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Trace metal accumulation in tissues of wedge clams from sandy habitats of the Bulgarian Black Sea coast

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Abstract
The aim of the present study was to carry out an initial screening of trace metals bioaccumulation in soft tissues of the wedge clam (*Donax trunculus* Linnaeus, 1758) from different localities of the Bulgarian Black Sea coastal area and to evaluate the bioindicator potential of this species. Wedge clams were collected in June and September 2020 from sublittoral sandy habitats at different localities of the Bulgarian Black Sea coast. Soft tissues of individual clams were digested with nitric acid followed by ICP-OES analytical determination. The content of trace metals in the wedge clams differed significantly amongst localities. Higher metal content was present in wedge clams from Sveti Vlas, Shkorpilovtsi, Slanchev Bryag, Ahtopol and Kranevo. The highest values of lead (Pb) (2.51 mg/kg) and cadmium (Cd) (0.32 mg/kg) were found in samples from Sveti Vlas and the highest concentration of copper (Cu) (34.12 mg/kg), iron (Fe) (269.52 mg/kg) and nickel (Ni) (0.32 mg/kg) were detected in wedge clams from Shkorpilovtzi. Maximum content of chromium (Cr) (0.58 mg/kg) was present in samples from Slanchev Bryag, together with high values of Fe. The highest concentration of zinc (Zn) (18.04 mg/kg) together with high values of Cr and Fe were measured in wedge clams from Irakli. In conclusion, the wedge clams from the localities known to have higher coastal inflows and touristic pressures, i.e. Varna, Shkorpilovtzi, Sveti Vlas, Slanchev Bryag and Ahtopol accumulated significantly higher metal elements in their tissues. Only few significant seasonal differences in the concentration of metal elements in wedge clams were present and the observed

seasonal variations were probably connected to the hydrological parameters of the ecosystems. The wedge clam *D. trunculus* is a suitable bioindicator for assessment and monitoring of metal pollution in the Bulgarian Black Sea environment.

Keywords
Bulgarian Black Sea, *Donax trunculus*, trace metals

Introduction

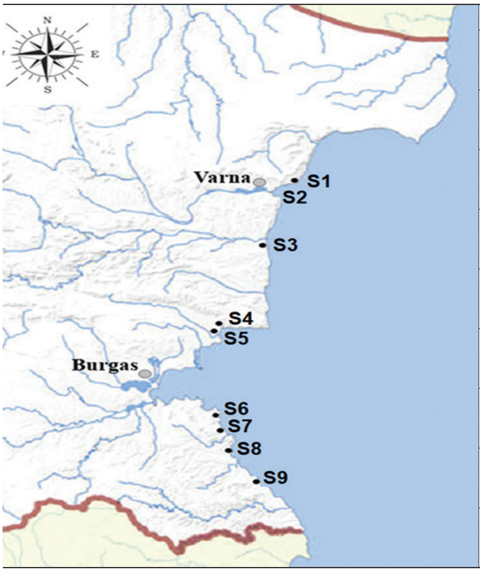
Trace metals constitute a significant proportion of the pollutants in the Black Sea (Mirinchev et al. 1999; Jitar et al. 2013; Bat et al. 2018a; Doncheva et al. 2020). The highest concentrations of metals are present in marine sediments from where they can be released back to water. Due to the migratory nature and bioaccumulation of metal elements, their discharge into the Black Sea can have profound effects on the entire marine environment (Islam and Tanaka 2004; Zaitsev 2008; Jitar et al. 2013). Metal pollution poses serious problems as it has a specific role in the impacts on marine ecosystems (Tchounwou et al. 2012). Unlike other pollutants, trace metals are characterised by their long time persistence in the environment (soils and waters) with periods ranging from hundreds to thousands of years (Bowen 1979). Being filter feeders and primary consumers in the food chain of the Black Sea, bivalves can accumulate metals and other pollutants in their tissues and shells (Romeo et al. 2005; Bat and Öztekin 2016). The capability of marine bivalves to accumulate metal elements makes them suitable pollution bioindicators (Bat and Öztekin 2016). Clam species are being reported as reliable bioindicators to monitor pollution in marine coastal areas (Adjei-Boateng et al. 2010; Bat et al. 2018b).

