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Effects of Vanadium on Population Growth and Na-K-ATPase Activity of the Brackish Water Hydroid *Cordylophora caspia*

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Vanadium, a relatively abundant heavy metal, enters the environment naturally through rock weathering. A large fraction of vanadium input is of human origin. The combustion of petroleum- and coal-products, which contain relatively high concentrations of vanadium, is one of the most important sources of the enrichment of vanadium in the environment. As it is used as an alloy, and vanadium rich iron-ores of various origin are used in steel production, the residual slag-stones of the steel industry can contain considerable vanadium concentrations. Wherever slag-stones serve as a cheap and convenient material in riverbank reinforcement, vanadium can leach into the aquatic environment.

Vanadium is regarded as an essential trace element for higher animals (Phillips et al. 1983). Cantley et al. (1977) indicated a regulatory function of vanadate *in vivo.* Although considerable information is available on the toxic effects of vanadium on humans, very little is known about the toxicity of vanadium towards aquatic organisms, especially invertebrates. Bell and Sargent (1979) have shown an inhibition of Na-K-ATPase activity in gills of the eel *Anguilla anguilla.* Holleland and Towle (1990) have demonstrated the inhibition of Na-K-ATPase activity by vanadium in the gills of the shore crab *Carcinus maenas.*

The aim of this study was to determine the toxicity of vanadium towards the brackish water hydroid *Cordylophora caspia.* Hydroids are known to be particularly sensitive to heavy metals and their asexual reproduction can be used in a well-established population growth test. Furthermore, the effects of vanadium on Na-K-ATPase activity in hydroids were studied in in vivo experiments, wherein the animals were exposed to sublethal concentrations of vanadium. In addition, the inhibition of Na-K-ATPase was measured *in vitro,* by adding vanadium to a microsomal preparation.

MATERIAL AND METHODS

The test species *Cordylophora caspia* was derived from the Nord-Ostseekanal, a brackish water canal connecting the North Sea and the Baltic in the northern part of Germany. Laboratory stocks are reared in glass vessels (pH 8, 10 ppm salinity, 20 °C) in the dark and are fed with live brine shrimp nauplii *(Artemia salina)* twice a week. The rearing water is used as control water in the toxicity test.

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One week before the test was started; some individuals were fixed on acid-washed glassplates and allowed to settle. During this week the hydroids were fed daily to make sure the test started in an exponential growth phase of the animals. At the beginning of the test, the colonies on each plate were reduced to 10 polyps. Four of the plates were put into an acid-washed glass aquarium containing 1.5l either one of the test solutions or control water. During the test, which lasted 10 days, *Cordylophora* were kept at 20 °C in the dark and fed daily. During feeding, the animals were counted under a binocular microscope, using 25 x magnification. An individual was counted when tentacles could be detected.

Vanadium was added as ammoniummetavanadate to the control water in nominal concentrations from 0.01 mg to 10 mgl^{-1}. Eight parallels were carried out for each test solution and the control water. Each day the mean number of individuals and its standard deviation was calculated for each test solution. The relative growth rate K (as the mean of the 8 parallels) was calculated for each day and for each test solution as following:

$K = (\ln n_2 - \ln n_1)/t_2-t_1$

(n_1, n_2 = number of individuals at day 1, day 2 respectively)

The LC_{50} value was calculated with probit analysis using the statistical PC-software StatEasy 3.5. The statistical evaluation of group differences over 10 days was performed with an ANOVA using the Apple Macintosh software STATVIEW II. Significance levels of K-values were evaluated by the Scheffe-test ($\alpha = 0.05$) using the same software.

For the measurement of Na-K-ATPase activity, hydroids were homogenized for 60 sec with a sonifier (60 sec 42 W, continuously) in 5 ml of ice-cold imidazole-buffer (250 mM sucrose, 6 mM EDTA, 20 mM imidazole, 0.1 % Na-deoxycholate, pH 7.5). The homogenate was centrifuged for 10 min at 5000 g. The supernatant was centrifuged twice (30 min x 10,000 g; 60 min x 110,000 g). After the last centrifugation, the pellet containing the microsomal fraction was resuspended in 3 ml imidazole-buffer. All steps were carried out at 4 °C. The protein concentration was determined according to Lowry et al. (195 1). Na-K-ATPase activity was measured in a coupled enzyme assay (Schwartz et al. 1969) at room temperature. The final incubation volume was 1.3 ml of following composition: 100 mM imidazole, 50 mM NaCl, 20 mM NH_4Cl, 4 mM $MgCl_2$, 0.5 mM NADH (NADH-Na_2), 1.2 mM PEP (tricyclohexylammoniumsalt), 1.4 mM ATP-Na_2 (vanadium free, Sigma), 6.75 U LDH, 0.36 U PK. For the measurement of total ATPase activity 0.2 ml distilled water was added. For ouabain-insensitive ATPases 0.2 ml ouabain (1.5 mM) was added. After preincubation for 5 min, the reaction was started by adding 200 µl homogenate. The oxidation of NADH was measured in a microplate reader (Anthos HT II) at 340 nm for 20 min. For calculating the Na-K-ATPase activity, the mean linear decrease of NADH was used. Na-K-ATPase activity was calculated as the difference between total ATPase activity and ouabain-insensitive ATPase activity.

