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Original article

The toxicity effect of vanadium on *Artemia Franciscanna* and *Artemia Urmiana*

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ABSTRACT

The presened research was carried out in order to study how the toxicity of such heavy metals as vanadium affects on the remains of nauplius and the growth and life cycle of Artemia urmiana and Artemia franciscanna. The LC_{50} in 24 h of A. urmiana and A. franciscana exposed to vanadium were 0.0107 ,0.011 mg/l respectively. In growth experiments, the length of animals was considered as growth index. Results indicates that the mean length of animals in (0.001, 0.002 and 0.003 mg/l) V on first, 5th, ٧th and ١١th days of life significantly decreases in comparison with control groups ($p<0.05$).Bioaccumulation of V in the same concentration, after 24 h in nauplius and also in adults of A. urmiana and A. fransicana were statistically significantly higher than of the control groups ($P < 0.05$). Both species accumulate vanadium in their bodies. However A. urmiana is more resistant to the heavy metals. Results show, vanadium is high toxic on Artemia.

1. Introduction

Metals are considered very important and highly toxic pollutants in the various environmental departments. Heavy metals naturally occur in seawater in very low concentrations, but their concentration levels have increased due to anthropogenic pollutants over time. Industrial activities as well as agriculture and mining create a potential

source of heavy metals pollution in aquatic environment. Pollution of aquatic ecosystems by heavy metals is an important environmental problem, as heavy metals constitute some of the most dangerous toxicants that can bioaccumulate (Agh et al., 2008).

Among pollutants of aquatic ecosystems, heavy metals have great importance, because of their toxicity and ability of bio-accumulation in various marine species and even their bio-magnification across the food chain. Heavy metals are not broken down in the same chemical or biological methods as other materials existing in the environment do and this puts animals` life at risk. Artemia and its nauplius are among live foods which widely used for feeding fish larvae, crustaceans and oyster (lowns et al., 2003).

Artemia is an important and relatively common species of crustaceans which lives in brakish to very salty waters containing such amounts of salt several times more than that of the sea. Artemia is uniquely capable of producing inactive foetus, called cyst and made its introduction possible as a comfortable, easy and excellent food source. Cysts are found in large amounts in those coastal band of very brine lakes, coastal swamps and salt-producing little pools, throughout five continents. After 24 hours of incubation, cysts are turned into free swimming nauplius which are directly used by larvae of marine species and also freshwater species, as nutrients. Various life stages and their easy culture in artificial environments are among factors that allow to study how heavy metals of industrial, manufacturing and power plant productions entering water bodies affect on them and to study the amount of bio-accumulation in human body. However, one of the problems in applying Artemia for studying pollution is its resistance against some contaminating agents (Akbari et al., 2004).

Vanadium are considered as essential elements for living species, although this metals in high concentrations are toxic and lethal and might be bio-accumulated in animal bodies (Hagezieh et al., 2003). In the research, the effect of vanadium as essential elements in lethal and damaging amounts has been studied in different stages of development and also the amount of bio-accumualtion of A. franciscanna and A. urmiana was studied.

2. Materials and methods

Artemia's cysts were hatched in a funnel shaped plastic container filled with synthetic seawater. Newly hatched nuclei were processed following the procedure described by (Larenz et al., 2003; Amat et al., 2005; Abatzopoulos et al., 2006). The larvae were transferred into separate aquaria, where they were cultured until adulthood (Del et al., 1995). The animals were cultured at 27 $\pm$ 1˚C under constant aeration. The salinity (35 and 75ppt for A.franciscana and A.urmiana, respectively) in each flask was checked twice a day in order to maintain salinities according to the experimental set up. Artemia were fed unicellular algae Dunaliella tertiolecta and chemically treated yeast (Martinez et al.,1998; Medina et al., 2007).

At first, LC50 of each species with Nauplii of less than 24 h were determined. In growth experiments, 0.5 g of hatched cysts were put in 0.5 lit of solution with 0.001, 0.002 and 0.003 mg/l of V. Experiments were carried out in triplicate (18 treatments and 3 control groups) and each replicate underwent 95% volume every 4 days. The aeration process was done continuously during the test (Hadjispyrou et al.,2000 ; Nejatkhah et al .,2007) .Longevity of Artemia carried out with animals that were fixed in lugol solution in first, fifth, seventh and eleventh days of life.

