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HEAVY METAL CONCENTRATIONS IN ZOOPLANKTON OF SINOP COASTS OF THE BLACK SEA, TURKEY

Estimation of level contamination of marine ecosystems with heavy metals is important task in the field of MSFD. Total zooplankton samples were collected using a standard zooplankton net (mesh size=112 µm, mouth diameter=50 cm) from Sinop coast of the Black Sea, Turkey in 2011 and 2012. Concentrations of Al, As, Cu, Zn, Hg, Fe, Cd and Pb were determined in the total zooplankton samples. The average concentration of heavy metals followed order: Fe > Al >Zn > Pb > Cu > As > Cd > Hg. Estimated Hg was below the detection limit among all heavy metals in all samples. Fe was the most common heavy metal in total zooplankton samples present in all, Al was the other heavy metal commonly present in the samples. Pb showed high value (210 mg · kg^{-1}) in total zooplankton. Similar to Pb, a high average concentration of As (40 mg · kg^{-1}) was observed in total zooplankton. Cd was 1.1 mg · kg^{-1}.

Key words: heavy metals, zooplankton, Copepod, Black Sea

Contamination of marine ecosystems with heavy metals continued attention in the field of Marine Environment Policy Marine Strategy Framework Directive (MSFD). The Black Sea is a unique marine environment, representing the largest land-locked anoxic basin in the world with a maximum depth of 2200 m, a surface area of $4.2 \cdot 10^5$ km^2 and a volume of $5.3 \cdot 10^5$ km^3. The basin is completely anoxic, containing an oxygenated upper layer (10–15 % of total sea volume) and anoxic deep water with hydrogen sulphide [6]. A permanent halocline separates the oxic and anoxic waters [30]. Its waters are almost completely isolated from the world ocean. There is a restricted exchange with the Mediterranean Sea through the Turkish Straits System: the Bosphorus, Dardanelles Straits and the Sea of Marmara. The temperature varies seasonally in the surface layer due to solar heating and decreases with depth to a minimum (50–100 m depth), which is identifiable by a cold intermediate layer. Below this layer, permanent halocline (50–200 m) separates the surface water from the deep water. The basin-wide distribution of oxygen-carrying cold intermediate layer waters has important implications on the health and ecology of the Black Sea [29, 22]. The aerobiotic waters of the Black Sea are biologically productive because of high run-off from many rivers including the Danube, Dnepr, Dnestr, Kizilirmak, Yesilirmak around the basin. Black Sea is being threatened by the discharge of untreated sewage wastes and industrial effluents which affects the sustainability of living resources. The coastal systems of the Black Sea have been increasingly impacted by heavy metals released from these anthropogenic activities [1, 13]. Moreover, some heavy metals enters the atmosphere from thermal plant, from burning, incinerating trash, things like that [12, 36]. These wastes carry enormous level of toxicants especially the heavy metals have the tendency to accumulate into the basic food chains and move up through the higher trophic level and results in negative impact on the marine resources thus causing economic loss.

Pollutants especially heavy metals bind preferentially to suspended particulate material and bottom sediments of rivers, estuaries and coastal waters. As a result, many toxic and persistent metals can be found at high concentrations in the sediment. This have caused to the Black Sea to deteriorate in terms of benthic and pelagic communities, fisheries, habitats, sediment and water quality etc. and potentially human health, through direct contact of organisms or re-suspension into the overlying water [13].

Zooplankton plays a pivotal role in shaping ecosystem structure because grazing by zooplankton is thought to influence or regulate primary production, and variations in zooplankton dynamics may affect biomass of many fish stocks. Zooplankton mainly Copepods are the most abundant forms in the Black Sea. They filter suspended matter and have important role in transferring the organic matter from primary producers to the higher trophic levels. It is also one of the most preferred foods for the fish [3, 14]. Zauke and Schmalenbach [40] pointed out that zooplankton play an important role in the biogeochemical cycling of metals in marine ecosystems.

