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The Effects of Thallium Acetate on Hepatopancreatic Cells of *Gammarus pulex* (Crustacea: Amphipoda)

Fatma OZGUL OZALP[1], Mehtap KUTLU[1]*, Arzu ISCAN[2]

[1]Anadolu University, Faculty of Science, Department of Biology 26470 Eskisehir-TURKIYE

[2]Anadolu University, Plant, Medicine and Scientific Research Center, 26470 Eskisehir-TURKIYE

*Corresponding author: hmkutlu@anadolu.edu.tr

Abstract

In our study, the toxic effects of thallium on *Gammarus pulex* a sensitive indicator organism for environmental pollution was studied for cytological changes. According to studies carried out with thallium acetate, this chemical was observed to lead to cellular changes in the hepatopancreas of the *Gammarus pulex*. In our studies, by using EPA acute toxicity tests, the LC_{50} value was found to be 0.244 mg/L. The LC_{50} value was calculated using the EPA Probit Analysis Program. The cytological changes to *Gammarus pulex* when exposed to thallium was examined with the Transmission Electron Microscope. Due to thallium intoxication, degenerative changes were frequently present in the cellular membranes; there were changes in the mitochondria as partial or total loss of cristae, there was an increase in the number of lipid droplets, lysosomes and autophagic vacuoles were found to have increased in the hepatocytesand the nucleus showed significant shrinkage and deformation. We also observed fragmentation and dilation of the rough endoplasmic reticulum (RER) and the number of lesions also increased in the inner and outer mitochondrial membranes and in the RER. A lot of lipid droplets were also observed in the hepatocytes.
Keywords: Cytopathology, *Gammarus pulex*, hepatopancreatic cell, thallium.

Talyum Asetat'ın *Gammarus pulex* Hepatopankreas Hücreleri Üzerindeki Etkileri

Özet

Çalışmamızda, talyumun toksik etkisinin, çevre kirliliği için hassas bir indikator canlı olan *Gammarus pulex*'de meydana getirmiş olduğu sitolojik değişiklikler çalışılmıştır. Talyum asetat ile yapılan çalışmalarda bu kimyasalın *Gammarus pulex*'in hepatopankreasında hücresel değişikliklere yol açtığı gözlenmiştir. Çalışmamızda, 96 saat süren EPA akut toksisite testi kullanılarak LC_{50} değeri 0,244 mg/L bulunmuştur. LC_{50} değeri EPA Probit Analiz Programı kullanılarak hesaplanmıştır.

Talyumun toksik etkisinin *Gammarus pulex*'de meydana getirdiği sitolojik değişiklikler Geçirimli Elektron Mikroskobu kullanılarak çalışılmıştır. Talyum kimyasalına maruz kalma, sık sık hücre membranında dejeneratif değişikliklere neden olmaktadır. Mitokondrilerde de kısmen yada tamamen krista kaybına, lipid damlacıklarında artışa neden olmaktadır. Bizim çalışmamızda hepatosit lizozomlarında ve otofajik vakuollerde bir artış olmuştur. Çekirdekte belirgin bir büzülme ve bozulma gözlenmiştir. Endoplazmik retikulumda parçalanma ve genişleme gözlemlenmiştir. Mitokondri iç ve dış zarları ile granüllü endoplazmik retikulumda ince yapı değişiklikleri belirlenmiştir. Hepatositlerdeki lipid damlacıklarında da azalma gözlemlenmiştir.
Anahtar Kelimeler: *Gammarus pulex*, hepatopankreas hücresi, sitopatoloji, talyum.

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INTRODUCTION

Thallium (Tl) has been identified to be an environmentally significant element because of its toxic effects. It is a heavy metal available in a number of soluable salts such as acetate, sulfate, and carbonate. Apart from Thallium there were a lot of studies about heavy metals being exposed to the aquatic ecosystem (Cicek and Koparal 2001, Dogan and Saygıdeger 2009, Ayas et al. 2009, Cigerci et al. 2010, Guner 2010). Thallium is extremely toxic in a aqueous solution (Arabinda et al. 2007). It is also known that this metal is still employed for many purposes such as optical, costume jewelry, cement, photographic, and the electronic industries, along with high-tech industries including semi conductors, scintillation counters, low-temperature thermometers ,and special glasses (Lan and Lin 2005). Despite the World Health Organization's suggestion to ban its usage as a pesticide in 1973, which many countries followed, thallium

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intoxication still occurs world wide (Lan and Lin 2005).

Tl compounds have also been used as medicines, rodenticides, and insecticides (Townshend et al. 1995). Water is probably the most studied environmental sample and in fact the major part of the Tl studies have been carried out on water.