Although the spatial variation of metal concentrations in sediments of the Bulgarian Black Sea coastal areas is well documented (Simeonov and Andreev 1989; Andreev and Simeonov 1992; Jordanova et al. 1999; Doncheva et al. 2020), no comprehensive studies on the bioaccumulation and concentration of metal elements in clams from the Bulgarian Black Sea coastal habitats were carried out. In addition to their key role in coastal ecosystems, clams recently obtained significant economic importance for Bulgaria and this is quickly increasing.

The aim of the present study was to carry out an initial screening of trace metals bioaccumulation in soft tissues of the wedge clam (*Donax trunculus* Linnaeus, 1758) from different localities of the Bulgarian Black Sea coastal area and to evaluate the bioindicator potential of this species to assess the level and distribution of metal contamination in sandy habitats.

Methods

Wedge clams (length 23–35 mm) were collected manually or were obtained from commercial providers from sublittoral sandy habitats at representative localities along the



Point	Locality
S1	Kranevo
S2	Varna Bay
S3	Shkorpolovtzi
S4	Sveti Vlas
S5	Slanchev Bryag
S6	Sozopol
S7	Primorsko
S8	Arkutino
S9	Ahtopol

Figure 1. Localities of wedge clam sampling in 2020 along the Bulgarian Black Sea coast.

Bulgarian Black Sea coast (Figure 1) in 2020, before and after the intensive touristic season (June and September). The soft tissues of individual clams were digested with nitric acid followed by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) determination of elements (Cd, Cr, Cu, Fe, Ni, Pb and Zn).

The measurements were carried out in the certified chemical laboratory at the Department of Chemistry of the Medical University-Varna. Significance of difference in means was estimated by the t-statistic and patterns of similarities between sites in metal bioaccumulation in clam tissues were studied by cluster analysis using the STATISTICA 10 package.

Results

The concentration of trace metals in the wedge clams collected in June 2020 differed significantly amongst the studied localities (Table 1). Higher metal content was present in tissues of *D. trunculus* from Sveti Vlas, Shkorpilovtzi, Slanchev Bryag, Ahtopol and Kranevo.

It can be seen from the data in Table 1 that the highest values of Pb (2.51 mg/kg) and Cd (0.32 mg/kg), together with high concentration of Cr (0.424 mg/kg) and Cu (29.7 mg/kg) were found in wedge clams sampled from Sveti Vlas. High metal bioaccumulation levels were established in wedge clams from Shkorpilovtzi – Cu (26.13 mg/kg), Fe (192.1 mg/kg) and Ni (0.187 mg/kg). Maximum concentration of Cr (0.504 mg/kg) was present in wedge clams from Slanchev Bryag, together with the highest content of Fe (244.4 mg/kg). The highest concentration of Zn was measured in wedge clams from Varna Bay (15.98 mg/kg). Significantly higher concentrations of

Table 1. Metal content (ME ± SD mg/kg wet weight) in tissues of wedge clams gathered in June 2020, from different localities of the Bulgarian Black Sea coastal area in (* - significance of difference at $p < 0.05$ from locality s^n; nd – not detectable).