So far very little work has been published on the heavy metal estimation in zooplankton from the Black Sea coast of Turkey. However, metal compounds have been extensively used and are widespread in the Black Sea. Therefore it is important to determine the heavy metal concentrations in zooplankton for a better understanding of metal pollution in the Black Sea. In this regard, the heavy metal concentrations have been measured in total zooplankton from Sinop coast of the Black Sea, Turkey in 2011 and 2012.

Materials and Methods. The sampling of the zooplankton was carried out between February – August 2011 (except April and June) and June – September 2012 (except July) near Sinop coasts of the Black Sea (42° 00'21" N ; 35° 09'32" E) (Fig. 1). The zooplankton samples were collected during daytime with 5 replicate vertical hauls by using standard plankton net (mesh size =112 µm, mouth diameter =50 cm) from the bottom to the surface (50–0 m). Following the vertical tows, contents of the cod ends were filtered using a 2 mm sieve to retain and subsequently to quantify gelatinous organisms. The zooplankton samples were placed in a nylon sieve (20 µm mesh size) and thoroughly rinsed with distilled water to remove salts. Remaining water was removed from animals by placing the nylon sieve. Collected samples were divided into two parts. One was transferred to 4% formalin for quantitative and qualitative taxonomical analyses of zooplankton.

The analyses of samples were carried out using sub-samples of 1 ml sampled with a Stempel pipette. Samples were counted in a Bogorov chamber and analyzed under a stereomicroscope. The results were then averaged and extrapolated to the whole sample. All samples of species, including rare groups like Chaetognatha, Decapod larvae, fish eggs and larvae were analyzed according to Harris et al. [21]).

All Cladocera, Copepoda, Appendicularia, Chaetognatha were identified to species. All other taxa were identified to phylum, class or order levels. The main references used for the identification of major zooplanktonic groups were Bradford-Grieve et al. [15] and Conway et al. [16]. Systematic classification and nomenclature of zooplankton species were made according to Appeltans et al. [2]. The abundance results were given in ind. · m^{-2}.

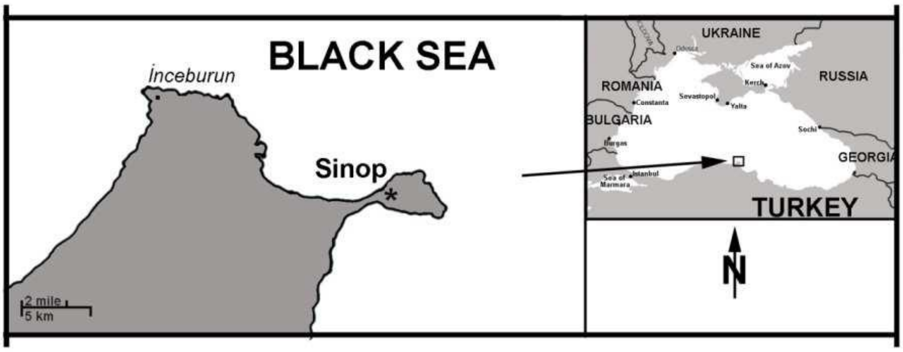

Fig. 1 Sampling region
Рис. 1 Район отбора проб

The other one was kept for heavy metals analyses in the laboratory. Subsequently, the samples were placed in petri dishes and wet weighed. The samples were dried in an oven at 100°C in 24 hours and weighted again then, dried sample were placed in Eppendorf tube. Sampling date, wet and dry weights and percentage of weight loss were shown in Table 1. All samples were stored deep frozen at –21°C until their analysis. Metal analysis in total zooplankton were performed using m-AOAC 999.10–ICP/MS method by accredited ÇEVRE Industrial Analysis Laboratory Services Trade Company (TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T). The method for determination of heavy metals, used acid, standard reference material,

Table 1 Sampling date, wet and dry weights (g) and percentage of weight loss (%)
Табл. 1 Дата отбора проб, сырой и сухой вес проб (г), доля потери веса (%)

