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Influence of soil contaminated with cadmium on cell death in the digestive epithelium of soil centipede *Lithobius forficatus* (Myriapoda, Chilopoda)

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Abstract

Cadmium is a heavy metal that is treated as an environmental pollutant (air, water, soil). In order to understand the potential effects of cadmium in soil and soil invertebrates, it is important to describe all alterations which appear at different levels in organisms. The main aim of this study was to investigate, analyze and describe the alterations caused by cadmium short- and long-term intoxication at different levels in the organisms: from tissues to cells and organelles. In addition, the activation of cell death mechanisms that take part in homeostasis maintenance according to cadmium has been studied. Therefore, as the species for this project, a terrestrial and well-known widespread European species – the centipede *Lithobius forficatus* (Myriapoda, Chilopoda, Lithobiomorpha) – was chosen. This omnivorous species lives under upper layers of soil, under stones, litter, rocks, and leaves, and it is also commonly found in human habitats. The animals were divided into three groups: **C** – the control group, animals cultured in a horticultural soil; **Cd1** – animals cultured in a horticultural soil supplemented with 80 mg/kg (dry weight) of CdCl₂, 12 days – short-term exposure; **Cd2** – animals cultured in a horticultural soil supplemented with 80 mg/kg (dry weight) of CdCl₂, 45 days – long-term exposure. The midgut was isolated from each specimen and it was prepared for analysis using some histological, histochemical and immunohistochemical methods. Our studies showed that short-term intoxication causes intensification of autophagy and digestion of reserve material, while long-term exposure to this heavy metal causes activation of cell death processes together with inhibition of autophagy connected with the lack of reserve material. Additionally, we can infer that autophagy and cell death are nutrient deprivation-induced processes. Finally, we can conclude that short- and long-term exposure of soil centipede to cadmium affects different mechanisms and processes of cell death.

Keywords: Digestive system, myriapods, apoptosis, cadmium, ultrastructure

1. Introduction

Many processes or mechanisms have been identified as the effects of exposure to xenobiotics (e.g., heavy metals, temperature, starvation, pathogens). Heavy metals are treated as environmental pollutants whose contamination and concentration in soil result from anthropogenic as well as natural activities. Cadmium is one of heavy metals which can be taken up by

organisms (plants or animals) from the soil more readily than other heavy metals (Descamps et al. 1996; Kramarz 1999; Satarug et al. 2003). Numerous studies have revealed the possibility of using many invertebrate species as bioindicators in analyzing environmental pollution, and one such invertebrate taxon is the Myriapoda (Hopkin et al. 1985; Triebskorn et al. 1991; Grgić & Kos 2005). It is connected with the fact that myriapods, feeding on detritus, leaves, plant

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and animal organic matter or other animals, take part in soil mineralization and its decomposition. Studies on this arthropod group have focused on different organs and tissues in which the toxic substances (e.g., heavy metals) could be accumulated (Köhler & Alberti 1992; Köhler et al. 1995; Vandenbulcke et al. 1998a; Köhler 2002; Perez & Fontanetti 2011; Souza & Fontanetti 2011).

The digestive system is a barrier against any effects of xenobiotics on the entire organisms (Malagoli et al. 2010; Franzetti et al. 2012; Teixeira et al. 2013; Bonelli et al. 2019). Its middle region, the midgut, is responsible for synthesis and secretion of many substances (e.g., enzymes, hormones), intracellular digestion, absorption, accumulation of reserve material, etc., and it is treated as a model organ in analysis of the effects of xenobiotics on organisms (Wilczek et al. 2014; Bonelli et al. 2019). Our previous studies on the midgut epithelium of Myriapoda have confirmed that this organ is sensitive to numerous stressors that originate from the external environment (Chajec et al. 2012, 2014; Sosinka et al. 2014; Rost-Roszkowska et al. 2015, 2016). Toxic metals and reserve material are accumulated in the digestive epithelia in myriapods (Lewis 1981, 2006; Hopkin 1989; Hopkin & Read 1992; Chajec et al. 2012). This phenomenon has been suggested as a mechanism of detoxification (Hopkin & Martin 1983; Vandenbulcke et al. 1998a; Rost-Roszkowska et al. 2015, 2016). Despite our studies, in the literature, we can find some data connected with the structure and ultrastructure of the midgut epithelium in myriapods as commonly distributed terrestrial animals. However, the papers present only a general view (Lewis 2006; Minelli 2011), while only some experiments have been conducted as the primary ones. Vandenbulcke et al. (1998a) described the general ultrastructure of the midgut digestive cells in animals treated with cadmium and lead – two elements which are common for polluted environments (Hopkin & Martin 1983). In order to better understand the potential effects of cadmium in soil and soil invertebrates, we decided to expand the preliminary experimental studies (Descamps 1971; Descamps et al. 1996; Vandenbulcke et al. 1998a) and to present the precise alterations which appear at the ultrastructural level with the emphasis on changes in the amount of the reserve material, activation/deactivation of cell death processes and level of ATP in the midgut epithelial cells. Therefore, the main aim of this study was to investigate, analyze and describe all alterations caused by cadmium short- and long-term intoxication at different levels in the organisms: from tissues to cells and organelles and ATP/ADP levels. In addition, the activation of cell death mechanisms that take part in homeostasis

maintenance according to cadmium has been studied. Therefore, as the species for this project, one of terrestrial centipede *Lithobius forficatus* (Myriapoda, Chilopoda, Lithobiomorpha) – was chosen. It is a well-known and widespread European species that lives under the upper layers of soil, under stones, litter, rocks and leaves. It is also commonly found in human habitats, e.g., gardens and parks. This species is a predator, but also feeds on litter with organic and inorganic matter (omnivorous species) (Eisenbeis & Wichard 1987; Ostrowska et al. 1991; Lewis 2009).

2. Materials and Methods

2.1. Material

Adult specimens of *L. forficatus* (males and females) were collected in fields, yards and forests of southern Poland, e.g., Żywiec (19°12'E, 49°42'N) and from parks near Poznań (16°55'E, 52°24'N) (no specific permission is required). The material was collected from May to October, because in November the specimens start to prepare for overwintering and hibernation. Then, the specimens were cultured in the laboratory of the Institute of Biology, Biotechnology and Environmental Protection (University of Silesia in Katowice) and acclimated to laboratory conditions for several weeks (Vandenbulcke et al. 1998a). They were placed in 30-l aquaria at room temperature and fed *ad libitum* with forest litter composed of leaves, roots, detritus, and *Chironomus* larvae bought from a local fishing goods supplier.

2.1.1. Soil properties. The soil used in laboratory culture and the experiments was commercial peat-based soil (Geolia, ref.no 45845884). The levels of macronutrients and microelements (K, Mg, Na, Ca, P, S, Fe, Zn, Mn, Cu) and Cd in soil were estimated according to Zheljzkov et al. (2008). The soil samples (0.5 g) were placed in digestion tubes, soaked overnight in 5 ml of concentrated HNO₃ at room temperature, then decomposed further on an aluminum digestion block at 150°C for 8 h, filtered and brought to a 25 ml volume in volumetric flasks with a deionized water. The elements were measured in the filtered extracts by inductively coupled plasma-atomic emission spectroscopy (Spectroblue ICP-OES, Spectro Analytical Instruments, Germany). Soil pH was measured using a 1:2.5 soil to water ratio according to Ostrowska et al. (1991). The levels of C and N were measured with a CNS analyzer (Variomax CNS, Elementar Analysensysteme GmbH, Germany). Before the experiment the soil was air dried, mixed by hand and passed through a 2 mm screen. Cd was added in the form of CdCl₂ as solution at rate of

80 mg Cd kg⁻¹. Soil without Cd was established as a control. The soil moisture content during the entire experiment was maintained at 30%. Equilibration was conducted for 1 month at room temperature after putting the soil into the tubes and adding animals according to experimental data described below.

2.1.2. Experiment. The animals were divided into experimental groups (Figure 1) according to preliminary studies (Descamps et al. 1996; Vandebulcke et al. 1998a, 1998b): **C** – control group, animals cultured in a laboratory conditions in a horticultural soil and fed with *Acheta domestica* and *Chironomus* larvae

maintained in distilled water; **Cd1** – animals cultured in a horticultural soil supplemented with 80 mg Cd kg⁻¹, fed with *A. domestica* and *Chironomus* larvae maintained in distilled water, 12 days – short-term exposure; **Cd2** – animals cultured in a horticultural soil supplemented with 80 mg Cd kg⁻¹, fed with *A. domestica* and *Chironomus* larvae maintained in distilled water, 45 days – long-term exposure (Table I). Periods of cadmium exposure and its concentration in the soil were determined from previous preliminary studies connected with accumulation-detoxification curves (Descamps et al. 1996). Animals were anesthetized with chloroform. The midgut was isolated from

