Effect of mercury contamination on the hepatopancreas ultrastructure of Armadillo officinalis duméril, 1816 (crustacea, isopoda)

EFFECT OF MERCURY CONTAMINATION ON THE HEPATOPANCREAS ULTRASTRUCTURE OF *ARMADILLO OFFICINALIS* DUMÉRIL, 1816 (CRUSTACEA, ISOPODA)

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ملخص

تأثير الزئبق على البنكرياس لدى *Armadillo officinalis* Duméril, 1816 : هدفت هذه الدراسة إلى تقييم تأثير تلوث التربة بواسطة الزئبق على البنكرياس في *Armadillo officinalis* Duméril, 1816 . جمعت عينات غير ملوثة من ضفاف بحيرة غار الملح ثم تعرضت لمدة ثلاثة أسابيع إلى ثلاثة تركيزات من محلول ملح الزئبق. بعد نهاية التعرض، تم مقارنة البنكرياس لدى الحيوانات المعرضة وغير المعرضة لتلوث للكشف عن التغيرات النسيجية.

أظهرت ملاحظات الفحص ألمجهري للإرسال الإلكتروني أن كبد الحيوانات المعرضة للزئبق أظهر تغيرات مورفولوجية ونسيجية مقارنة بحيوانات الغير معرضة لتلوث حتى عند أقل تركيز. لقد أظهرنا أن درجة هذه التعديلات كانت تعتمد على الجرعة. وكانت السمات السائدة هي: تعطل الحدود microvillus، وتكثيف بعض مناطق السيتوبلازم ، والكروماتين ، وشبكة الإندوبلازمية الخام والتغييرات في الميتوكوندريا ، وتعديل قطرات الدهن بالإضافة إلى زيادة عدد حبيبات B في الخلايا B وS.

الكلمات المفاتيح : قشريات ، زئبق ، بنية تحتية ، جهاز تخزين.

ABSTRACT

This study aimed to evaluate the effect of substrate contamination by mercury on the hepatopancreas of the Crustacean species; *Armadillo officinalis* Duméril, 1816. Uncontaminated specimens were collected from the banks of Ghar El Melh lagoon then exposed for three weeks to three concentrations of mercury salt solution. After the end of the exposure, the hepatopancreas of unexposed and exposed animals were compared to detect histological changes.

Transmission Electron Microscopy observations showed that the hepatopancreas of Hg-exposed animals showed morphological and histological changes compared with control animals even at the lowest concentration. The degree of these alterations was found to be dose-dependent. The global predominant features were: microvillus border disruption, condensation of some cytoplasm areas and of chromatin, rough endoplasmic reticulum and mitochondrial alterations, lipid droplets modifications in addition to the increasing number of B-granules in the B and S cells.

Keywords: Crustaceans, mercury, ultrastructure, storage organ.

RÉSUMÉ

Effet de la contamination au mercure sur l'ultrastructure de l'hepatopancreas chez *Armadillo officinalis* Duméril, 1816 (Crustacea, Isopoda) : Cette étude vise à évaluer l'effet de la contamination du substrat par le mercure sur l'hépatopancréas de l'espèce de Crustacés; *Armadillo officinalis* Duméril, 1816. Des échantillons non contaminés ont été recueillis sur les rives de la lagune de Ghar El Melh, puis exposés pendant trois semaines à trois concentrations de solution de sel de mercure. A la fin de l'exposition, l'hépatopancréas des animaux non exposés et exposés ont été comparés pour détecter les modifications histologiques.

Les observations en microscopie électronique à transmission ont montré que l'hépatopancréas des animaux exposés au mercure présentait des changements morphologiques et histologiques par rapport aux animaux témoins, même à la concentration la plus faible. Le degré de ces altérations s'est avéré dépendant de la dose. Les principales caractéristiques globales étaient: la rupture de la frontière des microvillosités, la condensation de certaines zones du cytoplasme et de la chromatine, l'altération du réticulum endoplasmique rugueux et des mitochondries, la modification des gouttelettes lipidiques en plus du nombre croissant de granules B dans les cellules B et S.

Mots clés: Crustacés, mercure, ultrastructure, organe de stockage.

