Journal of Trace Elements in Medicine and Biology 76 (2023) 127110

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Seasonal variations in mercury, cadmium, lead and arsenic species in Norwegian blue mussels (*Mytilus edulis* L.) – Assessing the influence of biological and environmental factors

Ana I. Gomez-Delgado [a], Jojo Tibon [a,b], Marta S. Silva [a], Anne-Katrine Lundebye [a], Antonio Agüera [a], Josef D. Rasinger [a], Tore Strohmeier [a], Veronika Sele [a,*]

[a] Institute of Marine Research, Nordnes, NO-5817 Bergen, Norway
[b] National Food Institute, Technical University of Denmark, Kemitorvet, Building 201, DK-2800 Kgs. Lyngby, Denmark

ARTICLE INFO

Keywords:
Potentially toxic elements (PTEs)
Inorganic arsenic
Arsenobetaine
Food and feed safety
Speciation analysis
HPLC - ICPMS

ABSTRACT

Background: Blue mussels (*Mytilus edulis* L.) can accumulate undesirable substances, including the potentially toxic elements (PTEs) cadmium (Cd), mercury, (Hg), lead (Pb), arsenic (As) and As species. In this study, the levels of PTEs and As species were determined in samples of blue mussels to assess the influence of environmental and biological factors, and evaluate the potential risk associated with blue mussels in terms of food and feed safety.

Methodology: Blue mussels were collected monthly from one location in Western Norway from February 2018 to December 2018, and from April 2019 to April 2020. Samples were analyzed for PTEs using inductively coupled plasma mass spectrometry (ICP-MS), and high-performance liquid chromatography (HPLC) coupled to ICP-MS. Temperature, salinity and fluorescence (chlorophyll a) were monitored in the seawater column by STD/CTD, to assess the potential influence of these environmental factors on the PTE levels in the mussels.

Results: The results showed seasonal variations in the PTEs, with somewhat higher concentrations in spring and winter months. Unusually high levels of total As (101.2 mg kg^{-1} dw) and inorganic As (53.6 mg kg^{-1} dw) were observed for some of the time points. The organic As species arsenobetaine was generally the major As species (17–82% of total As) in the mussels, but also simple methylated As species and arsenosugars were detected. Principal components analysis (PCA) did not show a consistent relationship between the environmental factors and the PTE concentrations, showing contrary results for some elements for the periods studied. The condition index (CI) could explain variations in element concentration with significant correlations for Cd ($r = -0.67$, $p = 0.009$) and Pb ($r = -0.62$, $p = 0.02$ in 2019/20 and $r = -0.52$, $p = 0.02$ in 2018), whereas the correlation between As and CI was not significant ($r = 0.12$ in 2018, and $r = -0.06$ in 2019/20). Higher concentrations of iAs and arsenosugars coincided with increased signals of chlorophyll a, suggesting that phytoplankton blooms could be a source of As in the blue mussels.

Conclusion: To our knowledge, this is the first study of As species in blue mussels collected over a time period of two years, providing an insight into the natural variations of these chemical forms in mussels. In terms of mussel as food and future feed material, concentrations of Cd, Hg and Pb were below the maximum levels (MLs) established in the EU food and feed legislation. However, levels of As and iAs in mussels at some time points exceeded the MLs for As in the feed legislation, and the margin of exposure (MOE) was low if these mussels were for human consumption, highlighting the importance of determining the chemical forms of As in feed and food.

1. Introduction

Blue mussels (*Mytilus edulis* L.) are bivalve mollusks widely consumed as seafood, with a high content of proteins, omega-3 fatty acids and vitamins [1]. These low trophic marine organisms are also being considered as a potential alternative feed material [2].

Although mussels contain high levels of essential nutrients, they also accumulate undesirable chemicals, including arsenic (As), cadmium

* Correspondence to: PO. Box 1870 Nordnes, NO-5817 Bergen, Norway.
E-mail address: Veronika.Sele@hi.no (V. Sele).

https://doi.org/10.1016/j.jtemb.2022.127110
Received 11 August 2022; Received in revised form 3 November 2022; Accepted 21 November 2022
Available online 23 November 2022

(Cd), lead (Pb) and, mercury (Hg) [3], characterized as potentially toxic elements (PTEs) [4]. Main sources of PTEs include industrial processes, fossil fuel combustion, erosion, and waste [5], and they have a tendency to end in the aquatic ecosystems, where they accumulate in organisms and can integrate into the food web [6]. The PTEs Hg, Pb, Cd, and As are linked to numerous adverse health effects, including neurodevelopment and cancer [7,8].

Cadmium and Pb concentrations in seawater vary, with generally lower levels in open ocean water than in coastal and estuarine waters [9]. Cadmium and Pb are taken up by marine organisms, including blue mussels, with concentrations often reflecting the anthropogenic sources [10,11]. In organisms, Hg is an element known to increase in concentration with size and age and bio-magnify in the food chain [12]. This element occurs in different chemical forms, where the organic form dimethylmercury (Me_2Hg) is the most toxic, and methylmercury (MeHg) the most bioavailable and abundant form in marine organisms [13,14]. Arsenic is a metalloid that is abundant in marine organisms [15], and which naturally occurs in a range of As species [16]. The toxicity of As species varies, with inorganic As (iAs) classified as carcinogenic, arsenobetaine (AB) as non-toxic, whereas some of the organic As species (e.g. dimethyl arsinate (DMA)) classified as possibly carcinogenic, and other organic As species as considered 'not classifiable' [17,18]. Due to the differences in toxicity of As species, characterization of As species is necessary for a better assessment of this element [19]. In marine fish, As is primarily present in the organic and non-toxic form of AB [20], and iAs normally represents less than 1% of the total As [21]. High concentrations of iAs have, however, been reported for some families of brown seaweed, including *Hizikia fusiforme* [22] and *Laminaria digitata* [23]. Also, high levels of iAs have been reported in blue mussels (*Mytilus edulis* L.) harvested from Norwegian fjords (up to 5.8 mg kg^{-1} wet weight (ww)) [24].

To protect consumers, farm animals and the environment, the European legislation defines maximum levels (MLs) for PTEs and other undesirable substances in feed and feed ingredients [25] and for foodstuffs [26]. Furthermore, the FAO/WHO Expert Committee on Food Additives (JEFCA) and the European Food Safety Authority (EFSA) have established tolerable (provisional) weekly intakes (PTWI or TWI) or bench mark doses (BMD) to estimate the amount of a potentially harmful contaminant that can be ingested over a lifetime without risk of adverse health effects [20,27]. Levels of PTEs are typically higher in mussels than in ambient water, and can be higher than in other marine organisms due to a limited detoxification capacity [28]. The levels of As, Cd, Hg and Pb in mussels have also been associated with a range of environmental factors, including season [29], temperature, pH [30], salinity [31], location, level of seawater contamination [32] or amount and type of food available [33]. In addition, biological factors, such as the reproductive state [34], sex [35] or size [36] can affect metal accumulation in mussels.

Despite numerous studies on metal levels in mussels, few studies have examined the inter-annual concentrations of heavy metals, As and As species in mussels from the same geographical position. The aim of this study was to examine the inter-annual concentrations PTEs and As species in mussels from the same geographical position over a longer time period, and to (1): assess the possible influence of environmental and biological factors on the levels of PTEs and As species in mussels, and (2): evaluate the risk associated with blue mussels in terms of food and feed safety.

2. Methods

2.1. Sample collection and preparation

Blue mussels (*Mytilus edulis* L.), of approximately two years of age, were collected in Austevoll at two different time-points, in January 2018 and in March 2019. For each batch of mussels, they were kept in lantern nets at 3 m depth, at the Institute of Marine Research's field station (60°05'16''N, 5°15'45''E, Fig. 1) for the sampling period. A total of 19–21 individual blue mussels were sampled monthly from the nets with one to three sampling times per month) from February 2018 to December 2018 and from April 2019 to April 2020 (Table S1, Supplementary).

The discontinuity in the sampling points, together with minor differences in sample preparation, and differences in mussel's size, led to grouping the data into two different periods; 2018 (period 1, February 2018 to December 2018), and 2019/2020 (period 2, April 2019 to April 2020). Individual mussels sampled in 2018 were cleaned, measured, freeze-dried and pooled (n = 19–21) to minimize individual (intraspecific) variation between mussels [37]. For 2018, there were a total of 20 samples, representing different time points (Table S1, Supplementary). Mussels sampled in 2019/2020 were stored in polyethylene bags, immediately frozen at − 20 °C prior to further analyses. Epiphytes and other physical impurities were removed from the mussels, and total weight, and shell lengths were measured. Soft tissues were pooled into one sample (n = 19–20). The pooled soft tissues were homogenized in a blender (Braun Multiquick 7, German, Kronberg, Germany), and freeze-dried for 48 h (FreeZone® 18 Liter, Kansas City, Labconco). For 2019/2020, there were a total of 14 samples, representing different time points (Table S1, Supplementary).

2.2. Environmental parameters

Environmental parameters were monitored at 3 m depth and next to the lantern nets. Measurements of temperature (°C), salinity (psu, practical salinity unit) and fluorescence (calibrated proxy of chlorophyll a, μg L^{-1}) were performed at time intervals of 1 h for 2018 and 10 min for 2019/20 by a STD/CTD (model SD-204, Saiv A/S, Bergen, Norway). Data was retrieved for the all months in 2018 and from January 2019 to April 2020, with a gap between March to June 2019.

2.3. Chemical analyses and quality control

2.3.1. Determination of Cd, Hg, Pb and As

Determination of As, Cd, Hg and Pb in soft tissue of blue mussels was performed by inductively coupled plasma mass spectrometry (ICP-MS) following assisted microwave digestion, with a multielement method [38]. In brief, approximately 0.2 g of freeze-dried sample (n = 3, technical replicates) was weighed into quartz tubes containing 0.5 mL of Milli-Q® water (18.2 MΩ-cm, Millipore RiOs, Burlington, USA), 2 mL concentrated nitric acid (HNO_3, 69% w/w, Suprapur, Merck, Darmstadt, Germany) was added, and samples were digested in an UltraWAVE digestion system (Milestone, Sorisole, Italy). The digest was diluted to 25 mL with Milli-Q® and stored in falcon tubes at room temperature until analyses by ICP-MS (Thermo iCap-Q, Thermo Fisher Scientific, Waltham, USA) equipped with a collision cell and FAST SC-4Q DX auto-sampler (Elemental Scientific, Omaha, USA). The elements were quantified using an external calibration curve (Multielement standard stock solution, Spectrascan Teknolab, Ski, Norway), and isotopes of rhodium, germanium and thulium were used as internal standards. Processing of data was performed with the software Q-tegra ICP-MS (Thermo Fisher Scientific, Waltham, USA). The limits of quantification (LOQ) were 0.01 mg kg^{-1} (As), 0.005 mg kg^{-1} (Cd and Hg), 0.03 mg kg^{-1} (Pb), when using standard dry weight.