Site	Locality	Pb	Zn	Cd	Cu	Cr	Fe	Ni
S1	Kranevo	**0.237** ± 0.14	**8.475** ± 0.13	**0.054** ± 0.008	**19.231** ± 3.83	**0.378** ± 0.16	**137.3** ± 62.41	**0.104** ± 0.04
S2	Varna Bay	**0.281** ± 0.01	**15.98** ± 1.04 $*s^{1,8}$	**0.062** ± 0.01	**24.201** ± 2.4	**0.24** ± 0.09	**68.6** ± 14.6	**0.062** ± 0.055
S3	Skorpilovtzi	**0.629*** ± 0.59 $s^{1,6,8}$	**13.884** ± 3.53	**0.158** ± 0.12 $*s^{1,2,6,7,8}$	**26.131** ± 10.1 $*s^{1,6,7,8}$	**0.313** ± 0.11	**192.1** ± 155.76	**0.187** ± 0.14 $*s^{2,6,8}$
S4	Sveti Vlas	**2.514** ± 2.36 $*s^{1,2,3,5,6,7,8}$	**14.455** ± 0.45 $*s^{1,8}$	**0.322** ± 0.203 $*s^{1,2,6,7,8}$	**29.754** ± 0.48 $*s^{1,6,7,8}$	**0.424** ± 0.13 $*s^{2,6,7,8}$	**186.6** ± 19.63 $*s^{2,6,7,8}$	**0.109** ± 0.012
S5	Slanchev Bryag	**0.267** ± 0.12	**14.375** ± 1.59 $*s^{1,8}$	**0.144** ± 0.02	**22.391** ± 2.91 $*s^{6,7,8}$	**0.504** ± 0.09 $*s^{2,6,7,8}$	**244.4** ± 49.92 $*s^{2,6,7,8,9}$	**0.147** ± 0.02 $*s^{2,6,8}$
S6	Sozopol	**0.068** ± 0.002	**12.613** ± 0.06	**0.066** ± 0.001	**5.774** ± 0.06	**0.066** ± 0.001	**57.01** ± 0.91	**0.015** ± 0.004
S7	Primorsko	**nd**	**12.224** ± 0.24	**0.057** ± 0.001	**5.150** ± 0.35	**0.121** ± 0.05	**36.4** ± 8.89	**nd**
S8	Arkutino	**0.065** ± 0.008	**10.222** ± 0.08	**0.054** ± 0.003	**5.982** ± 0.03	**0.053** ± 0.003	**46.01** ± 0.46	**0.016** ± 0.005
S9	Ahtopol	**0.249** ± 0.12	**12.988** ± 2.987	**0.151** ± 0.04 $*s^{1,2,6,7,8}$	**25.427** ± 7.2 $*s^{6,7,8}$	**0.204** ± 0.14	**74.03** ± 37.26	**0.168** ± 0.04 $*s^{2,6,8}$

Zn (14.573 mg/kg), Cr (0.504 mg/kg) and Fe (244.4 mg/kg) were measured in wedge clams from Slanchev Bryag.

The pattern of the similarities between the studied localities, based on the metal bioaccumulation in wedge clams sampled in June 2020, was studied by cluster analysis using Euclidean distance as a measure (Figure 2).

The analysis revealed the presence of two main clusters of localities, based on the similarity of the metal concentrations in wedge clams (Figure 2). The first cluster (upper part of the diagram) comprised localities situated in the northern coastal area - Shkorpilovtzi, Sveti Vlas, Kranevo and Slanchev Bryag. This cluster clearly indicated that the localities from the northern coastal part (first cluster) had similar metal bioaccumulation in the wedge clams amongst them, which however differed significantly from the metal concentrations in the wedge clams of the localities along the southern coastal part, grouped in the second cluster (lower part of the diagram). Amongst the northern localities (first cluster), Shkorpilovtzi and Sveti Vlas showed the highest similarity in the metal concentrations in the wedge clams sampled there.

The second cluster included the localities Primorsko, Arkutino, Sozopol and Ahtopol from the southern coastal area. In this cluster, Varna Bay was grouped together with Ahtopol as a result of the similarity of the metal concentrations in the wedge clams between them.