For *in vivo* studies, 300-400 hydroids were put into acid-washed bowls containing 100 ml of either culture water, 10 mgl^{-1}, 1 mgl^{-1} or 0.1 mgl^{-1} vanadium (added as ammoniummetavanadate). Na-K-ATPase activity was measured after 2, 24 and 72 hr of vanadium exposure. Each concentration was tested in 3 to 7 replicates. Measurements were carried out with each replicate in 4 parallels.

For the measurements of Na-K-ATPase activity *in vitro* vanadium was added during preincubation of the enzyme assay (as described above), resulting in the following final concentrations: 0.74 mM, 0.074 mM, 0.0074 mM, 0.74µM, 0.074 µM and 0.0074 µM. Each concentration was tested in 5 replicates. Each replicate consisted of a pool of 300 - 400 polyps and was measured in 4 parallels.

RESULT AND DISCUSSION

Population growth was significantly impaired over the 10 days with 2 mgl^{-1} vanadium (Fig. 2). At higher concentrations the animals ceased reproduction. Moreover, population levels decreased at 8 and 10 mgl^{-1}, as indicated by a negative K-value for 10 mgl^{-1} and for 8 mgl^{-1} (Fig. 1). Probit analysis indicated a 10-d LC_{50} of 5.8 mgl^{-1} vanadium.

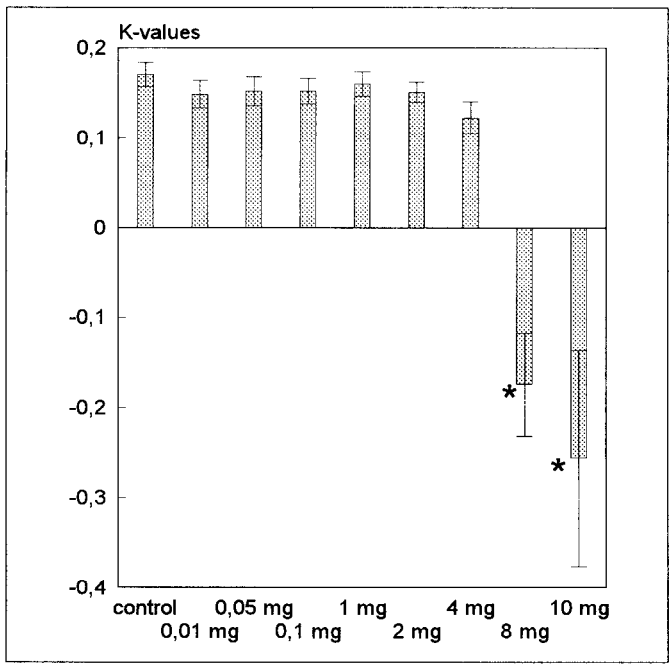

Figure 1. *Cordylophora caspia* toxicity test: K-values of different vanadium concentrations. The bars represent the mean of 8 parallels at the 10th day, the lines indicate the standard deviation. The K-values for 10 mgl-1 were calculated for the 5th day, as this was the last day of the experiment where living hydroids could be found. * = $p \leq 0.05$

