

COPPER, IRON, SELENIUM, ZINC AND MAGNESIUM CONCENTRATIONS IN OYSTERS (*Ostrea edulis*) FROM THE CROATIAN ADRIATIC COAST

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Summary: The aim of the study was to determine and compare the concentrations of essential elements (Cu, Fe, Se and Zn) and the macronutrient Mg in oysters (*Ostrea edulis*) from six different farming sites along the Croatian Adriatic coast. This is the first study to determine the accumulation of Cu, Fe, Mg and Se in oysters in Croatia. The concentrations of elements were determined in the ranges (mg kg⁻¹, wet weight): Cu 8.84–76.6, Fe 22.4–69.3, Se 1.66–4.88, Zn 129–737, Mg 607–1226. There were no significant differences in Fe, Mg and Se concentrations between sites. Significantly higher Zn and Cu levels were measured in oysters from Pag compared to those collected at localities in Mali Ston Bay. The results indicate an environmental influence on element levels in oysters. The estimated daily intakes (EDIs) calculated in oysters show a low contribution (0.008–2.52%) to the recommended dietary allowance (RDA) for these five elements. According to risk assessment results, it can be concluded that it is unlikely that the intake of Fe, Mg and Se through oysters would involve any risk for the average consumer. However, the high contribution of the EDI values to the provisional maximum tolerable daily intake (PMTDIs) obtained for Cu and especially for Zn suggests that oysters may pose a health risk to consumers.

Key words: Copper; Iron; Selenium; Zinc; Magnesium; Oysters; Croatia

Introduction

Ecological changes in water, as the result of human activity and pollution with heavy metals and other elements, has become a serious health concern due to the toxicity and accumulation of these elements by different aquatic organisms. Seafood products such as shellfish are known as accumulators of a number of pollutants, including

the most studied biomagnifying substances, i.e. PCBs or methylmercury. They may accumulate essential and non-essential elements, which may exceed dietary recommendations (1). Accordingly, it is important to determine the health risk assessment and establish the benefits of seafood consumption.

For example, Zn-rich seafood may contribute to nutritional requirements for the antioxidant prevention of xenobiotic-induced oxidative stress and associated toxic hepatitis (2). Zinc has multiple biochemical functions and is present

in a large number of proteins (3). Seafood can contribute to the attainment of the recommended intake levels of essential metals such as Cu and Zn (4). This may be important, particularly in developing countries with low socioeconomic development and nutritional deficiency of Zn. On the other hand, excessive Zn levels may cause acute and chronic toxicity and may lead to altered Fe function, reduced Cu status, reduced levels of high density lipid and reduced immune function (5). Acute Cu toxicity can cause nausea, vomiting, abdominal pain, headache, lethargy, diarrhoea, tachycardia, respiratory difficulties, haemolytic anaemia, gastrointestinal bleeding, liver and kidney failure and death. Chronic Cu toxicity can result in liver disease and severe neurological defects (6).

It is known that the oysters are an excellent source of copper (Cu), iron (Fe), selenium (Se) and zinc (Zn). Recently, the *Second French Total Diet Study* (7) indicated that the oysters species in the food group of “shellfish” are the group with the highest content of essential trace elements Cu, Zn and Se. Iron plays a major role as an oxygen carrier in haemoglobin in blood, or myoglobin in muscle, and it is also required for many metabolic processes (8). Selenium has roles in several critical metabolic pathways and in the immune and endocrine systems, and acts as an antioxidant showing enzymatic redox activity through essential enzymes as glutathione peroxidase (9). Magnesium (Mg) is required for more than 300 biochemical reactions in the body, including maintenance of normal muscle and nerve function and heart rhythm (10).

Although fish and seafood can contribute to achieving the recommended daily intake, they may also contribute to human exposure to environmental pollutants (11). Oysters and other marine species may serve as bioindicators for monitoring a variety of contaminants in the ecosystem. It has been confirmed that oysters originating from contaminated sites generally show significantly higher metal concentrations than controls (1).

