Studies on the effect of *Artemia franciscana* on the removal of Chromium by Bioaccumulation

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In the present work, the bioaccumulation of Cr (VI) by *Artemia franciscana* was investigated. Metallothionein protein plays a key role in the uptake of Cr (VI) by *Artemia* and it was estimated by the silver saturation method. *Artemia* (Brine Shrimp) was subjected to Cr (VI) initial concentration of 40 ppm which was reduced to 2 ppm on 4th day of experimental condition with removal efficiencies of 95%. The number of organisms were varied to find out the maximum removal efficiency. Physical parameters such as pH were maintained in alkaline condition of 9-10, temperature was maintained at room temperature and 30-35 ppt of salinity condition was found to be optimum for the survival and reduction of chromium by *Artemia*.

**Keywords:** *Artemia franciscana*, Chromium, Bioaccumulation, Synthetic waste water

**Introduction**

Chromium is a common pollutant and it is released into the environment through a large number of industrial operations, which include manufacturing of alloys, dyes and pigments, electroplating, metal finishing, film and photography, metal cleaning, mining, petroleum refining, leather tanning and as corrosion inhibitors in conventional and nuclear power plants. Chromium (III) occurs naturally in the environment and is an essential nutrient. Chromium (VI) and chromium (0) are generally produced by industrial processes. Cr (VI) is mobile in the environment and is highly toxic. Cr (VI) can easily penetrate the cell wall and exert its noxious influence in the cell itself, being also a source of various cancers diseases. Various techniques were used for the removal of chromium, such as absorption, filtration, Ion exchange and Electrochemical Techniques. However, these processes have significant disadvantages, including the requirement of expensive equipment, monitoring systems, high energy requirement and chemical cost. This has led to the development of alternative low-cost technologies for the reduction of Cr (VI) from industrial effluents. The present study evaluates the feasibility of using *Artemia* as a bioaccumulator in reducing the Cr (VI) from the synthetic waste water. This crustacean has been widely used since the early 1970s as a test organism in short-term toxicity testing and able to bioaccumulate quite large amounts of elements from the aquatic environment even when their concentration in this compartment is extremely low. It has the broad tolerance to the environmental factors such as salinity, temperature, and dissolved oxygen in the water. This organism possesses an uncommon adaptability to extreme conditions, thus being found in environments where other life forms are not sustainable.

The habitats in which the genus *Artemia* is found are characterized by the absence of predatory animal species. Therefore, in such environment the evolution of *Artemia* populations is favored by the abundance of bacteria, protozoa and algae that are the basis of the *Artemia* diet. There are several advantages of using *Artemia*, including their ready availability, ease of culturing, low cost and a large literature describing their morphological. Biochemical and molecular characteristics.

Bioaccumulation is a fundamental process in environmental toxicology and risk assessment, as it determines the internal dose of potential toxicants. In this present investigation, *Artemia* Nauplii has been used as a bioaccumulator in reducing the concentration of Chromium from the synthetic waste water. The effect of physical parameters such as pH and salinity has been observed in the removal of Chromium. The uptake capacity of the *Artemia* was found to be increased with the help of the MTs protein. MTs are superfamily of lower molecular mass metal thiolate cluster proteins, with an ability to bind metallic ions.
Materials and Methods

Artemia cysts were collected from the salt pans located at Kelambakkam, 12°47’N 80° South India. The cysts were processed and hatching of the cyst was carried out in the sea water with the 35ppt concentration with a pH of 8.0-8.5. Exactly 1.00 grams of Artemia Cysts are measured out for testing. Visible cysts were hatched out within the 24 hrs time period. 10 days old Artemia has been used for the experimental study.

The Cr (VI) stock solution was prepared by dissolving accurately weighed Potassium dichromate in synthetic seawater. Experimental solutions were obtained by diluting the stock solution in accurate proportions to initial concentrations. Cr (VI) concentrations were chosen for the experimental condition was 40 ppm respectively. Population of organisms varied from 300-700 for 1000ml of synthetic waste water for the experimental study. The Artemia was introduced into the synthetically prepared waste water solution to the different salinity in 30,35,40,45 and 50 ppt and different pH range of 8-14. Experimental studies were carried out in the room temperature. The synthetic waste water was analysed in Atomic Absorption Spectrophotometer for the reduction of Cr (VI) before and after treatment.

Estimation of Metallothionein Protein in Artemia

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. Homogenate was centrifuged at 20,000g for 20 min. at 4°C. Aliquots of 750µl supernatant were analyzed for MT content by the silver-saturation method18 with small modifications. Samples were incubated with 1ml of 20mg l⁻¹ silver 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.). Heat treatment caused precipitation of Ag⁺ bound hemoglobin and other proteins, except for MT which is heat stable. Denatured proteins were removed by centrifugation at 1000g for 5 min. Hemolysate addition, heat treatment and centrifugation were repeated three times in each sample. Amount of Ag⁺ in the final supernatant fraction is proportional to the amount of MT present. Silver concentrations were estimated by atomic absorption spectrophotometer and in a similar way Cr (VI) from supernatant was estimated using Atomic Absorption Spectrophotometer (Shimadzu, AA6300).

