0.004	0.04	0.04	0.01	0.006	0.29	0.011	0.63
	max	0.009	0.06	**0.25**	0.006	0.06	0.05	0.02	0.01	**0.74**	0.017	**1.23**
2	mean	0.002	0.03	0.07	0.003	0.07	0.04	0.02	0.003	0.18	0.013	0.43
	max	0.004	0.04	0.09	0.003	0.13	0.05	0.02	0.005	**0.27**	0.023	0.65
3	mean	0.003	0.03	0.07	0.003	0.12	0.06	0.02	0.005	**0.31**	0.014	0.64
	max	0.004	0.04	0.08	0.004	0.18	0.07	0.04	0.008	**0.43**	0.017	0.89
4	mean	0.008	0.07	0.14	0.006	0.12	0.08	0.01	0.016	0.17	0.009	0.64
	max	0.014	**0.21**	**0.26**	0.009	0.16	0.16	0.02	0.027	**0.2**	0.014	**1.09**
5	mean	0.005	0.03	0.09	0.005	0.12	0.1	0.01	0.012	**0.39**	0.015	0.79
	max	0.011	0.06	0.15	0.007	**0.33**	0.16	0.02	0.02	**0.61**	0.026	**1.43**
6	mean	0.008	0.02	0.09	0.001	0.04	0.04	0.01	0.004	0.07	0.005	0.29
	max	0.013	0.08	0.11	0.002	0.06	0.04	0.03	0.009	0.09	0.012	0.45
7	mean	0.021	0.06	0.17	0.009	0.03	0.03	0.02	0.006	0.15	0.007	0.51
	max	0.039	0.1	**0.24**	0.015	0.06	0.04	0.02	0.007	0.2	0.013	0.74
8	mean	0.041	**0.2**	**0.38**	0.008	0.04	0.03	0.02	0.013	0.19	0.01	0.95
	max	0.074	**0.28**	**0.53**	0.014	0.06	0.05	0.05	0.018	**0.26**	0.013	**1.39**
KW	P<	0.05	0.02	0.001	0.001	0.01	0.01	0.008	0.03	0.002	0.02	

The values with the highest contribution (THQ > 0.2) marked in bold.
The last case represents the significance values (p) among stations in the Kruskal-Wallis non-parametric test.

zones.

The PTWI of Al established by JECFA at of 2 mg/kg bw/week, but the maximum estimated weekly intake reached 60 % of this value according to our study. JECFA concluded that aluminium compounds could negatively affect the reproductive system and developing nervous system in rats, mice, dogs at 1 mg/kg bw of PMTDI (JECFA, 2011). In rats were noticed renal damage (hydronephrosis, urethral dilatation, obstruction, and/or presence of calculi) and reduced grip strength, but not cognitive impairment in the pups at the levels higher than the NOAEL of 30 mg/kg bw per day (JECFA, 2011). The levels of Al in mussels were in the range of 8–1088 (mean 209) μg/kg ww. The highest concentrations of aluminum were found for mussels from st. 8 (Durban), st. 4 (Waterfront).

4.2.2. Chromium

Chromium is associated with occupational exposures via numerous materials and processes, including chrome plating baths, colors and dyes, cement, tanning agents, wood preservatives, anticorrosive agents, welding fumes, lubricating oils and greases, cleaning materials, and textiles and furs.

While Cr^{3+} is a natural dietary constituent present in a variety of foods and also in dietary supplements, Cr^{+6} most commonly occurs in industrial processes and is present in drinking water usually as a consequence of anthropogenic contamination (EFSA, 2014). In humans, the dietary exposure levels of chromium absorption are relatively low (< 10 % of the ingested dose) and depend on its valence state and ligands. Most of the ingested Cr^{+6} is considered to be reduced in the stomach to Cr^{+3}, which is poorly bioavailable and presents low ability to enter cells (EFSA, 2014).