2.3.2. Determination of inorganic As

Inorganic As (iAs) was determined by anion-exchange high-performance liquid chromatography (AE-HPLC) coupled to ICP-MS as described in early studies [39] and according to CEN method 16802 [40]. Approximately 0.2 g of homogenized freeze-dried sample (n = 3, technical replicates) was weighed into 13 mL polypropylene centrifuge tubes (Sarstedt, Nümbrecht, Germany) and 10 mL of extraction solution (0.1 M HNO_3 and 3% (v/v) H_2O_2 (30%, EMSURE, Darmstadt, Germany), was added, with the addition of H_2O_2 for the oxidation of arsenite (As

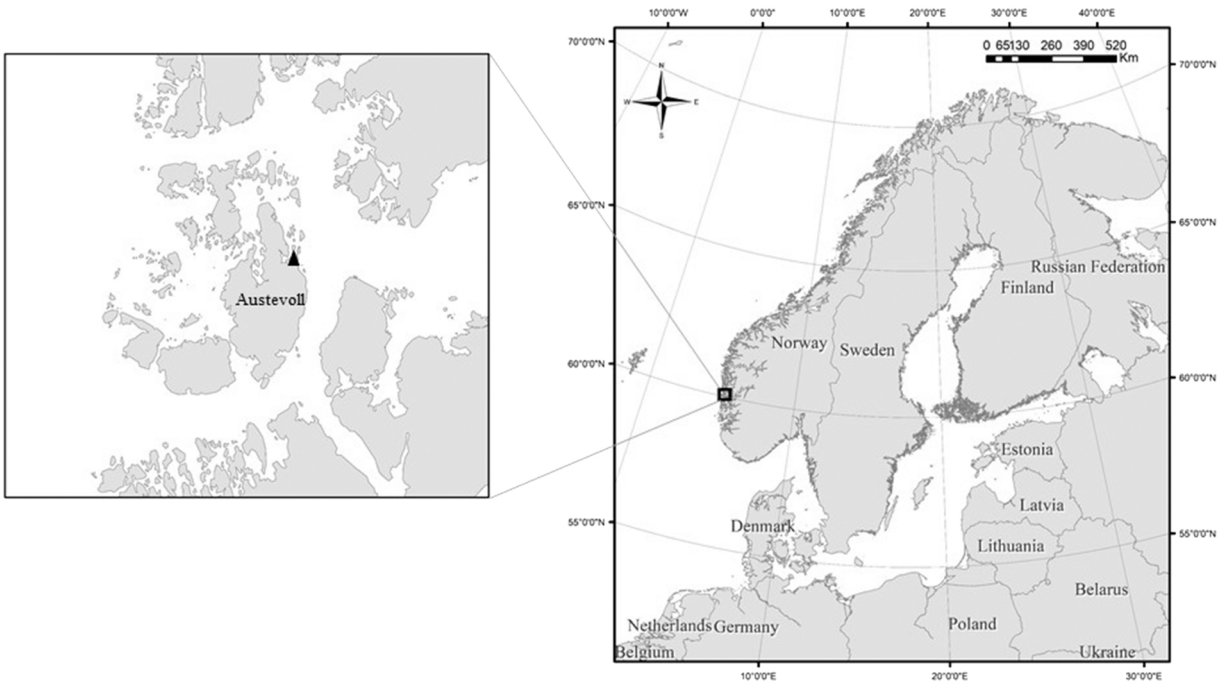

Fig. 1. Geographical location of sampling points of blue mussels (*Mytilus edulis* L.) on the western coast of Bergen, Norway (*1.5-column fitting image*).

(III)) to arsenate (As(V))). Samples were shaken in a vortex mixer (MS1 Minishaker, IKA, Staufen, Germany), and stored at room temperature overnight, then placed in a water bath (OLS200 orbita, Grant) at 90 ± 2 ºC for 60 min and shaken at 100 rpm. Samples were left to cool to room temperature and centrifuged (Centrifuge 5702, Eppendorf®, Hamburg, Germany) for 10 min at 3800 rpm. The supernatants were removed with 5 mL disposable needle syringes (SOFT-JECT 5 mL, Henke Sass, Tuttlingen, Germany + BD Microbalance 3, 1.25 ×50 mm, Becton) and filtered with disposable syringe filters (Minisart RC25 0.45 μm, Merck Millipore) into 1 mL propylene HPLC vials. Determination of iAs was performed using HPLC-ICP-MS (HPLC 1260 Infinity, Agilent 7900 ICP-MS, Agilent Technologies, Wilmington, USA) equipped with an analytical column IonPac AS7 2 × 250 mm (Dionex, Sunnyvale, USA) with a guard column (IonPac AG7 2 ×50 mm, Dionex, Sunnyvale, USA). The mobile phase utilized was 50 mM ammonium carbonate ($(NH_4)_2CO_3$, reagent grade, Merck, Darmstadt, Germany) in an aqueous methanol 3% (v/v) (MeOH, ≥ 99.97%, Merck), at a pH of 10.3 (adjusted with 25% (v/v) aqueous ammonia (NH3, 25%, Merck). Quantification of As(V) was based on chromatographic peak area of As(V) using an external calibration curve of arsenate standard solution (1000 mg L^{-1} As (V), Spectrascan TeknoLab, Oppegaard, Norway).

2.3.3. Determination of organic As species

Organic As species were quantified by ion-exchange HPLC coupled to ICP-MS using methods described in previous work [41]. Approximately, 0.2 g of sample (n = 3, technical replicates) was weighed into 13 mL polypropylene tubes. Five mL of aqueous methanol solution (50%, v/v) was added, thereafter homogenized by vortex mixing. The tubes were heated in a water bath set at 90 °C for 30 min (shaking speed at 100 rpm) and centrifuged (3800 rpm, 10 min). The supernatant was filtered through a 5 mL syringe connected to a 0.45 μm filter and transferred into new 13 mL polypropylene tubes. A 100 μL aliquot was transferred to an HPLC vial and diluted with 400 μL extraction solution. As speciation was performed using a 1260 Infinity HPLC coupled to a 7900 ICP-MS (Agilent Technologies, Wilmington, USA). Cationic As species were separated using a cation-exchange Metrosep C6 column (250 ×4.0 mm, 5 μm, Metrohm, Herisau, Switzerland) under gradient conditions using pyridine-based mobile phases, adjusted to pH 2.7 (mobile phase A: 0 mM pyridine, 0.5% ACN, and mobile phase B: 50 mM pyridine, 0.5% ACN). Anionic As species were eluted through an anion-exchange PRP-X100 column (250 ×4.6 mm, 5 μm) (Hamilton, Reno, NV, USA), also under gradient conditions, using carbonate-based mobile phases and adjusted to pH 9.3 with aqueous ammonia (mobile phase A: 0.5 mM $(NH_4)_2CO_3$, 3% MeOH, and mobile phase B: 60 mM $(NH_4)_2CO_3$, 3% MeOH), both gradients described in [41]. Quantification was based on external calibration curves generated from mixed calibration standard solutions of arsenic compounds, of AB, dimethylarsinate (DMA), monomethylarsonate (MA), tetramethylsineoxide (TMAO), trimethylarsoniopropionate (TMAP), tetramethylarsonium iodide (TETRA), and arsenocholine (AC).

2.3.4. Quality assurance and control

The method for determining elements (As, Hg, Pb and Cd) and the method for determining iAs are accredited by the Norwegian accreditation body according to NS-EN ISO/IEC 17025:2017. The method for water-soluble As species has been validated, and results presented in previous work [41].

For determination of As, Hg, Pb and Cd the following certified reference materials (CRMs); i.e. Oyster Tissue (SMR 1566b, National Institute of Standards and Technology, Gaithersburg, USA), Lobster Hepatopancreas (TORT-3, National Research Council Canada, Ottawa, Canada) and Mussel Tissue (ERM-CE 278 K, European Commission's Joint Research Centre, Geel, Belgium) were used for quality control (Table S2, Supplementary). For determination of iAs CRM Rice (ERM BC 211, Institute for Reference Materials and Measurements, Geel, Belgium) and an in-house control material of tuna fish tissue (BCR 627, Community Bureau of Reference) were included in all analysis (Table S1). For the analysis of organic As species BCR-627 (tuna fish tissue) and DORM-4 (fish protein) were used to verify accuracy of the results, with certified values for AB. The measured concentrations for all CRMs were in agreement with the certified values (Table S2, Supplementary).

2.4. Calculations and statistical analysis

Condition Index (CI) was used to determine the physiological con-

dition of the mussels. The CI calculation was based on the description of Walne and Mann [42], as follows:

$$CI = \frac{bodydw(g)}{shelldw(g)} * 100$$

Where body dw (g) is the weight of soft tissue after freeze-drying, and the shell dw (g) is the shell weight after drying on blotting paper. The CI was calculated for the mean blue mussel samples, since dry weights were obtained after pooling the sample.

2.4.1. Evaluation of risk

The concentrations of PTEs were determined in dried samples to minimize uncertainties related to the moisture content. For the food and feed risk evaluations, it was, however, necessary to convert the dry weight (dw) concentrations to wet weight (ww) concentrations to compare the levels to concentrations in blue mussel as a food item or as feed material, The following formula was used:

$$\frac{mg}{kg}ww = \left(\frac{100 - \%moisture}{100}\right) * \frac{mg}{kg}dw$$

where $mg\ kg^{-1}\ dw$ is the concentration obtained for freeze-dried samples, and the *%moisture* corresponds to the moisture content of the sample. For evaluating the risk associated with blue mussel as a feed material, a moisture content of 12% was applied in accordance with the EU Directive 2002/32/EC on undesirable substances in animal feed [25]. For the evaluation of risk associated with blue mussel as foodstuff, a fixed moisture content of 85% was applied, based on previous risk assessments [24]. The estimated intakes (EI, μg kg^{-1} bw) of the PTEs were calculated and compared to the Tolerable Weekly Intakes (TWI) established by EFSA for Cd (2.5 μg kg^{-1} bw [14]) and MeHg (1.3 μg kg^{-1} bw [14]) using the following equation [43];

$$EI = \frac{C(\mu g kg^{-1} ww) * IR}{bw}$$

Where *C* was the concentration of PTEs (μg kg^{-1} ww), *IR* was the intake rate (g mussel per day or per week) and *bw* was the body weight of an adult person. The consumer IR of the general European population was set to 1.17 kg $year^{-1}$ or 0.0032 kg day^{-1} [44]. The bw was set at 70 kg, similar to risk assessments by EFSA [20]. For Hg, it was assumed that 100% of the Hg present in mussels was in the form of MeHg.

For iAs and Pb, safety concern was assessed by calculation of individual margin of exposures (MOE), defined as the ratio between the respective benchmark dose lower bound BMDL (reference point) and the estimated intakes. BMDLs have been established for iAs ($BMDL_{01}$ from 0.3 to 8 μg kg^{-1} bw day^{-1}) [18] and for Pb ($BMDL_{01}$ from 0.50 to 1.50 μg kg^{-1} bw) [27].

The calculation of the MOE for iAs and Pb was calculated using the following formula:

$$MOE = \frac{BMDL}{EI(mgKg^{-1}bwday^{-1})}$$

2.5. Statistical analyses

Statistical analyses were performed using R v4.1.2 [45] in RStudio [46]. Differences in shell lengths among the years were compared using nonparametric Kruskal-Wallis test ($p \leq 0.05$). Correlations between PTEs and CI were assessed by Spearman`s correlation ($p \leq 0.05$). Principal components analysis (PCA) was used to determine tendencies and groups among concentrations and environmental variables. For the statistical analysis, the environmental variables were calculated as the mean of 15 days prior to the blue mussel sampling (mean, n = 15 days) to reflect a consistent time period that could potentially affect the uptake in blue mussels.