The development of new methods for selective separation, preconcentration, purification, and determination of this metal continues to be a challenging problem. The need for highly sensitive and reliable method for the determination of ultra trace level thallium has been recognized in analytical and environmental chemistry (Arabinda et al. 2007). Thallium has been applied as a rodenticide and as a medicine to treat ringworm, gonorrhea, syphilis, and tuberculosis. More recently Tl has been used in the diagnosis of myocardial infarction or ischemia (Braunwald et al. 1987) because, Tl scintigraphy has increased sensitivity in the detection of coronary diseases. Since this widely used metal is toxic to mammals, there have been many reported cases of Tl intoxication. For example, people are poisoned by the intake of rat poisons (homicidal and suicidal attempts), by chronic exposures in occupations exposed to Tl (such as the workers in cement factories or workers handling pyrites), and by contact to the ash from coal-combustion power plants (Thompson et al. 1981). Thallium is a cumulative poison and the retention in various tissues increases with age. Symptoms of Tl intoxication in humans include nausea, vomiting, abdominal pain, hair loss, alopecia, tachycardia, and cardiac arrhythmias. Death may result from cardiac failure (Anderson 1984) or respiratory failure (Mayfield et al. 1983). It is also a neurotoxin which causes tremor, ataxia, ptosis of the eyelids, painful lower extremities, paresthesias of hands and feet after a few days of intoxication (Thompson et al. 1981).

The amphipod *Gammarus pulex* (L) is a major component of the biomass of many streams (Welton 1979) and is sensitive to a wide range of pollutants (Williams et al. 1984, McCahon and Pascoe 1988, Kutlu et al. 2002). The fresh water amphipod *G. pulex* as a sensitive indicator organism for environmental pollution. It has been used as a test organism for aquatic toxicology for many years (Macek et al. 1976).

Recent studies show that thallium concentration in the water in some contaminated areas is very high (Lan and Lin 2005) and, there is very little available data on the aquatic toxicity of thallium. Biomagnification of thallium in aquatic ecosystems has been reported at very high levels (Lin et al. 2001).

Gammarus pulex has been particularly popular for this reason and it is why we choose *Gammarus pulex* for our study about the toxic effects of thallium.

This study suggests that the effects of acute thallium toxicity can be seen clearly as some cytopathological changes in the cells of the hepatopancreas.

MATERIAL AND METHODS

Collecting and Keeping Sample

The *Gammarus pulex* was collected from the Porsuk River in Eskişehir (Turkey). We selected male organism for this study with lengths from 6 mm to 10 mm. and then transferred them to the laboratory. The animals were them acclimated to laboratory conditions in a recirculating aquarium containing tap water for at least 7 days prior to use. Air was continuously supplied and the temperature was controlled at 10-12ºC) with a photoperiod of 12 hour light anf 12 hour dark . the control groups were maintained in conditioned tap water with a pH of 7.

Thallium solutions were prepared by dissolving thallium acetate in distilled water. For the determination of LC_{50}, 6 groups of 10 animals were exposed to six different thallium acetate concentrations (0.1 mg/L, 0.2 mg/L, 0.3 mg/L, 0.4 mg/L, 0.5 mg/L and 0.6 mg/L) for 96 h.

The test organism, *G. pulex* was exposed to thallium acetate for 96 h and they were not fed. Mortalities in each concentration were recorded and all experiments were carried out in triplicate.

The LC_{50} (96 h) value and its confidence limits were calculated using the EPA Probit Analysis Program (Horning and Weber 1985). The LC_{50} value was found to be 0.244 mg\L. This value is based on the mortalities after 96 h exposure to the toxicant (Table 1). The animals were exposed for a single time period (96 h) in an LC_{50} toxicant concentration. The control group was not exposed to the toxicant.

Dissection and Electron Microscopy

The same dissection and electron microscopy techniques and procedures were applied to both the experimental and control samples. Dissection was accomplished in a fixation medium consisting of a

4% glutaraldehyde solution buffered to a pH of 7.4 with an 0.1 M phosphate buffer. All extremites were taken out to accomplish the dissection easily. The first fixation was done in 4% glutaraldehyde for 24 h.

Tissue samples were washed with an 0.1 M phosphate buffer (pH 7.4) for 1 h. with the Buffer being changed every 15 min. Post fixatio continued with a 2% osmic acid at room temperature for 2 h in a rotator. The fixation tissue samples were them washed twice with a phosphate buffer for 15 min. Following the second washing the tissue samples were dehydrated twice through an ice-cold graded ethanol series; in 30, 50, 70 and 90% ethanol, for 15 min then twice in 96% ethanol for 30 min, and then twice in 100% ethanol for 30 min, at room temperature. The tissue samples were then placed propylene oxide for twice 30 min, and then were placed in 1:1 mixture of propylene oxide/araldite for 1 h. Samples were than embedded into pure araldite and kept overnight at room temperature. Polimerization was made at 60ºC for 48 h. Thick sections were stained with toluidine blue (Fig. 1) and ultrathin sections were stained for 1 h with a 2% uranile acetate/lead citrate (Bancroft and Gamble 2002). Observations were made using a Tecnai Bio Twin FEI Transmission Electron Microscope at 120 kV.

