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Comparative Studies on Trace Metal Levels in Marine Biota

II. Trace Metals in Krill, Krill Products, and Fish from the Antarctic Scotia Sea*

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Vergleichende Studien zum Spurenmetallgehalt in marinen Biota

II. Spurenmetalle in Krill, Krillprodukten und Fischen aus der antarktischen Scotia-See

Zusammenfassung. In Rohkrill, Krillfleisch, Krillprodukten und in Filets der antarktischen Fische *Notothenia Rossi Marmorata, Dissostichus Eleginoides* und *Notothenia Gibberifrons* wurden die Gehalte an Cd, Pb, Cu, Ni, Hg und As bestimmt. Als Methoden wurden elektrothermale AAS für Cd, Pb, Cu und Ni, Kaltdampf- bzw. Hydridtechniken für Hg und As nach HNO_3-Druckaufschluß, für As gefolgt von einer Naßveraschung mit $HClO_4/H_2SO_4$, eingesetzt. Die Qualitätskontrolle erfolgte durch die gleichzeitige Analyse geeigneter Standardreferenzmaterialien und Arbeitsstandards sowie durch Vergleich mit der differentiellen Pulsinversvoltammetrie (DPASV) für Pb und Cd. Die erhaltenen, auf Frischgewicht bezogenen, Mittelwerte für Krillfleisch (46 ng Cd/g; $\leqq$50 ng Pb/g; 380 ng Cu/g; 130 ng Ni/g; $\leqq$20 ng Hg/g und 340 ng As/g) und im Filet antarktischer Fische ($\leqq$3 ng Cd/g; $\leqq$100 ng Pb/g; $\leqq$200 ng Cu/g; $\leqq$150 ng Ni/g; $\leqq$50 ng Hg/g und 300–1500 ng As/g) bestätigen nach derzeitigem Kenntnisstand deren toxische Unbedenklichkeit für die menschliche Ernährung. Aufgrund höherer, aber nicht exzessiver Spurenmetallgehalte der Krillprodukte dürften sich diese eher als proteinreiches Tierfutter eignen.

Summary. In whole krill, krill muscle tissue, krill products and in fillets of the antarctic fish *Notothenia rossi marmorata, Dissostichus eleginoides,* and *Notothenia gibberifrons* the levels of Cd, Pb, Cu, Ni, Hg and As have been determined. The methods applied were electrothermal AAS for Cd, Pb, Cu, and Ni, cold-vapour and hydride-generation AAS for Hg and As, respectively, usually after HNO_3 pressure decomposition and for As followed by a $HClO_4/H_2SO_4$ treatment. Quality control was performed by analysis of appropriate Standard Reference Materials and working standards and by intercomparison with differential pulse anodic-stripping voltammetry (DPASV) for Pb and Cd. The mean values obtained related to fresh weight for krill muscle meat (46 ng Cd/g, $\leqq$50 ng Pb/g, 380 ng Cu/g, 130 ng Ni/g, $\leqq$20 ng Hg/g, and 340 ng As/g) and fillets of antarctic fish ($\leqq$ ng Cd/g, $\leqq$100 ng/Pb/g, $\leqq$200 ng Cu/g, $\leqq$150 ng Ni/g, $\leqq$50 ng Hg/g, and 300–1500 ng As/g) confirm the absence of toxic risks for human food according to the present knowledge. Due to the somewhat higher, but not excessive, trace metal contents of krill products, these should be more suitable as a protein rich animal feed.

Modern fishing technologies, increasingly applied in the fishing areas of the oceans resulted in a significant decrease in sea food yields due to overfishing. That situation, besides efforts to limit fishing campaigns, strongly stimulated the search for new marine protein sources.

