POLISH POLAR RESEARCH (POL. POLAR RES.) POLSKIE BADANIA POLARNE	7	4	371—376	1986

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The krill chitin and some aspects of metals transport in Antarctic sea-water

ABSTRACT: Concentration of Zn, Cu, Cd, Pb and Co have been determined in Antarctic water (South Shetland Islands) and in krill exoskeletons with the help of atomic absorption spectrophotometry. Concentrations of these metals both in sea-water and in krill exoskeleton are in order Zn > Cu > Cd > Ni > Pb > Co.

Comparing concentrations of these metals in sea-water to their concentrations in krill exoskeleton, the factors have been calculated giving a list of metals in the order of krill chitin ability, which is Ni > Cu > Zn > Cd > Pb > Co accumulation.

The highest accumulation factors for Ni and Cu point out to the special role played by these metals in krill life.

Key words: Antarctic, krill, chitin, metals.

1. Introduction

Chitin is the second, after cellulose, naturally occurring polymer. Chitin occurs widely in lower animals and fungi. The exoskeletons of crabs and lobsters contain from 20 to 50% chitin (Muzzarelli 1973). *Euphausia superba*, the shrimp-like crustacean (krill) is an extremely rich source of protein and fat, and is potentially a source of valuable by-products such as chitin and chitosan. Very little is known about the metabolism of chitin in animals, but it is clear that it acts as a carbohydrate and nitrogen reserve. Chitin is bound with proteins to form glycoproteins. The minor role of chitin seems to be the supporting and protecting function.

Chitin is a polysaccharide consisting of β — (1→4) 2-acetamido-2-deoxy--D-glucose units. This natural polymer can be called poly-N-acetyl-D-glucosamine (Fig. 1).

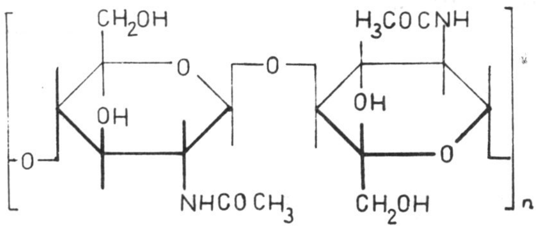

Fig. 1. Unit of chitin

In native chitin not all the glucosamine units are acetylated. For example chitin from lobster contains 82.5% N-acetylglucosamine, 12.5% glucosamine, 5% water (Giles et al. 1958). The free amino groups of glucosamine units may accept a proton and carry a positive charge. These are the zones where metal elements from oceanic water could be absorbed.

Chitin sediments in sea-water were estimated to amount to several billion tons per year, mostly due to moulted copepod exoskeletons (Brisou et al. 1964). The moulted cuticle contains more inorganic than organic constituents (Muzzarelli 1973). In sediments, chitin affects the C/N ratio which reaches sometimes value 10 and which is controlled by chitinolytic bacteria (Muzzarelli 1973).

Table 1

Concentration factors for the zooplankton (Martin 1970).

Element	μg/g wet zooplankton	ng/cm^3 sea-water	accumulation factor ($\times 10^3$)
Pb	5.9	0.03	197.0
Cd	0.6	1.00	6.0
Zn	51.0	10.00	5.0
Co	1.6	0.50	3.0
Ni	5.0	2.00	2.5
Cu	4.9	3.00	1.6

Zooplankton can be of great importance in the cycling of elements in the world's oceans (Martin 1970). Zooplankton can transport elements in oceanic water in various ways, and, what is most important, by the moulting of exoskeletons and by sinking of skeletal structures after death. It is certain that chitin with collected metals reaches the bottom in large amounts (Muzzarelli 1973). Accumulated residues of chitinous cuticles of Crustacea were found in oozes (Muzzarelli 1973). It is quite probable that chitin of living organisms (including krill) plays a major function in the transport of transition metal iones in the sea. Zooplankton accumulation factors were calculated by Martin (1970) by converting average elemental

Table II

Concentration factors for krill exoskeleton and Antarctic sea-water.

