



# Ecotoxicology of Trace Elements in European Oyster (*Ostrea edulis*), Seawater, and Sediments across Boston Harbor, Massachusetts, USA

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## Abstract

European oyster (*Ostrea edulis*) can be used for biological monitoring of water and sediment quality and serve as a conduit of trace elements to humans via consumption. Trace element concentrations in seawater, sediment, *O. edulis* edible tissues and shells from Boston Harbor were studied and found to be elevated relative to comparative studies in native ecosystems in the Adriatic Sea and Bay of Biscay. Average edible oyster tissues concentrations (mg/kg) were: arsenic 6, cadmium 1.7, cobalt 3.1, chromium 1.9, copper 153, mercury 0.265, nickel 1.8, lead 3.3, and zinc 2390. Arsenic was elevated in seawater and oyster shells. Mercury was elevated in sediments and oyster tissues. Lead was elevated in suspended sediments. Total Hazard Quotient (THQ) was < 1 but when summed across trace elements, THQ was nominally > 1 for all sites. Further study is warranted to determine mechanisms and spatial extent of bioaccumulation.

**Keywords** Mollusks · New England seafood · Bioaccumulation · Toxic elements · Total hazard quotient

Bivalves such as oysters are common in temperate coastal regions and help to form the foundations of benthic habitat and food webs in aquatic ecosystems (Grabowski et al. 2012). As a sessile organism that grows close to shore, oysters provide ecosystem services that include improved water quality by filtering out toxins as well as supporting fisheries through wild harvest and aquaculture (Grabowski et al. 2012). European oyster, *Ostrea edulis*, was consumed in its native range since the neolithic civilizations of Europe, Northern Africa, and Western Asia. Today, *O. edulis* is also present throughout the northeastern United States due to artificial introduction, with populations thriving in parts of Massachusetts.

Across its native range, *O. edulis* provide essential trace elements such as Cu, Co, Ni, and Zn and have been found to be permissible for human consumption using standards

such as intake levels and Total Hazard Quotients (THQ; Bilandžić et al. 2014, Bogdanović et al. 2014, Ong et al. 2012). Despite trace element concentration below regulatory limits in past studies, *O. edulis* could pose a hazard to specific human consumers as a result of interacting factors. Ecologically-linked organisms, including humans, could be exposed to toxic trace element levels from oysters in regions with degraded water quality where bioaccumulation occurs via near constant water filtration during respiration and suspension feeding. Filter-feeding, along with life spans up to 20 years, sessile behavior that enables linkages to local conditions, a wide distribution, and being targeted for human consumption, make *O. edulis* a top candidate for biological monitoring of water and sediment quality. In addition to tissue monitoring, the shell of *O. edulis* could be monitored as it is affected by toxic element availability; Cariou et al. (2017) found shifts in Pb/Ca ratios in shells in Brittany, France, likely corresponding with anthropogenic change in water quality.

Anthropogenic impacts on water quality are notable in Boston Harbor, Massachusetts which receives water input from a number of metropolitan watersheds (Bowen et al. 2019). Sewage was discharged into the harbor from the urban population center and surrounding towns starting in the late 19th century, leading to the first treatment plans being introduced in the 1950s (Zago et al. 2001). Enforcement of the

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Clean Water Act in the 1990s prompted a reduction in solids from the treated sewage outfall and a rerouting of the outfall discharge to outside of the harbor (Bowen et al. 2019). Metals associated with legacy, urban, and airport activities and arsenic in sediments within rivers discharging into the harbor cause water quality concerns after heavy rains. Thus, while great strides and financial resources have been taken to improve Boston Harbor water quality that have resulted in an improved environment and recovery of depleted species (Bowen et al. 2019), there are still contaminant exposure opportunities for organisms. Particularly, filter feeding species have a high potential risk, affecting other organisms through direct consumption or food web interactions.

