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Kinetics of Vanadium Bioaccumulation by the Brackish Water Hydroid *Cordylophora caspia* (Pallas)

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Vanadium is an abundant metal, which enters the environment via natural rock leaching. A large fraction however, enters the environment through the combustion of coal or petroleum products, which can contain high vanadium concentrations of biological origin. In steel industry vanadium is used as an alloy, therefore, the residual slag stones of steel production can contain rather high concentrations of this heavy metal. Since slag stones are increasingly used in riverbank reinforcement and since it is known that vanadium can leach from these artificial stones into the aquatic environment current interest arose on the toxicity of vanadium towards aquatic organisms. Vanadium, inhaled by humans as vanadium pentoxid, accumulates mainly in hair, liver, kidney and bones (Nechay et al. 1986) and causes several health hazards (for review see: Philipps et al. 1983). For the aquatic environment, very little is known about accumulation in biota and about toxic effects arising in invertebrates. Apart from studies with several ascidian species, which are known to accumulate vanadium from sea water up to the 10^6-fold (Michibata et al. 1989) sparse information exists on accumulation of vanadium by invertebrates. Ringelband & Karbe (1996) showed that vanadium inhibits the colonial growth of hydroids, and that it acts as a potent inhibitor of hydroidal Na-K-ATPase. Up to now, very little is known about the biokinetic behaviour of vanadium in hydroids. The aim of the present study is to determine accumulation kinetics of vanadium in the brackish water polyp *Cordylophora caspia*.

Cordylophora caspia is a colonial polyp (fam.: Clavidae) with optimal population growth in brackish water with salinity ranging between 10 and 16 ‰ (Kinne 1956). Hydroids are particularly sensitive to heavy metals and they serve as test organisms in a well-established population growth test (Stebbing 1976, Karbe et al. 1984).

MATERIALS AND METHODS

Cordylophora caspia polyps were derived from a brackish water canal connecting the Baltic Sea with the Northern Sea in the north of Germany. Laboratory stocks were reared in glass vessels in an artificial brackish water (10 ‰ salinity, pH 8, 20 °C, darkness). Twice a week they were fed with live brine shrimp nauplii *(Artemia salina)*.

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As the artificial brackish water contains the complexing agents EDTA and NTA (up to 4 $\mu g\ g^{-1}$ EDTA, 1 $\mu g\ g^{-1}$ NTA) diluted Atlantic Sea water was used in the accumulation experiment. The Atlantic water was sampled at the Celtic Sea west of Ireland in polyethylene cans avoiding contact with steel materials and transported to the laboratory. Before dilution and further use the water aged under aeration for two months. Before use it was filtered. The Atlantic water used in the experiment was diluted to salinities of 5, 10 and 20 ‰. Before the experiment was started the hydroids were adapted to the different salt water dilutions over 14 days. After this adaptation the animals were transferred to vanadium contaminated experiment water containing 4 mg L^{-1} ammonium metavanadate (NH_4VO_3). During accumulation phase animals were collected at various exposure times and the vanadium content of the polyps was measured photometrically using an atomic absorption spectral photometer (AAS). The accumulation phase was stopped by transferring the remaining hydroids to vanadium uncontaminated diluted sea water. During the phase of elimination animal samples were taken at different time intervals and analysed for their vanadium content. Temperature during the experiment was 20 °C.

Animal samples were rinsed in distilled water and dry-freezed immediately (0.05 mbar / 24 h). The dried tissue was disintegrated with 1 ml of HNO_3 (suprapur) in three steps: 1 h at 80 °C, 2 h at 120 °C and 11 h at 160 °C; every step was carried out under pressure. After disintegration the samples were added to 5 ml using distilled water.

For measurements of vanadium a Perkin Elmer AAS (Zeeman 3030) was used. Atomisation was carried out in four steps: 30 s at 140 °C; 15 s at 220 °C; 25 s at 1300 °C; 5 s at 2650 °C. Measurements took place at a wavelength of 318,4 nm. For measurement the disintegrated animal tissue samples were diluted 1:10; experiment water was diluted 1:50 from accumulation phase, and measured undiluted from elimination phase. Measurements were calibrated by means of standard addition. The detection limit was 2 mgL^{-1}. For both accumulation and elimination phase, the vanadium content of the hydroids was calculated as $\mu g\ g^{-1}$ tissue dry weight (tdw). Each sample was tested in two replicates. Each replicate was analysed twice. Results represent the mean of the replicates.