Results and Discussion

Populations of organisms varied from 300,400,500,600 and 700 for 1000ml of synthetic waste water used to improve the removal efficiency of Cr (VI) by the Artemia. Among these, 500 numbers of organisms were found to be very effective in the removal efficiency (Fig. 1).

Fig. 1—Effect of population of organisms on the removal of Cr (VI)

The 300 and 400 numbers of organisms were not much effective in removal efficiency due to its less number, whereas 600 and 700 numbers of organisms were also not effective due to its high number, brings out the suffocation to the organisms. So in the present study, the 500 numbers of organisms were found to be optimized condition for the removal of chromium.

Maximum removal of Cr (VI) was observed under the salinity condition in 30-35 ppt, the removal percentage of Cr (VI) was found to be 93.75-95% respectively. Whereas, at the salinity condition of 40, 45 & 50ppt, the removal percentage of Cr (VI) was found to be 75,70 and 62.5% respectively (Fig. 2).

Fig. 2—Effect of salinity on the removal of Cr (VI)

Optimized pH condition for the present study was found to be 9-10, it brings out the removal efficiency of 92.5-95%, whereas in other pH
conditions, the removal efficiency was found to be less (Fig. 3).

Artemia franciscana was found to reduce 95% of Cr (VI). 500 numbers of organisms were used for this experimental study with the optimized pH and salinity condition. As the initial concentration of 40 ppm Cr (VI) was reduced to the final concentration of 2 ppm on the 4 day. This day by day reduction 36,29,13 and 2 ppm on 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} days was mainly due to the accumulation of chromium in the body of the organism (Fig. 4).

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. MT content in Artemia increased in a time-dependent fashion. Metallothionein synthesis in Artemia is very high and one of the reasons of the high resistance of this creature to pollutants is attributed to this issue. 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\textsuperscript{19}. In the present study, Metallothionein Proteins were found to be one of the reasons for the Bioaccumulation of Cr (VI) in the Artemia. An increase in the MT content depends on the time-dependent fashion. Increased MT content of 640 and 660 µg MT/g wet weight can be observed on the 3\textsuperscript{rd} and 4\textsuperscript{th} day as it was very less in the first two days (350 and 450 µg MT/g wet weight) of exposure (Fig. 6).
So this increased MT content on 3rd and 4th day was responsible for the accumulation of Cr (VI) in Artemia, which results in 95% of Chromium removal.

Metallothionein synthesis and metal binding are among the mechanism, presumably involved in cadmium tolerance\(^{20}\). Likewise, in the present study Metallothionein plays the key role in the reduction of Cr (VI) by Artemia. 92% of chromium were removed by Aspergillus sp. and 90% of chromium removed by Micrococcus sp. by the process of bioaccumulation\(^{11}\). These organisms were effective in chromium removal, but the culturing and maintaining of the organism was found to be difficult. Alcaligenes eutrophus can be very useful for bioremediation of chromium from industrial wastewater\(^{22}\). The present investigation has been concerned with the removal of chromium from the bioaccumulation process rather than the bioremediation process.

Various agricultural byproducts have been used as the adsorbents in Cr (VI) uptake, such as wool, olive cake, sawdust, pine needles, almond shell, cactus and coal with the removal efficiency of 69.3%, 47.1%, 53.5%, 42.9%, 19.8% and 23.5% respectively. The removal efficiency of the above mentioned adsorbents was found to be very less compared to the removal efficiency of the present study\(^{23}\).

In the past, research has focussed on the use of Artemia Nauplii as test organism for a wide variety of contaminants, such as metals\(^{24,25,26}\), trace elements\(^{27}\), s-triazine herbicides\(^{27,28}\), acrylonitrile\(^{29}\), pollutants produced by incineration plants\(^{30}\), antifouling compounds\(^{31,24}\). Above mentioned literature showed the effect of Artemia in the toxicity studies, as in the case of present study Artemia acts as the bioaccumulator for the reduction of Cr (VI) from the synthetic waste water.

It was the first time, the organism Artemia franciscana has been used in the treatment process. As most of the previous work in Artemia was based on the toxicity studies and the present work is based on the treatment study. In the present study there are several advantages of using Artemia, including their ready availability, ease of culturing, low cost and a large literature describing their morphological, Biochemical and molecular characteristics\(^{13,14}\) and it is also reducing 95% of the Cr (VI). The present investigation has been concerned with the removal of Cr (VI) by bioaccumulation process rather than the other biological process.

**Conclusion**

Thus, the present study amply demonstrates the efficient use of Artemia franciscana, in the removal of Cr (VI), as this organism appears to be suitable as an environmental bio indicator. Easy culturing of Artemia proves this biological treatment to be an alternative for the various microbial treatments. Artemia reveals the superior nature in effective treatment and it would replace the cost effective digester systems.

Gradual decrease was observed from 40 ppm to 2 ppm with the removal efficiency of 95% with the optimized pH of 9-10 and salinity condition of 30-35. Metallothionein protein plays a key role in the uptake of Cr (VI) by Artemia. There was a gradual increase in metallothionein protein on the 3rd and 4th day and which is responsible for the bioaccumulation of Cr (VI) in Artemia, which results in 95% of removal.

**References**


