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ORIGINAL PAPERS

Condition Index of Mussel *Mytilus galloprovincialis* (Lamarck, 1819) as a Physiological Indicator of Heavy Metals contamination

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Abstract
The condition index (CI) is an ecophysiological indicator used to assess the response of mussels at different environmental pressures. The present paper aims to assess the heavy metals contamination of three polluted sites (Midia Port, Constanta Port, and Mangalia Port) and one reference site (2 Mai), using indigenous mussels *Mytilus galloprovincialis* as bioindicators of pollution. The concentrations of heavy metals (Cu, Cd, Pb, Ni, and Cr) in mussels' tissues, seawater and sediments from the 4 studied areas were evaluated in order to find a possible correlation with the physiological index (condition index) of the mussels. For this purpose, the condition index, Bioaccumulation Factor (BAF) and Biota-Sediment Accumulation Factor (BSAF) have been assessed and related to seasons and location. The study showed that mussels can bioaccumulate elements such as Cu, Cd, Pb, Ni, and Cr, certain differences being reported between sampling sites and season. Condition index showed a very significant correlation with heavy metals concentration in mussels (Cu, Pb, and Ni), with BAF (Pb) and with BSAF (Cd, Ni, and Pb). Also, CI correlates significantly with Cd concentration in seawater and Cr content in sediments. The highest values of CI and heavy metal concentrations were highlighted in mussels collected from the most polluted sites. Thus, any significant seasonal correlation between condition index and metal concentration in mussels may be related to food availability and the level of pollution in the studied sites.

Keywords: condition index, physiological indicator, heavy metals, bioaccumulation, *Mytilus galloprovincialis*, Romanian Black Sea coast.

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Introduction

Coastal ecosystems receive various inputs of contaminants, including heavy metals, derived from anthropogenic activities (e.g. industry, agriculture, shipping, navigation, and tourism).

Marine mussels *Mytilus galloprovincialis* are commonly used as bioindicators of environmental pollution in coastal waters due to their high filtration rates and their capacity to accumulate heavy metals in their soft tissues [24, 44, 6, 27, 28, 29, and 45].

Heavy metals are taken up from the aquatic environment through various routes of chemical exposure (*e.g.* dietary absorption, transport across the respiratory surface, dermal absorption, and inhalation). Bioaccumulation is the process where the chemical concentration in an aquatic organism achieves a level that exceeds the one recorded in the water as a result of chemical uptake [11].

Metal bioaccumulation is influenced by the interaction between many factors, such as: physiological (*e.g.* growth and filtration ratio), biochemical (e.g. lipid content), chemical (*e.g.* metal concentration, speciation and bioavailability) and environmental (*e.g.* temperature and food concentration) [2].

The concentration of chemicals in aquatic organism can be calculated by two different factors: bioconcentration factor (BCF) and bioaccumulation factor (BSAF). Both illustrate the partitioning of a chemical between water and aquatic organism. BCF refers to levels in organisms only due to the uptake by the organism from the surrounding water, while BAF also includes the uptake from food. BCF can therefore only be measured in laboratory studies, where uptake from food can be restricted, whereas the ratios measured in field are BAF [20, 10, 11]. Bioaccumulation is of great concern to authorities around the world. These organizations established threshold values for BAF [31, 38, and 39]. A bioaccumulation factor relative to the concentration of the chemical in the sediment (*i.e.* Biota-Sediment Accumulation Factor – BSAF) can be calculated, if the water concentrations are below the detection limit [11].

Changes in biochemical and physiologic responses can be used to assess the impact of heavy metals pollution [8]. The condition index (CI) of mussels has been used as a tool to evaluate the physiological and metabolic response to different environmental pressures [26, 47]. It can also be used as a nonspecific index to assess the relation between contaminants concentration and the mussel health [47, 35]. Condition index of mussels exhibit seasonal variations and is influenced by the interaction between environmental factors (*i.e.* mainly by water temperature and food availability) [34, 18, 19, 36].

The aims of the present study were:

(1) To evaluate the heavy metal concentrations (in seawater and sediments) and the bioaccumulation of various metals (Cu, Cd, Pb, Ni, and Cr) in the

indigenous mussels *Mytilus galloprovincialis*, collected from three polluted sites (Midia Port, Constanta Port, and Mangalia Port) and one reference site (2 Mai).

(2) To assess a seasonal variability of environment water quality and contamination gradient between the studied sites.

(3) To determine the Bioaccumulation Factor (BAF) and Biota - Sediment Accumulation Factor (BSAF) as a measure of chemical concentration of heavy metals in the mussels' soft tissue.