Sampling date	Wet Weight (g)	Dry Weight (g)	Loss of Weight (%)
24 February 2011	0.5479	0.0214	96.1
29 March 2011	0.9355	0.0938	90.0
4 May 2011	0.6244	0.0921	85.2
27 May 2011	2.5456	0.1014	96.0
7 July 2011	4.1859	0.1347	96.8
4 August 2011	15.7217	0.5974	96.2
13 June 2012	3.8718	0.1925	95.0
1 August 2012	6.9543	0.4123	94.1
4 September 2012	6.1195	0.3356	94.5
27 September 2012	6.205	0.3849	93.8

wet digestion was used by European Standard method with number EN 15763. Analytical quality control sample was routinely run through during the period of metal analysis. Standard solutions were prepared from stock solutions (Merck, multi-element standard). Certified Reference Material No: 414 trace elements in plankton (powder) were used for calibration. The results showed good agreement between certified and analytical values (recovery rates 95 – 110 %). The limits of detection used for analysis of aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead were 0.5, 0.05, 0.5, 0.5, 0.05, 0.5, 0.02 and 0.05, respectively.

Results and Discussion. Proportion of zooplankton groups in collected samples is given in Fig. 2. Copepoda dominated among zooplankton groups. The abundance percentage of Copepoda was about 60.5% of the total. Dinophyceae (*Noctiluca scintillans*) was the second most abundant group (16.5%), followed by Cladocera (9.5%) and meroplankton (7.9%) (Fig. 2). This finding is in agreement with general understanding of zooplankton classes spatial and temporal distribution in the Black Sea [7, 10, 39]. The list and mean abundance (ind. · m^{-2}) of zooplankton species are given in Table 2. Like the Black Sea, low salinity of the Baltic Sea water favours the development of Copepoda, which frequently dominate coastal waters and Copepoda proved to be enriched with heavy metals in comparison with Cladocera [32]. Martin [25] showed that many heavy metals had become adsorbed to copepod exoskeletons at greater depths because food-dependent moulting rates were lower; thus more time was available for elemental adsorption to take place.

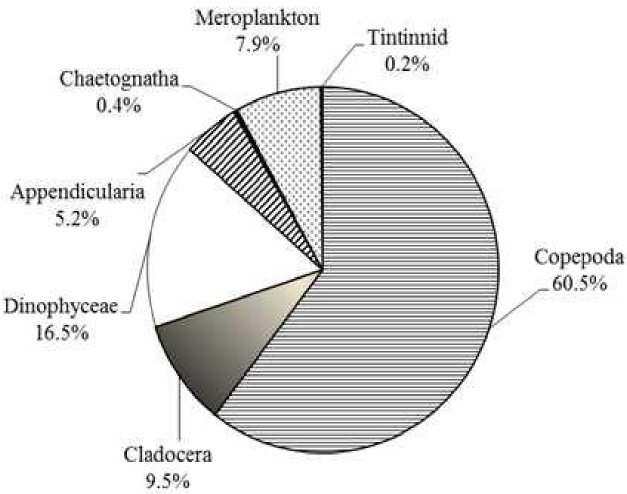

Fig. 2 Composition of zooplankton in coastal water near Sinop (in percents)
Рис. 2 Состав зоопланктона по группам у побережья Синопа (в процентах от общей численности)

The mean heavy metal concentrations in total zooplankton of the present study are given in Fig. 3. The extent of different heavy metals in zooplankton followed hierarchy, Fe> Al> Zn> Pb> Cu> As> Cd> Hg. All metals except Hg measured in the present study in detectable levels, indicating some degree of heavy metal pollution in this region. Fe was the most common heavy metal in total zooplankton samples present in all, Al was the other heavy metal commonly present in the samples. Al concentrations in the zooplankton samples of the southern Baltic Sea varied in the range from 20 to 1615 g · g^{-1} dry wt. [32].

