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Study on the toxicokinetics of Ni(II) on *Artemia franciscana*

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ABSTRACT

The toxicokinetic study was employed to evaluate the potential of an organism for Ni(II) bioaccumulation, and it was ensured using a bicompartmental model. The effect of Ni(II) concentration on kinetics parameter in *Artemia franciscana* were determined. In present investigation, the optimized population was observed to be 500, in which the uptake and elimination rate seems to be high with the time period of 120 h by the process of bioaccumulation. The bioconcentration factor (BCF) and metal influx rate (I) were also found to be higher in optimized population when compared to population range of other organism. Based on observations, BCF was inversely related to the concentration. These parameters help in easy prediction of effective bioaccumulation of Ni(II) by *Artemia franciscana*. The related kinetic parameters were also found to pertinent with bicompartmental model.

Keywords: *Artemia franciscana*; Bioaccumulation; Bicompartmental model; Metallothionein; Uptake and elimination rate

1. Introduction

Artemia is a lower crustacean form and has been widely used as a test organism in short-term toxicity testing for environmental and pharmacological purposes since 1970s. It is widely used in laboratory toxicity studies due to its body size and short lifespan together with its availability from dry cysts. This organism has an uncommon adaptability to extreme condition, thus being found in environments where other life forms are not sustainable. *Artemia*, like many other similar organisms, is able to bioaccumulate quite large amounts of elements from the aquatic environment, even when their concentrations are extremely low. The habitat of this organism was characterized by less predatory species, and abundance of bacteria, protozoa, and algae in such environment is the foundation of *Artemia* diet [1]. It has a broad tolerance to environmental factors such as salinity, temperature, and dissolved oxygen, allowing it to live in hypersaline waters [2].

Bioaccumulation is a fundamental cognitive process in environmental toxicology and risk assessment, as it determines the internal dose of potential toxicants [3]. Several studies reported *Artemia franciscana* as very tolerant organism based on accumulation and toxic effects. This investigation was based on bioaccumulation of Ni(II) with respect to the metallothionein (MT) protein which is synthesized in the organism.

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Toxicokinetic studies are often applied to evaluate the potential of an organism during bioaccumulation. The single-compartment model predicts about the partitioning between exposure medium and the organism. It is considered as one of the most simplified models for bioaccumulation and has proved to be very useful in predicting toxic accumulation due to small animals. Besides the different kinetic parameters that describe the accumulation model, bioaccumulation factor (BAF) and bioconcentration factors (BCF) have been widely used in ecotoxicology. These two factors mainly depend on metal exposure concentration [4,5]. MT protein is superfamily of lower molecular mass metal thiolate cluster proteins, with an ability to bind metallic ions [6,7]. MT content increases in a time-dependent fashion, and in this investigation, it played a central part in uptake and elimination process.

The main aim of this work was to study the accumulation and elimination patterns of Ni(II) in *A. franciscana* with respect to the MT protein synthesis by first-order toxicokinetics model for better understanding of strategies used by *A. franciscana* to elude Ni(II) toxicity.

2. Materials and methods

2.1. Collection, processing, and hatching of A. franciscana cyst

A. franciscana cysts were collected from the salt pans located at Kelambakkam, 12˚47′N 80˚ South India. The collected cysts were brought to the laboratory and subjected to washing with tap water to separate viable cysts. The viable cysts that settled at the bottom were flushed out through a fine sieve of 200-μm mesh. The cysts were then dried at 28 ± 12˚C and stored in airtight vials for further studies. In this study, exactly one gram of the cysts were incubated in 1,000 mL of seawater at 27 ± 1˚C in a beaker with lateral illumination for 500–1,000 Lux, salinity 35‰, pH of 8.5, and keeping them in suspension by gentle aeration.

The viable cysts were hatched out within 24-h time period. The hatching percentage was calculated by the following procedure: After 24 h with aeration and illumination still on, 1 ml of seawater was pipetted out and evenly spread across the filter paper. After spreading, only the fully hatched nauplii was counted and multiplied by 1,000 to arrive nauplii per gram (NPG). Hatching percentage was found by dividing NPG using cysts per gram (CPG) [8]. After hatching, acclimatization was carried out for about 10 d and it was used for the further experimental study.