(4) To estimate the condition index of mussels *M. galloprovincialis* and compare the values between seasons and sites.

(5) Finally, to assess the use of condition index (CI) of mussels *M. galloprovincialis* as a physiological indicator of bioaccumulation of heavy metals in their soft tissues.

Material and method

Sampling sites

The study was carried out in late November and December 2017 (winter season) and May – early June 2018 (spring), at four sampling sites along the Romanian Black Sea coast, in coastal shallow waters (Fig. 1). Three sampling sites were selected considering the main pollution sources - Midia Port (44^{0}20'32.76"N; 28^{0}40'53.87"E), Constanta Port (44^{0}09'39.03"N; 28^{0}39'23.36"E), and Mangalia Port (43^{0}48'20.98"N; 28^{0}34'50.05"E) and one reference site - 2 Mai (43^{0}46'44.33"N; 28^{0}34'57.60"E). The sampling sites were represented using Ocean Data View [33].

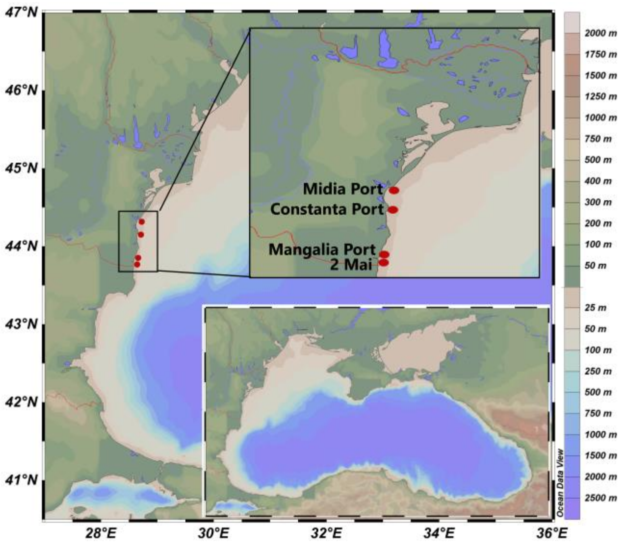

Fig. 1. Locations of sampling sites – Midia Port, Constanta Port, Mangalia Port, and 2 Mai

Environmental parameters

Physico-chemical parameters (temperature, salinity, pH, dissolved oxygen, and total dissolved solids) were measured *in situ* using a multiparameter sonde (HANNA HI 7698194). Triplicate seawater samples were collected for total suspended solids – TSS (0.5 L) analysis and for measurements of biological parameter – chlorophyll *a* (1 L). The samples were filtered through MF-Millipore filters (pore size 0.45 μm, diameter 47 mm) using a vacuum pump. After filtration, the filters used to retain chlorophyll *a* pigment were frozen until further analysis (at -20^{0}C). The pigments were extracted with 90% acetone and measured by spectrophotometry; concentrations being calculated using the SCORE-UNESCO trichromatic equation [43]. For the analysis of total suspended solids, the filters were dried (at 105^{0}C for 3h), weighed with an electronic balance (d=0.01 g) and the mean weights were converted to mg/L [13].

Heavy metal analysis

The concentration of copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) were measured in seawater, sediments, and whole soft tissues of mussels.

The concentration of total metals in seawater was measured in unfiltered seawater samples, acidified to pH = 2 with Ultrapure HNO_3 [13, 15].

Sediment processing consisted of lyophilization, homogenization and treatment with nitric acid (HNO_3 Suprapur), followed by the digestion process in the microwave. At the end of the mineralization, the samples were taken up in a 100 ml graduated flask with deionized water [42].

For heavy metals analysis, ten mussels were selected from each sampling site and frozen until further analysis. The soft tissue was removed from the shell, rinsed with seawater and deionised water. To determine the heavy metals, the mussel tissues were homogenized, weighed, and digested with 5 ml nitric acid (HNO_3 65%, Suprapur Merck), in sealed Teflon vessels, on hotplate at 120^{o}C. After mineralization, the samples were brought up to 100 ml volume with deionised water [40, 41].

The analytical determination of heavy metals content in all samples was performed by graphite furnace atomic absorption spectrometry method (GF-AAS), using a SOLAAR M6 Dual Thermo Electron. Three instrumental measurements were performed for each sample, with an average value reported.

