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TOXICITY OF NICKEL IN ARTIFICIAL SEDIMENT ON ACETYLCHOLINESTERASE ACTIVITY AND HEMOGLOBIN CONCENTRATION OF THE AQUATIC FLEA, *MOINA MACROCOPA*

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The water flea Moina macrocopa, *an important freshwater zooplankter, is a useful test species for the study of sensitivity to environmental toxicants, and is recognized as a general representative of other freshwater animals. The sublethal effect of nickel on hemoglobin concentration and acetylcholinesterase activity of* Moina macrocopa *was evaluated, as well as the usefulness of bioassays using artificial sediments. The results show that hemoglobin concentration and acetylcholinesterase activity in the* Moina macrocopa *test could become useful for routine monitoring to detect the presence of nickel in aquatic environments.*

INTRODUCTION

Heavy metals have been identified as one of the most dangerous pollutants of aquatic ecosystems, due to their persistence and elevated toxicity for many organisms. These elements affect acetylcholinesterase activity and hemoglobin concentration (Martínez-Tabche et al., 1999; Carson et al., 1991, Shanker et al., 1979). Nickel is a ubiquitous trace metal that occurs in soil, water, air and the biosphere. The average content in the earth's crust is about 0.008 percent. Levels in natural waters have been found to range from 2 to 10 µg/l (freshwater) and from 0.2 to 0.7 µg/l (seawater) (IPCS, 1991).

IPCS (1991) showed that exposure of *Daphnia magna* to nickel sulfate at concentrations ranging from 5 to 10 µg/l nickel from 3 generations resulted in extermination. It is necessary to detect and evaluate nickel toxicity (and other metals) through bioassays with sediments and different organisms which simulate the natural conditions of the activity or active change of the metal in the system. These bioassays can be evaluated if the heavy metals spiked in the sediments are bioavailable, that is to say if they are presented in a form or phase which causes a biological response in one or more species which are present (SETAC, 1993).

For this reason the use of artificial sediment (free of pollutants) is important to carry out standardized toxicity evaluations. It allows results to be obtained in a shorter time independent of seasonal variations. It also provides results that are reproducible.

The water flea *Moina macrocopa,* an important freshwater zooplankter, has proven to be a useful test species for the study of sensitivity to environmental toxicants, and is recognized as a general representative of other freshwater animals (Sujata and Lakshmipathi, 1991). The cladoceran is sensitive to heavy metals abundant in many water bodies in Mexico, and it contains acetylcholinesterase (AchE) (Martínez-Tabche et al., 1997) and hemoglobin (Martínez-Tabche et al., 1991). For this reason these parameters were chosen for the toxicity study of *Moina macrocopa.*

The aim of the present study was to evaluate the sublethal effect of nickel on hemoglobin concentration and acetylcholinesterase activity of the cladoceran *Moina macrocopa* as well as to demonstrate the usefulness of bioassays with artificial sediments.

MATERIAL AND METHODS

Experimental animals

The cladoceran *Moina macrocopa* was obtained from Texcoco Lake, Mexico. It was maintained in a synthetic freshwater medium ($NaHCO_3$ = 174 mg/l, $MgSO_4$ = 120 mg/l, KCl= 8 mg/l and $CaSO_4.2H_2O$ = 120 mg/l). The physicochemical characteristics of the freshwater medium were: hardness ($CaCO_3$ = 170 ± 10 mg/l, pH = 8.2 ± 1°C, dissolved oxygen = 5.48 ± 1°C and conductivity = 510 µhoms/cm. 20 g (wet weight) of cladoceran were put in 3 l of synthetic water at 20 ± 2°C. The cladoceran was fed with the algae *Ankistrodesmus falcatus* at a 1.5 x 10^{-6} cell/ml concentration.

Artificial sediment preparation

Artificial sediments were prepared mixing 70 percent sand (particle size 0.05-0.2 mm), 20 percent kaolin (particle size < 0.002 mm) and 10 percent organic matter (particle size 0.2 mm). The source of organic matter was cow manure. It was inactivated by means of heat at 55-60°C over a 3-day period. The artificial sediment was sterilized in an autoclave during 3 cycles of 15 minutes to 121°C, at 15 lbs of pressure, with separate intervals of 1 hour. Sediment was stored in a polyethylene container until its use.