2.2. Preparation of spiked salt water

The spiked salt water was prepared synthetically by dissolving Ni Cl_2 (CAS 7718-54-9) in one liter of seawater. The chemicals used in this work were obtained from Merck, India.

2.3. Estimation of MT protein in A. franciscana

For MT estimation, samples were weighed and placed in a homogenizing tube with a solution of 0.25 M sucrose, and the mixture was homogenized with a motor-driven Teflon pestle at 4˚C. The homogenate was centrifuged (Remi centrifuge, R244A, Chennai, Tamil Nadu, India) at 20,000 *g* for 20 min at 4˚C. Aliquots of 750 μL supernatant were analyzed for MT content by the modified silver-saturation method [9]. Samples were incubated with 1 ml of 20 mg/L silver (CAS 7440-22-4) solution for 15 min at 20˚C to saturate the metal binding sites of MT. Excess metal was removed by the addition of 200 μL human red blood cell hemolysate to the assay tubes followed by heat treatment in a water bath (100˚C for 2 min.). Since MT was a heat-stable protein, Ag^+ bound hemoglobin and other proteins were precipitated. The denatured proteins were removed by centrifugation at 1,000 RPM for 5 min. The hemolysate addition, heat treatment, and centrifugation were repeated three times in each sample. The amount of Ag^+ in the final supernatant fraction is proportional to the amount of MT present. Silver concentrations were estimated in an Atomic Absorption Spectrophotometer (Shimadzu Corporation, AA6300, Kyoto, Japan). In a similar way, Ni(II) from supernatant fraction obtained from homogenate was estimated by Atomic Absorption Spectrophotometer (Shimadzu Corporation, AA6300, Kyoto, Japan).

2.4. Bicompartmental model

It is the model used to evaluate the process of accumulation/elimination of Ni(II) in *A. franciscana* with respective to the time period at steady state, in which the spiked salt water as a source and the organism as recipient. This model depicts the changes in Ni(II) concentration in organism over time and with respect to MT synthesis, assuming that the flux of Ni (II) across boundaries depends on Ni(II) concentration in the spiked salt water [10]. The equation is defined as

$$dC_{a/dt} = (K_u.C_w) - (K_e.C_a) \quad (1)$$

where C_a is the concentration of MT protein synthesized in the organism (μg MT/g wet weight), t is the time of exposure (day), and C_w is the concentration of Ni(II) in solution (mg/L). K_u (mL/g h) is the uptake rate constant, and K_e (h^{-1}) is the elimination rate constant and describes the fractional decrease of toxicant concentration in the organism per day.

For time = 0, $C_a = 0$, and C_w = constant, the uptake period may be calculated using Eq. (2)

$$C_a = \left(K_u . \frac{C_w}{K_e}\right).(1 - e^{-K_{et}}) \quad (2)$$

and for the elimination period:

$$C_a = C_{as}.e^{-K_{et}} \quad (3)$$

where in Eq. (4), C_{as} is the concentration of MT protein synthesized in the organisms at steady state (the end of the uptake period)

$$C_{as} = \left(K_u/K_e\right).C_w.(1 - e^{-K_{et}}) \quad (4)$$

When time (t) approaches infinity ($t \rightarrow \infty$), the BCF can be obtained from the uptake and elimination capacity constants:

$$\text{BCF} = K_u/K_e = C_{as}/C_w \quad (5)$$

K_u and K_e were calculated from mean values of MT concentration in *A. franciscana* samples for each exposure time by means of the parametric data analysis [11]. Once values for K_u and K_e and their confidence interval (88%) had been obtained, mean values in μg MT/g wet weight for each exposure could be calculated according to the model. The goodness of fit was assessed through the coefficient of determination (R^2).

Half-lives of Ni(II) in *A. franciscana* tissues ($t_{1/2}$) were determined in terms of K_e by the following formula:

$$t_{1/2} = 0.693/K_e \quad (6)$$

We have also calculated metal influx rate (μg Ni(II) /g per day) according to [12] as:

$$I = K_u \times C_w \quad (7)$$

Influx rate is the amount of Ni(II) entering into the organism per gram weight of organism per day.

