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**Research Article**

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# Studies on the toxicological effects of bimetals on the cladoceran, *Daphnia magna* and examination of histopathological effects through Transmission Electron Microscopy (TEM)

**Akila Selvi Rajaretnam and Shaleesha A. Stanley***

*Department of Biotechnology, Jeppiaar Engineering College, Chennai, Tamil Nadu, India*

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**ABSTRACT**

*Daphnia magna is the only (OECD) approved aquatic study organism to experimentally study the hazardous level of the water bodies and therefore to prescribe the lethal dose of metals. A study is envisaged and carried out to evaluate the toxicity of bimetals namely, Potassium dichromate ($K_2Cr_2O_7$), Zinc Sulphate ($ZnSO_4.7H_2O$) and Cupric Sulphate ($CuSO_4.5H_2O$) on the cladoceran, Daphnia magna. The toxicity was observed to find the $LC_{50}$ at known physico-chemical parameters and at regular intervals of time (h). Results revealed that at a frequency of 24 h exposure, the $LC_{50}$ was found to be 3.5mg/L for potassium dichromate, 4mg/L for zinc sulphate and 4mg/L for cupric sulphate on Daphnia magna. The exposed Daphnia magna were subjected to histopathological studies using Transmission Electron Microscopy (TEM) and the pictograms revealed intense deposits of metal (Cr > Zn >Cu) around the arterial system which would have been the reason for mortality of Daphnia magna.*

**Key words:** *Daphnia magna*, toxicity, chromium, zinc, copper, $LC_{50.}$

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## INTRODUCTION

*Daphnia magna* is one of the most used bio-indicator organisms for both water and sediment toxicity bioassays. The extremely fast growth and high reproductive rates and short life cycles associated with daphnia were all perceived as positive features for an ideal test organism. Daphnids moult frequently [6] and lack the ability to store Calcium while moulting, hence their calcium demand is very high [12] and the majority of their calcium must be extracted from the external medium immediately after moulting [10,17]. An inadequate supply of calcium could threaten daphnid persistence. The daphnia is a freshwater Cladoceran used worldwide as test organism in aquatic toxicity assays.

The use of *Daphnia magna* as an experimental animal for such purposes is advantageous in many respects. Daphnids are small, reaching a size of five mm, so that a great many can be reared in a small space [13,15]. They have a relatively short life span, which reaches a maximum of about two months when they are reared at 25°C. Daphnids are easy to culture, requiring only water containing bacteria or their equivalent for food. They can be grown individually in small bottles or in mass culture in large aquaria. They mature early, giving birth to young within their first week of life [14]. After their first brood, they give rise to new broods every two or three days throughout the remainder of their lives.

Zooplankton is one of the most sensitive to toxic chemicals, and these organisms occupy the central position in the lentic food chain; therefore, they are most commonly used in ecotoxicological tests [8]. The cladocerans are an important link in the aquatic food chain. Daphnids are aquatic organisms also frequently used in toxicological studies worldwide. The use of *Daphnia* spp. as toxicity indicator organisms is well documented in the literature because of their fast growth rate, high reproductive rates, and short life cycles [1].

**Toxicological Aspects of Chromium**

Chromium VI is a toxic form of chromium, new toxicology data indicate that Cr VI may be carcinogenic to the gastrointestinal tract of laboratory animals when ingested at high doses over their lifetime through drinking water. At these very low concentrations (less than 1 ppb) non-cancer effects are unlikely [18]. At very high doses (greater than 100 ppb) Cr VI can irritate the skin and be a risk to the developing fetus. Hexavalent chromium is more toxic than the Cr III form because its oxidizing potential is high and it easily penetrates biological membranes. The members of the class Branchiopoda and the subclass, Cladocera share a two-valved carapace covering most of the body except the appendages. All Cladocerans have an unpaired compound eye which is the result of a fusion of two eyes in the late embryonic development [6].

**Toxicological Aspects of Copper**

Copper is present in normal human serum (the liquid part of blood) at concentrations of 120-140 μg/L. Signs of toxicity will be seen if the copper concentration rises significantly above this range [2,20]. When a person is exposed to copper levels above the essential levels needed for good health, the liver and kidneys produce metallothionein. Cupric ion is the main toxic form of copper. Cupric ion in water is bound (complexed) with inorganic and organic compounds, which reduces cupric ion concentrations (and its toxicity) substantially. Their toxicity varied with alkalinity, pH and hardness [19]. The presence of organic chelators generally decreased the toxicity of copper to aquatic organisms i.e., EDTA reduced the activity (concentration) of the toxic $Cu^{2+}$ species.

Copper in water with higher hardness was generally less toxic to fish than water with lower hardness. If species is not sensitive to pH, toxicity may be greater at higher pH due to diminished competition of $Cu^{2+}$ and $H^{+}$ ions at receptor sites compared to competition at lower pH [4, 9]. As a result, chelated copper compounds exhibit lower toxicity to fish and most invertebrates than copper sulfate. The high toxicity of copper to algae creates a ripple effect throughout the ecosystem and demonstrates that changing one part of an ecosystem will affect the entire ecosystem [20]. Copper is generally more toxic to organisms in freshwater than in saltwater. One of the reasons for this difference is that freshwater lacks cations, which compete with $Cu^{2+}$ at the biological action sites, thus reducing copper toxicity.

