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# Preliminary Study on Toxicity of Some Heavy Metals Towards the Ciliated Protozoan *Paramecium* sp.

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## ABSTRACT
In the present study, the ciliated protozoan *Paramecium* sp. obtained from the Ismailia Canal, Cairo, Egypt, was cultured in a laboratory and then exposed to heavy metal ions of cadmium, copper, and lead at various concentrations. The purpose of the present work was to assess the sensitivity of *Paramecium* sp. to heavy metals and to get predictive data for risk and hazard assessments in standards for water quality. The careful microscopic examination and measurements of cultured non-stressed *Paramecium* sp. revealed that the species could be a part of the *P. aurelia* complex since its morphological features matched those described by Sonneborn (1975). Further investigation is required to validate the present identification of *Paramecium* sp. Concerned with heavy metal toxicity against cultured *Paramecium* sp., copper at concentrations of 10, 30, and 60µg $ml^{-1}$ appeared highly toxic since the organisms became motionless and died indoors a few minutes post-exposure. No full mortality was seen in *Paramecium* sp. culture exposed to lead within concentrations up to 150µg $ml^{-1}$ before 3hr of incubation. All tested lead concentrations (30, 80, and 150µg $ml^{-1}$) induced full mortality at 6hr post-incubation. The LD50 of this pollutant was 19.11µg $ml^{-1}$. Furthermore, complete mortality wasn't noted in *Paramecium* sp. communities subjected to 10, 25, and 50µg $ml^{-1}$ of cadmium during the experiment. The LD50 values of this pollutant at 3, 6, and 12 hours of incubation were 13.9, 8.08, and 11.2µg $ml^{-1}$, respectively. Moreover, an unexpected observation was recorded since the mortality rate decreased as the cadmium concentrations and the exposed time increased. This exploratory work may be useful for ecotoxicological testing and applications, especially in heavy metal-contaminated aquatic ecosystems and systems. Furthermore, the presence of a consistent and distinguishable growth in contaminated samples might be employed as a quick qualitative test field.

## INTRODUCTION

Pollutant contamination of the aquatic environment has become a significant issue in recent years. Rivers and lakes, in particular, which receive agricultural, urban, and industrial wastewater, may have contaminants, such as heavy metals, pesticides, and phenols in high concentrations. Furthermore, surface runoff, industrial effluents, landfill leachates, and contaminated groundwater are all known to include a variety of chemicals. The uncontrolled use of pesticides and heavy metal salts is harmful to the environment, generating imbalances in aquatic ecosystems **(Twagilimana *et al.*, 1998)**. A number of studies have been conducted in

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recent years on the toxicity of many relevant hazardous chemicals in a series of biotests employing diverse test organisms as bioindicators. The ease and reliability of these tests make them appealing. However, test organisms for assessing environmental risk and impact must have several desirable characteristics, including being eukaryotic, having well-known biology and general responses, being easy to manage in the laboratory, and having a short generation time when studying long-term effects **(Nilsson, 1989)**.

Protozoa are considered biological indicators of pollution when their presence or absence can be linked to environmental conditions, and they are considered test organisms when a species or population is used to assess the toxicity of relevant toxic compounds. In the case of water pollution, protozoa are an excellent tool for assessing both toxicity and pollution **(Jeelani *et al.*, 2018)**. Furthermore, protozoa have proven to be helpful tools for assessing the occurrence of pollution during wastewater biological treatment, along with their role in the control of pollution itself through the grazing of dispersed bacteria and the maintenance of a healthy trophic web in those artificial ecosystems. The protozoan community in the aeration tank of activated sludge plants stays an innovative and useful instrument to monitor biological wastewater treatment **(Nicolau *et al.*, 2001)**.

Moreover, many investigations of protozoan populations in heavy metal-polluted streams have proved changes in their dynamics. The structural and functional diversity of these protozoan communities allows for an assessment of the effects and hazards that toxic metals have on various aspects of ecosystems, such as species diversity and food chain dynamics **(Ferandes-Leborans & Novillo, 1995)**.

Several researchers postulated diverse ways in which heavy metals affect microorganisms. Metals, according to scientists, can inhibit enzyme systems or interfere with critical cellular metabolites of protozoa and bacteria **(Morgan & Lackey, 1958)**. Metallothioneins are tiny molecular-weight proteins and polypeptides with a high metal and cysteine content that form metal-thiolate clusters. **(Carpene *et al.*, 2007)**. They are restricted to the Golgi apparatus membrane. Their function is to bind, store, transport, and detoxify metals, which in general means that they protect cells against the toxicity of heavy metals **(Dar *et al.*, 2013)**. Prokaryotes and mammals are among the many taxonomic groupings whose cells have metallothioneins **(Vašák, 2005)**.

