

Essential and Toxic Elements in Blood Samples of Harbor Seals (*Phoca vitulina*) from the Islands Helgoland (North Sea) and Anholt (Baltic Sea): A Comparison Study with Urbanized Areas

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Abstract The harbor seals (*Phoca vitulina*) from Helgoland (North Sea) and Anholt (Kattegat, Baltic Sea) are top predators within the marine food web and an indicator species of the environmental contamination. Furthermore, they are a main tourist attraction. Despite these important roles, little is known about the health and pollutant contamination of these seals. The objective of this study was therefore to investigate 18 essential and nonessential/toxic elements (Al, As, Be, Ca, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Pb, Rb, S, Se, Sr, and Zn) in blood samples using inductively coupled plasma mass spectrometry and total X-ray-fluorescence spectrometry. Blood concentrations of mineral nutrients, such as Ca, K, P, and S, were within the reference ranges described for harbor seals. Likewise, for the trace elements, As, Be, Rb, Se, and Sr, no significant differences were observed compared with previous studies. Interestingly, blood concentrations of nine nonessential as well as essential trace metals (Al, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn) measured significantly lower in the offshore living seals from Helgoland and Anholt compared with results obtained from animals living close to urbanized areas, such as the Wadden Sea and Elbe estuary. This suggests that industrial emissions, sewage deposition, shipping traffic and dredging tasks might be the cause of increased metal concentrations of inshore harbor seals.

The harbor seal (*Phoca vitulina*) is the most abundant marine mammal in the North and Baltic Sea. Seals living closed to coastal areas are strongly influenced by anthropogenic activities impacting on their habitat, such as fishery and environmental pollution. For these ecosystems, organic and metal contaminant concentrations have been analyzed in several tissues, e.g., in liver, kidney, or blubber from harbor seals, obtained during postmortem investigations (Boon et al. 2002; Das et al. 2008; Ahrens et al. 2009; Kuenstl et al. 2009; Weijs et al. 2009a, b, c; Agusa et al. 2011; Kakuschke et al. 2012; Dietz et al. 2012). Blood sampling of harbor seals is considered minimally invasive and allows the analyses of pollutants, biomarkers, and health parameters additionally to in vitro cell experiments from living animals. For the North Sea, several investigations have been performed to measure contaminants (Griesel et al. 2006; Das et al. 2008; Griesel et al. 2008; Kakuschke et al. 2009; Weijs et al. 2009a, b, c; Kakuschke et al. 2010b), assess immune cell functions (De Swart 1995; Ross 1995; Kakuschke et al. 2005; Das et al. 2008; Dupont et al. 2013), or analyse biomarkers (Fonfara et al. 2008; Hasselmeier et al. 2008; Kakuschke et al. 2010a; Grebe et al. 2010, 2011, 2012; Kakuschke et al. 2013) in blood samples of harbor seals. Most of these studies focused on animals closed to urbanized areas along the Wadden Sea coast.

Helgoland, which is located 46 km off the German coastline (54°10'58.9"N 7°53'52.2"E), is characterized by its unique flora and fauna. In particular, the presence of a harbor seal population with up to 300 animals makes Helgoland highly attractive for tourists. Live and dead seals are regularly counted and investigations of behavior and animal movements have been performed in previous studies (Adelung and Müller 2008; Abt 2011a, b; van Neer et al. 2015). However, only a few health and pollution

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studies have been realized. In three previous studies, we published acute phase protein blood concentrations of haptoglobin, C-reactive protein, and transferrin from harbor seals (Kakuschke et al. 2010a; Grebe et al. 2011; Kakuschke et al. 2013). Additionally, a postmortem study of seals from Helgoland described macroscopic findings, blood parameters as well as metal and organic contaminants of several body fluids and tissues (Kakuschke et al. 2012).

Anholt is located 50 km off the Danish coastline (56°42′50.7″N 11°33′16.9″E) in the Kattegat, Baltic Sea. Up to 1000 harbor seals use the island as a haul-out site (Chudzinska 2009). The seal population of Anholt was the first affected by the phocine distemper virus epidemics (PDV) in 1988 and 2002 (Härkönen et al. 2006; Hall et al. 2006). In 2007, another increase of dead harbor seals was reported (Härkönen et al. 2008). Researchers investigated the population status, movement patterns, diet, human interactions, and diving behaviour of seals in southern Scandinavia including Anholt (Chudzinska 2009; Andersen 2011). However, pollution studies of this area are limited (Olsson et al. 1994; Dietz et al. 2012).

The present study reports the first multielement analysis of electrolytes, essential, and toxic trace elements in blood samples from free-ranging, healthy harbor seals from Helgoland and Anholt. Furthermore, results were compared with published data obtained from animals living in different North Sea locations, and from diseased animals.

Materials and Methods

Animals Investigated and Blood Sample Collection

The blood samples analyzed during this study were collected in 2005 and 2006 from 13 free-ranging harbor seals

(10 from Helgoland and 3 from Anholt; Table 1; Fig. 1). The seals were captured in individual tube nets and manually restrained for physical examination, to assess sex, length and weight, and to collect blood samples. The maximum time for which the animals were kept for investigations and sampling was 1 h. During this time, the seals were under continuous veterinary observation. All animals appeared healthy on physical examination, showed no clinical signs of disease, and were of normal nutritional status. The seals were classified into two age groups, <1 year and >2 years, based on length and weight.