**Toxicological Aspects of Zinc**

The toxicity of zinc to aquatic organisms is affected by factors such as temperature, hardness, pH and dissolved organic carbon [11]. The toxicity of zinc to terrestrial organisms is similarly dependent upon its bioavailability, which in turn is determined by various factors such as the speciation of zinc, and the physicochemical and biological characteristics of the soil. The bioavailable fraction of zinc in soil has been calculated to range from < 1% to 10% of the total zinc concentration [5]. The total concentration of an essential element such as zinc, alone, is not a good predictor of its bioavailability or toxicity. Zinc is an essential element in the environment. The possibility exists for both a deficiency and excess of this metal. For this reason it is important that regulatory criteria for zinc, while protecting against toxicity, are not set so low as to drive zinc levels into the deficiency area (Environmental Health Criteria For Zinc).

## EXPERIMENTAL SECTION

**Aquatic Toxicity evaluation using the experimental procedure of $LC_{50}$**

**Experimental organisms**

The test organism *Daphnia magna* was obtained from a fish farm in south-western part of Chennai, Tamil Nadu, India and introduced to 30 L aquariums with de-chlorinated tap water, which serves as holding tanks, maintained at 26°C.

The water temperature and pH were measured regularly in the laboratory; the temperature was 25 ± 1.3 °C and pH was 7.7±0.4 providing aeration to the tanks**.** All young daphnids were withdrawn from the incubation containers with Pasteur pipette and used for testing.

**Experimental procedure**
Acute 24h toxicity tests for Cr (2.5, 3.0, 3.5 mg/L), Cu (2.0, 3.0, 4.0 mg/L ), Zn (2.0, 3.0, 4.0 mg/L) was taken as triplicates with control groups under static non-renewal conditions in 400mL of reconstituted water in 500mL beaker.

A total of 18 neonates obtained from the original culture were exposed to different concentrations of Cr, Zn and Cu. The containers were slightly aerated without disturbing the daphnids with air bubbles. The toxicity was expressed by the median lethal concentration, that is, the dose required to kill half of the daphnid members during a 24h period of $LC_{50}$ exposure. After 24h the live *D. magna* were counted, and after gently shaking the glass containers the ones that did not move were considered dead.

**Histopathological studies using Transmission Electron Microscopy (TEM)**
**1. Sections of Embedded Material**
Biological material contains large quantities of water. Since the TEM works in vacuum, the water must be removed. To avoid disruption as a result of the loss of water, you preserve the tissue with different fixatives. These cross-link molecules with each other and trap them together as stable structures. The tissue is then dehydrated in alcohol or acetone.

After that, your specimen can be embedded in plastic that polymerize into a solid hard plastic block. The block is cut into thin sections by a diamond knife in an instrument called ultra microtome. Each section is only 50-100 nm thick.

The thin sections of your sample is placed on a copper grid and stained with heavy metals. The slice of tissue can now be studied under the electron beam.

**2. Negative Staining of Isolated Material**
The isolated material (can be a solution with bacteria or a solution with isolated molecules) is spread on a support grid coated with plastic. A solution of heavy metal salt is added. The metal salt solution does not bind to the material but forms a "shadow" around it on the grid. The specimen will appear as a negative picture when viewing it in the TEM.

**Statistical analysis**
Exposure to the different concentrations was carried out in triplicate. $LC_{50}$ (median lethal concentration) values were calculated by probit analysis.

## RESULTS AND DISCUSSION

The toxicity study subjected for three bimetals at varying concentration exposed for 24h resulted in identifying the lethal as 3.5mg/L for potassium dichromate, 4mg/L for zinc sulphate and 4mg/L for cupric sulphate on *Daphnia magna*. The control experimental samples did not show any behavioral abnormalities and the mortality was nil [18]. Acute toxicity tests indicated chromium at 3.5mg/L had a detrimental effect on the survival of *D. magna* when viewed under Transmission Electron Microscopy. The results of the toxicity test using potassium dichromate are shown in (Table 1).

**Table 1: $LC_{50}$ values of Potassium dichromate observed at a frequency of 2, 4, 8 and 24 h on *D.magna***

| Concentration of Potassium dichromate | Control | 2 h | 4 h | 8 h | 24 h |
|---|---|---|---|---|---|
| 2.5mg/L | 18 | 2 | 14 | 15 | 18 |
| 3.0mg/L | 18 | 3 | 14 | 15 | 18 |
| 3.5mg/L | 18 | 6 | 15 | 16 | 18 |

**Table 2: $LC_{50}$ values of Zinc Sulphate observed at a frequency of 2, 4, 8 and 24 h on *D.magna***

| Concentration of Zinc sulphate | Control | 2 h | 4 h | 8 h | 24 h |
|---|---|---|---|---|---|
| 2mg/L | 18 | 2 | 10 | 13 | 14 |
| 3mg/L | 18 | 2 | 11 | 13 | 18 |
| 4mg/L | 18 | 3 | 13 | 14 | 18 |

Zinc is known as an essential metal and takes part in more than 300 enzymes [3]. However it is shown that zinc may be toxic at high levels [7] found that 24h $EC_{50}$ values for $ZnSO_4$ were between 3.65 and 7.32 mg/L, which is similar to our findings (Table 2).

