

Cytotoxic Effects of Mercury, Cadmium, Lead and Zinc on *Acanthamoeba Castellanii*

¹Nakisah Mat Amin*, ¹Rainee a/p Velamuthu and ²Noor Azhar Mohd Shazili

¹Department of Biological Sciences, Faculty of Science and Technology

²Institute of Oceanography,

Kolej Universiti Sains dan Teknologi Malaysia [KUSTEM]

Mengabang Telipot 21030 Kuala Terengganu

Abstract

Acanthamoeba spp are single-cell organisms that are commonly encountered in aquatic and terrestrial ecosystems. Their role in our natural environment is mainly as bacterial consumer although some of them can become pathogens and infect humans and animals. Heavy metals such as mercury, cadmium, lead and zinc are dangerous pollutants and often its dissolved form enters the aquatic environment and affects the organisms in the food web of aquatic ecosystem including amoebae. A study on the cytotoxic effect of mercury, cadmium, lead and zinc on *Acanthamoeba castellanii* was conducted in order to understand more about their toxicity on the amoeba by looking at their EC₅₀ and effect on the amoeba cell morphology. The results of this study indicated that mercury is the most toxic metal since its EC₅₀ is only 2.0 ppm, followed by Cd (13.4 ppm), Pb (23.8 ppm) and Zn (143.3 ppm). The amoeba cells were observed to be smaller in size and stained orange with AOPI indicating the cells lost its cell membrane integrity which eventually lead to the cell death by necrosis when being exposed to the heavy metals.

Keywords: *Acanthameoba castellanii*, cytotoxic effects, heavy metals, necrosis, AOPI

1.0 Introduction

Free-living amoebae are ubiquitous in nature; they can be found in aquatic and terrestrial environment. Their roles in the food web of the ecosystems are mainly as bacterial consumers although some of them have potential to infect animals and man. Any changes in the ecosystem due to the introduction of pollutants including heavy metals will affect the life of these amoebae, which later upset the ecosystem. Some information available on the lethal effects of cadmium (Cd), mercury (Hg), zinc (Zn) and lead (Pb) on marine and aquatic organisms [1,2]. These metals are dangerous pollutants and often its dissolved form enters the aquatic environment and affects the organisms including free-living amoebae. Little is known however, on the effects of these heavy metals on *Acanthamoeba* spp. Being single cells, the amoebae act both functional and adaptive unit to the environmental changes, so in case of heavy metal contamination in the aquatic ecosystem, the level toxicity of the metals on the amoebae can be investigated. Therefore, the purpose of this study is to see the toxicity effects of four heavy metals viz Hg, Cd, Pb and Zn on a free-living amoeba, *Acanthamoeba castellanii* by examining their EC₅₀ value against the amoebae, and by observing their effect on the amoeba's membrane integrity.

* Correspondence author: Tel:609-6683245; Fax:609-6694660, Email: nakisah@kustem.edu.my

2.0 Materials and Methods

2.1 *Amoeba cultivation and preparation*

Acanthamoeba castellanii used in this study was cultivated axenically in polypeptone medium at 30°C. The amoeba cells were harvested when confluence and 20 µL of the cell suspensions from that cultures containing amoebae at density of 5×10^5 cells/mL were transferred into 24-well plates for treatment with various concentration of metals. Six different concentrations of each metal solutions (Hg, Cd, Pb and Zn) were prepared from their stock solution (10 ppm for each metal solution) in order to get 3.2, 1.0, 0.5, 0.2 and 0.1 ppm of Hg solution, 32, 18, 10, 5.6 and 3.2 ppm of Cd solution, 32, 18, 10, 3.2 and 1.0 ppm for Pb solution, and 180, 100, 80, 40 and 20 ppm for Zn solution and added to the wells. The appropriate volume of fresh medium was later added to the wells to get the final volume of 1000µL. Controls in this experiment are wells containing amoebae without adding metal solution. The plates were incubated for 72h at 30°C before cell count and observation on the cell morphology after staining with acridine orange and propidium iodide were done.

