

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

H. KHEMAISSIA ^(1*), R. JELASSI ⁽¹⁾, C. GHEMARI ⁽¹⁾, M. RAIMOND ⁽²⁾, C. SOUTY-
GROSSET ⁽²⁾ et K. NASRI-AMMAR ⁽¹⁾

(1) University of Tunis El Manar, Faculty of Sciences of Tunis, LR18ES06 Laboratory of Diversity, Management and Conservation of Biological Systems, 2092, Tunis, Tunisia

(2) University of Poitiers, Laboratory of Ecology and Biology of Interactions (UMR CNRS 7267 EBI), Team Ecology Evolution Symbiosis, Poitiers Cedex 9, France

* hajer_kh@yahoo.fr

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 المجهر الإلكتروني أن كبد الحيوانات المعرضة للزئبق أظهرت تغيرات مورفولوجية ونسجية مقارنة بحيوانات الغير معرضة لتلوث حتى عند أقل تركيز. لقد أظهرنا أن درجة هذه التعديلات كانت تعتمد على الجرعة. وكانت السمات السائدة هي: تعطيل الحدود microvillus، وتكثيف بعض مناطق السيتوبلازم، والكروماتين، وشبكة الإندوبلازمية الخام والتغيرات في الميتوكوندريا، وتعديل قطرات الدهون بالإضافة إلى زيادة عدد حبيبات B في الخلايا B و S.

الكلمات المفتاحية: 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'hépatopancréas 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 ($20 \pm 2^\circ\text{C}$) 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 (Hg Cl_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, dome-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).

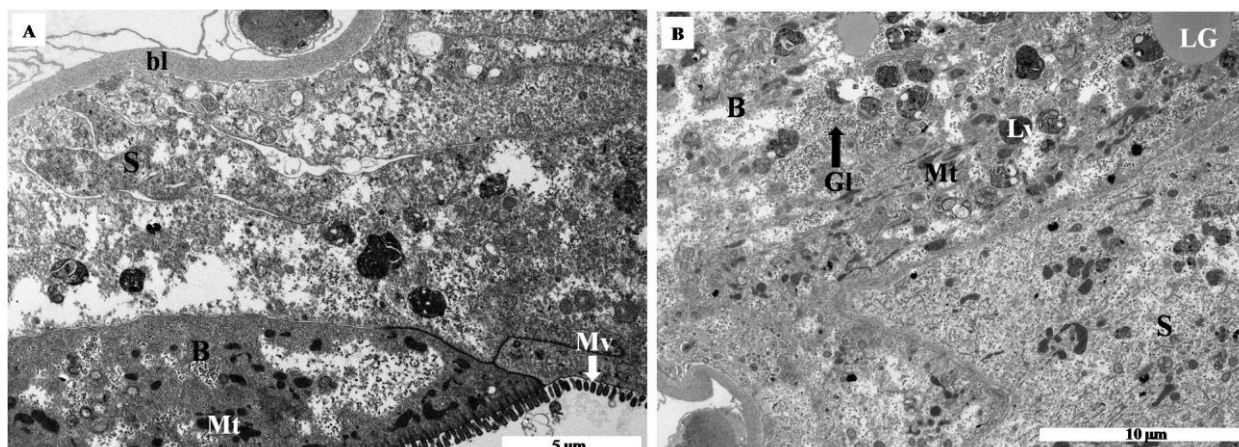


Figure 1: TEM micrographs of hepatopancreatic tubules of control specimens of *Armadillo officinalis*. (B) B cells; (S) S cells; (bl) basal lamina; (Mv) microvilli; (LG) lipid granules; (Mt) mitochondria; (Gl) glycogen granule; (Ly) lysosome.

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 microvilli 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.