The overarching goal of this study was to investigate contaminants and the linkages of dissolved and suspended sediment trace element concentrations on the accumulation within the filter feeding, introduced European oyster (*O. edulis*) in Boston Harbor. Local shellfish regulatory agencies are responsible for testing toxin levels against known human tolerances and determining actions (USFDA 2019), but may lack the resources to carry out testing. The objectives of this study were to opportunistically (1) evaluate dissolved and suspended sediment trace element concentrations in Boston Harbor, (2) compare the accumulation of trace elements in tissue and shells of *O. edulis*, and (3) evaluate risk from *O. edulis* trace element concentrations for human consumption. The results of this study shall provide insight on seawater

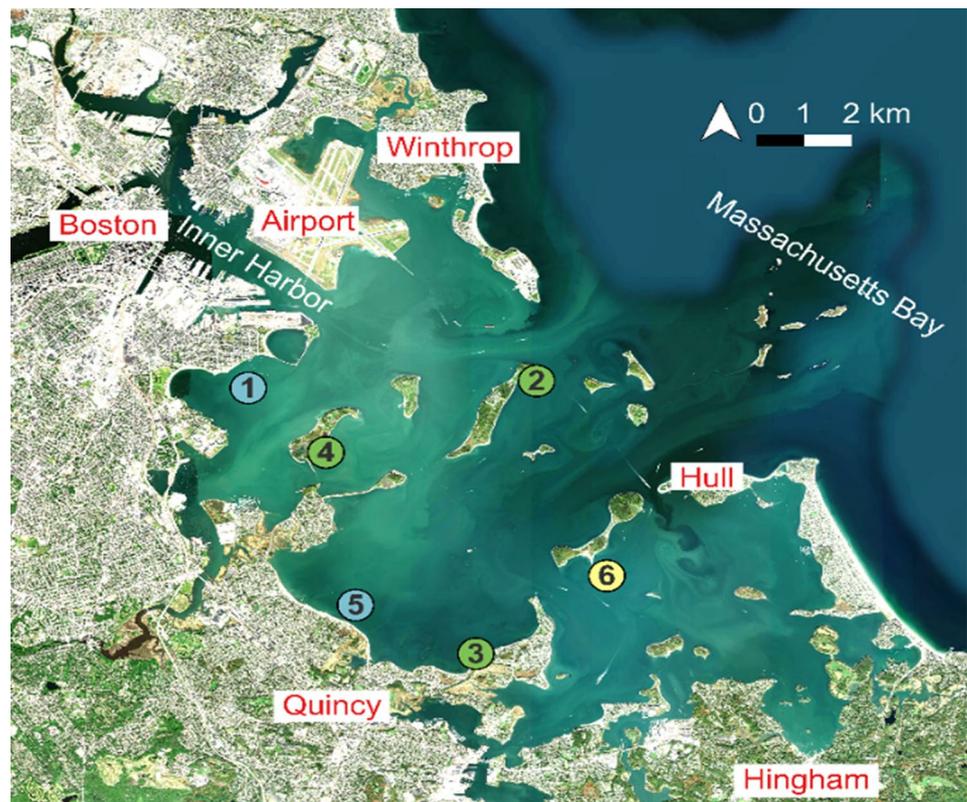
and suspended sediment contamination affecting non-native shellfish and contributes to knowledge of potential ecological and human health impacts.

## Materials and Methods

Sampling occurred opportunistically in October 2022 during a fisheries and biodiversity survey at random sites within three different depth strata in Boston Harbor. Six sites from the shallow stratum with a depth of 0–2 m at the mean lower low water line were sampled for this study (2.3–4.7 m depth at time of sampling; Fig. 1). At the arrival to each field site, “surface seawater” samples were collected by submerging an empty water bottle at the sea surface. A 1-m wide trawling net was then towed along the bottom for 300 m. Oysters collected in the catch were bagged by site. “Bottom seawater” and suspended sediment samples were taken from the drainage of slurry from the net and catch. Due to the single collection, seawater and sediment concentrations represent a current snapshot of seawater and sediment conditions. All samples were stored chilled in a cooler in the field and promptly brought to the Trace Metal Biogeochemistry Lab at the University of Massachusetts Amherst.

