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REVIEW

Quantitative determination of heavy metal contaminants in edible soft tissue of clams, mussels, and oysters

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Abstract Aquatic environments are important sources of healthy and nutritious foods; however, clams, mussels, and oysters (the bivalves most consumed by humans) can pose considerable health risks to consumers if contaminated by heavy metals in polluted areas. These organisms can accumulate dangerously high concentrations of heavy metals (e.g., Cd, Hg, Pb) in their soft tissues that can then be transferred to humans following ingestion. Monitoring contaminants in clams, mussels and oysters and their environments is critically important for global human health and food security, which requires reliable measurement of heavy-metal concentrations in the soft tissues. The aim of our present paper is to provide a review of how heavy metals are quantified in clams, mussels, and oysters. We do this by evaluating sample-preparation methods (i.e., tissue digestion / extraction and analyte preconcentration) and instrumental techniques (i.e., atomic, fluorescence and mass spectrometric methods, chromatography, neutron activation analysis and electrochemical sensors) that have been applied for this purpose to date. Application of these methods, their advantages, limitations, challenges and expected future directions are discussed.

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Abbreviations

HM	heavy metal
LOD	limit of detection
LOQ	limit of quantification
SPE	solid-phase extraction
CPE	cloud-point extraction
AAS	atomic absorption spectrometry
AFS	atomic fluorescence spectroscopy
ICP	inductively coupled plasma
MS	mass spectrometry
OES	optical emission spectrometry
EDXRF	energy dispersive X-ray fluorescence spectroscopy
ES	electrochemical sensor
NAA	neutron activation analysis
HPLC	high-performance liquid chromatography
GC	gas chromatography

Introduction

Clams, mussels, and oysters are important foods for human consumption, especially in coastal areas. The estimated production of these marine bivalves on the global market is more than 15 million tonnes per year with slightly more than 10% coming from wild fisheries (Wijsman et al., 2019). Marine bivalves are considered

as healthy food (Noman et al., 2022; Pastorelli et al., 2012; Qin et al., 2021), however, environmental contaminants and biotoxins may create food safety issues and decrease market demands. Food is the primary source of toxic element intake; therefore, food safety and consumer protection are of major concerns (Barchiesi et al., 2020; Chera-Anghel & Staden, 2023; Liao et al., 2020; Millour et al., 2012). Monitoring poisonous element concentration in food is therefore of high importance because bioaccumulation of harmful elements to toxic levels may occur even at low concentration of these elements at long-term exposure. Clams, mussels, and oysters are filter feeding organisms and are sensitive to their environment. They accumulate harmful compounds and heavy metals in their soft tissue depending on their surroundings, which can be 100 to 1000 times higher than that of water where they reside (Dang et al., 2022; Lehel et al., 2018). Concentrations of heavy metals in soft tissue are measures of the bioavailability of metals and are the results of complex and interrelated geological, hydrological, physico-chemical, and biological factors, including feeding habits and kind of organisms. Significant correlation has been established between heavy metals in water and sediments and in bivalves (Amiard-Triquet et al., 1998; Cao et al., 2023; Diwa et al., 2022; Griscom & Fisher, 2004; Kalman et al., 2014; Khoei, 2022; Luoma & Bryan, 1982; Shirneshan et al., 2013; Wang & Lu, 2017).

Heavy metals (metals and metalloids having a density of higher than 5 g cm^{-3}) are natural constituents of the lithosphere and occur in varying concentrations in all ecosystems. However, anthropogenic activity, especially in urban and industrial areas, has increased the concentration of both essential (e.g., Co, Cu, Fe, Mn, Mo, Zn) and non-essential (e.g., Cd, Hg, Pb) heavy metals in these systems, which generates environmental and health concerns (Andrade-Rivas et al., 2022; Jin et al., 2023; Millour et al., 2012; Sajwan et al., 2008). The most important anthropogenic activities in this respect are industrialization, urbanization, and agriculture, which include heavy metal sources such as industrial wastes and wastewater, wastes from smelting ores, leachates from mining sites, sewage sludge, vehicular traffic, marine transportation, fertilizers, and pesticides (Jahromi et al., 2021; Liu et al., 2022). Heavy metal pollution of rivers, estuaries and coastal areas possess risk to river and marine organisms due to adverse effects, and to consumers of aquatic animals, as heavy metals are toxic and not biodegradable. Bivalves are known to accumulate high concentrations of heavy metals in their tissues due to their very efficient dietary assimilation and exceedingly dissolved uptake (Chen et al., 2014). The ability of these organisms to accumulate heavy metals makes them excellent species for monitoring metal contamination in rivers and coastal waters but may possess threat to human health via the food chain if they are harvested in polluted environment (Sun et al., 2023).

Due to direct link of toxic heavy metal accumulation in bivalve soft tissues to human health issues, the tolerance limit of most toxic heavy metals in food is regulated by many countries and organizations around the world; examples are shown in Table 1.

Harmful heavy metal accumulation in soft tissues of clams, mussels, and oysters is receiving continuous interest due to its direct link to health issues and environmental pollution (Yap, Sharifinia, et al., 2021). Analysis of the relevant literature to date confirms the increasing number of publications on heavy metal quantification in these bivalves, namely, 19%

Table 1 Maximum permitted levels of heavy metals in bivalve foodstuff (in mg kg^{-1} wet tissue)

Regulator	As	Cd	Cr	Hg	Sn	Pb
EU[a]		1.0		0.5	200[b]	1.5
FAO[c]		1		0.5		1
FAO/WHO[d]		2			250[b]	
China[e]	0.5[f]	2.0[g]	2.0	0.5[h]	250[b]	1.5
AU/NZ[i]	1[f]	2		0.5	250[b]	2
India[j]	86	2.0	12	0.5 (0.25[h])	250[b]	1.5

[a] Commission of European Communities (CEC, 2006; CEC, 2008), [b] Canned foods, [c] Food and Agriculture Organization of the United Nations (FAO) (FAO, 2003), [d] FAO and World Health Organization (FAO-WHO, 2019), [e] Standardization Administration of the People's Republic of China (SCA, 2017), [f] Inorganic arsenic, [g] Viscera removed, [h] Methyl mercury, [i] Food Standards Australia New Zealand (ANZ-FSC, 2021), [j] Food Safety and Standards Authority of India (FSSAI, 2020)

of publications to date are published in the period of 2000 and before, 26% between 2001 and 2010, and 55% between 2011 and 2022 (Tables 4-6). Targeted heavy metals in previous works, and a comparison of the number of studies for quantification of each heavy metal is shown in Fig. 1.

Clams, mussels, and oysters, either as foods or sentinel organisms of environmental change, play an important role in aquatic ecosystems, and monitoring their soft tissue heavy metal concentration is of crucial importance for both food security and environmental pollution. Several methods have been developed and applied to date to test for heavy metal accumulation in these bivalves. Challenges for these techniques, in general, are the low concentration of heavy metals in the tissue and the need for quantifying multiple elements. There has been a clear trend over the last decades to improve the efficiency (recovery and analyte enrichment) of sample preparation and sensitivity of applied instrumentation techniques, as well as to reduce the total analysis time. Instruments which are sensitive and able to perform simultaneous multielement analysis are becoming more and more popular as shown for example by the increasing application of internally coupled plasma optical emission or mass spectrometry. Although only 20% of publications reported the quantification of heavy metals using these two techniques in year 2000 and before, this number increased to 55% between 2001 and 2010, and further increased between 2011 and 2022 up to 65% (based on publications listed in Tables 4–6). Despite developments, it is disadvantageous for the currently used analytical methods that they require laboratory conditions, trained workforce, expensive instruments, and complex and time-consuming analysis. It remains challenging to adopt or develop simple tools for field applications. The present review aims to summarize sample preparation and instrumentation techniques applied to date for the quantification of heavy metal content in tissues of clams, mussels, and oysters. The aim of the work is to give an overview of the field by discussing in consecutive sections the heavy metal extract preparation from tissue of these bivalves, including digestion/extraction and preconcentration, instrumentation used to date for heavy metal quantification in the extract, and the importance of quality assurance and control. Pros and cons and references to further reading of each technique, as well as future challenges of heavy metal testing in clams, mussels and oysters are also presented.

Quantification of heavy metals in soft tissues of clams, mussels, and oysters

The success of HM quantification in bivalves depends on both the sample preparation method and the instrumental technique used. For HM determination, the soft tissue matrix of bivalves needs to be digested to convert the solid sample into solution prior to analytical measurements. If the measurement of the total HM

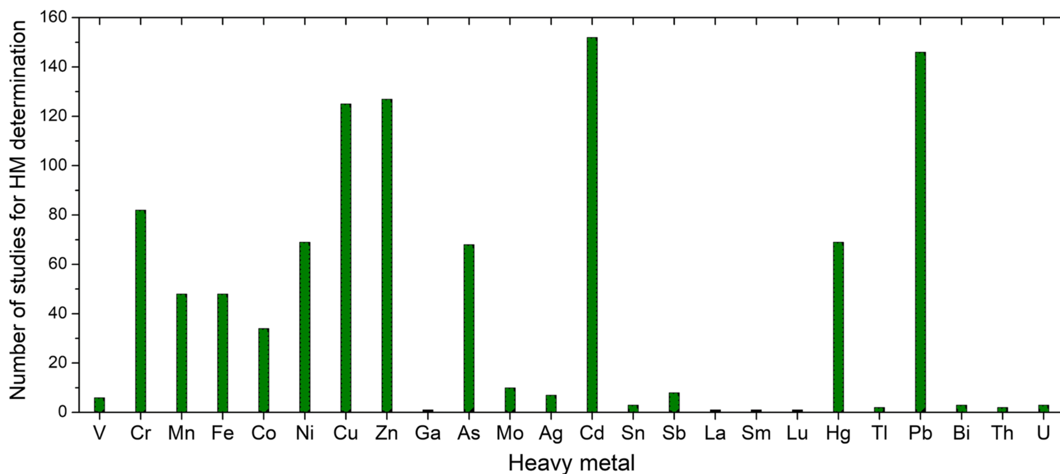


Fig. 1 Comparison of the number of studies for the determination of heavy metals in soft tissues of clams, mussels, and oysters

concentration in soft tissue is required, which is most often the case, the destruction of the matrix and compounds in the matrix is acceptable and the method of choice; special extraction techniques must be used if HM speciation is aimed. The two major steps of HM quantification, therefore, are 1.) liquid HM extract preparation, which may include drying, digestion, extraction, and pre-concentration, and 2.) instrumental determination of HM concentration in the extract, typically by utilizing atomic spectrometric methods, such as AAS, ICP-OES and AFS, mass spectrometry, such as ICP-MS, chromatographic methods, such as HPLC and GC, or other methods such as EDXRF, NAA or ES. Steps of HM quantification in soft tissue is summarized in Fig. 2 (only those methods are listed which have already been applied in HM evaluation in bivalves). Relatively large number of papers have addressed the HM determination in clams, mussels, and oysters to date, and details of HM extract preparation and instrumental HM quantification are collected in the Appendix in Tables 4, 5, and 6, respectively (Anagha et al., 2022; Araujo et al., 2019; Avelar et al., 2000; Bat et al., 2019; Belbachir et al., 2013; Blankson et al., 2022; Bray et al., 2015; Bryan et al., 1980; Camusso et al., 2001; Dabwan & Taufiq, 2016; Dahms et al., 2014; de Astudillol et al., 2005; Elvira et al., 2021; Esposito et al., 2022; Ferreira et al., 2004; Ferreira et al., 2005; Grant & Ellis, 1988; Griscom et al., 2002; Hursthouse et al., 2003; Intawongse et al., 2012; Januar et al., 2019; Jia et al., 2018; Kamaruzzaman et al., 2010; Katsallah et al., 2013; LaBrecque et al., 2004; Langston et al., 1999; Li et al., 2015; Lias et al., 2013; Lin et al., 2004; Liu et al., 2022; Lozano-Bilbao et al., 2018; Lu et al., 2005; Matos et al., 2021; Mauri et al., 2004; McDougall et al., 2020; Meng et al., 2014; Mtanga & Machiwa, 2007; Otchere, 2022; Ozden et al., 2009; Pakingking et al., 2022; Peake et al., 2006; Rohalin et al., 2019; Ruelas-Inzunza et al., 2009; Rule, 1985; Said et al., 2022; Santos & Boehs, 2021; Senez-Mello et al., 2020; Shenai-Tirodkar et al., 2017; Sheng et al., 2021; Silva et al., 2001; Siva et al., 2010; Sokolowski et al., 2007; Soto-Jimenez et al., 2001; Telahigue et al., 2022; Tessier et al., 1984; Tran et al., 2001; Tu et al., 2014; Vieira et al., 2022; Wang et al., 2022; Weston & Maruya, 2002; Yap et al., 2021; Zhang et al., 2022; Zhu et al., 2017).

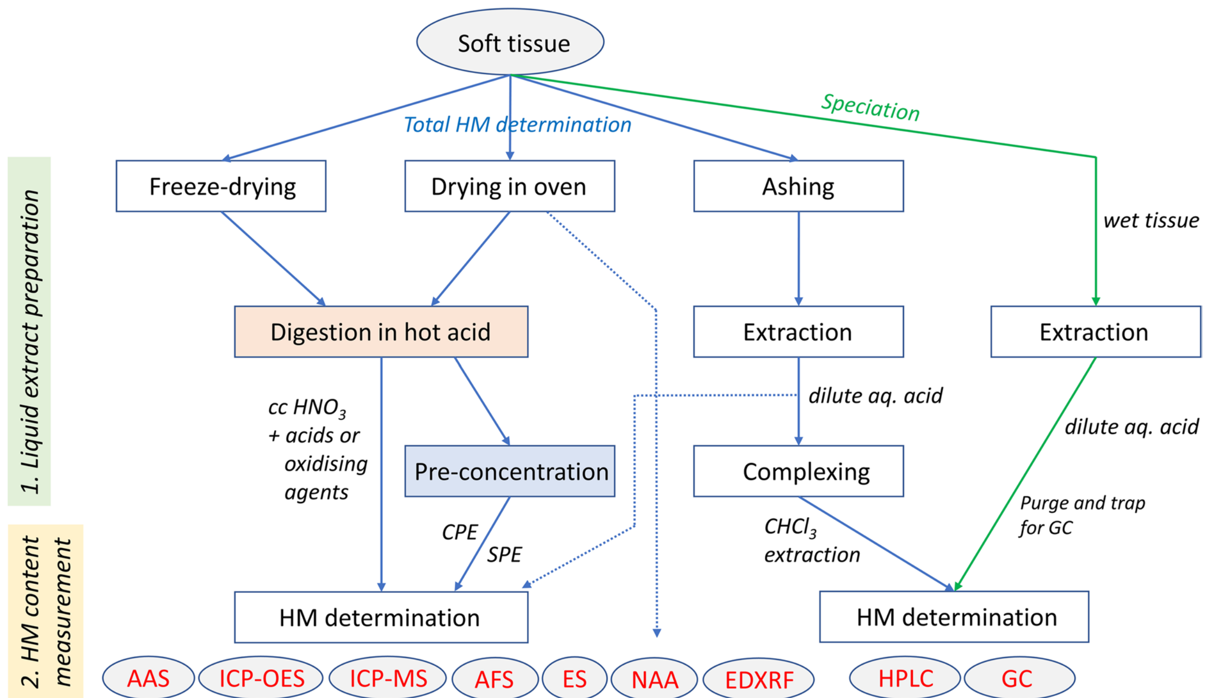


Fig. 2 Steps of heavy metal quantification in soft tissue of clams, mussels, and oysters

Heavy metal extract preparation

The liquid sample preparation is a key step for the accurate measurement of HM concentration in different soft tissue matrices of bivalves. Several procedures have been developed to date to minimize sample loss and maximize recovery, to minimize contamination and to shorten the extract preparation time, as well as to address specific requirements of consecutive HM determination in the extract, such as the need for total HM concentration or speciation or decreasing matrix effects. Selected examples are shown in Table 2, and more details including the name of bivalve species are collected in Tables 4–6.