**Table 3: $LC_{50}$ values of cupric sulphate observed at a frequency of 2, 4, 8 and 24 h on *D.magna***

| Concentration of Cupric sulphate | Control | 2 h | 4 h | 8 h | 24 h |
|---|---|---|---|---|---|
| 2mg/L | 18 | 2 | 3 | 3 | 10 |
| 3mg/L | 18 | 3 | 4 | 4 | 15 |
| 4mg/L | 18 | 3 | 4 | 4 | 18 |

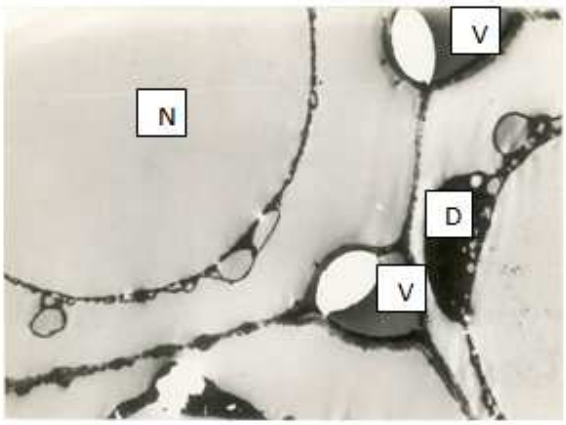
Copper does have some effect on the survival rate of *Daphnia magna*, the statistical data proves that copper sulfate solution at the different concentrations used in which all of the *Daphnia magna* were found dead 24 hours later. LC value of copper for *Daphnia* is given in (Table 3).

**Histopathological examination**

The maximum concentration at which the *Daphnia magna* was lethal was subjected to TEM for histopathological examination. The results revealed that metal was deposited on the walls of heart blocking the blood supply throughout the body, which could be the reason for its death.

**Figure 1: Transmission electron micrographs of the Cladoceran, *Daphnia magna* (X3000)**

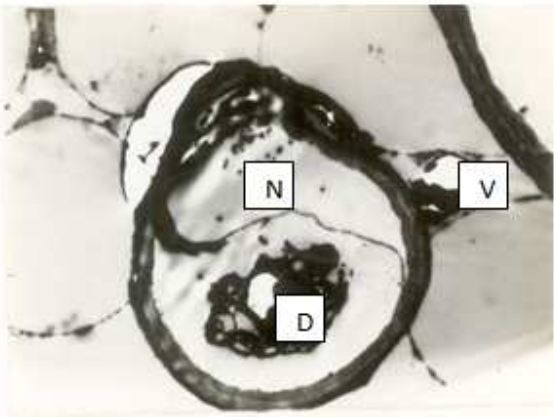
A




*A - Daphnia magna exposed to Potassium dichromate*
*N - cell with nucleus ;*
*V - loss of cytoplasmic density due to the were deteriorated, some of them appeared disorganized presence of numerous vacuoles;*
*D- Deposit of heavy metal as thickenings in the basal lamina.*

**Figure 2: Transmission electron micrographs of the Cladoceran, *Daphnia magna* (X4000)**

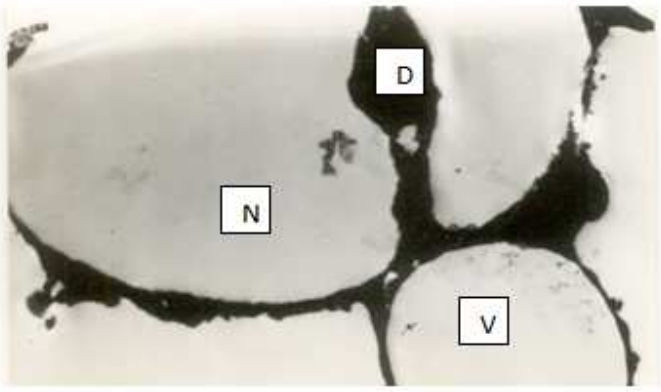
B




*B – Daphnia magna exposed to Zinc sulphate*
*N- disorganized nucleus;*
*V- lysis of cytoplasmic area appeared filamentous nucleus;*
*D- having abnormal changes than normal heterochromatin distribution due to the deposit of heavy metal.*

**Figure 3: Transmission electron micrographs of the Cladoceran, *Daphnia magna* (X4000)**

C




*C – Daphnia magna exposed to Cupric sulphate*
*N- disorganized nucleus;*
*V- lysis of cytoplasmic area appeared filamentous nucleus;*
*D- having abnormal changes than normal heterochromatin distribution due to the deposit of heavy metal.*

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