Chromium is suggested to be an essential trace element for humans, as it is involved in the metabolism of carbohydrates and lipids (Gu et al., 2016). Suboptimal dietary intakes of Cr are associated with increased risks of cardiovascular diseases and diabetes (Kobla and Volpe, 2000). The Codex Alimentarius commissions of Australia, Japan, New Zealand, Taiwan, and the United States have not set regulatory limits for Cr in foods. The Chinese national standard maximum Cr concentration in seafood is 2.0 μg/g ww (MPHC, 2012) (Gu et al., 2016). The levels of chromium in the soft tissue of mussels were in the range of 0.2−2.8 (mean 0.8) μg/kg ww. It means that some individuals from st. 5 and 8 (Durban) contained higher amounts of this element than recommended by national standards.

4.2.3. Cobalt

Anthropogenic emissions of cobalt into the aquatic environment include cobalt mining and processing activities, the production of alloys and chemicals, sewage effluents, urban run-off, and agricultural run-off (Nagpal, 2004). The principal pathway involves the binding of cobalt to suspended solids, which settle to the bottom of the water body and become part of the bed sediments (Nagpal, 2004). The mussels could intake the cobalt in solid particles from resuspended bottom sediments.

Oral exposure may potentially entail many adverse effects in humans (cardiac effects, effects on erythropoiesis, effects on the thyroid, developmental effects, and allergic dermatitis). Cobalt has been found to be a sensitizer in humans. A daily oral intake of 600 g cobalt (based on a LOAEL of 1 mg/kg for polycythaemia) appears minimum risk level for humans that would protect from the known threshold-related adverse effects (EFSA, 2009). The oral cobalt dose of 0.54 mg cobalt/kg-day represents a NOAEL for thyroid effects in humans. The point of departure of 1 mg cobalt/kg-day for decreased iodine uptake in human thyroid is the LOAEL, dividing this point of departure by a composite uncertainty (UF) of 300 yields a subchronic p-RfD of 0.003 mg/kg-day (USEPA, 2008). In our study the concentrations of cobalt in mussels were in the range of 0.04−0.46 μg/kg ww (mean 0.13) and maxima were noticed for st. 1 (Port Nolloth), st. 2 (Bok river), and st. 8 (Durban).

4.2.4. Zinc

Zinc is a ubiquitous metal present in the environment, as most rocks and many minerals contain zinc, which can be used for the zinc industry. Zinc is utilized as a protective coating of other metals, dye casting, construction industry, for alloys, dry cell batteries, dental, medical, and household applications, fungicide, topical antibiotics, and lubricants. Natural emissions results from erosion and forest fires.

Zinc is an essential trace element; the requirement for zinc changes throughout life and the health effects associated with zinc deficiency are numerous. Zinc occurs as a natural constituent in all plant and animal tissues and functions as an integral part of several enzyme systems. Protein foods are important dietary sources of zinc. The natural levels of this element are the highest in oysters, but in other seafood are lower. It was estimated that the average daily intake of zinc should not exceed 20 mg/day for adults, otherwise cause harmful effects could occur. Oral RfD of Zn in 300 μg/kg/day set as NOAEL (USEPA, 2005). According to our studies, the estimated daily intake of zinc ranged from 12 (mean from station 6) to 43 μg/kg bw/day (maximum at individuals from station 4).

4.2.5. Arsenic

The high levels of arsenic in air, soils could be associated with pesticides, manure, and mining and smelting activities. Groundwater contains higher levels of arsenic due to thermal activity or the dissolution of arsenic minerals (Chakrabarty, 2015). Arsenic enters into organisms mainly through water and food. Fish and meat are the main sources of arsenic among food, but the percentage of inorganic arsenic is low. Oral RfD for As is 0.3 μg/kg/day above which dermatitis, lowered neuron transmission, and liver carcinoma may develop (Korkmaz et al., 2017). In our study, the estimated daily intakes of arsenic in consumption of mussels' meat is ranged from 0.9 (st. 1 – Port Nolloth) to 1.9 μg/kg bw/day (maximum in individuals from st. 5 – Hout Bay).

The maximum permitted arsenic concentrations in marine crustaceans, fish, and shellfish are 0.5, 0.1, and 0.5 μg/g ww, according to the Chinese Food Codex (MPHC, 2012). The United States Food and Drug Administration and the WHO have specified a permissible As the concentration of 0.5 μg/g ww, and a comparable permissible concentration (specified in EC regulation 466/2001 and amended in EC regulation 221/2002) is used in the European Union (Gu et al., 2016).