According to the present knowledge promising species are, e. g., the small crustacean krill, which is a close relative to the shrimp, and some fish, e. g. *Notothenia rossi marmorata* (antarctic marmor perch), *Dissostichus eleginoides,* and *Notothenia gibberifrons* (antarctic gurnard). All these species live predominantly in antarctic seas. The high propagation of krill is probably due to the strongly decreasing population of whales, which are krill-feeding marine mammals. Now the antarctic oceanic regions are expected to produce annually several hundred million tons of krill [1]. This crustacean is regarded as a promising future protein source for feeding of animals, and could be also a valuable marine contribution to the human food basket [2,

* Reference [37] constitutes part I of thin series, references [8] and [38] are parts III and IV, respectively

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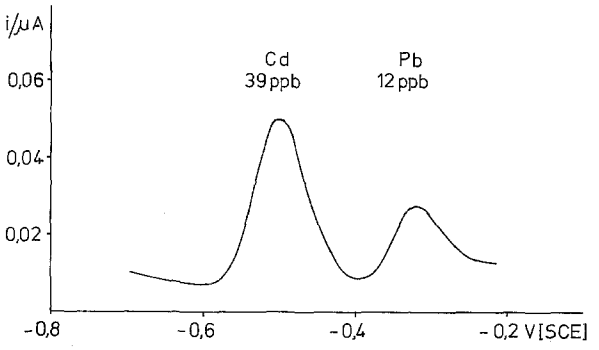

Fig. 1. Simultaneous DPASV determination of Cd and Pb in the analyte solution of a sample of krill tail after low temperature ashing. pH adjusted with HCl suprapur Merck to 1.5–2.0. Cathodic deposition potential −0.8 V, cathodic deposition time 3 min, rest time 60 s, scan rate 5 mV/s, pulse height 50 mV, pulse duration 57 ms, clock time of pulses 0.5 s. HMDE (hanging mercury drop) area $3.56 \times 10^{-2} cm^2$. Since the concentration of Cd and Pb in the solution was in the same order of magnitude, no instrumental sensitivity changes were necessary. Evaluation by two standard additions. For details of voltammetric procedure see [20]

3]. Thus, the Federal Republic of Germany carried out recently two research expeditions to investigate carefully the behavior and habitat of these hitherto relatively unknown marine organisms. Also remarkable amounts of krill and antarctic fish were caught for numerous particular studies ranging from food technology and feeding experiments to the analysis of particular trace elements [3–7].

The present study as part of ecotoxicological baseline studies on toxic trace metals in the marine environment and in food chains [8–11, 37] was devoted to the investigation of the mean levels of Cd, Pb, Cu, Ni, Hg, and As in five selected samples of krill and krill products and three samples of the above mentioned antarctic fish. The aim of our study was to contribute to the question of whether the concentration of toxic trace metals would generally permit the use of krill and other products for human nutrition and animal feeding.

Materials and Methods

Description of Materials

The samples came from the second expedition of the Federal Republic of Germany with the fishery research vessels *Walter Herwig and Julius Fock* in 1977/78 to the Scotia Sea and were provided by the Bundesforschungsanstalt für Fischerei, Hamburg; either as deep-frozen or dried material. They are characterized as follows [12]:

Deep Frozen:

Nr. 1 whole specimens (with shell).

Nr. 2 boiled, homogenized whole specimens, shells predominantly removed.

Nr. 3 krill muscle, i.e. krill tail; shell removed.

Nr. 6–8 fillets of *Notothenia rossi marmorata, Dissostichus eleginoides,* and *Notothenia gibberifrons* with skin.

Dried:

Nr. 4 spray-dried material, similar to 2.

Nr. 5 krill meat from complete specimens with shell; protein content 50–55%.

Sample Preparation and Sample Pretreatment

Sub-samples up to 2 g from wet material, and up to 0.5 g from dried material from the above described samples were taken randomly. For this purpose, quartz and plastic knives and pincers were used and clean laboratory conditions (clean benches) were maintained at the working place to avoid contamination.

These sub-samples were placed in Teflon crucibles of different sizes up to 100 ml in volume [13], up to 8.0 ml of concentrated nitric acid, Merck Suprapur, were added and several samples were decomposed simultaneously under pressure at temperature increasing up to about 160 °C in units containing up to 9 crucibles [13, 14].

The solutions obtained were used after an appropriate dilution for Cd, Pb, Cu, and Ni by electrothermal AAS and for Hg with cold-vapour AAS. If As had to be determined, the samples were treated after nitric acid, pressure digestion with $HClO_4/H_2SO_4$ until SO_3 fumes appeared [15].

