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Evaluating the Bioaccumulation of Nickel and Vanadium and their effects on the Growth of *Artemia urmiana* and *A. franciscana*

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Abstract

Although there is growing evidence that metals can be toxic to various aquatic species, there is still insufficient knowledge to integrate this information in environmental risk assessment procedures. In this study, we have investigated bioaccumulation and effects of nickel and **v**anadium on mortality and growth of *Artemia urmiana* and *Artemia franciscana*. The LC_{50} in 24 h of *A. urmiana* and *A. franciscana* exposed to nickel and vanadium were 0.0072, 0.0114 mg/l and 0.0107 and 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) Ni and V on first, 5th, 7th and 11th days of life significantly decreases in comparison with control groups ($p<0.05$).Bioaccumulation of Ni and 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 nickel and vanadium in their bodies. However *A. urmiana* is more resistant to the heavy metals. Results show, nickel is less toxic than vanadium on *Artemia*.

Keywords: Nickel, Vanadium, Bioaccumulation, *Artemia urmiana*, *Artemia franciscana*

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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). Metals that are deposited in the aquatic environment may accumulate in the aquatic species and in the food chain and cause ecological damage also posing a threat to human health due to biomagnifications over time (Agh et al.,2008; Arruda et al.,2010). The oxidative nature of metal-induced genotoxic damage has been provided by the detailed studies showing that metals (iron, copper, cadmium, chromium, mercury, nickel, vanadium, cobalt and others) possess the ability to produce the reactive radicals resulting in DNA damage, lipid peroxidation, carcinogenicity, depletion of protein sulfhdryls and others effects (Asadpour et al., 2006). Nickel is a common metal in most surface waters, with both natural sources (e.g., weathering of rocks) and anthropogenic ones (e.g., industrial discharges from electroplating and melting). In general, world consumption of refined Ni has declined, but aqueous Ni concentrations may be elevated near natural deposits, Ni mining and refining operations, and other industrial emissions.

Although vanadium is an abundant as nickel and zinc in the earth’s crust, it is not a common pollutant. Vanadium does not occur as the free metal, but as relatively insoluble minerals and organo-metallic complexes (Brix et al., 2004).Vanadium enters the environment through natural rock weathering or by combustion of oil products. A third pathway is the leaching of vanadium-rich building materials. Stones made from steel industry residual slags, so-called slag stones, contain rather large amounts of vanadium. The increasing use of these slag stones has therefore led to increased interest in the toxicity of vanadium to aquatic organisms (Brix et al., 2006).

The brine shrimp *Artemia* (crustacean, Anostraca) is distributed worldwide with the exception of Antarctica (Blust et al., 1992). *Artemia* lives in salt lakes and ponds. The Urmia Lake is the main habitat for the endemic Iranian brine shrimp, *A.urmiana* (Blust et al., 1993; Del et al., 1995) . *Artemia franciscana* was not an endemic organism in Iran and the first introduction of it in Iran took palace in 1998 (Environment Canada,2010).

Artemia is widely used in laboratory toxicity studies due to its small body size and short lifespan together with its availability from dry cysts

(Fichet et al., 1998). Early embryonic and larval stages of development can be clearly defined and it is possible to use progression from stage to stage as a parameter of normal biological function potentially disrupted by toxic substances. All things considered, *Artemia* has been used to study metal toxicity (Fichet et al., 1998; Hadjispyrou et al.,2000; Hafezieh,2003). Some studies have demonstrated that brine shrimp is moderately sensitive to insensitive to a wide range of metals (Laughlin et al .,1981; Karbassi et al., 2010). In this research, we have studied the bioaccumulation and toxicity effects of nickel and vanadium on growth of adults and nauplius of *A. urmiana* and were compared with *A. franciscana*.

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 ± 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, LC_{50} 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 Ni and 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 Ni and 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

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in different jars until machine analysis (Rainbow et al., 1987 ; Ringelband, 2001 ; Nejatkhah et al., 2007). Concentration of Nickel and 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. Ni and 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 LC_{50} in 24 h of nickel and vanadium in *Artemia urmiana* and *Artemia franciscanna* were 0.0072, 0.0114 mg/l and 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 Ni and 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 ($P > 0.05$).