Two experiments were performed. The first experiment was carried out in 10 ‰ salinity, only. Samples were taken at 0 min, 15 min, 30 min, 45 min, 4 h, and 24 h for accumulation phase. After transferring the remaining hydroids to uncontaminated water samples were taken at 15 min, 30 min, 45 min, 60 min, 3 h, 6 h, and 24 h for elimination phase. The results of this small time scale experiment were used to develop the mathematical model for bioaccumulation kinetics.

The aim of the second experiment was to determine accumulation and elimination of vanadium under various salinities. Parallel experiments were carried out in 5 ‰, 10 ‰ and 20 ‰ salinity. Samples were taken at 0 min, 1 h, 8 h, 24 h, and 48 h for accumulation phase. For sampling of the hydroids for elimination phase the same time scale (1 h, 8 h, 24 h, and 48 h) was used.

RESULTS AND DISCUSSION

The experimental study was designed to investigate the accumulation as well as the elimination of vanadium in hydroids. The simplest possible approach to describe the vanadium exchange behaviour of the polyps over time is the adaptation of a single exponential function. Because this simple model fitted the experimental data inadequately, an alternative mathematical model, which is able to describe the vanadium exchange behaviour of the hydroids, was developed. The model is described below, and the model results are shown in fig. 1.

Although the involved processes regarding the intake and withdrawal of vanadium to polyps are not well known, the model described in this paper is based on the simple assumption that the vanadium balance is controlled by the two distinct mechanisms: adsorption and incorporation. In detail: On the one hand, vanadium will be partly adsorbed at the epithelial surfaces, wherein epithelial surfaces envelope the outer surface of the hydroid and the gastrodermal interior of the hydroid. This uptake should depend both on the vanadium concentration in the brackish water and on the effective uptake rate. The vanadium withdrawal is assumed to be proportional to the already uptaken vanadium mass m_s. For convenience, all vanadium masses in this paper are divided by the tissue dry weight, i.e. all 'masses' are given in µg g^{-1}. From the above arguments the mass balance of m_s is controlled by:

$$\frac{\partial m_s}{\partial t} = C k_u - k_s m_s \quad , \tag{1}$$

where m_s = vanadium mass adsorbed at the hydroidal epithelial surfaces in µg g^{-1} tdw, C = vanadium concentration in the brackish water in µg m^{-3}, k_u = effective vanadium uptake rate in h^{-1}, k_s = outflow rate of vanadium out of the hydroid in h^{-1}, and t = time elapsed since start of experiment in h.

If it is assumed that there is no vanadium inside the hydroid at $t = 0$, integration of eq. (1) leads to:

$$m_s(t) = \frac{C k_u}{k_s}\left(1 - \exp(-k_s t)\right) \quad . \tag{2}$$

On the other hand a part of the uptaken vanadium will be incorporated in the cells of the polyps. It is assumed that in case of saturation there is a maximum mass m_i^*, which can be incorporated by the polyps, and that the mass incorporation rate is proportional to the mass deficit $m_i^* - m_i$, where m_i is the actual incorporated mass. The mass balance of m_i can then be written as:

$$\frac{\partial m_i}{\partial t} = k_i\left(m_i^* - m_i\right) \quad , \tag{3}$$

where k_i = incorporation rate of vanadium in h^{-1}.