INTRODUCTION

Invertebrates are usually used to assess the effects of anthropogenic activities on the terrestrial and coastal ecosystems, as they are in contact with toxic elements in the soil and the leaf litter (Heikens et *al.*, 2001). Among them, terrestrial isopods, important primary decomposers participating in the mineralization of organic matter and in the litter process (Paoletti et Hassall, 1999; Zimmer et Topp, 1999; Gongalsky et *al.*, 2005), play a crucial role in the early succession of the restoration process in polluted or damaged ecosystems (Frouz et *al.*, 2006, 2007). Due to their ability to cope with high amounts of heavy metals, isopods became favourite models for ecotoxicological studies (Hopkin et *al.*, 1993; Vijver et *al.*, 2005). These organisms are easily sampled and identified, commonly abundant in addition to their capacity to be reared under laboratory conditions and to survive even in heavily contaminated areas (Hussein et *al.*, 2006; Loureiro et *al.*, 2006; Mazzei et *al.*, 2013; Longo et *al.*, 2013).

Crustacean isopods have shown their ability to accumulate heavy metals from food or soil. Their tolerance to these elements is related to their physiological adaptation by compartmentalizing essential and nonessential metals in their hepatopancreas (Wieser, 1979; Hopkin et Martin, 1982; Hopkin, 1990; Raessler *et al.*, 2005). Although the hepatopancreas represents only 5% of isopod's body weight, it remains the main organ of metal storage as reported in the morphological and histological studies of Hopkin et Martin (1982, 1984) and Wieser (1979). Essential heavy metals like copper, manganese, nickel and zinc are required by organisms in small amounts (Epstein et Bloom, 2004). Contrastingly, nonessential metals such as aluminum, arsenic, cadmium and mercury are not required for normal biological function and may quickly lead to toxicity (Boyd et Rajakaruna, 2013). Recently, the effects of Cd, Cu and Zn on the growth of *A. officinalis* individuals as well as the histological hepatopancreas features have been studied under laboratory exposure conditions (Khemaissia *et al.*, 2019a). In continuity with the obtained data, we aim, in the present work, to evaluate the morphological and ultrastructural changes induced by mercury contaminated substrate in the hepatopancreas of this species.

MATERIALS AND METHODS

Isopod collection

Individuals of *A. officinalis* were sampled from the supralittoral banks of Ghar El Melh lagoon (N 37°10'12" E 010°12'6") situated in north-east Tunisia. This site was considered as a reference site since it is far from any source of contamination (Jelassi *et al.*, 2013).The collected specimens were held in transparent plastic containers. These later were weekly moistened to ensure constant soil humidity. Individuals were fed on a disk of carrots and maintained under laboratory conditions of temperature (20 ± 2°C) and photoperiod under the exposure.

Laboratory procedure

The sediment collected from the study site was sterilized and dried in an oven at 90°C for 24h. It was then impregnated homogeneously with 10 mL (1 mL/1g) of three mercury chloride solutions ($HgCl_2$) according to Köhler *et al.,* (1996) as follows: 0.3, 0.6 and 0.9 mg/L Hg. A soil sprayed with only distilled water has been prepared as a control. Once they were spiked, the substrate was acclimated at 25°C during three days. We prepared two replicates for each concentration using 10 isopods per replicate.

Transmission Electron Microscopy

For the transmission electron microscopy, we adopted the protocol described in Jelassi *et al.,* (2018, 2019) and Khemaissia *et al.,* (2019a, b). At the end of the exposure, individuals were sacrificed. The hepatopancreas was fixed in 3% glutaraldehyde, in sodium cacodylate 0.3 M and NaCl 3% at pH 7.3, for 6h at room temperature. Then hepatopancreas were washed (Sucrose 0.8 M, sodium cacodylate 0.3 M, NaCl 3% at pH 7.3) and post-fixed for 2h at room temperature in OsO_4 4%, sodium cacodylate 0.3 M and NaCl 5.5%. They undergo a series of acetone (50%, 70%, 90% and 100%). Samples were placed in an EPON resin-acetone mixture for some hours and then transferred to the pure EPON resin for 24h. Finally, samples were polymerized at 70°c for 24h. Semithin sections were cut on an Ultramicrotome Leica with a diamond knife (Diatome) and observed with an Olympus CX 31 light binocular microscope. The ultrathin sections (70nm) were transferred to carbon-coated films on 1 mm copper whole grids and stained with uranyl acetate 2% for 1 min and lead citrate for 10 min and examined in a Jeol-JEM 1010 TEM at 80 kV with an Ultrascan 894 GATAN digital camera (512*512 pixels) and Microphoton LC micro software.

RESULTS AND DISCUSSION

As shown in other oniscidean species, the hepatopancreas in untreated animals showed, globally, similar ultrastructure features (Wägele, 1992). We identified two types of cells (Fig. 1A): the B cells, drome-shaped, projects apically into the lumen of the hepatopancreas, whereas the S cells, conoidal in form, are much shorter than B cells (Marcaillou *et al.,* 1986; Witkus *et al.,* 1987; Hames et Hopkin, 1989; Leser *et al.,* 2008).