3. Results

A significant difference in shell length between mussels collected in 2018 and 2019/20 was observed (Kruskal Wallis, $p < 2.2e^{-16}$) (Table S1, Supplementary). Because size is known to affect concentrations of PTEs [47], and because there were also minor differences in the sample preparation within periods, statistical analyses were selectively performed for the periods 2018 and 2019/20.

3.1. Concentrations of PTEs

The concentrations of PTEs in the blue mussels varied within and between years (Fig. 2 and Table S2). The largest variations were seen for As, followed by Pb, Cd and Hg. Overall, higher concentrations of PTEs were seen for the samples collected in the period 2019/2020 compared to 2018, except for As, where the highest levels were detected in the mussels sampled in the period 2018. For all PTEs, a decline in concentrations was seen at the start of summer months for both periods studied. Higher concentrations of PTEs were seen in winter or spring, and this was particularly noticeable in mussels sampled in 2019/2020.

The concentration of Cd ranged from 0.41 to 0.64 mg kg^{-1} dw in 2018, and between 0.48 and 0.93 mg kg^{-1} dw in 2019/20. The highest Cd levels in 2018 were found in mussels collected in June (0.63 ± 0.04 mg kg^{-1} dw) and the lowest in July (0.41 ± 0.02 mg kg^{-1} dw), whereas for 2019/20 the highest levels were found in May (0.95 ± 0.03 mg kg^{-1} dw) and the lowest in November (0.48 ± 0.04 mg kg^{-1} dw) (Fig. 2A).

Concentrations of Hg ranged from 0.03 to 0.15 mg kg^{-1} dw in 2018 and from 0.07 to 0.18 mg kg^{-1} dw in 2019/20. The highest concentration was obtained in June for 2019 (0.18 ± 0.01 mg kg^{-1} dw), and in February for 2018 (0.15 ± 0.01 mg kg^{-1} dw). The lowest levels of Hg were obtained in May for 2018 (0.03 kg^{-1} dw mg kg^{-1} dw) and November 2019 (0.07 ± 0.01 mg kg^{-1} dw) (Fig. 2B).

For Pb, concentrations ranged between 0.2 and 0.7 mg kg^{-1} dw in 2018 and from 0.5 to 1.2 mg kg^{-1} dw in 2019/20. Concentrations were lowest in May 2018 (0.17 mg kg^{-1} dw), whereas the highest concentration was measured in May 2019 (1.25 ± 0.01 mg kg^{-1} dw). A second increase in Pb concentration was measured in mussels collected in February 2020 (0.98 ± 0.02 mg kg^{-1} dw). The lowest levels for 2019/20 mussels were measured in November (0.53 ± 0.04 mg kg^{-1} dw) (Fig. 2C).

The mean As concentration in blue mussels collected in 2018 (24.8 mg kg^{-1} dw) was higher than for the mussels collected in 2019/20 (14.4 mg kg^{-1} dw). The levels ranged from 12.3 to 101.2 mg kg^{-1} dw in 2018, and from 11.6 to 17.8 mg kg^{-1} dw in 2019/20. High concentrations of As were detected in November and December 2018 (101.2 and 61.2 mg kg^{-1} dw, respectively), while for 2019, the highest As level was measured in May (17.8 mg kg^{-1} dw). The lowest concentrations of As were seen in blue mussels collected in August, both in 2018 and 2019/20 (12.3 ± 0.1 and 11.6 ± 0.4 mg kg^{-1} dw, respectively) (Fig. 2D).

3.2. Arsenic species

3.2.1. Inorganic arsenic

The concentrations of iAs in the mussels varied from 0.07 to 53.6 mg kg^{-1} dw, but most samples were below 1 mg kg^{-1} dw (Fig. 3). For three of the sampling points, November 2018, December 2018 and September 2019, high levels of iAs were detected, with concentrations of 53.6 ± 9, 28.7 ± 0.6, and 12.0 ± 0.2 mg kg^{-1} dw, respectively. No correlation between iAs and total As was seen in samples taken in 2019/20, but a positive correlation was observed when concentrations of total As were above 3.5 mg kg^{-1} ww (R^2 =0.98, $p < 0.05$) for samples of 2018. The highest concentration of iAs (53.6 mg kg^{-1}, November 2018) corresponded to 53% of the total As. For 2019 the highest concentration of iAs (12 mg kg^{-1}, September 2019) corresponded to 77% of the total As, however, it was not the mussel sample with the highest total As

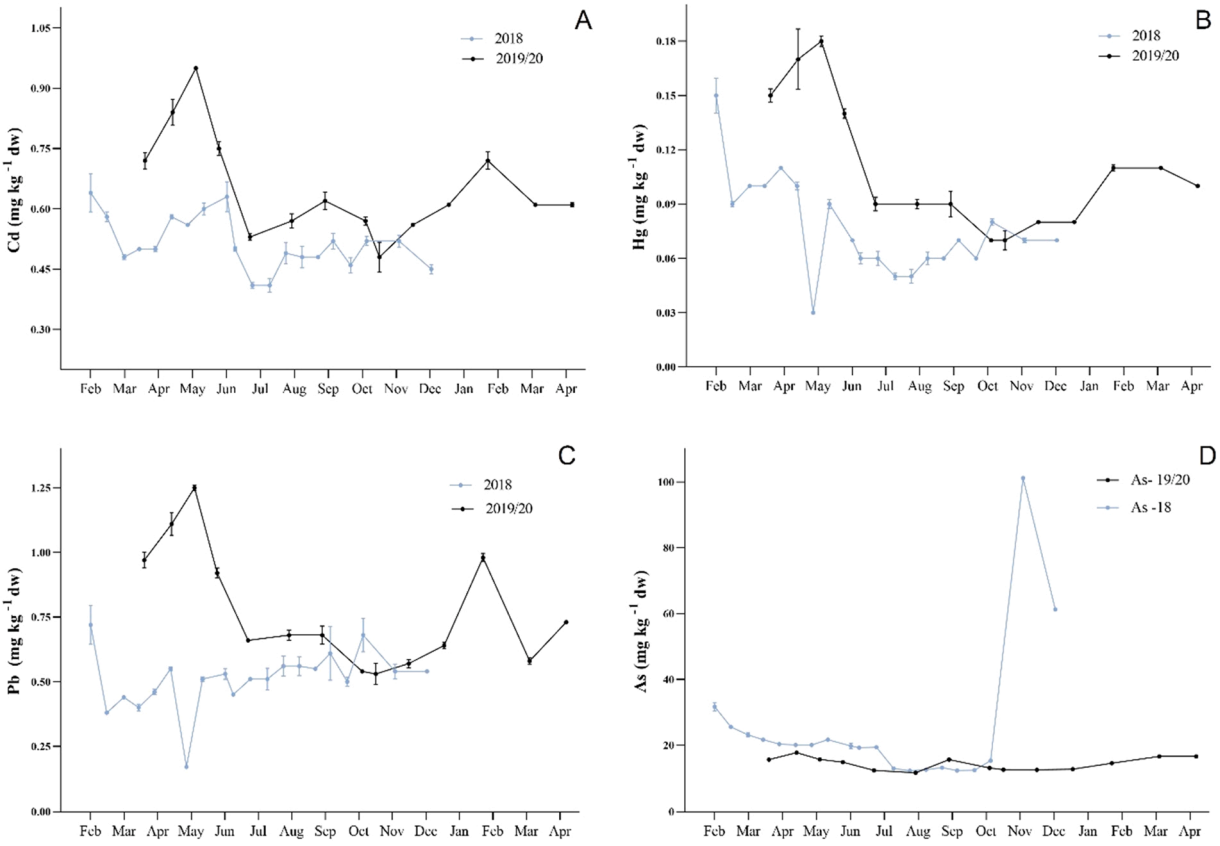

Fig. 2. Concentrations of cadmium (Cd) (A), mercury (Hg) (B) and lead (Pb) (C) and arsenic (As) (D) in pooled blue mussels (*Mytilus edulis* L., n = 20 ± 1) with sampling from February 2018 to December 2018 (period 1, blue line), and April 2019 to April 2020 (period 2, black line). Concentrations given in mg kg^{-1} dw. Error bars corresponds to standard deviations of technical replicates (n = 3).

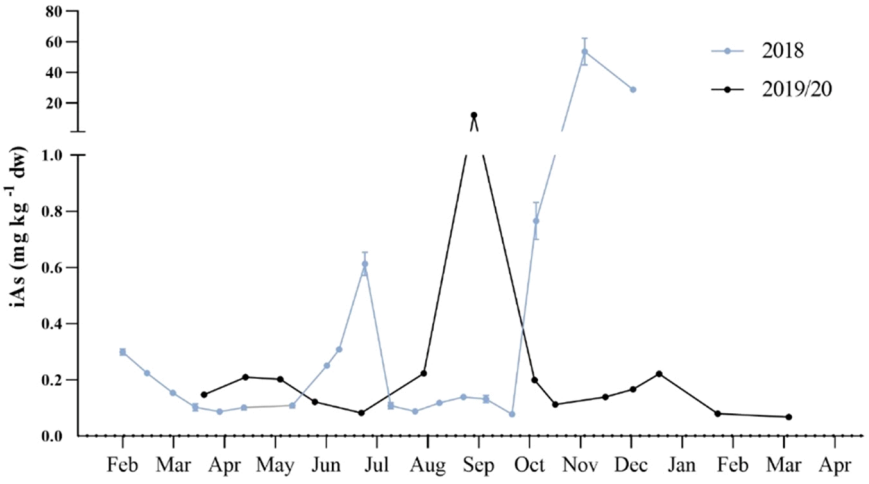

Fig. 3. Concentrations of inorganic arsenic (iAs, mg kg^{-1} dw) in blue mussels (*Mytilus edulis* L.) from February 2018 to December 2018 (blue line), and from April 2019 to April 2020 (black line). Error bars correspond to the standard deviations of technical replicates (n = 3).

concentration for that period. Besides the high concentrations detected for these three months, all other samples had iAs concentrations representing less than 5% of total As and concentrations from 0.07 to 0.8 mg kg^{-1} dw.