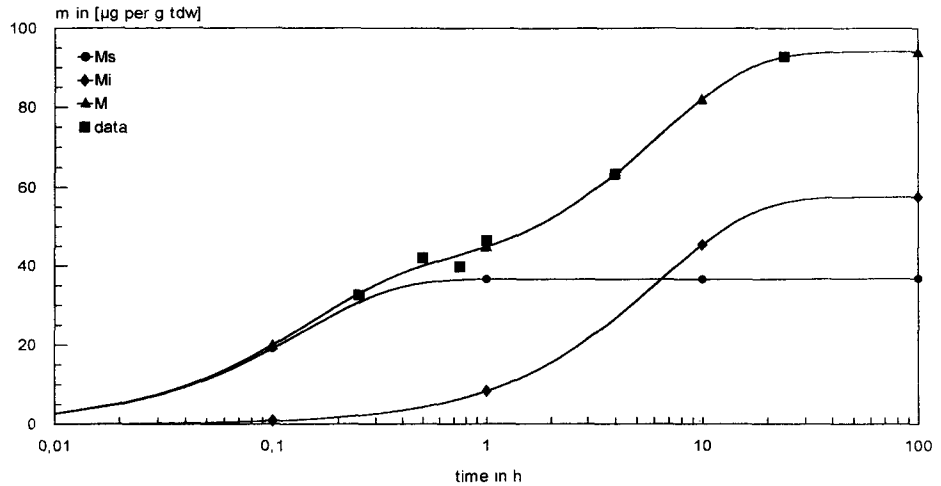

Figure 1. Uptake of vanadium by *C. caspia*: Vanadium content of the tissue. Experimental data and model calculation. The hydroids were exposed in 4 mg L^{-1} ammonium metavanadate. M = total V-mass of the hydroids; Ms = V-mass, adsorbed at epithelial surfaces; Mi = incorporated V-mass.

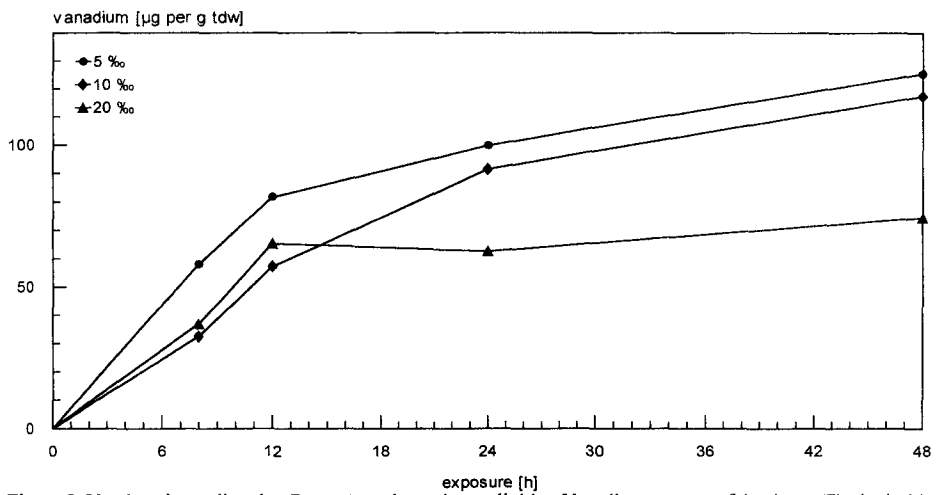

Figure 2. Uptake of vanadium by *C. caspia* under various salinities: Vanadium content of the tissue. The hydroids were exposed in 4 mg L^{-1} ammonium metavanadate.

Again starting with an uncontaminated polyp, i.e. $m_i = 0$ at $t = 0$, integration of eq. (3) leads to:

$$m_i(t) = m_i^*\left(1-\exp(-k_i t)\right) \quad . \tag{4}$$

Finally, combination of eq. (2) and (4) yields an equation for the total vanadium mass m in the polyps at time t:

$$m(t) = m_s(t) + m_i(t) = \frac{Ck_u}{k_s}\left(1-\exp(-k_s t)\right) + m_i^*\left(1-\exp(-k_i t)\right) \quad . \tag{5}$$