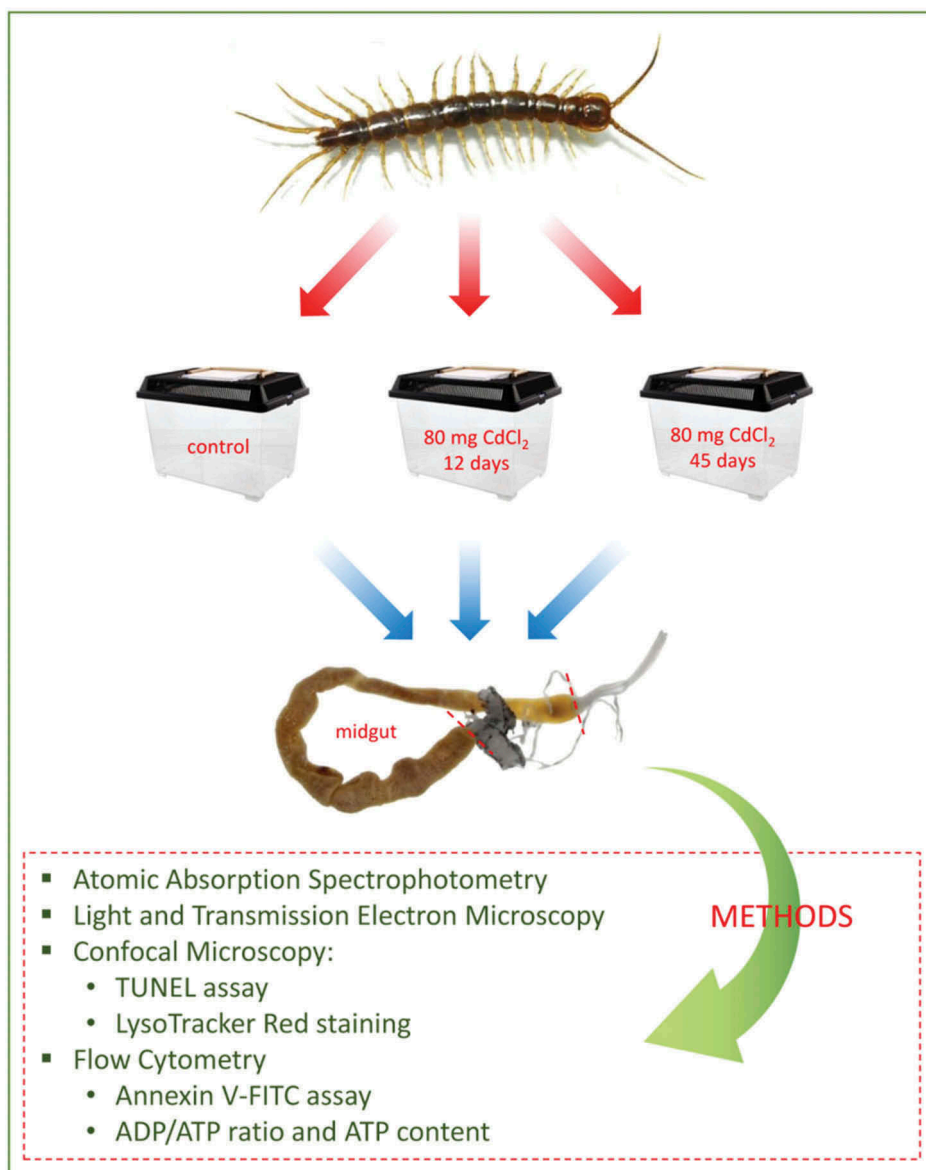


Figure 1. Scheme of experimental setup described in 2.1.2. chapter.

each specimen and it was prepared for the analysis using some histological, histochemical and immunohistochemical methods (Table I).

2.2. Methods

2.2.1. Atomic absorption spectrophotometry (AAS).

Cadmium concentration was assayed in the whole brown centipede bodies (Table I). The animals were dried at 70°C for about 72 h. Samples were digested in nitric acid (approx. 65%, Suprapur, Merck KGaA Darmstadt, Germany) at 150°C and dissolved in 2 ml of redistilled water. Digests were analyzed for metal content with an AAS ICE 3500 Thermo Scientific atomic absorption spectrophotometer in a PU-93 090X graphite furnace. Metal levels were calculated on the basis of similarly prepared Merck standards. The accuracy of determination was checked using spiked samples with BRC-185 bovine liver as reference material (IRMM, Geel, Belgium). Metal concentrations were expressed in µg Me/g dry weight. Mean values for each metal were calculated from five replicates for each group.

2.2.2. Light and transmission electron microscopy.

Isolated organs (Table I) were fixed with 2.5% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.4, 4°C, 2 h), postfixed in 2% osmium tetroxide in a 0.1 M phosphate buffer (4°C, 1.5 h) and dehydrated in a graded series of concentrations of ethanol (50, 70, 90, 95 and 4x100% each for 15 min) and acetone (15 min). Then the material was embedded in epoxy resin (Epoxy Embedding Medium Kit; Sigma). Semi- (0.8 µm thick) and ultra-thin (70 nm) sections were cut on a Leica Ultracut UCT25 ultramicrotome (Institute of Biology, Biotechnology and Environmental Protection). Semi-thin sections were stained with 1% methylene blue in 0.5% borax and observed using an Olympus BX60 light microscope (Institute of Biology, Biotechnology and Environmental Protection). After staining with uranyl acetate and lead citrate, ultra-thin sections were examined using a Hitachi H500 transmission electron microscope (Institute of Biology,

Biotechnology and Environmental Protection). Light and transmission electron microscopes were used in order to describe all alterations of the analyzed organs at the ultrastructural level.

Quantitative analysis - ultrathin sections were additionally used in order to count the number of cells that had signs of autophagy (autophagosomes, autolysosomes, residual bodies) in relation to the total number of cells in the midgut epithelium of animals from three experimental groups (TEM enables one to distinguish digestive, secretory and regenerative cells). Cells from different regions of the midgut were used for the study, because sections were selected randomly throughout the entire length of the midgut. The percentage of autophagic cells was determined by randomly counting cells in the midgut.

2.2.3. Confocal microscopy. TUNEL assay together with DAPI staining - detection of DNA fragmentation during apoptosis.

Midguts isolated from specimens of each experimental group (Table I) were incubated in a permeabilization solution (0.1% sodium citrate) (2 min on ice at 4°C), washed in TBS (Tris buffered saline) (3 × 5 min) and stained with a TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) reaction mixture (In Situ Cell Death Detection Kit, TMR red, Roche) (60 min at 37°C in the dark). After washing the material in TBS and labeling with DAPI (4',6-Diamidino-2'-phenylindole dihydrochloride) (30 min in darkness), the material was analyzed with an **Olympus FluoView FV1000** confocal microscope. Negative controls without terminal deoxynucleotidyl transferase (TdT) were prepared according to the labeling protocol (In Situ Cell Death Detection Kit, TMR red, Roche).

LysoTracker Red (LTR) staining - acid organelles labeling. LysoTracker Red is a red fluorescent dye that selectively accumulates in acidic organelles and can be used to investigate lysosomes and autolysosomes. Isolated midguts (Table I) from the group without fixation were incubated in 2.5 mM LysoTracker

Table I. Diagrammatic representation of the collection of adult specimens of *L. forficatus*. F - females, M - males.

Experimental group	Number of specimens examined					
	TEM F/M	Confocal microscope		Flow cytometry Annexin V-FITC	ADP/ATP ratio	AAS
		TUNEL assay	LysoTracker			
C	5/5	5	5	6	5	5
Cd1	5/5	5	5	6	5	5
Cd2	5/5	5	5	6	5	5

Red DND-99 (15 min, RT, in the dark) (Molecular Probes, L 7528) diluted in 500 ml of PBS (Phosphate-buffered saline). Next, the material was washed several times with PBS and DAPI (30 min in darkness), diluted in PBS and washed several times in PBS. The material was analyzed with an Olympus FluoView FV1000 confocal microscope.

2.2.4. Flow cytometry – quantitative analysis.

Preparation of cell suspension - for the flow cytometry study, the dissected organs isolated from specimens from each experimental group (Table I) were crushed mechanically and suspended in 100 μL of PBS (pH 7.4). Then, the intestine cells were separated by gentle shaking in a homogenizer (Minilys, Bertin Technologies). The cell suspension was washed using centrifugation at 1500 rpm for 5 min and the precipitate was suspended in 100 μL of a PBS buffer.

Viability assessment - quantitative measurement of viable, apoptotic and necrotic cells in the intestine of centipedes was performed with the Annexin V-FITC (fluorescein isothiocyanate) Apoptosis Detection Kit (Abcam, № ab14085) according to the manufacturer's protocols. Cells stained with the FITC-labeled Annexin V emit green fluorescence and they were classified as apoptotic, while the propidium iodide (PI) positive cells exhibit red fluorescence, enabling the distinction between necrotic cells (Annexin V-FITC-/PI+) and apoptotic cells (early apoptosis: Annexin V-FITC+/PI- or late apoptosis: Annexin V-FITC+/PI+). According to the procedure 5 μL of Annexin V-FITC, 5 μL of PI and 500 μL of binding buffer were added to the previously prepared cell suspension (50 μL of cell suspension in 100 μL of PBS, pH 7.4). The solution was incubated for 10 min. Then the samples were ready for acquisition using the Beckman Coulter Instrument FC 500 flow cytometer with a 488 nm argon laser. Results were expressed as the percentage of apoptotic (early and late) and necrotic cells.

2.2.5. *ADP/ATP ratio and ATP content.* In this study the ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (BioVision, № K254) and ApoSENSOR ADP/ATP Ratio Bioluminescence Assay Kit (BioVision, № K255) were used according to the manufacturer's protocols. Determination of ATP content is based on the reaction of oxidative decarboxylation of luciferin catalyzed by luciferase. Light intensity, measured by the luminometric method at a wavelength of 562 nm, is proportional to the ATP content in the examined sample. ADP level was measured by its conversion to ATP that is detected using the same reaction. The concentration

of ATP in materials was calculated based on the standard curve, which was prepared using the ATP standard provided in the kit. Results were expressed as nmol ATP \cdot mg⁻¹ protein.

Protein content was measured according to Bradford (1976), using bovine albumin (protein content >95%, Fluka) as a standard.

2.2.6. *Statistical analysis.* Statistical analyses were performed using the STATISTICA 10.0 software package (StatSoft, Inc. (2010) version 10.0. <http://www.statsoft.com>). Normality was checked using the Shapiro-Wilk test. Tukey test, $p < 0.05$, was used to evaluate the significance of differences among the experimental groups.

3. Results

3.1. Soil properties

The commercial soil properties were as follows: total C content 44.6%, total N 1.5%, total K 0.49 g kg⁻¹, total Mg 0.75 g kg⁻¹, total Na 0.49 g kg⁻¹, total Ca 20.2 g kg⁻¹, total P 1.566 g kg⁻¹ g kg⁻¹, total S 7.9 g kg⁻¹, total Fe 0.62 g kg⁻¹, total Zn 15.2 mg kg⁻¹, total Cu 7.8 mg kg⁻¹, total Mn 15.6 mg kg⁻¹, Cd not detected, pH 5.65.