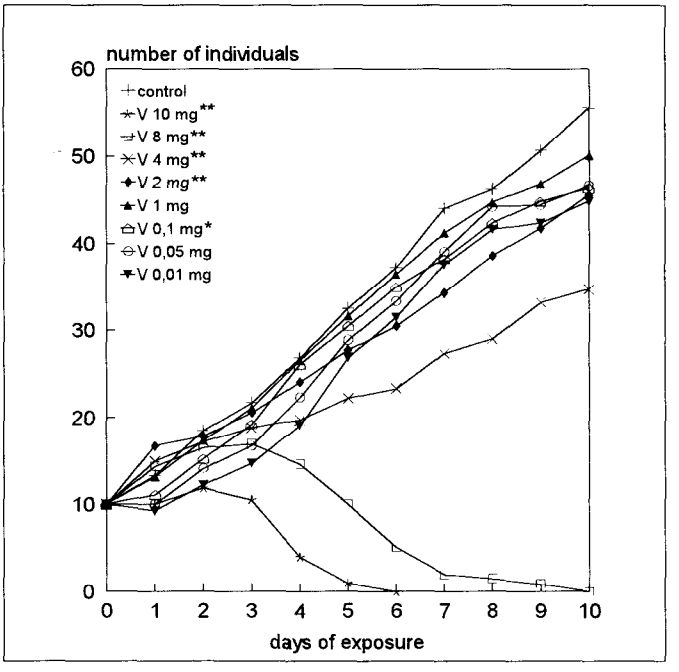

Figure 2. *Cordylophora caspia* toxicity test: population growth over 10 days. The animals were exposed to different vanadium concentrations. Each measurement represents the mean of 8 parallels. * = $p \leq 0.05$; ** = $p \leq 0.001$.

Very few toxicity tests with vanadium have been done on invertebrates. Miramand and Unsal (l978) found a 9-d LC_{50} for *Nereis diversicolor* at 10 mgl^{-1}, for *Carcinus maenas* at 35 mgl^{-1} and for *Mytilus galloprovincialis* at 65 mgl^{-1}. The hydroid *C. caspia* used in this study seems to be more susceptible to vanadium than the three benthic species mentioned above. For *Mytilus galloprovincialis,* Miramand et al. (1980) found a higher vanadium uptake at lower salinities. Therefore, the higher susceptibility of the brackish water species to vanadium compared with the three marine invertebrates tested by Miramand and Unsal (1978) may have been due to the reduced salinity required by *C. caspia.* As hydroids are known to be very sensitive to metal ions, they are a suitable group for testing the toxicity of metal contaminants (Stebbing and Pomroy 1978). Beusen and Neven (1987) determined a vanadium-LC_{50} for *Daphnia magna* at 2 mgl^{-1}. Although exposure time was prolonged in the cited study to 23 days, the 10-d LC_{50} for *C. caspia* in this study shows the same order of magnitude. A stimulatory effect of low metal concentrations on hydroids as described by Stebbing (1976) for the marine species *Campanularia flexuosa* could not be detected for vanadium in this study. Comparing the toxicity of vanadium towards hydroids with the toxicity of mercury, copper, cadmium (Karbe 1972; Stebbmg 1976; Stebbing and Santiago-Fandino 1983) and arsenic (Karbe et al. 1984) it appears about 10 times less toxic. Towards hydroids, vanadium can be considered as toxic as zinc, lead and selenium (Karbe et al. 1984).

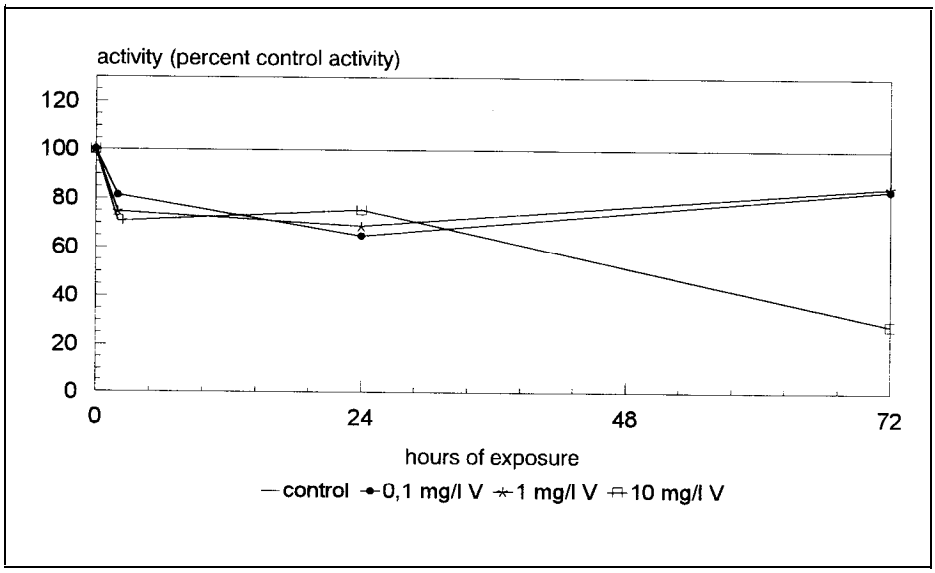

Figure 3. Inhibition of *in vivo* Na-K-ATPase activity: enzyme activity of *Cordylophora caspia* exposed to vanadium for 72 hr. Data are presented as a percentage of the control activity. Each measurement represents the mean value of 3 to 7 parallels. Each parallel consists of a pool of 300 to 400 individuals.