RESULTS AND DISCUSSION

In this study, the toxic effects of thallium acetate on *G. pulex* as a sensitive indicator organism for environmental pollution was conducted. The freshwater invertebrate has been used as test organism for aquatic toxicology for many years (Macek et al. 1976, Kutlu and Sumer 1998). According to the studies carried out with thallium acetate, it has been observed that this chemical lead to the cellular changes in the hepatopancreas of *G. pulex*. During previous studies the LC_{50} value of thallium was found to be 0.8 mg/kg in rats (Galvan-Arzate et al. 2000). In our studies, LC_{50} (96 h) value and its confidence limits were calculated by the EPA Probit Analysis Program (Horning and Weber 1985). This value is based on mortalities after a 96 h exposure to the toxicant and a further 24 h in toxicant free water. The LC_{50} value was found to be 0.244 mg/L using with EPA Probit Analysis Program.

Therefore, this study characterized thallium's relative hazard in aquatic ecosystem by evaluation of the acute toxicity of thallium acetate to the invertebrate *G. pulex* hepatopancreatic cell.

In this study, the toxic effect of thallium acetate on *G. pulex* was studied for cytological changes.

Light microscobic micrographs of the hepatopancreatic cell in the control and experimental groups are shown in Fig. 1. In the ultrastructural examination, there was no deformation in the nucleus and mitochondria of the hepatopancreatic cells of the control group and no cellular or metabolic changes were observed. The integrity of the nucleus and nuclear membrane was preserved and endoplasmic reticulum and the membrane integrity were normal. Cristae had normal appearance and dense microvilli. Furthermore, the level of the fat cells in the normal metabolism was low Fig. 2.

G. pulex was exposed to thallium acetate with an LC_{50} value for 96 h. Consequently, deformations were observed in the nucleus and particularly the nuclear membrane of the hepatopancreatic cells, and appearence of the microvilli. Fat cells increased, cristae of mitochondria deformed and the length and number of microvilli sharply decreased. Additionally, we observed degenerated mitochondria, dilation, and the disconnection of the endoplasmic reticulum when exposed to thallium acetate in the test organism Fig. 3 and Fig. 4.

Although this toxic element is rapidly distributed and accumulated in all organs and tissues (Rossi et al. 1987, Galvan-Arzate et al. 2000) its remains are unknown. Indeed toxicity caused by thallium has been widely investigated, but the thallium toxicity mechanism is yet unclear (Rios et al. 1989, Galvan-Arzate and Rios 1994, Appenroth and Gambaryan 1998, Repetto et al. 1998).

The composition of the hepatopancreatic tissue and the crustacean body in general is highly dependent on the molt stage in which the animal is analysed (Vonk 1960, Kutlu et al. 2002). In this study the male Gammarid sample was used.

The alimentary canal of Crustacea is essentially a straight tube with one to several pairs of caeca almost always present. Malacostraca has usually become modified to from the glandular tissue of the digestive apparatus commonly referred to as the hepatopancreas (Schultz 1974, Kutlu et al. 2002). The results of the bpresent study indicates theresults of an important organ, such as the hepatopancreatic cells,to thallium acetate.

Various mechanisms of thallium toxicity have

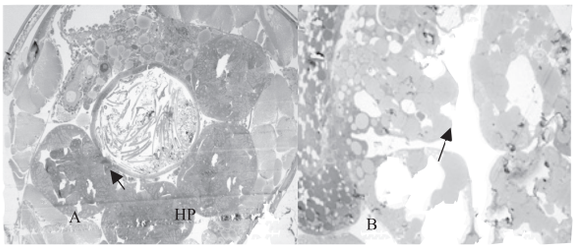


Fig 1. Semi thin section of hepatopancreatic cells (1000 nm)x 20
A. The hepatopancreatic cell of the control group. (MG: midgut, HP: hepatopancreatic cell).
B. The hepatopancreatic cell of the experimental group, exposed to thallium for 96 h. (L: lipid)

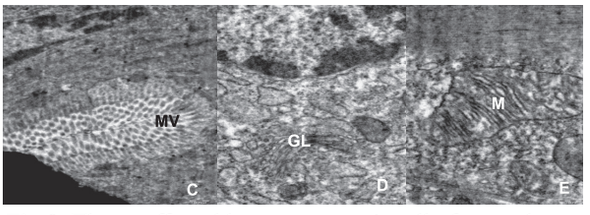


Fig 2. The uneffected hepatopancreatic cell of control organism's thin sections (70-80 nm).
C, D, E control; hepatopancreatic cell architecture showing normal appearance
(MV; microvillus, M; mitocondria, GL; golgi apparatus)