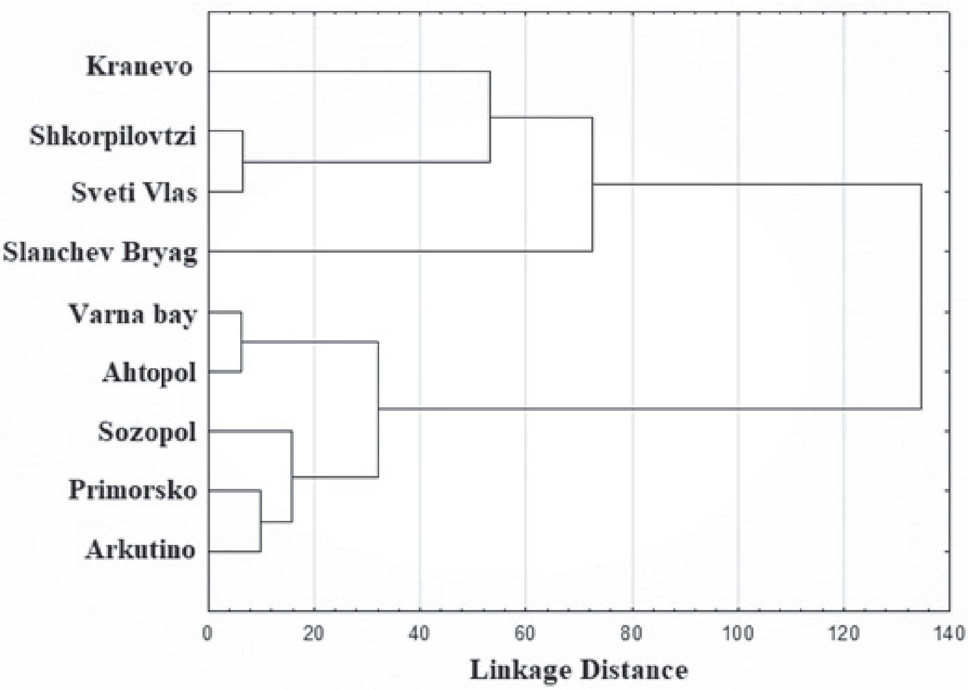


Figure 2. Cluster analysis of the similarity of localities based on metal microelements bioaccumulation in wedge clams sampled in June 2020.

Table 2. Seasonal variations (June and September 2020) in metal microelement concentration (ME ± SD mg/kg wet weight) in wedge clams from four localities (* - significant difference from June at p < 0.05; nd – not detectable).

Locality	Month	Pb	Zn	Cd	Cu	Cr	Fe	Ni
Kranevo	June	**0.237**	**8.47**	**0.054**	**19.20**	**0.37**	**137.20**	**0.104**
		± 0.14	± 0.13	± 0.01	± 3.83	± 0.16	± 62.4	± 0.037
	September	nd	**13.02***	**0.053**	**4.46***	**0.02***	**26.20***	nd
			± 0.08	± 0.01	± 0.1	± 0.01	± 0.32	
Varna Bay	June	**0.281**	**15.98**	**0.062**	**24.20**	**0.24**	**68.60**	**0.062**
		± 0.01	± 1.04	± 0.01	± 2.4	± 0.09	± 14.6	± 0.055
	September	**0.232**	**14.91**	**0.050**	**27.50**	**0.14***	**51.90**	**0.070**
		± 0.036	± 0.04	± 0.01	± 0.1	± 0.01	± 0.71	± 0.009
Primorsko	June	nd	**12.22**	**0.057**	**5.20**	**0.12**	**36.40**	nd
			± 0.239	± 0.001	± 0.3	± 0.05	± 8.89	
	September	nd	**10.09**	**0.077**	**6.40**	**0.06***	**55.80**	**0.202**
			± 0.017	± 0.001	± 0.1	± 0.01	± 0.99	± 0.008
Arkutino	June	**0.068**	**10.22**	**0.054**	**5.90**	**0.05**	**46.01**	**0.016**
		± 0.01	± 0.083	± 0.003	± 0.1	± 0.01	± 0.46	± 0.005
	September	nd	**13.10**	**0.058**	**5.60**	**0.04**	**38.10**	nd
			± 0.04	± 0.002	± 0.1	± 0.01	± 0.79	

Specifically, the levels of metal bioaccumulation in wedge clams from the northern localities was significantly higher, as a whole, than in the wedge clams from the southern localities, thus indicating different metal pollution levels of the northern and southern Bulgarian Black Sea coastal areas (see also Table 1).

Amongst the cluster of southern localities, Primorsko and Arkutino were most similar in the metal content in the wedge clams sampled there (Figure 2). The similarities between the southern localities seemed to be most probably due to the lower level of metal contamination of the marine environment at the southern localities compared to the northern ones. The Ahtopol locality was an exception showing the lowest similarity with the other southern localities, due to the relatively higher values of Cu, Cd and Ni in the wedge clams sampled there (Table 1) and, hence, was grouped together with Varna Bay.

Available data from four localities sampled in June and also September 2020 (before and after the intensive touristic season), were analyses for seasonal changes in the metal accumulation in the wedge clams (Table 2). As a whole, little seasonal differences were present in the concentration of metal elements in the wedge clams from the studied localities. Some exceptions, however, were present. In particular, in wedge clams from Kranevo, the concentration of Cu, Cr and Fe was significantly lower in September samples compared to June samples. The content of Cr in wedge clams from Varna Bay and Primorsko was also significantly lower in September (Table 2).