Oyster samples were frozen to  $-20^{\circ}\text{C}$  and freeze-dried to a constant mass. Oyster tissues were shucked from their shells and ground to a homogeneous material using

**Fig. 1** Sampling sites within Boston Harbor which extends from Winthrop to Hull and includes Quincy and Hingham Bays (ESRI Satellite basemap). Colors represent the depth at sites at the time they were sampled (yellow: ~2 m, green: ~3 m, blue: ~4 m)



a stainless steel coffee grinder. Oyster right shells (i.e. flat shells) were ground to < 2 mm fragments using an agate mortar and pestle. A 5 g subsample of the 2 mm fragments was further crushed to < 100 µm using a tungsten ball mill. To process oyster tissues and shells for trace elements following US Environmental Protection Agency (EPA) Method 3050B, a 1 g subsample was placed in centrifuge tubes with 5 ml of reverse aqua regia (9:1 ratio of 15.6 M HNO<sub>3</sub> and 12.1 M HCl), similar to (Ong et al. 2012). For the digestion process, the subsample and reverse aqua regia were heated at 70°C for 50 min. The digests were diluted to 50 ml using de-ionized water, at which point 3 g of each digested sample was further diluted with de-ionized water up to 15 g. Every 25 samples included a digestion blank, a sample duplicate, and National Institute of Standards and Technology (NIST) standard reference materials (SRM) 1566b Oyster Tissue, 1944 NY&NJ sediments, and 2711a Montana Soil for quality assurance/quality control (QA/QC).

To obtain suspended sediment samples, 0.237 L seawater was filtered using a 0.45 µm nylon membrane and hand-vacuum filtration system (Ong et al. 2012). Suspended sediments were collected from the nylon filter membranes by freezing the samples at -20 °C and freeze-dried to a constant mass. For the digestion based on EPA Method 3050B, between 0.100 and 0.300 g of sediments were transferred to centrifuge tubes and acidified with 5 ml of reverse aqua regia and heated to 70°C for 50 min. The sediment digests were diluted to 50 ml using de-ionized water and then further diluted by transferring 3.00 g of digest to 15.00 g. Every 25 samples included a digestion blank, a sample duplicate, and NIST SRM 1944 NY&NJ sediments, and 2711a Montana Soil for QA/QC. For microelement analysis of seawater, a filtered 1.00 g sample was diluted to 50.00 ml using 2.5% trace metal grade HNO<sub>3</sub>. For trace elements in seawater, an ion exchange resin was used to concentrate the dissolved ions based on Miyazaki and Reimer (1993). First, a 10 ml column containing 1 g of HCl washed 100–200 mesh Chelex resin was rinsed with 0.05 M NH<sub>4</sub>Ac buffered at pH 7.0. Next, 50 ml of seawater was adjusted to pH 6 with trace metal grade HNO<sub>3</sub> and eluted through the column followed by 10 ml of 18.2 MΩ deionized water. Trace elements were released from the Chelex resin using 10 ml of 5 M HCl, which was diluted with 10 ml of 2.5% HNO<sub>3</sub>.

The diluted digests of oyster tissues, oyster shells, seawater, and suspended sediment were analyzed for trace elements (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Se, Sn, Th, U, Zn) using an Agilent 7700x Inductively Coupled Plasma Mass Spectrometer (ICP-MS). SRM recovery rates for NIST 1944 NY&NJ sediments and Oyster Tissue 1566b were 81% to 113% of their certified values. Digestion blanks were the following: < 0.01 ng/g for Co, Cr, Pb, and < 0.1 ng/g for As, Cu, Ni, Zn. Duplicates were within < 11% CV. LOD were 0.01 ng/g for trace elements and 1 ng/g for macroelements.

Mercury concentrations were determined using a Milestone DMA-80 Direct Mercury Analyzer. SRM recovery rates were within 4% of their certified values, duplicates were < 6% CV, and Hg concentrations in combustion blanks were < 0.01 ng/g with an LOD of 0.01 ng/g. Seawater Hg concentrations were below the limit of quantification.

Average values are presented in text and in figures ± 1 standard error and an alpha of 0.05 was used for significance. Descriptive statistics and nonparametric statistical tests were calculated in Matlab (Mathworks, Natick, MA, USA). The *corrplot* package in R (Wei and Simko 2021) was used to generate the correlation matrix. Target hazard quotients (THQs) were used to determine the non-carcinogenic risk to human health from trace elements within *O. edulis* during consumption. THQ was calculated using the following variables: Cte is the trace element concentration in seafood (mg/kg wet weight, Eq. 1); EF is the exposure frequency (180 days/year); ED is the exposure duration (United States average life expectancy 77.3 years); IR is the ingestion rate (oyster consumption rate; mg/person/day assumed to be 12 oysters per week); AT is the average time (365; days); LT is the lifetime (equal to exposure duration; years); and BW is an average body weight of US men (90.7 kg) and women (77.5 kg); and RfD is the oral reference dose for each individual trace element. RfD values (mg/kg•day) used were: 0.0003 for As, 0.001 for Cd, 0.03 for Co, 0.003 for Cr, 0.04 for Cu, 0.0001 for Hg, 0.02 for Ni, 0.002 for Pb, and 0.3 for Zn (IRIS 2022).