In bioaccumulation experiment, about 2000 Nauplius and 100 adult Artemia were exposed to 0.001, 0.002 and 0.003 mg/l of V for 24 h. The experiment repeated 3 times (Nejatkhah et al.,2007). Afterwards, the separated Artemia samples were washed with distilled water and transferred to a container which had previously been completely cleaned and washed with distilled water and was then kept in freezer with a temperature of – 20ºC up to digestion and analysis phases (Rahimi et al., 2010). The samples were placed in oven for digestion in a temperature of 50 ºC for 24 h to be completely dried. After cooling the samples in desiccator, the dried samples were transferred to separate beakers and were weighted by a 0.0001g scale. At first, 1 ml nitric acid was added to dry samples and the samples were heated in a temperature of 60ºC for 10 min. Then, 1 ml of hydrochloride acid was added and they were heated for 30 min. Then the solutions were reached to a volume of 10 ml and were kept in different jars until machine analysis (Rainbow et al., 1987 ; Ringelband, 2001 ; Nejatkhah et al., 2007). Concentration of Vanadium were estimated by atomic absorption spectrophotometer (Shimadzu flameless 670 G) and graphic oven. This part of experiment was performed in the Atomic Energy Organization of Iran. V concentration measured in Artemia described above using SPSS software. All sets of data were tested for homogeneity of one way ANOVA and HSD test and all figures drew with excel program.

The atomic absoption machine was shimadzu flameless 670 G and a graphite oven was also used. Given the dry weight of samples and determined concentration of metal in the sample, the bio-accumulation might be achieved through the following formula, according to mL of metal in kg of Artemia (Hadjispyrou et al.):

$$\frac{\text{Mg}}{\text{kg}}\text{sample} = \frac{\text{A.V}}{\text{D}}$$

μg of metal, according to litre in the sample achieved form A calibration curve.

dry weight of sample according to gram D final volume of sample according to litre V.

$$\frac{\text{Mg}}{\text{kg}}\text{sample} = \frac{\text{A}((\mu\text{g}/\text{l}).\text{V(l)}}{\text{D(gr)}}$$

To measure the amount of absorption. Standard solutions of metal were firstly prepared and they had been injected before the main samples of prepared standards were analyzed and the real samples were tested following drawing calibration curve by the machine.

3.Results

The LC_{50} in 24 h of vanadium in Artemia urmiana and Artemia franciscanna were 0.0107 and 0.011 mg/l, respectively.

The length of Artemia was considered as growth index. The mean length of each species in different concentrations of V at first, fifth, seventh and eleventh days of life is shown in Tables 1, 2.

In both species, the growth in different treatments of metals indicated a significant increase compared to control group, but there were not significant difference in body length of treated groups ($P > 0.05$).

Table 1
Growth of A. urmiana in different concentrations of vanadium.

Concentration (mg/l)	Test day	Average length (mm) ± Standard deviation Exposure on V
0	1	26.6 ± 4.623
	5	34.8 ± 2.485
	11	119.2 ± 11.621
	17	166.9 ± 12.749
0.001	1	23.3 ± 2.945
	5	32.2 ± 2.097
	11	52.8 ± 3.583
	17	91.3 ± 4.922
0.002	1	23.2 ± 2.936
	5	33.7 ± 2.869
	11	52.3 ± 2.496
	17	92.1 ± 4.357
0.003	1	23.4 ± 2.913
	5	34.4 ± 2.412
	11	59.2 ± 6.124
	17	93.4 ± 3.204

Table 2
Growth of A. franciscana in different concentrations of vanadium.

Concentration (mg/l)	Test day	Average length (mm) ± Standard deviation Exposure on V
0	1	23.7 ± 3.128
	5	34.9 ± 2.601
	11	117.1 ± 12.114
	17	159.6 ± 8.959
0.001	1	21.4 ± 3.835
	5	30.6 ± 3.806
	11	40.9 ± 1.911
	17	88.9 ± 4.357
0.002	1	21.2 ± 3.224
	5	27.6 ± 2.547
	11	40.7 ± 1.702
	17	85.9 ± 3.928
0.003	1	21.1 ± 3.281
	5	26.2 ± 2.859
	11	40.6 ± 1.646
	17	81.7 ± 2.983

Bioaccumulation of V in 0, 0.001, 0,002 and 0,003 mg/l after 24 h in nauplius and also in adults of A. urmiana and A. fransicana are shown in Figures 1, 2. Bio-accumulation of treated groups with V were statistically significantly higher than of the control groups ($P < 0.05$).