Arsenic is often found at high concentrations in organic forms (Shiomi, 1994), especially in the marine environment up to 50 μg/g of arsenic on a wet weight basis in some seafood including seaweed, fish, shellfish, and crustaceans. The percentages of inorganic As in seafood are 1–5 % Munoz et al. (1999), while in bivalve molluscs, they are 1.9–6.5 %. Mussels usually contain approximately 1–2 % of inorganic As compounds (Sloth and Julshamn, 2008) but with high and potentially toxic levels of As, this percentage could increase to 50 % (Feldmann and Krupp, 2011; Chiesa et al., 2018). Arsenobetaine, which is the principal arsenic form in fish and crustaceans is considered non-toxic. Inorganic arsenic is usually classified as a human carcinogen. The intake of total arsenic in the human diet is dominated by organic arsenic derived from seafood (Gu et al., 2016).

4.2.6. Iodine

Iodine is an essential element for animals and humans because it is a key component in the thyroid hormones thyroxine and triiodothyronine. Deficiency can lead to many diseases, ranging from enlargement of the thyroid to severe cretinism with mental retardation (Goldhaber, 2003).

High consumption of iodine by humans can lead to goiter, hypothyroidism, or hyperthyroidism (Leung and Braverman, 2012). (Goldhaber, 2003) noticed that at levels of 200–500 mg/kg-day, the acute toxicity of iodine to animals resulted in death, and consumption of higher than 10 mg per day was toxic to some humans. EPA has not set an RfD for iodine. An upper level of 1.1 mg/day was set based upon thyroid dysfunction as a critical endpoint whereas the nutritional requirement for iodine (under review by WHO) is currently considered to be in the range of 0.10 to 0.14 mg per person per day. A LOAEL of 1.7 mg/day was selected based on two studies that reported elevated thyroid-stimulating hormone concentrations in men receiving iodine supplements. WHO had set a PMTDI for iodine of 1 mg/day (corresponding to 0.017 mg/kg bw) (JECFA, 2019). This was based on the observation that an iodine intake of 1 mg/ day or less is probably safe for the majority of the population, but may cause adverse effects for some individuals, e.g., people with thyroid disorders or those that are particularly sensitive to iodine (Goldhaber, 2003).

The concentrations of iodine in wet mussels ranged from 2.4 (minimum at st. 6 – Plettenberg Bay) to 36.2 (at st. 1 – Port Nolloth).

4.3. Regions with high risks according to risk quotients

According to the estimated consumption of mussel meat and the evaluated MPL, RQ, and THQ (HI) for the elements Cr, Fe, Co, Zn, and I, the riskiest zones (HI > 1) were st. 1 (Port Nolloth), st. 4 (Waterfront), st. 5 (Hout Bay) and st. 8 (Durban). St. 1 (Port Nolloth) revealed risks (HI > 1) based on the levels of iodine consumption due to the high concentration of this element and low values for the calculated limits (RfD and PTWI). It is interesting to note the high levels of THQ for iodine at the West Coast stations (1−5), which can be explained by the high hydrochemical levels in the surrounding waters, which should be investigated in the future.

The calculated ratios revealed the specificities of pollution on the West Coast (st. 4, 5 – Cape Town water area) and on the East Coast (st. 8 – Durban). The high concentrations of the elements Al, Sc, Ti, V, Cr, Mn, Fe, Co, Th, U in mussels which might be of terrigenous origin collected from the East Coast could be associated with the climatic features of the subtropical zone. (Erasmus et al., 2020) found that Fe, Co, Ni, Zn, and As in four species of marine organisms (including mussels) were higher at a subtropical site (Sheffield Beach) than at a warm temperate site (Tsitsikamma). The authors explained this by diffuse sources of metal input through estuaries and groundwater seepage into the marine environment. The Ti, Cr, and Cd were higher at Tsitsikamma and this was attributed to the metal-rich phytoplankton from the frequent upwelling events along the south coast.