If individual or summed THQ values were determined to be < 1, it can be assumed to pose a carcinogenic human health risk to an individual over their lifetime exposure while THQ values > 1 pose a non-carcinogenic risk to consumers. Since several assumptions are utilized to determine THQ, it is important to note that lower or greater consumption rates, additional sources of exposure, and individual biosorption rates of trace elements can differ.

$$THQ = (Cte \times EF \times ED \times IR) / (AT \times LT \times BW \times RfD) \quad (1)$$

## Results and Discussion

Seawater was collected from two depths (surface and bottom) at six sites across Boston Harbor (Table 1). Bottom seawater concentrations were not significantly different from surface seawater concentrations except for Cr and Zn, which were significantly higher for surface than bottom seawater. Comparing our observations with the Massachusetts Surface Water Quality Standards, average concentrations of As, Hg, and Ni exceeded their screening values of 69 µg/L, 1.8 µg/L, and 74 µg/L, respectively (Massachusetts CMR 314). Thus, the observed seawater concentrations are moderately

**Table 1** Seawater and suspended sediment concentrations across six sites and the associated ecological screening values. Each seawater and suspended sediment measurement reported is the arithmetic average of triplicate field samples and error bars are standard error

Sample	Site	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
		µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Surface seawater	1	77±9	0.2±0.0	1.9±0.2	2.5±1.1	3.78±2.1	20±1	107±11	188±49	49±17
	2	106±6	0.1±0.0	2.6±0.1	7.9±1.7	0.65±0.04	20±3	144±5	7±2	14±4
	3	107±2	0.1±0.0	2.9±0.2	7.1±1.3	0.79±0.38	25±2	152±7	39±16	17±6
	4	107±5	0.2±0.0	2.6±0.2	9.4±1.8	0.67±0.28	40±13	145±5	16±11	19±4
	5	109±2	0.1±0.0	2.4±0.1	4.1±0.7	0.80±0.23	12±3	143±4	67±15	13±4
	6	112±9	0.1±0.0	2.4±0.1	3.9±0.5	0.61±0.22	20±4	142±3	9±5	19±6
Bottom seawater	1	116±2	0.1±0.0	3.0±0.2	0.7±0.3	2.14±1.35	18±6	129±6	85±7	54±14
	2	104±5	0.1±0.0	2.3±0.1	0.6±0.3	0.43±0.09	7±2	127±9	14±4	16±7
	3	101±9	0.2±0.0	2.2±0.1	0.5±0.2	1.71±0.74	15±4	121±23	62±7	25±4
	4	106±4	0.1±0.0	2.6±0.1	0.8±0.3	3.47±1.87	22±6	120±6	135±33	47±7
	5	110±9	0.3±0.1	2.8±0.3	1.6±0.5	2.28±1.43	19±6	127±12	89±13	41±12
	6	113±6	0.1±0.0	2.7±0.2	1.4±0.6	0.69±0.05	13±7	141±15	22±7	9±2
MA CMR 314 Standard		69	33	N/A	1,100	4.8	1.8	74	210	90
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Suspended sediment	1	19±4	0.9±0.1	8.6±1.4	29±8	25±2	651±46	13±3	23±8	52±9
	2	22±2	1.0±0.2	4.7±0.4	64±14	47±10	336±36	20±3	41±9	118±16
	3	11±5	0.4±0.1	3.3±0.4	42±8	35±7	579±23	12±2	31±5	91±10
	4	23±2	0.8±0.2	5.1±0.5	75±15	57±10	343±11	21±4	45±6	156±36
	5	20±3	0.9±0.2	7.1±1.3	91±16	64±8	733±32	21±3	59±12	141±12
	6	18±3	0.7±0.2	8.1±1.5	69±8	51±7	498±16	21±2	46±8	127±10
Friday (1999) Standard		8.2	1.0	20	52.3	18.7	0.13	15.9	30.2	124

elevated compared to concentrations observed to negatively impact the health of aquatic invertebrates. Stanković et al. (2014) examined Boka Kotorska Bay in Montenegro near southern Croatia and seawater concentrations of As, Pb, Zn were 2–5x times higher in our study while Cd, Co, Cu, Hg, Ni were 2–10x higher in their study. Stanković et al. (2014) also observed some significantly higher surface than bottom seawater concentrations, though collected 2005–2007, and water quality may have substantially improved.