Results

Table 1: Growth of *A. urmiana* in different concentrations of nickel and vanadium

Concentration (mg/l)	Test day	Average length (mm) ± Standard deviation Exposure on Ni	Average length (mm) ± Standard deviation Exposure on V
0	1	26.9 ± 4.175	26.6 ± 4.623
	5	34.2 ± 2.347	34.8 ± 2.485
	11	119.1 ± 11.493	119.2 ± 11.621
	17	162.6 ± 12.946	166.9 ± 12.749
0.001	1	21.1 ± 3.281	23.3 ± 2.945
	5	32.7 ± 2.359	32.2 ± 2.097
	11	57.8 ± 4.022	52.8 ± 3.583
	17	110.9 ± 10.826	91.3 ± 4.922
0.002	1	22.6 ± 4.742	23.2 ± 2.936
	5	33.8 ± 3.084	33.7 ± 2.869
	11	58.6 ± 5.460	52.3 ± 2.496
	17	117.9 ± 9.036	92.1 ± 4.357
0.003	1	23.8 ± 3.119	23.4 ± 2.913
	5	34.9 ± 2.601	34.4 ± 2.412
	11	59.9 ± 5.152	59.2 ± 6.124
	17	117.0 ± 4.106	93.4 ± 3.204

Table 2: Growth of *A. franciscana* in different concentrations of nickel and vanadium

Concentration (mg/l)	Test day	Average length (mm) ± Standard deviation in Exposure on Ni	Average length (mm) ± Standard deviation Exposure on V
0	1	21.6 ± 3.864	23.7 ± 3.128
	5	34.8 ± 2.043	34.9 ± 2.601
	11	115.1 ± 15.249	117.1 ± 12.114
	17	160 ± 21.807	159.6 ± 8.959
0.001	1	21.2 ± 3.966	21.4 ± 3.835
	5	32.3 ± 1.766	30.6 ± 3.806
	11	49.0 ± 1.825	40.9 ± 1.911
	17	89.2 ± 4.131	88.9 ± 4.357
0.002	1	21.5 ± 3.836	21.2 ± 3.224
	5	31.8 ± 32.973	27.6 ± 2.547
	11	47.0 ± 2.357	40.7 ± 1.702
	17	82.0 ± 6.536	85.9 ± 3.928
0.003	1	21.0 ± 3.651	21.1 ± 3.281
	5	30.9 ± 4.121	26.2 ± 2.859
	11	43.1 ± 2.183	40.6 ± 1.646
	17	86.3 ± 4.831	81.7 ± 2.983

Bioaccumulation of Ni and 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, 3 and 4. Bio-accumulation of treated groups with Ni and V were statistically significantly higher than of the control groups ($P < 0.05$).