The model constants k_u, k_s, k_i, and m_i^* in eq. (5) were fitted against the experimental data of the small time scale experiment performed at 10 ‰ salinity by a least-square curve-fitting algorithm, i.e. the model constants are varied in order to minimise the squared differences between the masses m obtained from experiment and model. The values of the fitted model constants are $k_u = 14\ h^{-1}$, $k_s = 7.4\ h^{-1}$, $k_i = 0.16\ h^{-1}$, and $m_i^* = 57$ µg vanadium per g tdw. As mentioned above the vanadium concentration in the brackish water $C = 4$ mg L^{-1} is given by the experimental design. The experimental data agree very well with the model results (fig. 1). The correlation coefficient r and the standard error s amount to 0.998 and 1,4 µg g^{-1} tdw, respectively. As expected, the adsorption and diffusion processes are acting on a considerably smaller time scale compared to the incorporation of vanadium in the cells of the hydroids. This can also be seen from the biological half-life times $t_{1/2}$ (defined as $t_{1/2} = \ln(2)/k$ with the appropriate rate k) which amount to three minutes, six minutes and four hours for $t_{1/2}u$, $t_{1/2}s$, $t_{1/2}i$, respectively.

Vanadium uptake varied inversely with salinity (fig. 2). Hydroids exposed to vanadium contaminated water at 5 ‰ salinity accumulated 100 µg Vanadium per g tdw after 24 h and 125 µg g^{-1} tdw after 48 h, respectively. Hydroids exposed to vanadium contaminated water at 20 ‰ accumulated significantly less vanadium (62 µgg^{-1} after 24 h and 74 µgg^{-1} after 48 h, respectively). After 48 h of accumulation bioconcentration factor (BCF) was: 31,3 for 5 ‰, 29,3 for 10 ‰, and only 18,6 for 20 ‰.

The decreased uptake of vanadium under higher salinities could be explained by the speciation of vanadate ions in aqueous solutions. In low salinities vanadate ions exist mainly as mono- and divanadate. With increasing salinity tetra- and pentavanadates are formed (Pettersson 1994). The increased uptake of vanadate in water of decreased salinity indicates that the uptake of mono- and divanadates is facilitated in comparison to the uptake of polyvanadate ions. Further investigations should focus on the uptake mechanisms of vanadate. Up to now, very little is known about cellular uptake mechanisms of vanadate. Possibly monovanadates permeate passively through cell membranes and polyvanadates are incorporated by an active uptake mechanism. This hypothesis has yet to be tested.

Only few authors investigated accumulation of vanadium by aquatic organisms. Miramand et al. (1980) found BCF for vanadium accumulation by *Mytilus galloprovincialis* of 5 for soft tissue, and Bell et al. (1980) for vanadium accumulation by *Anguilla anguilla* of 5 (kidney), 32 (liver), 15 (bones), respectively. In general the BCF for vanadium is lower than for the heavy metals cadmium, lead and zinc BCF of 10^3 to 10^4.

In contrast to accumulation, which appeared to be salinity dependent, the elimination was independent of the salinity of the diluted Atlantic Sea water used in the experiment (fig. 3). Within the first six hours of elimination vanadium content of the hydroids decreased to 50 %, regardless of the salinity of the experiment water. After 48 h of elimination the vanadium content decreased to 20 µgg^{-1} tdw (5 ‰ and 10 ‰) and to 12 µgg^{-1} tdw (20 ‰), respectively.