2.2 *Evaluation of viable cells and Determination of EC₅₀*

The viable cells of amoebae after treatment with heavy metals were determined using trypan blue exclusion technique [3] and the cell count was done on a haemocytometer. The number of viable cells obtained was used to determine the EC₅₀ of each metals against *Acanthamoeba*

2.3 *Observation on the cytotoxic effects of heavy metals on amoebae*

The cells after treatment with the heavy metals at concentrations of their EC₅₀ values were stained with Acridine Orange and Propidium Iodide (AOPI) and visualized under fluorescence microscopy.

3.0 Results and Discussion

Toxicity is the study of the effects of toxic foreign compounds on living organisms including amoebae. The relationship between dose of foreign compounds and response of the organism are always become of primary important in toxicity study. In this study, various concentration of Hg, Cd, Pb and Zn were added to the multi-well plates containing amoebae and culture medium for 72h at 30°C. The presence of heavy metals' ions in the amoeba culture medium either in form of complex inorganic or organic compounds, perhaps disturb the physiology, biology and growth of amoebae. All heavy metals, whether biologically essential or not, have the potential to be toxic to organisms at threshold bioavailability.

The sensitivity of *Acanthamoeba castellanii* to various concentrations of Hg, Cd, Pb and Zn conducted in this study was indicated by percentage of inhibition by the metals and their EC₅₀ (the effective concentration that inhibit 50% of amoeba population) values (Table 1, Figure 1). Mercury is predicted as the most toxic metals compared to cadmium, lead and zinc (based on their position in the Periodic Table) and this prediction was reflected by their EC₅₀ values against *Acanthamoeba*. The most toxic metal will have the lower value of EC₅₀. Only 2.0 ppm of mercury and 13.4 ppm of cadmium needed to inhibit 50% of the amoeba population whereas for lead and zinc, their concentration at 23.8ppm and 143.3ppm, respectively was required to give such inhibition (Figure 1). Toxicity of the metals decrease with an increase in

the stability of electron configuration [4] thus brings variation in values of EC₅₀ obtained in this study.

Table 1 Number of amoebae (x 10⁵ cells.mL⁻¹) and percentage of inhibition in metals at various concentration

	Concentration of metals (ppm)					
	0	0.1	0.2	0.5	1.0	3.2
Hg						
No of amoeba (mean ±S _D)	13.65±0.47	10.71±0.61	10.30±0.18	9.60±0.22	9.08±0.09	4.35±0.09
% of Inhibition	0.0	21.5	24.5	30.8	33.5	68.1
Cd						
No of amoeba (mean ±S _D)	10.50 ±0.44	9.15±0.85	6.40±0.43	5.25±0.34	4.22±0.20	0.25±0.10
% of Inhibition	0.0	13.7	18.9	47.5	59.8	97.6
Pb						
No of amoeba (mean ±S _D)	12.75±0.44	11.85±00.19	11.48±00.38	9.95±00.49	7.90±0.19	4.30±00.26
% of Inhibition	0.00	7.05	9.96	21.90	38.00	66.27
Zn						
No of amoeba (mean ±S _D)	14.0±0.33	9.8±0.82	9.45±0.19	9.08±0.22	8.54±0.21	6.15±0.24
% of Inhibition	0.0	30.00	32.50	35.17	39.00	56.07

Data are from four replicate

Heavy metals have a high affinity to negatively charged protein groups that cause the change in protein conformation which will result inhibition of enzymatic activity and biochemistry of the cells [5]. The heavy metals cause disturbance to the structural proteins, damage the cell cytoskeleton and disrupt the cell movement. In the present study, the amoebae were not only observed as smaller in size but they were less active in the presence of heavy metals. The interaction of heavy metal ions with actin and myosin that form cytoskeleton of the amoeba cell probably cause inhibition to the amoeba motility in this study. And also, the growth of *A. castellanii* was affected by the presence of these metals in its growth media since these metals inhibit the enzymes that are involved in fatty acid metabolism which control the physical state and fluidity of membranes, and other physiological and biochemical characteristic displayed by this amoeba [6].