Physicochemical characterization

Sediments were characterized by means of a determination of organic matter content (concomitant organic carbon was determined). It was measured using the Walkley and Black method (Secretaria de Agricultura y Recursos Hidraúlicos, 1988). pH was measured using a potentiometer (glass electrode), dissolved oxygen and conductivity were determined using an oxymeter (Simpson-Electric Co. YST Model 51-B), and cation exchange capacity was determined (Gillman, 1979). The physicochemical properties are summarized in Table 1.

Table 1. Physicochemical Characteristic of Artificial Sediments

Parameter	Value
pH	7.9 ± 0.10
Dissolved oxygen ($mg.L^{-1}$)	5.6 ± 0.32
Organic matter (%)	7.6 ± 0.25
Organic carbon (%)	4.4 ± 0.17
Conductivity µmho/cm	0.6 ± 0.02
Moisture (%)	41 ± 0.56
Cation exchange capacity (CEC) (meq/100 g sediment)	66.4 ± 0.98

Note: Each value represents the median of three replicates ± S.E.

Equilibrium time

Nickel was spiked to the artificial sediments at six concentrations (3.16, 4.89, 7.76, 12.30 and 19.49 mg/kg). The analytical grade chemical used for spiking was $NiSO_4.H_2O$. The spiked sediments were mixed for 20 minutes. The systems were shaken at 0, 1, 2, 3, 4, 6, 24 and 48 h. Finally the nickel concentration in water and sediment was measured using an atomic absorption spectrophotometer to determine equilibrium time. The basis for the choice of concentrations was EPA 600/8-83-012FF (U.S. Environmental Protection Agency) and NOM-001-ECOL (1996) (Secretaria del Medio Ambiente, Recursos Naturales y Pesca).

Nickel analysis

One-gram samples of sediment and 1 ml of water were digested with 10 ml of concentrated nitric acid during 1h in an autoclave (15 lbs of pressure and 121°C). The nickel concentration was determined by flame atomic absorption spectrophotometry (air-acetylene, Varian Model AA-1475).

Acute toxicity

Water system: three replicate groups of 10 newborn organisms (one day) were added to polyethylene recipients that contained 50 ml of synthetic water with 5 nickel concentrations (1.65, 2.63, 4.16, 6.60 and 10.47 mg/l) as well as the control group.

Sediment system: three replicate groups of 10 newborn organisms (one day) were added to polyethylene recipients that contained 10 grams of sediment spiked with 5 nickel concentrations (3.16, 4.89, 7.76, 12.30 and 19.49 mg/kg) reconstituted in 40 ml of synthetic water as well as the control group. The median lethal concentrations for both systems after 48 h (48-h LC_{50}) were calculated by computerized log-probit analysis.

Exposure sublethal

Three replicate groups of 1 gram (wet weight) organisms were put in polyethylene recipients that contained 150 grams of sediment and were reconstituted with 600 ml of synthetic water spiked with 3 nickel concentrations (0.01, 0.1 and 1.2 mg/kg). The choice of nickel concentrations was based on the threshold limit effect (TEL) for Ni^{+2} in water (0.01 mg/kg), LC_{10} (1.2 mg/kg), and the intermediate concentration between TEL and LC_{10} (0.1 mg/kg). Exposure time was 48 hours. After the exposure time, 0.5 g of cladoceran (wet weight) were homogenized with 2 ml of buffer tris pH 7.0. The homogenate was centrifuged at 7000 g x 30 min at –5°C. The pellet was discarded and the supernatant was used for biochemical analysis.

Acetylcholinesterase activity

The supernatant was used to determine acetylcholinesterase activity. It was assayed by means of Hestrin methods (1949). 1 ml of supernatant was added with 2 ml of buffer tris pH 7.0 and 1 ml of AchE standard (3.50, 7.01, 10.52, 14.03 and 17.54 µmols). This system was incubated at 25°C for 35 min. The reaction was stopped by adding 2 ml alkaline hydroxilamine 2M, 1 ml HCl 4 N and 1 ml $FeCl_3$ 0.37 M in 0.1 N HCl. The developed color was assayed at 540 nm in a spectrophotometer (Varian DMS90). Protein concentration was estimated by the method of Bradford (1976) using bovine albumin as the protein standard. Enzyme activity was expressed as µmol AchE hydrolyzed/ mg protein/min.