Evaluation of heavy metals bioaccumulation factor (BAF) and Biota-Sediment Accumulation Factor (BSAF)

The extend of metal bioaccumulation is usually expressed in the form of a bioaccumulation factor (BAF) which is the ratio of the chemical concentration in the organism (C_B) (mass of chemical per kg of organism/dry weight) and the water (C_W) (mass of chemical/l) [20, 10, 11]:

$$BAF = C_B / C_W \quad (2)$$

According to the measured values of BAF, the soft tissues of mussels can be classified as: bioaccumulative (BAF ≥ 1000) [31], bioaccumulative (BAF ≥ 2000) [38] or very bioaccumulative (BAF ≥ 5000) [31, 38, 39].

To estimate the proportion in which metal occurs in mussel and in the associated sediment, Biota-Sediment Accumulation Factor (BSAF) was calculated for the five metals in the whole tissue of mussels. BSAF is calculated using the following equation, where C_B is the chemical concentration in the biota (mass of chemical per kg of biota/dry weight), while C_S is the concentration in the related sediment (mass of chemical per kg of sediment/dry weight) [37]:

$$BSAF = C_B / C_S \quad (3)$$

Based on measured values of BSAF, the soft tissues of mussels can be classified as: macroconcentrator (BSAF > 2), microconcentrator (1 < BSAF > 2), or deconcentrator (BSAF < 1) [5].

Determination of the condition index (CI)

A number of 50 mussels (*M. galloprovincialis*) were collected manually from each sampling site, from 1-1.5 m depth. The samples were transported in a cold box after collection in the field. Upon arrival, mussels were inspected and the dead ones were removed.

For condition index determination, thirty mussels were cleaned of epibionts, external byssus, washed, and measured (shell length, shell width, and shell heights) with a Vernier calliper (UNIOR 270A) to an accuracy of 0.02 mm. Each mussel was opened with a stainless scalpel and placed with its ventral edge on tissue papers to remove the internal water. Then the total tissue of mussels was detached from the shell. The mussels' meat and shell were dried until constant weight for 24h at 105°C, in a hot air oven. Dry meat and dry shell weights of mussels were kept in the desiccator for 12-14h and then measured with an electronic balance (d=0.01 g).

The condition index (CI) was calculated based on the coefficient of dry meat weight (DMW) and the dry shell weight (DSW), multiplied by a constant of 100 [7, 4].

This index was calculated as follows:

$$CI\ (g) = (DMW/DSW) * 100 \tag{1}$$

Data analysis

All measured environmental parameters are presented as the mean±standard error of the mean. Normal distribution and variance homogeneity for each group of data were checked using Shapiro-Wilk Normality Test and Levene Test. Due to lack of normality in data distribution, non-parametric tests were used to analyze the site and season variability, between median values of mussels' populations level.

A non-parametric Kruskal-Wallis one-way analysis of variance, was used to compare the mussel's population medians between sampling sites and seasons.

To see if there is a difference in condition index between sampling sites, correlation test (Spearman's rank correlation test) was used to determine the relationships between condition index and heavy metals concentration of seawater, sediments, and mussels. Another series of correlations were made between CI and BAF and BSAF. The statistical analysis was carried out using SPSS Statistics 20 Software. Also, for data analysis, contaminant and condition index records were input into the Microsoft Excel spreadsheets.

Results and discussions

- **Environmental parameters of seawater**

The environmental parameters of seawater recorded in Midia Port – MDP, Constanta Port – CTP, Mangalia Port – MGP and 2 Mai – 2MI, were shown in Table 1, as means±standard error values.

The temperature varied seasonally and recorded similar values for all stations. The maximum mean temperatures were measured in port areas (MDP, CTP and MGP) with 12.140±0.000°C (winter) and 19.430±0.000°C (spring). The salinity recorded the highest value in CTP (16.324±0.018 PSU) and the lowest value in MGP (9.028±0.003 PSU), in spring. The dissolved oxygen (DO) varied between 8.475±0.013 – 9.058±0.000 mg/L (winter) and 1.417±0.004 – 6.999±0.017 mg/L (spring). The pH values measured in the two seasons recorded similar values (between 8.270±0.002 and 8.698±0.003). The TDS did not show any clear trend, varying around 7.681±0.169 ppt to 13.193±0.061 ppt. The TSS lowest and highest mean values were recorded at 2 Mai, 0.103±0.003 mg/L (winter) and 75.267±5.591 mg/L (spring). The Chl *a* concentration varied between 0.517±0.003 – 3.663±0.003 mg/L (in winter), and between 4.470±0.192 mg/L – 26.633±0.212 mg/L (in spring). The maximum Chl *a* concentration was measured in CTP (26.633±0.212 mg/L).