There are various physiological and ecological processes of Protista that may be altered by heavy metals, such as reduced food absorption, suppression of growth, and decreased endocytosis rate, which may affect survival **(Nilsson, 1979, 1981)**. The relative sensitivity of protozoa to various heavy metals will not always be the same. Heavy metals are toxic to most microorganisms at certain concentrations. The characteristics and intensity of damage depend on the nature and level of the metal, i.e., there are differences between the effects of essential and non-essential metals **(Irato & Piccinni, 1996)**. Sediment and water properties, such as pH and organic matter content, will also affect the bioavailability of the metals. Heavy metal exposure causes the production of low-molecular-weight, thiol-rich proteins in a range of species

including protists. The absence of metal-binding molecules may explain some microbes' high sensitivity **(Coppellotti, 1994)**.

The study of ciliate sensitivity to a variety of toxic chemicals could supply an indicator for finding the magnitude and potential for ecological damage caused by manufactured contaminants discharged into surface waters. Since the ciliate community is a complex assemblage of interacting organisms, species that are sensitive, resistant, or intermediate in their susceptibility to pollutants are typically present **(Madoni & Romeo, 2006)**. Ciliated protozoa are abundant in aquatic environments and in all forms of biological treatment systems **(Madoni *et al.*, 1996; Amann *et al.*, 1998)**; they play a significant role in the purification and overall regulation of the aquatic community. It has been established that ciliates improve effluent quality by regulating bacterial biomass and removing most of the scattered bacteria **(Madoni, 2003)**. In addition, the ciliate assay has proven to be an effective technique for detecting environmental disturbances and determining trophic conditions **(Cairns & Pratt, 1989)**.

*Paramecium* spp. is a useful ciliated assay organism that has been verified across decades. **Wang (1963)** used *Paramecium aurelia* to prove the harm of cigarette smoke to the present, while **Zahara *et al.* (2023)** used *Parameciu multimicronucleatum* to explore the bioremediation ability of this ciliate for heavy metals.

In the present study, the ciliated protozoan *Paramecium* sp. from the Ismailia canal, Cairo, Egypt, was exposed to heavy metal ions (cadmium, copper, and lead) under laboratory conditions. The aim of this preliminary study was to evaluate the sensitivity of *Paramecium* sp. to heavy metals and to develop a prediction tool for risk and hazard assessment in water quality standards.

## MATERIALS AND METHODS

### Sample collection, purification, and enrichment

Samples were collected in screw-capped sterile bottles of four liters from the Ismailia canal in Cairo, Egypt (30°06'59.7"N, 31°17'07.3"E) from a depth of 1- 2 meters. Temperature (°C), oxygen (mg/ L), and water pH were all measured at once after the sample was collected, and their values were 21, 5.5, and 7.7, respectively. The collected water sample was analyzed using a Flame Atomic Absorption Spectrophotometer (Savant AA Atomic Absorption Spectrophotometer from GBC Scientific Equipment in the Central Laboratory, Faculty of Sciences, Ain Shams University) for copper (Cu), lead (Pb), and cadmium (Cd) at mg/ l to confirm the purity of the water sample, which was addressed for three heavy metals at 0.01mg/ l.

Following that, the water samples were extensively checked under the microscope on a regular basis. The ciliates were examined with 10x and 40x magnification. Several species were examined, photographed, and recognized. *Paramecium* species were isolated and grown on wheat grain medium. The medium's pH was set at 7.2 at room temperature. Algae were removed from the samples by exposing them to semi-darkness. The physical characteristics, specific body

form, behavior, and motions of *Paramecium* species were used for identification **(Shakoori *et al.*, 2014)**.

To purify the culture, many procedures were applied. Mechanical methods for *Paramecia* isolation include selecting *Paramecium* from water one by one with a micropipette under a microscope **(Ishida & Hori, 2017)**. The procedures were repeated until the needed paramecia was obtained. A depression slide was also employed for purification. A ten µl drop of water was put on a glass slide and focused under the light microscope's 40X power. After much washing with distilled water, the drop with only one *Paramecium* was placed on the depression slide. In each depression, one grain of wheat and 200µl of distilled water were added. Every day, a little drop of 10µl of water was collected from the depression slide, and the number of cells was observed under the microscope **(Hyman, 1931)**.