Table 2 The list and mean abundance (ind · m^{-2}) of zooplankton groups during this study
Табл. 2 Список видов и средняя численность (экз. · $м^{-2}$) групп зоопланктона в районе исследований

SPECIES	Abundance (ind · m^{-2})
APPENDICULARIA	
Oikopleura (Vexillaria) dioica Fol, 1872	5603
CLADOCERA	
Penilia avirostris Dana, 1849	9089
Pleopis polyphaemoides (Leuckart, 1859)	463
Pseudevadne tergestina (Claus, 1877)	303
Eavdne spinifera P. E. Müller, 1867	401
CHAETOGNATHA	
Parasagitta setosa (Müller, 1847)	401
COPEPODA	
Acartia (Acartiura) clausi Giesbrecht, 1889	13888
Calanus euxinus Hulsemann, 1991	271
Centropages ponticus Karavaev, 1895	465
Oithona similis Claus, 1866	4515
Oithona davisae Ferrari F. D. & Orsi, 1984	10118
Paracalanus parvus (Claus, 1863)	4840
Pseudocalanus elongatus (Boeck, 1865)	5705
Harpacticoida	38
Copepoda nauplii	24450
Copepoda eggs	1250
DINOFLAGELLATA	
Noctiluca scintillans (Macartney) Kofoid & Swezy, 1921	17838
TINTINNIA	213
MEROPLANKTON	
Bivalvia larvae	6263
Cirripedia larvae	229
Decapoda larvae	124
Gastropoda larvae	1463
Medusae planula	16
Microniscus sp.	3
Polychaeta larvae	401
Fish larvae	26
Fish eggs	3

Pempkowiak et al. [31, 32] suggested that this can be caused by varying composition of zooplankton species and/or contribution of mineral particles, since Al is a major component of particulate mineral matter [32].

Studies on metals in zooplankton have been conducted for decades, but there is little information on concentrations of heavy metals in zooplankton from the Black Sea are available [8, 37, 38] and these studies were carried out with certain metals. The levels of metals were compared to those found in the Black Sea and other areas for the mixed zooplankton (Table 3). Regarding Zn and Cu the reported patterns are in good agreement with the literature obtained in the present study. High accumulation of Zn in zooplankton may be due to coprecipitation of Zn with calcium carbonate [34].

Zooplankton samples from the Black Sea coast show Pb and As concentrations in detectable levels (210 and 40 mg · kg^{-1} dry weight, respectively), suggesting that these metals might not serve as a regional background value. Martin [25] indicated that three main factors govern adsorption rates of the metals. They are concentration of the available metal for uptake, physical factors and the amount taken up will also depend on duration. It is known that salinity played a major role in the depletion of the dissolved Pb in the coastal water. If salinity decreased, the concentrations of dissolved Pb increased [35]. Pb is known to form colloids in seawater, and such colloids would have adsorbed onto planktonic debris, which consequently might have resulted in higher concentration of the metals in zooplankton from the coastal waters [34]. Marine organisms accumulate As by uptake from the surrounding water and foods and retain assimilated As mainly in the exoskeleton and muscle tissues, but its compounds are efficiently and rapidly excreted reducing the possibility of accumulation and trophic transfer in food chains [28]. However, in view of the large surface-to-volume ratio of the zooplankton, it is assumed that differences in the amounts taken up by adsorption-exchange may be largely responsible for the observed variations of the metals [25]. This phenomenon may be true for the Black Sea.

The high values of these heavy metals in zooplankton from the Black Sea may be due to industrial and domestic effluents into the harbor and its passage to the coastal areas, together with land based sourses from rivers [1, 9]. Possible explanations for this may be due to larger availability of these heavy metals in the Black Sea. Many Cu ores are processed at the Black Sea coast of Turkey. The Zarbana River bed close to Etibank Küre mine and Zarbana river mouth located at central part of Northern Anatolia are highly contaminated with respect to metals including Cu, Zn, As, Fe, and Pb [17].

