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Heavy metals in bones from Harbour Porpoises *Phocoena phocoena* from the Western Black Sea Coast

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Abstract. During the last few years, the Western Black Sea coast has documented increase in the number of stranded marine mammals, particularly the harbour porpoise (*Phocoena phocoena*). This species is a subject to threats such as exposure to contaminants, fishery by-catch and introduced new marine species. The aim of this study was to analyse spatial and age trends in bone metal concentration in harbour porpoises from the Western Black Sea Coast. Selected heavy metals (Cu, Pb, Zn, Cd and Ni) were measured in bones of 33 harbour porpoises stranded along the Bulgarian Black Sea Coast from 2017. Spatially, we found higher metal levels in the harbour porpoises stranded in the Northern region compared with those from Southern region. The effect of aging was evident only for Zn content – the levels were higher in juveniles than in adults. The obtained results suggested that heavy metal contamination represent an important threat encountered by harbour porpoises.

Key words: Heavy metals, Harbour Porpoises, Western Black Sea.

Introduction

The harbour porpoise (*Phocoena phocoena relicta* Linnaeus, 1758) is one of the three species of cetaceans found in the Black Sea. It is the second to abundance marine mammal inhabiting the Black Sea and adjacent waters (Evtimova *et al.* 2015, Evtimova *et al.* 2016, Evtimova *et al.* 2018). Other studies on the distribution of cetaceans in the Black Sea revealed that harbour porpoise is widely distributed in coastal areas (Panayotova *at al.* 2017), which are subject to contamination from various pollutants.

Studies on cetacean strandings in this area showed increase in stranding events during the last few years (Evtimova *et al.* 2015, Evtimova *et al.* 2016, Evtimova *et al.* 2018). Due to their role as top predators within the marine food web, marine mammals such as porpoises have been used as indicators for ecosystem changes. Since chemical contaminants may affect the health of harbour porpoises, contamination by particular heavy metals may be associated with the increased stranding of harbour porpoises in the Western Black Sea along the Bulgarian coast. Therefore, the aim of this study was to assess

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for the first time the bone heavy metals concentration in harbour porpoises along the Bulgarian Black Sea Coast.

Material and Methods

Bones were collected from 33 harbour porpoises (15 males and 18 females) stranded in 2017 along the Bulgarian Black Sea coast (Fig. 1). The samples were processed in a laboratory by drying, grinding and mixing with concentrated acids. Metal concentrations (Cu, Pb, Zn, Cd and Ni) were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES) Optima 7000 DV (PerkinElmer, USA). Statistical analysis was performed using SPSS v.21. The harbour porpoises were aged using dentinal Growth Layer Group (GLG) method (Boutiba 2012).

Fig. 1. Map of the Western Black Sea showing the sampling sites along the Bulgarian Black Sea Coast.

Results and Discussion

Mean bone concentrations of metals determined in harbour porpoises from the Southern and Northern Bulgarian Black Sea coast are presented in Table 1. The results show that, Zn had the highest mean levels in bones of porpoises followed by Pb, Cd, Cu and Ni.

For the geographical comparison, Cu, Pb, Zn, Cd and Ni concentrations displayed significantly higher levels in the harbour porpoises stranded in the Northern Black Sea coast compared with those from Southern Black Sea coast ($p<0.05$). In addition, harbour porpoises found in Aheloy (Southern region) and Shabla (Northern region) had the highest levels of Cu (3.75 mg/kg and 3.5 mg/kg, respectively) while animals found in Krapets (Northern region) had significantly higher bone concentrations of Ni (1.87 mg/kg) in comparison with all other sites.

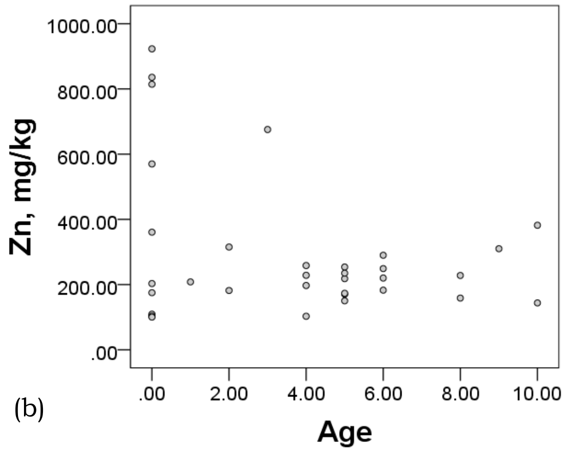
Table 1. Trace metal (mg/kg) concentrations in bones of harbour porpoises stranded between 2006 and 2013 along the Southern and Northern Bulgarian Black Sea Coast. Mean ± SD; range of concentrations (minimum – maximum); n - number of samples.