**Estimation of optimum growth conditions**

The optimum growth of *Paramecium* was checked at different concentration ratios between the sample and media for 15 days. The purified culture in its log phase was used as the inoculum for both parameters. Wheat grain medium (~20gm boiled wheat grains in one-liter distilled $H_2O$, used day after tomorrow) was used to check the growth patterns of *Paramecium* **(Hyman, 1931; Twagilimana *et al.*, 1998; Li *et al.*, 2022)**. A certain volume (100ml) of medium and water sample with optimum pH 7.1± 0.1 and 25± 1°C was kept at different ratios (1:1; 1:2) with and without yeast for 15 days. The *Paramecia* cells per mL were counted after every 24 hours under the lens of a light microscope at 40X for both parameters. All the experiments described were performed in triplicate.

**Growth assay under different metal stress**

A purified culture of *Paramecium* (100 cells) was inoculated in a 250-ml conical flask with 30ml of wheat grain medium. In the log phase of the organism, different concentrations of salts of heavy metals were added to the flasks to determine the minimum inhibitory concentration of these metals and enumerate the living cells at 3, 6, 12, 24, and 48hr. Lead stress was presented as $Pb_2(CH_3O_2)_2$ with varying concentrations of 30, 80, and 150µg $ml^{-1}$; copper stress was obtainable as $CuSO_4.5H_2O$ with concentrations of 10, 30, and 60µg $ml^{-1}$, and cadmium stress was accessible as $CdCl_2$ with varying concentrations (10, 25, and 50µg $ml^{-1}$). Flasks were kept at optimum temperature and pH. No more metal ions were added when *Paramecium* species became dead, which decided the minimum inhibitory concentration (MIC) for each metal. The morphological changes occurring due to metal stress were detected, and the number of diving cells was counted by taking a drop of 10µl observed under the light microscope. The growth patterns with metal stress, without metal stress (+ve control), and without media (-ve control) samples were plotted with the mean values estimated for 1ml of the medium. The experiment was performed in triplicate **(Aubusson-Fleury *et al.*, 2015)**.

**Calculation of median lethal concentration ($LC_{50}$)**

$LC_{50}$ is the abbreviation used for the exposure concentration of a toxic substance that is lethal to half of the test animals. In the present study, $LC_{50}$ was calculated by the probit analysis method using SPSS® software version 26.

**Statistical analysis**

Three independent replicates were used to record each value. Error bars are used to display means and standard deviations (SD).

## RESULTS AND DISCUSSION

**Growth characteristics of *Paramecium***

A growth pattern of *Paramecium* of different concentration ratios between the sample and media with and without yeast was observed under optimum conditions of pH 7.1± 0.1 and 25± 1°C for 15 days **(Zahara *et al.* 2023)**. The best growth rate of *Paramecium* species was noted without yeast in different concentration ratios (Table 1). **Pritchard *et al.* (2016)** in their study observed that *P. aurelia, P. caudatum,* and *Euplotes patella* cannot grow on yeast. On the other hand, the difference in concentration ratios showed a definite fall in the growth of organisms with ratio (1:2) which appeared as the most adverse concentration ratio in the case of both media. Furthermore, the organism showed better growth and behavior in concentration ratios between the sample and media (1:1) of wheat grain medium without yeast as compared to the addition of yeast with concentration ratios (1:1), hence we preferred to use the concentration ratios (1:1) of wheat grain medium without yeast for further experiments.

**Table 1.** Growth of *Paramecium* at different concentration ratios (A) with yeast and (B) without yeast

| Time (Days) | (1:1) Count/ mL | | (1:2) Count/ mL | |
|---|---|---|---|---|
| | A | B | A | B |
| 1 | 60 | 90 | 36 | 56 |
| 2 | 110 | 240 | 71 | 123 |
| 3 | 230 | 530 | 168 | 485 |
| 4 | 580 | 960 | 450 | 870 |
| 5 | 985 | 1520 | 732 | 1480 |
| 6 | 1350 | 1880 | 1204 | 1750 |
| 7 | 1612 | 2312 | 1470 | 1940 |
| 8 | 1760 | 2560 | 1540 | 2130 |
| 9 | 1853 | 2653 | 1655 | 2340 |
| 10 | 1770 | 2130 | 1530 | 2050 |
| 11 | 1463 | 1763 | 1410 | 1580 |
| 12 | 1210 | 1390 | 1150 | 1260 |
| 13 | 1030 | 1160 | 943 | 1030 |
| 14 | 843 | 960 | 760 | 870 |
| 15 | 580 | 670 | 488 | 560 |