The aims of this study were: I) to determine the levels of essential elements (Cu, Fe, Se and Zn) and the macronutrient Mg in oysters from six different farming sites along the Croatian Adriatic coast, II) to compare element levels between the different farming sites, III) to compare data of this study with previously reported data for these elements in

oysters, and IV) to compare the estimated intakes with reference toxicological and nutritional values for each element.

Materials and methods

Sample collection

European flat oysters (*Ostrea edulis*) from six farming sites were collected during September and October 2011. Locations were sampled on different days. Ten oysters were collected at each sampling site, and three individual oysters ($n=3$, per site) were used for the analyses.

Oysters were collected from five oyster farms in the Mali Ston Bay and one farm from the island of Pag. Mali Ston Bay is situated between the mainland and the Peljesac Peninsula near the Neretva River delta on the southern Adriatic coast. Five oyster cultivation areas in Mali Ston Bay located near the village Mali Ston were: Brijesta, Mali Ston, Bistrina, Bjejevica and Sutvid (Figure 1).

The island of Pag is situated centrally on the eastern Adriatic coast. The oyster farm Stara Povljana is located in the closed Pag Bay near the main settlement on the island, the town of Pag. The town of Pag is known for sea salt production and is the island's main port.





Figure 1: Map of the sampling locations in Pag Island and Mali Ston Bay

Reagents and standards

All reagents were of analytical reagent grade, HNO_3 and H_2O_2 (Kemika, Croatia). Ultra high purity water processed through a purification system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia) was used for all dilutions. Plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9, v/v) and by subsequent rinsing with double deionised water and drying prior to use. Calibrations were prepared with Cu, Fe, Mg, Se and Zn standard solutions of 1 g L^{-1} (Perkin Elmer, USA). The stock solution was diluted in HNO_3 (0.5%).

Sample preparation

Shellfish meat samples (0.5 g) were digested with 4 mL of HNO_3 (65% v/v) and 2 mL of H_2O_2 (30% v/v) in a microwave oven. A high-pressure laboratory microwave oven (Multiwave 3000, Anton Paar, Germany) was employed to perform the acid digestion of samples. The digestion program began at a potency of 500 W, then ramped for 1 min, after which samples were held for 4 minutes. The second step at a potency of 1000 W (ramp 5 min) was held for 5 minutes. The third step at a potency of 1400 W (ramp 5

min) was held for 10 minutes. A blank digest was carried out in the same way.

Digested samples were diluted to a final volume of 50 mL with double deionised water. Concentrations of Cu, Fe, Mg, Se and Zn were determined on a wet weight basis as mg kg^{-1} . All samples were run in batches that included blanks, a standard calibration curve and two spiked specimens. The limits of detection (LODs, mg kg^{-1}) were determined as the concentration corresponding to three times the standard deviation of ten blanks in oyster samples: Cu 0.0021, Fe 0.005, Mg 0.008, Se 0.0018 and Zn 0.0015. The quality of data was checked by an analysis of the recovery rate using certified reference material: dogfish liver (DOLT-4, National Research Council, Canada). The reference material was treated and analysed under the same conditions as the samples. The results showed good accuracy with a recovery rate for tested elements (%): Cu 102.2, Fe 99.6, Mg 104.9, Se 105.2 and Zn 101.3.

Analysis of elements

An inductively coupled plasma optical emission spectrometer with axial and radial viewing plasma configuration (ICP-OES Model Optima 8000, Perkin-Elmer, USA) and an S10 autosampler

(Perkin Elmer, Waltham, Massachusetts, USA) was utilized. The instrumental operating conditions used are shown in Table 1.

Calculation of estimated daily intake

The estimated daily intake (EDI) was calculated by the equation (12):

EDI (mg kg⁻¹ BW day⁻¹) = [(zinc concentration; mg kg⁻¹) per (meal size or daily intake of food; kg)] divided by [adult body weight (60 kg)]

In the calculation, the average consumption of shellfish in Croatia was set as 20 g day⁻¹ per adult (13). The values of EDI were used to calculate contributions of each element to the reference nutritional (RDA, recommended dietary allowance, for females and males) and toxicological values (PMTDI, provisional maximum tolerable daily intake; TDI, tolerable daily intakes).