	Metal concentrations (mg/kg)				
	Cu	**Pb**	**Zn**	**Cd**	**Ni**
Southern region	2.61 ± 0.88	11.45 ± 1.30	275.02 ± 177.86	2.17 ± 0.40	0.76 ± 0.34
n=14	0.97-3.82	9.18-13.82	100.24-814.20	1.57-3.07	0.41-1.45
Northern region	2.09 ± 1.07	15.53 ± 3.55	309.25 ± 247.38	2.66 ± 0.71	1.13 ± 0.60
n=19	(0.21-3.91)	(8.84-25.23)	102.80-922.90	1.44-3.96	0.50-2.56
Total	2.31 ± 1.01	13.80 ± 3.46	294.73 ± 218.11	2.45 ± 0.64	0.98 ± 0.53
n=33	(0.21 - 3.91)	(8.84-25.23)	(100.24-922.90)	(1.44-3.96)	(0.41-2.56)

For harbour porpoises stranded in the Bulgarian Black Sea coast in 2017, correlations in bones between metals are presented in Figure 2. There was a significant correlation between Cd and Pb concentrations (Spearman correlation value R=0.576, p<0.05) (Fig. 2a). A significant negative correlation was detected between Zn concentration and the age of the harbour porpoise (R=-0.318, p<0.05) (Fig. 2b).

	Cu	**Pb**	**Zn**	**Cd**	**Ni**
Cu	1				
Pb	.113	1			
Zn	-.061	-.324	1		
Cd	.078	**.576**	-.035	1	
Ni	-.268	.231	-.122	-.120	1
Age	.183	.023	**-.318**	-.027	.078

(a)



(b)

Fig. 2. (a) Correlation matrix between heavy metals and age in bone tissue of harbour porpoises (*Phocoena phocoena*) beached at Black Sea, Bulgaria (significant correlations highlighted in bold). (b) Zink (Zn) concentration in the bones of common harbour porpoises (mg/kg) as a function of age (years).

Heavy metals, such as Cu and Zn are essential elements, and thus they are homeostatically regulated and their concentration can significantly change for a particular tissue in different specimens (Marcovecchio *et al.* 1990). Cu concentration in bone harbour porpoises tissues from Shabla and Aheloy were the highest registered. This result should be related with possible contamination in these regions. Our study also revealed a significant negative correlation of Zn bone concentration in harbour porpoises with age. Similarly, Agusa *et al.* (2008) found a negative correlation in striped dolphins. In addition, Cd concentration was related with Pb levels. We did not found an expected significant correlation between Cu and Zn. Neither the Cd-Cu nor the Cd-Zn relationships of this study were significant. A possible explanation for this result could be that Cd concentrations found in harbour porpoises are probably not enough to induce Cu or Zn ion displacement, leading to co-accumulation with Cd. Similar results were found by Lahaye *et al.* (2007).

Present evidence suggests a possible enrichment of the studied heavy metals in the Northern Black Sea Coast, where farming activities are present. These novel results fulfill the information gap existing about heavy metals pollutants presence in the Western Black Sea waters. Nevertheless, further studies are necessary in order to clarify the contamination and bioaccumulation process of heavy metals in the marine mammals from this region.

Several authors reported different feeding habits and diet related to age in harbour porpoises (Das *et al.* 2004). Some prey species are more important in the diet of young porpoises, such as gobies and shrimps, compared to adult ones (Santos *et al.* 2004). Santos and Pierce suggested that juveniles cannot dive as deep as adults and could be limited by their small size from catching big prey. Our data suggest that the different Zn levels between juveniles and adults may also be related to the maturity status.

In the present study a passive monitoring of stranded animals was presented, which can provide insight into environmental impacts on marine mammals. Our findings indicate that we cannot reject the hypothesis that metallic contaminants may influence the health of harbour porpoises and contribute to the increased stranding events.

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