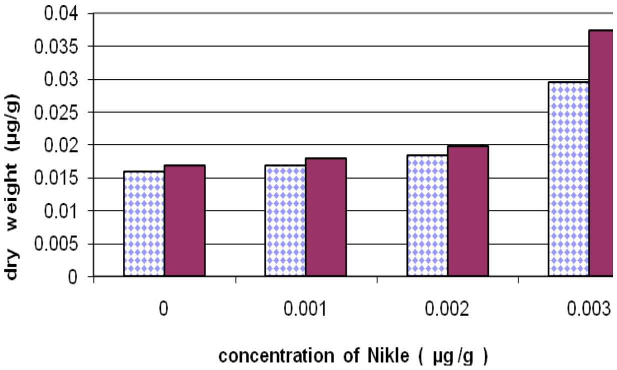

Figure 1. Bioaccumulation of nickel in nauplius and adult of *Artemia urmiana*

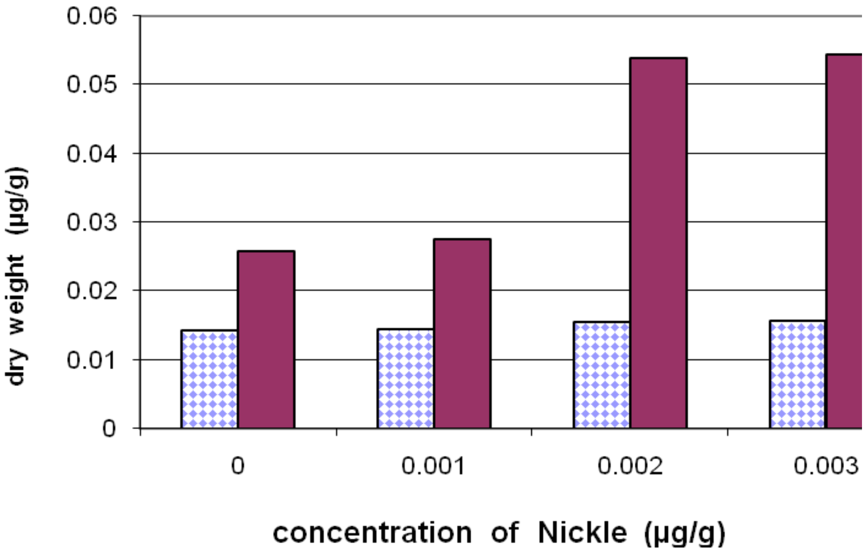

Figure 2: Bioaccumulation of nickel in nauplius and adult of *Artermia franciscana*

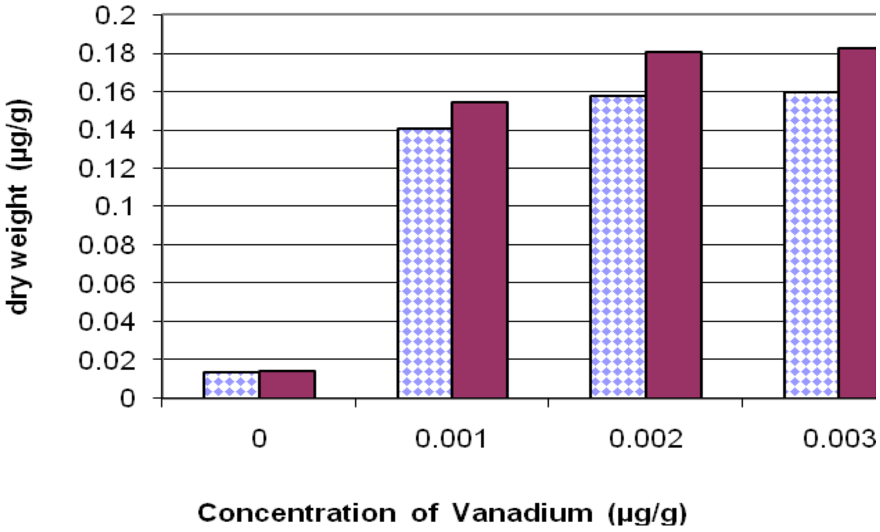

Figure 3: Bioaccumulation of vanadium in nauplius and adult of *Artemia urmiana*

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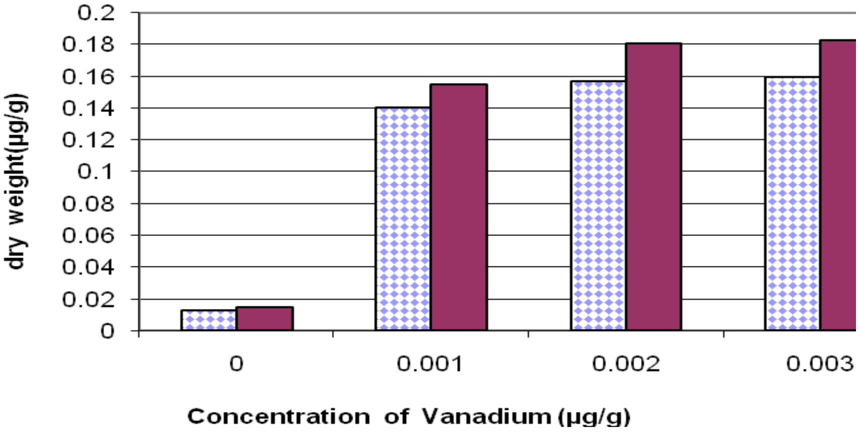

Figure 4: Bioaccumulation of vanadium in nauplius and adult of *Artemia franciscana*

Nickel and Vanadium decreased *Artemia`s* growth rate in comparison with control group ($p<0.05$). However there was no significant difference between days of experiment and treatments ($p>0.05$). There was no difference in all treatments, too ($p>0.05$).

Discussion

Increasing concentration of nickel in the environment led to the increasing bioaccumulation of nickel 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 Ni and 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 nickel 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, nickel and 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. In this study toxicity of vanadium was more than nickel.

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