3.2.2. *Organic arsenic species*

Arsenobetaine was generally the major As species found in mussels (Fig. 4), with concentrations ranging from 3.8 to 17.5 mg kg^{-1} dw (Table S3 and Fig. S1, Supplementary), and accounting for 17–82% of total As in the mussels. The highest concentrations of AB were detected in November and December 2018 (17.5 ± 4.7 and 16.5 ± 0.3 mg kg^{-1} dw) and in April 2019 and 2020 (9.2 ± 0.7 and 9.6 ± 0.5 mg kg^{-1} dw, respectively). Lower concentrations of AB were seen in September 2018 and 2019 (6.3 ± 0.2 and 3.8 ± 1.0 mg kg^{-1} dw, respectively). The lowest percentage of AB (in ratio to total As) was detected in mussels sampled in November 2018 (17%), which was the sample having the highest total As. No consistent pattern for the amount of AB to total As could be seen for the mussels, although samples with high

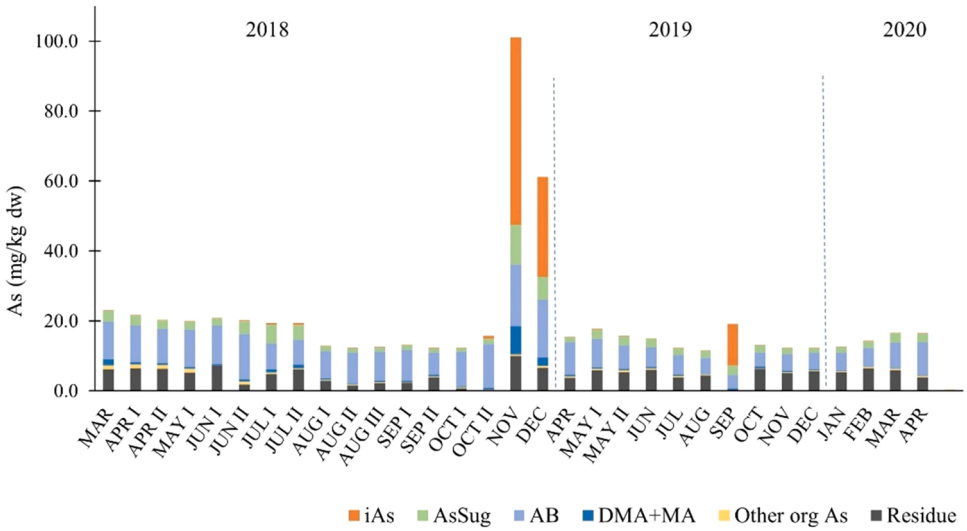

Fig. 4. Partitioning of As species, including inorganic arsenic (iAs), arsenosugars (AsSug; sum of $AsSugPO_4$, AsSugSO3, AsSugSO4, AsSugOH), dimethylarsinate (DMA)+ methylarsonate (MA), arsenobetaine (AB), other organic As (TMAO, TMAP, AC and TETRA) and the calculated unidentified fraction (Residue) in blue mussels (*Mytilus edulis* L.) collected from March 2018 to April 2020.

concentrations of As generally contained higher concentrations of AB (Fig. S2, Supplementary).

The methylated species MA and DMA were present in all samples (Table S4, Supplementary). Concentrations of DMA were higher than those of MA. Levels of DMA ranged from 0.3 to 6.9 mg kg^{-1} dw in 2018 and from 0.1 to 0.5 mg kg^{-1} dw in 2019/20, whereas for MA, concentrations ranged from 0.03 to 1.30 mg kg^{-1} dw in 2018 and 0.02–0.2 mg kg^{-1} dw in 2019/20.

Different species of AsSugars were detected, where glycerol arsenosugar (AsSugOH) and phosphate arsenosugar ($AsSugPO_4$) were the major ones, followed by sulphate arsenosugar ($AsSugSO_4$) and sulphonate arsenosugar ($AsSugSO_3$) (Table S5, Supplementary). Overall AsSugars represented between 8.5% and 28% of the total As, with higher fractions in June-July for 2018 (18–28%) and August-September for 2019 (17%) (Table S5, Supplementary).

Other minor organic As species detected in the mussels were tetramethyl arsonium ion (TETRA), tetramethylsine oxide (TMAO), trimethylarsonio propionate (TMAP), and arsenocholine (AC) (Table S5, Supplementary), accounting for 0.5–5.3% of total As concentrations. Of these organic As species, AC and TMAP were found with highest concentrations, ranging from 0.04 to 0.6 mg kg^{-1} and from 0.07 to 0.3 mg kg^{-1}, respectively (Table S5, Supplementary). The mass balance of As was determined by comparing the sum of As species with the total As for each sample. The mean recovery was 74 ± 15% (data not shown). This shows an overall acceptable recovery, and comparable to the extraction recovery seen for As in blue mussels [41].

3.3. Condition index

The fluctuations in the CI for *M. edulis* L. for the time periods 2018 and 2019/20 are shown in Fig. 5. The CIs were generally higher for the mussels collected in 2018 (17 ± 3%) compared to 2019/20 (7.7 ± 1.2%) indicating less soft tissue for the mussels from 2019/20 compared to their shell weight (dw), i.e. the proportion of soft tissue /shell weight decreased with shell length. The CI pattern for both collection periods was similar in terms of having a decreased CI for the months of April and May and a following increase in CI for the months of June and July. For 2018 a decrease in the CI value was observed from

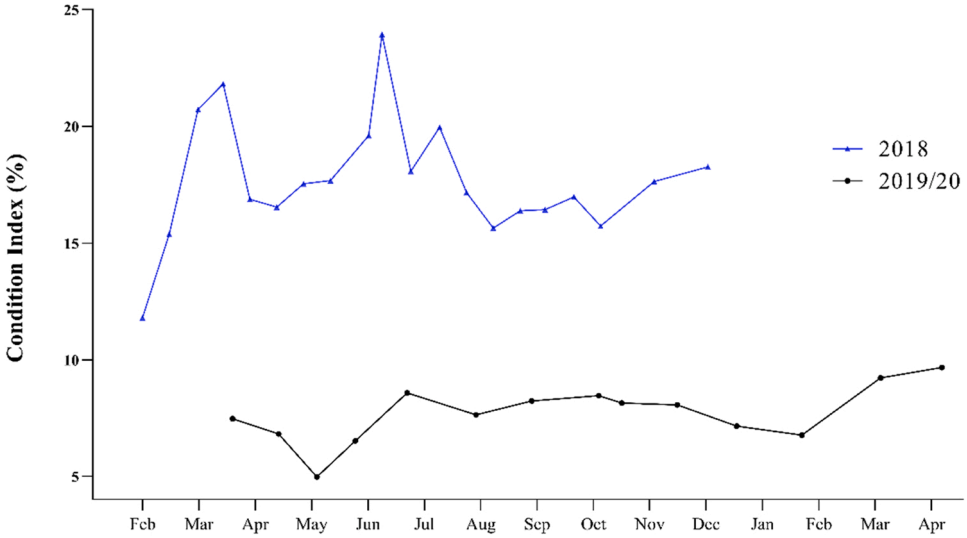

Fig. 5. Seasonal variations in the condition index (CI, %) for the blue mussels (*Mytilus edulis* L.) from February 2018 to December 2018 (blue line) and from April 2019 to April 2020 (black line).

June, and the 2018 CI data had larger variations particularly for the summer months than the 2019/2020 mussels.

Using Spearman correlation ($p < 0.05$) CI showed a significant but weak negative correlation with Pb for the 2018 data ($r = -0.52$, $p = 0.02$), whereas no significance correlation was found for Cd ($r = -0.32$, $p = 0.16$), Hg ($r = -0.18$, $p = 0.45$), and As ($r = 0.12$, $p = 0.61$). For samples collected in 2019/20, CI was significantly and negatively correlated with Cd ($r = -0.67$, $p = 0.009$) and Pb ($r = -0.62$, $p = 0.02$), no significant correlation was found for As ($r = -0.06$, $p = 0.84$) and Hg ($r = -0.50$, $p = 0.07$).

3.4. Environmental variables

Seawater temperatures were highest in summer months, with June-September (15.3 ± 1.7 °C) for the 2018 period and August (18.3 ± 0.3 °C) for the 2019 period (Fig. 6A). A gradual decrease was then seen towards winter months. For 2018, the lowest temperatures were in February and March (from 2.4 to 6.6 C°) and for 2019/20 period in February 2020 (5.9 ± 0.1 °C).

Seawater salinity fluctuated for the sample period, with lower salinity in the months of August to October (27–30 psu) for both periods studied (Fig. 6B). In 2018 the highest salinity was recorded in seawater in December (33.5 ± 0.9 psu). For 2019/20, salinity also followed a similar pattern to 2018, with the lowest value (28 ± 0.5 psu) at the end of the warmer months and the highest in winter.

Highest levels of fluorescence in 2018 was observed in the summer months July and August for 2018, with levels between 2.7 and 13 chlorophyll a (Fig. 6C). Higher fluorescence signals were also observed in late winter months (i.e. February-March), with up to 5.0 ± 3.8 chlorophyll a. For 2019/20 fluorescence measurements were lowest in late autumn and winter months (November-January) and highest in late summer and early autumn, with a maximum in September (3.6 ± 0.8 chlorophyll a).

3.5. Relationship between environmental variables and PTEs

The PCA plots shows an association between Cd and Hg for both year periods. A negative correlation with temperature and fluorescence was seen for Cd and Hg in both periods 2018 (Fig. 7A) and 2019/20 (Fig. 7B), however, in the case of fluorescence this correlation was lower for the 2019/20 data. In terms of salinity no consistency can be determined from both periods regarding these elements. A positive correlation was observed for Pb and temperature and fluorescence for 2018, contrary to 2019/20. Nevertheless, a positive influence of salinity was found for Pb in both year periods. For As and iAs, a positive correlation with salinity was seen for 2018 data, but for 2019/20 no correlation was observed. Similarly, no clear correlation between the levels of As and temperature were seen as data for 2018 were opposing the 2019/20 data. Inorganic As, was correlated with the fluorescence levels for the 2019/20 data, but no correlation was seen for 2018.

