Revista Digital del Departamento
El Hombre y su Ambiente
ISSN: 2007-5782

2013. Vol.1 (3): 6-11

Effect of inorganic mercury concentration on biometric, survival and LC50 of *Artemia franciscana* Kellogg, 1906 (Crustacea:Anostraca).

Bhattacharjee D,[1] Asem A,[2] Eimanifar A,[3] Castro MJ,[4*] Castro MG,[4] Orozco DI[4]

[1]CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. 608502.
[2]Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China
[3]Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany
[4]Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso No.1100. Col. Villa Quietud. México, 04960, D.F. Tel (Office): 5255 54837151.

*Corresponding author: camj7509@correo.xoc.uam.mx

ABSTRACT

This research presents the effect of different concentrations of inorganic mercury (0, 4, 8, 12, 16, 20 and 24 ng L^{-1}) on the biometry, survival and LC50 of nauplius stages I and II of *Artemia franciscana*. The biometry of N-I and N-II shows significant differences by different concentration of inorganic mercury ($P<0.001$). Mortality rate reached to more than 70%, when inorganic mercury concentration increased to 24 ng L^{-1}. The biometric data of N-I and N-II stages were disturbed, even at the lower concentrations of inorganic mercury. With respect "control" organisms, total body length showed a reduction of 4.16% in N-I and 7.81% in N-II; whereas, for abdomen width, a reduction of 14.48% and 15.38% were observed, respectively. The LC50 for N-I and N-II nauplius stage were estimated to be 16.13, 12.46 ng L^{-1}. The real problem is not only the biometric reduction and quickly LC50 values, but it is the incorporation of inorganic mercury in the tissues of nauplii. That's why is necessary to make these chemical determinations to complete the information.

Key words: *Artemia franciscana*, growth, inorganic mercury, LC50, nauplius stages, survival.

INTRODUCTION

Mercury in ground and surface waters has been indicated as a health problem in several regions of India which caused by industrial activities across the country. Such levels of contamination have been reported to 600 to 700 times higher than the limits permitted by Indian standards (Bureau of Indian Standards (BIS). This problem represents a danger, not only to human health, but also to aquatic organism. The effect of toxicants on animal development and growth have an enormous inherent value, since there is an increasing interest for the ecological aspects of the physiological responses to toxic stress (Sarabia et al. 1998).

Brine shrimp *Artemia* exhibits several biological characteristics as a model organism in toxicological studies and has been used to evaluate the disruption of heavy metals in the biological function of different life stages (Gebhardt 1976; Browne 1980; Jayasekara et al. 1986; Bragshaw et al. 1986; Macrae & Pandey, 1991; Brix et al. 2003; Brix et al. 2006). However, little information is available regarding the effects of mercury on the development, survival and growth of *Artemia*. Sarabia et al. (1998) reported the effects of low concentrations of mercury affected the adult survival rate and increased the reproductive performance. Previous experiments in our laboratory showed that the nauplii and metanauplii stages are differentially sensitive to inorganic mercury. The objective of the present research is to assess the effect of mercury on the biometry and the LC_{50} of *Artemia* larval stages (N-I & N-II).

MATERIAL AND METHODS

Cysts, nauplii and mercury solutions preparation

The *Artemia* cysts (Red Jungle Brand, O. S. I. Inc., Snowville, UT, USA) were separated from debris or cysts shells by density in tap water for three hrs. Then, the cysts were hatched in filtered sea water (40 g L^{-1} salinity) at 25 $\pm$ 2°C, continuous

Receive: February 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

aeration and illumination provided by fluorescent lamps.

After hatching, nauplii were collected and transferred to fresh sea water and cultured until nauplius stage I (N-I) conditions over. One half of the nauplii were used immediately for the experiments, while the rest was further incubated in separate beakers for another 18 hours under similar conditions. At the end of incubation period, most nauplii attained metanaupliar (N-II) stage, and were used for the experiments.