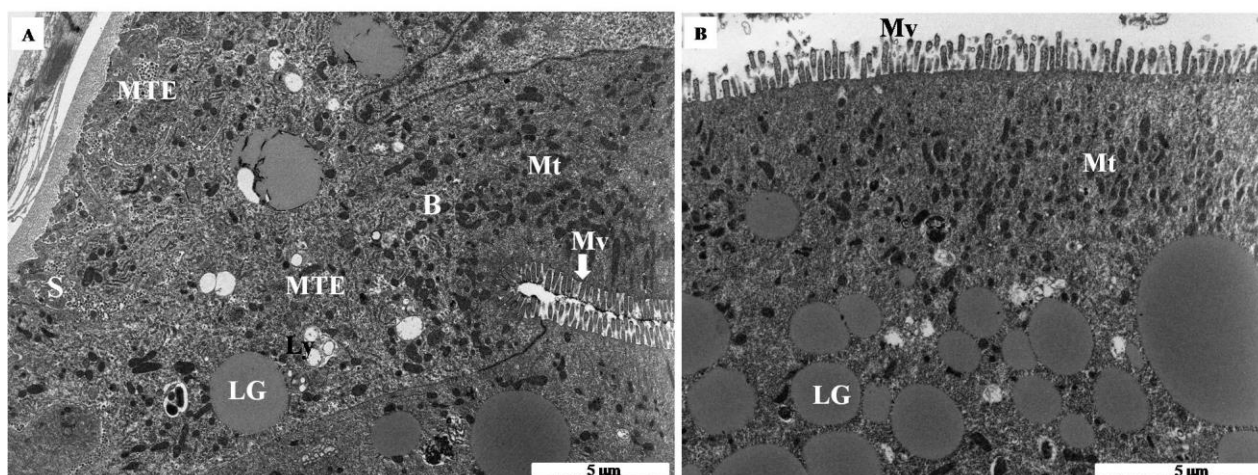


Figure 2: TEM micrographs of hepatopancreatic tubules of specimens of *Armadillo officinalis* submitted to 0.3 mg/L Hg exposure. (B) B cells; (MTE) metal trace element; (Mv) microvilli; (Ly) lysosome; (LG) lipid granule; (Mt) mitochondria.

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).

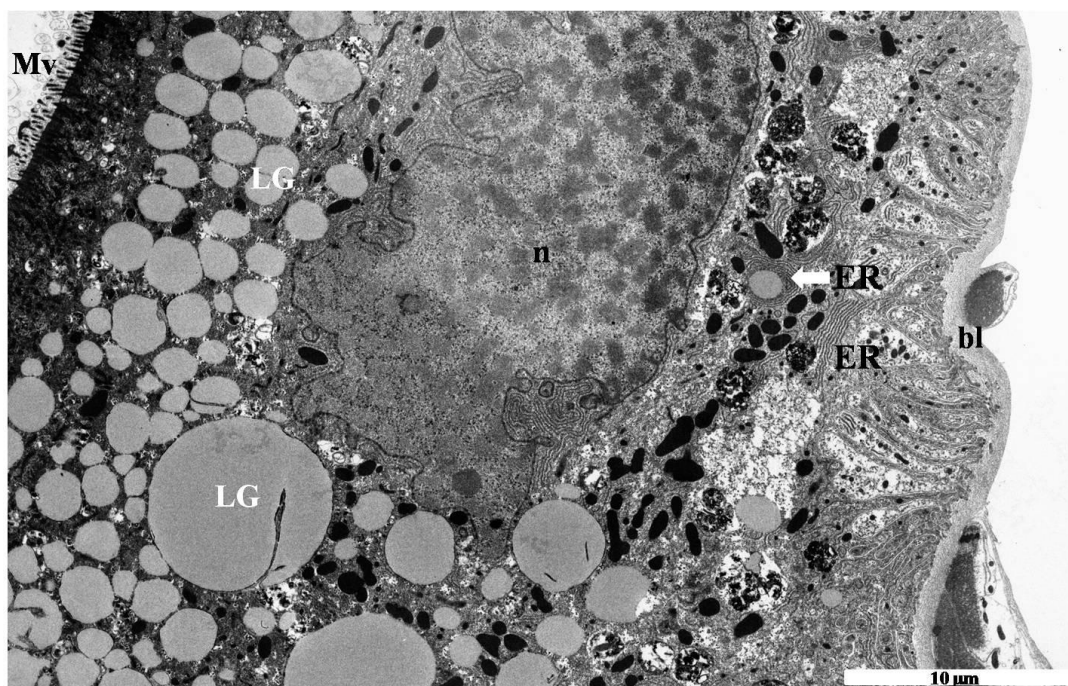


Figure 3: TEM micrographs of hepatopancreatic tubules of specimens of *Armadillo officinalis* submitted to 0.6 mg/L Hg exposure. (ER) endoplasmic reticulum; (LG) lipid granule; (bl) basal lamina; (n) nucleus.