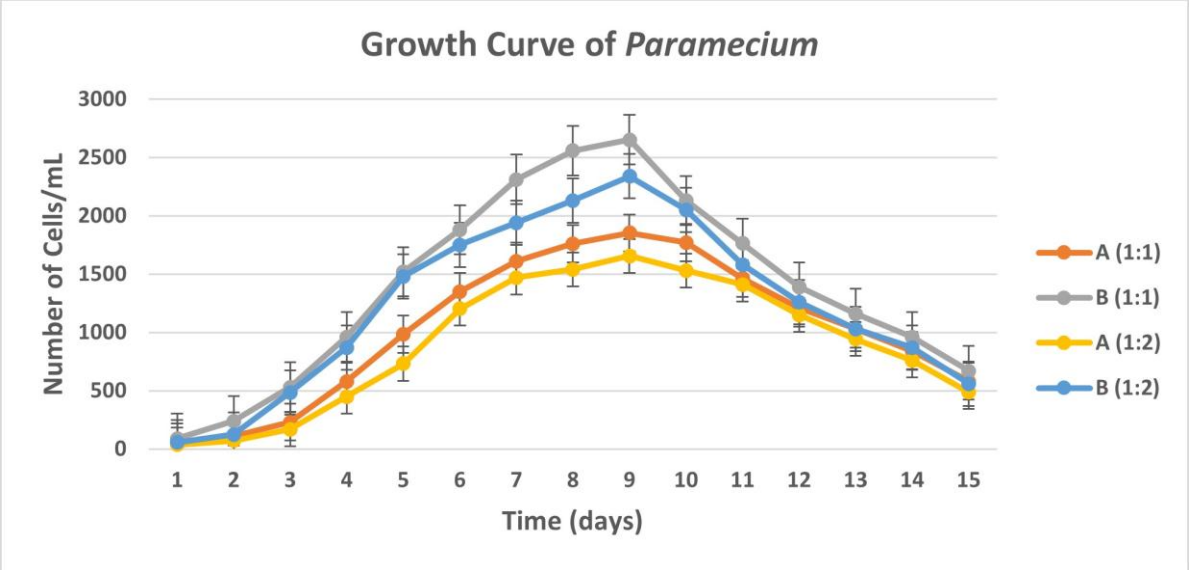


**Fig. 1.** Growth curve of *Paramecium* at different ratios (A) with yeast and (B) without yeast

**Non-stressed *Paramecium* sp.**

The careful microscopic examination and measurements of cultured *Paramecium* sp. revealed that the body length is short (100– 150µm long); the attenuated posterior end is rounded, and the macronucleus is large, rounded, with 33– 36 µm in diameter, located close to the anterior dorsal border of the buccal cavity. A clear region between the nuclear membrane and the chromatic area appeared. The micronuclei are two in number and about 3µm in diameter. The cytoplasm contains many types of symbiotic bacteria (Figs. 2, 3, 4). The present *Paramecium* species could be a member of the *P. aurelia* complex since its morphological features matched those described by **Sonneborn (1975)** for the *P. aurelia* complex. *Paramecium aurelia* complex consists of fourteen sibling species; he characterized and named the former syngens 1– 14 of *P. aurelia* as follows: *P. primaurelia, P. biaurelia, P. triaurelia, P. tetraurelia, P. pentaurelia, P. sexaurelia, P. septaurelia, P. octaurelia, P. novaurelia, P. decaurelia, P. undecaurelia, P. dodecaurelia, P. tredecaurelia,* and *P. quadecaurelia.* **Sonneborn (1975)** distinguished each new species based on a few characteristics, such as morphological features, geographical distribution, bacterial endosymbiont species, mating reactions, breeding relations, and mode of inheritance of mating type, in addition to zymograms by starch gel electrophoresis for one or more groups of enzymes. Following the guidelines and criteria established by **Sonneborn (1975)**, a new member of the *P. aurelia* complex was added by **Aufderheide *et al.* (1983)**, who described *Paramecium sonnehorni* sp. as the latest member of the *aurelia* complex in honor of the late Dr. Tracy M. Sonneborn of Indiana University. Therefore, further investigation is needed to confirm the present identification of *Paramecium* sp. **Beale *et al.* (1969)** reported that the endosymbionts live within *Paramecium aurelia* and consist of several different Gram-negative bacteria, and it is not known whether in nature this relationship is mutually beneficial or not. The symbiont isolated from one *Paramecium* can kill other Paramecia that lack this type of symbiont.