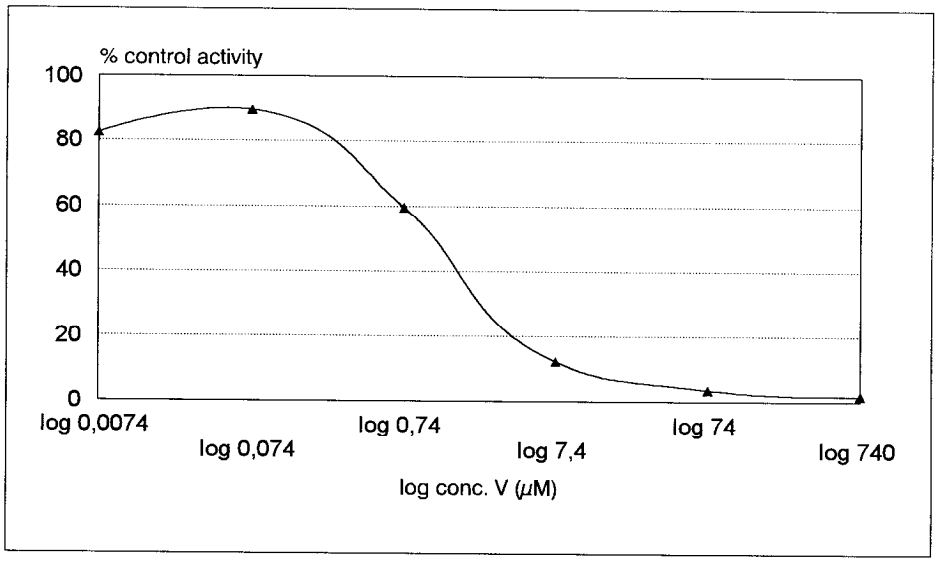

Figure 4. Inhibition of *in vitro* Na-K-ATPase activity: enzyme activity of microsomes extracted from *Corcylophora caspia* after addition of different concentrations of vanadium. Data are presented as a percentage of the control activity and the log transformation of metal concentration. Given are mean values of 5 parallels. Each parallel consists of a pool of 300 to 400 individuals.

After 2 hours of exposure, the *in vivo* Na-K-ATPase activity decreased to 70 to 80 %. After 24 hr only 65 to 75 % of the control activity was measured in all tested vanadium concentrations (Fig 3). The animals exposed to 0.1 and 1 mgl^{-1} vanadium showed 80 % of the control activity after 72 hr. On the other hand, Na-K-ATPase activity was distinctly reduced at 10 mgl^{-1} vanadium (30 % of control activity) after 72 hr exposure.

In vitro-Na-K-ATPase activity was totally inhibited with 740 µM vanadium (Fig. 4). Probit analysis indicated an *in vitro* EC_{50} of 0.49 µM vanadium. Compared with control activity, the addition of 0.0074 µM vanadium caused a 20 % *in vitro* inhibition of Na-K-ATPase activity.

Although the concentrations 0.1 and 1 mgl^{-1} vanadium in the toxicity test did not show a significant reduction of K-values, the Na-K-ATPase activity was considerably reduced *in vivo* at these concentrations. The hydroids seem to adapt to the vanadium exposure after 72 hr. The results of the *in vitro* test suggest a higher vanadium toxicity than the *in vivo* results. On the one hand the hydroids may have reacted *in vivo* to the vanadium contamination with an increased number of enzymes to maintain membrane processes. On the other hand Michibata et al. (1989) found that the activity of myosin ATPase extracted from the mantle of ascidians was only inhibited by pentavalent vanadium ions but not by tetra- or trivalent vanadium ions. He discussed the possible reduction of the toxic pentavalent vanadium ion to the non-toxic tetravalent ion *in vivo* by agents like ascorbic acid, glutathion, cystein or NADH. Whether a similar detoxification pathway exists in hydroids has yet to be tested.

In vitro inhibition of Na-K-ATPase activity is described by several authors. Holleland and Towle (1990) found a 50 % inhibition of Na-K-ATPase in membrane vesicles extracted from crab gill *(Carcinus maenas)* by vanadate at approximately 1 µM. Bell and Sargent (1979) showed an inhibition of microsomal Na-K-ATPase extracted from eel gills in the same order of magnitude. Nechay and Saunders (1978) described a 50 % inhibition of microsomal Na-K-ATPase of mammalian tissue preparations (kidney, brain and heart) in the range of 0.01 to 0.1 µM vanadium.

Taking the cited results into account, mammalian Na-K-ATPase appears to be the most sensitive to *in vitro* vanadium inhibition. *C. caspia* ATPase appears to be as sensitive as the enzymes in the other aquatic non-mammalian species.
Further investigations should focus on the accumulation and elimination of vanadium in hydroids. Special attention should be paid to a possible detoxification metabolism and the effect of salinity on the uptake of vanadium.

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