Table 1. Seasonal variations of environmental parameters in coastal waters from Midia Port – MDP, Constanta Port – CTP, Mangalia Port – MGP, and 2 Mai – 2MI, during 2017 – 2018.

Season	Parameter	Site			
		MDP	CTP	MGP	2MI
Winter	T (ºC)	12.140±0.000	10.155±0.000	10.972±0.000	9.151±0.003
	S (PSU)	15.040±0.000	15.040±0.000	14.555±0.000	16.257±0.002
	DO (mg/L)	8.475±0.013	9.058±0.000	8.941±0.005	8.653±0.113
	pH	8.510±0.000	8.482±0.000	8.452±0.001	8.602±0.003
	TDS (ppt)	13.095±0.039	12.051±0.056	13.193±0.061	11.192±0.104
	TSS (mg/L)	0.303±0.003	0.597±0.003	0.397±0.003	0.103±0.003
	Chl *a* (mg/L)	1.527±0.003	3.663±0.003	2.507±0.003	0.517±0.003
Spring	T (ºC)	19.159±0.040	19.430±0.000	19.111±0.001	18.849±0.007
	S (PSU)	13.284±0.008	16.324±0.018	9.028±0.003	16.184±0.002
	DO (mg/L)	4.550±0.007	6.999±0.017	1.417±0.004	6.089±0.005
	pH	8.270±0.002	8.832±0.003	8.698±0.003	8.429±0.007
	TDS (ppt)	7.681±0.169	7.689±0.0123	11.113±0.170	11.358±0.002
	TSS (mg/L)	3.133±0.851	6.933±0.352	11.000±1.724	75.267±5.591
	Chl *a* (mg/L)	4.470±0.192	26.633±0.212	4.933±0.063	6.477±0.504

T= Temperature (n=30); S= Salinity (n=30); DO= Dissolved oxygen (n=30); TDS= Total dissolved solids (n=30); TSS= Total suspended solids (n=3); Chl *a*= Chlorophyll *a* (n=3). Results are expressed as mean±standard error.

- **Heavy metals concentration**

The heavy metals concentration (Cu, Cd, Pb, Ni, and Cr) in seawater, sediments, and mussels, in MDP, CTP, MGP, and 2MI, are illustrated in Figure 2.

The mean concentration of heavy metals in seawater recorded the highest values in spring. The Cu concentration in seawater showed the maximum value at 2 Mai (23.360 μg/L). Heavy metals concentrations of seawater showed a decreasing sequence: Cu > Ni > Pb > Cd > Cr (in winter) and Cu > Ni > Pb > Cr > Cd (in spring).

The highest concentrations of heavy metals in sediments were noticed in the port areas. The Cu concentration in sediment recorded the maximum value in winter, at Midia Port (85.760 μg/g dry weight). The order of heavy metals concentrations in sediments was: Cu > Ni > Cr > Pb > Cd (in winter) and Ni > Cr > Cu > Pb > Cd (in spring).

Higher concentrations of heavy metal in mussels were observed in both seasons, for Cu and Ni. The Cu concentration reached the maximum level in spring, at MGP (4.250 μg/g wet weight). In the case of Ni, the highest value was observed also in spring season (3.080 μg/g wet weight), at 2 Mai. The Cu, Cd and Cr concentrations showed similar mean values in winter and spring. The bioaccumulation potential of metals in mussel's tissue follows a decreasing sequence of Cu > Cd > Cr > Ni > Pb (in winter) and Cu > Ni > Cd > Cr > Pb (in spring).