Numerous tubular invaginations characterizing the membranous labyrinth of the basal plasmalemma of S cells were shown (Fig. 1A). Compared with S cells, the cytoplasm of B cells was softly darker, quite denser and containing lipid droplets of different sizes, abundant mitochondria distributed throughout the cell, lysosomes and various granule's glycogen (Fig. 1B). Apically, the B cells were surmounted by well-developed and thick microvilli (Fig. 1A).

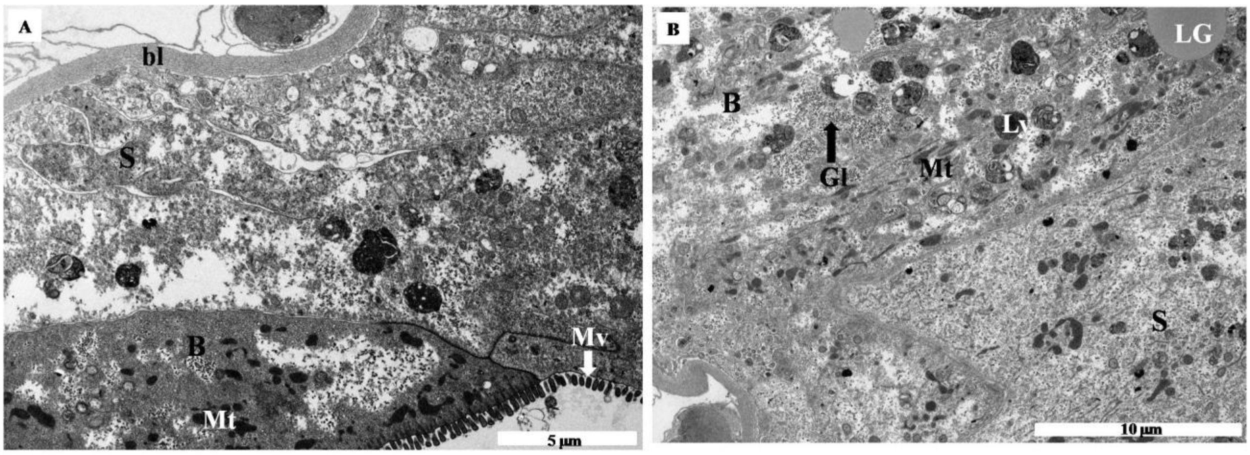

Figure 1: TEM micrographs of hepatopancreatic tubules of control specimens of *Armadillo officinalis*. (B) B cells; (S) S cells; (bl) basal lamina; (Mv) microvilli; (LD) lipid granules; (Mt) mitochondria; (Gl) glycogen granule; (Ly) lysosome.

Mercury-exposed animals showed morphological and histological changes even at the lowest concentration. The observed changes depend on the metal dose. When exposed to 0.3 mg/L Hg, the microvilli border was affected (Fig. 2A and B). The disorganization of the microvillus border, resulting in a drastic reduction in the absorption of nutrients (Köhler *et al.,* 1996; Longo *et al.,* 2013), was the most significant cellular target affected by metals. A similar effect was described for *Armadillidium granulatum* and *Ligia italica* subjected to mercury contamination (Khemaissia *et al.*, 2019b; Longo *et al.*, 2013) and for *Porcellio scaber* exposed to Cd, Pb and Zn contaminations (Köhler *et al.*, 1996). The accumulation of trace metal elements was highlighted in S cells at C1 Hg. Additionally, the condensation of mitochondria on the side of the microvilli was observed (Fig. 2B). Mitochondrial alterations were marked by the swelling, reduction or complete disappearance of their cristae. Such changes were detected in *L. italica* consequentially to Hg accumulation (Longo *et al.,* 2013), in *P. scaber* under food contamination (Znidarsic *et al.*, 2003) and in *A. granulatum* when contaminated with Cd, Hg, and Ni (Khemaissia *et al.*, 2019b). Tarnawska *et al.* (2007) noted that metals exposure caused mitochondrial dysfunctions suggesting respiratory metabolism.