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).

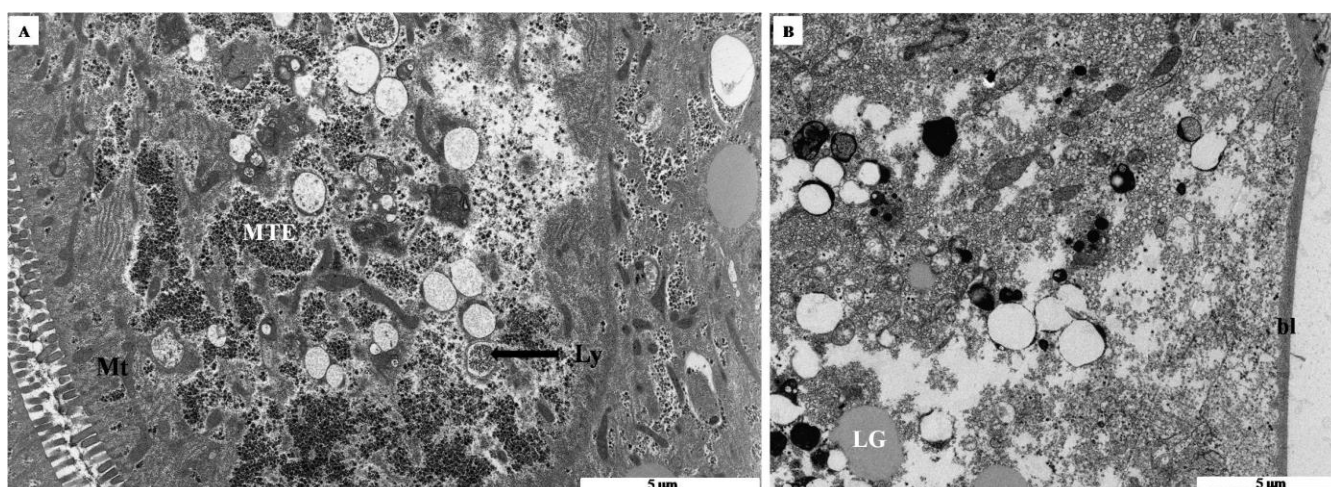


Figure 4: TEM micrographs of hepatopancreatic tubules of specimens of *Armadillo officinalis* submitted to 0.9 mg/L Hg exposure. (MTE) metal trace element; (Ly) lysosome; (LD) lipid droplet; (Mt) mitochondria; (LG) lipid granule.

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.

Acknowledgements

The present study was funded by the Laboratory of Diversity, Management and Conservation of Biological Systems (LR18ES06), University of Tunis El Manar, Faculty of Sciences of Tunis. TEM observations were made in "Image UP" service at the University of Poitiers. Financial support by the Erasmus Mundus Al Idrisi 2015 program for a postdoctoral position at the University of Poitiers is gratefully acknowledged.