Discussion

The pollution of the Black Sea with metals is a major environmental challenge (Strezov 2008). Metal elements are introduced into the marine environment by natural geological processes, rivers or direct discharge of industrial waste (Mitryasova et al. 2020). Recent studies have revealed variable average concentrations of metals in seawater and surface sediments from the Bulgarian Black Sea coastal zone and pollution hotspots were designated (Peycheva et al. 2017; Doncheva et al. 2020). Bivalves, in particular the mussel *Mytilus galloprovincialis* (Lamarck, 1819), have been traditionally used as biomonitors of Black Sea metal pollution (Bat 2012; Bat and Öztekin 2016). However, available data on the spatial and temporal distribution of metal accumulation in benthic clam species from the Bulgarian Black Sea coast is quite scarce.

In this paper, we present the first comprehensive study on the distribution and bioaccumulation of metal elements in the wedge clam *D. trunculus* from different localities of the Bulgarian Black Sea coastal area. The content of trace metals in the wedge clams differed significantly amongst the studied localities. Higher metal content was present in the tissues of wedge clams gathered from several resort beaches at Sveti Vlas, Shkorpilovtzi, Slanchev Bryag, Ahtopol and Kranevo. Highest values of Pb (2.51 mg/kg) and Cd (0.32 mg/kg) were found in wedge clams from Sveti Vlas and of Cu (34.12 mg/kg), Fe (269.52 mg/kg) and Ni (0.32 mg/kg) in the wedge clams from Shkorpilovtzi.

Patterns in the similarity amongst localities, based on metal bioaccumulation in the wedge clams, were studied by cluster analysis using Euclidean distance. The analysis showed the presence of two main clusters of localities according to the metal content in the wedge clams. The first one included the localities Shkorpilovtzi, Sveti Vlas, Kranevo and Slanchev Bryag which are situated in the northern coastal area and appeared more polluted since the wedge clams had higher concentrations of metal elements. Amongst these localities, Shkorpilovtzi and Sveti Vlas showed the highest similarity, obviously due to the high accumulated levels Pb, Cd and Cu in the wedge clams sampled there. The second cluster was formed by the localities Primorsko, Arkutino and Sozopol which are situated along the southern coastal area and appeared to have less accumulated metal elements in the wedge clams living there. Our findings correspond, at least in part, with the data on the concentration of metal elements in sediments from these regions (Peycheva et al. 2017).

We did not find many significant seasonal differences in the concentration of the studied in June and September 2020 trace metal elements in wedge clams with the exception of Kranevo where the content of Zn, Cu, Cr and Fe was significantly lower in September compared to June. The observed seasonal variations could have been mainly connected with local changes in the hydrological parameters of the ecosystem.

Recent studies on trace elements (Cd, Cr, Cu, Fe, Ni, Pb and Zn) concentration in commercial wedge clams from the Bulgarian Black Sea coast were carried out with respect to consumption risks for humans and indicated that this species is, as a whole, safe for human consumption (Peycheva et al. 2021). In general, our data also showed that the concentrations of the accumulated metal elements in wedge clams from the studied in 2020 localities were below the maximum residual levels prescribed by different local and international regulations for seafood. There was only one exception connected with the established high concentration of Pb (2.51 mg/kg) in wedge clams from Sveti Vlas in June which significantly exceeded the national and European regulations (1.5 mg/kg, Commission of the European Communities 2008) for seafood.

Conclusion

The wedge clam *D. trunculus* plays a significant role in maintaining functionality of marine ecosystems and, as a filter feeder, can accumulate considerable amounts of trace metal elements. Wedge clams from localities with high coastal inflows and touristic pressures, i.e. Varna Bay, Shkorpilovtzi, Sveti Vlas, Slanchev Bryag and Ahtopol accumulated significantly higher metal elements in their tissues. No significant seasonal variations in the concentration of accumulated metal elements in wedge clams were present. The few observed significant seasonal differences were probably connected with changes in the local hydrological parameters of the ecosystems. Our data showed that concentrations of accumulated metal elements in wedge clams from the studied localities were below the maximum residual levels prescribed by local and international regulations for seafood. The wedge clam *D. trunculus* proved to be a suitable bioindicator for assessment and monitoring of the metal pollution levels of the Bulgarian Black Sea environment.

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