Mean Cd level is 1.1 mg · kg^{-1} dry weight in this study and lower than those in many studies presented in Table 3. Pempkowiak et al. [31] indicated that low Cd concentrations, both dissolved and in particulate matter rich with organic carbon. It is predicated to absorption of dissolved Cd by algae and dilution of particulate Cd by high loads of organic matter originating in the process of primary production and resulted in Cd depletion in zooplankton [32]. These results may be explained by any, or a combination of the different factors [32]. High amount of organic matter [4, 5] and primary production loads [1, 23, 26] in the Black Sea decrease bioavailability of dissolved metals and decreased concentrations of metals in particulate organic matter [31]. However, high Cd concentrations in Copepods were found in Barrent Sea [40]. These differences were explained that these may be related to changing accumulation strategies of the copepods or to seasonally changing Cd absorption in copepods from food or to local upwelling [40]. A study by Gama-Flores et al. [19] demonstrated that even at low concentrations of Cd with longer exposure periods was detrimental to zooplankton.

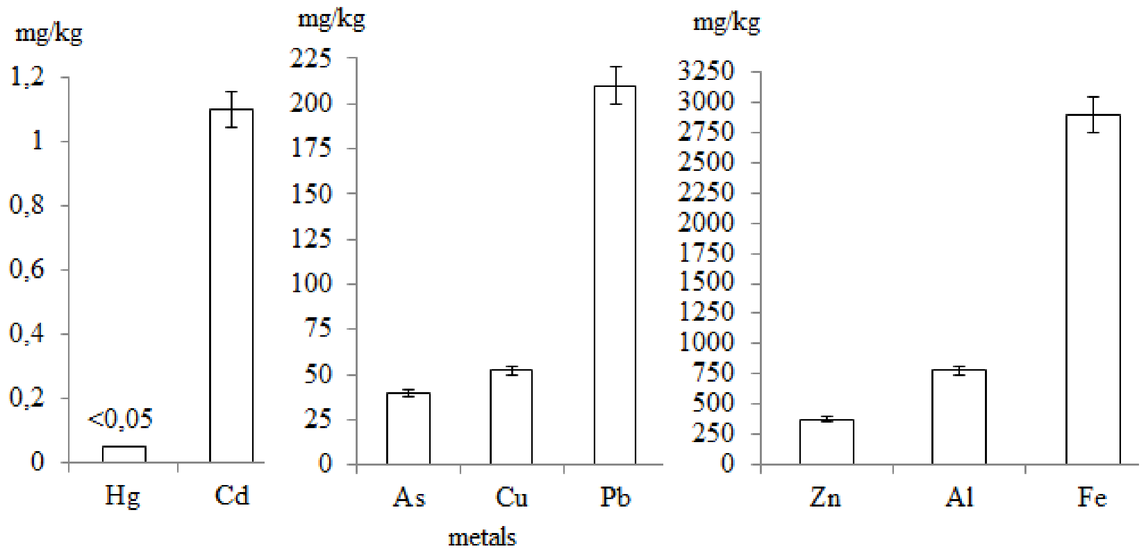

Fig. 3 Heavy metal concentrations (mean±SE) in total zooplankton collected from Sinop coasts of the Black Sea during 2011–2012
Рис. 3 Концентрация тяжёлых металлов (mean±SE) в тотальных сборах зоопланктона у побережья Синопа (Чёрное море) в 2011–2012

Zooplankton groups mostly feed on phytoplankton and may contribute to the transfer of heavy metals to higher trophic levels. They have been chosen as one of the recommended groups for the base line studies of metals in marine ecosystems [33]. Moreover, Ferdous and Muktadir [18] reviewed many studies and recommended that potentiality of zooplankton as bioindicator is very high. However these results of the present study could be considered as basic elementary information for future investigations. It is important note that, there is an urgent need for a better understanding of background information on heavy metal concentrations with respect to space and time in the Black Sea.

