

**CADMIUM AND VANADATE OLIGOMERS COMPARATIVE  
EFFECTS ON THE TOADFISH ERYTHROCYTE**

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**Introduction**

The increasing levels of toxic metals in the environment, specially in marine environments, due to anthropogenic activities over the last years, make it important to study its toxicity mechanisms. Heavy metals, such as cadmium and vanadium, are known to cause extremely harm on biological systems. Cadmium presents a high potential as a toxic substance, even in low concentrations (Hu, 2000). On contrary, vanadium is considered benefit to live organisms in vestigial concentrations (1-10 nM), although it becomes toxic in higher concentrations (>100 µM) (Harland and Harden-Williams, 1994). Several haematological changes, compromising oxygen transport efficacy (cell destruction, hemolytic anemia and methaemoglobinemia), have been described upon metal intoxication. In teleost species this is specially important since 10% of haemoglobin is in the form of methaemoglobin (Lewis and Morris, 1986). In the present study, biochemical and morphological *in vivo* toxic effects of cadmium and vanadium were evaluated in red blood cells from *Halobatrachus didactylus* (Schneider, 1801), in order to: 1) compare cadmium and vanadium effects in methaemoglobin reductase activity; 2) evaluate the contribution of different vanadate oligomeric species to vanadium toxicity; 3) study cadmium and vanadium interaction with haemoglobin and 4) determine morphological changes induced in red blood cells by cadmium and vanadium.

## Material and Methods

*H. didactylus* – Lusitanian toadfish – individuals were collected in Ria Formosa lagoon (Portuguese south coast) and divided into five groups: Control 1, non injected; Control 2, injected intraperitoneously (i.p.) with 0.9% NaCl (placebo); Cd, injected i.p. with 5 mM of Cd (CdCl<sub>2</sub>); Meta, injected i.p. with a “metavanadate” solution containing 5 mM of total vanadium; and Deca, injected i.p. with “decavanadate” containing 5 mM of total vanadium. Metal solutions were diluted into final concentration (5 mM) in 0.9% NaCl. “Metavanadate” and “decavanadate” solutions were prepared from ammonium metavanadate, according to described elsewhere (Aureliano and Madeira, 1994). All solutions were administrated in a dosage of 1 mL solution/Kg of body weight. Subgroups of 4 individuals of each group were sacrificed after 1 and 7 days. Blood samples were collected using heparin as an anticoagulant.

Methaemoglobin reductase activity was determined by Board (1981) method.

Cadmium and vanadium interactions with haemoglobin were studied in red blood cells haemolysates, from a group of 6 non-injected fishes. The obtained supernatant was incubated with metal concentration ranging from 0.5 to 5 mM, at 25 °C, up to 90 minutes and haemoglobin spectrum changes were analyzed by spectroscopy.

Erythrocyte number, haemoglobin level and haematocrit were determined, in blood samples, by routine methods. Morphological changes, induced by cadmium and vanadium on red blood cells were studied on haematological preparations with Giemsa stain.

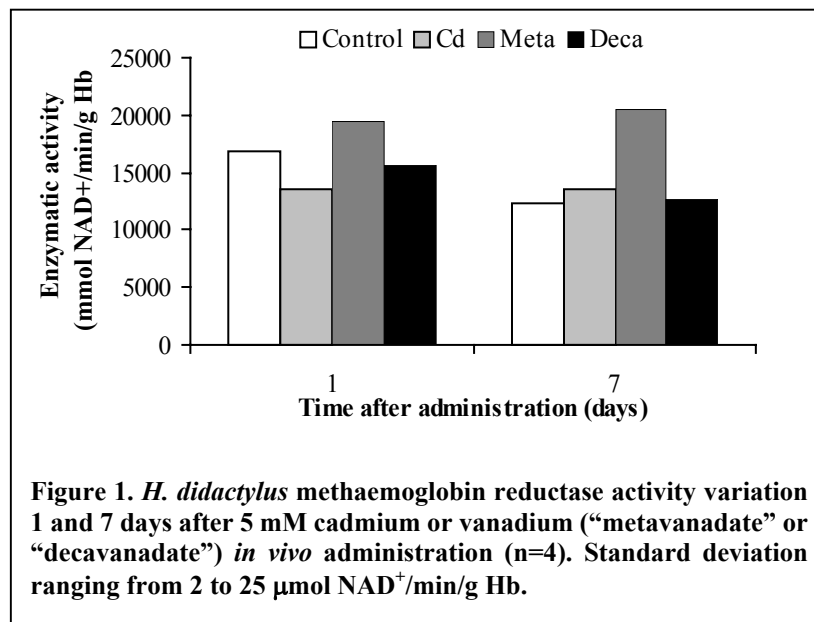
The Mann-Whitney test was applied to test differences between groups, on all the parameters analysed. The significant level used was  $p < 0.05$ . Control 1 and Control 2 showed no significant differences and for result analysed were considered together as Control.