Determination

Cd, Pb, Cu, and Ni were determined by automated electrothermal AAS directly or after solvent extraction (Pb) using either matrix-matched calibration graphs or the standard-addition technique [16, 17]. The instruments used were Perkin-Elmer M 400 instruments equipped with HGA 74 graphite furnaces and AS-1 autosamplers for Pb, Cd, and Cu, and a M 430 with a HGA 500 and an AS-1 autosampler for Ni. Arsenic was determined in the clear solution of the above described $HClO_4/H_2SO_4$ digestion by hydride generation with $NaBH_4$ [18]. In the latter case a Perkin-Elmer M 410 instrument equipped with a MHS-1 system of the same manufacturer was used. Mercury determinations were performed with an automated cold-vapour AAS system after Hg preconcentration on silver wool [19].

Quality Control

The analytical procedures were controlled by the current analysis of the NBS Standard Reference Materials 1577 bovine liver and 1571 Orchard leaves with satisfactory agreement between the certified and found values [21].

Further, the accuracy of Cd and Pb determinations was checked by fish homogenate standards and independent analysis with differential pulse anodic-stripping voltammetry (DPASV) at the hanging mercury-drop electrode after low temperature ashing [20] of sample aliquots or $HClO_4$ or H_2O_2 treatment of the nitric acid solution from pressurized decomposition [20, 21]. Figure shows a typical voltammogram of a krill tail sub-sample. Lower nickel levels were confirmed by NaDDC/MIBK extraction at about pH 5 and subsequent electrothermal AAS measurement of the obtained IMBK phase [17, 21].

Results and Discussion

The results obtained for Cd, Pb, Cu, Ni, Hg, and As are summarized in Table 1. Despite the observed scatter of results in some materials, probably due to inhomogeneity, and probably also contamination (Pb), distinct conclusions can be drawn according to the present knowledge about probable future uses of the materials studied.

Table 1. Trace metal concentrations and sampling dates for krill, krill products and antarctic fishes caught 1977/78 in the antarctic Scotia Sea. Values are given in ng/g (ppb) FW = fresh weight, DW = dry weight

No.	Species or sample	Sampling location and date	Cd (*n*) *average* (range)	Pb	Cu	Ni	Hg	As	Remarks
1	Whole krill with shells	61°43′S; 61°85′W 11.01.78	(10) *170* (70–360)	(8) *340* (130–550)	(8) *6500* (2,900–11,000)	(8) *300* (240–370)	(10) *≦20*	(10) *2,000* (1,170–3,500)	FW
2	Boiled, homogenized krill without shells	61°10′S; 59°02′W 08.01.78	(7) *220* (170–275)	(7) *310* (240–530)	(7) *4700* (3,400–5,900)	(7) *270* (220–340)	(9) *≦20*	(5) *540* (250–840)	FW
3	Krill muscle meat	—	(20) *48* (36–66)	(20) *≦50* (<10–90)	(8) 380 (340–520)	(9) *130* (70–200)	(9) *<20*	(5) *340* (270–600)	FW
4	Spray dried as sample 2	59°42′S; 49°47′W 25.01.78	(8) *500* (470–570)	(7) *580* (440–700)	(7) *51,500* (49,400–54,900)	(7) *1,700* (140–1,860)	(9) *≦20*	(5) *3650* (3,200–4,100)	DW
5	Krill meal from whole krill	—	(8) *1200* (1,080–1,600)	(6) *610* (450–960)	(8) *32,000* (30,000–37,000)	(7) *1,200* (1,100–1.300)	(9) *≦20*	(5) *2,690* (2,580–2,770)	DW
6	*Notothenia rossi* fillet	54°48,5′S; 35°20′W 12.11.77	(3) *3* (2–5)	(3) *80* (70–90)	(3) *200* (180–220)	(2) *≦100*	(5) *25* (12–35)	(5) *470* (330–620)	FW
7	*Dissostichus* fillet	53°35′S; 41°08′W	(3) *3*	(3) *150*	(3) *170*	(2) *≦100*	(5) *50* (25–70)	(5) *1,520* (1,200–1,650)	FW
8	*Notothenia gibberifrons* fillet	54°48,5′S; 35°20′W 12.11.77	(3) *3*	(3) *100*	(3) *160* (130–170)	(3) *≦100*	(5) *40* (20–70)	(5) *820* (580–900)	FW

In *krill* and *krill products* elevated Cd values in comparison to, e. g. teleost fish, were observed as had been also found usually in the course of our comparative surveys for crustaceans, mussels and algae [8]. Similar tendencies were also reported by other authors [22–27]. Also elevated Cd levels were found in fish meal products [28].