Extract for total heavy metal determination

Clams, mussels, and oysters are consumed without removing organs or viscera; therefore, the HM analysis usually aims to determine the HM content in the whole body and all tissues. Samples for analysis are prepared by removing the whole body from the shell using a metal-free tool, cutting and homogenizing. There are a limited number of studies, however, which are aimed at determining HM distribution among bivalves' various tissues and organs. Vadopivez et al. observed that the kidney tissue of *Laternula elliptica* contained much higher concentration of Cd, Mn, Pb and Zn than the digestive gland or the gills, but the digestive gland contained the highest amount of Cr and Cu (Vodopivez et al., 2015). Couillard et al. detected higher Cd, Cu and Zn concentration in gills of *Anodonta grandis* than in hepatopancreas and the mixture of remaining tissues (Couillard et al., 1993). Shaari et al. studied the Cd, Cu, Pb, Co and Zn content of different organs of *Psammotaea elongate*, namely stomach, ligament, and leg, from different sampling sites and found that the variation of HMs concentration in the different organs followed the variation of the HMs concentration in the whole-body tissue. The distribution of HMs among organs was dependent on the sampling site (Shaari et al., 2015). Zheng et al. determined the distribution of Cd in visceral, mantle, gill, and muscle tissues of *Crassostrea gigas* (Zheng et al., 2021). The total Cd content was the highest in the viscera, but the Cd concentration was the highest in gill followed by viscera, mantle, and muscle (see Fig. 3).

The soft tissue of bivalves contains large amounts of water, which is disadvantageous for concentrated acid digestion, dilutes the sample and reduces sensitivity; therefore, the soft tissue is usually dried to constant weight and grounded to powder as the first step of extract preparation. High temperature drying or especially ashing results in loss of volatile analytes, e.g., Hg and As, therefore the freeze-drying at around −50 °C or drying at slightly elevated temperature between 60 and 130 °C are the most popular methods (Tables 2 and 4–6).

Digestion of the dried tissue, as the next step of liquid extract preparation, is critical concerning analysis time and analyte recovery. The hot acid digestion method using concentrated oxidizing acids, typically HNO_3, offers a simple and relatively rapid sample decomposition for total HM analysis. The advantage of this method is in its simplicity, and that it does not require any special equipment and concentrated acidic solutions after appropriate dilution can be directly injected into AAS, ICP-OES and ICP-MS instruments. Concentrated HNO_3 is either used alone or in combination with other acids, such as $HClO_4$, HCl and H_2SO_4, or oxidizing agents such as H_2O_2 and $KMnO_4$ (Tables 2 and 4–6). Elevated temperature always presents a risk for sample loss, and typically the loss of mercury might become a major problem in the hot acid sample preparation procedure due to its volatility. To avoid this problem, it was shown that the presence of oxidizing agents could prevent complete mercury loss even under severe heating conditions (Akagi & Nishimura, 1991). Digestion time is critical for sample throughput. Combination of acid treatment with microwave or ultrasound irradiation has been shown to provide a significant speeding up in the pre-treatment step; the typically 6–24 hours hot acid treatment time, in general, can be reduced to 15–60 min using irradiation (Tables 2 and 4–6). An additional advantage of the microwave digestion is that it is carried out in a closed vessel, which latter can reduce the amount of environmental contamination and contamination from the digestion vessel. Microwave digestion can be effective without a pre-drying step (Tables 4–6) and requires, in general, less acid for the digestion than do conventional wet digestion methods (Sheppard et al., 1994). Concerning different microwave digestion techniques, the cavity-microwave acid digestion method was shown to be more advantageous than the focused-microwave

Table 2 Selected examples for the quantification of HM content in soft tissues of clams, mussels, and oysters (see Tables 4–6 for more details)

Extraction and digestion method	HM Determination[a]			Ref.
	Method	LOD / $\mu g\ g^{-1}$	HM monitored	
Freeze-dried; ultrasound + centrifugation, dilute HCl, HNO_3 and CH_3COOH, 25–30 °C	ICP-OES	0.012–2.62	Cd, Cu, Mn, Zn	(dos Santos et al., 2010)
Freeze-dried for 48–72 h; microwave, cc HNO_3	ICP-OES	0.05–0.3	Cd, Cr, Cu, Fe, Mn, Pb, Zn	(Vodopivez et al., 2015)
Dried at 60 °C for 48 h; microwave, cc HNO_3 + cc H_2O_2	ICP-OES	0.01–0.03[b]	Cd, Cu, Zn	(Wanick et al., 2012)
Freeze-dried; Microwave, cc HNO_3 + cc H_2O_2, 100–140 °C, 50 min	ICP-OES	0.002–0.03[b]	As, Cd, Co, Cr, Cu, Hg, Fe, Mn, Mo, Ni, Pb, Zn	(Zheng & Yan, 2011)
Microwave, cc HNO_3 + cc $HClO_4$, 200 °C, 40 min	ICP-OES	0.00033–0.01518	Co, Cu, Fe, Mn, Ni, Pb, Zn	(Chinnadurai et al., 2022)
Microwave, cc HNO_3, 125–155 °C, 1 h	ICP-OES ICP-MS	0.2–2.7 0.0002–0.0028	As, Cd, Pb	(Sheppard et al., 1994)
Freeze-dried; cc HNO_3 for 6 h, + cc H_2O_2, microwave, 180 °C, 30 min	ICP-MS	0.0013–0.0135[b]	As, Cd, Cr, Cu, Hg, Pb, Ni, Zn	(Liao et al., 2020)
Freeze-dried; microwave, cc H_2O_2 + cc HNO_3, 70–180 °C, 1 h; SPE using MGO@SiO_2-APTES-IL	ICP-MS	0.00242–0.00375[b]	Cd, Cu, Pb	(Dong et al., 2021)
Dried at 50 °C for 24 h; cc HNO_3, 1 h at room temp, 4–6 h at 110 °C; cc H_2O_2, 1 h	ICP-MS	0.01–0.2	Cd, Cu, Ni, Pb, Zn	(Thomas & Bendell-Young, 1998)
Microwave, cc HNO_3 + cc H_2O_2 + cc HF	ICP-MS	0.0015–0.0076	Cd, Hg, Pb	(Barchiesi et al., 2020)
Freeze-dried; cc HNO_3 + cc H_2O_2, microwave for 1.5 h	ICP-MS	0.01–3.67	As, Cd, Cr, Cu, Ni, Pb, Zn	(Chen et al., 2022)
Microwave, cc HNO_3 + cc H_2O_2, 120–190 °C, 60 min	ICP-MS	0.001–0.004	Cd, Hg, Pb	(Miedico et al., 2015)
cc HNO_3 at room temp. For 8 h then at 85 °C for 8 h	ICP-MS	0.02–0.05	As, Cd, Hg, Pb	(Falco et al., 2006)
Microwave, cc HNO_3 + cc H_2O_2	ICP-MS	0.02–0.05	Cd, Pb	(Pastorelli et al., 2012)
Freeze-dried; microwave, cc HNO_3 + cc H_2O_2, 24 min	ICP-MS	0.006–0.08	As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Tl, V, Zn	(Culotta et al., 2008)
Dried at 60 °C; n.a.	ICP-MS	0.0038–0.0461	As, Cd, Cr, Cu, Fe, Ni, Pb, Zn	(de Souza et al., 2021)
Dried at 105 °C; cc HNO_3 at r.t., cc HNO_3 + cc H_2O_2 at 180 °C	ICP-MS	0.03–1.66	As, Cd, Cr, Cu, Pb, Zn	(Liu et al., 2022)
Freeze-dried; Microwave, cc HNO_3 + cc H_2O_2	ICP-MS	0.00047–0.00236	As, Cd, Cr, Pb	(Jin et al., 2023)
Microwave, cc HNO_3 at 195 °C for 20 min	ICP-MS	0.0003–0.005	As, Cd, Cr, Hg, Ni, Pb	(Pan & Han, 2023)

Table 2 (continued)

Extraction and digestion method	HM Determination[a]			Ref.
	Method	LOD / µg g^{-1}	HM monitored	
Dried in vacuum at 80 °C overnight; microwave, cc HNO_3, 20 min; + cc H_2O_2, 20 min	AAS	0.004–0.05	Cd, Cu, Pb, Zn	(Yaru et al., 1999)
Dried at 50 °C for 24 h; cc HNO_3, 1 h at room temp, 4–6 h at 110 °C; cc H_2O_2, 1 h	AAS	0.004	Hg	(Thomas & Bendell-Young, 1998)
Freeze-dried for 18 h; microwave, aq. H_3PO_4/ KH_2PO_4 buffer, SDS, Triton X-100 surfactant	AAS	0.02–0.05	Cd, Co, Cr, Ni, Pb	(Hernandez-Martinez et al., 2016)
Microwave, cc HNO_3 + cc H_2O_2	AAS	0.5	Hg	(Pastorelli et al., 2012)
Wet sample; cc HNO_3 + cc H_2O_2, microwave for 46 min	AAS	0.01–0.12	As, Cd, Cu, Hg, Pb, Zn	(Garcia-Rico et al., 2001)
Freeze-dried; cc HNO_3 + cc $HClO_4$, microwave for 2 h	AAS AFS	0.005–0.4 0.002–0.2	Cd, Cr, Cu, Pb, Zn As, Hg	(Noman et al., 2022)
Dried at 105 °C; cc HNO_3 + cc $HClO_4$	AFS	0.002	Hg	(Liu et al., 2022)
Freeze-dried; Microwave, cc HNO_3 + cc H_2O_2	AFS	0.001	Hg	(Jin et al., 2023)
Dried at 120 °C for 24 h; cc HNO_3 at 90–130 °C for 6 h	AFS	n.a.	Hg	(Maanan, 2008)
Dilute HNO_3, ultrasound, 1 h	HPLC-ICP-MS	0.0083, 0.0052[b]	H_3AsO_3, H_3AsO_4	(Liao et al., 2020)
2-mercaptoethanol, L-cysteine, aq. HCl, ultrasonicated at 40 °C for 30 min	HPLC-ICP-MS	*n.a.*	MeHg	(Ferraris et al., 2021)
Ashing at 500–550 °C; aq. HCl; HMA-HMDC; extraction	HPLC	*n.a*	Cd, Ni, Pb, Zn, Co, Cu, Bi	(Ichinoki et al., 1984)
Microwave, 1 M HCl, 70 °C, 3 min; ethylation	GC-MS	0.0007	MeHg	(Valsecchi et al., 2021)
Freeze-dried; microwave, 4 M HNO_3, 55 °C overnight; purge and trap preconcentration	GC-ICP-MS	0.000072	MeHg	(Taylor et al., 2008)
Freeze-dried; microwave, cc HNO_3; buffered, eluted on a Chelex-100 resin	NAA	*n.a.*	As, Co, Cr, Hg, Sb, Zn	(Yusof et al., 1994)
Dried at 40 °C; no chemical pretreatment	NAA	*n.a.*	As, Ag, Co, Cr, Fe, Mn, Mo, Sb, Th, U, V, Zn	(Bezuidenhout et al., 2015)
Freeze-dried for 48 h; no chemical pretreatment	EDXRF	*n.a.*	As, Fe, Cu, Zn	(Santos et al., 2014)
Dried at 45 °C for 24 h; cc HNO_3 + cc H_2SO_4, 140 °C, 3 h	ES	*n.a.*	Cu, Cd, Pb, Zn	(Locatelli, 2000)
Freeze-dried for 24 h; microwave, cc HNO_3 + cc H_2O_2	ES	*n.a.*	Cd, Pb	(Pizarro et al., 2020)

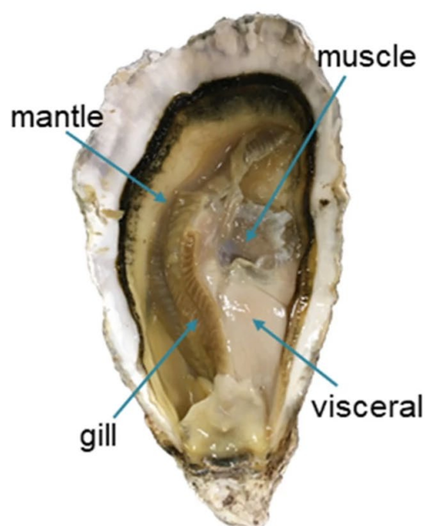
Table 2 (continued)

Extraction and digestion method	HM Determination[a]			Ref.
	Method	LOD / µg g^{-1}	HM monitored	
Without drying; microwave, cc HNO_3	ES	0.0005–0.0040	Cu, Cd, Pb, Zn	(Skiba et al., 2023)

[a]n.a. = not available; LOD in µg g^{-1} (ppm) unless otherwise indicated, [b]LOD in µg L^{-1}

acid digestion because the latter was prone to losses of certain volatile elements and compounds (Costa et al., 2009). Combination of ultrasound and centrifugation has been reported to be effective under mild conditions using dilute acids and room temperature (dos Santos et al., 2010). Although different variants of the acid digestion method are fast exclusively used in this field, a disadvantage of acid digestion is the application of highly corrosive and toxic acids. It is important to note in this respect that Hernandez-Martinez at al. have recently developed an oxidizing acid free extraction method based on biodegradable mixed-micelles (Sodium Dodecyl Sulphate (SDS) and Triton X-100) dissolved in aqueous phosphate buffer and microwave irradiation (Hernandez-Martinez et al., 2016).

Sample pre-treatment can cause analyte loss and influence the detected amounts of HMs if not carefully performed. Open vessels and high temperature for digestion always present a risk; however, this strongly depends on the oxidized form of the HM and the digestion medium. Best choices for sample acid digestion are the use of closed vessels either heating in a cavity-microwave oven or digesting at low temperature. It is worth to note that, in general, good recoveries can be obtained by the acid digestion method if properly performed (see references in Tables 4–6). Each instrumental technique used to detect and quantify HMs have specific requirements concerning the MH extract. Chromatographic methods, for example, may require the derivatization of the analyte (see below).