3.2. Cadmium concentration in *L. forficatus*

The concentration of cadmium in the Cd2 group was not significantly different from the control value (animals cultured in non-polluted soil). The cadmium concentration measured in the Cd1 group was significantly, about 7 times higher than in the control and Cd2 groups (Figure 2).

Based on the results of our previous studies (Chajec et al. 2012; Rost-Roszkowska et al. 2015, 2016), where we found no differences in the structure of midgut epithelial cells in females and males, we present the general changes described for both sexes. In addition, we analyzed only specimens of *L. forficatus* that were devoid of *Rickettsia*-like microorganisms, which have been described in about 10% of animals (Chajec et al. 2012).

3.3. Ultrastructural changes in the midgut epithelium of *L. forficatus*

3.3.1. *Control group.* The precise ultrastructure of the pseudostratified midgut epithelium (Figures 3 and 4(a)) with the digestive, secretory and regenerative cells has been presented in our previous paper (Figure 3) (Chajec et al. 2012). The apical cytoplasm of the digestive cells has numerous mitochondria, cisterns of rough

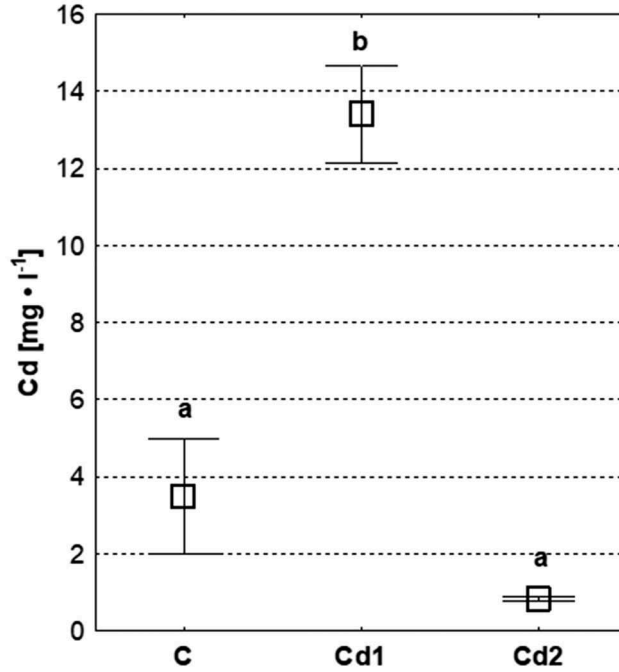


Figure 2. Cadmium concentration [mg · l⁻¹] in the experimental groups: control, Cd1 and Cd2. Mean ± SE. Different letters (a, b) mean statistically significant differences at $p \leq 0.05$. One-way ANOVA, Tukey's test, $n = 5-6$.

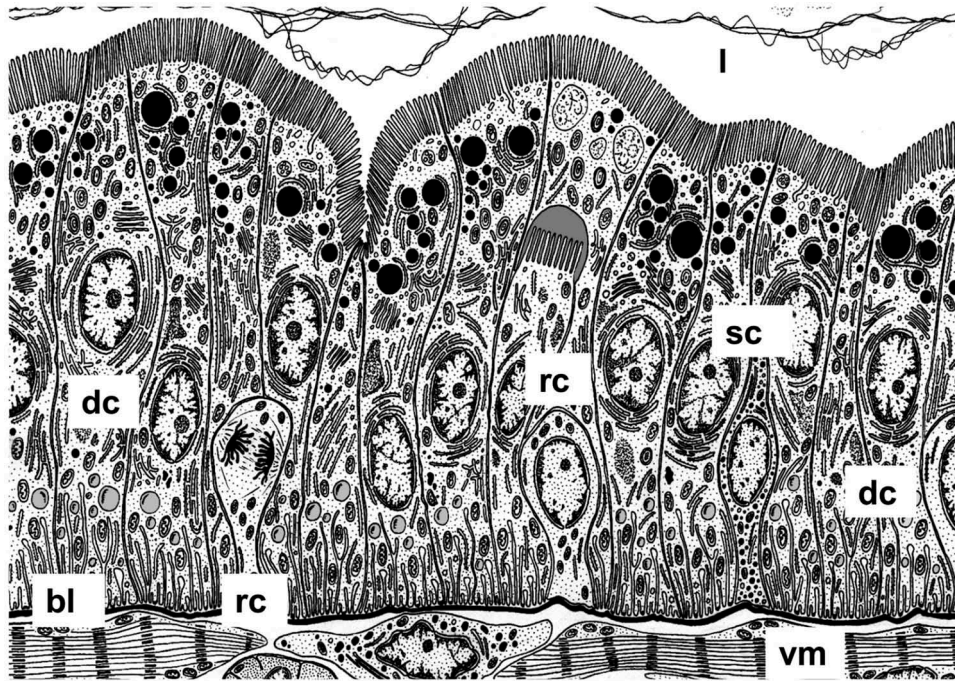


Figure 3. Diagrammatic representation of the midgut epithelium in *L. forficatus*. Digestive cells (dc), secretory cells (sc), regenerative cells (rc), basal lamina (bl), visceral muscles (vm), midgut lumen (l).

endoplasmic reticulum, and spherites (Figure 4(b-d)). Some small vesicles with electron lucent content accumulate in the neighborhood of the apical cell membrane which forms the microvilli (Figure 4(c)). Exocytosis

(merocrine secretion) has been observed. The perinuclear cytoplasm possesses many mitochondria, Golgi complexes, and cisterns of rough endoplasmic reticulum surrounding the oval nucleus (Figure 4(e-f)). Folds

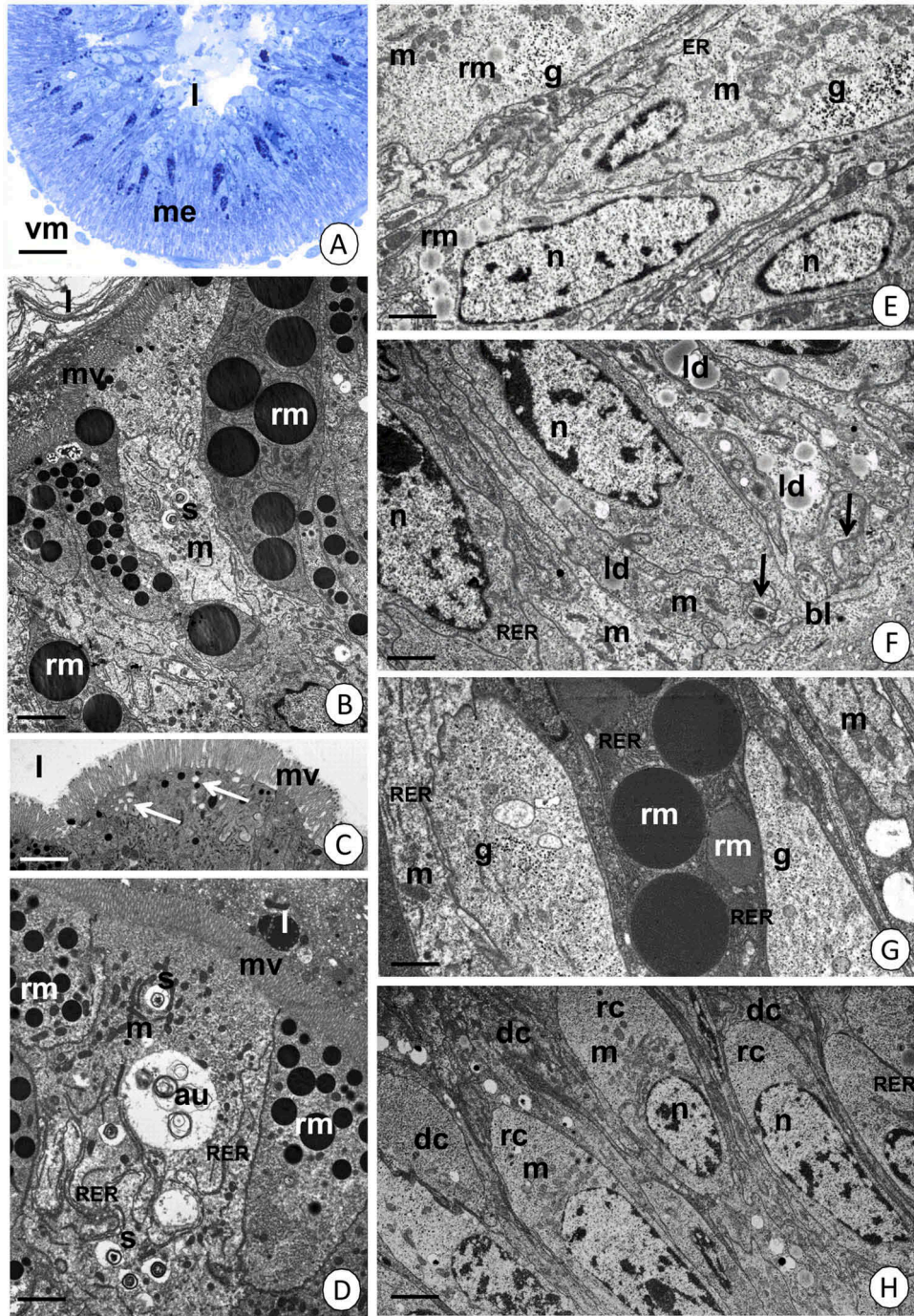


Figure 4. The midgut epithelium of *L. forficatus* – the control group (C group).