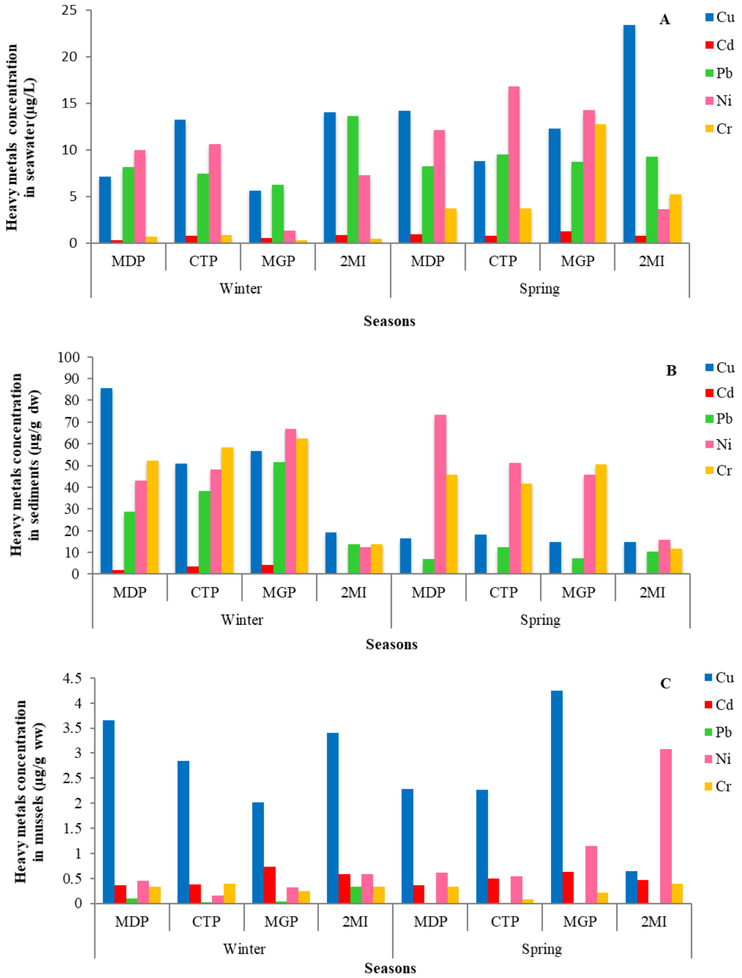

Fig. 2. Seasonal variations of heavy metals concentration in seawater (A), sediments (B) and mussels *Mytilus galloprovincialis* (C), from Midia Port – MDP, Constanta Port – CTP, Mangalia Port – MGP, and 2 Mai – 2MI, during 2017 – 2018.

- **Bioaccumulation Factors**

The BAFs and BSAFs values compiled in this study were calculated based on field data.

The seasonal variations of heavy metals bioaccumulation factors (BAFs) and Biota-Sediment Accumulation Factor (BSAFs) in mussels at the four sites MDP, CTP, MGP and 2MI, were shown in Table 2. Whatever the site, the decreasing sequence of metal BAFs and BSAFs was as follows: Cd > Cu > Cr > Ni > Pb.

The BAF_{Cu} recorded higher values in all sites, both in winter and spring, with a maximum value at MGP (10697.430 ≥ 5000). BAF_{Cr} showed the highest value at MGP (5961.359 ≥ 5000), followed by 2MI (5495.371≥ 5000). BAF_{Cu} showed higher values in all sites, particularly in winter. BAF_{Ni} recorded the maximum value at MGP (1849.828 ≥ 1000), in winter. BAF_{Pb} showed the lowest value, both in winter and spring. The $BSAF_{Cd}$, exceeded the threshold value in all sampling sites (in spring), and in winter only in MDP and 2MI. The $BSAF_{Cd}$ recorded the maximum value at MGP (31.847 > 2), in spring. The $BSAF_{Cu,}$ showed a high value in winter, at 2MI (1.357 > 2), and one in spring at MGP (1.490 > 1). The measured values of $BSAF_{Pb}$, $BSAF_{Ni}$ and $BSAF_{Cr}$, are within the threshold's values, both in winter and spring.

Table 2. Seasonal variations of heavy metals bioaccumulation factors (BAFs) and Biota-Sediment Accumulation Factor (BSAFs) in mussels *Mytilus galloprovincialis*, from Midia Port – MDP, Constanta Port – CTP, Mangalia Port – MGP, and 2 Mai – 2MI, during 2017 – 2018.

Seasons	Bioaccumulation Factors	Heavy metals	Sites			
			MDP	CTP	MGP	2MI
Winter	BAF	Cu	**3782.542[b]**	**1642.154[a]**	**2732.924[b]**	**1851.623[a]**
		Cd	**8300.278[c]**	**3885.528[b]**	**10697.430[c]**	**5533.284[c]**
		Pb	81.800	30.878	41.778	183.787
		Ni	329.597	107.926	**1849.828[a]**	616.501
		Cr	**3400.882[b]**	**3418.895[b]**	**5961.359[c]**	**5495.371[c]**
	BSAF	Cu	0.312	0.426	0.272	**1.357[d]**
		Cd	**1.472[d]**	0.806	1.352	**19.744[e]**
		Pb	0.023	0.006	0.005	0.180
		Ni	0.076	0.024	0.037	0.362
		Cr	0.046	0.051	0.031	0.190
Spring	BAF	Cu	703.732	**1470.888[a]**	**1790.351[a]**	116.458
		Cd	**1750.598[a]**	**3799.259[b]**	**2619.688[b]**	**2669.552[b]**
		Pb	2.658	3.004	7.079	2.265
		Ni	220.022	183.457	413.367	**3570.850[b]**
		Cr	385.092	122.232	84.993	311.502
	BSAF	Cu	0.599	0.705	**1.490[d]**	0.185
		Cd	**7.162[e]**	**72.089[e]**	**31.847[e]**	**8.741[e]**
		Pb	0.003	0.002	0.008	0.002
		Ni	0.036	0.060	0.128	0.809
		Cr	0.032	0.011	0.021	0.140