Mercury (II) chloride (Product Number 203777, Sigma-Aldrich, and St. Louis, MO, USA) was used to prepare a $1gL^{-1}$ stock solution of mercury, by dissolving 1.3535 g in 1 L of Milli Q water. This standard stock solution was diluted with filtered-sterilized seawater in order to obtain the final concentrations of 4, 8, 12, 16, 20 and 24ng L^{-1}. The concentration ranges were determined according to Sarabia et al. (1998). A control group was also set up with any concentration of mercury

Bioassay to determine the biometry

The bioassays for each naupliar stage were carried out with five organisms stocked in 200 mL glass beakers. The larvae were acclimated in the test beakers for 5 hours in filtered sterilized seawater, and then the contaminant was introduced at the various concentrations. Each concentration was run in triplicates. The nauplii were incubated for 24 hours after the introduction of the contaminant under the mentioned conditions above. The biometry values (total length and abdomen wide), were taken with and Ocular Micrometer (Bio Slides Laboratory, Chandigarh, India) in the two stages for the control group and the different inorganic mercury concentrations.

LC_{50} bioassays

The LC_{50} values were determined according to the method described by Meyer et al. (1982). Briefly, 5 organisms at N-I and N-II were transferred to 200 ml beaker containing fresh seawater. Final concentrations of 0, 4, 8, 12, 16, 20 and 24 ng/L were used to determine LC_{50}. The lethality experiments were carried out with 3 replicates for each concentration, and their average percent mortalities were recorded after 24 hours of exposure. During the exposure, the nauplii were not fed.

Statistical analysis

A descriptive statistical analysis was carried out for total body length and abdomen width for each stage (N-I and N-II). The mean values of total body length and abdomen width were analyzed using one-way ANOVA as implemented in SPSS package. Significant differences among treatments were evaluated with Tukey multiple comparisons test. Statistical significance of differences was determined by setting the probability at 5% ($P<0.05$) for each set of comparisons.

The percentage rate of mortality was calculated from the total number of dead animals (N-I and N-II) for each concentration. LC_{50} were calculated using the probit analysis (Finney 1953), and higher R^2 regression curve value.

RESULTS

Table 1 shows the mean values of total body length and abdomen width with standard deviation of *A. franciscana* nauplius (N-I & N-II) in different concentrations of inorganic mercury culture medium. The highest values are in "control" test organisms with total length values of 960.80 µm to N-I and 998.70 µm to N-II. The smallest values were found in the 24 ng L^{-1} inorganic mercury; with 920.60 µm and 920.80 µm respectively.

Regarding to abdomen width, the "control" organisms were the highest values with 283.60 µm in N-I and 286.30 µm in N-II. The smallest values were also found in 24 ngL^{-1} inorganic mercury concentration estimated to be 242.40 and 242.80 µm, respectively.

The ANOVA and Tukey test (multiple mean values comparison), shows significant differences in the biometry parameters between the inorganic mercury concentrations ($P < 0.001$).

The comparison of biometrical values between "control" and different mercury concentrations showed, a low growth development in total body length and abdomen width in two *Artemia franciscana* stages.

Receive: February 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

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Table 1. Mean values and standard deviation of total body length and abdomen width of N-I and N-II *Artemia franciscana* nauplius stage in different inorganic mercury concentrations

Treatments (ng L^{-1})	Total body length (µm)		Abdomen width (µm)	
	N-I	N-II	N-I	N-II
Control	960.80 ±0.42	998.70 ±1.70	283.60 ±0.52	286.30[a] ±0.67
4	951.80 ±1.03	966.20[a] ±2.10	280.80 ±0.42	286.90 ±1.45
8	942.00 ±2.31	954.90 ±1.10	277.60 ±0.52	280.70[a] ±0.48
12	954.70 ±1.25	952.20 ±0.63	278.70 ±1.16	279.00 ±0.94
16	938.20 ±2.15	951.60[a] ±1.26	274.90 ±0.74	278.90 ±1.45
20	922.80 ±1.03	923.30 ±0.67	256.60 ±0.84	257.20 ±1.14
24	920.60 ±0.97	920.80 ±1.23	242.50 ±0.53	242.80 ±0.92

Note: Similar letters (in column) shows no significant differences (P>0.05).