(a) The transverse section through the midgut epithelium (me). Midgut lumen (l), visceral muscles (vm). Light microscope. Scale bar = 12 μ m. (b–d) The apical cytoplasm of the digestive cells. Midgut lumen (l), microvilli (mv), mitochondria (m), reserve material (rm), spherites (s), autophagosome (au), cisterns of rough endoplasmic reticulum (RER), vesicles with electron lucent content (white arrows). TEM. (b) Scale bar = 2 μ m. (c) Scale bar = 3 μ m. (d) Scale bar = 2.2 μ m. (e) The perinuclear cytoplasm of the digestive cells: mitochondria (m), glycogen granules (g), nuclei (n), reserve material (rm), cisterns of RER (RER). TEM. Scale bar = 1.4 μ m. (f) The basal and perinuclear cytoplasm of the digestive cells. Nuclei (n), mitochondria (m), basal lamina (bl), lipid droplets (ld), folds of the basal cell membrane (black arrows). TEM. Scale bar = 1.4 μ m. (g) The perinuclear and apical cytoplasm of the digestive cells with electron dense spheres of reserve material (rm) and glycogen granules (g). Mitochondria (m), cisterns of RER (RER). TEM. Scale bar = 1.25 μ m. (h) Regenerative cells (rc) located among basal regions of digestive cells (dc). Mitochondria (m), nuclei (n), cisterns of RER (RER). TEM. Scale bar = 1.6 μ m.

of the basal cell membrane are surrounded with mitochondria and cisterns of rough endoplasmic reticulum (Figure 4(f)). The cytoplasm of the digestive cells is rich in reserve material, but regionalization in their distribution has been described (Chajec et al. 2012): the apical cytoplasm has electron-dense spheres (Figure 4(b,d,g)), numerous glycogen granules and some lipid droplets accumulate in the perinuclear cytoplasm (Figure 4(e,g)), while numerous lipid droplets have been mainly described in the basal cytoplasm (Figure 4(f)). However, some of the digestive cells, as newly differentiated cells, are devoid of reserve material (Figure 4(b,d)).

Regenerative cells are individually located among basal regions of digestive cells. Their organelle-poor cytoplasm has only some mitochondria and cisterns of rough endoplasmic reticulum (Figure 4(h)). The cytoplasm of secretory cells acquires some mitochondria, cisterns of endoplasmic reticulum, and numerous electron-lucent and electron-dense vesicles (Chajec et al. 2012).

3.3.2. Midgut epithelium ultrastructure in animals from Cd1 group. The apical and perinuclear cytoplasm of some of the digestive cells is electron lucent (Figure 5(a-d)), while in others (Figure 5(d)) the cytoplasm does not show any changes in comparison to the control group. The apical cytoplasm is poor in organelles: only some mitochondria, some spheres with reserve material and vacuoles have been observed (Figure 5(b)). The apical cell membrane forms microvilli, while the apical membrane of some digestive cells is devoid of these structures (Figure 5(b)). Exocytosis (merocrine secretion) was not detected. This cytoplasmic region is full of autophagosomes (Figure 5(b)). The perinuclear region in digestive cells with electron lucent cytoplasm possesses only some mitochondria, cisterns of rough endoplasmic reticulum, autophagosomes and vacuoles (Figure 5(c-d)). The basal cytoplasm does not show any changes: folds of the basal cell membrane are surrounded with mitochondria and cisterns of rough endoplasmic reticulum (Figure 5(e-f)). A distinct decrease in the amount of the reserve material has been detected: the cytoplasm of some digestive cells contains a small amount of electron-dense spheres located in the apical cytoplasm (Figure 5(b)). Only sporadic lipid droplets have been described in the basal cytoplasm (Figure 5(e-f)), while no glycogen granules occur. However, numerous vacuoles with electron lucent content (Figure 5(c-d)) and spherites (Figure 5(g)) appear in the entire cytoplasm of digestive cells. The above-described changes at the ultrastructural level concern the digestive cells of the midgut epithelium,

while no changes were noticed in the cytoplasm of regenerative (Figure 5(h)) or secretory cells (Figure 5(i)).

3.3.3. Midgut epithelium ultrastructure in animals from Cd2 group. The entire cytoplasm of the majority of digestive cells of the midgut epithelium (Figure 6(a)) is electron lucent with a small amount of organelles (Figures 6(b,c) and 7(c)). The apical cell membrane forms microvilli; however, in the majority of digestive cells, it forms large bulges lacking microvilli (Figure 6(d)). Near the apical cell membranes numerous small vesicles with electron lucent content appear (Figure 6(d)). They fuse to form large vesicles, and eventually the vacuoles could be observed in the entire cytoplasm (Figure 6(e-f) and 7(c)). The process of exocytosis, where the vesicles fuse with the apical cell membrane, was detected (Figure 6(e)). The basal cell membrane forms small folds which protrude into the cytoplasm that is electron lucent and devoid of mitochondria and cisterns of endoplasmic reticulum (Figure 6(c)). The cytoplasm of some digestive cells contains small amount of spheres of reserve material with different electron density, while the majority of them are devoid of reserve material, and contain large amounts of vacuoles with electron lucent content (Figure 6(b-c,g)). Small patches of glycogen have been noticed in the apical cytoplasm of some of the digestive cells (Figure 6(d)). The cytoplasm is poor in cisterns of endoplasmic reticulum, Golgi complexes, mitochondria (Figures 6(b-c,f-g) and 7(c)) and some spherites (Figure 6(h)), so we noted a distinct decrease in the number of such organelles and structures. Numerous digestive cells possessed a nucleus with electron lucent nucleoplasm that was devoid of heterochromatin (Figure 6(h)). The above-described changes at the ultrastructural level concern the digestive cells of the midgut epithelium, while no changes were observed in the cytoplasm of regenerative (Figure 6(i)) and secretory cells (not shown).

3.4. Autophagy

TEM revealed that autophagic structures (autophagosomes, autolysosomes and/or residual bodies) were present in the apical and perinuclear cytoplasm of some of the digestive cells in animals from the control group (Figures 4(d) and 7(a)) and in animals treated with cadmium for 45 days (Figure 7(c)). However, in animals treated with cadmium for 12 days, autophagic structures were detected in the entire cytoplasm of the majority of digestive cells (Figures 5(b-c) and 7(b)). No autophagic structures were observed in