Comparing our observations with the ecological screening values for sediment, average suspended sediment concentrations of As, Cr, Cu, Hg, Ni, and Pb exceeded their screening values of 8.2 mg/kg, 52.3 mg/kg, 18.7 mg/kg, 0.13 mg/kg, 15.9 mg/kg, and 30.2 mg/kg, respectively (Friday 1999). Thus, these concentrations are moderately elevated compared to concentrations observed to negatively impact the health of sediment-dwelling invertebrates. For example, in marine sediments within a Croatian aquaculture area in the Adriatic, Žvab Rožič et al. (2012) measured As, Cd, Co, Cr, Cu, Ni, Pb and Zn sediment concentrations to be 5%–60% of the concentrations in our study. Marine sediments in Brittany, France studied by Ong et al. (2012) found Cr, Cu, and Zn concentrations were less than 50% of the concentrations in our study but Cd, Co, and Pb concentrations were comparable. Suspended sediment concentrations were

compared with surface and bottom seawater concentrations using Pearson Correlations. We found that As, Cu, and Pb were not significantly correlated for both surface and bottom seawater whereas surface seawater Cd concentrations were significantly correlated with suspended sediment. Bottom seawater Co and Zn were negatively correlated while bottom seawater Cr and Ni were positively correlated with suspended sediment concentrations. These limited correlations suggest a limited relationship between seawater and suspended sediment concentrations at the scale of daily samples. Since rain likely brings sediments with contamination into Boston Harbor, follow-up work should focus on the source and fate of sediments in the source watersheds and how the fate of sediments varies associated with precipitation patterns.

Edible tissues, including the mantle, gills, adductor muscle, digestive and circulatory systems, were freeze-dried, milled, and homogenized from six sites across Boston Harbor and analyzed for trace elements (see Ong et al. 2012). The FDA and EPA do not have regulatory limits on trace elements in shellfish, thus we have compared our observations with the Food and Agriculture Organization (FAO) and World Health Organization (WHO) permissible values. Copper and Zn exceeded the FAO/WHO regulator limits of 30 mg/kg and 1000 mg/kg limits, respectively. Oyster

tissue concentrations of As, Cd, Co, Cr, Hg, Ni, and Pb were within FAO and WHO permission values.

*O. edulis* edible tissue trace element concentrations in our study were significantly higher than values from previous studies (Table 2). Copper and Zn in our study were 2–20 times higher than Bilandžić et al. (2014) observed in the Adriatic coast of Croatia. Similarly, As, Cd, and Pb were 2–20 times higher and Hg was 0.9–6 times higher than measured by Bogdanović et al. (2014). These results highlight that introduced *O. edulis* bioaccumulated higher trace element concentrations than in their European range, which we directly attribute to the elevated sediment and seawater concentrations of their localized habitat. *O. edulis* shells, specifically the whole flat shell, was crushed and homogenized at the six sites across Boston Harbor. *O. edulis* shell concentrations were significantly higher in our study than observations by Martinčić et al. (1986); they measured Cu at 2.8 mg/kg, Cd at 0.01 mg/kg, Pb at 0.48 mg/kg, and Zn at 10 mg/kg. These findings strongly support that seawater and suspended sediment quality in Boston Harbor are much lower than the Adriatic Sea of Croatia, most plausibly due to historical anthropogenic pollution of trace elements. Further study is needed to determine seasonal and interannual effects on trace element concentrations in seawater and suspended sediments.