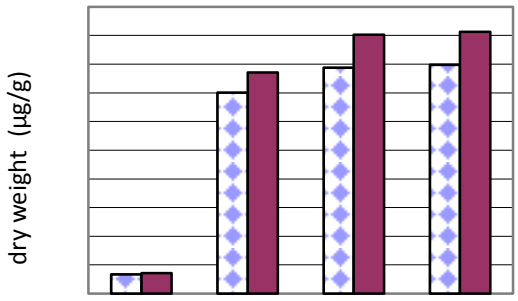

Fig. 1. Bioaccumulation of vanadium in nauplius and adult of Artemia urmiana.

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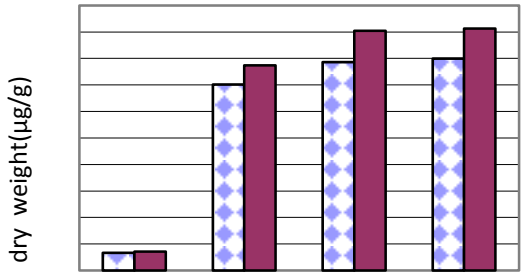

Fig. 2. Bioaccumulation of vanadium in nauplius and adult of Artemia franciscana.

4.Discussion

Increasing concentration of vanadium in the environment led to the increasing bioaccumulation of vanadium in A.urmiana and A.franciscana. There was a significant difference between control group and treatments ($p<0.05$). Our Results indicated, with increase of V concentration, therefore the accumulation and concentration of these metals are also increased in Artemia's body. Compared to the adults, elder individuals had more vanadium in their bodies.(Hadjispyrou et al., 2000) proved that Nauplius of A.franciscana had an ability to accumulate of tin, potassium, cadmium and chrome (Nejatkhah et al., 2007). They also proved that Artemia is more resistant to the heavy metals. They compared the amounts of bio-accumulation of those metals in nauplius of A. franciscana with fish, they results indicated that metals bio-accumulation in Artemia was more than in fish and this causes high resistance of this animal against heavy metals. (Fichet et al., 1998) reported that small amount of V had not affected growth of A. Salina and toxic effects appeared only after 8 days of exposing to 4 times more concentration of V (Sarabia et al., 1998). The growth of A. parthenogenetica and A.franciscana increased in compared to control group when were exposed to mercury, zinc and copper (Sarabia,1998; Karbassi et al.,2010). Difference in results can be explained by existing difference in various effects of heavy metals on species of Artemia and difference in metabolism and physiology among strains and also the different concentration of metals. This kind of effects on growth in such studies explained in terms of hormesis (Sarabia,2002).

The processes through which different aquatics can regulate the concentrations of different metals in their bodies are quite diverse and complicated. For example, accumulators are creatures that store the metals on a non-toxic basis in high amounts. These creatures change the metals somehow to a non-toxic form and store them by granulating them and combining them with metallothionein. Metallothioneins are a class of low- molecular-weight, cytoplasmic, metal-binding proteins, that have a high affinity for various toxic heavy metals. Elevated levels of such proteins have been suggested as indicating involvement in uptake, storage, transport, and elimination of toxic metals and in the routine metabolism of metal. (Del Ramo et al .,1995) showed the MT content in Artemia increased in a time- dependent fashion. Metallothionein synthesis in Artemia is very high and one of the reasons of high resistance of this creature to pollutants is attributed to this issue. The other mechanism in crustaceans is increasing the excretion of heavy metals as the concentration of the metals increases in the environment (Soegianto et al.,2008). These mechanism acts only in sub lethal concentration of metal and any disorder in these mechanisms may lead to the death of animals. Also, there are a variety of mechanisms may be involved in the effects of metals exposure, such as temperature, sex, salinity and other compounds (Triantaphyllidis et al.,1995;Valavanidis et al.,2010). To sum up, vanadium are toxic to A.urmiana and A. fransicana, so that they can influence the species' lifespan and growth rate. However both species especially A.urmiana is resistant to heavy metals.

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