Element	Concentration of metal in krill exoskeleton ng/g ($\times 10^3$)	Concentration of metal in Antarctic sea-water $\mu g/dm^3$	accumulation factor ($\times 10^3$)
Ni	127.9	0.7	182.7
Cu	406.8	2.3	176.8
Zn	971.9	12.0	80.9
Cd	141.2	1.9	74.3
Pb	25.7	0.4	64.2
Co	23.1	less than 0.5	46.3

Concentrations of metals in krill exoskeleton has been calculated on chitin-like substance.

concentrations for analyzed zooplankton samples divided by the amount of the given element found in an equal weight of sea-water. They are listed in Table I.

The intention of this investigation was to determine some metals (Ni, Co, Pb, Cd, Zn, Cu) in exoskeleton of Antarctic krill and in surrounding waters to find a correlation between them.

2. Material and methods

Studies were carried out on krill and oceanic water from South Shetland Islands area in 1980 year. The krill caught and water samples were kept (out of contact with metal parts) at −25°C for 3 months. Krill was prepared by removing of the edible parts and viscera by mechanical peeling, giving shell waste. The shell waste was washed with water and dried on Buchner funnel under vacuum. Such chitin-protein exoskeletons were used for analysis.

This analytical material contains 11.27% dry mass and 6,76% insoluble substance in 5% NaOH after 2 hours boiling. It gives 60.23% chitin-like substance and rest of minerals.

2.1. Sample preparation

Mineralisation

Determination of Zn and Cu was carried out directly from dissolved and mineralized samples. Determination of Ni, Co, Pb and Cd was carried out by concentration of complexed metals and extraction to MIBK (methyl isobutyl ketone).

As a complexing mixture (Wilczewski, unpubl. data) a solution of sodium diethyldithiocarbamate (NaDDC), ammonium pyrrolidyne dithiocarbamate (APDC), cupferron ($C_6H_5N(NO)ONH_4$) and 4 — (2-pyridylazo) resorcinol (PAR) has been used.

Reagents

NaDDC	— A.R. grade, POCH Gliwice, Poland
APDC	— A.R. grade, Fluka, Switzerland
Cupferron	— A.R. grade, PAN Warszawa, Poland
PAR	— A.R. grade, POCH Gliwice, Poland
Citric acid	— A.R. grade, POCH Gliwice, Poland
NH_4OH (29%)	— A.R. grade, POCH Gliwice, Poland

2.2. Apparatus

An atomic absorption spectrophotometer Varian Techtron Model 1200 was used. The parameters ensuring the reliable operation of the apparatus were determined experimentally by the criterium of the absorbance maximum of the signal.

Solutions

Mixed complexing solution. To 5 g of sodium diethyldithiocarbamate (NaDDC), 1 g of ammonium pyrrolidine dithiocarbamate (APDC), 3 g of cupferron and 0.05 g of 4-(2-pyridylazo)resorcinol (PAR) were added to 250 cm^3 of ethanol, 250 cm^3 of water and two drops of NH_4OH (29%). The solution remained stable for several weeks if stored in the dark.

1.5 M aqueous citric acid solution. 315 g of citric acid was dissolved in water and filled up with water to 1000 cm^3.

Buffer solution. To a 1000 cm^3 measuring flask containing 500 cm^3 of 1.5 M of citric acid solution, about 175 cm^3 of concentrated ammonia solution under vigorous stirring and cooling in ice-water was added slowly portionwise. The solution was cooled and filled up with water to about 950 cm^3. The pH of the solution was checked by diluting 1 cm^3 of buffer in 20 cm^3 of the investigated water. The pH should remain within 6.5—7.2 with ca 7.0 as optimum. The pH of the whole buffer portion was adjusted with citric acid or diluted ammonia solution and filled with water to 1000 cm^3. The buffer solution was purified by adding 30 cm^3 of mixed complexing solution and 100 cm^3 of MIBK to the whole portion. The mixture was shaken moderately for 5 min. in a 2000 cm^3 separatory funnel. The organic layer was separated and 70 cm^3 of MIBK was added and extraction was repeated. After several hours aqueous phase was poured into a polyethylene container. The pH for the aqueous solution was higher after extraction by 0.6—0.7 units.