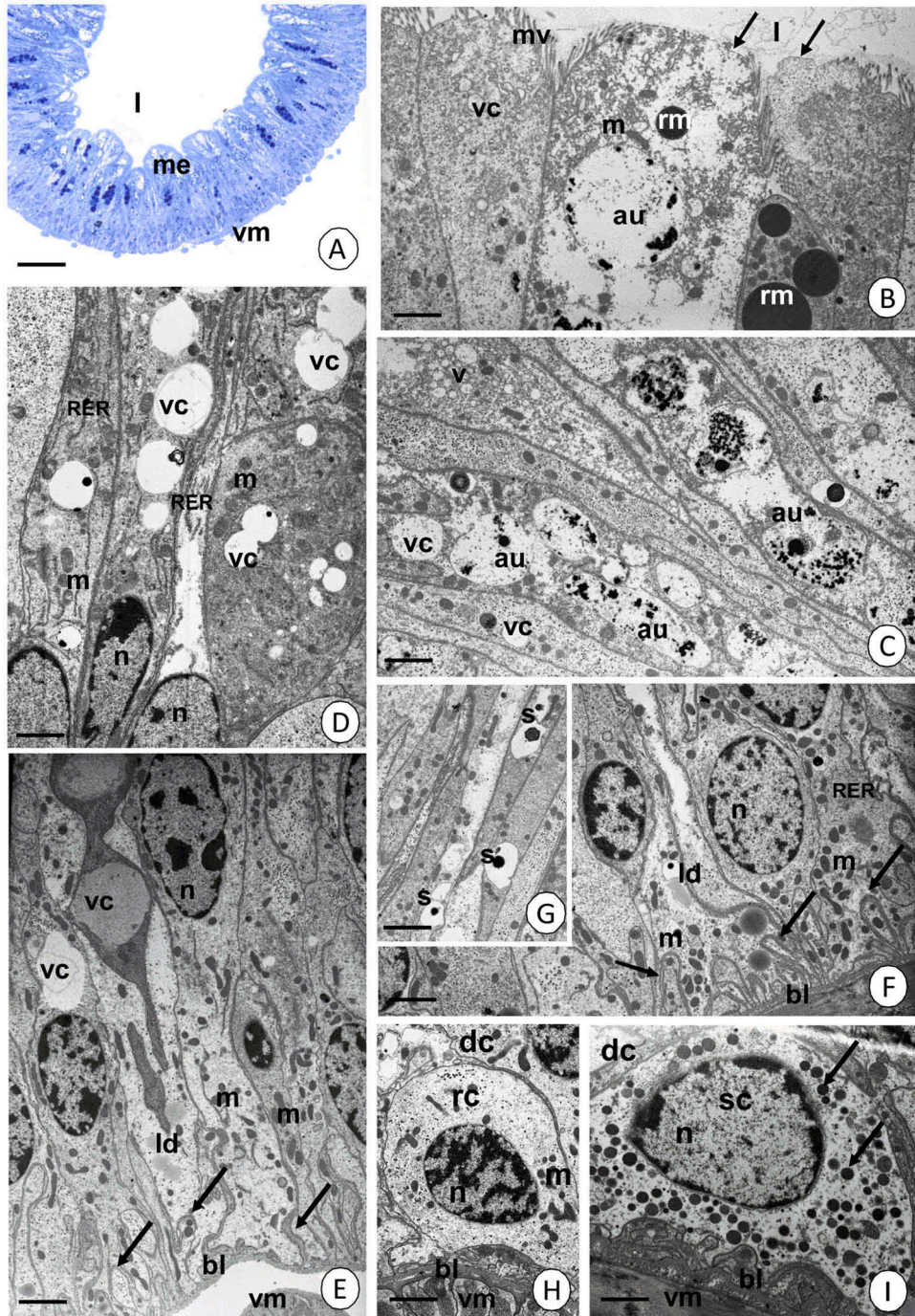


Figure 5. The midgut epithelium of *L. forficatus* – animals treated with cadmium for 12 days (Cd1 group). (a) The transverse section through the midgut epithelium (me). Midgut lumen (l), visceral muscles (vm). Light microscope. Scale bar = 20 μ m. (b) The apical cytoplasm of the digestive cells. Microvilli (mv), midgut lumen (l), mitochondria (m), reserve material (rm), autophagosome (au), vacuoles (vc), apical cell membrane devoid of microvilli (black arrows). TEM. Scale bar = 2 μ m. (c–d) The perinuclear region in digestive cells with some mitochondria (m), cisterns of RER (RER), vacuoles (vc) and autophagosomes (au). Nuclei (n). TEM. C. Scale bar = 1.1 μ m. (d) Scale bar = 1.4 μ m. (e–f) Basal cytoplasm of digestive cells. Basal lamina (bl), visceral muscles (vm), nuclei (n), mitochondria (m), lipid droplets (ld), cisterns of RER (RER), vacuoles (vc), folds of the basal membrane (black arrows). TEM. (e) Scale bar = 2.2 μ m. (f) Scale bar = 2 μ m. (g) Spherites (s) accumulated in the cytoplasm of digestive cells. TEM. Scale bar = 1.5 μ m. (h) Regenerative cell (rc) located among basal regions of digestive cells (dc). Mitochondria (m), nucleus (n), basal lamina (bl), visceral muscles (vm). TEM. Scale bar = 1.5 μ m. (i) Secretory cell (sc) located among basal regions of digestive cells (dc). Nucleus (n), electron dense granules (black arrows), basal lamina (bl), visceral muscles (vm). TEM. Scale bar = 1.1 μ m.

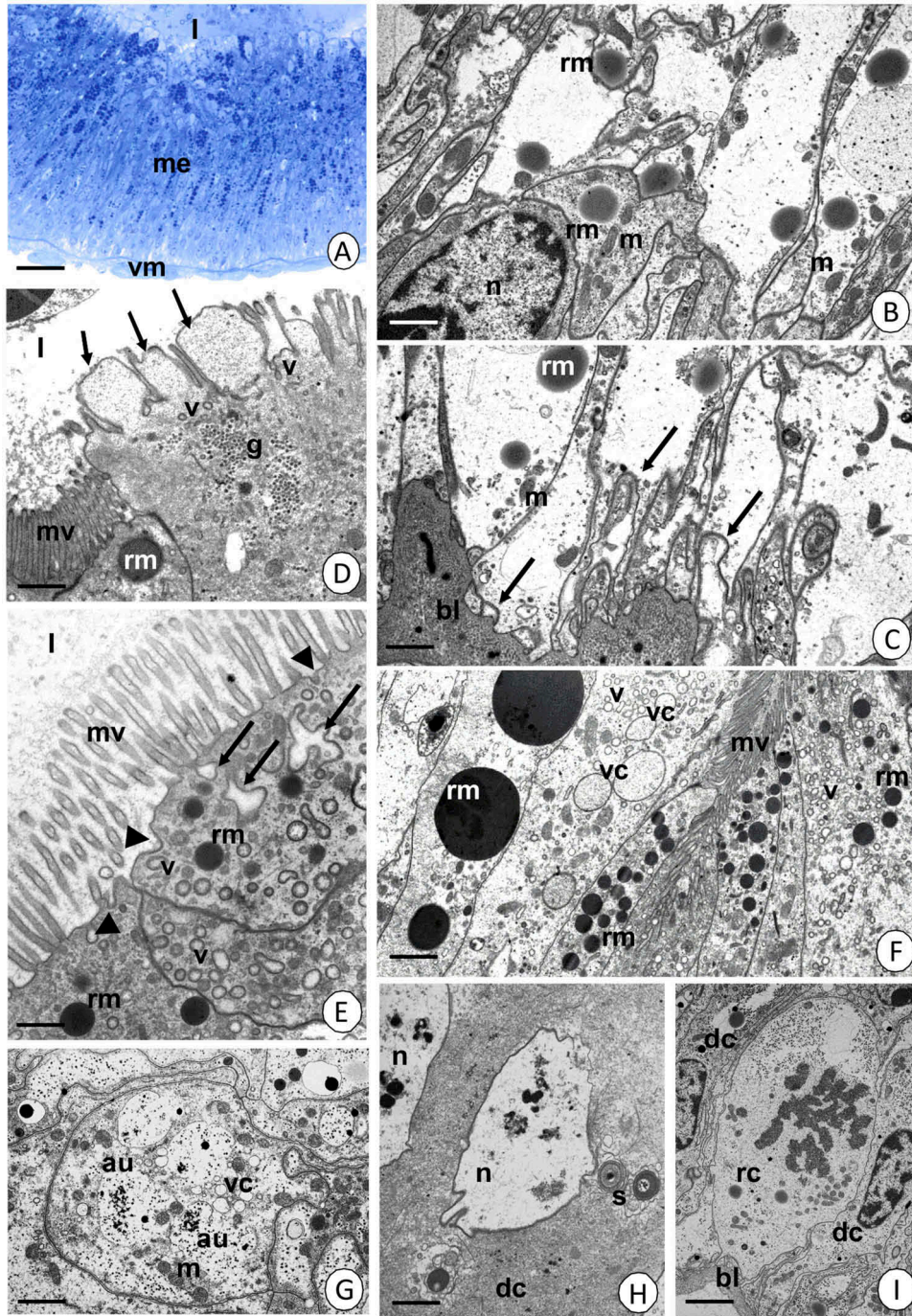


Figure 6. The midgut epithelium of *L. forficatus* – animals treated with cadmium for 45 days (Cd2 group).

(a) The transverse section through the midgut epithelium (me). Midgut lumen (l), visceral muscles (vm). Light microscope. Scale bar = 7.5 μ m. (b) The cytoplasm of numerous digestive cells is electron lucent with small amount of organelles. Mitochondria (m), nucleus (n), reserve material (rm). TEM. Scale bar = 1 μ m. (c) The electron lucent basal cytoplasm of digestive cells with small amount of organelles. Mitochondria (m), reserve material (rm), basal lamina (bl), folds of the basal cell membrane (arrows). TEM. Scale bar = 1 μ m. (d) The apical cytoplasm of the digestive cells with microvilli (mv) and large bulges lacking microvilli (black arrows). Glycogen granules (g), midgut lumen (l), vesicles (v), reserve material (rm). TEM. Scale bar = 2 μ m. (e) The apical cytoplasm of the digestive cells with microvilli (mv). Midgut lumen (l), vesicles (v), fusion of the vesicles (black arrows), exocytosis (black arrowheads), reserve material (rm). TEM. Scale bar = 0.8 μ m. (f) The cytoplasm of the digestive cells with the reserve material (rm) and the cytoplasm of the medium-electron density. Vesicles (v) and vacuoles (vc) in the digestive cells with the electron lucent cytoplasm. Microvilli (mv). TEM. Scale bar = 2 μ m. (g) Electron lucent cytoplasm of the digestive cells with vacuoles (vc) and autophagic structures (au). Mitochondria (m). TEM. Scale bar = 1.2 μ m. (h) Nuclei (n) in the digestive cells (dc). Spherites (s). TEM. Scale bar = 1.2 μ m. (i) Regenerative cell (rc) among basal regions of the digestive cells (dc). Basal lamina (bl). TEM. Scale bar = 2.3 μ m.

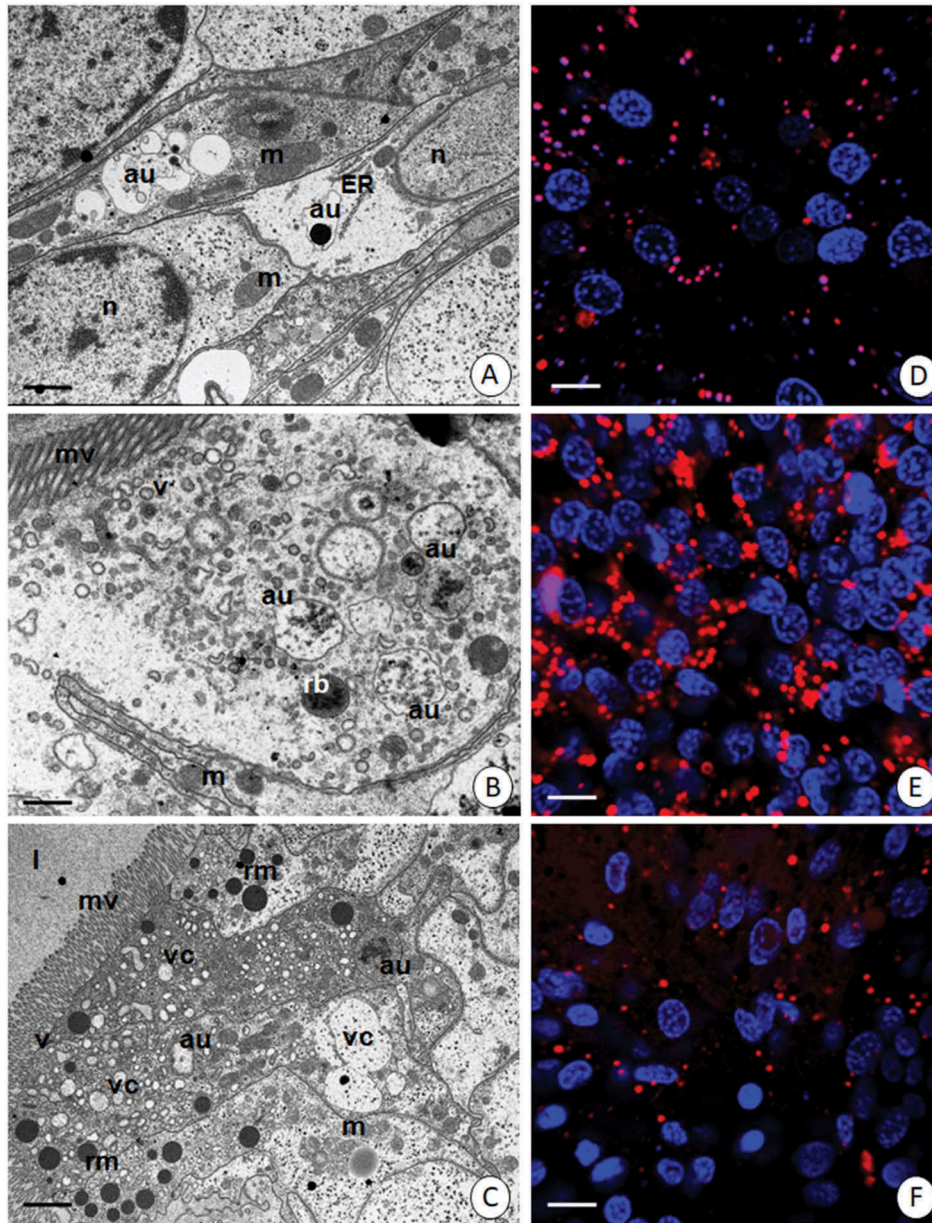


Figure 7. Autophagy in the midgut epithelium of *L. forficatus*.