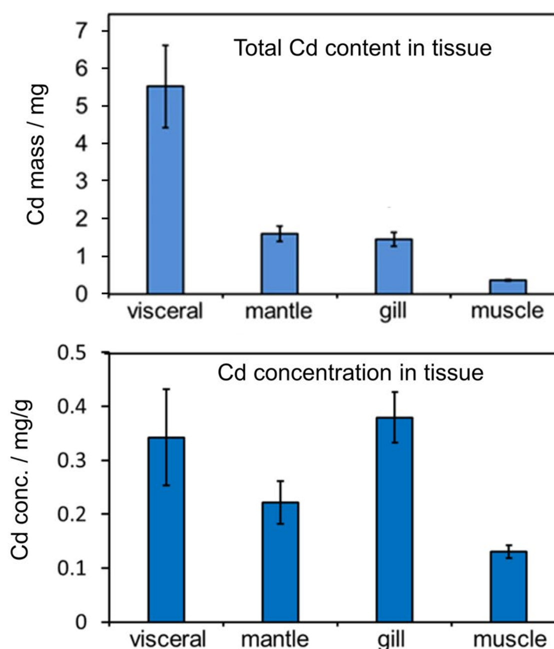


Fig. 3 Distribution of Cd in visceral, mantle, gill, and muscle tissues in an oyster (Zheng et al., 2021). Reproduced from the original article according to the Creative Commons Attribution 4.0 International License

Extract for the quantification of specific compounds in soft tissue

The speciation analysis of heavy metal compounds in soft tissue of bivalves is still challenging due to the lability of these compounds in vitro and the scarce of the reference metalorganic compounds. Not surprisingly, most of the speciation analysis to date has been focused on known small molecules such as MeHg (methyl mercury). The determination of specific compounds in the soft tissue requires the extraction of these compounds from the tissue without decomposition, therefore aggressive concentrated acid digestion methods cannot be used. HPLC using ICP-MS as the detection technique is typically used for this purpose, however, knowledge about the metalorganic compound is essential as the HPLC-ICP-MS coupled technique can only acquire the elemental messages of the species; unknown compounds and the metal-binding ligands need to be further characterized. GC is rarely used for speciation in clams, mussels, and oysters to date due to its limitation concerning volatility and lability of metal compounds; it is mainly used for MeHg speciation. To avoid breaking the metal-ligand bond, extraction of HM-compounds from bivalves requires a low temperature leach. MeHg is usually extracted from the wet soft tissue using ultrasound assisted dilute HCl extraction (Liao et al., 2020). Alternatively, thiol compounds may be added to the aq. HCl solution for the extraction of mercury species (Ferraris et al., 2021). Arsenious and arsenic acid can be extracted similarly using dilute HNO_3 (Liao et al., 2020). Extraction with a mixture of dilute HNO_3 and H_2O_2 leads to the oxidation of arsenous acid and thereby to the speciation of the inorganic arsenic content as arsenic acid (Ferraris et al., 2021). After neutralization and mixing with mobile phase carrier solution, the extract can be injected onto the HPLC column, and compounds can be identified with various detection techniques, typically UV or ICP-MS.

Bioaccumulation of HMs in bivalves' soft tissue is expected to lead to the formation of various metal organic compounds and identifying these species could provide important information for the understanding of bivalve's metabolism and the toxicology of heavy metals. Analytical procedure for HM speciation, however, is more complex than that of total HM quantification, which partially explains the paucity of such research on clams, mussels, and oysters to date. A notable example is the work of Li et al. who investigated Cd species in short necked clam by extracting the tissue with aqueous tris (hydroxymethyl) aminomethane-HCl buffer containing dithiothreitol and identified Cd species using size exclusion chromatography (SEC) coupled with ICP-MS. Three Cd species were observed and further characterized after pre-fractionation of the extract with Sephadex column using SEC coupled with electrospray ionization quadrupole - time of flight mass spectrometry (ESI-Q-TOF-MS) (Li et al., 2021).

Preconcentration

It is, in general, a great challenge for quantitative analysis to increase the sensitivity of the analytical method and decrease the LOD and LOQ. All these performance parameters depend on both the sample preparation and the instrumental technique used. Analyte preconcentration is an effective method to improve sensitivity and reduce matrix interference. Preconcentration is based on separating HMs from the sample into a small volume. The preconcentration factor can be defined as the ratio of the highest initial sample volume and the lowest final volume. Applying preconcentration is relatively new in HM quantification in clams, mussels, and oysters. Methods that are already used in this field are the Cloud Point Extraction (CPL), the Solid-Phase Extraction (SPE), and the 'purge and trap' method for MeHg quantification (see below). The trial of potential complexant and surfactant for CPL and adsorbent for SPE, as well as the development of green methods seem to be the main trend in this field.

Cloud point extraction CPE procedure is based on the property of a surfactant to form micelles in aqueous media after changing experimental conditions such as temperature or inclusion of additives. The surfactant-rich phase extracts and preconcentrates HMs, which can be subsequently separated by centrifugation (Fig. 4). Adjusting the pH of the solution is very important, as the extraction of HMs is quantitative only at the optimum pH range of the CPE procedure (Mortada, 2020; Sunder et al., 2020). High acidity of the soft tissue extract can prevent preconcentration. CPE is

considered as a green method as it consumes no or minimum amounts of organic solvent. A CPE method in the investigated field was developed by Dos Santos Depoi et al. for As, Bi, Cd, and Pb quantification using ICP-OES. The method was based on the application of O,O-diethyldithiophosphate (DDPP) as complexant and octylphenoxypolyethoxy-ethanol (Triton X-114) as surfactant (dos Santos Depoi et al., 2012); LODs were 0.055, 0.063, 0.047 and 0.28 µg L^{-1}, respectively. To obtain an extract with low acid concentration, oyster samples were digested in dilute aqueous nitric acid using microwave induced combustion (MIC).

Solid-phase extraction SPE is one of the most frequently used preconcentration techniques due to its simplicity, high preconcentration factors, and availability of a wide variety of sorbent materials. It is based on the consecutive steps of 1) adsorption of HMs on an adsorbent, 2) separation of adsorbent, and 3) desorption of HMs from adsorbent by using an eluent (Fig. 4) or simply the heat of the atomizer of an instrument after injection. Since SPE does not require or only small amounts of organic solvents, it is a green technique (Sunder et al., 2020). There is a continuous development in this field to improve efficiency and operational aspects by introducing novel extractants and new adsorbents, reducing manual

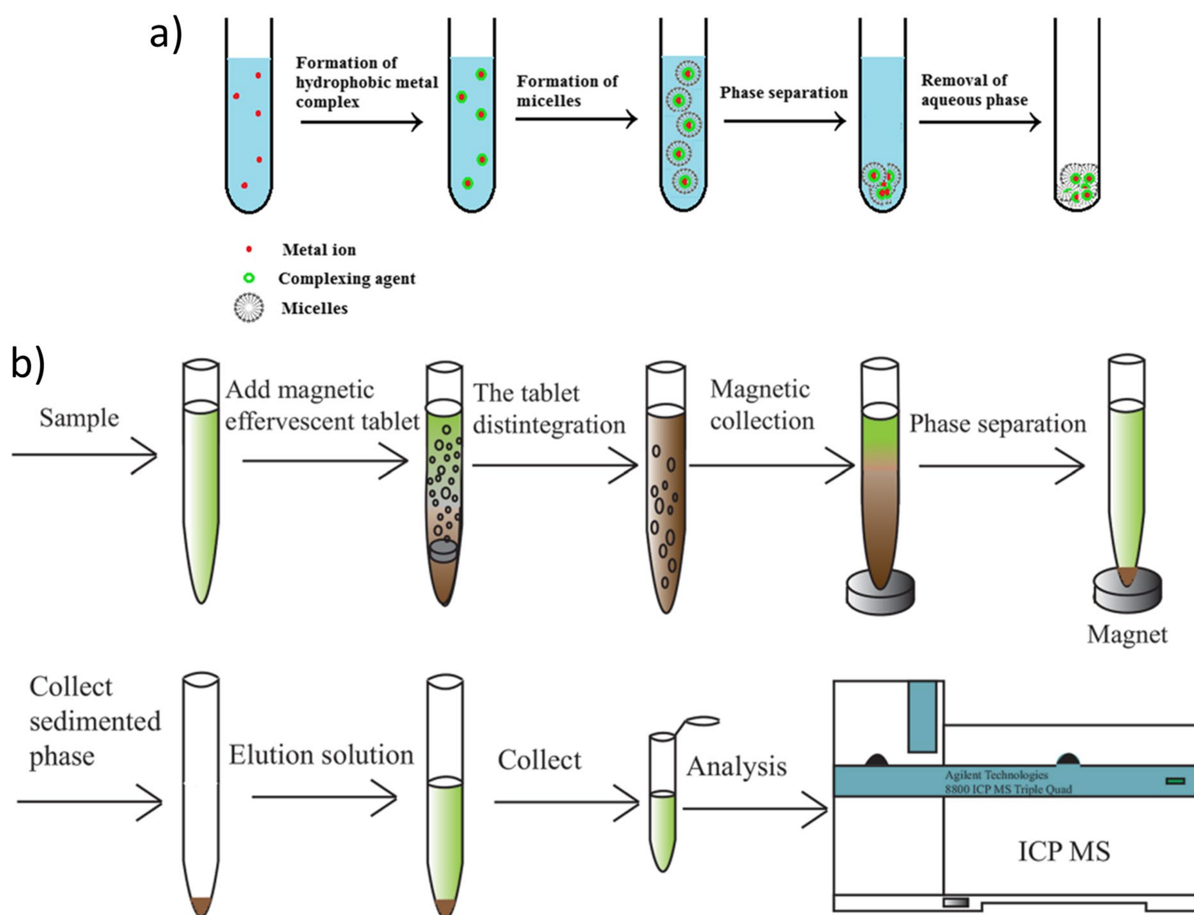


Fig. 4 **a**) Preconcentration of HMs using CPE, reproduced with permission from ref. (Mortada, 2020). Copyright of Elsevier Ltd., 2020; **b**) SPE using effervescent tablets. Reproduced with permission from ref. (Zhou et al., 2019). Copyright of The Royal Society of Chemistry, 2019

operations, and speeding up the analysis time (Faraji et al., 2019; Madikizela et al., 2019; Schettino et al., 2022; Song & Huang, 2022; Su et al., 2022).

Concerning developments in the field of HM quantification in clams, mussels, and oysters, Benvidi et al. developed recently a SPE separation method for Ni determination in oyster tissue (Benvidi et al., 2020). The method was based on collecting nickel ions after digestion of the soft tissue on magnetic Silk Fibroin-Fe_3O_4-EDTA nano adsorbent, separating the nano-adsorbent from the solution using a magnet and injecting the nano-adsorbent into the furnace of an AAS instrument. An extremely low LOD of 0.0017 μg L^{-1}, a linearity range of 0.0030–5.0 μg L^{-1}, a preconcentration factor of 243, and a mean recovery percent of 100.1% could be achieved (Benvidi et al., 2020). Yekrangi et al. synthesized and applied magnetic nano-adsorbents for the SPE of Pb^{2+} ions for AAS quantification. The nano-sorbent consisted of humic acid as the lead-collecting ligand anchored to graphene oxide (GO) as the substrate. GO was magnetized with iron oxide (Yekrangi et al., 2021). LOD of 0.07 μg L^{-1} and a linearity range of 0.2–12 μg L^{-1} was achieved. Other examples of SPE include the development of Dong et al. on a dispersive magnetic solid phase microextraction method using ionic liquid-coated amino silanized magnetic graphene oxide (MGO@SiO2-APTES-IL) as adsorbent for enriching and extracting Cd, Cu, and Pb ions in shellfish digests; LODs for the three target HM ions were 3.75, 3.36, and 2.42 ng L^{-1}, respectively, with recoveries between 95.4 and 99.5%, and microextraction time of 6.4 min (Dong et al., 2021). Zhou et al. developed an effervescent tablet SPE method using $NiFe_2O_4$-based magnetic nanoparticles for preconcentration of Cu, Mn, Zn and Cd ions prior to ICP-MS analysis of shellfish (Fig. 4). LODs for the four metal ions were as low as 0.007–0.018 μg g^{-1} (Zhou et al., 2019). Ghanemi et al. applied sulfur nanoparticle (NP) loaded alumina for the determination of trace amounts of Cd, Cu, Zn, and Pb and low LODs of 0.30, 0.24, 0.21 and 0.63 μg L^{-1} were achieved, respectively, using flame AAS (Ghanemi et al., 2011).

Purge and trap Purge and trap method refers to the preconcentration technique in which volatile compounds are purged out of the sample matrix by an inert gas or by suction and carried onto a trap, where they are collected and concentrated, and consequently introduced into an instrument for analysis. The sample matrix may contain the volatile compound, or this latter is generated by derivatization of matrix components. The trap can be a sorbent material or volatiles may be collected in a low temperature trap without sorbents. The most frequently used instrumental techniques for analysis are the GC and GC-coupled techniques with various detectors. The purge and trap method was introduced in the 1970s for headspace analysis, and has been gone through improvements and modifications over the last half century concerning instrumentation (purging device, sorbent trap, sorbent type, inlet and outlet systems), operational procedures (performance of thermal desorption or solvent elution, application of secondary trap and analyte refocusing), and quantification methods (external standards and internal standards using stable isotopically labeled compounds) to broaden the application field (Harries & Bruno, 2019; Kou & Mitra, 2005). The overall performance of the method depends on the efficiency of each of the three main individual steps, namely purging (extraction), trapping (adsorption), and subsequent desorption (thermal desorption or elution using a solvent). Although the purge and trap method is widely used in various fields of analytical chemistry, it has hardly been applied in HM quantification in clams, mussels and oysters to date. A notable example is the method developed by Taylor et al. for MeHg quantification, which involves the aqueous derivatization of Hg(II) species in the acid leachate with $NaBEt_4$, purging the solution with He and collecting volatile mercury compounds in a trap, thermal desorption of mercury compounds from the trap, and determination of MeHg and Hg(II) species on a GC-ICP-MS. A very low LOD of 0.000072 μg g^{-1} was achieved for MeHg determination (Taylor et al., 2008).

Quantification of heavy metals in the soft tissue extract

In principle, there are numerous spectroscopic and chromatographic techniques that could be used for the determination of HM content in tissue extract. The applied instrumental method, however, depends

on several factors, such as the HM concentration in the extract, viz. required sensitivity of the instrument and LOQ, sample throughput number, and the availability and running and investment costs of the instrument. The application of an instrumental technique is also not independent of extract properties, e.g., solvent, electrolyte, and pH, and extracts are prepared to fulfill instrumental requirements, and vice versa. In general, the method of standard additions and using internal standards are widely used to allow for matrix interferences, however, each techniques require specific conditions to counter matrix effects. General techniques like preconcentration (see above) and column chromatography may be used to prevent matrix effects (Shiel et al., 2012). A brief introduction of the instrumental techniques that have been used to date for HM quantification in clams, mussels and oysters is presented in this section. The advantages and disadvantages of each technique are summarized at the end.