3. Results and discussion

Bicompartmental model was chosen in the present investigation based on the bioaccumulation of Ni(II) in *A. franciscana*. This established model provided flexibility of changing parameter values based on the physiological differences in the process.

3.1. Hatching percentage of the A. franciscana cyst

Only the fully hatched nauplii was counted and found to be 268. Considering dilution factor the counted nauplii was multiplied with to obtain NPG (i.e. 268 nauplii × 1,000 = 268,000 NPG.). About 97.45% ((268,000/275,000) × 100) of hatching was observed.

3.2. Optimization of organism population

The accumulation of Ni(II) in the organism was determined by the MT protein synthesis. MT protein synthesis was directly proportional to the number of organisms present in seawater. High-MT protein was synthesized in the population range of 500 and least can be observed at 300 and 700, as shown in Fig. 1. This can be explained by the less availability of organisms to take up metal at the population range of 300. Lack of organisms to synthesis protein at population

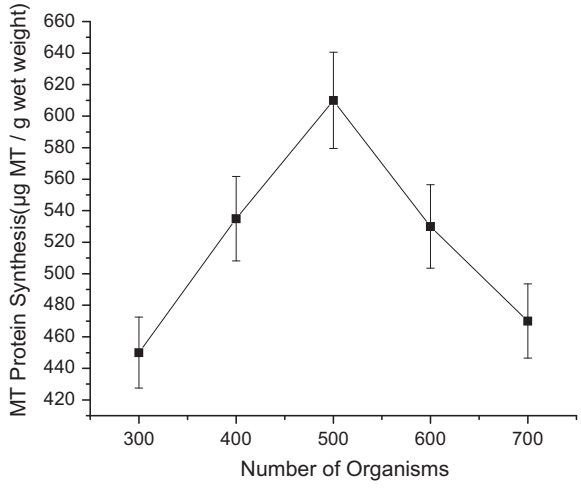

Fig. 1. Optimization of population of the *A. franciscana* with respect to the MT protein synthesis (μg MT/g wet weight).

range of 700 may be explained as due to the effect of suffocation on organisms. The 500 numbers of organisms showed high-MT protein synthesis and were found to be very effective in the accumulation of Ni (II). Hence, in this study, the 500 numbers of organisms were found to be optimized condition for accumulation and elimination process. Accumulation of metal and synthesis of MT protein were observed till 120 h, and beyond that time period the population of organisms started to decline.

3.3. Bicompartmental model parameters

The bicompartmental kinetic model to estimate the uptake and elimination rate of the Ni(II) using *A. franciscana* is as follows:

3.3.1. Determination of coefficient for the concentration of MT protein in the organism

A graph is drawn between the concentrations of MT protein in the organism with respect to time (Fig. 2). The fitness of experimental values to those estimated by the model gave coefficients of determination which range from 0.96 to 0.99 as indicated in Table 1. Thus, it confirms the goodness of fit and allowed to accept that a high proportion of the variables can be explained by the bicompartmental model.

3.3.2. Uptake (K_u) and elimination (K_e) rate of the organism with respect to population

The different model variables obtained from bicompartmental model (Table 1), in which the uptake (K_u) and elimination (K_e) rates were obtained for all the population ranges starting from 300 to 700. In the case of least population of organisms, the uptake and elimination rate ranged as 0.010–0.065 mL/g h and 0.0033–0.0041 h^{-1}, respectively. As per Figs. 3 and 4, the rates were found to be low when compared to optimized population, whereas the range was 0.024–0.433 mL/g h and 0.0049–0.012 h^{-1}, respectively. The optimized population was found to be efficacious in the uptake and elimination rate which depend on the MT protein synthesis in the organism. MT protein synthesis was found to be higher in the optimized population when compared to other populations which in turn brings out the maximum reduction in the Ni(II) concentration [13]. MT content in *A. franciscana* increased with respect to time, which clearly indicated the high resistance of this creature against pollutants [14]. In case of high range of population, the range falls into negative value due to maximum accumulation. Beyond the limit of accumulation, the uptake rate becomes slow in comparison with other population.