(a) Digestive cells in animals from the control group. Autophagosomes (au), cisterns of endoplasmic reticulum (ER), mitochondria (m), nuclei (n). TEM. Scale bar = 1 μm . (b) Digestive cells in animals from Cd1 group. Autophagosomes (au), microvilli (mv), mitochondria (m), residual bodies (rb), vesicles with electron lucent content (v). TEM. Scale bar = 0.8 μm . (c) Digestive cells in animals from Cd2 group. Autophagosomes (au), microvilli (mv), mitochondria (m), midgut lumen (l), reserve material (rm), vesicles with electron lucent content (v), vacuoles (vc). TEM. Scale bar = 1.7 μm . (d–f) 3D representation of the accumulation of lysosomes and autolysosomes (red signals) in the *L. forficatus* midgut. Nuclei (blue signals). LysoTracker Red and DAPI staining. Confocal microscope. (d) Control group. Scale bar = 6 μm . (e) Animals treated with cadmium for 12 days. Scale bar = 7 μm . (f) Animals treated with cadmium for 45 days. Scale bar = 7 μm .

the cytoplasm of the regenerative and secretory cells in any of the experimental groups. The qualitative analysis showed that the signals from acidic organelles (lysosomes and autolysosomes) in the midgut epithelium were stronger in individuals treated with

cadmium for 12 days according to the control group. However, after 45 days of exposure of the animal to metal, the signals were weaker than after 12 days of exposure and the results were similar to the control group (Figure 7(d–f)). The percentage of

autophagic cells in the intestine of individuals treated with cadmium was depended on the duration of exposure. The quantitative analysis revealed that the degree of severity of autophagy in the midgut epithelium of individuals treated with cadmium for 12 days (Cd1 group) was nearly 45% higher than in the control group ($P = 0.0002$), while in the intestine of individuals from the Cd2 group nearly a twofold decrease in the number of autophagic cells compared to the control group was observed ($P = 0.0002$) (Figure 8).

3.5. Viability assessment

The qualitative analysis with the use of the confocal microscopy showed that the signals from apoptotic cells in the midgut epithelium were stronger in individuals treated with cadmium for 12 and 45 days compared to the control group (Figure 9(a–c)).

The quantitative analysis using the flow cytometry revealed that regardless of the time of exposure to Cd, the decrease in cell viability was about 13%. More than threefold and a nearly fivefold increase in the number of early apoptotic cells, respectively, in Cd1 ($P = 0.03$) and Cd2 ($P = 0.006$) groups were observed in the intestine of individuals treated with cadmium compared to the control (Figure 9(d)). The degree of severity of apoptosis in the midgut epithelium treated with cadmium for 45 days was 25% higher than that

of individuals treated for 12 days (Figure 9(d)). Additionally, an increase in the number of late apoptotic and necrotic cells in both experimental groups (12 and 45 days of cadmium treatment) relative to the control was noted (Figure 9(e–f)), but only in the Cd1 group were there statistically significant differences relative to the control group ($P = 0.03$, $P = 0.005$, respectively). The percentage of necrotic cells in intestine derived from the individuals from Cd1 and Cd2 groups was similar, and on average 12 times higher relative to the control (Figure 9(f)).

3.6. ADP/ATP index and ATP content

With the increase of the exposure time of the organism to the metal, an increase in the ADP/ATP index was recorded in the midgut of the specimens from each experimental group. The level of this indicator from animals treated with cadmium was about fivefold and 10-fold higher than in the control group, respectively for Cd1 ($P = 0.150$) and Cd2 ($P = 0.003$) groups. An increase in the ADP/ATP index indicates a decrease in ATP concentration and an increase in ADP concentration, demonstrating the possibility of inhibiting the growth and/or intensification of degenerative processes in the cell. The concentration of ATP was significantly reduced in midgut epithelial cells after both 12 and 45 days of exposure to Cd, respectively 13 times ($P = 0.016$) and 25 times ($P = 0.013$)

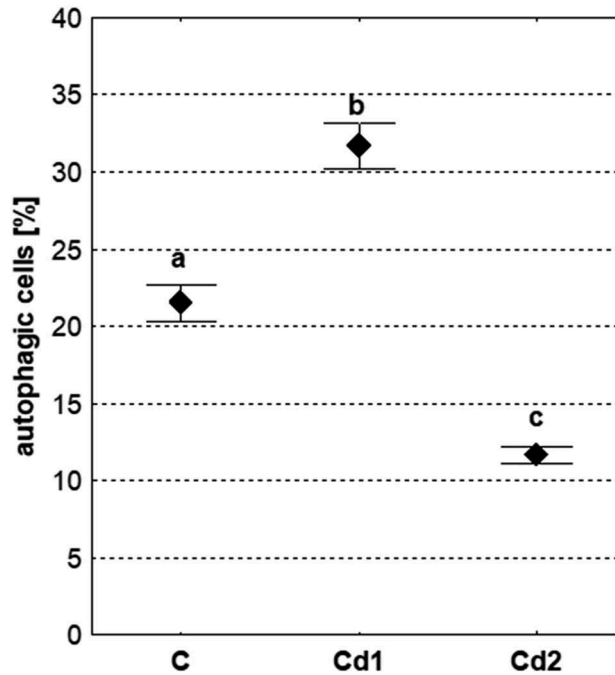


Figure 8. Percentage of autophagic cells in intestine of *L. forficatus* from the control group (C) and exposed to cadmium (Cd1, Cd2). Different letters (a, b, c) indicate significant differences between experimental groups ($x \pm SE$; Tukey's test, $p < 0.05$); $n = 6$.

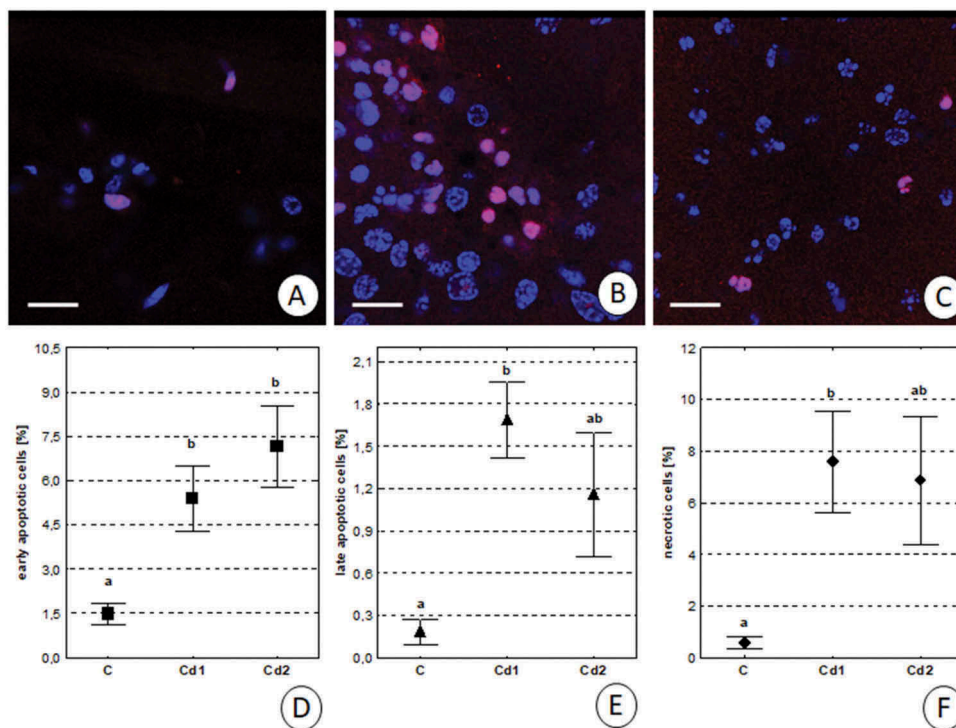


Figure 9. Apoptosis and necrosis in the midgut epithelium of *L. forficatus*.

(a-c) 3D representation of the TUNEL assay and DAPI staining. Nuclei of apoptotic cells (red signals), nuclei (blue signals). Confocal microscope. (a) Control group. Scale bar = 6 μm . (b) Animals treated with cadmium for 12 days. Scale bar = 6 μm . (c) Animals treated with cadmium for 45 days. Scale bar = 6 μm . (d-f) Diagrammatic representation of quantitative analysis of apoptotic and necrotic cells in the midgut epithelium of *L. forficatus* from the control group (C) and exposed to cadmium (Cd1, Cd2). Different letters (a, b) indicate significant differences between experimental groups within each parameter ($x \pm \text{SE}$; Tukey's test, $p < 0.05$); $n = 5-6$. Flow cytometry. (d) Percentage of early apoptotic cells (Annexin⁺, PI⁻). (e) Late apoptotic cells (Annexin⁺, PI⁺). (f) Necrotic cells (Annexin⁻, PI⁺).

compared to the control group. There were no statistically significant differences in ATP concentration in intestines of individuals exposed to cadmium depending on the time of exposure to metal (Table II).