Spectroscopic methods

ICP-OES and ICP-MS ICP-OES and ICP-MS are based on atomizing and ionizing the sample in an argon plasma, introduced as a fine aerosol, and detecting emitted lights of excited state atoms (ICP-OES) or extracting ions and detecting them with a mass analyzer (ICP-MS) (Donati et al., 2017; Wilschefski & Baxter, 2019). Both methods require a liquid sample for nebulizing (note that there is capability for direct solid sampling, but it is not applied for routine analysis or HM determination in bivalves). The analytical sensitivity of an analyte in ICP-OES is linked to its overall atomization and excitation; in ICP-MS to its overall ionization and isotopic abundance of the measured isotope. The detection sensitivity therefore differs considerably according to the analyte and operating conditions. Both methods can simultaneously measure multiple elements, and strongly acidic digested samples can be introduced directly into these instruments; these are huge advantages in HM analysis in bivalves. Challenges in analysis, namely spectral and chemical interferences, are well documented and avoidable for both methods (Sneddon & Vincent, 2008; Wilschefski & Baxter, 2019). Using internal standards is essential to correct for matrix effects and instrumental drifts, which latter are smaller in ICP-OES than in ICP-MS (Sheppard et al., 1994). The determination of some elements by ICP-MS is known to suffer from polyatomic isobaric interferences. The MS/MS mode combined with collision reaction cell using He, O_2 or NH_3 as collision gas is proved to be an effective method for relieving such isobaric interferences (Culotta et al., 2008). LODs of most sensitive analytical methods for HM quantification in clams, mussels, and oysters using ICP-OES or ICP-MS are now reaching 0.0003–0.015 μg g^{-1} (Chinnadurai et al., 2022) and 0.0003–0.005 μg g^{-1} (Jin et al., 2023; Pan & Han, 2023), respectively (this includes the efficiency of both sample preparation and instrumental HM quantification; see Tables 2 and 4–6).

AAS In AAS, the sample is desolvated and then atomized in an atomizer. The gas-phase analyte atoms are then irradiated with photons emitted by a narrow line profile light source and the intensity of the absorbed light is detected. Measurement of each element requires a different light source (either line source or selected wavelength of a continuum source), therefore only one analyte can be measured at a time. The sensitivity of the technique is linked to the overall atomization of the analyte and the amount of ground state analyte atoms (Bings et al., 2010; Hill & Fisher, 2017a). The two major types of atomizers are the flame and electrothermal. In flame AAS (FAAS) the sample is nebulized as a fine spray into a high-temperature flame where metal ions are reduced to their atoms. The advantage of FAAS is that it is a robust technique, however, its sensitivity is relatively low due to the spectral noise created by the flame and because a large part of the sample is lost in the flame. The electrothermal graphite furnace AAS (GFAAS) has much higher sensitivity than FAAS. In GFAAS the sample is atomized and vaporized within seconds in a hollow graphite tube and the absorption of the vapor is measured above the heated surface. Other specialized atomizing techniques for HM analysis are the hydride-generating atomization and the cold-vapor atomization. Hydride-generation (HGAAS) is mainly used for HM atoms which form volatile and thermally unstable hydrides, like As, Sb, Bi, Sn, Se. Metal-hydrides are generated in acidified sample solutions using sodium borohydride, and then transferred to the flame or furnace of the atomization

chamber by an inert gas to produce atoms. Cold-vapor atomization (CVAAS) is specifically developed for Hg analysis as Hg does not atomize well in a flame or furnace. In CVAAS the sample containing Hg ions is acidified and reduced to Hg, with e.g., sodium borohydride, and then purged by an inert gas. The absorption of the Hg in the gas is then determined. The detection sensitivity of AAS depends on the analyte, method of atomization, and operating condition of the instrument. Reported LODs of HM quantification in clam, mussel, or oyster samples are between 0.004 and 0.5 μg g^{-1} (see Tables 2 and 4–6) (Hernandez-Martinez et al., 2016; Noman et al., 2022; Yaru et al., 1999).

AFS In AFS, gaseous atoms are excited to a higher energy level with a light source and the emitted photons, which formed in the deactivation process, are detected. The instrumentation of AFS is very similar to that of AAS, and the LOD of classical AFS instruments is about the same as AAS (Butcher, 2019; Hill & Fisher, 2017b; Stchur et al., 2001). This similarity may explain why AFS has not been so heavily commercialized than AAS in the past, and this latter may explain the limited number of applications of AFS in HMs quantification in bivalves. The introduction of modern intensive lasers as light sources for AFS, however, has strongly increased sensitivity and selectivity of the technique. Laser excited AFS (LEAFS) instruments exhibit much smaller detection limit than AAS, and have LODs for many elements lower than those of ICP-MS. The disadvantage of LEAFS is that it is a sequential multielement technique, namely only one element at a time can be measured, as well as the technical complexity of tunable lasers used to excite the wide variety of atomic energy levels. AFS may become a routine analytical tool in the future, but it is not expected to compete with the multielement analysis capability of ICP-OES or ICP-MS. AFS has been used recently for Hg quantification in clams, mussels and oysters, and the analytical method exhibited LOD of 0.001–0.002 μg g^{-1} for this element (Jin et al., 2023; Liu et al., 2022; Maanan, 2008; Noman et al., 2022).

EDXRF EDXRF spectroscopic method is a multielement technique where the sample is irradiated with X-ray photons and the emitted X-ray fluorescence, characteristic of atoms in the target, is detected. EDXRF spectrometry has notable advantages compared to other techniques, namely the ability to measure both solid and liquid samples, nondestructive, and all the elements (with atomic numbers larger than eleven) present in the sample can be simultaneously determined without chemical pre-treatment of the dried tissue. Minimum sample treatment may involve homogenizing, pulverizing, or pelletizing. The main disadvantage of EDXRF which limits its application is that the technique is limited by its LOD of larger than 1 ppm (Marguí et al., 2022; Navas et al., 2016; Santos et al., 2014; Yao et al., 2015). EDXRF, for example, was used by Santos et al. for the determination of HMs in soft tissue of mussels (Santos et al., 2014).

Chromatographic methods

HPLC HPLC, in general, is one of the most powerful and widely used techniques for separation and quantitative determination of various analytes in solutions. It is based on the principle of affinity chromatography having a stationary and a liquid mobile phase. Molecules in the sample mixture separate as they travel the length of the column due to their different chemical properties and retention on the stationary phase. Selecting the most appropriate stationary and mobile phases as well as instrumental parameters to solve an analytical problem are key issues for method developments (Moldoveanu & David, 2017). HPLC has capability for simultaneous multielement HM quantification in bivalves, however, it also has limitations which prevent its widespread application in this field. If HPLC is used for the total HM analysis, HMs must be converted into mobile phase soluble metal complexes with an appropriate chelating reagent; the sample preparation is therefore complex and binding ligand properties strongly influence analytical performances. For example, in the case of photometric detection of a metal chelate, the detection sensitivity is proportional to the molar absorptivity of the chelate. In contrast to ICP-OES and ICP-MS, HPLC columns don't tolerate strongly acidic digests. Considering the equipment and running costs, however, HPLC can be more economical than ICP-OES/MS, therefore, simultaneous determination methods for various metals by HPLC have been developed. Ichinoki et al., for example, developed a simple method for the simultaneous

determination of HMs at the ppm level in oyster tissue by reversed phase HPLC using hexamethyleneammonium hexamethylenedithiocarbamate (HMA-HMDC) as chelating agent. The method is based on the consecutive steps of ashing the tissue, extracting the ash with dilute aq. HCl, neutralizing and buffering the extract, HMA-HMDC addition, extracting the HMs-HMA-HMDC complexes with chloroform, and injecting the chloroform phase onto a reversed phase column (Ichinoki et al., 1984). In general, application of HPLC in speciation of HM compounds is more important than in total HM quantification.

GC GC utilizes a capillary column for the separation of gas-phase analyte molecules. Retention on the column depends on the column's dimensions and phase properties, as well as on analyte properties and experimental conditions. Only compounds which can be vaporized on the GC column can be analyzed by GC. Many other compounds with low vapor pressures, however, can be investigated if they are chemically derivatized (converted into a volatile derivative) (Gawale et al., 2022; Laajimi et al., 2022). Identification of a particular molecule by GC is not possible using traditional detectors (e.g., flame ionization, flame photometric, thermal conductivity, electron-capture, chemiluminescence); this challenge is best addressed by the coupled GC-MS technique (see below). Traditional GC techniques are not applied for HM quantification in bivalve tissue due to known limitations (limited number of volatile compounds, derivatization is not possible for all metals, lack of reference materials, lack of sensitive detectors for metal-organic compounds). Application of GC-MS in this field is discussed below.

Spectroscopy coupled with chromatography

HPLC-ICP-MS HPLC-ICP-MS is an emerging technique in HM speciation. The advantage of coupling HPLC and ICP-MS is to combine an effective separation of the species under examination with a sensitive and versatile detector (Favilli et al., 2022). The method can be used to quantify limited number of leachable compounds from bivalves' soft tissue, e.g., MeHg, H_3AsO_3, or H_3AsO_4 (Ferraris et al., 2021; Liao et al., 2020). Quantifying multiple elements simultaneously, like ICP-OES and ICP-MS, or identifying molecules, like MS, is not possible due to solubility issues of organometallic or metal-organic compounds in the HPLC mobile phase and the inability of the ICP-MS detector to provide a molecular message as compounds are atomized in the plasma. Application of this coupled technique is rather in speciation than in total HM determination in bivalves, as ICP-MS can fulfill the requirements of total HM quantitation, and both running and instrument costs are cheaper for ICP-MS than for HPLC-ICP-MS.

GC-MS and GC-ICP-MS GC-MS combines the features of gas chromatography and mass spectrometry and is generally considered a versatile analytical platform which offers robustness, high chromatographic resolution, high sensitivity, selectivity, and reproducibility. It can be used to separate and identify volatile and low molecular weight (ca. 50–600 Da) compounds. The GC unit of the instrument is used to separate the volatile components of a sample and MS identifies separated analytes based on their mass and fragmentation patterns. MS is a specific detector of GC using most commonly electron impact or chemical ionization methods. There is a relatively large variability in combining the GC and MS units; GC-MS instruments may combine one or two GC columns or one or three tandem mass analyzers depending on application fields. The two-dimensional GC is a novel technique, and its combination with MS is very powerful for analyzing volatile and semi-volatile compounds (Bhavyasri et al., 2022; Lakshmi HimaBindu et al., 2013; Upadhyay et al., 2023). Valsecchi et al. recently applied the static headspace GC-MS for MeHg quantification in freshwater mussel tissue using internal standard isotope dilution quantification, after microwave acid digestion and aqueous phase $NaBEt_4$ ethylation. An excellent LOD of 0.0007 μg g^{-1} was achieved (Valsecchi et al., 2021).

GC-ICP-MS combines GC, ICP, and MS into one integrated system and allows even lower LOD and LOQ for HM quantification than GC-MS. Cavalheiro et al. compared the performance of GC-MS and GC-ICP-MS in the quantification of organomercury and organotin compounds in certified bivalve reference samples using isotope dilution. Both techniques were found to exhibit excellent precision and linearity, but

LOQs determined for GC-MS were higher than those of GC-ICP-MS by a factor of nine. Although the higher sensitivity favors GC-ICP-MS, this technique is more expensive than GC-MS considering instrumental, operational, and maintenance costs (Cavalheiro et al., 2014). Combining GC-ICP-MS with preconcentration further increases the methods sensitivity; a LOD of 0.000072 μg g^{-1} (72 ppt) for MeHg quantification was achieved by Taylor et al. using this technique (Taylor et al., 2008).

Other methods

Neutron activation analysis NAA is a nondestructive multielement analytical technique that is based on nuclear reactions and measurement of γ-rays emitted from a sample that was irradiated by neutrons. The method can be applied to the quantification of over sixty elements in a wide range of matrices. The two main steps of NAA, namely activation of the sample in a source of neutrons (e.g., in a reactor) and the γ-ray spectrometry, can be separated (by transferring irradiated samples from the reactor to another laboratory). This latter is used to identify and quantify the induced activity which is proportional to the concentration of elements in the irradiated sample. The activity is compared to known standards irradiated and counted under the same conditions. The advantages of NAA are the simultaneous detection of multiple elements, very low LOD, and ability to analyze solid samples without chemical pre-treatment. The major disadvantage is that it requires a radioactive source, the measurement for long-lived isotopes may take several weeks, and the limitation for certain elements (e.g., Pb cannot be determined by this method) (Bezuidenhout et al., 2015; Hamidatou, 2019; Minc, 2008; Parry, 2019). NAA is rarely used for HM detection in bivalves due to radioactive source requirements (examples are provided in Table 2 and Tables 4–6).

Electrochemical sensors The quantification of HMs in bivalves' soft tissue is not a routine task, and requires proper laboratories, specialists, and the investment for purchasing and running expensive analytical instruments. If currently used instruments could be replaced by fast, selective, reliable, and cheap analytical tools, HM analysis could become a cost effective and routine task, at least for a certain group of HMs. Electrochemical sensors and lab-on-chip electrochemical devices may fulfill these requirements. In addition, these tools have the potential to be miniaturized, which could open the door for field applications by manufacturing portable instruments. An electrochemical sensor is an analytical tool that converts a chemical signal into quantifiable signal that can be monitored. A typical sensor contains three key elements, namely a receptor that directly interacts with and recognizes the analyte, a transducer that translates the physical or chemical change induced by the receptor-target interaction into measurable current, and a signal-processing unit. In principle, the variability of sensor platforms is extremely large, and the aim of the sensor research is to find the right sensor platform which fits the analytical purpose (Karthik et al., 2022; Krebsz et al., 2017; Pasinszki et al., 2017; Simões & Xavier, 2017; Tung et al., 2017). The application of ES for HM determination in bivalves, however, is currently in its infancy, and only a very limited number of applications are published to date. The challenges are finding the right receptor and transducer which can recognize and selectively quantify large number of HMs simultaneously. It is important to note in this respect that Locatelli et al. applied differential pulse anodic stripping voltammetry in 1999 to simultaneously determine the Cd, Cu, Pb and Zn concentration in clam, mussel, and oyster samples (Locatelli, 2000; Locatelli et al., 1999). Pizarro et al. developed in 2020 an inexpensive ES for the quantification of Cd and Pb in clams at the trace level by using square wave anodic stripping voltammetry. The sensor was based on a glassy carbon electrode modified with graphene quantum dots and Nafion and showed very good results when compared with certified samples and the ICP-OES method (Pizarro et al., 2020). Under optimized conditions, the sensor exhibited a linearity range of 20–200 μg L^{-1} with a LOD of 11.30 μg L^{-1} for Cd and 8.49 μg L^{-1} for Pb, respectively. Skriba et al. developed in 2023 a rapid and inexpensive electrochemical sensor for the simultaneous quantification of Cd, Cu, Pb, and Zn in mussel tissue based on anodic stripping voltammetry and using thick film modified graphite electrodes (Skiba et al., 2023). The sensor exhibited a LOD of 0.0005 and 0.0040 μg g^{-1} for Cd and Pb, respectively.