They were identified to seven classes of endosymbiotic organisms: *Kappa*, *mu*, *lambda*, *sigma*, *gamma*, *delta*, *nu*, and *alpha* particles **(Stevenson, 1972)**. All are cytoplasmic symbionts except *alpha* ones, which are nuclear symbionts found in the macronucleus.

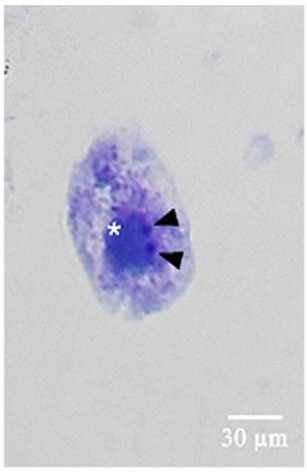


Fig. 2


Fig. 3

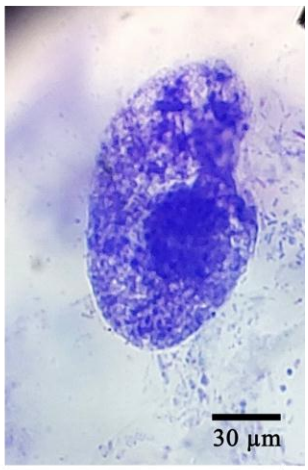


Fig. 4

**Fig. 2.** Photomicrograph of a *Paramecium* sp. showing the spherical macronucleus (asterisk) with two small round micronuclei (black arrowheads), Giemsa stain

**Fig. 3.** Photomicrograph of *Paramecium* sp. showing the macronucleus (white arrowhead) and the contractile vacuole (black arrowhead), Giemsa stain

**Fig. 4.** Photomicrograph of *Paramecium* sp. showing many symbiotic bacteria into the cytoplasm, Giemsa stain

**Heavy metals stressed *Paramecium* sp.**

After exposing *Paramecium* sp. to soluble compounds of cadmium, copper, and lead, at several selected concentrations, the mortality rate was recorded after 3, 6, 12, 24 and 48 hours of incubation in different concentrations under constant conditions. Additionally, the $LC_{50}$ values were determined and the results are represented in Table (2).

Microscopic observations revealed that all the concentrations (10, 30 and 60µg $ml^{-1}$) of copper were highly toxic to *Paramecium* sp. since the organisms became motionless and died within a few minutes (Fig. 5). The animals appeared to have suffered from no gross deformity of any kind apart from a slight shrinkage, and the dead organisms remained intact, little disintegrated if at all. According to **Shunmugam *et al.* (2021)**, the incidence of heavy metals rapidly affects the movement of Paramecia, resulting in a considerable decrease in their speed even when copper sulphate concentrations are just half of those regarded as harmful for drinking water. Furthermore, this observation was corroborated by **Modoni and Romeo (2006)**, who reported that copper was more toxic to ciliate species than Cd, Cr, or Pb in their work on the severe toxicity of some heavy metals toward four freshwater ciliates (*Colpidium colpoda*,

*Dexiotricha granulosa*, *Euplotes aediculatus*, and *Halteria grandinella*). **Al-Rasheid and Sleigh (1994)** investigated the effects of copper and other heavy metal toxicity on the feeding rate of the marine hypotrichous ciliate *Euplotes mutabilis*. They subjected cells to two different concentrations of the metal close to the lethal limit for one hour and found that *Euplotes mutabilis* survived in 0.25mg $l^{-1}$ Cu for 60% of the time. Only 25% survived after one hour in a 1.25mg $l^{-1}$ copper solution, and this concentration caused a significant decrease in the feeding rate of the survivors. In experiments with the ciliate *T. pyriformis*, copper