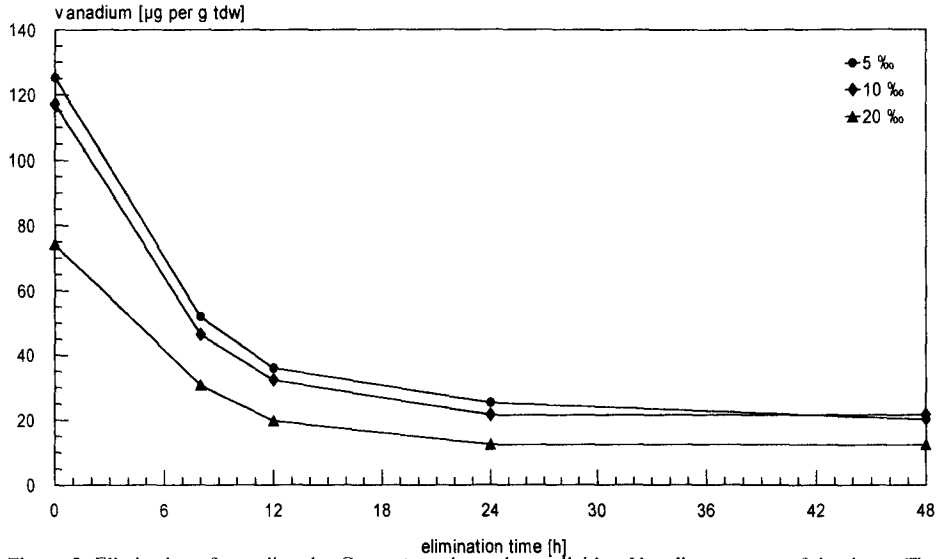

Figure 3. Elimination of vanadium by *C. caspia* under various salinities: Vanadium content of the tissue. The hydroids were exposed in 4 mg L^{-1} ammonium metavanadate for 48 h and then transferred to uncontaminated diluted sea water.

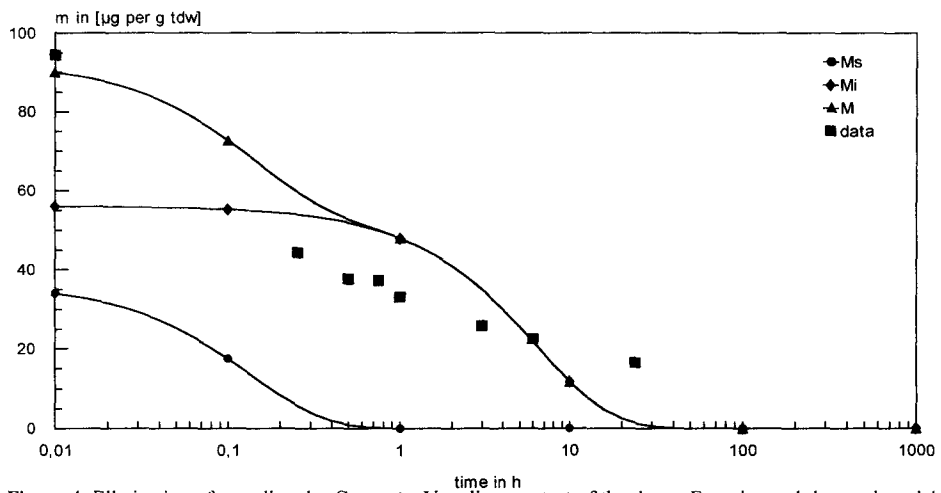

Figure 4. Elimination of vanadium by *C. caspia*: Vanadium content of the tissue. Experimental data and model calculation. The hydroids were exposed in 4 mg L^{-1} ammonium metavanadate for 24 h and then transferred to uncontaminated diluted sea water. M = total V- mass of the hydroids; Ms = V-mass, adsorbed at epithelial surfaces; Mi = incorporated V-mass;

The model presented above describes the process of vanadium uptake of polyps by adsorption and incorporation. The experiment was also carried out to investigate the elimination of vanadium. After time $T = 24$ h the polyps were removed from the contaminated water and planted into fresh brackish water without any vanadium. If the same kinetics control the vanadium uptake as well as the elimination, the same basic eqs. (1) and (3) should also apply to the elimination process, except for adapted initial and boundary conditions. The initial adsorbed and incorporated masses $m_{\backslash}$

and m_l, respectively, can be obtained from the uptake model for time T at the end of the accumulation experiment. The initial masses from eqs. (2) and (4) at $t = T$ amount to 36.6 µg g^{-1} tdw and 56.1 µg g^{-1} tdw for m_s and m_l, respectively.