Blood samples were collected by venipuncture of the epidural vertebral vein using a 1.2 mm × 100 mm needle (TSK-Supra, TSK Laboratory, Japan) and filled into specific lithium heparin (LH) monovettes for metal analysis (Sarstedt AG & Co). The tubes were carefully agitated and kept at ambient temperature during the capture event. Afterwards, the blood samples were transported to the laboratories of the Biologische Anstalt Helgoland, Research and Technology Centre in Büsum, and Helmholtz-Zentrum Geesthacht, Germany, for further processing and storage at −80 °C.

Multielement Analysis

Eighteen elements (Al, As, Be, Ca, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Pb, Rb, S, Se, Sr, and Zn) were analyzed in whole blood according to the procedure described in our previous study (Griesel et al. 2008). For multielement measurements, a microwave digestion system (MarsXpress, CEM GmbH, Kamp-Lintfort, Germany) was used. 500 µL of whole blood was pipetted into perfluoralkoxy (PFA) container; 2 mL sub-boiled nitric acid, 1 mL of hydrogen peroxide, and 50 µL internal standard (1 mg L^{−1} of yttrium, Merck, Darmstadt, Germany) were added, and the container were heated in a three step program up to 180 °C.

Table 1 Details of harbor seals from the islands Helgoland and Anholt investigated in this study

Seal code	Date of sampling	Sex	Age (years)	Location	Length (cm)	Weight (kg)
Pv 01	Sept 27, 2005	Male	>2	Helgoland	153	45
Pv 02	Sept 27, 2005	Male	>2	Helgoland	169	78
Pv 03	Sept 27, 2005	Male	>2	Helgoland	163	69
Pv 04	April 10, 2006	Male	>2	Helgoland	142	57
Pv 05	April 10, 2006	Male	>2	Helgoland	160	95
Pv 06	April 10, 2006	Male	>2	Helgoland	170	86
Pv 07	April 11, 2006	Male	>2	Helgoland	144	55
Pv 08	Sept 26, 2006	Male	>2	Helgoland	165	73
Pv 09	Sept 26, 2006	Male	>2	Helgoland	165	69
Pv 10	Sept 26, 2006	Male	>2	Helgoland	165	71
Pv 11	Sept 14, 2005	Female	<1	Anholt	114	24
Pv 12	Sept 14, 2005	Female	<1	Anholt	102	19
Pv 13	Sept 14, 2005	Male	<1	Anholt	102	22

Fig. 1 Seals were sampled on the islands Helgoland, Germany and Anholt, Denmark, and compared to seals investigated in our previous studies (Wadden Sea: Rømø and Lorenzenplate; Neuwerk, Elbe estuary)



The elements were measured with two different analytical methods. Al, Be, Cr, Mn, Mo, Ni, and Pb were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) equipped with a collision cell (Agilent 7500c ICP-MS, Agilent Technologies, Tokyo, Japan). Measurements of As, Ca, Cu, Fe, K, P, Rb, S, Se, Sr, and Zn were performed by total X-ray-fluorescence spectrometry (TXRF) (Atomika TXRF 8030 C, FEI Company, Oberschleissheim, Germany).

For internal quality control, the reliability of the analytical procedures was confirmed with the human reference material Seronorm™ Trace Elements Whole Blood L-2 (SERO AS, Billingstad, Norway) and/or Clin Check® Whole Blood Control Level II (Recipe, Chemicals + Instruments, Munich, Germany). Additionally, reference material was measured in connection with the participation in the NIST/NOAA 2007 Interlaboratory Comparison Exercise for trace elements in blood of marine mammals (Table 2).

Data Analysis

We used the nonparametric Mann–Whitney *U* test for comparing element blood concentrations between animals from the island Helgoland with the animals from Rømø and Lorenzenplate (data obtained from Griesel et al. 2008). In case of nondetectable concentrations, the 0.5 LOD-value was used for statistical analyses and graphs. The animals from both studies were within the same age group

(>2 years). Due to the fact that no gender-related differences had been found in former studies (Griesel et al. 2008; Dupont et al. 2013), the results for males and females were pooled. The results from Anholt seals were not statistically tested due to the small number and different age of animals. All indicated *p* values are two-tailed. Statistical significance was designated as $p \leq 0.05$.

Results and Discussion

To the authors' knowledge, our study is the first to report 18 element concentrations, including electrolytes, essential and nonessential/toxic elements measured in blood samples of free-ranging, healthy harbor seals from the islands Helgoland and Anholt (Table 3).

Local Differences of Element Concentrations in Blood Samples from Harbor Seals

Median, minimum, and maximum element concentrations were compared between seals of the islands Anholt (Kattegat, Baltic Sea) and Helgoland (North Sea, Germany) with our previous studies on seals from Rømø (Wadden Sea, Denmark), Lorenzenplate (Wadden Sea, Germany), and Elbe estuary (Neuwerk, Germany) (Table 4; Griesel et al. 2008, Kakuschke et al. 2010a).