[a] **BAF ≥ 1000** (bioaccumulative) (REACH); [b] **BAF ≥ 2000** (bioaccumulative) (TSCA); [c] **BAF ≥ 5000** (very bioaccumulative) (REACH, TSCA, TSMP); [d] **BSAF > 1** (microconcentrator) (Dallinger, 1993); [e] **BSAF > 2** (macroconcentrator) (Dallinger, 1993); **BSAF < 1** (deconcentrator) (Dallinger, 1993).

- **Condition index of mussels**

The seasonal variations of condition index (CI) of the mussels *M. galloprovincialis*, at the four sampling sites (MDP, CTP, MGP, and 2MI), were presented in Figure 2.

The CI highest mean values were recorded in MDP (9.881 g, in winter) and at MGP (29.486 g, in spring). The maximum CI was observed during spring, in MDP (35.387 g) and the lowest in winter, in CTP (4.190 g).

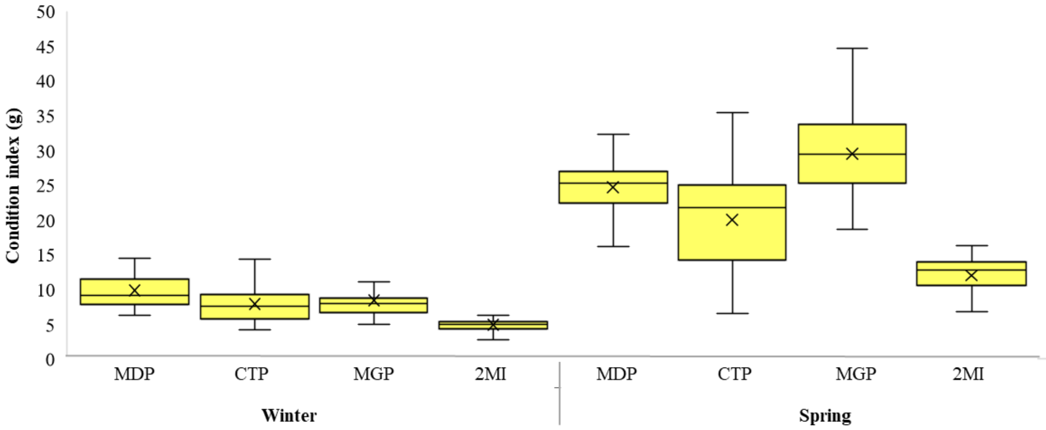

Fig. 3. Seasonal variation of condition index in mussels *Mytilus galloprovincialis*, from Midia Port – MDP, Constanta Port – CTP, Mangalia Port – MGP, and 2 Mai – 2MI. The horizontal line inside the box represents the median value and the asterix the mean value, the box represents the data range of 25-75%, and the whiskers are the minimum and maximum values.

The condition index data from all seasons were tested for normal distribution, with Shapiro-Wilk test, and for homogenity of variance (Levene test). The results showed that the condition index values are not symmetrically distributed and that the values are not dispersed homogenous. Thus, a non-parametric test (Kruskal-Wallis test) was chosen to test the condition index differences between sites and seasons.

Kruskal-Wallis test showed that was a statistically significant difference in condition index between sites and seasons. The results showed that there is a significant difference in condition indices between the sites, in winter ($p = 0.001 < \alpha = 0.05$; df = 3, $Chi^2 = 55.609$). The mean rank of condition index decreases from MDP to 2MI (MDP – 86.20, MGP – 70.53, CTP – 63.17, and 2MI – 22.10). The values recorded in spring, also showed that a significant difference in condition indices between the sites exist ($p = 0.001 < \alpha = 0.05$; df = 3, $Chi^2 = 68.848$). The mean rank of condition index decreases in the following order: MGP – 92.45, MDP – 74.12, CTP – 54.03, and 2MI – 21.40. The test results showed a statistically significant difference in condition index between winter and spring winter (p = 0.001 < α = 0.05; df = 1, Chi^2 = 142.231). The mean rank of CI increases from winter (67.05) to spring (173.95).