Table 2. Mortality values of N-I of *A. franciscana* with different concentrations of inorganic mercury.

Inorganic mercury concentrations (ngL^{-1})	Inorganic mercury concentration (ln)	Mortality (%)
4	1.39	6.66
8	2.08	13.33
12	2.48	26.66
16	2.77	46.66
20	3.00	66.66
24	3.18	73.33

The respective 24-hour mortality values of N-I and N-II nauplii stages are also showed in Table 2 and 3. Figure 1 and 2 presents the logarithmic relationship between mortality rates and increasing concentration of inorganic mercury in N-I and N-II nauplii stages, respectively.

The LC_{50} values (x) of N-I *Artemia* was determined by the equation of $y=13.272 \cdot \ln(x)^2 - 19.365$ and for N-II *Artemia* by $y=41.527\ \ln(x)^2 - 53.109$. For N-I, the LC_{50} was 16.14 ng L^{-1} and 12.46 ng L^{-1} for N-II (Figure 1 and 2).

Table 3. Mortality values of N-II of *A. franciscana* with different concentrations of inorganic mercury.

Inorganic mercury concentrations ($ngmL^{-1}$)	Inorganic mercury concentration (ln)	Mortality (%)
4	1.3863	6.66
8	2.0794	33.33
12	2.4849	46.66
16	2.7726	60.00
20	2.9957	66.66
24	3.1781	86.66

DISCUSSION

The industrial city of Bhopal, India, has been reported to have as increased level of mercury concentration in the ground water

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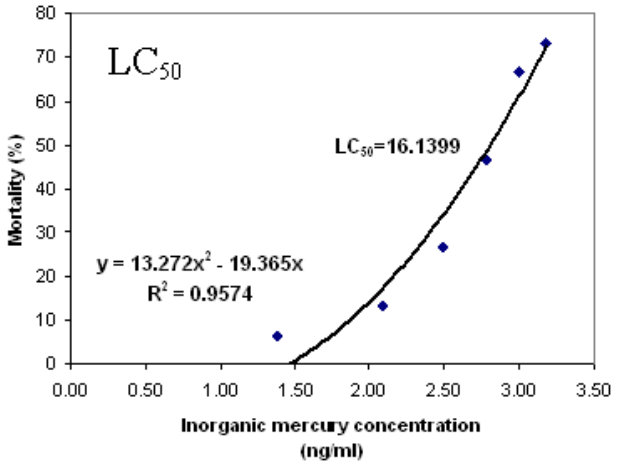

Fig. 1. Logarithmic relationship between mortality rates of N-I *Artemia* stage with increasing concentrations of Hg (inorganic mercury).

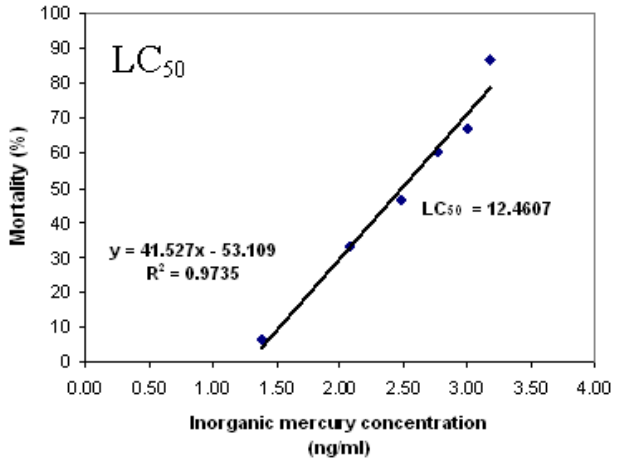

Fig. 2. Logarithmic relationship between mortality rates of N-II *Artemia* stage with increasing concentrations of Hg (inorganic mercury).

Dehra Doon based research organization, India on the "Groundwater contamination near the Union Carbide plant at Bhopal, 2001–2002").