Helgoland seals showed significantly (Mann–Whitney *U* test) lower blood concentrations for Cr ($p < 0.01$), Fe

Table 2 Trace element concentration in California sea lion (*Zalophus californianus*) blood, results of the NIST/NOAA Interlaboratory Comparison Exercise 2007

Element	Recommended NIST/NOAA			Measured ($n = 3$)		
	Mean value	\pm SD	Range	Mean value	\pm SD	Range
Mineral nutrients (mg L^{-1})						
Fe	550	67.0	483–617	550	3.00	547–553
Zn	4.93	0.60	4.33–5.53	4.02	0.13	3.89–4.14
Trace elements ($\mu\text{g L}^{-1}$)						
As	42.0	15.0	27.0–57.0	29.0	5.00	25.0–34.0
Cu	768	79.0	689–847	773	59.0	730–840
Mn	68.0	12.0	56.0–80.0	60.0	1.00	59.0–60.0
Mo	4.00	1.00	3.00–5.00	5.00	0.50	4.50–5.50
Ni	1.20	0.20	0.20–1.40	1.10	0.60	1.00–1.10
Rb	124	9.00	115–133	121	2.00	119–123
Se	2240	320	1920–2560	1730	60.0	1690–1790

Table 3 Element profiles in whole blood samples of harbor seals caught at the islands Helgoland, North Sea, Germany ($n = 10$, Pv 01–10) and Anholt, Kattegat, Baltic Sea, Denmark ($n = 3$, Pv 11–13)

Element	Pv 01	Pv 02	Pv 03	Pv 04	Pv 05	Pv 06	Pv 07	Pv 08	Pv 09	Pv 10	Pv 11	Pv 12	Pv 13
Mineral nutrients (mg L^{-1})													
Ca	35.6	46.1	39.7	52.1	31.4	40.6	43.8	63.3	41.8	46.1	43.9	38.8	38.3
Fe	494	507	560	712	546	457	511	196	393	526	510	651	575
K	137	148	143	168	133	149	167	149	154	145	154	171	139
P	360	419	424	489	349	351	438	304	397	390	401	391	336
S	1185	1372	1300	1538	1170	1215	1355	1058	1220	1298	1285	1252	1159
Zn	2.60	2.75	2.75	3.12	2.30	1.81	3.04	1.10	1.74	3.18	2.70	2.90	3.16
Trace elements ($\mu\text{g L}^{-1}$)													
Al	0.75	2.12	0.44	6.85	4.03	1.27	1.33	1.99	2.87	2.14	0.56	0.26	0.32
As	167	246	220	230	202	147	215	358	130	159	148	175	633
Be	<0.08 ^a	<0.08 ^a	<0.08 ^a	0.17	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a
Cr	0.20	0.30	0.27	1.39	1.03	0.63	0.71	0.12	0.06	0.53	0.66	0.59	0.88
Cu	742	738	850	674	605	525	683	740	634	929	703	807	762
Mn	6.26	8.05	6.14	13.5	9.20	8.96	8.66	3.71	4.70	6.41	4.91	4.00	5.20
Mo	1.08	1.14	1.50	0.98	0.72	0.76	0.68	1.19	0.78	1.42	0.70	0.78	0.13
Ni	0.78	0.84	0.85	1.00	0.88	0.90	0.88	0.82	0.79	0.77	0.83	0.82	0.84
Pb	0.23	0.06	0.13	<0.02 ^b	<0.02 ^b	<0.02 ^b	<0.02 ^b	<0.02 ^b	0.29	0.18	<0.02 ^b	<0.02 ^b	<0.02 ^b
Rb	104	114	92	73	70	65	68	67	81	112	50	102	90
Se	839	970	947	975	761	692	1063	488	527	778	659	585	773
Sr	58	32	33	119	50	51	73	77	54	45	75	63	37

^a Be blood concentration LOD 0.08 $\mu\text{g L}^{-1}$ ^b Pb blood concentration LOD 0.02 $\mu\text{g L}^{-1}$

($p < 0.01$), Mn ($p < 0.01$), Mo ($p < 0.01$), Ni ($p < 0.01$), Pb ($p < 0.05$), and Zn ($p < 0.01$) than seals from Rømø and the Lorenzenplate. Furthermore, results for Al ($p < 0.01$) were lower in comparison to Rømø seals, Cu ($p < 0.01$) was lower in comparison to seals from the Lorenzenplate (Fig. 2).

For the trace elements As, Be, Rb, Se, and Sr and the mineral nutrients Ca, K, P, and S, no significant differences were observed between animals from Helgoland and the Wadden Sea locations. Measurements for Ca, K, P, and S were within the normal range described for harbor seals (Griesel et al. 2006).