Hemoglobin concentration

Hemoglobin concentration was measured using the cyanmethemoglobin method described by Wintrobe (1956). 500 µl of supernatant was added with 2 ml of Drabkin reagent. After 20 min these samples were analyzed in a spectrophotometer (600 nm).

Statistical analysis

The experimental data were analyzed by unifactorial variance analysis (ANOVA) and the mean differences of each group were compared using the Tukey test for multiple comparisons against a single control. Differences were considered significant at $P < 0.05$.

RESULTS

Physicochemical characterization

The general physicochemical characterization of artificial sediment is given in Table 1. The pH mean value is 7.9, which is alkaline. Organic matter and organic carbon in artificial sediments are high for aquatic ecosystems; this last parameter represents 57.89 percent of the total organic matter. The artificial sediment had a high percent humidity. The artificial sediment was slightly anoxic and its conductivity allowed it to be classified as a saline sediment (Colli, 1990). These properties were determined by the equilibrium time, which was 24 hours.

Acute toxicity

Water

In the control group (synthetic water) the aquatic flea displayed typical behavior. At different nickel concentrations after 48 h of exposure, the fleas were presenting a white coloration. The 48-h LC_{50} value for nickel in a water test against *Moina macrocopa* was 6.84 mg/l (Figure 1A). The 48-h LC_{50} value for nickel in sediment was 12.09 mg/kg (Figure 1B), this value is 76.75 percent larger than the value of LC_{50} of water.