In several coastal regions, the ground water has been used for salt extraction and therefore, brine shrimp *Artemia* could grows naturally there. Although in some cases, the mercury can concentrate in *Artemia* biomass and then biomagnified the problem in food web system. Moreover, the coastal waters used in some other salt works are contaminated with mercury which ultimately increases the chances of mercury accumulation in food web too.

Other studies were carried out with nickel and zinc ion metals with the possibility of bioaccumulation of ions in organisms' tissues and eventually transport into food web. Chen and Liu (1987) found that *Artemia* nauplii can accumulate nickel and zinc 10 to 100 times higher in their bodies than aquatic medium which has effect to other organisms feeding *Artemia*. This phenomenon demonstrated with organ chlorine pesticides in trout larvae (*Salvelinus fontalis*). Wang and Simpson (1996) found an accumulation of dichlorodiphenyltrichloroethane (DDT) through food chain organisms, by feeding *Artemia* nauplii exposed to DDT for 24 hours

Many environmental pollutants have toxic effects which could alter normal molting process in crustaceans. The most common effect of heavy metals is the delay of ecdysis (Weis et al. 1992). In the present study, however, molting cycle was not changed at the examined concentration. On the other hand, growth inhibitory effect of mercury is very obvious, since the data reveals the reduction of total body length (4.16%) and abdominal width (14.48%) in N-I and accordingly 7.81% and 15.38% in N-II stages.

The biometric variation in N-I to N-II in total length was 3.65% and in abdomen width was only 0.9%. It has been well documented that total body length and abdomen width are regulated by environmental and genetically conditions (Criel and Macrae 2002;

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Triantaphyllidis et al. 1998; Gajardo et al. 1998).

Ochi et al. (1985) has described the growth suppression could be due to the blockage of SH-groups in proteins. Benova et al. (2007) described for cadmium that lower level of metallothionein production might have led to an increase in cells cadmium pool and has given rise to a much dangerous situation, resulting in toxicity at cellular level. This can be possible occurred with mercury too.

High concentrations of essential and non-essential metals dissolved in aquatic medium, are considered to be toxic for organisms tissues, because they cause interferences in intracellular enzymatic systems (Páez-Osuna, 1996). The problem is not only restricted to their concentration in water, but their combination with organic compounds, in coastal silts and their deposition in food webs can cause bioaccumulation in organism's tissues (Ponce-Vélez and Vázquez-Botello 1991). Another factors like pH, microorganisms concentration and type of waste waters, can also affect the toxicity of ion metals (Albek et al. 1997).

Report as early as 2003 (Mercury menace, mercury pollution in India, Nov. 7, 2003) indicated mercury levels in fish were calculated to be 0.03–0.82 mg total Hg kg^{-1} dry weight (dw) and crabs 1.42–4.94 µg total Hg kg^{-1} (dw) mercury compared to the permissible limit of 0.5 µg kg^{-1}. Mercury levels in oysters in Karwar were ranged between 0.18–0.54 µg kg^{-1} (dw). The North Koel River showed mercury concentrations almost 600–700 times above the limits. Mercury in ground water and surface water was detected across the country including Delhi, Mumbai, Vadodara, Vapi, Ankleshwar, Bhopal, Panipat, Singhrauli, Ganjam, Dhanbad, Durgapur, Howrah and Medak. Higher levels (permissible limits) of mercury were found near chlor-alkali, cement and chemical units and thermal power plants.

The present study is unique and it solely considered the initial larval biometry as a parameter to check the secondary effect of inorganic mercury on *Artemia*. Our observations partially support earlier findings by others, being insufficient in details due to lack of comparison in different developmental stages of *Artemia*.

Therefore, it's recommended to assess the secondary toxicity of mercury on the *Artemia* population. As in this case we do not consider their mortality as the ultimate end point but their impairments or even their biometry as a correlation to aquaculture application. Application of such studies will also enable us to use initial larval stages as model organism to find out safety in surrounded environment.

ACKNOWLEDGEMENT

The authors are appreciated to Dr. T. Balasubramanian, Director of Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, India 608502, for providing working facilities.

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Receive: February 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.

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Receive: February 1st 2013. Accepted: May 1st 2013. Published: July 1st 2013.