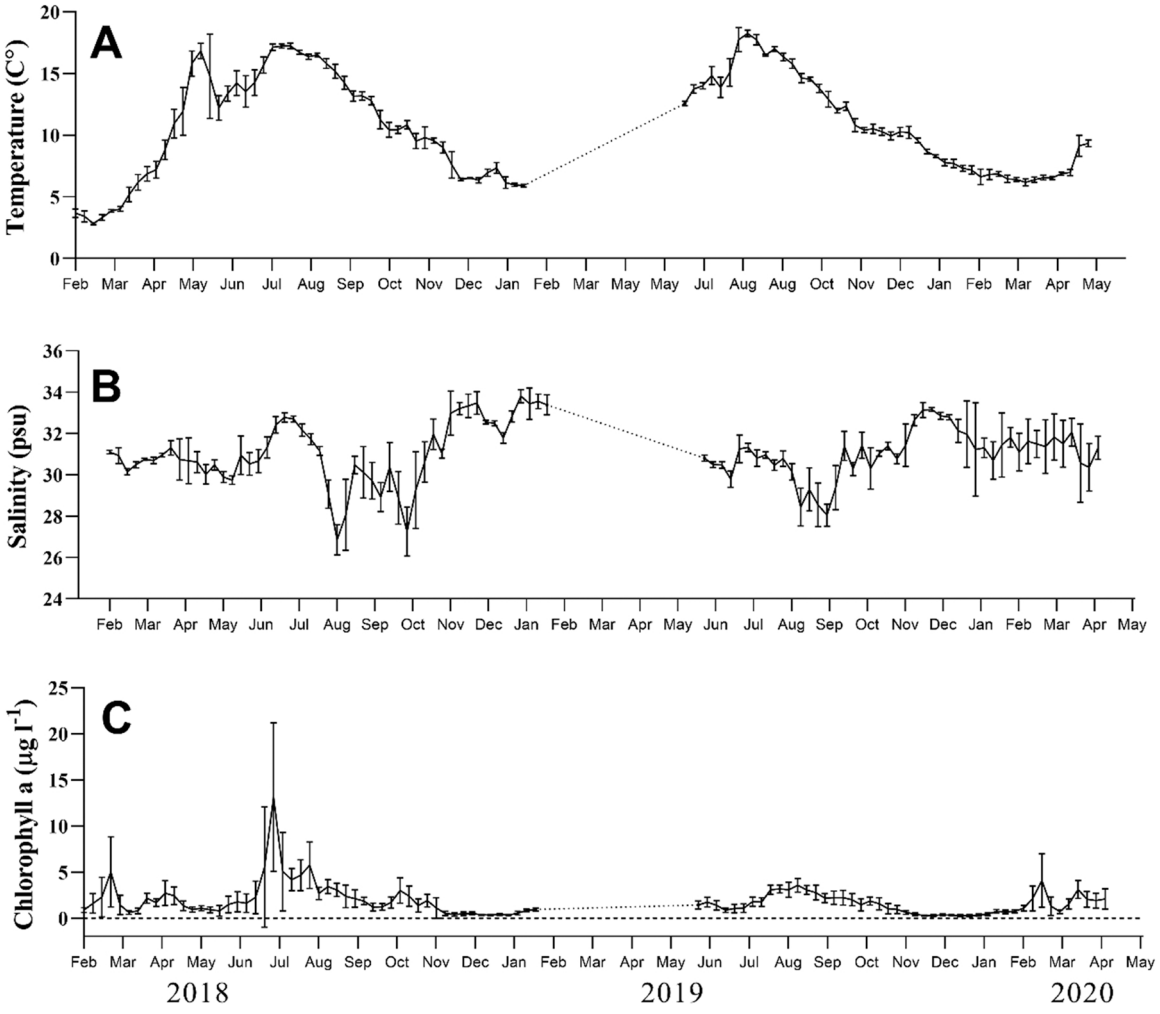

Fig. 6. Seasonal data for temperature (a), fluorescence (chlorophyll a) (b) and salinity (c) measured in the seawater at the collection site of the blue mussels, in the period of February 2018 to January 2019, and from May 2019 to May 2020. Dotted line represents data not retrieved.

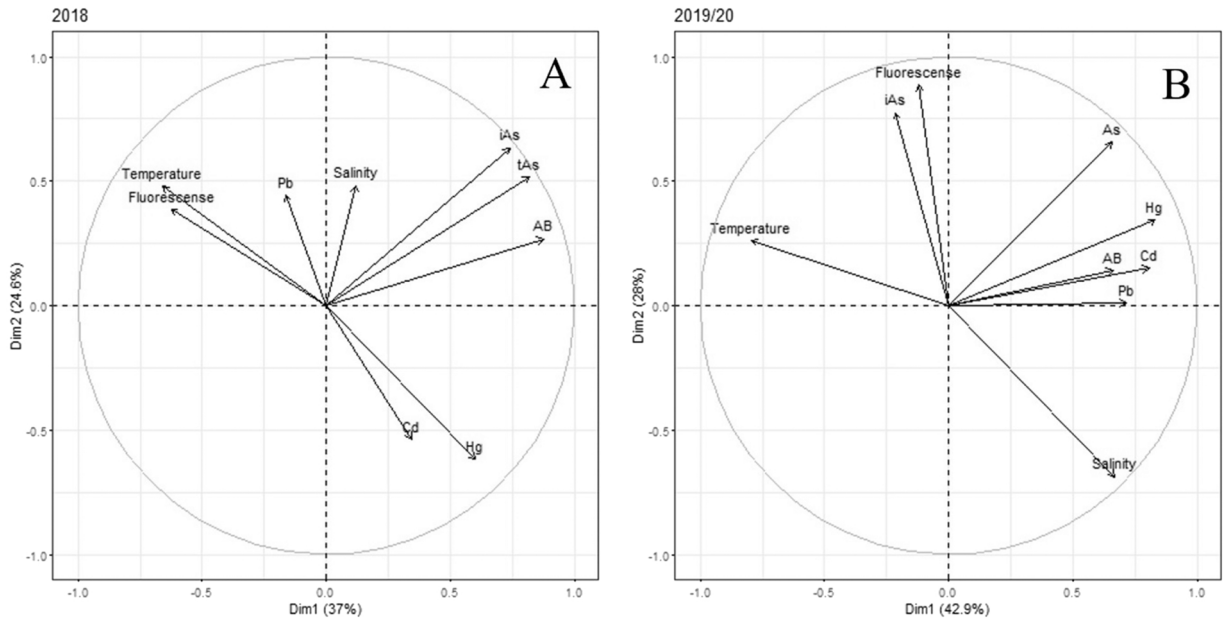

Fig. 7. Principal component analyses (PCA) of temperature, salinity and fluorescence (chlorophyll a), and the concentrations of cadmium (Cd), mercury (Hg), lead (Pb), total arsenic (tAs), inorganic arsenic (iAs) and arsenobetaine (AB) in blue mussels (*Mytilus edulis* L.) collected in 2018 (a) and 2019/20 (b). The variation explained by the two firsts components was 57% for 2018 and 69% for 2019/20.

3.6. Evaluating the PTEs levels in terms of food and feed safety

The mean and maximum levels of Cd, Hg, Pb, As and iAs in the blue mussels analysed are summarized in Table 1 and are compared with the MLs established for food and feed materials in the EU, for an evaluation of the PTEs levels in terms of food and feed safety.

Table 1
Concentration of arsenic (As), inorganic arsenic (iAs), cadmium (Cd), mercury (Hg) and lead (Pb) in blue mussels (mg kg^{-1} ww, mean ± SD and min-max, n = 34), compared to the maximum levels (MLs) in foodstuffs (bivalve molluscs, EC 1881/2006 and amendments) and in feed material (EC 2002/32 and amendments). Range in estimated daily intakes (EDI, μg kg^{-1} bw) and margin of exposure (MOE, mg kg^{-1} bw day^{-1}) are given.

Food safety[a]	**As**	**iAs**	**Cd**	**Hg**	**Pb**
Mean ± SD (n = 34)	3.1 ± 2.5	0.5 ± 1.6	0.09 ± 0.02	0.013 ± 0.005	0.09 ± 0.03
Min – Max (mg kg^{-1} ww)	1.7 – 15.2	0.01 – 8.0	0.06 – 0.14	0.005 – 0.027	0.03 – 0.18
MLs			1	0.5	1.5
EDI (Min – Max) (μg kg^{-1} bw day^{-1})	1. – 1	0.0005 – 0.4	0.02 – 0.04	0.001 – 0.01	0.001 – 0.01
MOE[b] (Min – Max)		817 – $6.5e^5$ $2.2e^4$ – $1.7e^7$[c]			$7.5e^4$ – $5.4e^5$ $1.8e^5$ – $1.3e^6$[d]
Feed safety[e]					
Mean ± SD (n = 34)	18 ± 15	2.7 ± 9.3	0.5 ± 0.1	0.08 ± 0.03	0.5 ± 0.2
Min – Max	10.2 – 89.0	0.1 – 10.5	0.4 – 0.8	0.06 – 0.16	0.2 – 1.1
MLs	25	2[f]	2	0.5	10

[a] Wet weight concentrations were calculated using an 85% moisture content.
[b] MOE value of 10 000 or higher indicate low safety concern of public health.
[c] For iAs, the lower and higher $BMDL_{0.1}$ limit is 0.3 and 8 μg kg^{-1} bw day^{-1}, respectively.
[d] For Pb, the lower and higher $BMDL_{0.1}$ limit is from 0.50 to 1.5 μg kg^{-1} bw day^{-1}, respectively.
[e] Dry weight transformed to 12% moisture content, according to EU directive 2002/32/EC.
[f] Upon request, the responsible operator must demonstrate that inorganic As is lower than 2 mg kg^{-1} (EU directive 2002/32/EC).

4. Discussion

Potentially toxic elements (PTEs) naturally occur in seawater, and their concentrations in mussels are dependent on different factors. This study aimed to assess the seasonal variations in PTEs in mussels for periods between 2018 and 2020, as well as to describe the potential correlation with environmental and biological variables, with the overall aim to evaluate the risk associated with blue mussels in terms of food and feed safety.

4.1. PTEs concentrations

The concentrations of Cd, Hg and Pb in blue mussels collected from the West-coast of Norway were in the same, or lower concentration range compared to previous studies of Norwegian blue mussel [10,48]. The levels of Pb in the mussels were generally lower than the levels seen in mussels collected in nearby areas, including the areas surrounding Bergen, Norway [49,50]. This could be explained by lower inputs of Pb from urban activities, i.e. road runoff, as the samples were taken at a rural location. The levels of Cd and Hg in the present study were relatively low, within the range seen in the National monitoring program on mussels in Norway [48], and lower than those reported in mussels from the genus *Mytilus* in Spain [51], Poland [52] and Algeria [34]. The present study found seasonal variations in levels of Pb, Hg and Cd in mussels, with somewhat higher levels in February to May, although not consistent for both years. It is known that the basal levels of trace elements in tissues of marine organisms can be affected by marked seasonal fluctuations [11,53]. In most temporal studies of mussels, the concentrations of Cd, Hg, and Pb can vary, being higher in the spring and summer months as a result of the combination of biotic and abiotic factors [29,54].

For As, most samples had concentrations below 3 mg kg^{-1} ww, which is in accordance with levels seen in the National monitoring program in Norway, where levels between 1.4 and 4.5 mg kg^{-1} ww are normally seen [55,56]. However, a notably high concentration of As of 15.2 mg kg^{-1} ww was detected in one sample (November). Also, the iAs concentrations was notably higher for this specific sample, with a

concentration of 8.0 mg kg^{-1} ww. To our knowledge, these concentrations of total As and iAs are among the highest concentrations measured in mussels in Norway. This is not the first time that high levels of As have been detected in Norwegian mussels. Sloth & Julshamn [24] reported high concentrations of total As and iAs (13.8 and 5.8 mg kg^{-1} ww, respectively) in mussels from the Sognefjord. In Mediterranean mussel (*Mytilus galloprovincialis*), similarly, 16.5 mg kg^{-1} ww was found in samples from an area close to an oil refinery site in the North Adriatic Sea [57]. The fluctuations in levels of total As and iAs in M.*edulis* from the Norwegian fjord were not linked to pollution but suggested to be caused by responses to natural phenomena, such as phytoplanktonic bloom, or weathering of minerals [24].

4.2. Inorganic and organic As species

In the marine environment, iAs in seawater is taken up by phytoplankton or other microorganisms, which then are consumed by other marine organisms, and iAs is biotransformed to organic As species [58]. The highest concentrations of iAs in mussels were seen in mussels with the highest concentrations of total As, collected in November and December 2018. It has been suggested that there is a threshold in the biotransformation of As, i.e. when reaching a certain concentration of iAs, the mussels would not have the capacity to transform it to organic forms, causing iAs to accumulate [24]. This has also been observed in a study where Zhang and colleagues [59] exposed marine clams (*Asaphis violacens)* to different concentrations of iAs (1, 3, 10 and 20 mg L^{-1}). The clams were able to bio-transform iAs efficiently when exposed to low concentrations but not that efficiently when exposed to higher concentrations (over 1 mg L^{-1}). However, for some sample points it was seen in this study that the highest concentrations of total As did not necessarily mean highest percentage of iAs. Also, the sample with the highest percentage of iAs (77%) did not correlate with the highest concentration of total As. These results show that the level of total As cannot be used to extrapolate the levels of iAs.