The application of the fluorescent dye such as acridine orange and propidium iodide (AOPI) to stain the amoeba cells after exposure to heavy metals appears to be suitable for morphological analysis especially to study the membrane integrity of amoebae. Results obtained in the present study indicated that heavy metals (Hg, Cd, Pb and Zn) induce morphological changes such as nuclear vacuolization and cytoplasmic condensation in *Acanthamoeba* (Figure 2). The stained nuclear and cytoplasmic components in *Acanthamoeba* cells by AOPI are to indicate the amoeba lost its membrane integrity after being exposed to heavy metals which result the cell lost its ability to maintain homeostasis leading to influx of water and extra cellular ions. The amoeba cells will lyse and this manner of cell death is termed as necrosis [7].

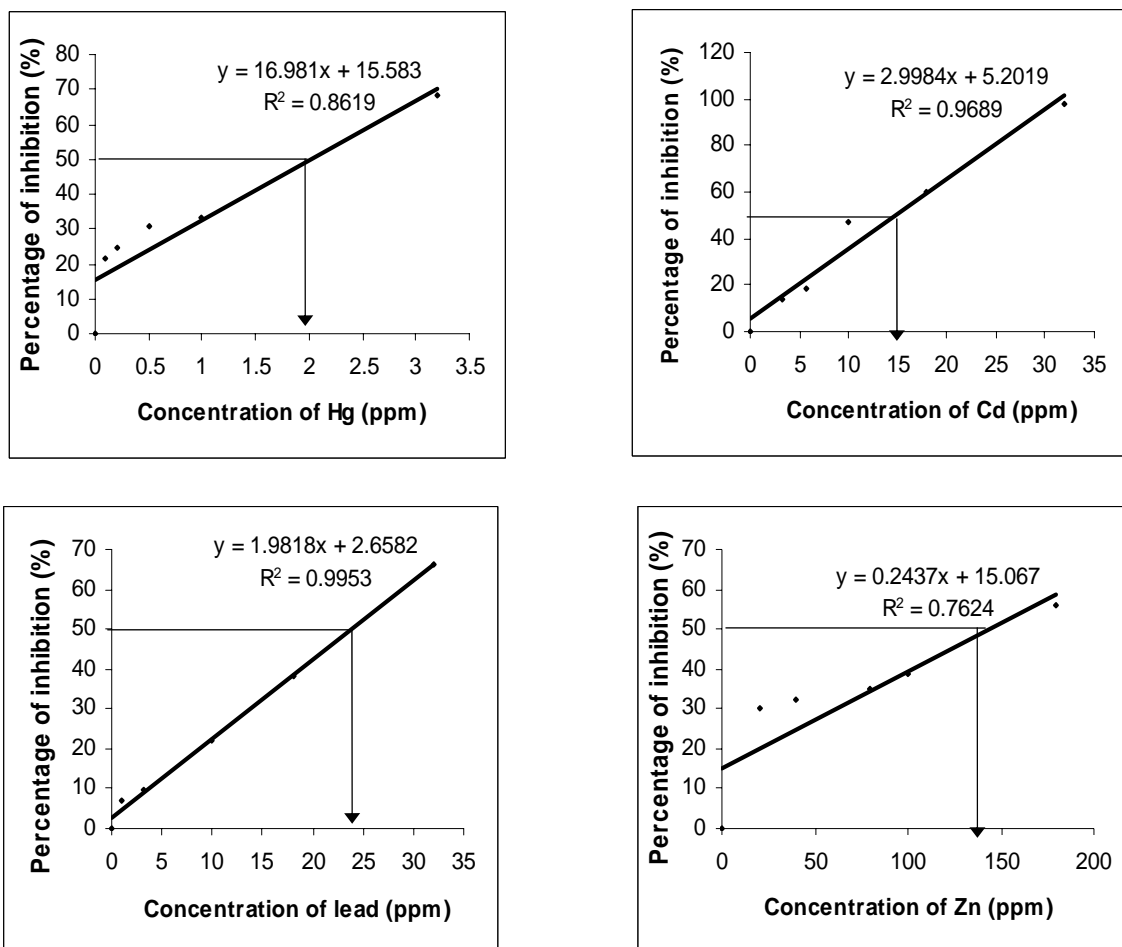


Figure 1 Percentage of inhibition of amoebae in various metals with different concentration. The EC₅₀ values of each metals were derived from this Figure. The EC₅₀ values for Hg is 2.0 ppm, for Cd is 13.4 ppm, for Pb is 23.8 and for Zn is 143.3 ppm.