Comparison of the performance of instrumental techniques

There is no doubt that the most widely used methods in the quantification of HMs in clams, mussels, and oysters are AAS, ICP-OES and ICP-MS (see Tables 4–6) (Donati et al., 2017; Hill & Fisher, 2017a, b; Wilschefski & Baxter, 2019). These methods are sensitive, although the detection sensitivity differs considerably according to the metal. As a rule of thumb, ICP-MS can detect HMs at the ppt and ICP-OES at the ppb levels. The sensitivity of AAS using electrothermal or cold vapor/hydride generation techniques is between those of ICP-OES and ICP-MS. LOD is higher for flame atomization AAS than that of ICP-OES (Wilschefski & Baxter, 2019). It is important to note that the performance of these instruments strongly depends on operating conditions. Although AAS methods, using cold vapor technique (typically for Hg), hydride generation method (e.g., for As and Se), flame or electrothermal atomization, are sensitive, the major disadvantage of AAS is that AAS only allows single-element determination at a specific wavelength, thus providing slow analytical performance if several elements are to be quantified. ICP-OES and ICP-MS, on the other hand, can simultaneously determine multiple elements. It may be considered by selecting the method that the running costs of ICP-OES/MS are higher than that of AAS. If only one element is the target of the analysis, the application of AAS is advantageous, as the throughput for one element is faster than that of ICP-OES/MS. Similarly, problematic elements for ICP methods can be determined by AAS. Mercury is well-known to cause memory effects for ICP methods, and in severe cases may prevent the quantification (Pasinszki et al., 2020). Memory effects may be overcome by using strongly oxidizing solutions, or by adding Au to the sample at its final dilution stage before the analysis to obtain a gold-mercury amalgam. Ferraris et al. reported recently that the optimized concentration of Au additive (100 μg L^{-1}) prevented mercury losses and memory effects (Ferraris et al., 2021). Nonetheless, it is important to note that several research groups are still using AAS for Hg determination and ICP-OES/MS for the simultaneous determination of all other HM elements (see Tables 4–6). ICP-MS is clearly the most sensitive technique and provides the isotope distribution of elements. The major drawback is the cost of the instrument (roughly two times higher than that of ICP-OES, which latter is more expensive than AAS instruments), high degree of maintenance requirement, and high level of staff expertise. ICP-OES is more robust than ICP-MS, tolerate higher level of total dissolved solids or suspended solids, and as a simpler method, relative maintenance and operator skill requirements are lower. If the analyte concentration is above about 10 ppb and the measurement of elemental isotope ratios, quantification of lanthanide and actinide elements, and speciation capability are not required, which are best addressed by ICP-MS, ICP-OES is the best option to use in routine analysis. A comparison of major advantages and disadvantages of instrumental techniques that have been used to date for HM quantification in clams, mussels and oysters is shown in Table 3.

Quality assurance and control

Quality and reliability of the final analytical results is of key importance for the credibility of measured HM contamination data; therefore, laboratories are required to use validated analytical protocols and methods for HM quantification (Lakshmi HimaBindu et al., 2013; Taverniers et al., 2004; Taverniers et al., 2010). Several protocols have been published in the literature for HM quantification to date based on in-house validation (see Tables 4–6) and/or using standardized methods approved by regulatory bodies (for example the U.S. Environmental Protection Agency or the Association of Official Analytical Chemists). If the laboratory is using a previously fully validated international protocol, extensive validation studies are not required, and the laboratory must verify only that it can reproduce the same performance characteristics as outlined in the validated protocol. In general, in-house validation involves in this case the determination of performance characteristics such as accuracy (precision and bias), LOQ, LOD, sensitivity, specificity, reproducibility, stability, linearity of the operational range, and recovery of analytes, as well as the applicability of the method for the sample matrix. Certified reference materials (CRMs) from scientific bodies and certified standard solutions (CSSs) of

Table 3 Comparison of instrumental techniques used for HM quantification in clams, mussels, and oysters

Method	Advantage	Disadvantage
ICP-OES	Multiple element determination More robust than ICP-MS Large analytical range High sample throughput	Less sensitive than ICP-MS (ppb) High level of staff expertise
ICP-MS	Multiple element determination Isotope distribution of elements Sensitive (low LOD, ppt) Large analytical range High sample throughput	Vulnerable instrument Expensive High maintenance requirement High level of staff expertise
AAS	Cost effective Relatively simple operation Low LOD for graphite furnace and cold vapor/hydride generation AAS Relatively interference free	Single element determination Low sample throughput Limited analytical range High LOD for flame AAS
AFS	Sensitive (low LOD) if combined with laser source Equipment cost of flame AFS	Single element determination Limited analytical range Expensive with laser sources High LOD for flame AFS
HPLC	Multiple element determination Can be coupled with ICP-MS	HMs must be converted into mobile phase soluble metal complexes
GC	Can be coupled with ICP-MS	Only for volatile or derivatized volatile compounds of HMs
NAA	Multiple element determination Nondestructive Chemical pre-treatment of samples is not necessary	Requires a radioactive source Very long measurement time Limitation for certain elements
EDXRF	Multiple element determination Nondestructive Chemical pre-treatment of samples is not necessary	Limited by its LOD > 1 ppm
ES	Cheap tool Large variability of potential electrochemical methods and sensor platforms	Limited for multiple element determination Field for HM quantification in bivalves is not developed yet

elements, both single and multi-element, from chemical suppliers are available on the market to assist in-house validation. CSSs are also used as internal standards and matrix spikes. Measuring both matrix spike and laboratory control samples is important for quality assurance as the former demonstrate the applicability of the overall analytical method to the specific sample matrix and the latter prove that the analytical system is performing well in a matrix free of interferences from the sample. The determination of both performance characteristics and HM concentration in biota samples require sample replicates or split samples for reliable statistical data analysis. In practice, three replicates are typically used, but the number of replicates must be higher for samples which have high natural variability of the analyte. Instrument performance must be verified over the course of the instrumental run by including quality control standards in the sample analysis sequence, for example one after twenty samples (5% frequency). Sample set for example for ICP-MS and ICP-OES typically consists of reagent blank, negative control, positive control(s) and samples, as well as one quality control sample/twenty samples.

Conclusions

Heavy-metal pollution of the aquatic environment is a global problem, which creates environmental hazard and therefore requires continuous monitoring. Filter-feeder bivalves such as clams, mussels and oysters have a natural tendency to accumulate heavy-metals

in their soft tissue. This accumulation creates health risk for humans as HMs can be transferred via the food chain. HM accumulation in bivalves results in multi-HM-element profiles, reflecting their habitat conditions, and can be used to monitor pollution in the environment where they reside. There is therefore a high demand, for both food security and environmental monitoring, to test bivalves for HMs. The determination of HMs in bivalves is, however, challenging because of their trace levels and complex sample matrices. Several methods, including the digestion of the soft tissue and measuring the HM concentration in the digest, have been developed to date to screen for the presence of HMs in bivalves. Although these methods are sensitive and able to detect low concentrations of HMs in bivalves' soft tissue (from the ppm down to ppt level), they are labour-intensive, time-consuming, and require laboratory facilities with well-trained technicians. Depending on the method, the HM quantification may take several days, including one to three days for preparing the tissue extract and additional half to two days for instrumental determination of HMs' concentration in the extract depending on the instrumental technique and analyte numbers. There is therefore a high demand to reduce analysis time and to increase sensitivity of the method.

Determination of total HMs and metal organic species in tissue are performed in two separate analysis as total HM determination allows the complete dissolution of the sample matrix, whereas metal speciation requires mild conditions for not to break the metal-ligand bond. Previous studies, namely 96% of publications (Tables 4–6), have mainly focused on total metal content, and little was concerned on the metal-organic species, which latter may be more important for understanding the toxicity and metabolic characteristics of HMs than the total HM concentration. For total HM determination, the acid digestion is currently the method of choice and provides good recoveries for HMs. Microwave digestion is gradually replacing the conventional lengthy hot-plate digestion procedures because it substantially shortens the digestion time, prevents contamination as it is performed in a closed vessel, and reduces analyte loss due to volatilization. Bivalves contain large amounts of water, therefore, drying the tissue is required to reach low LOD. Drying, however, is time consuming. Improving instrumental detection limit and/or applying preconcentration could help in leaving out the drying step and speeding up the process. Preconcentration, however, is still not routinely applied in laboratories for HM quantification in clams, mussels, and oysters and might be a promising future direction. Similarly, if a relatively higher LOD is acceptable, due to relatively high concentration of HMs in tissue, the drying step may become unnecessary. Measuring HMs simultaneously provides a drastic reduction in analysis time, therefore instruments able to perform simultaneous multielement analysis, namely ICP-OES and ICP-MS, are becoming more and more popular. New development in this field, such as ICP-OES/MS with reduced argon consumption, contributes to decreasing instrument running costs. It is worth to note that if the number of target analytes is limited, AAS is expected to continue wide scale application in this field as it is highly sensitive and instrumental costs are lower than those of ICP-OES and ICP-MS. The development of multielement AAS would certainly boost the application of this technique. AFS utilizing laser sources is an emerging technique due to its superior sensitivity. Recent developments and application of coupled techniques such as HPLC-ICP-MS, GC-ICP-MS and GC-MS are pointing toward increasing interest in speciation of HMs in soft tissue. Considering cost-effectiveness, sensitivity, and analysis time, electrochemical sensors and the lab-on-chip concept are very attractive. It is expected that gaps in these technologies, such as simultaneous detection of large number of analytes, can be filled in the future. Research in this field is expected to move toward shorter multielement analysis, replacing the corrosive and toxic acids in digestion, and reduced instrumental running costs, as well as speciation of metal compounds in soft tissue. It is also reasonable to expect that manual sample preparation methods will be replaced by automatic systems, as well as the coupling of these systems with analytical instruments to improve reproducibility, decrease analysis time, and reduce labor.

Appendix

Table 4 Determination of heavy metal (HM) content of clam's soft tissue

Clam[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Anomalocardia brasiliana	Freeze-dried; ultrasound (15 min) + centrifugation (30 min), dilute HCl, HNO_3 and CH_3COOH, 25–30 °C	ICP-OES[4]	Cd, Cu, Mn, Zn (Ca, K, Mg, Na, Sr)	(dos Santos et al., 2010)
Anomalocardia brasiliana	Freeze-dried; microwave, cc HNO_3, cc H_2SO_4, cc H_2O_2, 30 min, 90–220 °C	ICP-OES	As, Cd, Pb, Zn (Se)	(Costa et al., 2009)
Austrovenus stutchburyi	Freeze-dried for 48 h; HNO_3 at 70 °C, ashing at 450 °C for 24 h, HNO_3 + HCl at 80 °C	ICP-OES	Cu, Cr, Mn, Ni, Zn	(Peake et al., 2006)
Laternula elliptica	Freeze-dried for 48–72 h; microwave, cc HNO_3	ICP-OES[5]	Cd, Cr, Cu, Fe, Mn, Pb, Zn	(Vodopivez et al., 2015)
Venus antiqua *Mesodesma donacium*	Freeze-dried for 24 h; microwave, cc HNO_3 + cc H_2O_2	ICP-OES	Cd, Pb	(Pizarro et al., 2020)
Meretrix lusoria *Ruditapes variegata*	Freeze-dried; cc HNO_3, reflux for 1 h, + cc $HClO_4$, reflux, 0.5 h or + H_2SO_4, reflux, 1 h	ICP-OES	As, Cd, Cr, Cu, Ni, Pb, Zn	(Lin et al., 2004)
n.a.	cc HNO_3 overnight, then boiling	ICP-OES	Cd, Cr, Cu, Ni, Pb, Zn	(Grant & Ellis, 1988)
Ensis directus	Drying at 70 °C, 24 h; mineralization at 450 °C for 48 h; dissolved in aq. HNO_3	ICP-OES	Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn (Al, B, Ba, Ca, K, Li, Mg, Na, Sr)	(Lozano-Bilbao et al., 2018)
Macoma balthica	cc HNO_3 + cc HCl + cc H_2O_2, overnight; microwave for 30 min	ICP-OES	Cd, Cu, Ni, Pb, Zn	(Thomas & Bendell-Young, 1998)
Macoma balthica	Dried; reflux in cc HNO_3	ICP-OES	Ag, Cd, Co	(Griscom et al., 2002)
Trivela mactroidea	Drying at 80 °C for 24 h; cc HNO_3 at 70 °C for 8 h, + cc H_2O_2 at 70 °C for 4 h	ICP-OES	Cd, Cr, Cu, Ni, Zn	(LaBrecque et al., 2004)
Donax obesulus	Microwave, cc HNO_3 + cc HCl, 170 °C for 7 min	ICP-OES	Cd, Cu, Pb	(Many et al., 2021)
Paphia malabarica	Microwave, cc HNO_3 + cc $HClO_4$, 200 °C, 40 min	ICP-OES[6]	Co, Cu, Fe, Mn, Ni, Pb, Zn	(Chinnadurai et al., 2022)
Venerupis philippinarum *Glycymeris glycymeris*	Microwave, cc HNO_3 + cc H_2O_2, 200 °C, 50 min	ICP-OES	As, Cd, Hg, Pb	(Lehel et al., 2018)
n.a.	Microwave, cc HNO_3, 125–155 °C, 1 h	ICP-OES[7] ICP-MS[8]	As. Cd, Pb	(Sheppard et al., 1994)
n.a.	Freeze-drying; microwave, cc H_2O_2 + cc HNO_3, 70–180 °C, 1 h; SPE using MGO@SiO_2-APTES-IL	ICP-MS[9]	Cd, Cu, Pb	(Dong et al., 2021)

Table 4 (continued)