Arsenobetaine was the major organic As species in the mussels, and within the range of concentrations seen in other studies of the genus *Mytilus* (40–60% of the total As) [41,60,61]. For samples from 2019/20 concentrations of AB were relatively consistent. Stable concentration of AB in mussels sampled in areas with different levels of total As has been linked to the osmotic capacity of this compound due to its similarity with the organic osmolyte glycine betaine [62]. Other organic As species, i.e. DMA, MA and AsSugars, as well as minor levels of TMAO, TMAP, TETRA, AC were detected in the mussels. These results are in agreement with previous studies, where several As species where detected in a sample of blue mussel [41]. Besides AB, AsSugars were the organic As species present in higher concentrations in the mussels in the current study. The concentrations of AsSugars fluctuated, and slightly different patterns were seen for the different AsSugars species. A possible explanation for these variations could be the type of diet, i.e. that different planktonic organisms could have different uptake and transformation efficiencies of As [63]. Different algae blooms could further reflect the accumulated As species and concentrations in the blue mussels. Whereas AB is considered non-toxic for humans and animals, the simple methylated As species (i.e. DMA) have been characterized as toxic species and possibly carcinogenic [16], and AsSugars [64] have been characterized as species of potential toxicity [65]. This highlights the importance of speciation studies in risk assessments of As.

4.3. Factors affecting PTEs and As species concentrations in blue mussels

The generally higher concentrations of Cd, Hg and Pb for the 2019/20 samples compared to samples collected in 2018, can be linked to the size of the blue mussels, since mussels from 2019/20 were significantly larger than in those of 2018. This is in agreement with previous studies where the accumulation of Cd and Pb were positively correlated with the size in blue mussels *(M. edulis)* [66]. Higher concentrations of PTEs were measured in the early months of spring (March - June) for both periods studied, and these levels coincided with lower levels in CI, as seen in previous studies [53]. This correlation was clearer for samples collected in 2019, where the lowest CIs in mussels (May 2019) coincided with highest metal concentrations, especially for Cd and Pb. For both periods studied, the largest variations in CI were observed in the spring months, when increased temperature and fluorescence were also observed. Although CI can be affected by availability and type of food, as well as the temperature or salinity of the water [37], it is recognized that the reproductive cycle is one of the most decisive variables [67]. Decreased CI in species of the genus *Mytilus* have been related to biomass loss, and lower CIs have been associated with spawning events [32,54,68]. The highest concentrations of PTEs in mussels are usually found in the gills and digestive gland [69] and are generally low in gonads, assuming a low loss of PTEs due to spawning and loss of gametes [70]. In the West-Norwegian coast, most mussels undergo the pre-spawning stage during early spring, and start spawning during spring or summer months [71]. Lower CI levels in winter months, especially for 2019, may be related to a decreased metabolic rate, as a response to lower food intake due to low temperature and food availability [69,72]. The results from the present study indicate that higher concentrations of PTEs in the mussels could be linked to spawning periods, as the lower CIs during spawning due to release of gametes and thereby loss of biomass, causing an up-concentration of the PTEs. However, this association was less clear for As, seeing a low correlation with CI for both of the periods studied. and where the high levels in September 2019 did not coincide with lower CI values. Also, high levels of As were detected for some of the months, but not observed for the other elements.

A multitude of studies have linked environmental variables, such as pH, salinity or temperature with PTEs uptake [30,73]. For both years, temperature seemed to be influencing the concentrations of Hg and Cd. For Pb, an opposite correlation to temperature was, however, seen for 2018, and not showing a consistent relationship for the two periods studied. Salinity has previously been seen to influence the heavy metal uptake and the species of metals [31,74], but no clear effect of salinity on the concentration of PTEs was seen in the present study. The location of this study was a coastal position, and therefore, the salinity in seawater is less affected compared to in the fjords, where e.g. river runoff would have a larger influence during the seasons., explaining why the effect of salinity was not clear. Similarly, no consistent seasonality was seen for metal and metalloids in mussels collected multiple times per year over several years in a mussel monitoring program in Germany [75]. It was further emphasized that finding representative sampling times that would best reflect the annual mean concentration is challenging.

Arsenic and As species showed different concentration patterns compared to the other PTEs, with high levels found in mussels for some months. For As and iAs, salinity was positive correlated with the element and its species in mussels for the 2018 period, whereas a negative correlation was seen for 2019 data. Accumulation of AB, and thereby also total As, have previously been shown to decrease with lower salinity [76]. This effect was ascribed to the chemical structure of AB, which is similar to glycine betaine, and may therefore have similar osmotic properties [76]. In addition, the conversion from arsenate (As(V)) to arsenite (As(III)) and further methylation into organic As species decreases with increased salinity in microalgae, leading to a lower conversion of iAs and, thus higher exposure to iAs for mussels [77].

The unusually high levels of As and iAs detected in mussels (November-December 2018), could be related to local contamination of the seawater, but there is no data to supporting this. A previous study showed no accumulation of iAs in mussels when exposed to arsenate in the seawater, even at an exposure concentrations of 100 μg As L^{-1} [78]. On a global scale, As concentration in seawater are typically 1–2 μg L^{-1} [79,80]. This indicate that at even high As levels in seawater, the uptake of As in blue mussels is limited, and that As in the mussels in the present study would likely be from other sources. The highest As concentrations

in mussels detected in November and December 2018, could be related to phytoplankton blooms, indicated by elevated fluorescence measurements at the end of October 2018. The high As concentrations in September 2019 could also be correlated with increased fluorescence of the water column in August 2019. Furthermore, increased concentrations of AsSugars in mussels for September 2019 could be a result of phytoplankton consumption by the mussels, since AsSugars are related to algae and primary producers [81]. This theory is also supported by the increased level of AsSugars and iAs in mussels from June-July and November-December 2018, were also higher levels of fluorescence signals are seen. However, the high As concentration in mussels from March 2020 do not correlate with higher levels in iAs, hence, not giving a consistent explanation for As accumulation in blue mussels. A possible explanation can be that different planktonic species could have different uptake and transformation efficiencies of As, which is further reflected in the As species and concentrations in the blue mussels.

4.4. Food and feed safety

The levels of Cd, Hg and Pb were all below the MLs established for bivalve molluscs in the EU (EC 1881/2006 and amendments) (Table 1). No EU ML has been established for total As in food or seafood, and for iAs MLs have only been establish for rice-based foodstuffs (Regulation (EU) 2015/1006) [82]. EFSA has established TWIs for Cd [83] and methylmercury (MeHg) [84]. The mussels from this study would contribute to less than 2% of the TWI for Cd and less than 1% of the TWI for MeHg, based on consumption figures for mussels by the European adult population. The previous TWIs for Pb and As were no longer considered appropriate [20,27], but instead BMDLs and MOEs [20,27] were used to assess the risk associated with the consumption of mussels in adults with regards to these elements. The estimated daily intakes (EDI) of iAs, based on data from this study, were below the $BMDL_{01}$ range set by EFSA (from 0.3 to 8 μg As kg^{-1} bw day^{-1}), with the exception of mussels collected in November 2018, which would lead to exposure to 0.4 μg of iAs kg^{-1} bw day^{-1}. The EDIs for Pb were all below the BMDL values derived by EFSA ($BMDL_{01}$ 0.5 μg kg^{-1} bw day^{-1} (Table 1). The MOE for Pb were above 10000 for all samples, however for the MOE for iAs for three samples were below 10000, indicating that a possibility of risk to some consumers cannot be excluded. Overall, Hg, Cd, Pb and As levels in most of the blue mussels in the present study are not considered a risk for consumers. However, the results show the potential of blue mussel to contain high levels and proportion of iAs, which is of potential concern for consumers.

In terms of feed safety assessment, the mean concentrations of PTEs were all below the MLs established for feed materials in EU Directive 2002/32/EC and amendments [25] (Table 1). The exception was for As, where some mussels (i.e. January, November and December 2018) contained levels which exceeded the ML for total As in feed materials, which is set at 25 mg kg^{-1} for feed materials of fish or other aquatic animals. Moreover, the concentrations of iAs exceeded the ML specified in the footnote of the feed legislation, of 2 mg kg^{-1}, for some of the months. It is, however, recognized that the production processes for feed ingredients could affect the concentrations of PTEs and As species [85,86]. Results from this study shows that the levels of As and iAs can be of concern in terms of using blue mussels as a feed ingredient, and further highlight the importance of monitoring this element and its species.

5. Conclusions

Variations in the levels of Pb, Hg, Cd, As and iAs were seen for the blue mussels collected monthly throughout the two periods studied, and particularly large variations were seen in levels of As and iAs. The concentrations of Pb and Cd in blue mussels were influenced by the CI but this was less evident for As. Data suggest that increased fluorescence signal in seawater, related to chlorophyll a abundance, is associated with increased concentrations of As and iAs in mussels. Several organic As species were detected in the samples, where the non-toxic AB was generally the most abundant As species. In terms of food and feed safety, the levels were below the MLs established in the EU for Cd, Hg and Pb in food and feed materials. However, for As and iAs, high levels were detected for some of the sampling times, indicating that this element can be of concern and must be monitored. High levels of iAs emphasize the importance of speciation studies, for a better assessment of As in seafood.

Funding

This work was part of the project “Metals, arsenic and arsenic species in novel marine feed resources” (Project no. 15333), which was funded by the Ministry of Trade, Industry and Fisheries (NFD), Norway.

CRediT authorship contribution statement

Ana-Isabel Gomez Delgaodo: Formal analysis, Investigation, Validation, Writing – original Draft. **Jojo Tibon:** Methodology, Validation, Investigation, Writing – review & editing. **Marta Silva:** Supervision, Writing – review & editing. **Anne-Katrine Lundebye:** Conceptualization, Supervision, Writing – review & editing. **Antonio Aguera Garcia:** Formal analysis, Writing – review & editing. **Josef Daniel Rasinger:** Formal analysis, Writing – review & editing. **Tore Strohmeier:** Resources, Formal analysis, Writing – review & editing. **Veronika Sele:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Acknowledgments

We would like to thank Cathinka Krognæss at the Institute of Marine Research`s research station at Austevoll for the sampling and shipment of samples, and to thank Snorri Gunnarsson at the inorganic chemistry laboratory, IMR Bergen, for the assistance in analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtemb.2022.127110.