Table 4 Element concentrations (median, min–max) in blood of healthy harbor seals from Helgoland and Anholt (this study) compared with animals of the Wadden Sea, Rømø, and Lorenzenplate (Griesel et al. 2008) and Elbe estuary (Kakuschke et al. 2010b)

Element	Anholt (<i>n</i> = 3)	Helgoland (<i>n</i> = 10)	Rømø (<i>n</i> = 12)	Lorenzenplate (<i>n</i> = 16)	Elbe estuary (<i>n</i> = 5)
Mineral nutrients (mg L ⁻¹)					
Ca	38.8 (38.3–43.9)	42.8 (31.4–63.3)	41.6 (32.7–52.9)	41.7 (29.9–55.0)	59.1 (45.3–74.3)
Fe	575 (510–651)	509 (196–712)	738 (599–936)	760 (520–1137)	797 (244–993)
K	154 (139–171)	149 (133–168)	155 (138–183)	161 (131–197)	244 (194–323)
Zn	2.90 (2.70–3.16)	2.67 (1.10–3.18)	3.42 (2.73–4.35)	3.44 (2.91–4.57)	3.97 (1.36–4.98)
Trace elements (µg L ⁻¹)					
Al	0.32 (0.26–0.56)	2.06 (0.44–6.85)	36.8 (3.97–500)	3.34 (<0.17–126)	16.2 (13.1–29.9)
As	175 (148–633)	209 (130–358)	185 (118–316)	169 (42.1–592)	283 (190–316)
Be	<0.08 (all seals)	– (<0.08–0.17) ^a	– (<0.08–0.18) ^a	– (<0.08–1.80) ^a	1.20 (1.04–1.39)
Cr	0.66 (0.59–0.88)	0.41 (0.06–1.39)	11.0 (1.88–84.9)	6.97 (1.52–21.5)	5.96 (4.24–7.56)
Cu	762 (703–807)	711 (525–929)	770 (527–986)	878 (604–1371)	1092 (704–1545)
Mn	4.91 (4.00–5.20)	7.23 (3.71–13.5)	79.1 (67.7–105)	95.8 (67.4–151)	127 (23.2–146)
Mo	0.70 (0.13–0.78)	1.03 (0.68–1.50)	6.16 (1.27–15.0)	4.78 (1.52–7.07)	7.82 (6.26–8.88)
Ni	0.83 (0.82–0.84)	0.84 (0.77–1.00)	3.72 (<0.38–25.7)	2.34 (0.94–9.48)	3.78 (3.34–5.92)
Pb	<0.02 (all seals)	– (<0.02–0.29) ^b	0.40 (<0.02–4.52) ^b	0.86 (<0.02–1.82) ^b	7.81 (3.63–11.4)
Rb	90.0 (50.0–102)	77.0 (65.0–114)	72.5 (52.0–99.0)	67.5 (36.0–149)	80.7 (65.6–115)
Se	659 (585–773)	809 (488–1063)	940 (518–1372)	899 (591–2261)	1054 (579–1847)
Sr	63.0 (37.0–75.0)	52.5 (32.0–119)	47.0 (34.0–70.0)	42.0 (25.0–63.0)	73.2 (43.4–125)

^a Be blood concentration was below 0.08 µg L⁻¹ (LOD) in 9 of 10 seals (Helgoland), in 11 of 12 seals (Rømø), and in 11 of 16 seals (Lorenzenplate)

^b Pb blood concentration was below 0.02 µg L⁻¹ (LOD) in 5 of 10 seals (Helgoland), in 1 of 12 seals (Rømø), and in 1 of 16 seals (Lorenzenplate)

Despite the small number of animals investigated, the element blood concentrations obtained from Anholt seals were comparable to Helgoland seals (Table 4; Fig. 2). Interestingly, seals living in the Elbe estuary showed the highest blood concentrations for most elements in comparison to other locations (Table 4; Fig. 2; Kakuschke et al. 2010b). However, the animals of Anholt and the Elbe estuary were younger, and it is likely that both, age and localization, have an influence on blood element concentrations.

Despite a reduction of contaminants over the recent years, the Elbe River is still the primary contributor to the contamination of its estuary and the German Bight (Loewe et al. 2006). The Elbe water has four times higher Cu concentrations than the Lake Issel (Western Dutch Wadden Sea), the rivers Ems and Weser (Lower Saxony), as well as Eider (Schleswig–Holstein) are in between (Bakker et al. 2009). Similarly, Pb and Zn concentrations are 3–4 times higher in the rivers Elbe and Weser in comparison to Eider and Ems (Bakker et al. 2009). The high blood concentrations of most elements, including Cu, Pb, and Zn, in seals living in the Elbe estuary are therefore not surprising.

The Wadden Sea areas around Lorenzenplate and Rømø are dominated by North Sea coastal water; however, rivers like Eider, Ribe A, or Rejsby A influence the contamination of these areas (Bakker et al. 2009). This is reflected by Wadden Sea seal blood concentrations of most elements, which were lower than in Elbe seals but higher compared with Helgoland seals. Interestingly, Ni concentrations in the sediment of the northeastern Wadden Sea regions of Dithmarschen, North-Frisia, or Sylt-Rømø were higher and showed an increasing trend over the past years compared with southwest regions of the Wadden Sea (Bakker et al. 2009). Seals from Rømø showed besides elevated Ni blood levels, Ni-specific hypersensitivity reactions, potentially as a result of a chronic metal influence (Kakuschke et al. 2005). Similar immunological reactions were found for the metals Al, Cr, Mo, Pb, Sn, and Ti. These elements (Al, Cr, Mo, Ni, and Pb) also were significantly higher in blood samples from the Wadden Sea seals in comparison to Helgoland seals.