Statistical analysis

Statistical analysis was performed using the SPSS Statistics 17.0 software. Concentrations were expressed as mean ± standard deviation, median, minimum and maximum values. One-way analysis of variance was used to test for differences in elements levels in samples. Firstly, the normality of distribution was tested using Shapiro-Wilk normality test. Since the data was not normally distributed, a non-parametric test, Kruskal-Wallis ANOVA test, was used in defining the difference significance between the element concentrations from different locations. The difference between the groups was determined according to the test results. Thus, a multiple comparison by a Tukey HSD was also conducted to describe the differences and point out which groups differed significantly from others.

Results

The concentrations of five elements in the meat of oysters collected from six study sites are shown in Table 2.

Elements concentrations in oysters were measured in ranges (mg kg⁻¹): Cu 8.84 - 76.6; Fe 22.4 - 69.3; Mg 607 - 962; Se 1.66 - 4.88; Zn 129 - 737. There was a statistically significant difference between groups as determined by Kruskal-Wallis test for Zn levels on specific locations ($H(2) =$

13.505, $p = 0.019$). Thus, a Tukey post-hoc test was conducted and it revealed that the Zn levels of samples from Pag were significantly higher than those collected at locations in Mali Ston Bay, i.e. Brijesta, Mali Ston and Sutvid ($p < 0.01$, all), including Bjejevica and Bistrina ($p < 0.04$). Furthermore, Cu levels of the samples collected on Pag were statistically significantly higher than those from other locations ($p < 0.021$, all).

There were no significant differences in Fe, Mg and Se concentrations between samples taken from different sites.

Table 3 shows the estimated daily intake (EDI) of elements based on the lowest and highest mean concentrations determined for each element. Also, the contribution of EDI values to the recommended dietary allowance (RDA), provisional maximum tolerable daily intake (PMTDIs) and tolerable daily intakes (TDI) were calculated for each element. The EDIs in oysters ranged from the lowest range of 0.0008 to 0.0011 mg day⁻¹ for Se to the highest range of 0.242 to 0.314 mg day⁻¹ calculated for Mg. The EDIs contributing to the RDA values were from the lowest percentage 0.008–0.024% for Cu to the highest contribution of 1.84–2.53% for Zn. Furthermore, contribution of the EDIs calculated for the lowest and highest mean of elements to PMTDIs values (%) were lowest for Se (0.0055–0.0076%) and highest for Zn (40.4–404%).

Discussion

To the extent of our knowledge, this is the first study to determine the accumulation of Cu, Fe, Mg and Se, alongside Zn, in oysters (*Ostrea edulis*) harvested in Croatia. In this study, Zn concentrations ranged from 129–737 mg kg⁻¹, with the highest mean concentration of 605 mg kg⁻¹ measured at the Pag site. Among the localities in Mali Ston Bay, the highest level of 365 mg kg⁻¹ was determined at Bjejevica. The results measured at the location Brijesta in Mali Ston Bay were much lower than those obtained in an earlier survey in Mali Ston Bay in oysters collected in February 2000 (> 600 mg kg⁻¹; 20). This may be explained by seasonal variations in element levels related to freshwater supply to the bay from the nearby Neretva River delta, which may be a key factor influencing element concentrations, particularly in winter and spring due to increased rainfall conditions (20). Increased freshwater inflow from

Table 1: Operating conditions for ICP-OES

Element / Parameter	Mg	Fe, Cu, Zn, Se
Plasma viewing mode	Radial	Axial
Read time	1-5 s	1-5 s
Measurement replicates	3	3
RF incident power	1000 W	1300 W
Plasma argon flow rate	8 L min ⁻¹	15 L min ⁻¹
Nebulizer argon flow rate	0.85 L min ⁻¹	0.55 L min ⁻¹
Auxiliary argon flow rate	0.2 L min ⁻¹	0.2 L min ⁻¹
Sample uptake rate	1.5 mL min ⁻¹	1.5 mL min ⁻¹
Inner diameter of the torch injector	2.0 mm	2.0 mm
Nebulizer type	Concentric glass (Meinhard)	Concentric glass (Meinhard)
Spray chamber type	Glass cyclonic spray chamber	Glass cyclonic spray chamber