3.7. Hemocytes

Hemocytes were observed as ameboid-shaped cells which accumulated near the visceral muscles and beneath the basal lamina of the midgut epithelium in the animals from the control group (Figure 10(a)) and from experimental group Cd2 (45 days of cadmium treatment) (Figure 10(b)). However, in animals treated with cadmium for 12 days hemocytes were observed among digestive cells of the midgut epithelium (Figure 10(c)). They have an irregular nucleus located in the central cell region. Their cytoplasm possessed numerous electron-dense granules, mitochondria and cisterns of endoplasmic reticulum (Figure 10(a-c)).

Table II. Concentrations of ATP ($x \pm \text{SE}$) and ADP/ATP ratio ($x \pm \text{SE}$) in intestine of *L. forficatus* from the control group (C) and groups exposed to cadmium (Cd1, Cd2). The different letters (a, b) indicate significant differences between experimental groups within each parameter (Tukey's test, $p < 0.05$); $n = 4-5$.

Groups	ATP	
	[nmol·mg protein ⁻¹]	ADP/ATP
C	0.321 \pm 0.085a	0.375 \pm 0.085a
Cd1	0.025 \pm 0.011b	1.934 \pm 0.326ab
Cd2	0.013 \pm 0.005b	3.839 \pm 0.925b

4. Discussion

Centipedes are considered to be able to survive in soils contaminated with heavy metals; hence it is believed that they must have developed some mechanisms that allow them to preserve homeostasis (Hopkin & Martin 1984; Hopkin et al. 1985; Read & Martin 1990; Descamps et al. 1996). Like other invertebrates, they absorb many substances via their epidermis or from food through the midgut

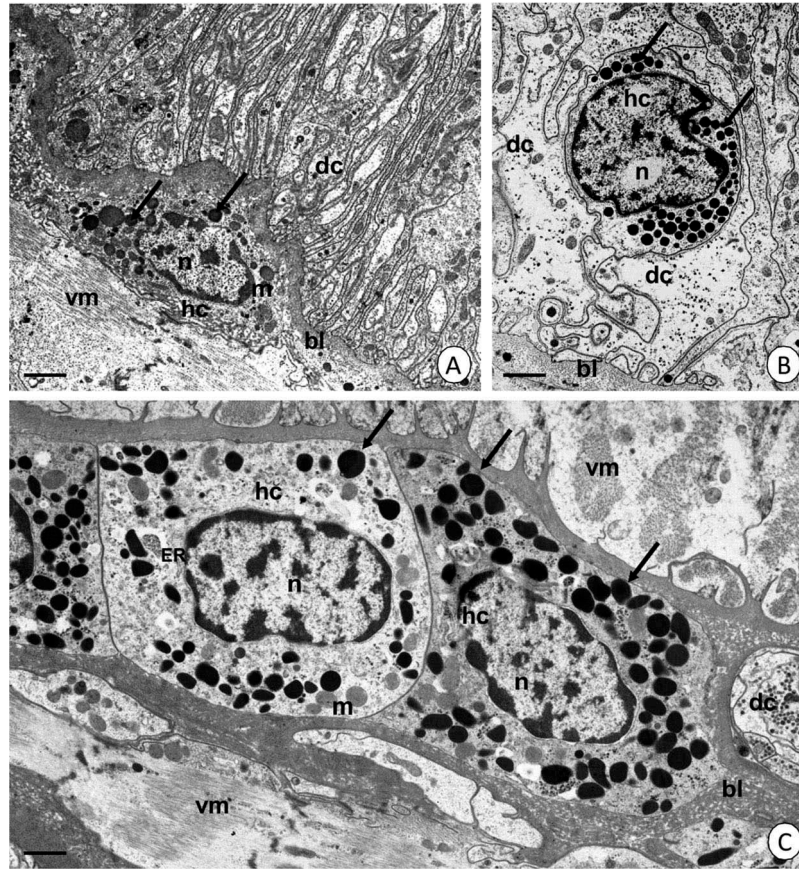


Figure 10. Hemocytes in the neighborhood of *L. forficatus* midgut. (a) Hemocytes (hc) in animals from the control group. Digestive cells (dc), basal lamina (bl), electron dense granules (black arrows), mitochondria (m), visceral muscles (vm), nucleus (n). TEM. Scale bar = 2 μ m. (b) Hemocytes (hc) located among digestive cells. Animals treated with cadmium for 12 days. Digestive cells (dc), basal lamina (bl), nucleus (n), electron dense granules (black arrows). TEM. Scale bar = 1.5 μ m. (c) Hemocytes (hc) located in the hemocoel in the neighborhood of visceral muscles (vm) and basal lamina (bl). Animals treated with cadmium for 45 days. Digestive cells (dc), electron dense granules (black arrows), mitochondria (m), cisterns of ER (ER). TEM. Scale bar = 0.6 μ m.

epithelium (Wieser 1967; Gräff et al. 1997). However, there are some data indicating that centipedes can prevent the uptake of some heavy metals (e.g., cadmium), even if their concentration in food is high. Metals could be bound as insoluble intracellular granules or spherites in midgut epithelium (Humbert 1978; Hopkin & Martin 1983). Descamps et al. (1996) stated that when animals were intoxicated with cadmium, a natural detoxification process took place. Hopkin et al. (1985) observed a high level of mortality among lithobiids collected in an unpolluted environment and fed with a polluted diet. These results, however, were different from those presented by Descamps et al. (1996). The periods of cadmium exposure and its concentration in the soil were established according to previous experimental data reported by Vandebulcke et al. (1998a, 1998b). Therefore we could compare and develop our studies on successive and more

advanced analyses. The experiment was performed in a non-polluted soil (see section 2.1.1. *Soil Properties*) and adding cadmium to the soil caused the increase in the accumulation of this metal in animals bodies after a short-term experiment, while the long-term exposure to cadmium showed a distinct decrease of cadmium concentration in animals' bodies in comparison to the control group. It can be caused by activation of numerous mechanisms that protect cells and neutralize the toxic substances. Therefore, some of cellular defence tools against cadmium could be involved, e.g., metallothioneins, superoxide dismutase (Włostowski et al. 2014, 2016; Polykretis et al. 2019). Additionally, Cd-induced oxidative stress causes the activation of autophagy and/or apoptosis (Hödl et al. 2010; Sheir et al. 2013; Liu et al. 2016; Włostowski et al. 2016; So et al. 2018; Mugnano et al. 2018).

Studies on the *L. forficatus* and changes in its midgut epithelium in animals living in metal-contaminated soils originate from the studies of Vandembulcke et al. (1998a). Appearance of vacuoles with electron lucent content was detected in 12-day treated animals, while in 45-day treated animals, some concentrically structured granules and sponge granules were described, while no information on vacuoles in this experimental group was presented. Our studies revealed more ultrastructural alteration which appeared after short- (12 days) and long-term (45 days) cadmium exposure. Changes which appear in 12-day treated animals such as the decrease in the number of organelles in the apical and perinuclear cytoplasm without any changes in the basal cytoplasm, the decrease in the amount of reserve material together with the appearance of vacuoles and intensive autophagy suggest that short-term intoxication causes damage in the epithelial cells at the ultrastructural level. The reserve material is exploited *via* autophagy as the source of energy (Fontanetti et al. 2006; Liu & Czaja 2013; Lipovšek et al. 2014, 2018; Lipovšek & Novak 2016; Rost-Roszkowska et al. 2019; Włodarczyk et al. 2019), so autophagy can play a role as a survival factor (Bernales et al. 2006; Cebollero et al. 2012; Włostowski et al. 2014, 2016). In *L. forficatus* midgut such changes are probably reversible if many organelles are still present in the cytoplasm and the synthesis of many substances can take part. It was suggested that the level of, e.g., metallothionein and glutathione has an impact on the visible toxicity of cadmium in digestive organs in some arthropods (Włostowski et al. 2014, 2016). However, in 45-day treated animals a strong reduction in organelles number has been noted. Additionally, the amount of reserve material has been strongly reduced, and only some glycogen has been described. It may originate from nourishments located in the midgut lumen, because the animals were fed *ad libitum*. Numerous vacuoles as the remains of the reserve material accumulated in the entire cytoplasm that is electron-lucent. It suggests that the new reserve material is not synthesized because of the lack of organelles (Rost-Roszkowska et al. 2019). Intensity of autophagy decreased in comparison to 12-day treated animals. It suggests that autophagy is a process that could help cells in the fight against a stressor (e.g., cadmium) for a precise period of time (Cebollero et al. 2012; Bednarska et al. 2016). If there is a lack of substances (e.g., enzymes) autophagy does not participate in cell survival, so processes of cell death as degeneration are activated intensively (Tettamanti et al. 2008; Franzetti et al.