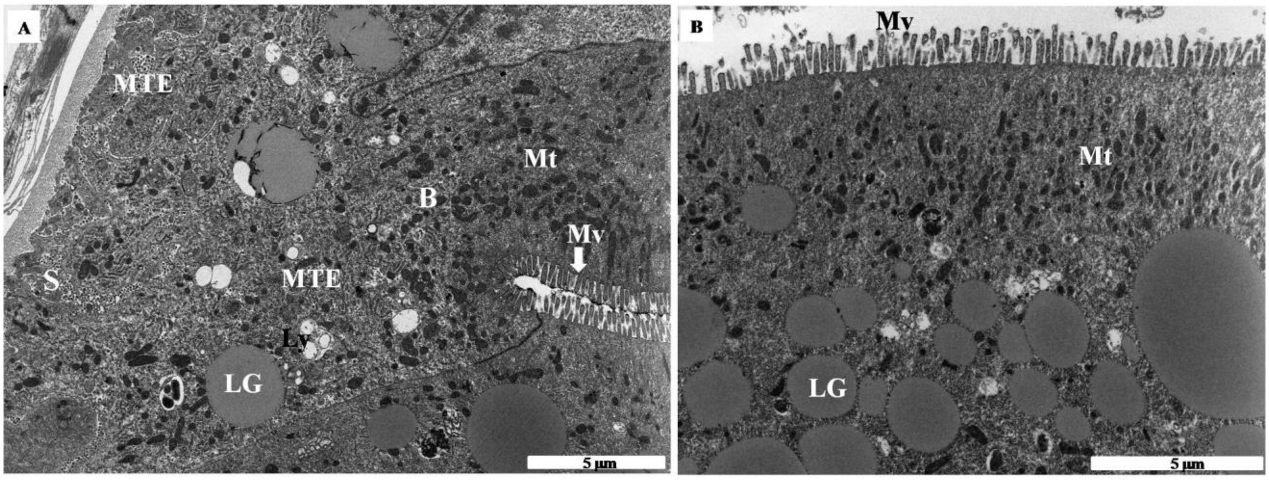

Figure 2: TEM micrographs of hepatopancreatic tubules of specimens of *Armadillo officinalis* submitted to 0.3 mg/L Hg exposure. (B) B cells; (MTE) metal trace element; (Mv) microvillus; (Ly) lysosome; (LG) lipid granule; (Mt) mitochondria.

When contaminated with 0.6 mg/L Hg, an increase in the number of lipid droplets occurred, some of them, non-homogeneous, contained electron-lucent areas which ultrastructurally looked similar to lipofuscin (Fig. 3). According to Hames et Hopkin (1991), the accumulation and the subsequent release of lipid droplets were related to the daily digestive cycle. Leser *et al.* (2008) reported that during fasting, lipids within the hepatopancreas were used as a source of energy in *P. scaber*. However, for other species, lipid droplets number tend to reduce or to disappear (Drobne et Strus, 1996; Strus et Blejec, 2001) due to the reduction of nutrient absorption by the hepatopancreatic epithelium resulting from the destruction of the brush border (Mazzei *et al.,* 2014). Additionally, we observed chromatin condensation resulting from a nuclear volume reduction and to cell osmolarity alteration (Köhler *et al.,* 1996). This feature was already described in the hepatopancreas of *P. scaber* exposed to Cd and Zn contaminated substrate (Znidarsic *et al.,* 2003), as well as of *A. granulatum, A. vulgare* and *P. laevis* exposed to Cd and Pb contamination (Mazzei *et al.,* 2014). Another change resulting from C2 Hg was the increase in the amount of endoplasmic reticulum. This last was arranged into regular rows or into concentric circles (Fig. 3).