References

[1] I. Aakre, S. Naess, M. Kjellevold, M.W. Markhus, et al., New data on nutrient composition in large selection of commercially available seafood products and its impact on micronutrient intake, Food Nutr. Res. 63 (2019), https://doi.org/10.29219/fnr.v63.3573.
[2] K. Tan, H. Ma, S. Li, H. Zheng, Bivalves as future source of sustainable natural omega-3 polyunsaturated fatty acids, Food Chem. 311 (2020), 125907, https://doi.org/10.1016/j.foodchem.2019.125907.
[3] G. Zhelyazkov, T. Yankovska-Stefanova, E. Mineva, D. Stratev, et al., Risk assessment of some heavy metals in mussels (Mytilus galloprovincialis) and veined rapa whelks (Rapana venosa) for human health, Mar. Pollut. Bull. 128 (2018) 197–201, https://doi.org/10.1016/j.marpolbul.2018.01.024.
[4] O. Pourret, A. Hursthouse, It’s Time to Replace the Term "Heavy Metals" with "Potentially Toxic Elements" When Reporting Environmental Research, Int. J. Environ. Res. Public Health 16 (22) (2019) 4446, https://doi.org/10.3390/ijerph16224446.
[5] T. Ansari, I. Marr, N. Tariq, Heavy metals in marine pollution perspective-a mini review, J. Appl. Sci. 4 (1) (2004) 1–20, https://doi.org/10.3923/jas.2004.1.20.
[6] H. Ali, E. Khan, Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health, Hum. Ecol. Risk Assess.: Int. J. 25 (6) (2019) 1353–1376, https://doi.org/10.1080/10807039.2018.1469398.
[7] J. Barbosa, T. Cabral, D. Ferreira, L. Agnez-Lima, et al., Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals, Ecotoxicol. Environ. Saf. 73 (3) (2010) 320–325, https://doi.org/10.1016/j.ecoenv.2009.10.008.

[8] M.S. Sankhla, K. Sharma, R. Kumar, *Heavy metal causing neurotoxicity in human health.* International Journal of Innovative Research in Science, Eng. Technol. 6 (5) (2017), https://doi.org/10.15680/IJIRSET.2017.0605054.

[9] J.M. Neff, in: J.M. Neff (Ed.), Bioaccumulation in Marine Organisms. Effect of Contaminants from oil well produced water, Elsevier, Oxford, 2002.

[10] K. Julshamn, E.K. Torpe, C. Børnes, L.J. Sæthre, et al., Cadmium, lead, copper and zinc in blue mussels (Mytilus edulis) sampled in the Hardangerfjord, Norway, J. Environ. Monit. 3 (5) (2001) 539–542, https://doi.org/10.1039/B103047G.

[11] D.J.H. Phillips, Common mussel edulis as a an indication of pollution by zinc, cadmium and copper. 1. Effects of environmental variables on uptake of metals, Mar. Biol. 38 (1) (1976) 59–69, https://doi.org/10.1007/bf00391486.

[12] R.A. Lavoie, T.D. Jardine, M.M. Chumchal, K.A. Kidd, et al., Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis, Environ. Sci. Technol. 47 (23) (2013) 13385–13394, https://doi.org/10.1021/es403103t.

[13] B. Henriques, L.S. Rocha, C.B. Lopes, P. Figueira, et al., Study on bioaccumulation and biosorption of mercury by living marine macroalgae: prospecting for a new remediation biotechnology applied to saline waters, Chem. Eng. J. 281 (2015) 759–770, https://doi.org/10.1016/j.cej.2015.07.013.

[14] EFSA, The European Food Safety Authority. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. Panel on Contaminants in the Food Chain (CONTAM), EFSA J. 10 (12) (2012) 2985, https://doi.org/10.2903/j.efsa.2012.2985.

[15] J.S. Edmonds, K.A. Francesconi, Arsenic in seafoods: Human health aspects and regulations, Mar. Pollut. Bull. 26 (12) (1993) 665–674, https://doi.org/10.1016/0025-326X(93)90549-Y.

[16] C. Luvonga, C.A. Rimmer, L.L. Yu, S.B. Lee, Organoarsenicals in seafood: occurrence, dietary exposure, toxicity, and risk assessment considerations - a review, J. Agric. Food Chem. 68 (4) (2020) 943–960, https://doi.org/10.1021/acs.jafc.9b07532.

[17] EFSA, European Food Safety Authority. Dietary exposure to inorganic arsenic in the European population, EFSA J. 12 (3) (2014) 3597, https://doi.org/10.2903/j.efsa.2014.3597.

[18] EFSA, Eur. Food Saf. Auth. Sci. Rep. Chronic Diet. Expo. Inorg. Arsen. 19 (1) (2021), e06380, https://doi.org/10.2903/j.efsa.2021.6380.

[19] L.S. Kato, R.G. Ferrari, J.V.M. Leite, C.A. Conte-Junior, Arsenic in shellfish: A systematic review of its dynamics and potential health risks, Mar. Pollut. Bull. 161 (2020), 111693, https://doi.org/10.1016/j.marpolbul.2020.111693.

[20] EFSA, The European Food Safety Authority. Scientific Opinion on Arsenic in Food. Panel on Contaminants in the Food Chain (CONTAM), EFSA J. 7 (2009) 1351, https://doi.org/10.2903/j.efsa.2009.1351.

[21] J.J. Sloth, K. Julshamn, A.K. Lundebye, Total arsenic and inorganic arsenic content in Norwegian fish feed products, Aquac. Nutr. 11 (1) (2005) 61–66, https://doi.org/10.1111/j.1365-2095.2004.00334.x.

[22] G.-y Park, D.-e Kang, M. Davaatseren, C. Shin, et al., Reduction of total, organic, and inorganic arsenic content in Hizikia fusiforme (Hijiki), Food Sci. Biotechnol. 28 (2) (2018) 615–622, https://doi.org/10.1007/s10068-018-0501-3.

[23] J.M. Ronan, D.B. Stengel, A. Raab, J. Feldmann, et al., High proportions of inorganic arsenic in Laminaria digitata but not in Ascophyllum nodosum samples from Ireland, Chemosphere 186 (2017) 17–23, https://doi.org/10.1016/j.chemosphere.2017.07.076.

[24] J.J. Sloth, K. Julshamn, Survey of Total and Inorganic Arsenic Content in Blue Mussels (Mytilus edulis L.) from Norwegian Fiords: Revelation of Unusual High Levels of Inorganic Arsenic, J. Agric. Food Chem. 56 (4) (2008) 1269–1273, https://doi.org/10.1021/jf073174+.

[25] EU, Commission Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. 2002, Official Journal of the European Union. European Commission: Brussel, Belgium.

[26] EU, Commission *Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs* . 2006, Official Journal of the European Union. European Commission.

[27] EFSA, Scientific Opinion on Lead in Food. Panel on Contaminants in the Food Chain (CONTAM), EFSA J. 8 (4) (2010) 1570, https://doi.org/10.2903/j.efsa.2010.1570.

[28] J. Beyer, N.W. Green, S. Brooks, I.J. Allan, et al., Blue mussels (Mytilus edulis spp.) as sentinel organisms in coastal pollution monitoring: A review, Mar. Environ. Res. 130 (2017) 338–365, https://doi.org/10.1016/j.marenvres.2017.07.024.

[29] D. Fattorini, A. Notti, R. Di Mento, A.M. Cicero, et al., Seasonal, spatial and inter-annual variations of trace metals in mussels from the Adriatic sea: A regional gradient for arsenic and implications for monitoring the impact of off-shore activities, Chemosphere 72 (10) (2008) 1524–1533, https://doi.org/10.1016/j.chemosphere.2008.04.071.

[30] M. Belivermis, M. Besson, P. Swarzenski, F. Oberhaensli, et al., Influence of pH on Pb accumulation in the blue mussel, Mytilus edulis, Mar. Pollut. Bull. 156 (2020), https://doi.org/10.1016/j.marpolbul.2020.111203.

[31] V. Kumar, A.K. Sinha, P.P. Rodrigues, V.K. Mubiana, et al., Linking environmental heavy metal concentrations and salinity gradients with metal accumulation and their effects: A case study in 3 mussel species of Vitoria estuary and Espirito Santo bay, Southeast Brazil, Sci. Total Environ. 523 (2015) 1–15, https://doi.org/10.1016/j.scitotenv.2015.03.139.

[32] S. Casas, J.L. Gonzalez, B. Andral, D. Cossa, Relation between metal concentration in water and metal content of marine mussels (Mytilus galloprovincialis): impact of physiology, Environ. Toxicol. Chem.: Int. J. 27 (7) (2008) 1543–1552, https://doi.org/10.1897/07-418.1.

[33] W.-X. Wang, N.S. Fisher, Assimilation of trace elements and carbon by the mussel Mytilus edulis: Effects of food composition, Limnol. Oceanogr. 41 (2) (1996) 197–207, https://doi.org/10.4319/lo.1996.41.2.0197.

[34] O. Rouane-Hacene, Z. Boutiba, B. Belhaouari, M.E. Guibbolini-Sabatier, et al., 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 (4) (2015) 362–374.

[35] J. Richir, S. Gobert, 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 (2014) 33–47, https://doi.org/10.1016/j.ecolind.2013.06.021.

[36] V.K. Mubiana, K. Vercauteren, R. Blust, The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in Mytilus edulis, Environ. Pollut. 144 (1) (2006) 272–279, https://doi.org/10.1016/j.envpol.2005.12.017.

[37] E. Orban, G. Di Lena, T. Nevigato, I. Casini, et al., Seasonal changes in meat content, condition index and chemical composition of mussels (Mytilus galloprovincialis) cultured in two different Italian sites, Food Chem. 77 (1) (2002) 57–65, https://doi.org/10.1016/S0308-8146(01)00322-3.

[38] K. Julshamn, A. Maage, H.S. Norli, K.H. Grobecker, et al., Determination of Arsenic, Cadmium, Mercury, and Lead in Foods by Pressure Digestion and Inductively Coupled Plasma/Mass Spectrometry: First Action 2013.06, J. AOAC Int. 96 (5) (2013) 1101–1102, https://doi.org/10.5740/jaoacint.13-143.

[39] K. Julshamn, B.M. Nilsen, S. Frantzen, S. Valdersnes, et al., Total and inorganic arsenic in fish samples from Norwegian waters, Food Addit. Contam. Part B-Surveill. 5 (4) (2012) 229–235, https://doi.org/10.1080/19393210.2012.698312.

[40] *CEN, E.C.f.S., EN 16802:2016. Foodstuffs. Foodstuffs. Determination of elements and their chemical species. Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS following water bath extraction,* 2016.

[41] J. Tibon, M. Silva, J.J. Sloth, H. Amlund, et al., Speciation analysis of organoarsenic species in marine samples: method optimization using fractional factorial design and method validation, Anal. Bioanal. Chem. (2021), https://doi.org/10.1007/s00216-021-03341-4.

[42] P. Walne, R. Mann, Growth and biochemical composition in Ostrea edulis and Crassostrea gigas. Ninth European Marine Biology Symposium, Aberdeen University Press Scotland,, 1975.

[43] USEPA, *U.S. Environmental Protection Agency. Risk-based concentration table*. 2000, United States Environmental Protection Agency Philadelphia, PA.

[44] EU, The EU fish market: 2016 edition. Directorate - General for Maritime Affairs and Fisheries, European Commission,, Brussel, Belgium, 2017.

[45] RCore Team, R Core Team. R: A language and environment for statistical computing, R Foundation for Statistical Computing,, *Vienna, Austria*, 2020.

[46] RStudio Team, *RStudio: Integrated Development Environment for R*, 2019.

[47] Y. David LaTouche, M.C. Mix, The effects of depuration, size and sex on trace metal levels in bay mussels, Mar. Pollut. Bull. 13 (1) (1982) 27–29, https://doi.org/10.1016/0025-326X(82)90494-5.