Table 2: Concentrations of Cu, Fe, Mg, Se and Zn in oysters from six different locations from the southern and central Adriatic coast

Location	N	mg kg ⁻¹ wet weight					
		Statistics	Cu	Fe	Mg	Se	Zn
Brijesta (MS Bay)	3	Mean ± SD	26.4±5.89	54.2±3.63	828±139	3.15±1.50	196±37.1
		Minimum	22.9	52.1	668	2.28	175
		Maximum	33.2	58.4	909	4.88	239
Mali Ston (MS Bay)	3	Mean ± SD	29.2±14.4	44.5±11.8	880±84.3	2.74±1.16	168±34.0
		Minimum	19.6	33.5	793	1.66	129
		Maximum	45.7	57.1	962	3.97	189
Bistrina (MS Bay)	3	Mean ± SD	37.8±8.03	38.2±13.8	727±104	2.43±0.59	336±121606
		Minimum	28.5	22.4	607	2.05	216
		Maximum	42.9	47.4	795	3.12	417
Bjejevica (MS Bay)	3	Mean ± SD	31.4±6.44	52.1±13.0	804±120	2.49±0.69	365±72.0
		Minimum	26.9	44.4	676	1.82	289
		Maximum	38.8	67.1	914	3.20	431
Sutvid (MS Bay)	3	Mean ± SD	21.2±12.1	41.4±13.1	784±149	2.48±0.49	207±49.8
		Minimum	8.84	26.2	621	1.92	169
		Maximum	33.0	49.5	916	2.86	263
Pag (Pag Island)	3	Mean ± SD	65.1±13.6	44.2±22.1	1024±238	3.11±1.05	605±142
		Minimum	50.5	27.7	761	1.99	454
		Maximum	76.6	69.3	1226	4.08	737

Table 3: Estimation of daily intakes (EDIs) of elements based on the lowest and highest mean concentrations and contribution to reference nutritional (RDA) and toxicological (PMTDI) values.

Food	EDI ^a (mg kg ⁻¹ BW day ⁻¹)	Contribution of mean to RDA ^b (%)	Contribution of mean to PMTDI ^c or TDI ^d (%)
Cu			
Lowest mean	0.007	0.008 (F/M)	14.0 1.40
Highest mean	0.0217	0.024 (F/M)	43.4 4.34
Fe			
Lowest mean	0.0127	0.071 (F) 0.165 (M)	0.026
Highest mean	0.0181	0.10 (F) 0.23 (M)	0.038
Mg			
Lowest mean	0.242	0.078 (F) 0.061 (M)	-
Highest mean	0.341	0.11 (F) 0.085 (M)	-
Se			
Lowest mean	0.0008	1.45 (F/M)	0.0055
Highest mean	0.0011	2.00 (F/M)	0.0076
Zn			
Lowest mean	0.056	0.70 (F) 0.51 (M)	112 11.2
Highest mean	0.202	2.52 (F) 1.84 (M)	404 40.4

^aEDI was calculated by the equation: [(element concentration; mg kg⁻¹) per (meal size or daily intake of food; kg)] divided by [adult body weight (60 kg)] (12). Meal size (g day⁻¹ per adult): 20 g (13).

^bRDA for female (F) and male (M): Cu 0.9 mg day⁻¹ (F/M); Fe 18 mg day⁻¹ (F), 8 mg day⁻¹ (M); Mg 310 mg day⁻¹ (F), 400 mg day⁻¹ (M); Se 0.055 mg day⁻¹ (F/M); Zn 8 mg day⁻¹ (F), 11 mg day⁻¹ (M) (14, 15).

^cPMTDI (provisional maximum tolerable daily intake): Cu 0.05-0.5 mg day⁻¹ BW day⁻¹ (16); Fe 48 mg day⁻¹ (17); Zn 0.3-1 mg day⁻¹ BW day⁻¹ (18).

^dTDI (tolerable daily intake): Se 14.4 mg kg⁻¹ (19).

various sources decreases salinity, which in turn significantly increases Zn levels in shellfish (21, 22). In comparing Zn levels with studies in other regions, Mali Ston Bay may be considered an area with low anthropogenic impact (1, 23).