(Wepener and Degger, 2020) during a summer survey conducted in 2008, which aimed to determine metal concentrations in brown mussels (*Perna perna*) from buoys along the coast of South Africa, found that concentrations of Al, Fe, Mn, Zn, and U at Port Elizabeth were higher than at Cape Town and Durban harbors, while differences in Cr, As, Se and Sr concentrations were insignificant. Our data, which are based on blue mussels (*Mytilus galloprovincialis*) at different spots of the Durban harbor, revealed that concentrations of Zn and As were lower and those of Fe were higher (610–1800 vs 210−280 ppm) than data presented by (Wepener and Degger, 2020). In comparison with (Wepener and Degger, 2020) at the Port Elizabeth the concentrations of Cr, Zn, and As were lower and of Fe was at the same level or lower (230–630 vs 420−750 ppm), and in Cape Town, the concentrations of Cr and As were lower, while of Fe and Zn they were almost equal or higher (260–1320 vs 245–340). However, the data obtained by the same authors in the 2009 survey are lower than the data obtained in 2008 by about one or two orders of magnitude and could not be compared correctly (Fe and Zn at Durban harbor were at the level of 3 ppm).

The high content of elements (Cr, Co), which could reflect a terrigenous component (and confirmed by other non-volatile elements Sc and Th), was found at the Durban station that indicates the pollution originated from the coastal runoff. The mussels from this site also accumulated high concentrations of V, Cr, Mn, Fe, and Ni, which are connected with this factor, based on the suspended material of bottom sediments (Naimo, 1995). Durban Harbour is the busiest container port in South Africa and is influenced by runoff from three contaminated rivers that transport waters from highly industrialized regions of the city of eThekwini (Moodley et al., 2016). The port is also exposed to significant quantities of domestic and industrial sewage discharges from the Umgeni River and a submarine outfall (McClurg et al., 2006).

The maximum levels of concentrations of the elements Zn, As, Se, Br and I at st. 5 (Hout Bay) reflected the anthropogenic component with low contents of Al and Fe. The mussels from this site experienced chemical pressure from a fishmeal factory and a vessel repair facility (sources mentioned in Brown, 1964 and Fru, 2020).

According to our approaches and results, the safest region for the consumption of mussels among the studied sites was in the area of Plettenberg Bay. The Bok River (Saldana Bay) and Port Elizabeth are designated as relatively safe, however, their proximity to greywater outlets is unfavourable. The mussels from these stations in terms of accumulation of the studied elements revealed a high potential for self-purification.

5. Conclusions

According to the estimated hazardous quotients and indexes, the levels of Na, Mn, Fe, Ni, Se, Sr, Sb, and U determined in local mussels in the studied South African cities can be considered relatively safe and the mussels are suitable for consumption.

Al, Cr, As, and I can increase the health risk when the consumption of mussel meat exceeds 250 g/week per person and the maximum concentrations in mussels exceed the limits in the studied coastal zones (at stations 4 and 8). Arsenic could affect health risk when a high percentage of inorganic form is present in the total amount.

Station 1 (Port Nolloth), st. 4 (Waterfront), st. 5 (Hout Bay), and st. 8 (Durban) were characterized by high risks (HI > 1) for Al, Cr, Co, Zn, As, and I in the case of a consumption rate of 200 g/week per person.

According to the intake of the studied elements, the Plettenberg Bay, Saldanha Bay, and Port Elizabeth could be considered relatively safe cities for regular consumption of local mussels.

CRediT authorship contribution statement

P.S. Nekhoroshkov: Conceptualization, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **J. Bezuidenhout:** Conceptualization, Funding acquisition, Methodology, Supervision. **M.V. Frontasyeva:** Conceptualization, Methodology, Writing - review & editing. **I.I. Zinicovscaia:** Methodology, Writing - review & editing. **N.S. Yushin:** Data curation, Formal analysis. **K.N. Vergel:** Data curation, Formal analysis. **L. Petrik:** Conceptualization.

Declaration of Competing Interest

The authors report no declarations of interest.

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