The general picture of a higher contamination of near-urban sites also was found for western Wadden Sea areas, and includes next to metal elements, several organic pollutants. Laane et al. (1999) described the distribution of

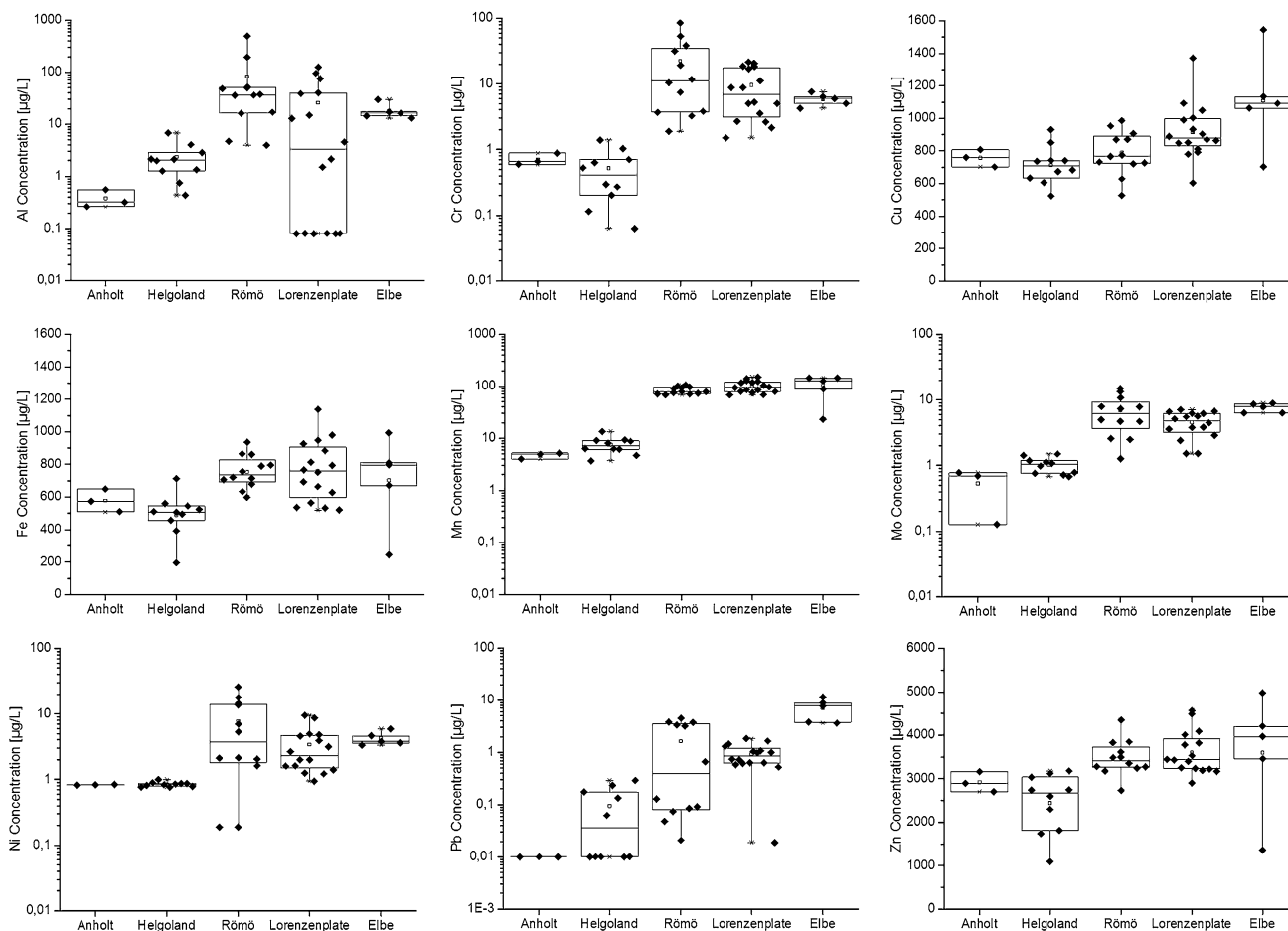


Fig. 2 Blood concentrations of Al, Cr, Cu, Fe, Mn, Mo, Ni, Pb, and Zn of free-ranging, obviously healthy harbor seals from Anholt and Helgoland (this study) compared with seals from the Wadden Sea—Römø and Lorenzenplate (Griesel et al. 2008), and the Elbe estuary (Kakuschke et al. 2010b). The width of the boxes are the 25–75

percentile range, the *line* inside the *box* is the Median, the *open dot* the Mean. The whiskers show the Min and Max values. *Black dots* are the measured values, whereas the 0.5 LOD-value was used for nondetectable levels

trace metals in the sediment of the Dutch coastal zone with highest concentrations close to the coast. Dietz et al. (2012) investigated perfluorinated chemicals (PFCs) in seal liver tissue from seven locations ranging from the Wadden Sea of the southern North Sea to the Western Baltic Sea and found the highest concentration of perfluorooctanesulfonic acids (PFOS) in animals from the Wadden Sea.