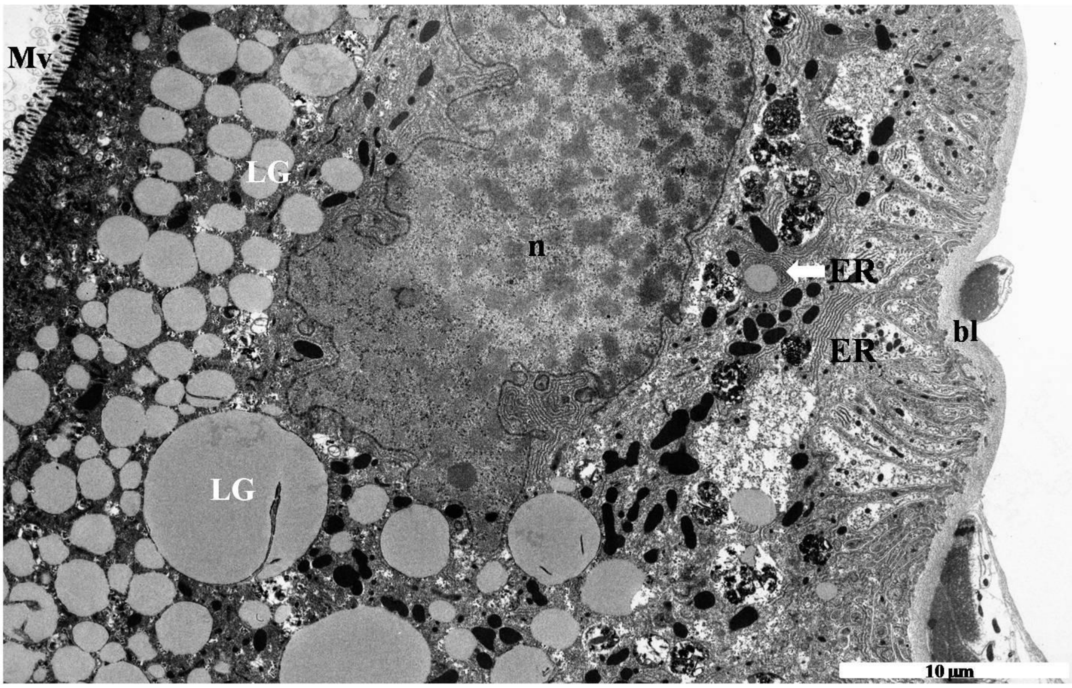

Figure 3: TEM micrographs of hepatopancreatic tubules of specimens of *Armadillo officinalis* submitted to 0.6 mg/L Hg exposure. (ER) endoplasmic reticulum; (LG) lipid granule; (bl) basal lamina; (n) nucleus.

After 0.9 mg/L Hg, exposure, we observed a major alteration principally represented by an increase in the number of vesicles of different shape and size where the trace elements were accumulated (Fig. 4A) additionally to cell remoteness (Fig. 4B).

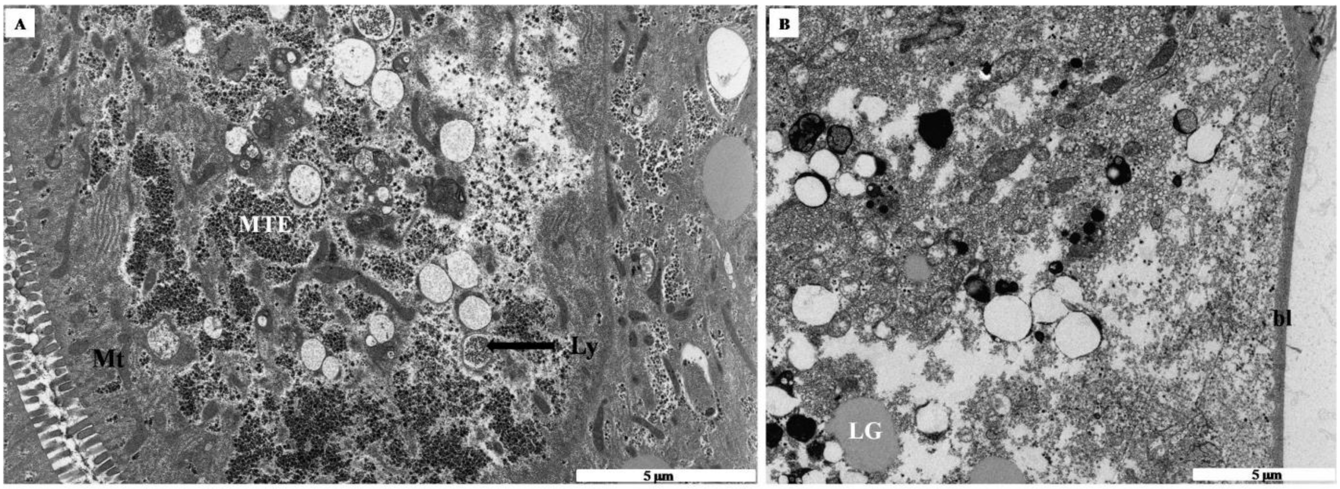

Figure 4: TEM micrographs of hepatopancreatic tubules of specimens of *Armadillo officinalis* submitted to 0.9 mg/L Hg exposure. (MTE) metal trace element; (Ly) lysosome; (LD) lipid droplet; (Mt) mitochondria; (LG) lipid granule.

In fact, once metals were incorporated, isopods were able to regulate trace elements by storing them in the form of granules (Hopkin, 1989), by detoxification or elimination, and by faecal and urinal excretion (Donker, 1992) to avoid their toxicity.
Our data confirm, once more, what is already known in the literature on the different ability of terrestrial isopods to be adequate biomarkers and a good candidate for monitoring pollution by heavy metals because of their capacity to accumulate heavy metals. The ultrastructural changes could be considered as a good indicator for evaluating the effects of metal exposure in the target cells (Znidarsc *et al.,* 2003). Furthermore, the observed changes were found to be dose-dependent and a function of the metal.

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