**Table 2.** The mortality rate of paramecia at the different concentrations of pollutants (copper, lead, cadmium) along period intervals from 3 to 48hr

| Time | Copper (Cu) | | | Lead (Pb) | | | Cadmium (Cd) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 ppm | 30 ppm | 60 ppm | 30 ppm | 80 ppm | 150 ppm | 10 ppm | 25 ppm | 50 ppm |
| **3hr** | 100% | 100% | 100% | 63.1% (±7.9) | 82.2% (±6.1) | 92.2% (± 0.5) | 51.8% (± 9.8) | 47.8% (± 4.5) | 18.8% (± 6) |
| **6hr** | 100% | 100% | 100% | 100% | 100% | 100% | 42% (± 9) | 42% (± 4) | 19% (± 1) |
| **12hr** | 100% | 100% | 100% | 100% | 100% | 100% | 52% (± 5) | 30% (± 11) | 14% (± 3) |
| **24hr** | 100% | 100% | 100% | 100% | 100% | 100% | 48% (± 2) | 23% (± 3) | 16% (± 4) |
| **48hr** | 100% | 100% | 100% | 100% | 100% | 100% | 45% (±0.07) | 20% (±0.02) | 22% (± 0.03) |

inhibited growth significantly above 200mg$l^{-1}$ **(Nicolau *et al.*, 1999)**.

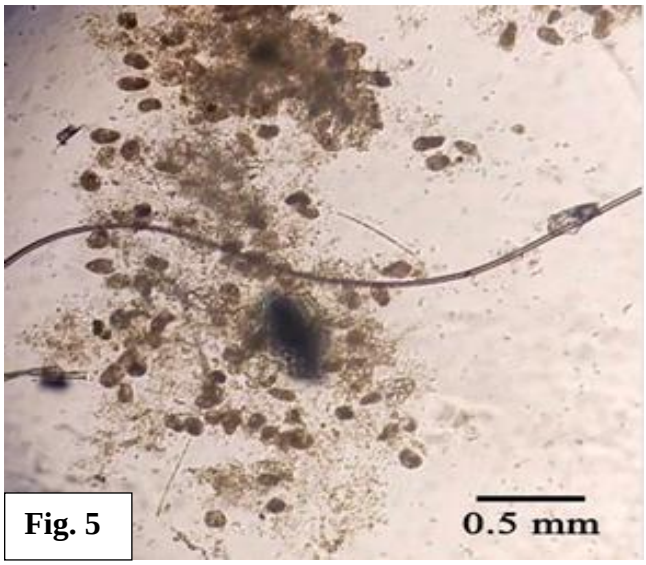


**Fig. 5.** Photomicrograph of *Paramecium* sp. treated with 30µg $ml^{-1}$ of copper showing died organisms

Concerned with *Paramecium* sp. exposed to lead, no full mortality was observed in animals within concentrations up to 150ppm before 3hr incubation. All tested concentrations (30, 80, 150µg $ml^{-1}$) induced full mortality at 6hr incubation (Fig. 7). The value of $LD_{50}$ of this pollutant was 19.11µg $ml^{-1}$. The morphological alterations included protrusion of a single ectoplasmic blister which occurred at one pole of some animals (Fig. 6). A considerable degree of body swelling coupled with some endoplasmic clumping took place as evidence for the opposite effect of the pollutant on plasma membrane permeability, which was noted by **Wang (1963)** on *P. aurelia* exposed to cigarette components. **Al-Rasheid and Sleigh (1994)** discovered that exposure to lead at a concentration of 0.65mg $l^{-1}$ for 10min resulted in slight (not significantly) decrease in the feeding uptake rate of the ciliate *E. mutabilis*. In a low-protein medium, *Tetrahymena* exhibits a decrease in food vacuole formation of 4mM lead acetate, and cell growth stops **(Nilsson, 1979)**.

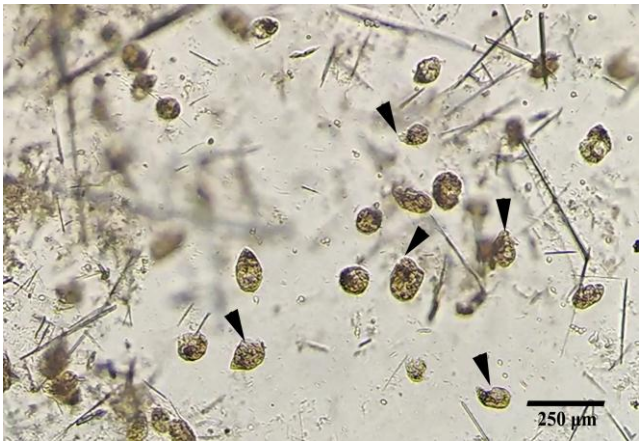


**Fig. 6.**

**Fig. 6.** Photomicrograph of *Paramecium* sp. treated with 80µg $ml^{-1}$ of lead showing a morphological alteration which includes protrusion of a single ectoplasmic blister at one pole of some animal (black arrowheads)