[48] J. Duinker, B.T. Storesund, Lunestad, M. Sanden, *National surveillance programme for the production of shells and other molluscs (in Norwegian)*. Rapport fra Havforskningen, Havforskningsinstituttet/Mattilsynet,, 2020, p. 24.

[49] S. Airas, A. Duinker, K. Julshamn, Copper, Zinc, Arsenic, Cadmium, Mercury, and Lead in Blue Mussels (Mytilus edulis) in the Bergen Harbor Area, Western Norway, Bull. Environ. Contam. Toxicol. 73 (2) (2004) 276–284, https://doi.org/10.1007/s00128-004-0424-y.

[50] V. Andersen, A. Maage, P. Johannessen, Heavy metals in blue mussels (Mytilus edulis) in the Bergen Harbor area, western Norway, Bull. Environ. Contam. Toxicol. 57 (4) (1996) 589–596, https://doi.org/10.1007/s001289900231.

[51] V. Besada, J. Manuel Andrade, F. Schultze, J. José González, Monitoring of heavy metals in wild mussels (Mytilus galloprovincialis) from the Spanish North-Atlantic coast. Cont. Shelf Res. 31 (5) (2011) 457–465, https://doi.org/10.1016/j.csr.2010.04.011.

[52] M. Protasowicki, M. Dural, J. Jaremek, Trace metals in the shells of blue mussels (Mytilus edulis) from the Poland coast of Baltic sea, Environ. Monit. Assess. 141 (1) (2007) 329, https://doi.org/10.1007/s10661-007-9899-4.

[53] P.B. Lobel, C.D. Bajdik, S.P. Belkhode, S.E. Jackson, et al., Improved protocol for collecting mussel watch specimens taking into account sex, size, condition, shell shape and chronological age, Arch. Environ. Contam. Toxicol. 21 (3) (1991) 409–414, https://doi.org/10.1007/bf01060364.

[54] C. Lacroix, E. Duvieilbourg, N. Guillou, J. Guyomarch, et al., Seasonal monitoring of blue mussel (Mytilus spp.) populations in a harbor area: A focus on responses to environmental factors and chronic contamination, Mar. Environ. Res. 129 (2017) 24–35, https://doi.org/10.1016/j.marenvres.2017.04.008.

[55] Duinker, A., J. Storesund, B.T. Lunestad, and M. Sanden, *Nasjonalt tilsynsprogram for produksjon av skjell og andre bløtdyr. Prøver analysert i 2018 for kjemiske forurensende stoffer og mikroorganismer*, in *Rapport fra havforskningen*. 2019, Havforskningsinstituttet: Havforskningsinstituttet.

[56] A. Duinker, J. Storesund, B.T. Lunestad, M. Sanden, National supervision programme for the production of shells and other molluscs– samples analyzed in 2019 for chemical pollutants and microorganisms. Rapport fra Havforskningen, Havforskningsinstituttet/Mattilsynet,, 2020, p. 24.

[57] S. Klaric, D. Pavicic-Hamer, C. Lucu, Seasonal variations of arsenic in mussels Mytilus galloprovincialis, Helgol. Mar. Res. 58 (3) (2004) 216–220, https://doi.org/10.1007/s10152-004-0188-0.

[58] F. Ardini, G. Dan, M. Grotti, Arsenic speciation analysis of environmental samples, J. Anal. At. Spectrom. 35 (2) (2020) 215–237, https://doi.org/10.1039/C9JA00333A.

[59] W. Zhang, Z.Q. Guo, Y. Wu, Y.L. Qiao, et al., Arsenic Bioaccumulation and Biotransformation in Clams (Asaphis violascens) Exposed to Inorganic Arsenic: Effects of Species and Concentrations, Bull. Environ. Contam. Toxicol. 103 (1) (2019) 114–119, https://doi.org/10.1007/s00128-018-2493-3.

[60] G. Esposito, A.G. Mudadu, M.C. Abete, S. Pederiva, et al., Seasonal accumulation of trace elements in native Mediterranean mussels (Mytilus galloprovincialis) collected in the Calich Lagoon (Sardinia, Italy), Environ. Sci. Pollut. Res. 28 (20) (2021) 25770–25781, https://doi.org/10.1007/s11356-021-12380-4.
[61] T. Kanduc, Z. Slejkovec, I. Falnoga, N. Mori, et al., Environmental status of the NE Adriatic Sea, Istria, Croatia: Insights from mussel Mytilus galloprovincialis condition indices, stable isotopes and metal (loid), Mar. Pollut. Bull. 126 (2018) 525–534, https://doi.org/10.1016/j.marpolbul.2017.09.052.
[62] K.J. Whaley-Martin, I. Koch, M. Moriarty, K.J. Reimer, Arsenic Speciation in Blue Mussels (Mytilus edulis) Along a Highly Contaminated Arsenic Gradient, Environ. Sci. Technol. 46 (6) (2012) 3110–3118, https://doi.org/10.1021/es203812u.
[63] E.G. Duncan, W.A. Maher, S.D. Foster, F. Krikowa, The influence of arsenate and phosphate exposure on arsenic uptake, metabolism and species formation in the marine phytoplankton Dunaliella tertiolecta, Mar. Chem. 157 (2013) 78–85, https://doi.org/10.1016/j.marchem.2013.08.004.
[64] F. Ebert, S. Meyer, L. Leffers, G. Raber, et al., Toxicological characterisation of a thio-arsenosugar-glycerol in human cells, J. Trace Elem. Med. Biol. 38 (2016) 150–156, https://doi.org/10.1016/j.jtemb.2016.04.013.
[65] J. Feldmann, E.M. Krupp, Critical review or scientific opinion paper: Arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? Anal. Bioanal. Chem. 399 (5) (2011) 1735–1741, https://doi.org/10.1007/s00216-010-4303-6.
[66] F. Riget, P. Johansen, G. Asmund, Influence of length on element concentrations in blue mussels (Mytilus edulis), Mar. Pollut. Bull. 32 (10) (1996) 745–751, https://doi.org/10.1016/0025-326x(96)00067-7.
[67] R. Seed, T.H. Suchanek, Population and community ecology of Mytilus, in: E. Gosling (Ed.), The Mussel Mytilus: Ecology, Physiology, Genetics and Culture, Elsevier, London, 1992, pp. 87–170.
[68] O. Rouane-Hacene, Z. Boutiba, B. Bethaouari, M.E. Guibbotini-Sabatier, et al., 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 (4) (2015) 362–374, https://doi.org/10.1016/j.oceano.2015.07.004.
[69] E. Giarratano, O.A. Amin, Heavy metals monitoring in the southernmost mussel farm of the world (Beagle Channel, Argentina), Ecotoxicol. Environ. Saf. 73 (6) (2010) 1378–1384, https://doi.org/10.1016/j.ecoenv.2010.06.023.
[70] D.C. Firth, K. Salie, B. O'Neill, L.C. Hoffman, Monitoring of trace metal accumulation in two South African farmed mussel species, Mytilus galloprovincialis and Choromytilus meridionalis, Mar. Pollut. Bull. 141 (2019) 529–534, https://doi.org/10.1016/j.marpolbul.2019.03.007.
[71] A. Duinker, L. Haland, P. Hovgaard, S. Mortensen, Gonad development and spawning in one and two year old mussels (Mytilus edulis) from Western Norway, J. Mar. Biol. Assoc. U. Kingd. 88 (7) (2008) 1465–1473, https://doi.org/10.1017/s0025315408002130.
[72] G.G. Thorarinsdottir, H.G. Gudfinnsson, S. Egilsdottir, J.O. Palsson, The gametogenic cycle and spawning in Mytilus edulis in two fjords in north-western Iceland, J. Mar. Biol. Assoc. U. Kingd. 93 (6) (2013) 1609–1615, https://doi.org/10.1017/s0025315413000040.
[73] H. Fischer, Influence of temperature, salinity, and oxygen on the cadmium balance of mussels Mytilus edulis, Mar. Ecol. Prog. Ser. 32 (2–3) (1986) 265–278, https://doi.org/10.3354/meps032265.
[74] D.J.H. Phillips, Common mussel mussel Mytilus edulis as an indicator of trace-metals in Scandinavian waters. 1. Zinck and Cadmium, Mar. Biol. 43 (4) (1977) 283–291, https://doi.org/10.1007/bf00396922.
[75] B. Knopf, A. Fliedner, G. Radermacher, H. Rudel, et al., Seasonal variability in metal and metalloid burdens of mussels: using data from the German Environmental Specimen Bank to evaluate implications for long-term mussel monitoring programs, Environ. Sci. Eur. 32 (1) (2020), https://doi.org/10.1186/s12302-020-0289-7.
[76] L.A. Clowes, K.A. Francesconi, Uptake and elimination of arsenobetaine by the mussel Mytilus edulis is related to salinity, Comp. Biochem. Physiol. C. -Toxicol. Pharmacol. 137 (1) (2004) 35–42, https://doi.org/10.1016/j.cca.2003.11.003.
[77] R.I. Papry, K. Ishii, M.A. Al Mamun, S. Miah, et al., Arsenic biotransformation potential of six marine diatom species: effect of temperature and salinity, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-46551-8.
[78] J. Gailer, K.A. Francesconi, J.S. Edmonds, K.J. Irgolic, Metabolism of arsenic compounds by the blue mussel Mytilus edulis after accumulation from seawater spiked with arsenic compounds, Appl. Organomet. Chem. 9 (4) (1995) 341–355, https://doi.org/10.1002/aoc.590090408.
[79] M.O. Andreae, Determination of arsenic species in natural waters, Anal. Chem. 49 (6) (1977) 820–823, https://doi.org/10.1021/ac50014a037.
[80] M.O. Andreae, Distribution and speciation of arsenic in natural waters and some marine algae, Deep-Sea Res. 25 (4) (1978) 391–402, https://doi.org/10.1016/0146-6291(78)90565-9.
[81] A.M. Featherstone, E.C.V. Butler, B.V. O'Grady, Seasonal cycling of arsenic species in a stratified, microtidal estuary (Huon River, Tasmania), Estuaries 27 (1) (2004) 18–35, https://doi.org/10.1007/bf02803557.
[82] EU, Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs T.E. Commission, Editor. 2015, Official Journal of the European Union: Brussel, Belgium. p. 3.
[83] EFSA, Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food, EFSA J. 980 (2009) 1–139, https://doi.org/10.2903/j.efsa.2009.980.
[84] EFSA, The European Food Safety Authority. Scientific opinion on the risk for public health realted tot eh presence of mercury and methylmercury in food. Panel on Contaminants in the Food Chain (CONTAM), EFSA J. 10 (12) (2012) 2985, https://doi.org/10.2903/j.efsa.2012.2985.
[85] J. Tibon, H. Amlund, A.I. Gomez-Delgado, M.H.G. Berntssen, et al., Arsenic species in mesopelagic organisms and their fate during aquafeed processing, Chemosphere 302 (2022), https://doi.org/10.1016/j.chemosphere.2022.134906.
[86] M.H.G. Berntssen, L. Thoresen, S. Albrektsen, E. Grimaldo, et al., Processing Mixed Mesopelagic Biomass from the North-East Atlantic into Aquafeed Resources; Implication for Food Safety, Foods 10 (6) (2021) 1265, https://doi.org/10.3390/foods10061265.