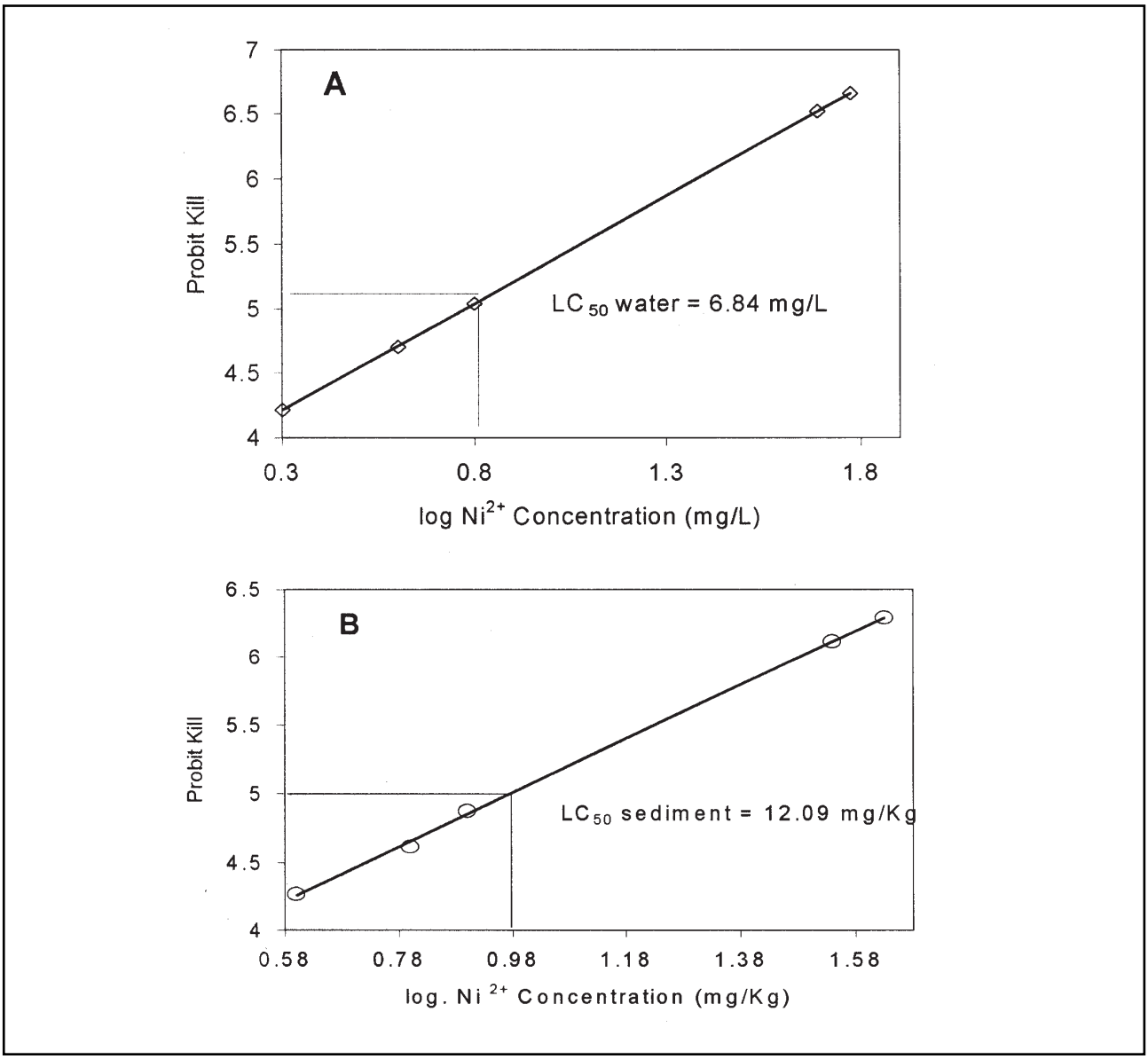

Figure 1. **A**.- Mortality of *Moina macrocopa* exposed to 1.65, 2.63, 4.16, 6.60 and 10.47 mg Ni^{+2}/l for 48 hours. **B.**- Mortality of *Moina macrocopa* exposed to 3.16, 4.89, 7.76, 12.30 and 19.49 mg Ni^{+2}/kg for 48 hours. Mortality expressed as probit kill.

Acetylcholinesterase activity

Moina macrocopa controls (0 mg/kg Ni^{+2}) produced an average of 0.042± 0.001 µmol/mg protein per hour of AchE hydrolyzed. When aquatic fleas were intoxicated with 0.01 and 0.1 mg/kg nickel, acetylcholinesterase activity was increased 11.90 and 26.19 percent with respect to the control group (Figure 2). On the other hand, when nickel concentration was enhanced (1.2 mg/kg), the acetylcholinesterase activity was inhibited 11 percent with respect to the control group. Both inhibiting and stimulating effects are significantly different with the control group ($P < 0.05$)

Hemoglobin concentration

Similarly to the acetylcholinesterase activity, hemoglobin concentration increased with increasing nickel concentration. Maximum hemoglobin concentration was found in the 0.1 mg/kg Ni^{+2} group, where this biomolecule was 40.34 percent higher compared to the control group (significantly