Clam[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Anadara granosa	Dried at 40 °C; microwave, cc HNO_3	ICP-MS	As, Cd, Cr, Co, Hg, Pb	(Januar et al., 2019)
Corbicula fluminea	Freeze-dried; microwave, cc HNO_3 + cc H_2O_2, 120–190 °C, 1 h	ICP-MS	Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb	(Jia et al., 2018)
Corbicula fluminea	Dried at 60 °C for 8–16 h; n.a.	ICP-MS	As, Cd, Cr, Cu, Ni, Pb, Zn	(Diwa et al., 2022)
Corbicula fluminea	Dried at 60 °C for 16 h; 7 M HNO_3, 125 °C, 2 h; cc H_2O_2, 120 °C; HNO_3, 115 °C, 3–5 h	ICP-MS	As, Cd, Cu, Cr, Ni, Pb, Zn	(Elvira et al., 2021)
Corbicula javanica	Dried at 60 °C for 72 h; microwave, cc HNO_3 + cc H_2O_2 at 200 °C for 25 min	ICP-MS	As, Co, Cd, Cr, Mn	(Yap et al., 2021)
Macoma balthica	Dried at 50 °C for 24 h; cc HNO_3, 1 h at room temp, 4–6 h at 110 °C; cc H_2O_2, 1 h	ICP-MS[10]	Cd, Cu, Ni, Pb, Zn	(Thomas & Bendell-Young, 1998)
Macoma nasuta	Freeze-drying; microwave, cc HNO_3	ICP-MS[11]	As, Cd, Cr, Cu, Hg, Ni, Pb, Zn	(Weston & Maruya, 2002)
Macoma nasuta	Freeze-drying; cc HNO_3, 120 °C, 6 h	ICP-MS	Ag, Cd, Cr, Cu, Mn, Fe, Ni, Pb, Zn (Be)	(Lu et al., 2005)
Mactra veneriformis	Freeze-dried for 72 h; microwave, cc HNO_3 + cc H_2O_2	ICP-MS	As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Sb, Pb, Zn (Se)	(Li et al., 2015)
Marcia Marmorata	Drying at 80 °C for 72 h; cc HNO_3 + cc H_2O_2, 160 °C for 5 h	ICP-MS	As, Cd, Cu, Zn	(Lias et al., 2013)
Mya arenaria *Nuttallia obscurata*	Drying at 105 °C for 30 min; nitric acid	ICP-MS	As, Cd, Pb	(Andrade-Rivas et al., 2022)
Ruditapes decussatus *Ruditapes phylippinarum* *Chamelea gallina* *Venus verrucosa*	Microwave, cc HNO_3 + cc H_2O_2 + cc HF	ICP-MS[12]	Cd, Hg, Pb	(Barchiesi et al., 2020)
Ruditapes philippinarum *Paphia undulata* *Meretrix meretrix* *Sinonovacula constricta* *Meretrix lyrata*	Wet sample, cc HNO_3 + cc H_2O_2, r.t. overnight, 120 °C for 30 min, 140 °C for 20 min, 160 °C for 60 min, 140 °C for 100 min	ICP-MS	As, Cd, Cu, Cr, Hg, Pb, Zn	(Qin et al., 2021)
Meretrix lyrata	Microwave, cc HNO_3 + cc H_2O_2, 46 min	ICP-MS	As, Cd, Hg, Pb	(Dang et al., 2022)
Scapharca subcrenata *Meretrix meretrix* *Amusium* *Mactra veneriformis* *Mya arenaria*	Freeze-dried; cc HNO_3 overnight at r.t., + cc H_2O_2, microwave for 1.5 h	ICP-MS[13]	As, Cd, Cr, Cu, Ni, Pb, Zn	(Chen et al., 2022)

Table 4 (continued)

Clam[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Sinonovacula constricta	1:1 aq. HNO_3 at 80 °C; microwave, cc HNO_3 + cc H_2O_2, 10 min	ICP-MS	As, Cu, Fe, Ni, Pb, V	(Tu et al., 2014)
Meretrix meretrix *Panopea abrupta* *Paphia undulate*	Drying at 105 °C; cc HNO_3, r.t. for 30 min, microwave, 130–190 °C, 30 min	ICP-MS	As, Cd, Pb	(Zhu et al., 2017)
Chamelea gallina *Donax trunculus*	Microwave, cc HNO_3 + cc H_2O_2, 200 °C, 20 min	ICP-MS	As, Hg, Cd, Co, Cr, Cu, Fe, Ni, Sn, Pb, Mn, Zn (Al)	(Ozden et al., 2009)
Polymesoda expansa	Dried at 80 °C, overnight; cc HNO_3 overnight, 100 °C for 2 h, + cc H_2O_2, heating	ICP-MS	Cd, Ni	(Rohalin et al., 2019)
Potamocorbula laevis	Freeze-drying; cc HNO_3 at 80 °C for 8 h	ICP-MS	Cu	(Cao et al., 2023)
Psammotaea elongata	Freeze-drying, 24 h; cc HNO_3 at 95 °C, 75 min	ICP-MS	Cd, Cu, Co, Pb, Zn	(Shaari et al., 2015)
Tapes philippinarum	Microwave, cc HNO_3 + cc H_2O_2, 120–190 °C, 60 min	ICP-MS[14]	Cd, Hg, Pb	(Miedico et al., 2015)
Tapes decussatus	cc HNO_3 at r.t. for 8 h + at 85 °C for 8 h	ICP-MS[15]	As, Cd, Hg, Pb	(Falco et al., 2006)
Tapes decussates	Microwave, cc HNO_3 + cc H_2O_2,	ICP-MS[16]	Cd, Pb	(Pastorelli et al., 2012)
Tapes decussates, *Chamelea gallina,* *Cardium edule*	Freeze-drying; microwave, cc HNO_3 + cc H_2O_2, 24 min	ICP-MS[17]	As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Tl, V, Zn (Be, Se)	(Culotta et al., 2008)
Circenita callipyga *Barbatia helblingii* *Solen brevis* *Amiantis umbonella*	Freeze-drying for 24 h; microwave, cc H_2SO_4 + cc HNO_3 + cc H_2O_2, 90 °C, 30 min	ICP-MS[18]	As, Cd, Co, Cr, Cu, Fe, Mo, Mn, Ni, Pb, Sb, Zn (Al)	(Jahromi et al., 2021)
Solen brevis	Drying at 65 °C for 72 h; heating with a mixture of cc HNO_3, cc H_2O_2, cc HCl and cc H_2SO_4	ICP-MS	Cd, Cu, Fe, Mn, Pb, Zn	(Kamaruzzaman et al., 2010)
Solen marginatus	Wet sample; microwave, cc HNO_3 + cc H_2O_2 + H_2O	ICP-MS	As, Bi, Cd, Co, Cr, Cu, Ga, Mn, Ni, Pb, Tl, U, V, Zn (Be, Cs, Se, Sr)	(Esposito et al., 2022)
Ruditapes philippinarum	Freeze-dried for 72 h; cc HNO_3 at r.t. for 24 h, 125 °C for 3 h, evaporated; cc HNO_3 + cc H_2O_2 at 125 °C for 24 h	ICP-MS	Co, Ni, Cu, Mn, Fe, Zn, Cr, As, Pb, Cd (Se)	(Vieira et al., 2022)
Meretrix meretrix *Ruditapes philippinarum*	Dried at 105 °C for 24 h; cc HNO_3 at r.t., cc HNO_3 + cc H_2O_2 at 180 °C	ICP-MS[19]	As, Cd, Cr, Cu, Pb, Zn	(Liu et al., 2022)

Table 4 (continued)

Clam[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Ruditapes philippinarum *Mactra chinensis* *Scapharca subcrenata*	Microwave, cc HNO_3 at 195 °C for 20 min	ICP-MS[20]	As, Cd, Cr, Hg, Ni, Pb	(Pan & Han, 2023)
Venerupis decussatus	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	ICP-MS	Pb	(Maanan, 2008)
Venerupis decussatus	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	AAS	Cd, Cr, Cu, Mn, Zn, Ni	(Maanan, 2008)
Arca noae	Dried at 80 °C for 48 h; microwave, cc HNO_3, 17 min	AAS	Cd, Cu, Fe, Pb, Zn, (Se)	(Said et al., 2022)
Barbatia decussate	Heating in cc HNO_3 + cc H_2O_2 at 130 °C	AAS	Cd, Cr, Hg Ni, Pb	(Khoei, 2022)
Circenita callipyga *Barbatia helblingii* *Solen brevis* *Amiantis umbonella*	Freeze-dried; aqua regia at 90 °C	AAS	Hg	(Jahromi et al., 2021)
Corbula gibba	Dried; hot cc HNO_3	AAS	Cu, Cr, Fe, Mn, Zn, Pb	(Mauri et al., 2004)
Corbicula javanica	Dried at 60 °C for 72 h; microwave, cc HNO_3 + cc H_2O_2 at 200 °C for 25 min	AAS	Cu, Fe, Ni, Pb, Zn	(Yap et al., 2021)
Chamelea gallina	Dried at 80 °C; microwave, HCl + HNO_3	AAS	Cd, Cu, Fe, Zn	(Belbachir et al., 2013)
Spisula solida *Venus striatula*	Freeze-dried; microwave, cc HNO_3	AAS	Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn	(Langston et al., 1999)
Scrobiculuria plana	Dried at 80 °C; refluxing hot HNO_3; evaporated; dissolved in dilute aq. HCl	AAS	Ag, Cd, Co, Cu, Pb, Zn	(Luoma & Bryan, 1982)
Mactra veneriformis *Ruditapes philippinarum*	Freeze-drying for 72 h; cc HNO_3 + H_2O_2, heating	AAS	Cd, Cr, Cu, Pb, Zn	(Meng et al., 2014)
Mactra sp. *Anadara inaequivalvis* *Villorita cyprinoides*	Sun-dried; HNO_3 (35%) + $HClO_4$ (70%) + H_2SO_4 (98%), heated at 60 °C	AAS	Cd, Cr, Cu, Pb, Zn	(Anagha et al., 2022)
n.a.	Drying in vacuum at 80 °C overnight; microwave, cc HNO_3, 20 min; + cc H_2O_2, 20 min	AAS[21]	Cd, Cu, Pb, Zn	(Yaru et al., 1999)
Tivela mactroidea	Drying at 80 °C for 24 h; cc HNO_3 at 70 °C for 8 h, + cc H_2O_2 at 70 °C for 4 h	AAS	V, Pb	(LaBrecque et al., 2004)

Table 4 (continued)

Clam[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Tivela tripla	Dried at 60 °C; cc $HNO_3 + HClO_4 + H_2SO_4$	AAS	Cu, Cr, Mn, Zn	(Blankson et al., 2022)
Scrobiculuria plana	Dried at 80 °C; refluxing hot HNO_3; evaporated; dissolved in dilute aq. HCl	AAS	Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb	(Bryan et al., 1980)
Scrobiculuria plana	Digestion with HNO_3, H_2SO_4 and H_2O_2	AAS	Hg	(Bryan et al., 1980)
Scrobiculuria plana	Mixed with $MgO/Mg(NO_3)_2$, dry ashed at 500 °C; dissolved in aq. HCl, reduced with $NaBH_4$	AAS	As, Sn	(Bryan et al., 1980)
Macoma balthica	Dried at 50 °C for 24 h; cc HNO_3, 1 h at room temp, 4–6 h at 110 °C; cc H_2O_2, 1 h	AAS[22]	Hg	(Thomas & Bendell-Young, 1998)
Macoma balthica	Freeze-drying; microwave, cc HNO_3	AAS	Cu, Fe, Mn, Ni, Pb, Zn	(Sokolowski et al., 2007)
n.a.	cc HNO_3 overnight, then boiling	AAS	Cd, Cr, Cu, Ni, Pb, Zn	(Grant & Ellis, 1988)
Corbicula fluminea	cc HNO_3, 95 °C for 3 h	AAS	Cd	(Tran et al., 2001)
Polymesoda Expansa *Anadara granosa*	Dried at 60 °C for 48 h; $HClO_4 + HNO_3$, 120 °C for 3 h	AAS	Cd, Cu, Fe, Pb, Zn	(Dabwan & Taufiq, 2016)
Corbicula fluminea *Polymesoda caroliniana*	Freeze-drying for 72 h; cc HNO_3, 3 h at 120 °C	AAS	Cd, Cu, Pb, Zn	(Ruelas-Inzunza et al., 2009)
Donax trunculus *Cerastoderma edule*	Freeze-dried for 18 h; microwave, aq. H_3PO_4/KH_2PO_4 buffer, SDS, Triton X-100 surfactant	AAS[23]	Cd, Co, Cr, Ni, Pb	(Hernandez-Martinez et al., 2016)
Mecenaria mecenaria	Dried at 60 °C; cc HNO_3 + cc H_2O_2	AAS	Cr, Cu, Fe, Mn, Ni, Pb, Zn	(Rule, 1985)
Macoma nasuta	Freeze-drying; cc HNO_3, 120 °C, 6 h	AAS	Hg	(Lu et al., 2005)
Meretrix lusoria *Ruditapes variegata*	Freeze-dried; cc HNO_3 at 80 °C	AAS	Hg	(Lin et al., 2004)
Tapes decussates	Microwave, cc HNO_3 + cc H_2O_2	AAS[24]	Hg	(Pastorelli et al., 2012)
Tapes philippinarum	aq. $K_2Cr_2O_7$ + cc H_2SO_4, 180 °C for 60 min; H_2O added, boiling for 30 min	AAS	Hg	(Locatelli et al., 1999)
Tapes philippinarum	Drying at 45 °C for 24 h; aq. $K_2Cr_2O_7$ + cc H_2SO_4, 180 °C for 60 min; H_2O added, boiling for 30 min	AAS	Hg	(Locatelli, 2000)
Anadara granosa	Freeze-dried; microwave, cc HNO_3	AAS	Cd, Cu, Pb	(Yusof et al., 1994)

Table 4 (continued)

Clam[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Potamocorbula laevis	Freeze-dried; cc HNO_3 + cc $HClO_4$, microwave for 2 h	AAS[25] AFS[26]	Cd, Cr, Cu, Pb, Zn As, Hg	(Noman et al., 2022)
Venerupis decussatus	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	AFS	Hg	(Maanan, 2008)
Meretrix meretrix *Ruditapes philippinarum*	Dried at 105 °C; cc HNO_3 + cc $HClO_4$	AFS[27]	Hg	(Liu et al., 2022)
n.a.	Dilute HNO_3, ultrasound, 1 h	HPLC-ICP-MS[28]	H_3AsO_3, H_3AsO_4	(Liao et al., 2020)
n.a.	Dilute HCl, ultrasound, 1 h	HPLC-ICP-MS	MeHg	(Liao et al., 2020)
Anadara granosa	Freeze-dried; microwave, cc HNO_3; buffered, eluted on a Chelex-100 resin	NAA	As, Co, Cr, Hg, Sb, Zn	(Yusof et al., 1994)
Tapes philippinarum	cc HNO_3 + cc H_2SO_4, 150 °C for 4 h	ES	Cu, Cd, Pb, Zn	(Locatelli et al., 1999)
Tapes philippinarum	Dried at 45 °C for 24 h; cc HNO_3 + cc H_2SO_4, 140 °C for 3 h	ES	Cu, Cd, Pb, Zn	(Locatelli, 2000)
Venus antiqua *Mesodesma donacium*	Freeze-dried for 24 h; microwave, cc HNO_3 + cc H_2O_2; formate buffer pH 4.5	ES	Cd, Pb	(Pizarro et al., 2020)

[1] n.a. = not available; [2] non-heavy-metals are in parenthesis; [3] LOD in μg g^{-1} (ppm): [4] 0.012–2.62 μg g^{-1}; [5] 0.05–0.3 μg g^{-1}; [6] 0.00033–0.01518 μg g^{-1}; [7] 0.2–2.7 μg g^{-1}; [8] 0.0002–0.0028 μg g^{-1}; [9] 0.00242–0.00375 μg L^{-1}; [10] 0.01–0.2 μg g^{-1}; [11] ca. 1 μg g^{-1}; [12] 0.0015–0.0076 μg g^{-1}; [13] 0.01–3.67 μg g^{-1}; [14] 0.001–0.004 μg g^{-1}; [15] 0.02–0.05 μg g^{-1}; [16] 0.02–0.05 μg g^{-1}; [17] 0.006–0.08 μg g^{-1}; [18] 0.01–1 μg g^{-1}; [19] 0.03–1.66 μg g^{-1}; [20] 0.0003–0.005 μg g^{-1}; [21] 0.004–0.05 μg g^{-1}; [22] 0.004 μg g^{-1}; [23] 0.02–0.05 μg g^{-1}; [24] 0.5 μg g^{-1}; [25] 0.005–0.4 μg g^{-1}; [26] 0.002–0.2 μg g^{-1}; [27] 0.002 μg g^{-1}; [28] 0.0083 and 0.0052 μg L^{-1}