Decreasing concentrations from estuary to coastal waters and the open sea could be explained by the dilution of estuarine with sea water. This is consistent with the reduction of water metal concentrations in the Germany Bay with an increasing distance from the coast line (Laane et al. 2013). Our results reporting different blood concentrations depending on the localization of the seals support these findings. However, as expected, essential elements physiologically regulated, e.g., Ca, K, P, and S were comparable for all seals investigated, independent of their location.

Differences of Blood Element Concentrations Comparing Healthy and Diseased Harbor Seals

For most elements no differences were detected between Helgoland seals, which were suspected to be healthy, and four diseased animals (Table 5; Kakuschke et al. 2012).

However, Pb blood concentrations of the diseased harbor seals were higher than the maximum value measured in healthy seals. Pb is known to be toxic and a health risk for marine mammals. A case study (Shlosberg et al. 1997) described progressive liver damage and finally death of a bottlenose dolphin resulting from Pb intoxication. Frouin et al. (2010) reported a decreased lymphoblastic proliferation of Pb incubated lymphocytes obtained from blood and lymph node samples. However, in the present study a correlation of health condition and Pb blood concentrations could not conclude unambiguous. First, the blood concentrations of diseased seals from Helgoland were

Table 5 Blood element concentrations comparing healthy (this study) and diseased seals (Kakuschke et al. 2012) from Helgoland

Element	Healthy seals ($n = 10$) Median (Min–Max)	Diseased seals ($n = 4$) Median (Min–Max)
Mineral nutrients (mg L ⁻¹)		
Ca	42.8 (31.4–63.3)	56.4 (45.8–70.2)
Fe	509 (196–712)	375 (147–457)
K	149 (133–168)	193 (139–216)
Zn	2.67 (1.10–3.18)	2.09 (1.36–2.50)
Trace elements (µg L ⁻¹)		
Al	2.06 (0.44–6.85)	1.25 (0.80–1.72)
As	209 (130–358)	370 (287–732)
Be	– (<0.08–0.17) ^a	<0.08 (all seals)
Cr	0.41 (0.06–1.39)	0.29 (0.09–0.89)
Cu	711 (525–929)	693 (668–779)
Mn	7.23 (3.71–13.5)	4.29 (2.68–8.12)
Mo	1.03 (0.68–1.50)	1.22 (1.09–1.54)
Ni	0.84 (0.77–1.00)	0.82 (0.72–0.85)
Pb	– (<0.02–0.29) ^b	1.41 (1.24–2.47)
Rb	77.0 (65.0–114)	94.5 (55.0–127)
Se	809(488–1063)	448 (330–530)
Sr	52.5 (32.0–119)	76.0 (61.0–126)

^a Be blood concentration was below 0.08 µg L⁻¹ (LOD) in 9 of 10 animals

^b Pb blood concentration was below 0.02 µg L⁻¹ (LOD) in 5 of 10 animals

comparable to those of healthy seals living in the Wadden Sea area and Elbe estuary (Griesel et al. 2008; Kakuschke et al. 2010b). Second, the liver, kidney, and muscle Pb concentrations of these diseased animals were mostly below detection limit (Kakuschke et al. 2012).

Conclusions

Although harbor seals are an indicator species of the ecosystem and a tourist attraction, little is known about the seals from the islands Helgoland and Anholt. Our multi-element investigation provides a data set of essential and nonessential/toxic elements in blood samples from these animals. For several metals, our findings support other studies showing higher levels of pollutants in animals living in estuary and inshore habitats, which are influenced by industrial emissions and sewage, shipping traffic and dredging tasks, in comparison to seals living further away from the mainland.

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Compliance with Ethical Standards