No full mortality was seen in *Paramecium* sp. communities exposed to all tested cadmium concentrations (10, 25, 50µg $ml^{-1}$) during the time of the experiment. The values of $LD_{50}$ of this pollutant at 3, 6, 12hr of incubation were 13.9, 8.08, 11.2µg $ml^{-1}$, respectively. Moreover, an unexpected observation was recorded since the mortality rate decreased as the cadmium concentrations and the exposed time increased (Table 2 & Fig. 8). This agrees with the observation of **Zahara *et al.* (2023)** who reported that, *paramecia* detached cadmium ions from the culture medium in a highly interesting pattern, with the absorption percentage of metal increasing with cumulative metal concentration, reaching a maximum uptake of 90% at 30µg $ml^{-1}$ after 96 hours of exposure. In some circumstances, the growth of *Paramecium* at higher concentrations is faster than at lower ones. On the other hand, *Paramecium triaurelia*, is more tolerant of high temperatures and cadmium than *Paramecium primaurelia*. Furthermore, the

organism is expected to gain resistance and the ability to thrive and reproduce in a stressful environment. This might be related to the microorganism's tolerance to the presence of chemical contaminants in the natural environment **(Shunmugam *et al.*, 2021)**. In addition, **Fernandes-Leborans and Antonio-Garcia (1988)** reported that *Colpidium colpoda* lived for 39 days at a concentration of 0.5mg Cd $l^{-1}$, and this verifies the high tolerance to Cd and other heavy metals shown in the study of **Madoni and Romeo (2006)**. Such tolerance to Cd could be due to the balance achieved between the oxidative stress induced by Cd and the antioxidant defense stimulated by the same metal, such balance is usually found in aerobic systems **(Benlaifa *et al.*, 2016)**. In addition, compartmentalization of metals in cytoplasmic granules or membrane-bound vesicles is widespread in organisms and is one of the tolerance mechanisms against metal toxicity **(Piccinni, 1989; Viarengo & Nott, 1993)**.

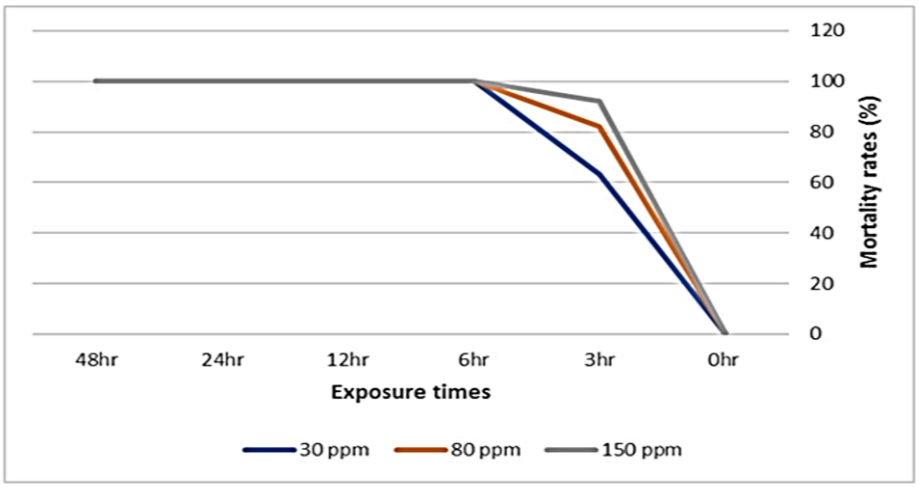


**Fig. 7.** The mortality rates caused by differ **Fig. 7.** ntrations (30, 80, 150µg $ml^{-1}$) of lead pollutant along time scale (from 3 to 48hr)