## Results

### *Cadmium and vanadium effects on methaemoglobin reductase activity*

It was observed a 20% decrease, relatively to Control, in methaemoglobin reductase activity 1 day after cadmium injection, while “metavanadate” induced

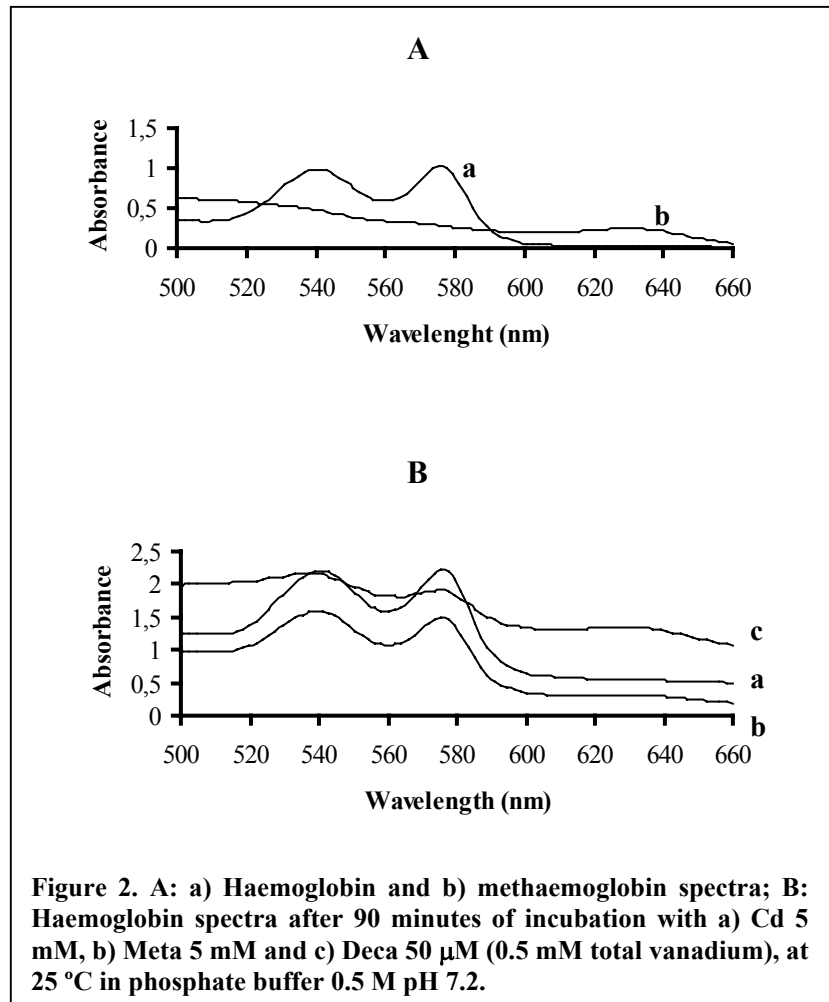
a 15 to 67% increase. “Decavanadate” had no significant effects on methaemoglobin reductase activity (Figure 1).



*Cadmium and vanadium interactions with haemoglobin*

A 0.5 mM “decavanadate” solution (which contains only 50  $\mu\text{M}$  of decameric species) induced haemoglobin oxidation to methaemoglobin after 30 minutes

incubation, whereas a slight spectrum change was detected for 5 mM of “metavanadate” and 5 mM of cadmium does not affect haemoglobin spectrum (Figure 2).



### *Cadmium and vanadium effects on haematological parameters*

After 7 days of metals administration distinct changes were found in the haematological parameters. Only “decavanadate” solution induced a decrease in erythrocytes count and haematocrit relatively to Control – 14% and 31%, respectively, whereas the haemoglobin concentration decreased 29, 22 and 36% in Cd, Meta and Deca groups, respectively, as it was observed an increase of erythrocytes cellular volume. In Cd group, the cell hypertrophy was followed by a nuclear volume increase.

### **Conclusions**

It is concluded that different vanadate oligomers contributed differently to vanadium toxicity in *H. didactylus*. Stronger effects were observed for decameric species in causing haematological changes in the toadfish erythrocyte. Upon administration, apparently cadmium induced an enzymatic activity decrease of the methaemoglobin reductase *in vivo*, while “metavanadate” solution promoted its stimulation and “decavanadate” had no effect. Vanadate species seems to be more effective causing haemoglobin oxidation *in vitro* than cadmium and, among vanadate species, “decavanadate” promoted a stronger effect in this process. Moreover, cadmium and vanadate oligomers induce a reduction of haemoglobin concentration, as well as the increase of erythrocytes cellular volume. However, decameric species of vanadate exhibit a more deleterious effect, promoting the reduction of erythrocytes count and hematocrit.

### **Acknowledgements**

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