BIBLIOGRAPHY

- Boyd R.S., Rajakaruna N., 2013. - Heavy metal tolerance. In: Gibson D (ed). Oxford bibliographies in ecology. Oxford University Press, New York.
- Donker M.H., 1992. - Energy reserves and distribution of metals in populations of the isopod *Porcellio scaber* from metal-contaminated sites. *Funct. Ecol.* 6: 445-454.
- Drobne D., Štrus J., 1996. - The effect of Zn on the digestive gland epithelium of *Porcellio scaber* (Isopoda, Crustacea). *Pflügers Arch. Eur. J. Physiol.* 431: 247-248.
- Epstein E., Bloom A.J., 2004. - Mineral nutrition of plants: principles and perspectives. Sinauer, Sunderland.
- Frouz J., Elhottova D., Kuraz V., Sourkova M., 2006. - Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites: Results of a field microcosm experiment. *Appl. Soil Ecol.* 33: 308-320.
- Frouz J., Vaclav P.I., Tajovsky K., 2007. - The effect of earthworms and other saprophagous macrofauna on soil microstructure in reclaimed and un-reclaimed post-mining sites in central Europe. *Eur. J. Soil Biol.* 43: 184-189.
- Gongalsky K.B., Savin FA., Pokarzhevskii A.D., Filimonova Z.V., 2005. - Spatial distribution of isopods in an oak-beech forest. *Eur. J. Soil Biol.* 41: 117-122.
- Hames C.A.C., Hopkin S.P., 1989. - The structure and function of the digestive system of terrestrial isopods. *J. Zool. Lond.* 217: 599-627.
- Hames C.A.C., Hopkin S.P., 1991. - Assimilation and loss of ¹⁰⁹Cd and ⁶⁵Zn by the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. *Bull. Environ. Contam. Toxicol.* 47: 440-447.
- Heikens A., Peijnenburg W.J.G.M., Hendriks A.J., 2001. - Bioaccumulation of heavy metals in terrestrial invertebrates. *Environ. Pollut.* 113: 385-393.
- Hopkin S.P., 1989. - Ecophysiology of metals in terrestrial invertebrates. London: Elsevier, pp.366.
- Hopkin S.P., 1990. - Critical concentrations, pathways of detoxification and cellular ecotoxicology. *Funct. Ecol.* 4: 321-327.
- Hopkin S.P., Martin M.H., 1982. - The distribution of zinc, cadmium, lead and copper within the woodlouse *Oniscus asellus* (Crustacea, Isopoda). *Tissue & Cell.* 14: 703-715.
- Hopkin S.P., Martin M.H., 1984. Heavy metals in woodlice. *Symp. Zool. Soc. Lon.* 53: 143-166.
- Hopkin S.P., Jones D.T., Dietrich D., 1993. - The isopod *Porcellio scaber* as a monitor of the bioavailability of metals in terrestrial ecosystems: Towards a global "woodlouse watch" scheme. *Sci. Total Environ.* 357-365.
- Hussein M.A., Obuid-Allah A.H., Mohammad A.H., Scott-Fordsmand J.J., Abd El-Wakeil K.F. 2006. - Seasonal variation in heavy metal accumulation in subtropical population of the terrestrial isopod, *Porcellio laevis*. *Ecotoxicol. Environ. Saf.* 63: 168-174.
- Jelassi, R., Zimmer, M., Khemaissia, H., Garbe-Schonberg, D., Nasri-Ammar, K. 2013. - Amphipod diversity at three Tunisian lagoon complexes in relation to environmental conditions. *J. Nat. Hist.*, 47: 2849-2868
- Jelassi R., Ghemari C., Khemaissia H., Raimond M., Souty-Grosset C., Nasri-Ammar K., 2019. - An assessment of copper, zinc and cadmium contamination and their ecotoxicological effects in *Orchestia mediterranea* Costa, 1853(Amphipoda, Talitridae). *Chem. Ecol.* 35: 361-378.
<https://doi.org/10.1080/02757540.2018.1554062>.
- Jelassi R., Khemaissia H., Ghemari C., Raimond M., Souty-Grosset C., Nasri-Ammar K., 2018. - Ecotoxicological effects of trace element contamination in talitrid amphipod *Orchestia montagui* Audouin, 1826. *Environ. Sci. Pollut. Res.* 26: 5577-5587. <https://doi.org/10.1007/s11356-018-3974-y>.