- **Condition index correlation with heavy metals concentration and bioaccumulation factors**

Correlation factors are shown in Table 3. Condition index showed a good positive correlation, statistically significant (r = 1.000, p = 0.001 < α = 0.05), with mussels' Pb and Ni concentrations and bioaccumulation factors – BAF_{Pb}, $BSAF_{Cd}$ and $BSAF_{Ni}$ (in winter) and with Cr concentration in sediment and Cu concentration in mussels (in spring). Also, a very strong correlation (r = 0.949, p = 0.04 < α = 0.05) was observed in spring, with BAF_{Pb} and $BSAF_{Pb}$.

Table 3. Spearman's rank order correlation of seasonal condition index (CI) with heavy metals concentration in seawater, sediments, mussels' soft tissue, BAF and BSAF.

		Cu	**Cd**	**Pb**	**Ni**	**Cr**
CI - Winter	HMsw	0.400	0.200	0.800	-0.400	-0.400
	HMs	-0.200	-0.800	-0.800	-0.800	-0.800
	HMm	0.600	0.000	1.000*	1.000*	-0.200
	BAF	0.400	0.200	1.000*	0.400	0.000
	BSAF	0.400	1000*	0.800	1.000*	0.400
CI - Spring	HMsw	-0.400	0.949*	-0.600	0.400	0.316
	HMs	0.200	-0.400	-0.600	0.400	1.000*
	HMm	1.000**	0.400	0.775	-0.200	-0.400
	BAF	0.800	-0.600	0.949*	-0.200	-0.400
	BSAF	0.800	0.000	0.949*	-0.400	-0.400

* Correlation is significant at the 0.05 level. HMsw: heavy metal concentration in seawater; HMs: heavy metal concentration in sediments; HMm: heavy metal concentration in mussels *Mytilus galloprovincialis*; BAF: Bioaccumulation Factor; BSAF: Biota Sediment Accumulation Factor.

Mussels are known as bioindicator organisms for monitoring chemical pollutants including heavy metals, due to their capacity to accumulate and concentrate pollutants in their soft tissues [44, 6, 28, 27, 29, 45]. The physico-chemical characteristics of water can influence the bioavailability of pollutants and therefore affect their bioaccumulation and the biological responses of organisms to these pollutants [2].

In this context, the seasonal variations of environmental parameters in the four sites (Midia Port – MDP, Constanta Port – CTP, Mangalia Port – MGP, and 2 Mai – 2MI) were assessed.

The temperatures recorded in the study are comparable with the monthly mean of temperature reported for surface seawater at Constanta [23]. Overall, high salinity values were recorded at 2 Mai because the site is in an open sea area and is further from the Danube influence. Low values of salinity were noted in port area, mainly because the sites are close to freshwater sources and wastewater treatment plants, and therefore the mixing with freshwater is higher. The minimum value of salinity 9.028 PSU (±0.003) was recorded in spring (MGP). This could be due to wastewater discharges from Mangalia treatment plant and significant rainfall recorded in that period. This support previous findings that show high salinity values in autumn and winter, and reduced values in spring and summer [23].

The highest concentrations of dissolved oxygen (DO) were recorded in winter and the lowest in spring. Because of the semi-enclosed nature of the ports, hypoxia occurs periodically, especially in warm season [9]. The DO dropped drastically to 1.417 mg/L in Mangalia Port due to high temperature and oxidative phenomena of organic matter decomposition. This organic enrichment may be due to phytoplankton blooms and/or discharge of wastewater. This confirms the previous findings of other authors [9].

The highest value of TSS was 75.267 mg/L (±5.591) and it was observed in spring. This value was reached due to the high amount of sediments washed by waves from the coastal terrace, during a strong storm. Large amounts of total suspended solids affect the seawater transparency. The obtained values agree with the findings of other author that showed that transparency has a seasonal trend, being low in spring and autumn, and high in winter and summer (Lazar, 2017).

Surface concentrations of chlorophyll *a* (Chl *a*) were measured as an indicator abundance and biomass of phytoplankton, that serves as food source for mussels. The highest concentration of Chl *a* (26.633±0.212 mg/L) was observed in spring (in CTP), and was correlated with the maximum value of temperature, salinity, and pH. The high temperature and salinity ensured perfect conditions for the occurrence of phytoplankton blooms.