2012; Park et al. 2013; Karpeta-Kaczmarek et al. 2016; Romanelli et al. 2016). Ultrastructural and structural changes caused by cadmium have been detected in different organs in invertebrates, e.g., muscles, gonads, suprapharyngeal and subpharyngeal ganglions, and digestive system (Dallai 1966; Lauverjat et al. 1989; Vega et al. 1989; Siekierska & Urbańska-Jasik 2002; Siekierska 2003; Hödl et al. 2010; Takacs et al. 2016; Yuan et al. 2016; Bednarska et al. 2016, 2019). A similar phenomenon has been observed in 12- and 45-day treated animals of *L. forficatus* because quantitative analysis revealed that regardless of the time of exposure to Cd, the decrease in cell viability was about 13%. We showed that both short- and long-term exposure of the animal to metal-contaminated soils triggers activation of cell death processes. During short-term exposure, autophagy is involved in alignment of homeostasis, while long-term exposure inhibits autophagy by the lack of reserve material. We observed non-specific autophagy that is mainly involved in different organelles and structures of digestion (Cebollero et al. 2012; Franzetti et al. 2012; Rost-Roszkowska et al. 2012, 2018, 2019), so disrupted organelles, the reserved material (e.g., lipids, proteins, saccharides), or heavy metals (e.g., cadmium, lead, copper, etc.) can be neutralized in order to maintain homeostasis. In this way, autophagy plays a key role in reducing intracellular stress by supplying components to replace decreased nutrient uptake (Kamitsuji et al. 2008; Bellodi et al. 2009; Altman et al. 2011; Lipovšek et al. 2014, 2018; Lipovšek & Novak 2016; Włodarczyk et al. 2019). Here we suggest that non-specific autophagy as a survival factor against cadmium exposure was activated in order to enclose organelles damaged by cadmium and utilize reserve materials inside the autophagosomes and remove them from the midgut epithelium. A similar mechanism has been described in the midgut epithelium of other invertebrates (Lipovšek et al. 2014, 2018; Wilczek et al. 2014; Lipovšek & Novak 2016; Włodarczyk et al. 2019). However, when too many autophagic structures occur in the cytoplasm, the cell death processes are activated (Levine & Klionsky 2004; Giusti et al. 2007; Rost-Roszkowska et al. 2010, 2015, 2018, 2019; Franzetti et al. 2012). Eventually, damaged cells could be eliminated from the tissue/organ and the inflammation is not activated (Chandra et al. 2009; Franzetti et al. 2012; Rost-Roszkowska et al. 2012, 2018, 2019; Bednarska et al. 2016; Mugnano et al. 2018). The relationship between autophagy and apoptosis has been described as commonly occurring in the

midgut epithelium of Myriapoda, both the centipedes (Chilopoda) (Rost-Roszkowska et al. 2015, 2016) and millipedes (Diplopoda) (de Godoy & Fontanetti 2010; Nogarol & Fontanetti 2011; Rost-Roszkowska et al. 2018, 2019) as well as in other species of Arthropoda (Malagoli et al. 2010; Franzetti et al. 2012; Lipovšek & Novak 2016). Apoptotic and autophagic features may appear and coexist in the same cells, and in addition, these two processes may depend on each other in some tissues/organs (Gozuacik & Kimchi 2007; Kamitsuji et al. 2008; Altman & Rathmell 2012). Due to the fact that there was a clear correlation between the amount of reserve material and the activation of autophagy and apoptosis in the midgut epithelium of *L. forficatus*, we can conclude that both these processes are nutrient deprivation-induced (Gozuacik & Kimchi 2007; Shang et al. 2011; Altman & Rathmell 2012; Włodarczyk et al. 2019). In the digestive epithelia of many invertebrates, apoptosis is a natural process which due to the neutralization of epithelial cells protects the tissue against inflammation (Vaidyanathan & Scott 2006; Maghsoudi et al. 2012; Romanelli et al. 2016; Rost-Roszkowska et al. 2016; Sonakowska et al. 2016; Lipovšek et al. 2018; Włodarczyk et al. 2019).

Our previous studies on autophagy, apoptosis and necrosis in the centipedes *L. forficatus* and *S. cingulata* revealed that autophagic structures (autophagosomes, autolysosomes, residual bodies) accumulated mainly in the apical and perinuclear cytoplasm of the digestive cells in *L. forficatus*, while in *S. cingulata* they were distributed in the entire cytoplasm (Rost-Roszkowska et al. 2015, 2016). Additionally, the description of autophagy, apoptosis and necrosis showed no differences between males and females, so the results are presented here independently of the sex. We stated that the cell death in the midgut epithelium of *L. forficatus* is presumably responsible for protecting the organism against infections, because it was intensive in animals infected with *Rickettsia*-like pathogens (Rost-Roszkowska et al. 2015, 2016). Due to the fact that we used only individuals not infected with pathogens for the study, it was possible to confirm that these processes will also intensify in the course of the operation of another stressor. We can conclude that both autophagy and apoptosis are activated according to the presence of a stressor (cadmium exposure) as has been described for other invertebrates (Siekierska & Urbańska-Jasik 2002; Siekierska 2003; Hödl et al. 2010; Amachree et al. 2013; Sheir et al. 2013; Włostowski et al. 2014). However, short-term

exposure activates mainly autophagy, while long-term cadmium intoxication involves mainly apoptosis and necrosis in functioning of the midgut epithelium, while autophagy is inhibited. Short-term exposure to cadmium caused an increase in the cadmium concentration in the animal body, whereas after long-term exposure, the cadmium concentration in the organism of the reared animals decreased below the level described for the control group. At the same time, it should be mentioned that there was a decrease in ATP concentration and an increase in ADP concentration. The concentration of ATP was significantly reduced in midgut epithelial cells after both 12 and 45 days of exposure to Cd. It demonstrates the possibility of inhibiting the growth and/or intensification of degenerative processes in the cell. The decrease in cadmium concentration in these animals is probably related to intense cell death (especially late apoptosis and necrosis), due to which damaged cells, as well as those having accumulated numerous toxic substances, are removed from the body. After 45 days of exposure to cadmium, distinct necrotic changes were observed in the midgut epithelium, as well as the number of spherites corresponding to the accumulation of heavy metals in animals (Humbert 1978; Hopkin & Martin 1983; Gräff et al. 1997). The physiological difference in the course of apoptosis and necrosis is the levels of intracellular ATP. ATP depletion induces necrosis, indicating that necrosis is ATP independent process, while apoptosis is ATP-dependent. High ATP levels direct a cell to undergo apoptosis, whereas low ATP levels favor necrosis (Eguchi et al. 1997; Leist et al. 1997; Nikolettou et al. 2013). Cadmium has been detected as accumulated in numerous granules/spherites in arthropods midgut (Dallai 1966; Humbert 1974; Lauerjat et al. 1989; van Straalen & Roelofs 2005; Leonard et al. 2009). It has been suggested that such granules could be excreted with gut pellets (van Straalen & Roelofs 2005). In myriapods, toxic metals are accumulated in the digestive cells as spherocrystals called spherites. This phenomenon has been suggested as the way that heavy metals are neutralized as a mechanism of detoxification (Hopkin & Martin 1983; Hopkin et al. 1985; Köhler & Alberti 1992; Köhler et al. 1995; Vandenbulcke et al. 1998b). If cadmium is not removed from the midgut epithelial cells either by excretion, degeneration processes or by, e.g., metallothioneins, it will pass through the hemocoel to different organs of the body and cause a toxic effect (Leonard et al. 2009; Włostowski et al. 2016; Bednarska et al. 2019).

Hemocytes, which form a kind of “immune system”, participate in pathogens’ phagocytosis and their opsonization, synthesis of antibacterial proteins, and regeneration of any damage caused by mechanical injuries or external factors by hemolymph coagulation (Gupta 1986; Xylander 1992, 2009a, 2009b; Lavine & Strand 2002). They have been described in both millipedes (de Godoy & Fontanetti 2010) and centipedes (Nevermann et al. 1991; Xylander 1992, 2009a, 2009b) as ameoboid-shaped cells which gather in the hemocoel in the vicinity of visceral muscles, the fat body, the digestive system and gonads. During our previous studies on *L. forficatus* and *S. cingulata* midgut, we observed that hemocytes accumulated just beneath the basal lamina in the vicinity of visceral muscles (Chajec et al. 2012, 2014). Additionally, numerous hemocytes that entered the midgut epithelium through the basal lamina have been detected in *S. cingulata* in all of the animals examined (infected and non-infected) (Chajec et al. 2014). However, in *L. forficatus* they have been observed between the digestive, secretory and regenerative cells only in specimens that had been infected with *Rickettsia*-like microorganisms (Chajec et al. 2012). We suggested that probably in *S. cingulata* hemocytes play a role not only against infection, but also against toxic substances that may enter the organism from the midgut lumen. In *L. forficatus* when the pathogens infect the midgut epithelium, hemocytes are responsible for protection of the organism against the migration of these pathogens into the body cavity (Chajec et al. 2012). In millipedes, hemocytes have been described as accumulating among cells of the fat body that surrounds the digestive system in animals treated with heavy metals. The number of hemocytes increased when a number of degenerated cells of the fat body occurred (de Godoy & Fontanetti 2010; Perez & Fontanetti 2011). The increase in circulating hemocyte number caused by heavy metals has been described for some arthropods (Lorenzon et al. 2001; Liu et al. 2013; Stalmach et al. 2015). Our study revealed that hemocytes migrate through the midgut epithelium and could be observed among digestive cells in animals treated with cadmium for 12 days. However, they did not occur inside the midgut epithelium when the period of cadmium exposure was longer – 45 days. We can assume that when the stressor is weak, the hemocytes will participate in maintaining the balance in the tissue, but when the stressor is strong or long-lasting, other mechanisms will have to be included in the protection of the entire organism – here, nutrient deprivation-induced autophagy and apoptosis. In general, short- and long-term exposure of soil centipede to

cadmium affects different processes that take part in homeostasis maintenance. To obtain a complete set of results, it is necessary to analyze the activation of proteins and enzymes (e.g., dismutases, peroxidase) involved in homeostasis maintenance after cadmium treatment.

5. Conclusions

Our studies showed that: (1) short-term intoxication causes intensification of autophagy and digestion of reserve material as survival factors, (2) long-term exposure to cadmium causes inhibition of autophagy connected with the lack of reserve material and the activation of cell death processes; (3) autophagy and cell death are nutrient deprivation-induced processes; (4) cell death is a mechanism that allows removal of cells with excess heavy metal from the tissue; (5) hemocytes are involved in tissue protection after short-term exposure to a stressor.

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Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This article does not concern any studies with human participants that were performed by any of the authors.

Disclosure statement

No potential conflict of interest was reported by the authors.

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