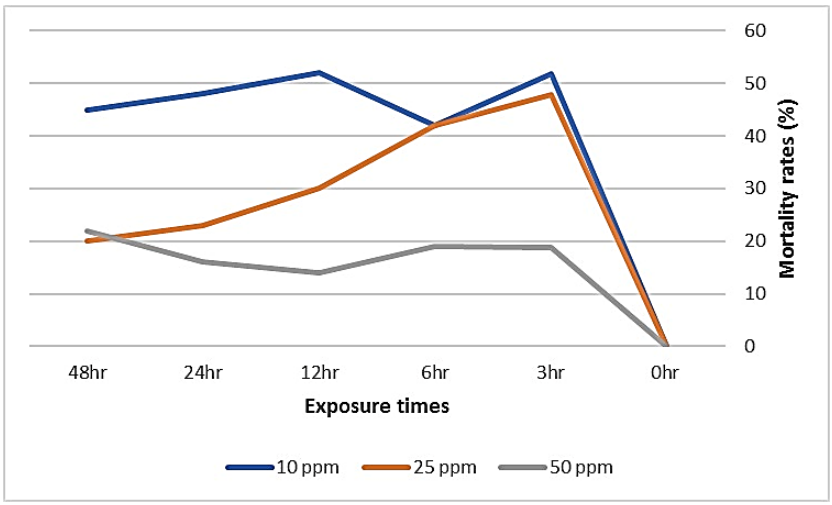


**Fig. 8.** The mortality rates caused by different concentrations (10, 25, 50µg $ml^{-1}$) of cadmium pollutant at time interval (from 3 to 48 hr)

## CONCLUSION

The purpose of the study was to determine the heavy metal sensitivity of *Paramecium* sp. and to supply prognostic information for water quality standards. The ciliated protozoan was cultivated and subjected to varying amounts of lead, copper, and cadmium. The species may belong to the complex *P. aurelia*. Lead resulted in complete mortality, while copper displayed moderate toxicity. No mortality was observed with cadmium at any quantity. With increasing cadmium contents and exposure duration, the death rate dropped. This preliminary study exploring the toxicity of certain heavy metals against *Paramecium* sp. would be helpful for further investigation using the same organism in eco-toxicological tests and applications, and specifically in the case of contamination of aquatic environments and systems, with heavy metals and Cd in particular. In the same context, the present authors will conduct additional studies to further investigate this promising research subject.

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## Arabic Summary

### دراسة مبدئية عن سمية بعض العناصر الثقيلة تجاه الحيوان الاولي الهدبي "برامسيوم"

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**في هذه الدراسة تم العثور على الحيوان الاولي الهدبي "برامسيوم" في عينات من المياه العذبة من ترعة الإسماعيلية، القاهرة، مصر. تم تنمية مستعمرته في المختبر، ثم تم تعريضها لأيونات المعادن الثقيلة من الكادميوم والنحاس والرصاص بتركيزات مختلفة. وتهدف هذه الدراسة الي تقييم حساسية البرامسيوم للمعادن الثقيلة، وذلك للحصول على أداة تنبؤ بيولوجية لتقييم جودة المياه. الفحص المجهري الدقيق والقياسات المورفوميترية كشفت أن نوع البرامسيوم موضوع البحث قد ينتمي الى مجموعة *Paramecium aurelia* نظرًا لأن سماته المورفولوجية تتطابق مع تلك التي وصفها عالم الاوليات Sonneborn عام 1975، ولذا يلزم إجراء مزيد من الفحوصات والدراسات للتحقق من صحة التعريف لنوع البرامسيوم موضوع الدراسة الحالية. أما فيما يتعلق بسمية المعادن الثقيلة ضد *Paramecium* sp المستزرع، ظهر أن النحاس بتركيزات 10 و30 و60 ميكروجرام/مللي شديد السمية على البرامسيوم، حيث أصبحت الكائنات الحية بلا حراك وماتت في غضون بضع دقائق بعد التعريض. لم يلاحظ وفيات كاملة في البرامسيوم المعرض لتركيز 150 ميكروجرام/مللي من الرصاص قبل 3 ساعات من الحضانة، ولكن تسببت جميع تركيزات الرصاص المختبرة 30، 80، 150 ميكروجرام/مللي في النفوق الكامل لمستعمرات البرامسيوم بعد 6 ساعات من التعريض. أيضا لم يلاحظ أي وفيات كاملة في مستعمرات البرامسيوم التي تعرضت لتركيزات الكادميوم 10 و25 و50 ميكروجرام / مللي طوال فترة التجربة التي امتدت الي 48 ساعة. تم تسجيل ملاحظة غير متوقعة وهي إنخفاض معدل الوفيات مع زيادة تركيزات الكادميوم وأمتداد فترة التعريض. هذه الدراسة الأولية لاستكشاف سمية بعض المعادن الثقيلة ضد البرامسيوم سوف ستكون مفيدة عند إجراء المزيد من البحوث باستخدام نفس الكائن الحي في اختبارات وتطبيقات السمية البيئية، وتحديداً في حالة تلوث البيئات والأنظمة المائية بالمعادن الثقيلة وخاصة الكادميوم.**