Table 5 Determination of heavy metal (HM) content of mussel's soft tissue

Mussel[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Mytella guyanensis	Freeze-dried; ultrasound (15 min) + centrifugation (30 min), dilute HCl, HNO_3 and CH_3COOH, 25–30 °C	ICP-OES[4]	Cd, Cu, Mn, Zn (Ca, K, Mg, Na, Sr)	(dos Santos et al., 2010)
Mytella guyanensis	Freeze-dried; microwave, cc HNO_3, cc H_2SO_4, cc H_2O_2, 30 min, 90–220 °C	ICP-OES	As, Cd, Pb, Zn (Se)	(Costa et al., 2009)
Mytella guyanensis	Freeze-dried; microwave, cc HNO_3 + cc H_2O_2	ICP-OES	As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn (Ba)	(Santos & Boehs, 2021)
Mytilus galloprovincialis	Drying at 70 °C, 24 h; mineralization at 450 °C for 48 h; dissolved in aq. HNO_3	ICP-OES	Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn (Al, B, Ba, Ca, K, Li, Mg, Na, Sr)	(Lozano-Bilbao et al., 2018)
Mytilus galloprovincialis *Mytilus edulis*	Microwave, cc HNO_3 + cc H_2O_2, 200 °C, 50 min	ICP-OES	As, Cd, Hg, Pb	(Lehel et al., 2018)
Mytilus galloprovincialis	Freeze-dried for 48 h; HNO_3 at 70 °C, ashing at 450 °C for 24 h, HNO_3 + HCl at 80 °C	ICP-OES	Cu, Cr, Mn, Ni, Zn	(Peake et al., 2006)
Mytilus galloprovincialis	Freeze-dried for 48 h; cc HNO_3 + cc H_2O_2	ICP-OES	Cr, Cd, Hg, Pb (Se)	(Santos et al., 2014)
Mytilus edulis	Dried at 60–80 °C; microwave, cc HCl + cc HNO_3, 30 min	ICP-OES	Cd, Cu, Pb, Zn	(Bray et al., 2015)
Perna perna *Choromytilus meridionalis*	Air dried; microwave, cc HNO_3 + cc H_2O_2, 200 °C, 70 min	ICP-OES	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn (Al)	(Dahms et al., 2014)
Anodonta grandis	Microwave, cc HNO_3	ICP-OES	Cd, Cu, Zn	(Couillard et al., 1993)
n.a.	cc HNO_3 overnight, then boiling	ICP-OES	Cd, Cr, Cu, Ni, Pb, Zn	(Grant & Ellis, 1988)
Brachidontes pharaonis	Microwave, HNO_3	ICP-OES	Cd, Cu, Pb, Zn	(Telahigue et al., 2022)
Perna viridis	Microwave, cc HNO_3 + cc $HClO_4$, 200 °C, 40 min	ICP-OES[5]	Co, Cu, Fe, Mn, Ni, Pb, Zn	(Chinnadurai et al., 2022)
Perna perna	Dried at 60 °C; cc HNO_3	ICP-OES	Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	(Ferreira et al., 2004)
Perna perna	Dried at 60 °C; n.a.	ICP-MS[6]	As, Cd, Cr, Cu, Fe, Ni, Pb, Zn	(de Souza et al., 2021)
Perna viridis	Dried at 60 °C; cc HNO_3, 100 °C, 8 h	ICP-MS	Cd, Cu, Pb, Zn	(Sheng et al., 2021)
Perna viridis	Dried at 105 °C for 24 h; cc HNO_3 at r.t., cc HNO_3 + cc H_2O_2 at 180 °C	ICP-MS[7]	As, Cd, Cr, Cu, Pb, Zn	(Liu et al., 2022)
Perna canaliculus	Dried overnight at 60 °C; microwave, cc HNO_3 + cc H_2O_2, 180 °C, 70 min	ICP-MS	As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Zn	(McDougall et al., 2020)

Table 5 (continued)

Mussel[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Species n.a.	Freeze-drying; microwave, cc H_2O_2 + cc HNO_3, 70–180 °C, 1 h; SPE using MGO@SiO_2-APTES-IL	ICP-MS[8]	Cd, Cu, Pb	(Dong et al., 2021)
Mytilus galloprovincialis *Mytilus edulis* *Modiolus barbatus*	Microwave, cc HNO_3 + cc H_2O_2 + cc HF	ICP-MS[9]	Cd, Hg, Pb	(Barchiesi et al., 2020)
Mytilus edulis	Freeze-dried; cc HNO_3 overnight at r.t., + cc H_2O_2, microwave for 1.5 h	ICP-MS[10]	As, Cd, Cr, Cu, Ni, Pb, Zn	(Chen et al., 2022)
Mytilus galloprovincialis	Microwave, cc HNO_3 + cc H_2O_2, 120–190 °C, 60 min	ICP-MS[11]	Cd, Hg, Pb	(Miedico et al., 2015)
Mytilus galloprovincialis	Microwave, cc HNO_3 + cc H_2O_2,	ICP-MS[12]	Cd, Pb	(Pastorelli et al., 2012)
Mytilus galloprovincialis	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	ICP-MS	Pb	(Maanan, 2008)
Mytilus galloprovincialis	Wet sample, cc HNO_3 + cc HCl	ICP-MS	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sn, Zn (Al, Ba, Se)	(Bezuidenhout et al., 2015)
Anodonta woodiana	Freeze-dried; microwave, cc HNO_3 + cc H_2O_2, 120–190 °C, 1 h	ICP-MS	Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb	(Jia et al., 2018)
Mytilus galloprovincialis	cc HNO_3 at r.t. for 8 h + at 85 °C for 8 h	ICP-MS[13]	As, Cd, Hg, Pb	(Falco et al., 2006)
Mytilaster lineatus	Microwave, cc HNO_3	ICP-MS	Cd, Cu, Hg, Pb, Zn	(Bat et al., 2019)
Mytilus edulis	Freeze-drying; hot cc HNO_3, cc HNO_3 + cc H_2O_2; anion exchange chromatography	ICP-MS	Cd, Pb, Zn	(Shiel et al., 2012)
Mytilus edulis	Microwave, cc HNO_3 + cc H_2O_2, 240 °C, 33 min	ICP-MS	As, Cd, Cu, Fe, Hg, Ni, Pb, Zn (Ca, I, Se)	(Ferraris et al., 2021)
Mytilus edulis	Microwave, cc HNO_3 at 195 °C for 20 min	ICP-MS[14]	As, Cd, Cr, Hg, Ni, Pb	(Pan & Han, 2023)
n.a.	Freeze-dried; microwave, 4 M HNO_3, 55 °C overnight; microwave, cc HNO_3, 150 °C, 30 min	ICP-MS	Zn, As, Se, Cd, Hg, Pb	(Taylor et al., 2008)
Dreissena polymorpha	Freeze-dried for 24 h; microwave, cc HNO_3, 17 min	AAS	Cd, Co, Cr, Cu, Hg, Ni, Pb, Zn	(Camusso et al., 2001)
Perna perna	Wet sample; cc H_2SO_4 + cc H_2O_2 (1:4) at ca. 100 °C for 40–60 min	AAS	Cd, Cr, Cu, Pb, Zn	(Avelar et al., 2000)
Perna perna	Dried; cc HNO_3 at 70–90 °C, + H_2O_2 at 135 °C	AAS	Cd, Cu, Fe, Mn, Hg, Zn	(Otchere, 2022)

Table 5 (continued)

Mussel[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Perna viridis	Freeze-dried for 72 h; cc $HNO_3 + H_2O_2$, heating	AAS	Cd, Cr, Cu, Pb, Zn	(Meng et al., 2014)
Perna viridis	Drying at 70–80 °C; cc. HNO_3, overnight at r.t., reflux at 130 °C, 6 h	AAS	Cd, Cr, Cu, Fe, Pb, Ni, Zn	(de Astudillol et al., 2005)
Perna viridis	cc. HNO_3, overnight at r.t., reflux at 130 °C, 3 h; H_2SO_4, HCl, reflux, 3 h; $KMnO_4$	AAS	Hg	(de Astudillol et al., 2005)
n.a.	aq. $K_2Cr_2O_7$ + cc H_2SO_4, 180 °C for 60 min; H_2O added, boiling for 30 min	AAS	Hg	(Locatelli et al., 1999)
Mytilus galloprovincialis	Drying at 45 °C for 24 h; aq. $K_2Cr_2O_7$ + cc H_2SO_4, 180 °C for 60 min; H_2O added, boiling for 30 min	AAS	Hg	(Locatelli, 2000)
Mytilus galloprovincialis	Microwave, cc HNO_3 + cc H_2O_2	AAS[15]	Hg	(Pastorelli et al., 2012)
Mytella strigata *Megalana bilineata* *Perna perna*	Sun-dried; HNO_3 (35%) + $HClO_4$ (70%) + H_2SO_4 (98%), heated at 60 °C	AAS	Cd, Cr, Cu, Pb, Zn	(Anagha et al., 2022)
Mytilus edulis	Drying at 85 °C; hot cc HNO_3	AAS	Cd, Cu, Zn	(Amiard-Triquet et al., 1998)
Mytilus edulis	Freeze-dried; cc HNO_3	AAS	Cr, Fe, Mn	(Hursthouse et al., 2003)
Elliptio complanata	Dried at 105 °C; cc HNO_3 overnight at r.t.; evaporated; cc H_2O_2 at 85 °C for 6 h	AAS	Cu, Fe, Mn, Pb, Zn	(Tessier et al., 1984)
n.a.	cc HNO_3 overnight, then boiling	AAS	Cd, Cr, Cu, Ni, Pb, Zn	(Grant & Ellis, 1988)
Perna viridis	Freeze-dried; microwave, cc HNO_3	AAS	Cd, Cu, Pb	(Yusof et al., 1994)
Mytilus galloprovincialis	Freeze-dried for 18 h; microwave, aq. H_3PO_4/ KH_2PO_4 buffer, SDS, Triton X-100 surfactant	AAS[16]	Cd, Co, Cr, Ni, Pb	(Hernandez-Martinez et al., 2016)
Mytilus galloprovincialis	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	AAS	Cd, Cr, Cu, Mn, Zn, Ni	(Maanan, 2008)
Mytilus galloprovincialis	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	AFS	Hg	(Maanan, 2008)
Perna viridis	Dried at 105 °C; cc HNO_3 + cc $HClO_4$	AFS[17]	Hg	(Liu et al., 2022)
Mytilus edulis	2-mercaptoethanol, L-cysteine, aq. HCl, ultrasonicated at 40 °C for 30 min	HPLC-ICP-MS	MeHg	(Ferraris et al., 2021)

Table 5 (continued)

Mussel[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Mytilus edulis	Diluted aq. $HNO_3 + H_2O_2$, standing overnight; heating at 95 °C for 1 h	HPLC-ICP-MS	H_3AsO_3/H_3AsO_4	(Ferraris et al., 2021)
Mytilus edulis	Microwave, 1 M HCl, 70 °C, 3 min; ethylation	GC-MS	MeHg	(Valsecchi et al., 2021)
n.a.	Freeze-dried; microwave, 4 M HNO_3, 55 °C overnight; purge and trap preconcentration	GC-ICP-MS	MeHg	(Taylor et al., 2008)
Perna viridis	Freeze-dried; microwave, cc HNO_3; buffered, eluted on a Chelex-100 resin	NNA	As, Co, Cr, Hg, Sb, Zn	(Yusof et al., 1994)
Anodonta anatine *Anodonta marginate* *Anodonta implicata*	Dried at 60 °C for 36 h; cc HNO_3 + cc $HClO_4$	NAA	As, Cr, Co, Fe, Lu, Mn, La, Sb, Sm, Th, Zn, U (Ba, K, Na, Rb, Sc, Y)	(Katsallah et al., 2013)
Mytilus galloprovincialis	Dried at 40 °C; no chemical pretreatment	NAA	As, Ag, Co, Cr, Fe, Mn, Mo, Sb, Th, U, V, Zn (Ba, Br, Ca, Cl, Cs, I, Na, Mg, K, Rb, Se, Sr)	(Bezuidenhout et al., 2015)
Mytilus galloprovincialis	Freeze-dried for 48 h; pressed into pellet, no chemical pretreatment	EDXRF	As, Fe, Cu, Zn, (K, Ca, Sr)	(Santos et al., 2014)
n.a.	cc HNO_3 + cc H_2SO_4, 150 °C for 4 h	ES	Cu, Cd, Pb, Zn	(Locatelli et al., 1999)
Mytilus galloprovincialis	Dried at 45 °C for 24 h; cc HNO_3 + cc H_2SO_4, 140 °C for 3 h	ES	Cu, Cd, Pb, Zn	(Locatelli, 2000)

[1] n.a. = not available; [2] non-heavy-metals are in parenthesis; [3] LOD in μg g^{-1} (ppm): [4] 0.012–2.62 μg g^{-1}; [5] 0.00033–0.01518 μg g^{-1}; [6] 0.0038–0.0461 μg g^{-1}; [7] 0.03–1.66 μg g^{-1}; [8] 0.00242–0.00375 μg L^{-1}; [9] 0.0015–0.0076 μg g^{-1}; [10] 0.01–3.67 μg g^{-1}; [11] 0.001–0.004 μg g^{-1}; [12] 0.02–0.05 μg g^{-1}; [13] 0.02–0.05 μg g^{-1}; [14] 0.0003–0.005 μg g^{-1}; [15] 0.5 μg g^{-1}; [16] 0.02–0.05 μg g^{-1}, [17] 0.002 μg g^{-1}