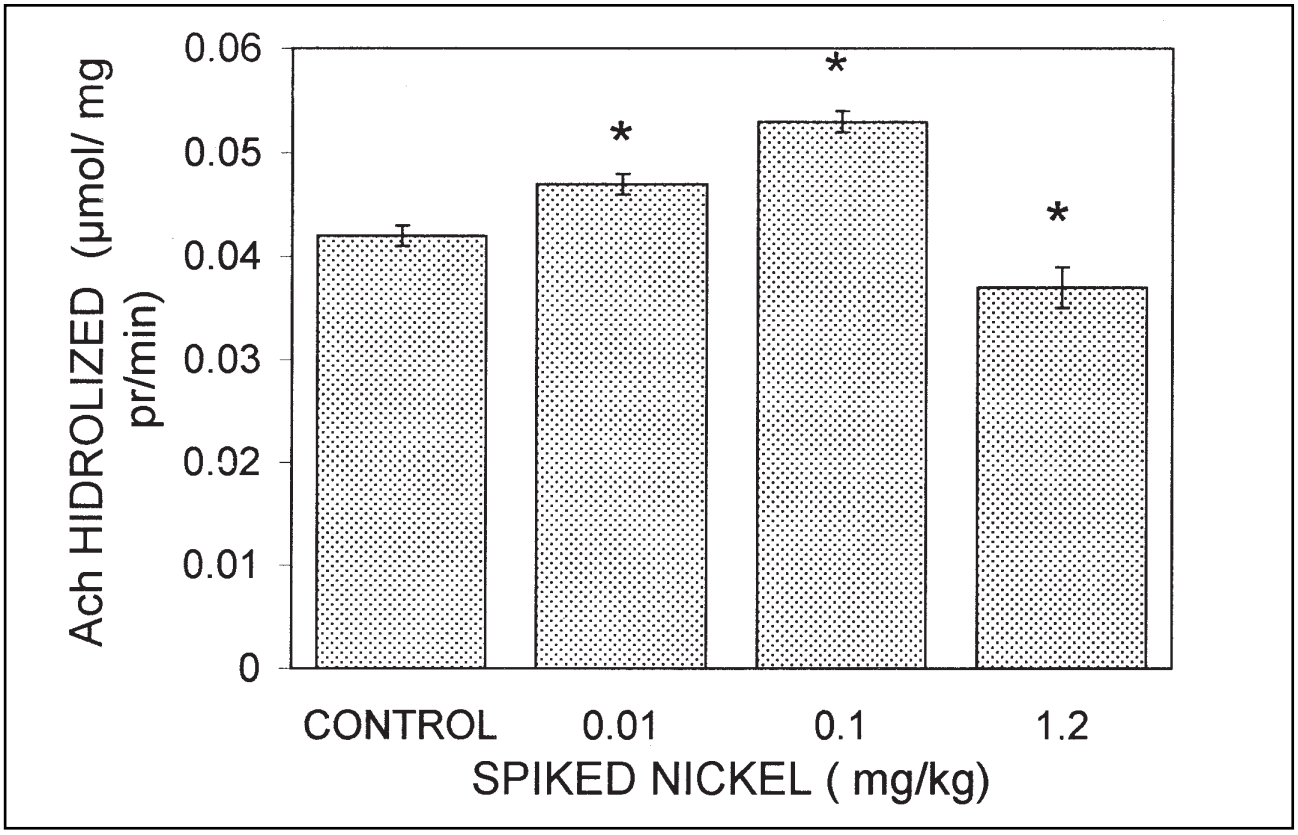

Figure 2. Toxic effect of nickel spiked in artificial sediment on acetylcholinesterase activity in *Moina macrocopa*. Each value represents an average of nine replicas ± standard deviation; *$P < 0.05$.

different). However, when the aquatic flea was intoxicated with 1.2 mg/kg $Ni^{+2,}$ an inhibiting effect was found on the hemoglobin concentration (a reduction of 4.19percent with respect to thecontrol group, $P < 0.05$) (Figure 3).

DISCUSSION

The accumulation of heavy metals depends on the physicochemical properties of the sediment, including clay minerals, organic matter and particle size, and pH. The composition of artificial sediment in this study was based on the guides OECD (1984) and SETAC (1993). This sediment contains a high percent of sand that allows the displacement of the nickel vertically and horizontally in the water column. The elevated percent of organic matter favors joining of nickel with both humic and fulvic acids of organic matter (Bonh et al., 1993).

The cation exchange capacity of artificial sediments in this study was 66.49 meq/100 g sediment. This is in large part due to the quantity of cations originating from organic and inorganic matter (kaolin). In general, nickel compounds are relatively soluble at pH below 6.5. At pH above 6.7 the pH of studied sediment (7.9) determines the nickel insolubility in the water column and favors trapping by the sediment. The physicochemical properties in this study determine the low availability of nickel in the water column.

The 48-h LC_{50} value for nickel in the water test against *Moina macrocopa* was 6.84 mg/l (Figure 1). This value is similar to that reported by Wong (1992): at 48-h LC_{50} Ni^{+2} was 6.48 mg/l. As can be observed the necessary minimal concentration to produce 50 percent mortality of the population of cladocera is lower and can be found in many water bodies in Mexico (Martínez-Tabche, 1999).