Table 3 Comparison of heavy metals in mixed zooplankton in this study with previous studies (expressed in mg · kg^{-1} dry wt., *= mg · kg^{-1} wet wt.) (LOD= Limit of Detection)
Табл. 3 Сравнение концентрации тяжелых металлов в зоопланктоне с данными предыдущих исследований (выражено в мг · кг$^{-1}$ сухого веса, *= мг · кг$^{-1}$сырого веса) (LOD=предел определения)

Region	Fe	Zn	Al	Cu	As	Pb	Cd	Hg	References
Sinop	2902	375	778	52	40	210	1.1	<0.05	Present study
Puerto Rico	3600	1285		123		148	16		[25]
North Pacific		61–160					1.66–14.55		[20]
Pacific Ocean						0.15–7.4			[27]
Black Sea*				0.24-17		0.08–21.6			[37]
Black Sea*				2.8-200		3.8–100			[38]
Southern Baltic	160	163		3.5		0.7	1.9		[32]
Gdansk Bay	2263	806		20.5		12.9	1.3		[32]
Barents Sea		108–509		4–9		LOD	0.8–6.3		[40]
Black Sea	17.21–28.2	28–34		9.58–13.54		LOD	0.177–0.55		[8]
Bay of Bengal	1350–50999	175–8162		19.2–89.5		1.4–21.1	8.2–49.4		[33]
Arabian Sea	833–14583	81–1891		3.4–65.5		0.2–23.3	4.4–29.7		[34]
Aegean (Izmir)	682–62593	81.4–2534		26,9–293					[24]
Baltic Sea	12.6–28.7	7.15–65.3	1.73–13.40	0.71–1.2	0.20–0.95	0.01–0.49	0.21–0.6	0.003–0.017	[28]

Conclusion. The current lack of comparable data will make it impossible to measure future trends in contamination heavy metals in zooplankton at the Black Sea coast. However anthropogenic activities and their type of waste in Sinop coast of the Black Sea region were food manufacturing such slaughtering, dairy products, canning of fruits/vegetables/fish, grain mill and bakery products, sugar factories, etc. [4]. However, Bat and Gökkurt Baki [11] showed that the research area of Sinop coasts, affected with intensive land-based pollution and organic matter originating from domestic discharge. Although some metal levels were found high in zooplankton of the Black Sea coast, Turkey, the level of most often of the heavy metal levels were not extremely enriched in Sinop coast and did not present a serious problem. It is recommended that further investigations would be useful to assess long term effects of anthropogenic inputs on the Black Sea.

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Conflict of interest. The authors declare that they have no conflict of interest.

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Концентрация тяжелых металлов в зоопланктоне у побережья Синопа, Чёрное море, Турция. Л. Бат, Ф. Устюн, Х. К. Озтекин. Оценка уровня загрязнения тяжёлыми металлами морских экосистем является важной задачей деятельности Морской охраны окружающей среды в области Морской стратегии рамочной директивы (MSFD). Работа основана на тотальных сборах зоопланктона, собранных стандартной зоопланктонной сетью (размер ячеи=112 µm, диаметр устья=50 см) в 2011 и 2013 гг. у побережья Синопа, Турция. В пробах определена концентрация Al, As, Cu, Zn, Hg, Fe, Cd и Pb. Средний уровень концентрации тяжелых металлов ранжирован в порядке: Fe > Al >Zn > Pb > Cu > As > Cd > Hg. Из всех тяжелых металлов концентрация Hg находилась ниже допустимого уровня определения в пробах. Наибольшая концентрация была присуща соединению Fe, присутствие которого отмечено во всех пробах. Следующим по уровню концентрации отмечен Al, так же присутствующий во всех пробах. Высоких значений в тотальных пробах зоопланктона достигала концентрация Pb (210 мг · кг$^{-1}$) и As (40 мг · кг$^{-1}$). Содержание Cd в пробах достигало 1.1 мг · кг$^{-1}$.

Ключевые слова: тяжёлые металлы, зоопланктон, копеподы, Чёрное море