3.3.3. Uptake and elimination rate of the organism with respect to time

There was a gradual increase in the uptake of Ni (II) up to 120 h (Fig. 5), while the overall experimental time was calculated as 120 h based on the saturation point. The elimination rate was found to increase gradually after 48 h. Thereafter, the increasing trend was found to be decreasing. Maximum elimination was observed at the end of experimental period. MT protein synthesized from the organisms plays a major role in uptake and elimination of heavy metal. MT protein synthesized directly relates to the uptake and

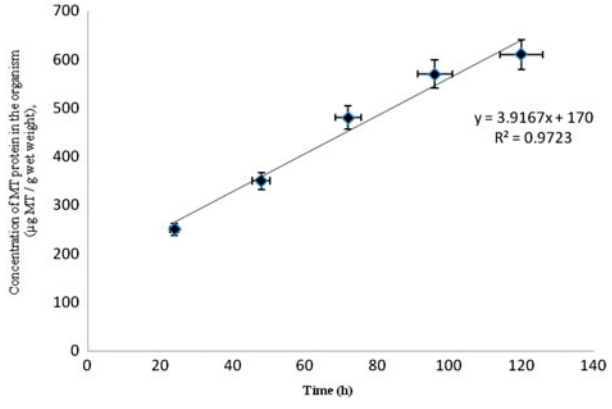

Fig. 2. Concentration of MT protein synthesized in *A. franciscana* with respect to time (µg MT/g wet weight).

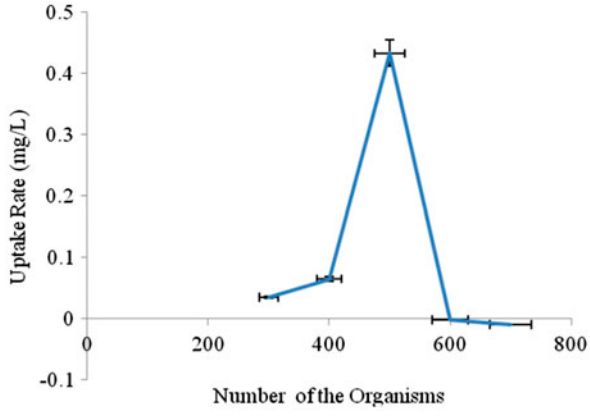

Fig. 3. Maximum uptake rate of the *A. franciscana* with respect to population in mL/g h.

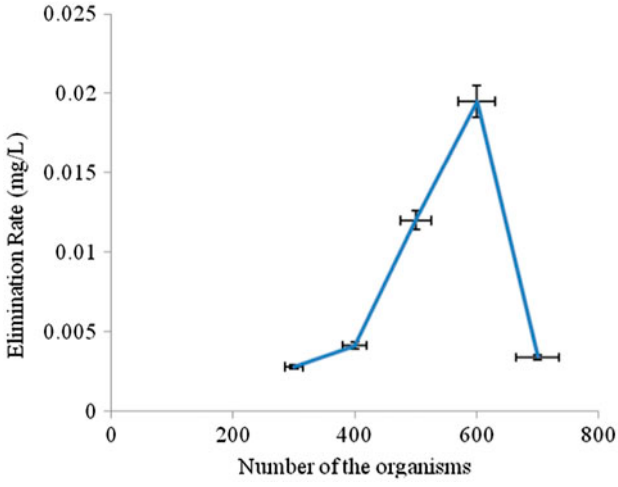

Fig. 4. Maximum elimination rate of the *A. franciscana* with respect to population in h^{-1}.