The three sites (Midia Port, Constanta Port and Mangalia Port) seem to be the most affected by heavy metals pollution, due to the various pressures associated with harbour activities. The reference site (2 Mai) appears to be the less contaminated by heavy metals because is located far from major urban and anthropogenic sources. One possible cause of the seasonal variation of metal concentrations in mussel tissues could be temporal and spatial variability of water and sediments concentrations. These results agree with some studies that demonstrated the influence of season on metal bioaccumulation in mussels [22, 12, 28, 27]. The enrichment in heavy metals content (Cu, Cd, Pb, Ni, Cr) in mussel tissues, collected in spring, may correspond to a period of high storage of energy reserves before the reproduction period. Similar results were found by another author [30].

The calculated BAF value rated: Cd as “very bioaccumulative” (in winter – MGP, MDP, and 2MI) and “bioaccumulative” (in winter – CTP; in spring – in all sites); Cu as “bioaccumulative” (in winter – in all sites; in spring – CTP and MGP); Cr was rated as “bioaccumulative” (in MDP and CTP) and “very bioaccumulative” (in MGP and 2MI), in winter; Ni as “bioaccumulative” (in winter – MGP and spring – 2MI); Pb as “non-bioaccumulative” in all studied seasons. The BSAF calculated value rated: Cd as “microconcentrator” (in winter – MDP) and “macroconcentrator” (in winter – 2MI; in spring – in all sites); Cu “microconcentrator” (in winter – 2MI; in spring – MGP); Cr, Ni and Pb as “deconcentrator”, in both seasons. Similar results were found by other authors [16, 32].

The condition index (CI), considered an integrated indicator of organisms physiological condition, was also measured. The condition index reflects the effect of pollution and the environment quality at which the mussels were exposed during their development. This response may be influenced by both seasons and sites [6, 26].

The condition index of mussels was the highest in spring because the environmental conditions were more favourable (*e.g.* high temperature and salinity, high level of organic matter, and food availability that is indicated by the increased concentration of Chl *a*). These results are in accordance with studies described by other authors [25, 14, 3, 17].

The condition index variations are related to the mussels’ reproductive condition and fluctuations of environmental parameters (temperature, salinity, and nutrition). Also, Schneider (1992) showed the impact of temperature and food availability on condition index values [34]. Strohmeier et al. (2008) declared that food concentration induces an increase of mussels’ condition index [36]. The chlorophyll *a*, an indicator of phytoplankton biomass, was correlated with the condition index during the present study. This shows that the variation of this index is related to food availability. Similar results can be found in literature [18, 19]. Lachowicz (2005) declared that mussels’ condition varied according to location and was linked to environmental parameters [21]. These findings are similar with the results discussed in the present study.

Physiological condition is one of the main factors capable to control internal distribution and retention of contaminants in mussels [46]. The recorded values of the condition index of *M. galloprovincialis* mussel show that it is higher in polluted area (in ports). These values can be explained by the presence of high phytoplankton densities (and probability of frequent phytoplanktonic blooms), and by high temperature values. At the reference station (2MI), a low condition index was recorded, caused probably by poor trophic conditions. Similar results were found by another author [1].

Conclusions

(1)The study show that the metal concentrations measured in mussel *Mytilus galloprovincialis*, collected from the four sites of the Romanian Black Sea coast during 2017 – 2018, presented temporal and spatial variations.

(2)The present study highlights that the mussels' condition index (CI) was correlated with heavy metal concentration in tissues and with bioaccumulation factors (BAF and BSAF).

(3)Overall, the assessment of bioaccumulation factors (BAF) showed that mussels can be classified as "bioaccumulative" and "very bioaccumulative" for Cd, Cu, Cr, and Ni. According to BSAF, the mussel can be considered "macroconcentrators" and "microconcentrators" (for Cd and Ni), and "deconcentrators" (for Cr, Ni, and Pb). High values of BAF and BSAF were recorded due to the high pollution degree in the sampling sites, mainly in the port areas (PMD, CTP, and MGP).

(4)The highest values of CI and heavy metal concentrations were revealed in mussels from the most polluted sites (ports). The native *M. galloprovincialis* populations living in chronic contaminated areas may have developed adaptative mechanisms of protection against environmental stressors. Any significant seasonal correlation between condition index and metal concentration accumulated in mussels may be related to the seawater and sediments levels in the studied sites. These finding confirmed that condition index of mussels can be used as a bioindicator of acute and chronic pollution of the marine environment.

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