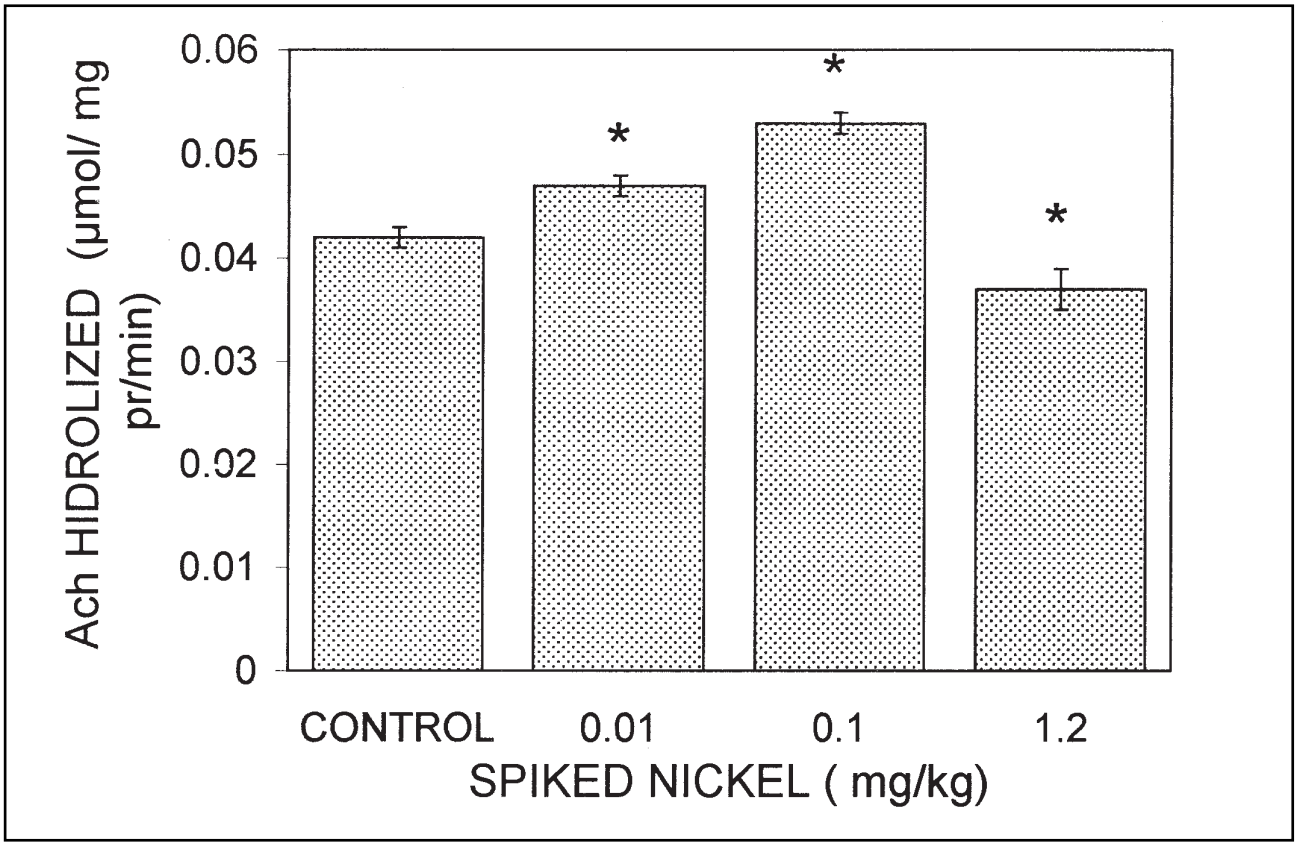

Figure 3. Toxic effect of nickel spiked in artificial sediment on hemoglobin concentration in *Moina macrocopa*. Each value represents an average of nine replicas ± standard deviation; *$P < 0.05$.

other species to delimit a new threshold limit effect in freshwater ecosystems in Mexico.

The 48-h LC_{50} value of nickel in sediment was 12.09 mg/kg[1]. As can be observed the value of LC_{50} obtained for water is less than the LC_{50} obtained for sediment. This fact can be due to sediment physicochemical characteristics: the higher organic matter and kaolin content favor interactions between the nickel and organic and inorganic compounds present in these substrates.

The usefulness of biochemical parameters, which are frequently classified as early warning, is to determine the action mechanism of chemical substances, under laboratory conditions and in this way know the probable causes of sublethal or lethal effects under field conditions (Martínez-Tabche, 1998; Giesey and Graney, 1989).