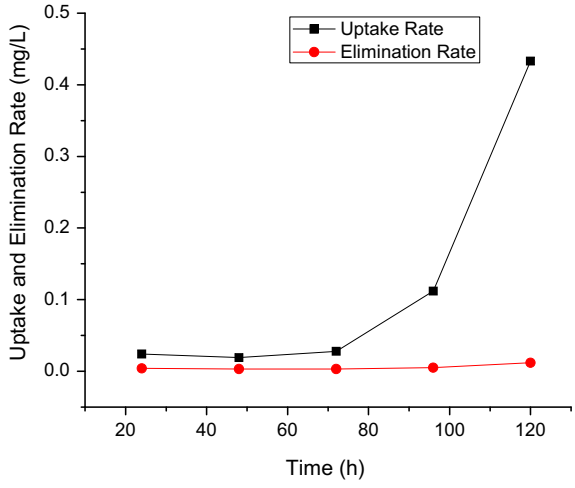

Fig. 5. Uptake and elimination rate of the optimized population of *A. franciscana* with respect to time.

elimination of nickel, and maximum removal was obtained at higher population range.

3.3.4. Effect of half-life period of Ni(II) accumulation in A. franciscana

In toxicology, the biological half-life has been used to compare the persistence of a substance in different organisms. In this study, the half-life of Ni(II) accumulation in the *A. franciscana* was determined by elimination capacity (Eq. (6)) and the values calculated from 35 to 241 h. The relationship between the elimination capacity and concentration of Ni(II) in the tissue was found to be positive and obeys the mechanism of first-order kinetics.

3.3.5. Effect of concentration of Ni(II) at steady state

The concentration of Ni(II) in the organism is considered at steady state (C_{as}), i.e. at the end of uptake rate. Steady state (C_{as}) for the least population was found to be 0.35–0.58 (μg/g). The above value was observed to be low compared to the value of an optimized population condition. It was concluded from the result that steady state (C_{as}) value of the optimized population was measured to be 2.16 (μg/g).

3.3.6. Effect of influx rate

Influx rate is the amount of Ni(II) entering into *A. franciscana* per weight of organism per day. In this case, the influx rate (I) was found to be 2.16 (μg Ni(II) /g per day) for optimized population. This influx rate of organism infers that optimized population was very effective in uptake and elimination of nickel.

3.3.7. Effect of bioconcentration on the uptake and elimination rate

BCF can be defined as the ratio of the concentration of a chemical in an organism to the concentration of that chemical in the surrounding environment. BCF is the measure of extent of chemical sharing between an organism and the surrounding environment [15].

The BCF_{kin} of optimized population was observed as 33.9 mL/g. This value range shows that optimized population was very good in the uptake and elimination rate, thereby supporting the previous finding of this study.

Similar type of toxicokinetic work was done with different types of *Artemia* populations, such as *A. franciscana* from USA and Brazil and *A.persimilis*, *A.Salina*, and *A. parthenogenetica* from two different regions of Spain. These different species were exposed to the different concentration of cadmium [2]. The uptake rate of the *A. franciscana* from both the USA and Brazil were observed to be as low as 0.14 and 0.2 mL/g h (Table 2) when compared to the uptake rate of the *A. franciscana* of optimized population from Kelambakkam (Table 1). The cadmium uptake rate of *A. persimilis* and *A. Salina* was found to be high when compared to *A. franciscana*. In the present investigation the concentration of nickel used was high about 40 mg/L compared to the concentration of cadmium (0.1 mg/L) used in that study. On comparison of *A. parthenogenetica* with *A. franciscana* from Kelambakkam, the uptake rate of latter organism was found to be effective. Though the BCF_{kin} of *A. franciscana* from Brazil and *A. persimilis*, *A. Salina*, and *A.parthenogenetica* from Spain is higher (Tables 1 and 2), the

Table 1
Bicompartmental kinetic parameters: K_u = rate constant of uptake; K_e = rate constant of elimination; $t_{1/2}$ = elimination half-life; R^2 = determination coefficient; C_{as} = concentration in tissues at equilibrium; t_{88} = 88% of the time needed to reach the steady state; I = metal influx rate; BCF_{kin} = bioconcentration factor = K_u/K_e; Confidence interval between brackets