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

References

- Abt K (2011a) Meeressäugerfunde an den Küsten Schleswig-Holsteins 2010. Bericht an Nationalparkverwaltung im Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz Schleswig-Holstein, Kiel
- Abt K (2011b) Robbenzählungen im schleswig-holsteinischen Wattenmeer 2010. Bericht an Nationalparkverwaltung im Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz Schleswig-Holstein, Kiel
- Adelung D, Müller G (2008) Forschungsverbund MINOSplus – Weiterführende Arbeiten an Seevögeln und Meeressäugern zur Bewertung von Offshore – Windkraftanlagen, Teilvorhaben TP6 – „Seehunde in See“ – Untersuchungen zur räumlichen und zeitlichen Nutzung der Nordsee durch Seehunde im Zusammenhang mit der Entwicklung von Offshore-Windenergieanlagen. Ministerium für Umwelt, Naturschutz und Reaktorsicherheit, Universität Kiel, Germany
- Agusa T, Yasugi S, Lida A, Ikemoto T, Anan Y, Kuiken T, Osterhaus A, Tanabe S, Iwata H (2011) Accumulation features of trace elements in mass-stranded harbor seals (*Phoca vitulina*) in the North Sea coast in 2002: the body distribution and association with growth and nutrition status. *Mar Pollut Bull* 62:963–975
- Ahrens L, Siebert U, Ebinghaus R (2009) Temporal trends of polyfluoroalkyl compounds in harbor seals (*Phoca vitulina*) from the German Bight, 1999–2008. *Chemosphere* 76:151–158
- Andersen SM (2011) Harbour seals and human interactions in Danish waters. Dissertation. University of Southern Denmark and Aarhus University
- Bakker J, Lüerßen G, Marencic H, Jung K (2009) Hazardous Substances. Thematic Report No. 5.1. In: Marencic H, de Vlas J (eds) Quality status report 2009. Wadden Sea Ecosystem No. 25. Common Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany
- Boon JP, Lewis WE, Tjoen-A-Choy MR, Allchin CR, Law RJ, de Boer J, ten Hallers-Tjabbes CC, Zegers BN (2002) Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. *Environ Sci Technol* 36:4025–4032
- Chudzinska M (2009) Diving behaviour of harbour seals (*Phoca vitulina*) from the Kattegat. Masterthesis. University of Aarhus, Denmark
- Das K, Siebert U, Gillet A, Dupont A, Di-Poi C, Fonfara S, Mazzucchelli G, De Pauw E, De Pauw-Gillet MC (2008) Mercury immune toxicity in harbour seals: links to in vitro toxicity. *Environ Health* 7:52
- De Swart (1995) Impaired immunity in seals exposed to bioaccumulated environmental contaminants. Dissertation. Erasmus University Rotterdam, The Netherlands
- Dietz R, Riget FF, Galatius A, Sonne C, Teilmann J, Bossi R (2012) Spatial trends of perfluorochemicals in harbor seals (*Phoca vitulina*) from Danish waters. *Sci Total Environ* 414:732–737
- Dupont A, Siebert U, Covaci A, Weijs L, Eppe G, Debier C, De Pauw-Gillet MC, Das K (2013) Relationships between in vitro lymphoproliferative responses and levels of contaminants in