Using Pearson correlations, we found that trace elements in seawater and suspended sediments were not strongly related to accumulation in edible tissues and shells (Fig. 2). Instead, there were mixed results for seawater and suspended sediment relationships among the trace elements. Edible tissue and shell trace element concentrations were compared

with surface seawater, bottom seawater, and suspended sediment concentrations. Surface and Bottom seawaters were positively correlated with edible tissue or shell concentrations for Co, Cr, Pb, and Zn. Suspended sediment was not positively correlated with edible tissue or shell concentrations. Conversely, surface seawater was negatively correlated with edible tissue or shell concentrations only for Ni. Bottom seawater was negatively correlated with edible tissue or shell concentrations for As, Cd, and Co. Suspended sediment concentrations were negatively correlated with edible tissue or shell concentrations only for As, Co, and Ni. From these results, we hypothesize that the dissolved trace elements were likely easier for uptake by the oysters and suspended sediments may act as sinks through adsorption that prevent accumulation.

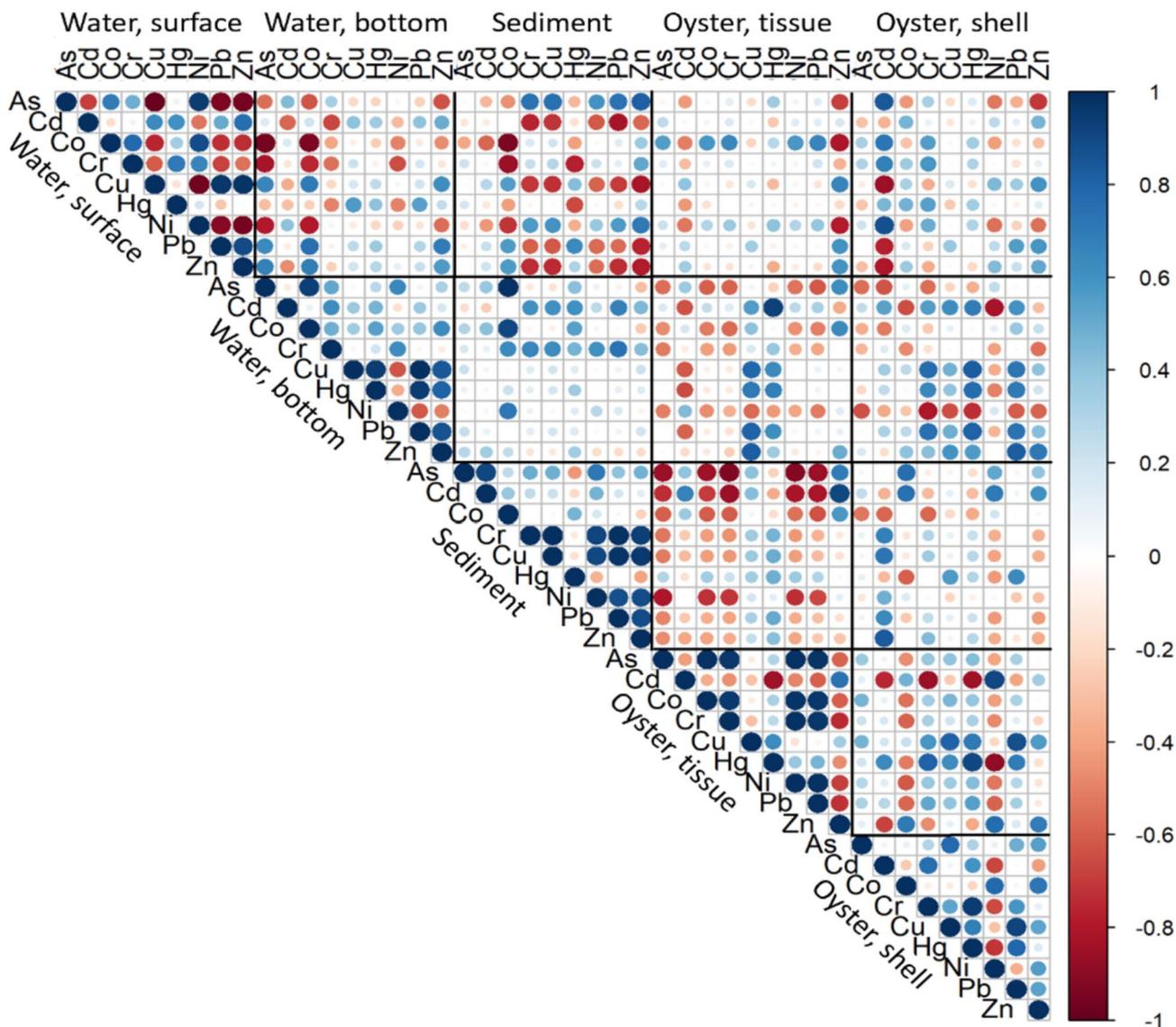
The THQ assessment was used to estimate non-carcinogenic human health risk from *O. edulis* consumption from Boston Harbor (Table 3). The THQs for the individual trace elements show that As poses the most serious hazard to human health, followed by Zn. Only As at Site 3 posed a threat to human health, as it is nominally > 1. Interestingly, the elevated concentrations of Pb and Hg were not high enough to pose a direct, non-carcinogenic human health risk. THQ value is a parameterized estimate for assessing human health risks from trace element contamination. Human consumers eating *O. edulis* with THQ value above 1 could have deleterious effects on their health, but it is important to note the assumptions of lifetime consumption, for half the year, eating at least ~ 12 oysters weekly. Data from our study suggests that the average consumption may pose a risk to human health and supports the known advisory to avoid excessive