2.3. Analytical procedure

— 10 g of krill exoskeletons (shell waste) dissolved in 25 cm^3 conc. HNO_3 was boiled for about 4 hours. After complete dissolving the sample was diluted with deionized water to 1000 cm^3.
— Sea-water. 800 cm^3 sea-water and 20 cm^3 of buffer solution have been shaken with 10 cm^3 of mixed complexing solution and 15 cm^3 of MIBK. After 15 min. the layers were separated and the aqueous phase was extracted again with 5 cm^3 of MIBK. The combined organic phases have been used for the spectrophotometric measurements.
— Shell waste (exoskeletons). 50 cm^3 of mineralyzed sample was shaken with 1 cm^3 of complexing solution and 7 cm^3 of MIBK for 15 min. The organic layer was separated and have been used to the spectrophotometric measurements.
— Standard solution. The mixed solutions to be used in investigations were obtained by dilution of the stock solutions. In case of Ni, Co, Pb, Cd standard solutions were extracted with MIBK in the same way as samples.

3. Results and discussion

The results of the investigations show that concentrations of these metal elements in Antarctic sea-water as well in krill exoskeleton are in order as follow: Zn > Cu > Cd > Ni > Pb > Co.

Concentrations of these metals in Antarctic water are similar to data from literature (Brzezińska, Samp 1981) for Admiralty Bay: 0.110—0.825 $\mu g/dm^3$ Cd, 0.356—0.462 $\mu g/dm^3$ Cu, 0.164—0.220 $\mu g/dm^3$ Pb, 1.745—2.565 $\mu g/dm^3$ Zn, and for Arctic water (Urbański et al. 1980): 0.06—1.15 $\mu g/dm^3$ Pb, 0.04—0.13 $\mu g/dm^3$ Cd, 8.2—16.8 $\mu g/dm^3$ Zn, 0.6—2.5 $\mu g/dm^3$ Cu, 0.6—0.8 $\mu g/dm^3$ Ni.

Comparing concentrations of these metals in krill exoskeleton to their concentrations in surrounding Antarctic sea-water, the accumulation factors have been calculated. The coefficients are shown in Table II.

The highest accumulation factors in case of Ni and Cu indicate that these metals play a special function in krill life. They are the most selectively collected metals by exoskeleton (chitin) of krill, probably through chitin-metal complex. Concentrations of these metals both in sea-water and in krill exoskeleton are in the same order: Zn > Cu > Cd > Ni > Pb > Co. Comparing concentrations of these metals in sea-water to their concentrations in krill exoskeleton, the factors have been calculated giving a list of metals in the order of krill chitin accumulation ability, which is Ni > Cu > Zn > Cd > Pb > Co. By collecting of all these metals, krill chitin plays an important role in natural migration of these elements in the Antarctic water zone. Because of a big population of krill in the Antarctic water zone such way of metals migration has to be significant.

4. Резюме

Определено содержание металлов Zn, Cu, Cd, Ni, Pb и Co в морской воде и в панцире криля в районе Южных Шетландских островов при помощи техники спектрофотометрии атомной абсорбции. Концентрация этих металлов как в антарктической морской воде, так и в панцире криля была определена в следующем ряде: Zn > Cu > Cd > Ni > > Pb > Co. Сравнивая концентрации металлов в панцире криля и их концентрации в морской воде, были вычислены коэффициенты аккумуляции исследуемых металлов в следующем ряде: Ni > Cu ≫ Zn > Cd > Pb > Co.

Способность к накоплению этих металлов приписывается хитину криля. В случае Ni и Cu наблюдаются наибольше коэффициенты аккумуляции металлов, что указывает на то, что именно эти металлы играют важную роль в жизни криля.

5. Streszczenie

Określono zawartość metali Zn, Cu, Cd, Ni, Pb i Co w wodzie morskiej i w pancerzu kryla z rejonu Szetlandów Południowych za pomocą techniki spektrofotometrii absorpcji atomowej. Stężenie tych metali zarówno w antarktycznej wodzie morskiej, jak i w pancerzu kryla oznaczono w szeregu: Zn > Cu > Cd > Ni > Pb > Co. Porównując stężenia metali w pancerzu kryla do ich stężeń w wodzie morskiej obliczono współczynniki akumulacji badanych metali, które zestawiono w szeregu: Ni > Cu > Zn > Cd > Pb > Co.

Zdolność gromadzenia tych metali przypisuje się chitynie kryla. W przypadku Ni i Cu zaobserwowano najwyższe współczynniki akumulacji metali, co wskazuje na to, że właśnie metale odgrywają ważną funkcję w życiu kryla.

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Paper received: 5 March 1986