The results of the krill products investigated indicate that the Cd levels in the tail muscle (sample no. 3) are somewhat higher than in fillets of the teleost fish [8, 29, 30] but about as high as Cd levels in pork meat [31]. Thus, they are sufficiently low to permit the use of this material for human nutrition, and they are also well below the threshold value for a weekly Cd intake of 0.4 mg by the standard man, as recommended by a Joint FAO/WHO Expert Committee [32]. These recommendations seem to be according to the present knowledge on Cd toxicity still on the safe side [33]. Hence, the potential use of krill meat products as protein source in food appears with respect to Cd levels to be possible without health hazard.

If, however, samples 1, 2, 4, and 5 are considered, remarkably elevated Cd levels were found, probably due to the accumulation of Cd in organs and the digestive glands, as is also known from recent studies on other marine organisms [8, 27, 35]. If sample 1, whole specimes, and sample 2, whole specimens with removed shells, are compared it seems that the shells do not contain very high Cd amounts. These materials may still be used in principle as protein-rich animal feed, since it was observed that even feeding of rainbow trout *(Salmo gairdneri)* with 10 ng Cd/g feed leads to low carry-over rates particularly for muscle tissue. Also no severe toxic effects were reported, and it was suggested that protein feed may contain up to 1 µg/g Cd (dry weight) [6]. That should in general permit the substitution of some hitherto applied fish-meal products by krill meal in order to balance the decreasing fish catches.

The same as discussed for Cd also in principle applies for the krill muscle meat (sample 3) with respect to Pb, Cu, Ni, Hg, and As. From preliminary determinations of Pb in an about 10-kg krill tail sample it was supposed that this sample could be contaminated with Pb from the outside. Carefully taken sub-samples only from the inner parts of this material showed much lower, but scattered values in comparison with the quite constant Cd values for the whole sample. Thus, it is obvious that this behaviour is still due to contami-

nation, and that the natural Pb level in krill meat may be well below 50 ng/g, which seems to be very close to Pb amounts reported for, e.g. beef meat [34], possibly nearly in the range recently found for the muscle meat of several teleost fishes [8]. Hence, Pb remains well within acceptable ranges, if compared with the FAO/WHO recommendations of a threshold for the weekly Pb intake of 3 mg [32].

Samples 1, 2, 4, and 5 contain, in general, slightly higher Pb amounts than sample 3, but this may be also explained by contamination during preparation, more than by natural content.

In the case of Cu the rather high values for these samples correspond to the results obtained for other crustaceans, as e.g. *portunus spec.* [8]. About the same applies to Ni showing also significant contents in organs and digestive glands. The Hg values are comparatively low, which is in good agreement with the fact that Hg values of marine species in colder oceans are comparatively lower than, e.g. in the Mediterranean Sea [9, 29, 30, 36–39]. Also the levels for As are comparatively low and show about the same values as has been earlier reported for some marine organisms from the North Sea [30].

The muscle meat (fillets) of the investigated *antarctic fish species*, sample 6–8, shows rather low values for Cd, Pb Cu, Ni and As, well within the ranges of the respective values for other comparable species [8, 23, 29, 30, 36–38] and thus constitute from this aspect no problems for human nutrition.

The observed somewhat elevated Pb levels in the analyzed skin containing fillet samples, if compared with other data from fish muscle tissue, may be also as discussed for krill tail muscle, partly due to a distinct contamination with Pb during the preparation of these materials on board the research vessels, and to the fact that the skin of fish usually contains appreciable amounts of lead [8, 39, 40].

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Note added in proof: The analyzed krill materials were *not centrifuged*. The effect of the removal of apart of the digestive tract by centrifuging the krill as done during the german expedition in 1977/78 (12) on trace metal levels has to be awaited.