**Table 2** Edible tissue concentrations for *O. edulis* across six sites. Values are arithmetic average and error bars are standard error

Site	n	Shell length	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
			mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
1	9	10.9	6.5±0.9	1.9±0.1	2.9±0.4	1.6±0.1	162±19	0.23±0.02	1.7±0.3	2.7±0.4	3250±448
2	10	10.1	5.9±0.5	2.0±0.2	3.0±0.4	1.6±0.2	137±23	0.20±0.02	1.3±0.2	2.4±0.4	2770±380
3	8	9.4	9.7±1.2	1.4±0.2	6.1±1.3	3.9±1.2	139±17	0.31±0.02	4.1±1.4	7.0±2.0	1413±263
4	10	9.8	5.5±0.4	1.5±0.1	2.0±0.2	1.3±0.1	191±25	0.30±0.04	1.1±0.3	2.4±0.3	2524±295
5	10	8.2	6.1±0.7	1.6±0.1	3.3±0.6	1.6±0.2	195±22	0.35±0.02	1.8±0.4	3.3±0.4	2485±441
6	10	10.9	5.3±0.4	1.7±0.1	2.1±0.2	1.6±0.2	111±8	0.24±0.04	1.4±0.2	2.3±0.3	2051±243

**Table 3** Total Hazard Quotient (THQ) values for non-carcinogenic risk to humans from consumption of *O. edulis* across the six sites

Site	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn	ΣTHQ
1	0.70	0.06	0.00	0.02	0.13	0.07	0.00	0.04	0.35	1.39
2	0.64	0.07	0.00	0.02	0.11	0.06	0.00	0.04	0.30	1.25
3	1.05	0.04	0.01	0.04	0.11	0.10	0.01	0.11	0.15	1.63
4	0.60	0.05	0.00	0.01	0.16	0.10	0.00	0.04	0.27	1.23
5	0.66	0.05	0.00	0.02	0.16	0.12	0.00	0.05	0.27	1.33
6	0.57	0.05	0.00	0.02	0.09	0.08	0.00	0.04	0.22	1.08



**Fig. 2** The correlation matrix for trace metals in seawater, sediment, and oyster samples. Blue represents positive correlations and red represents negative correlations. Color saturation and size of the circles are proportional to the correlation coefficients

consumption of these seafood in coastal areas to prevent adverse health effects.

**Conclusions**

Seawater and suspended sediment trace element concentrations in Boston Harbor were elevated with respect to guidelines and other studies globally, especially for As, Ni, Pb and Zn. This is reflected in elevated concentrations of As and Zn within the edible tissues of *O. Edulis*. Oyster As is likely sourced from seawater while Zn appear sourced from sediments. A continued reconciliation of the watershed contributions at present compared to legacy

contamination in the harbor is needed to identify pathways to humans and diminish the impact on resident biota. Our findings represent a modern snapshot of conditions and further studies are needed to capture the temporal nature of changes in seawater, sediment, and oyster trace element concentrations.

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## Declarations

**Conflict of interest** The authors declare no financial or non-financial interests.

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