The results obtained for all six sites were higher than Zn concentrations found in oysters *Ostrea edulis* from France (97 and 500 mg kg⁻¹ (1); 181 mg kg⁻¹ (7)). Previously, it was assumed

that the Zn content in marine animals is dependent on the contamination of the water at the specific sampling sites, coastal region or open sea. The Zn content of molluscs and crustaceans is approximately four times higher than that of marine fish (3). The fact that oysters are strongly influenced by habitat contamination was previously confirmed by the determination of extremely high Zn concentrations of >900 mg kg⁻¹

(24). A good example of high variation regarding habitat contamination was found for the oyster species *Ostrea edulis* (British and French coast) and *Crassostrea gigas* (French Atlantic coast) at non-contaminated and contaminated locations, i.e. (mg kg^{-1}): *Ostrea edulis* 97 and 500; *Crassostrea gigas* 92 and 217, respectively (1). In the same study, maximal Zn values ranged from 214–800 mg kg^{-1} in the oyster *Saccostrea cucullata* from the rocky shores of Hong Kong (1). Extremely high Zn concentrations were found in oysters from Cleveland Bay (2080 mg g^{-1}) and from Orpheus Island (2547 mg kg^{-1}) in Australia (21). Previously, it was concluded that oysters accumulate high concentrations of Zn in detoxified granules (1). The present findings confirm high Zn concentrations in oysters and that oyster is a good alternative dietary source of Zn.

In the present study, the highest mean Cu content of 65.1 mg kg^{-1} was also determined in oysters from the Pag location (Table 2). The highest Zn and Cu levels at the site Stara Poveljana on the island of Pag can be explained by the fact that this cultivation area is situated between the islands of Pag and Vir. In the summer months, this region has a high tourism load, which is not the case for the oyster cultivation area in Mali Ston Bay.

In French studies, the mean Cu concentrations measured in oysters were 12.9 and 11.7 mg kg^{-1} (7,11). These concentrations were 1.6–5.6 times lower than those found in the present study. Copper concentration differences in oysters from non-contaminated and contaminated locations from the coasts of Great Britain and France were previously determined (mg kg^{-1}): *Ostrea edulis* 6.4 and 78; *Crassostrea gigas* 13 and 58 (1).

There are few literature data providing other essential elements concentrations in oysters. In this study, Fe levels ranged from 22.4–69.3 mg kg^{-1} and the highest mean Fe level (54.2 mg kg^{-1}) was determined at the Brijesta location (Table 2). There were no significant differences in element contents between the locations from Pag Island and Mali Ston Bay. The results obtained were higher than those previously found (19.5 mg kg^{-1} ; 11) but lower than recently reported levels in France (103 mg kg^{-1} , 25).

Magnesium concentrations were in the range from 621 to 1226 mg kg^{-1} . The highest mean Mg of 1024 mg kg^{-1} was determined at the Pag site, though there were no significant differences in Mg levels between the six sites. These results were

similar to previously measured Mg concentrations of 1088 mg kg^{-1} in oysters from France (25). Selenium concentrations in this study were similar at all six sites and ranged from 1.66–4.88 mg kg^{-1} . The mean levels obtained were 5–17 times higher than those reported in studies from France (0.440 mg kg^{-1} , 11; 0.177 mg kg^{-1} , 7).

To evaluate the measured element levels in oysters from the nutritional and risk assessment perspectives, the estimated daily intake (EDI) was calculated and compared with recommended reference and toxicological values for each element. The sufficient recommended adequate intake of elements expressed as the recommended dietary allowance (RDA) for females and males are (mg day^{-1}): Cu 0.9; Fe 18 and 8; Mg 310 and 400; Se 0.055; Zn 8 and 11 (14, 15). As presented in Table 3, the EDIs calculated for the lowest and highest mean (chosen among means from all six sampling sites) for Cu, Fe and Mg represented very low percentages of the reference RDA values (ranging from 0.008% to 0.23% for a person weighting 60 kg). The EDIs calculated for Se and Zn showed a contribution to the RDA above 1.8% for highest measured mean values (%): Se 2.00; Zn 1.84 and 2.52.