Integration of eq. (1) for uncontaminated brackish water, i.e. $C = 0$, then leads to an expression for the adsorbed mass $\tilde{m}_s$ during the elimination process:

$$\tilde{m}_s(t) = m_s(T)\ \exp(-k_s t) \tag{6}$$

Assuming that the incorporated mass $\tilde{m}_l$ will decrease to zero after a sufficient long period in uncontaminated water (i.e. $m_l^* = 0$ in eq. (3)), $\tilde{m}_l$ can be obtained from eq. (3) by integration:

$$\tilde{m}_l(t) = m_l(T)\ \exp(-k_l t) \tag{7}$$

where k_s and k_l are the rate constants already obtained from the vanadium uptake model as given above. Finally, the total vanadium mass $\tilde{m}$ inside the polyps during the elimination process is given by:

$$\tilde{m}(t) = \tilde{m}_s(t) + \tilde{m}_l(t) = m_s(T)\ \exp(-k_s t) + m_l(T)\ \exp(-k_l t) \tag{8}$$

Data did not agree sufficiently with the developed model (fig. 4). Elimination processes seem to differ from accumulation processes. Nevertheless, the model neither contradicts nor confirms the assumption that desorption of vanadium ions from hydroidal epithelial surfaces follows the same mechanisms as adsorption. The model shows that elimination of incorporated vanadate ions from the cells follows different mechanisms than the incorporation of the ions, as the model does not fit for this assumption. However, there is no evidence that residual vanadium ions will remain in the hydroid's cells, but elimination process is slow. Further investigations should focus on the elimination kinetic of vanadium. Possibly hydroids reduce the incorporated toxic vanadate ions to less toxic vanadyl ions, like ascidians do (Michibata 1996). Intracellular NADH, glutathione or ascorbic acid could serve as reducing agents. This might represent a simple hydroidal detoxification pathway for heavy metals.

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REFERENCES

Bell MV, Kelly KF, Sargent JR (1980) The uptake of orthovanadate into various organs of the common eel, *Anguilla anguilla,* maintained in fresh water. Sci Tot Environ 16: 99-108

Karbe L, Borchardt T, Dannenberg R, Meyer E (1984a) Ten years of experience using marine and freshwater hydroid bioassays. In: Persoone G, Jaspers E, Claus C (eds) Ecotoxicological testing for the marine environment. State University of Ghent and Inst Mar Scient Res, Bredens, Belgium 2: 99-129

Kinne O (1956) Über den Einfluß des Salzgehaltes und der Temperatur auf Wachstum, Form und Vermehrung bei dem Hydroidpolypen *Cordylophora caspia* (Pallas), Thecata, Clavidae. I. Mitteilung über den Einfluß des Salzgehaltes auf Wachstum und Entwicklung mariner, brackischer und limnischer Organismen. Zoologische Jahrbücher 66: 574-638

Michibata H (1996) The mechanism of accumulation of vanadium by ascidians: some progress towards an understanding for this unusual phenomenon. Zool Sci 13: 489-502

Michibata H, Zenko Y, Yamada K, Hasegawa M, Terada T, Numakuani T (1989) Effects of vanadium ions in different oxidation states on myosin ATPase extracted from the solitary ascidian, *Halocynthia roretzi* (Drasche). Zool Sci 6: 289-293

Michibata H, Nishiyama I, Gualtieri R, de Vincentiis M (1985) Inhibition by vanadate of actomyosin ATPase extracted from ascidians. Comp Biochem Physiol 80B: 247-250

Miramand P, Guary JC, Fowler SW (1980) Vanadium transfer in the mussel *Mytilus galloprovincialis*. Mar Biol 56: 281-293

Nechay BR, Ninninga LB, Nechay PSE, Post RL, Grantham JJ, Macara IG, Kubena LF, Phillips TD, Nielsen FH (1986) Role of vanadium in biology. Fed Proc (FASEB) 45: 123-132

Pettersson L (1994) Equilibria of Polyoxometalates in aqueous solution. In: Pope MT, Müller A (eds.): Polyoxometalates: From platonic solides to anti-retroviral activity. Kluwer Dordrecht, pp 1-31

Phillips TD, Nechay BR, Heidelbaugh ND (1983) Vanadium: chemistry and the kidney. Fed Proc (FASEB) 42: 2969-2973

Ringelband U, Karbe L (1996) Effects of vanadium on population growth and Na-K-ATPase activity of the brackish water hydroid *Cordylophora caspia*. Bull Environ Contam and Toxicol 57: 118-124

Stebbing ARD (1976) The effects of low metal levels on a clonal hydroid. J Mar biol Ass United Kingdom 56: 977-994