- Khemaissia H., Jelassi R., Ghemari C., Raimond M., Souty-Grosset C., Nasri-Ammar K., 2019a. - Evaluation of trace element contamination using *Armadillo officinalis* Duméril, 1816 (Crustacea, Isopoda) as a tool: An ultrastructural study. *Microsc. Res. Tech.* 1-12. <https://doi.org/10.1002/jemt.23371>.
- Khemaissia H., Jelassi R., Ghemari C., Raimond M., Souty-Grosset C., Nasri-Ammar K., 2019b. - Effects of trace metal elements on ultrastructural features of hepatopancreas of *Armadillidium granulatum* Brandt, 1833 (Crustacea, Isopoda). *Microsc. Res. Tech.* 1-13. <https://doi.org/10.1002/jemt.23349>.
- Köhler H.R., Hüttenrauch K., Berkus M., Gräff S., Alberti G., 1996. - Cellular hepatopancreatic reactions in *Porcellio scaber* (Isopoda) as biomarkers for the evaluation of heavy metal toxicity in soils. *Appl. Soil Ecol.* 3: 1-15.
- Leser V., Drobne D., Vilhar B., Kladnik A., Znidarsic N., Strus J., 2008. - Epithelial thickness and lipid droplets in the hepatopancreas of *Porcellio scaber* (Crustacea: Isopoda) in different physiological conditions. *Zoology.* 111: 419-432.
- Longo G., Trovato M., Mazzei V., Ferrante M., Oliveri Conti G., 2013. - *Ligia italica* (Isopoda, Oniscidea) as bioindicator of mercury pollution of marine rocky coasts. *PLoS One.* v8 (3). <http://dx.doi.org/10.1371/journal.pone>.
- Loureiro S., Sampaio A., Brandão A., Nogueira A.J.A., Soares A.M.V.M. 2006. - Feeding behavior of the terrestrial isopod *Porcellionides pruinosus* Brandt, 1833 (Crustacea, Isopoda) in response to changes in food quality and contamination. *Sci. Total Environ.* 369: 119-128.
- Marcaillou C., Truchet M., Martoja R., 1986. - Rôle des cellules S de l'épithélium caecal des Crustacés Isopodes dans la capture et la dégradation de protéines hémolymphatiques, et dans le stockage de catabolites (acide urique, sulfure de cuivre, phosphates). *Can. J. Zool.* 64: 2757-2769.
- Mazzei V., Longo G., Brundo M.V., Copat C., Oliveri Conti G., Ferrante M., 2013. - Effects of heavy metal accumulation on some reproductive characters in *Armadillidium granulatum* Brandt (Crustacea, Isopoda, Oniscidea). *Ecotoxicol. Environ. Saf.* 98: 66-73.
- Mazzei V., Longo G., Brundo M.V., Copat C., Oliveri Conti G., Ferrante M., 2014. - Bioaccumulation of Cadmium and Lead and its effects on hepatopancreas morphology in three terrestrial isopod Crustacean species. *Ecotoxicol. Environ. Saf.* 110: 269-279.
- Paoletti M.G., Hassal M., 1999. - Woodlice (Isopoda: Oniscidea): Their potential for assessing sustainability and use as bioindicators. *Agr. Ecosys. Environ.* 74: 157-165.
- Raessler M., Rothe J., Hilke I., 2005. - Accurate determination of Cd, Cr, Cu and Ni in woodlice and their skins - is moulting a means of detoxification? *Sci. Total Environ.* 337: 83-90.
- Strus J., Blejec A., 2001. - Microscopic anatomy of the integument and digestive system during the molt cycle in *Ligia italica* (Oniscidea). In: R. Vonk and F.R. Schram (Eds). *Crustacean issues* 13.
- Tarnawska M., Migula P., Przybyłowicz W., Mesjasz-Przybyłowicz J., Augustyniak M., 2007. - Nickel toxicity in the hepatopancreas of an isopod *Porcellio scaber* (Oniscidea). *Nucl. Instrum. Methods Phys. Res.* 260: 213-217.
- Vijver M.G., Wolterbeek H.T., Vink J.P.M., Van Gestel C.A.M., 2005. - Surface adsorption of metals onto the earthworm *Lumbricus rubellus* and the isopod *Porcellio scaber* is negligible compared to absorption in the body. *Sci. Total Environ.* 340: 271-280.
- Wägele J.W., 1992. - Isopoda. In: F.W. Harrison and A.G. Humes (Eds). *Microscopic Anatomy of Invertebrates*. Wiley-Liss, New York, pp. 529-617.
- Wieser W., 1979. - The flow of copper through a terrestrial food web. In: J.O. Nriagu (Ed). *Copper in the Environment*, Part 1. John Wiley and Sons Inc., New York, pp. 325-355.
- Witkus R., Horgan M.J., Dowling P., Klein M., Faso L., 1987. - Comparative elemental analysis of the S and B cells of the hepatopancreas of *Armadillidium vulgare*, a terrestrial isopod. *Comp. Biochem. Physiol.* 87: 149-151.
- Zimmer M., Topp W., 1999. - Relations between woodlice (Isopoda: Oniscidea), and microbial density and activity in the field. *Biol. Fert. Soils.* 30: 117-123.
- Znidarsic N., Štrus J., Drobne D., 2003. - Ultrastructural alterations of the hepatopancreas in *Porcellio scaber* under stress. *Environ. Toxicol. Pharmacol.* 13: 161-174.