For risk assessment purposes, the calculated EDIs of Cu, Fe, Mg, Se and Zn were compared with their toxicity reference values. Permissible human exposure as a result of the natural occurrence of elements in food were defined as the provisional maximum tolerable daily intake (PMTDIs) and tolerable daily intakes (TDI) and is used for risk assessment purposes associated with food consumption. Defined PMTDIs for elements were: Cu 0.05–0.5 mg kg^{-1} BW day^{-1} (16), Zn 0.3–1 mg kg^{-1} BW day^{-1} (18), Fe 48 mg kg^{-1} (17). For Se, the tolerable daily intake (TDI) is defined as 14.4 mg kg^{-1} (19). No PMTDI or TDI for Mg is given by World Health Organisation.

Contribution of the EDIs for Fe and Se calculated for the lowest and highest mean of elements to PMTDIs values were very low and below 0.05%. However, EDIs calculated at low and high mean level for Cu contribute to PMTDIs values in higher percent (%): low 1.40–14.0, high 4.43–43.4. The EDIs determined for Zn contribute to PMTDIs values at the lowest mean in the range 11.1–112% and at the highest mean in the range 40.4–404%. Therefore, the high values for Cu and Zn in comparison to the toxicity reference values suggest a risk from the consumption of oysters from all six sites.

To conclude, element variations in oysters were established between all collection sites. Significantly higher concentrations of Zn and Cu were determined in oysters from Pag compared to those from Mali Ston Bay, indicating an environmental impact on element levels. In order to investigate the environmental impact on the concentrations of elements, future studies should include element analysis in seawater and a survey of the monthly or seasonal variations in element concentrations at all six sites.

From the nutritional perspective, the estimated daily intake calculated for these five elements in oysters show a low contribution in accordance to the reference daily requirements for these elements. Following risk assessment results, it can be concluded that it is unlikely that the intake of Fe, Mg and Se through oysters would involve any risk for the average consumer. However, the intake of Cu and Zn through oysters may pose a health risk to consumers.

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KONCENTRACIJA BAKRA, ŽELEZA, SELENA, CINKA IN MAGNEZIJA V OSTRIGAH (*Ostrea edulis*) S HRVAŠKE JADRANSKE OBALE

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Povzetek: Cilj raziskave je bil ugotoviti in primerjati koncentracije bistvenih elementov (Cu, Fe, Se in Zn) in makronutrienta Mg v ostrigah (*Ostrea edulis*) iz šestih različnih gojitvenih lokacij vzdolž hrvaške obale Jadranskega morja. To je prva študija o kopičenju Cu, Fe, Mg in Se v ostrigah na Hrvaškem. Koncentracije elementov so bile določene v mg/kg mokre teže in so se gibale v razponu: Cu 8,84 - 76,6, Fe 22,4 - 69,3, Se 1,66 - 4,88, Zn 129 - 737 in Mg 607 - 1226. Koncentracije Fe, Mg in Se se niso značilno razlikovale med različnimi gojitvenimi lokacijami, raven Zn in Cu pa je bila značilno višja v ostrigah s Paga v primerjavi z ostrigami iz zaliva Mali Ston. Naši rezultati kažejo pomemben okoljski vpliv na vsebnost ravni elementov v ostrigah. Ocenjeni dnevni vnos (EDIS) preiskovanih elementov z ostrigami predstavlja za človeka nizek prispevek (0,008 - 2,52 %) k priporočenemu dnevnomu vnosu (RDA). Glede na rezultate ocene tveganja je mogoče sklepati, da je malo verjetno, da vnos Fe, Mg in Se z ostrigami pomeni tveganje za povprečnega potrošnika. Nasprotno pa gre za visok delež vrednosti EDI pri najvišjem še sprejemljivem dnevnom vnosu (PMTDIs) za Cu in še posebej za Zn, kar kaže, da vnos Cu in Zn z ostrigami lahko predstavlja tveganje za zdravje potrošnikov.

Ključne besede: baker; železo; selen; cink; magnezij; ostrige; Hrvaška