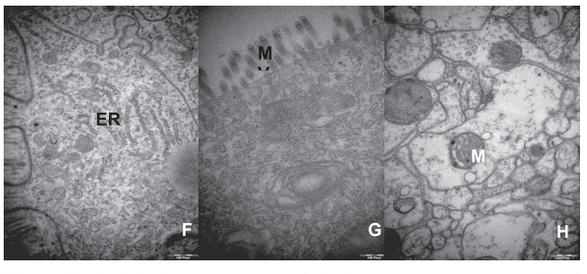


Fig 3. F, G, H ultrastructure of the hepatopancreatic cell of *Gammarus pulex* in the experimental group.
Endoplasmic Reticulum (ER); disconnected endoplasmic reticulum, G.MV; irregular microvillus H.M; swollen mitochondria cristea exposed to thallium acetate (x 26500)

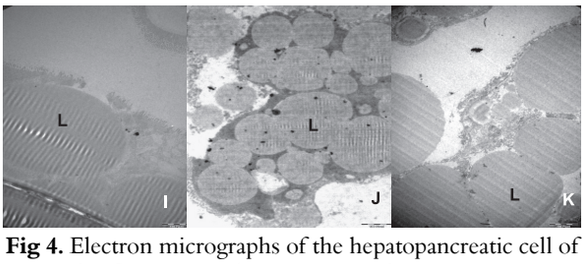
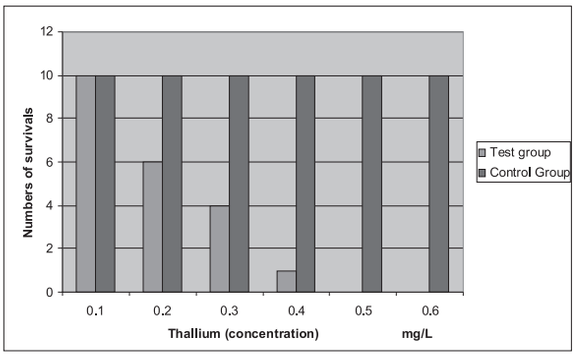


Fig 4. Electron micrographs of the hepatopancreatic cell of the *Gammarus pulex* experimental group.
Increased vacuolization and lipid materials were seen in test organism exposed to thallium. L; lipid (Ix2550, Jx16500, Kx4200).

been suggested one of them being thalliums ability to potassium ions, in its ionic charge and crystal

Table 1. Survival data for Gammmarus pulex exposed to several TI (thallium acetate) concentrations for 96 h.



radius (Lohmann and Wiegand 1996). Its toxicity can be its interaction with the membrane, membrane proteins and membrane dependent enzymes (Diaz and Montreal 1994).

Hasan and Ali (1981) and Aoyama et al. (1988) report TI induced peroxidation in various tissues. Results show that thallium toxicity is closely related with increased reactive oxygen species that play an important role in cell and tissue damage (Hasan and Ali 1981, Aoyama et al. 1988).

Depending on those changes in organelles, their special functions are surely affected. Mitochondrial damages affect ATP synthesis, endoplasmic reticulum (ER) and changes influence protein synthesis (Kutlu et al. 2002, 2005) an increase in the number of vacuoles, lipid droplets, and a decrease in the number of microvilli are often considered as nonsprecific stress responses. The accumulation of lipid droplets indicates that the decline of protein synthesis that accompanies cellular injury. When heavy metals enter a cell, they stimulate the cell to produce some defensive stress induced organelles. Thallium acetate can with a negative charge present on the organelle membrane, result in the change in the cell membrane permeability and integrity.

In their study similir to ours, Yang and Chen (2003), studied the hepatocyte ultrastructure of the common carp after gallium exposure and observed lipid inclusions, nucleus deformation, increase in the number of lysosomes, and ultrastructural changes of the organelles. The metal may consequently inhibit the physiological functions of some organs. Invertebrates are generally more sensitive to pollutants than either fish or algae (Yang and Chen 2003). We chose the freshwater amphipod *Gammarus pulex* as a sensitive indicator organism for

enviromental pollution.

Woods and Fowier (1986) have reported that the hepatocytes of the liver of TI-intoxicated rats caused ribosomes to be lost from the endoplasmic reticulum, swelling of the mitochondria and others cellular injuries (Wood and Fowier 1986).

Consequently, further studies must be carried out in order to characterize the precise mechanism of thallium toxicity. It can be useful to perform more detailed histopathological studies on the hepatopancreas of *G. pulex* exposed to thallium toxicity in order to support the findings of this study.

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