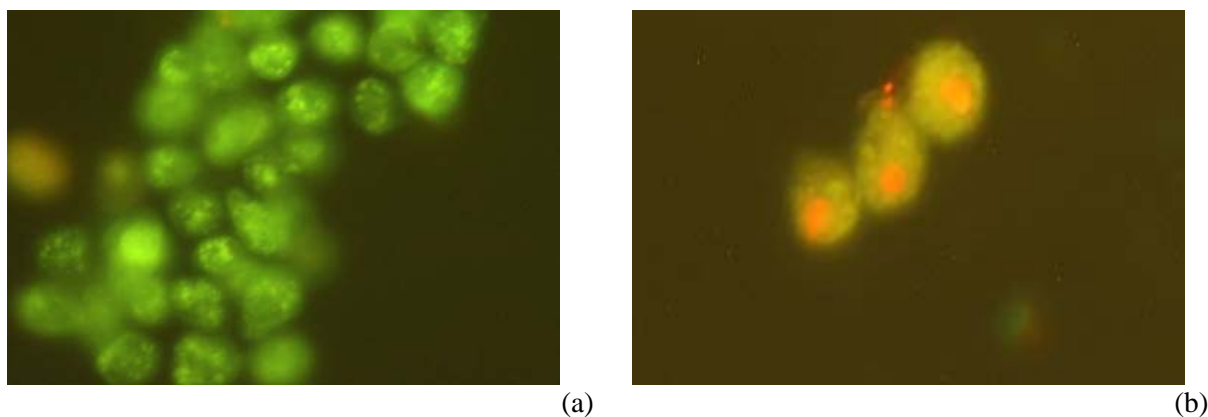


Figure 2 *Acanthamoeba* cells after stained with Acridine Orange and Propidium Iodide. A. Control cells. B. Cells after treatment with metal solutions Mag x 400.

In transmission electron microscopy study of ciliated protozoa under Cd or Zn exposure, the main cytoplasmic alteration in the protozoan cells were observed such as mitochondrial degeneration, cytoplasmic vacuolization, accumulation of membranous debris and autophagosome formation [8].

In natural environment, heavy metal can enter a water supply by industrial and consumer wastes or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers and ground water. Hg, Cd and Zn are often deposited with natural sediment in the bottom of streams and may become incorporated in plants and animals especially shellfish [9]. To single-celled organisms such as free-living amoebae, the cytotoxic effect of heavy metals on them are so severe since the metals affect the amoeba growth (at low dosage, in case of Hg) and its membrane integrity as observed in the present study. The metals probably inhibit the activity of the important enzymes for amoeba growth and other biological processes, affect the cytoskeleton proteins of the amoebae to move, and change the protein conformation in the cell membrane of amoebae which lead to lose its integrity. Therefore, the presence of metal pollutants in their environment will affect the life of amoebae which later affect the balance of the ecosystem since these amoebae are mainly bacterial consumers. Amoeba grazing also enhances remineralization and recycling of nutrients that increase nutrient availability to other organisms in aquatic environment.

4.0 Conclusion

Mercury (Hg), Cadmium (Cd), lead (Pb) and Zinc (Zn) are toxic the amoeba cells (*Acanthamoeba castellanii*) and these are reflected by the EC₅₀ values of each metal against this amoeba and also by loss of membrane integrity of the amoeba after exposure to these metals. The EC₅₀ values for Hg, Cd, Pb and Zn are 2.0 ppm, 13.4 ppm, 23.8 ppm and 143.3 ppm, respectively. The most toxic metal is Hg followed by Cd, Pb and Zn. The amoeba cells after treated with all metals (at concentration of their EC₅₀s) were observed to have lost its membrane integrity after staining with AOPI. The nuclear and cytoplasmic condensation occurred in the amoeba cells are to indicate that the cell death by necrosis.

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