Population of Organism	K_u (mL/g h)	K_e (h^{-1})	$t_{1/2}$ (h)	R^2	C_{as} (μg/g)	I (μg Ni(II) /g per day)	BCF_{kin}
300	0.0351 (0.010–0.0351)	0.0028 (0.0033–0.0028)	241	0.974	0.35	0.35	12.2 (3.2–12.2)
400	0.0650 (0.015–0.065)	0.0041 (0.0041–0.0041)	167	0.966	0.58	0.58	15.7 (3.8–15.7)
500	0.4333 (0.024–0.433)	0.012 (0.0049–0.012)	54	0.972	2.16	2.16	33.9 (4.8–33.9)
600	−0.0019 (−0.000069 –(−0.0019))	0.0195 (0.005–0.019)	35	0.995	−0.009	−0.009	−0.009 (−0.013–(−0.009))
700	−0.009 (−0.0042–(−0.0098))	0.0034 (0.004–0.0034)	199	0.992	−0.117	−0.17	−2.8 (−0.8–(−2.8))

Table 2
Bicompartmental kinetic parameters: K_u = rate constant of uptake; K_e = rate constant of elimination; R^2 = determination coefficient; BCF_{kin} = bioconcentration factor = K_u/K_e

Name of the organism	Concentration of the cadmium (mg/L)	K_u (mL/g h)	K_e (h^{-1})	R^2	BCF_{kin}
A. franciscana (S. Francisco Bay Brand USA)	10	0.1492 (0.042–0.25)	0.0231 (0.006–0.0402)	0.66	6.5
A. franciscana (Macau, Brazil)	1	0.2964 (0.192–0.40)	0.0108 (0.0067–0.0199)	0.79	31.2
A. persimilis	0.1	1.0564 (0.655–1.45)	0.005 (0.0018–0.008)	0.87	211.3
A. salina	0.1	1.84 (1.51–2.18)	0.0062 (0.0047–0.0077)	0.90	298.3
A. parthenogenetica (Petrola, Spain)	0.5	0.5223 (0.205–0.83)	0.005 (−0.0001 to 0.0112)	0.78	93.0
A. parthenogenetica (La mata Lagoon, Spain)	10	0.1098 (−0.0019 to 0.2214)	0.021 (−0.0017 to 0.0449)	0.62	5.1

concentration of metal used in that study was very low compared to the present study.

The size of *A. franciscana* makes it compatible with bicompartmental model. This limits the use of multi-compartmental model in the accumulation kinetics of Ni(II). Bicompartmental model is widely used for metal uptake in small-sized species using first-order kinetics [16]. Nonetheless, the results obtained from the application of bicompartmental kinetic models have been highly useful from a practical standpoint, taking into account the fact that the models are a mathematical description and not a description of the biological processes. The application of these models for the kinetics of metals in other crustaceans of a small size was reported [17,18].

As proposed by other authors [19–21], that the surface of *A. franciscana* is extremely impermeable to metals, that these have the digestive epithelium as the main route of entry, and that food is taken up along with the water (as corresponds to an animal with a filter feeding mechanism), the uptake rate of the metals would depend on the drinking rate (DR) and absorption efficiency of metals from food or from the dissolved phase (a), with the relationship between the uptake being

$$Ku = \alpha \times DR \tag{8}$$

Taking into account the fact that the uptake constant is related to the metal influx rate (I) and the

concentration in water (C_w) according to Eq. (8), our results suggest that there is an effect of the toxicant on DR, in such a way that at higher concentrations of Ni (II) in water, the aforementioned rate reduces in the organism. Organisms follow three different strategies for avoiding the toxic effect of Ni(II), such as (1) reducing the entry of the metal, (2) increasing the excretion, or (3) capturing the metal within the tissues in a way that is non-toxic to the organism [2]. This work belongs to the third category in which the Ni(II) becomes non-toxic by binding with MT protein.

4. Conclusion

In this investigation, the optimized population of *A. franciscana* was observed to be 500. The uptake and elimination rate of this organism for a time period of 120 h was found to be higher in the range of 0.024–0.433 mL/g h and 0.0049–0.012 h^{-1}, respectively. The BCF was found higher in optimized population of *A. franciscana* as 33.9 mL/g. The metal influx rate (I) determined was also higher in optimized population as 2.16 (μg Ni(II)/g per day). These parameters clearly indicate that *A. franciscana* organisms can be used for effective removal of Ni(II) contaminated seawater and the kinetic parameters best suits for bicompartmental model.

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