The present study evaluated acetylcholinesterase activity. The choice of this parameter is due to the fact that it is an biomarker for pollutants and the parameter has gained wide acceptance in environmental studies of both fresh and marine waters over the past few years (Martínez-Tabche, 1998). The results of acetylcholinesterase activity (Figure 2) showed that in the first two nickel concentrations spiked in artificial sediment (0.01 and 0.1 mg/kg) a stimulation was observed. Similar results were obtained by Kufcsak et al. (1994). They found that after exposing carp intestine to 5 mg/l of $CuSO_4$, the acetylcholinesterase activity increased. It is probable that the effect of nickel is bound in the alosteric site of the enzyme, increasing its activity by the subtract (AchE). These results are supported by Repetto (1995) and Sanz et al. (1991), which refer to activation of the alosteric type in the case of aluminum and other divalent metals. The alosteric regulation model of AchE activity reported by the same authors postulates the existence of three anionic places: (a) catalytic anionic places and (b) and (c) peripheral places, (b) being specifically cationic. Therefore Ni^{+2} would be

joined to the catalytic site (b) to stimulate enzyme activity.

On the other hand when aquatic flea was exposed to1.2 mg/kg, a decrease of 11 percent of acetylcholinesterase activity was observed. Similar effects were observed by Aziz et al. (1993). They demonstrated that *Tilapia massambica* exposed to $CdCl_2$ (10µg/15l) during two days showed a decrease in acetylcholinesterase activity. The decrease in acetylcholinesterase activity agrees with that reported by Carson et al. (1991), who state that nickel exerts a variety of effects on the enzymes due to the fact that it can substitute for other divalent metallic ions. Therefore the metal is capable of inhibiting and activating the same enzyme depending on the employed concentration. The explanation of this phenomenon would be that at highest concentrations the metal can be joined to the enzyme and therefore reduce its affinity to the sustrate. In addition some divalent metals such as the zinc, copper, lead and mercury are inhibitors of the cholinesterases (Repetto, 1995).

Several studies have used hemoglobin concentration to evaluate the effects of environmental stresses on aquatic organisms. Nickel toxicity on the hemoglobin concentration of fish has been reported by Agrawal (1979). Also Tabche et al. (1990) showed a similar effect on hemoglobin concentration of tilapia fish *Oreochromis hornorum.* Tilapia exposed to 31 and 47 mg/l Pb^{+2} had a significant increase in hemoglobin levels. However, the fish exposed at greater lead concentration had a decrease in hemoglobin concentration.

Similarly for acetylcholinesterase activity, the hemoglobin concentration was increased when the flea was exposed to low Ni^{+2} concentration. The increase in hemoglobin concentration agrees with Shanker et al. (1979). They demonstrated that after exposing *Colisa fasciatus* to 45 mg/l of $NiSO_4$ they presented leukopenia due to a reduction in the number of small lymphocytes and polycithemia with concomitant increases in the hematocrit and hemoglobin concentration. Increases in the hemoglobin concentrations are most probably due to nickel capacity to replace other divalent metallic ions in enzymes and proteins. It is possible that in anoxic environments, nickel is binding to acid d-aminolevulinic synthetase stimulating heme synthesis, and therefore increasing hemoglobin levels.

When artificial sediments were spiked with 1.2 mg/l of nickel, a decrease in hemoglobin concentration with respect to the control group was observed. Data from this study are supported by Martínez-Tabche et al. (1999), who demonstrated an inhibitor effect on hemoglobin concentration of tubificid *Limnodrilus hoffmeisteri* exposed to different nickel concentrations (153.4, 179.9 and 183 µg/kg Ni^{+2}) contained in rustic ponds of three trout farms. The explanation for this fact is supported by Tkeshelashvili et al. (1989). Their study found that Ni compounds affect the erythrocyte membrane lipid bilayer, as well as membrane proteins, depending on the type of compounds used and the exposure time.

CONCLUSIONS

The results obtained in the present study of Ni^{+2} allow us to conclude that hemoglobin concentration and acetylcholinesterase activity in the*Moina macrocopa*test could become useful in routine monitoring to detect the presence of nickel contaminant in aquatic environments.

The use of artificial sediments provides a relevant control on environmental variables as well as replication of treatments with various pollutants. This facilitates the establishment of cause and effect relationships.

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