Table 6 Determination of heavy metal (HM) content of oyster's soft tissue

Oyster[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Crassostrea angulata	Freeze-dried; 65% HNO_3, 200 °C	ICP-OES	Cu, Zn, Pb, Cd, As	(Chen et al., 2014)
Crassostrea virginica	Freeze-dried; HNO_3, $HClO_4$, H_2SO_4; wet sample with hot acid	ICP-OES	As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Zn	(Sajwan et al., 2008)
Crassostrea virginica	Drying at 115 °C, 24 h; microwave, cc HNO_3, 7 min	ICP-OES	Cd, Cr, Cu, Fe, Pb, Zn	(Siva et al., 2010)
Crassostrea belcheri	Drying at 60 °C, 72 h; microwave, cc HNO_3 + cc H_2O_2, 21 min	ICP-OES	Cd, Hg, Pb	(Intawongse et al., 2012)
Crassostrea gasar *Crassostrea rhizophorae*	Freeze-dried; microwave, cc HNO_3 + cc H_2O_2	ICP-OES	As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn (Ba)	(Santos & Boehs, 2021)
Crassostrea rhizophorae	Drying at 80 °C; cc HNO_3, 100 °C	ICP-OES	Cd, Cr, Cu, Pb, Ni, Zn	(Senez-Mello et al., 2020)
Crassostrea rhizophora	Freeze-dried; ultrasound (15 min) + centrifugation (30 min), dilute HCl, HNO_3 and CH_3COOH, 25–30 °C	ICP-OES[4]	Cd, Cu, Mn, Zn (Ca, K, Mg, Na, Sr)	(dos Santos et al., 2010)
Crassostrea rhizophora	Freeze-dried; microwave, cc HNO_3 + cc H_2O_2, 90–180 °C, 30 min	ICP-OES	Cd, Cu, Mn, Zn (Ca, K, Mg, Na, Sr)	(dos Santos et al., 2010)
Crassostrea rhizophora	Freeze-dried; microwave, cc HNO_3, cc H_2SO_4, cc H_2O_2, 30 min, 90–220 °C	ICP-OES	As, Cd, Pb, Zn (Se)	(Costa et al., 2009)
Crassostrea gigas	Dried at 60–80 °C; microwave, cc HCl + cc HNO_3, 30 min	ICP-OES	Cd, Cu, Pb, Zn	(Bray et al., 2015)
Crassostrea gigas	Drying at 80 °C; cc HNO_3, 150 °C, 10 h	ICP-OES	Cd	(Zheng et al., 2021)
Crassostrea gigas	Freeze-dried; cc HNO_3, reflux for 1 h, + cc $HClO_4$, reflux, 0.5 h or + H_2SO_4, reflux, 1 h	ICP-OES	As, Cd, Cr, Cu, Ni, Pb, Zn	(Lin et al., 2004)
Ostrea equestris	Drying at 60 °C; cc HNO_3	ICP-OES	Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	(Ferreira et al., 2005)
Saccostrea cucullata	Dried at 50 °C; cc HNO_3 + cc $HClO_4$	ICP-OES	Cd, Cr, Cu, Pb, Zn	(Mtanga & Machiwa, 2007)
Crassostrea gigas *Crassostrea angulata*	Microwave, cc HNO_3 + cc H_2O_2, 200 °C, 50 min	ICP-OES	As, Cd, Hg, Pb	(Lehel et al., 2018)
Crassostrea rhizophorae	Dried at 60 °C for 48 h; microwave, cc HNO_3 + cc H_2O_2	ICP-OES[5]	Cd, Cu, Zn	(Wanick et al., 2012)
n.a.	cc HNO_3 overnight, then boiling	ICP-OES	Cd, Cr, Cu, Ni, Pb, Zn	(Grant & Ellis, 1988)
Crassostrea madrasessis	Microwave, cc HNO_3 + cc $HClO_4$, 200 °C, 40 min	ICP-OES[6]	Co, Cu, Fe, Mn, Ni, Pb, Zn	(Chinnadurai et al., 2022)
n.a.	Dilute HNO_3, MIC, 20 min; CPE, DDPP + Triton X-114	ICP-OES[7]	As, Bi, Cd, Pb	(dos Santos Depoi et al., 2012)
n.a.	Freeze-dried; Microwave, cc HNO_3 + cc H_2O_2, 100–140 °C, 50 min	ICP-OES[8]	As, Cd, Co, Cr, Cu, Hg, Fe, Mn, Mo, Ni, Pb, Zn (Al, Mg, Se, Sr)	(Zheng & Yan, 2011)
n.a.	Microwave, cc HNO_3, 125–155 °C, 1 h	ICP-OES[9] ICP-MS[10]	As. Cd, Pb	(Sheppard et al., 1994)

Table 6 (continued)

Oyster[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
n.a.	Freeze-dried; microwave, cc H_2O_2 + cc HNO_3, 70–180 °C, 1 h; SPE using MGO@SiO_2-APTES-IL	ICP-MS[11]	Cd, Cu, Pb	(Dong et al., 2021)
n.a.	Freeze-dried; cc HNO_3 for 6 h, + cc H_2O_2, microwave, 180 °C, 30 min	ICP-MS[12]	As, Cd, Cr, Cu, Hg, Pb, Ni, Zn	(Liao et al., 2020)
n.a.	Dried; Microwave, cc HNO_3 + cc H_2O_2 + aq. HF, 180 °C, 20 min	ICP-MS	Cu, Zn, As, Cr, Cd, Pb	(Zhang et al., 2022)
Crassostrea gigas	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	ICP-MS	Pb	(Maanan, 2008)
Crassostrea gigas	Freeze-dried; microwave, cc HNO_3, 120–180 °C, 85 min	ICP-MS	As, Cd, Cr, Cu, Ni, Pb, Zn	(Liu, Lv, et al., 2022)
Crassostrea gigas	Microwave, cc HNO_3 at 195 °C for 20 min	ICP-MS[13]	As, Cd, Cr, Hg, Ni, Pb	(Pan & Han, 2023)
Crassostrea gigas *Crassostrea virginica* *Ostrea sandvicensis*	Freeze-dried; hot cc HNO_3, cc HNO_3 + cc H_2O_2; anion exchange chromatography	ICP-MS	Cd, Pb, Zn	(Shiel et al., 2012)
Crassostrea virginica	Microwave, cc HNO_3	ICP-MS	Cd, Pb	(Matos et al., 2021)
Ostrea edulis	Microwave, cc HNO_3 + cc H_2O_2, 120–190 °C, 1 h	ICP-MS[14]	Cd, Hg, Pb	(Miedico et al., 2015)
Saccostrea glomerata	Microwave, cc HNO_3 + cc H_2O_2, 46 min	ICP-MS	As, Cd, Hg, Pb	Dang et al., 2022
Saccostrea cucullata	Freeze-drying for 24 h; microwave, cc H_2SO_4 + cc HNO_3 + cc H_2O_2, 90 °C, 30 min	ICP-MS	As, Cd, Co, Cr, Cu, Fe, Mo, Mn, Ni, Pb, Sb, Zn (Al)	(Jahromi et al., 2021)
n.a.	Freeze-dried; microwave, 4 M HNO_3, 55 °C overnight; microwave, cc HNO_3, 150 °C, 30 min	ICP-MS	Zn, As, Cd, Hg, Pb (Se)	(Taylor et al., 2008)
n.a.	Freeze-dried; cc HNO_3 overnight at r.t., + cc H_2O_2, microwave for 1.5 h	ICP-MS[15]	As, Cd, Cr, Cu, Ni, Pb, Zn	(Chen et al., 2022)
Ostrea edulis *Crassostrea gigas* *Crassostrea angulata*	Microwave, cc HNO_3 + cc H_2O_2 + cc HF	ICP-MS[16]	Cd, Hg, Pb	(Barchiesi et al., 2020)
Ostrea rivularis Gould	Dried at 105 °C for 24 h; cc HNO_3 at r.t., cc HNO_3 + cc H_2O_2 at 180 °C	ICP-MS[17]	As, Cd, Cr, Cu, Pb, Zn	(Liu et al., 2022)
Crassostrea rhizophorae	Dried at 60 °C; n.a.	ICP-MS[18]	As, Cd, Cr, Cu, Fe, Ni, Pb, Zn	(de Souza et al., 2021)
Crassostrea iridescens	Dried at 70–80 °C; cc. HNO_3	AAS	Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	(Soto-Jimenez et al., 2001)
Crassostrea rhizophorae *Crassostrea virginica*	Dried at 70–80 °C; cc. HNO_3, overnight at r.t., reflux at 130 °C, 6 h	AAS	Cd, Cr, Cu, Fe, Pb, Ni, Zn	(de Astudillol et al., 2005)
Crassostrea rhizophorae *Crassostrea virginica*	cc. HNO_3, overnight at r.t., reflux at 130 °C, 3 h; H_2SO_4, HCl, reflux, 3 h; $KMnO_4$	AAS	Hg	(de Astudillol et al., 2005)
Crassostrea rhizophorae	Dried at 60 °C; cc. HNO_3, 100 °C	AAS	Ag, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	(Silva et al., 2001)

Table 6 (continued)

Oyster[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Crassostrea rhizophorae	Freeze-drying for 48 h; $HNO_3 + H_2O_2$ at room temp.; microwave, 10 min, 180 °C	AAS	Hg	(Araujo et al., 2019)
n.a.	aq. $K_2Cr_2O_7$ + cc H_2SO_4, 180 °C for 60 min; H_2O added, boiling for 30 min	AAS	Hg	(Locatelli et al., 1999)
n.a.	Drying at 45 °C for 24 h; aq. $K_2Cr_2O_7$ + cc H_2SO_4, 180 °C for 60 min; H_2O added, boiling for 30 min	AAS	Hg	(Locatelli, 2000)
Crassostrea iredalei	cc. HNO_3, overnight at r.t., reflux at 130 °C, 3 h; cc H_2SO_4, cc HCl, reflux, 3 h; $KMnO_4$	AAS	Hg	(Pakingking et al., 2022)
Crassostrea iredalei	Dried at 105 °C for 72 h; cc HNO_3 + cc HCl at 40 °C for 1 h and at 140 °C for other 3 h	AAS	Cd, Cr, Cu, Pb, Zn	(Pakingking et al., 2022)
Crassostrea tulipa	Dried; cc HNO_3 at 70–90 °C, + H_2O_2 at 135 °C	AAS	Cd, Cu, Fe, Mn, Hg, Zn	(Otchere, 2022)
n.a.	cc HNO_3 overnight, then boiling	AAS	Cd, Cr, Cu, Ni, Pb, Zn	(Grant & Ellis, 1988)
Saccostrea cucullata	Dried at 105 °C; hot cc $HNO_3 + HClO_4$	AAS	Cd, Cu, Pb, Zn	(Shirneshan et al., 2013)
Saccostrea cucullata	Freeze-dried; aqua regia at 90 °C	AAS	Hg	(Jahromi et al., 2021)
Saccostrea cucullata	cc HNO_3 + cc $H_2SO_4 + HClO_4$, 170 °C, 30 min	AAS	Hg	(Mtanga & Machiwa, 2007)
n.a.	Freeze-dried; $HNO_3 + HClO_4$; Silk Fibroin-Fe_3O_4-EDTA nano-adsorbent	AAS	Ni	(Benvidi et al., 2020)
n.a.	Freeze-dried at −40 °C, 11 h; cc $HNO_3 + HClO_4$; GO-Fe_3O_4-humic acid	AAS[19]	Pb	(Yekrangi et al., 2021)
Crassostrea rivularis	Dried in oven for 72 h; ashed around 450 °C; digested in cc HNO_3 + cc H_2O_2	AAS	As, Cd, Cu, Hg, Pb, Zn	(Wang et al., 2022)
Crassostrea madrasensis	Dried at 60 °C; soaking in cc HNO_3 overnight, heating at 80 °C	AAS	Pb	(Shenai-Tirodkar et al., 2017)
Crassostrea gigas	Freeze-dried; cc HNO_3 at 80 °C	AAS	Hg	(Lin et al., 2004)
Crassostrea gigas	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	AAS	Cd, Cr, Cu, Mn, Zn, Ni	(Maanan, 2008)
Crassostrea gigas	Dried at 85 °C; hot cc HNO_3	AAS	Cd, Cu, Zn	(Amiard-Triquet et al., 1998)
Crassostrea gigas	Wet sample; cc HNO_3 + cc H_2O_2, microwave for 46 min	AAS[20]	As, Cd, Cu, Hg, Se, Pb, Zn	(Garcia-Rico et al., 2001)
Crassostrea gigas	Dried; ashing at 450 °C for 3 h; dissolved in aq. HNO_3 (1:1); SPE using sulfur-NP/alumina	AAS	Cd, Cu, Pb, Zn	(Ghanemi et al., 2011)

Table 6 (continued)

Oyster[1]	Extraction and digestion method	HM Determination[2]		Ref.
		Method[3]	HM monitored	
Crassostrea gigas	Dried at 120 °C for 24 h; cc HNO_3 at 90 °C for 2 h and at 130 °C for other 4 h	AFS	Hg	(Maanan, 2008)
Ostrea rivularis Gould	Dried at 105 °C; cc HNO_3 + cc $HClO_4$	AFS[21]	Hg	(Liu et al., 2022)
n.a.	Ashing at 500–550 °C; aq. HCl; HMA-HMDC; extraction, $CHCl_3$	HPLC	Cd, Ni, Pb, Zn, Co, Cu, Bi	(Ichinoki et al., 1984)
n.a.	Dilute HNO_3, ultrasound, 1 h	HPLC-ICP-MS[22]	H_3AsO_3, H_3AsO_4	(Liao et al., 2020)
n.a.	Dilute HCl, ultrasound, 1 h	HPLC-ICP-MS	MeHg	(Liao et al., 2020)
n.a.	Freeze-dried; microwave, 4 M HNO_3, 55 °C overnight; purge and trap preconcentration	GC-ICP-MS	MeHg	(Taylor et al., 2008)
n.a.	cc HNO_3 + cc H_2SO_4, 150 °C for 4 h	ES	Cu, Cd, Pb, Zn	(Locatelli et al., 1999)
n.a.	Dried at 45 °C for 24 h; cc HNO_3 + cc H_2SO_4, 140 °C for 3 h	ES	Cu, Cd, Pb, Zn	(Locatelli, 2000)

[1] n.a. = not available; [2] non-heavy-metals are in parenthesis; [3] LOD in μg g^{-1} (ppm): [4] 0.012–2.62 μg g^{-1}; [5] 0.01–0.03 mg L^{-1}; [6] 0.00033–0.01518 μg g^{-1}; [7] 0.055–0.28 μg L^{-1}; [8] 0.002–0.03 mg L^{-1}; [9] 0.2–2.7 μg g^{-1}; [10] 0.0002–0.0028 μg g^{-1}; [11] 0.00242–0.00375 μg L^{-1}; [12] 0.0013–0.0135 μg L^{-1}; [13] 0.0003–0.005 μg g^{-1}; [14] 0.001–0.004 μg g^{-1}; [15] 0.01–3.67 μg g^{-1}; [16] 0.0015–0.0076 μg g^{-1}; [17] 0.03–1.66 μg g^{-1}; [18] 0.0038–0.0461 μg g^{-1}; [19] 0.07 μg L^{-1}; [20] 0.01–0.12 μg g^{-1}; [21] 0.002 μg g^{-1}; [22] 0.0083 and 0.0052 μg L^{-1}

Author contribution Tibor Pasinszki was responsible for conceptualization, methodology, data collection, interpretation of data, writing the original draft, and editing. Shilvee S. Prasad was responsible for data collection, interpretation of data, review, and editing. Melinda Krebsz was responsible for data collection, interpretation of data, review, and editing.

Data availability The data used in this study are available in the main body of the article, in appendices, and in referenced articles.

Declarations

Ethics statement All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests The authors declare no competing interests.

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