- blood of free-ranging adult harbour seals (*Phoca vitulina*) from the North Sea. *Aquat Toxicol* 142(143):210–220
- Fonfara S, Kakuschke A, Rosenberger T, Siebert U, Prange A (2008) Cytokine and acute phase protein expression in blood samples of harbour seal pups. *Mar Biol* 3:337–345
- Frouin H, Menard L, Measures L, Brousseau P, Fournier M (2010) T Lymphocyte-proliferative responses of a grey seal (*Halichoerus grypus*) exposed to heavy metals and PCBs in vitro. *Aquat Mamm* 36:365–371
- Grebe M, Proefrock D, Kakuschke A, Broekaert JAC, Prange A (2010) Metallomics approach for the identification of the iron transport protein transferrin in the blood of harbour seals (*Phoca vitulina*). *Metallomics* 2:683–693
- Grebe M, Proefrock D, Kakuschke A, Broekaert JAC, Prange A (2011) Absolute quantification of transferrin in blood samples of harbour seals using HPLC-ICP-MS. *Metallomics* 3:176–185
- Grebe M, Proefrock D, Kakuschke A, del Castillo Busto ME, Montes-Bayon M, Sanz-Medel A, Broekaert JAC, Prange A (2012) Comparison of different methods for the absolute quantification of harbour seal transferrin glycoforms using HPLC-ICP-MS. *J Anal Atom Spec* 27:440–448
- Griesel S, Mundry R, Kakuschke A, Fonfara S, Siebert U, Prange A (2006) Mineral elements and essential trace elements in blood of seals of the North Sea measured by total-reflection X-ray fluorescence analysis. *Spectrochim Acta B* 61:1158–1165
- Griesel S, Kakuschke A, Siebert U, Prange A (2008) Trace element concentrations in blood of harbor seals (*Phoca vitulina*) from the Wadden Sea. *Sci Total Environ* 392:313–323
- Hall AJ, Jepson PD, Goodman SJ, Harkonen T (2006) Phocine distemper virus in the North and European Seas: data and models, nature and nurture. *Biol Conserv* 131:221–229
- Härkönen L, Dietz R, Reijnders P, Teilmann J, Harding K, Hall A, Brasseur S, Siebert U, Goodman SJ, Jepson PD, Rasmussen TD, Thompson P (2006) A review of the 1988 and 2002 phocine distemper virus epidemics in European harbour seals. *Dis Aquat Organ* 68:115–130
- Härkönen T, Backlin BM, Barrett T, Bergman A, Corteyn M, Dietz R, Harding KC, Malmsten J, Roos A, Teilmann J (2008) Mass mortality in harbour seals and harbour porpoises caused by an unknown pathogen. *Vet Rec* 162:555–556
- Hasselmeier I, Fonfara S, Driver J, Siebert U (2008) Differential hematology profiles of free-ranging, rehabilitated, and captive harbor seals (*Phoca vitulina*) of the German North Sea. *Aquat Mamm* 34:149–156
- Kakuschke A, Valentine-Thon E, Griesel S, Fonfara S, Siebert U, Prange A (2005) Immunological impact of metals in harbor seals (*Phoca vitulina*) of the North Sea. *Environ Sci Technol* 39:7568–7575
- Kakuschke A, Griesel S, Fonfara S, Rosenberger T, Prange A (2009) Concentrations of selected essential and non-essential elements in blood of harbor seal (*Phoca vitulina*) pups of the German North Sea. *Biol Trace Elem Res* 127:28–36
- Kakuschke A, Erbsloeh HB, Griesel S, Prange A (2010a) Acute phase protein haptoglobin in blood plasma samples of harbour seals (*Phoca vitulina*) of the Wadden Sea and of the isle Helgoland. *Comp Biochem Physiol B Biochem Mol Biol* 155:67–71
- Kakuschke A, Valentine-Thon E, Griesel S, Gandrass J, Luzardo OP, Boada LD, Pena MZ, Gonzalez MA, Grebe M, Proefrock D, Erbsloeh HB, Kramer K, Fonfara S, Prange A (2010b) First health and pollution study on harbor seals (*Phoca vitulina*) living in the German Elbe estuary. *Mar Pollut Bull* 60:2079–2086
- Kakuschke A, Gandrass J, Luzardo OP, Boada LD, Zaccaroni A, Griesel S, Grebe M, Proefrock D, Erbsloeh HB, Valentine-Thon E, Prange A, Kramer K (2012) Postmortem health and pollution investigations on harbor seals (*Phoca vitulina*) of the islands Helgoland and Sylt. *ISRN Zoology*. Article ID 106259
- Kakuschke A, Proefrock D, Prange A (2013) C-reactive protein in blood plasma and serum samples of harbour seals (*Phoca vitulina*). *Mar Mamm Sci* 29:E183–E192
- Kuenstl L, Griesel S, Prange A, Goessler W (2009) Arsenic speciation in bodily fluids of harbor seals (*Phoca vitulina*) and harbor porpoises (*Phocoena phocoena*). *Environ Chem* 6:319–327
- Laane R, Sonneveldt HLA, Van der Weyden AJ, Loch JPG, Groeneveld G (1999) Trends in the spatial and temporal distribution of metals (Cd, Cu, Zn and Pb) and organic compounds (PCBs and PAHs) in Dutch coastal zone sediments from 1981 to 1996: a model case study for Cd and PCBs. *J Sea Res* 41:1–17
- Laane R, Vethaak AD, Gandrass J, Vorkamp K, Köhler A, Larsen MM, Strand J (2013) Chemical contaminants in the Wadden Sea: sources, transport, fate and effects. *J Sea Res* 82:10–53
- Loewe P, Becker G, Brockmann U, Dick S, Frohse A, Herrmann J, Klein B, Klein H, Nies H, Schmolke S, Schrader D, Schulz A, Theobald N, Weigelt S (2006) Nordseezustand 2004. Berichte des Bundesamtes für Seeschifffahrt und Hydrographie. Nr.40. Bundesamt für Seeschifffahrt und Hydrographie (BSH): Hamburg, Rostock
- Olsson M, Karlsson B, Ahnland E (1994) Diseases and environmental contaminants in seals from the Baltic and the Swedish West-Coast. *Sci Total Environ* 154:217–227
- Ross PS (1995) Seals, pollution and disease: environmental contaminant-induced immunosuppression. Dissertation, University Utrecht, The Netherlands
- Shlosberg A, Bellaiche M, Regev S, Gal R, Brizzi M, Hanji V, Zaidel L, Nyska A (1997) Lead toxicosis in a captive bottlenose dolphin (*Tursiops truncatus*) consequent to ingestion of air gun pellets. *J Wildl Dis* 33:135–139
- Van Neer A, Jensen LF, Siebert U (2015) Grey seal (*Halichoerus grypus*) predation on harbour seals (*Phoca vitulina*) on the island of Helgoland, Germany. *J Sea Res* 97:1–4
- Weijls L, Das K, Siebert U, van Elk N, Jauniaux T, Neels H, Blust R, Covaci A (2009a) Concentrations of chlorinated and brominated contaminants and their metabolites in serum of harbour seals and harbour porpoises. *Environ Int* 35:842–850
- Weijls L, Dirtu AC, Das K, Gheorghe A, Reijnders PJH, Neels H, Blust R, Covaci A (2009b) Inter-species differences for polychlorinated biphenyls and polybrominated diphenyl ethers in marine top predators from the Southern North Sea: Part 1. Accumulation patterns in harbour seals and harbour porpoises. *Environ Pollut* 157:437–444
- Weijls L, Dirtu AC, Das K, Gheorghe A, Reijnders PJH, Neels H, Blust R, Covaci A (2009c) Inter-species differences for polychlorinated biphenyls and polybrominated diphenyl ethers in marine top predators from the Southern North Sea: Part 2. Biomagnification in harbour seals and harbour porpoises. *Environ Pollut* 157:445–451