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Effect of lead and cadmium ions upon the pupariation and morphological changes in *Calliphora vicina* (Diptera, Calliphoridae)

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Abstract

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Modelling the influence of different concentrations of lead and cadmium ions upon a laboratory culture of insects has not been adequately studied. In our research, we assessed the influence of cadmium and lead nitrates at different concentrations (10^{-2}–10^{-9} M) upon the development of larvae, pupae and imagines of *Calliphora vicina* Robineau-Desvoidy, 1830 (Diptera: Calliphoridae). We found an acceleration in the development of larvae and an increase in mass of puparia when lead ions were added to the food of the larvae, and decrease in the mass of puparia when cadmium ions were added. We registered nanism and malformation of the fly imagines in experiments with lead and cadmium in the food substrate. We observed that under the influence of the studied heavy metal ions there was a reduced motor activity of the fly larvae at all stages of development, a delay in formation of puparia and a delay in the emergence of imagines in comparison with the control group.

Keywords

heavy metal pollution, larvae, morphological changes: nanism pupariation

Introduction

Urbanization on the whole and technogenic pollution in particular are the main causes of the saturation of the environment by different chemical elements (Li et al., 2011; Safaee et al., 2014). Contamination of the environment with toxic and dangerous substances causes serious problems for living organisms (Bessonova et al., 2015; Kulbachko et al., 2015). Among these polluting substances, heavy metals (HM) are the most significant. HM have a tendency to accumulate in living organisms, and most ions have toxic or cancerogenic properties (Fu, 2011; Brygadyrenko and Ivanyshin, 2014, 2015).

Heavy metals are common in the air, water and soil. As a result of the increasing level of usage of these metals in industry, they negatively affect human health (Ahamed and Siddiqui, 2007; Zhang and Pu, 2011; Li et al., 2011). When intruding into the biogeochemical processes in natural ecosystems, heavy metal compounds disrupt the stability of elementary compounds of biological systems at different levels of their organization. Specialists in environmental conservation have distinguished a priority group among toxic metals, in which cadmium, copper, arsenium, nickel, mercury, lead, zinc and chromium have been identified as dangerous for living organisms, lead and cadmium being the most toxic among them (Bobyliov et al., 2014).

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Heavy metals are a group of elements with a density of pure metal higher than 5 g cm^{-3}. Some ions of heavy metals are indispensible elements in the structure of proteins (Fe, Zn, Cu), the rest have no biological function and therefore they are the so-called "insignificant" metals (Cd, Pb, Hg, Ni). These "unnecessary" metals exist in the environment together with biologically necessary metals, e.g. in volcanic rocks, ores, various deposits created by industrial activity (Sharma and Agrawal, 2005; Florea and Busselberg, 2006). Both biologically necessary and undesirable metals can be toxic for living organisms if they are present in doses which exceed the critical level (Janssens et al., 2009).

For example, lead can cause the following human diseases: renal failure, hypertension, anemia, disorders of genital organs, nervous system disorders (Ahamed and Siddiqui, 2007). Lead has a tendency to accumulate in bones and concentrate in the bloodstream during hemopoietic processes, thus affecting not only the condition of mature organisms, but also the development of embryos. Lead can affect the genital organs of foetuses (Apostoli and Catalani, 2011). Therefore, research into the determination of the toxicity of heavy metals during the period of ontogenesis of different organisms is highly significant.

Another ecologically significant metal is cadmium (Bessonova et al., 2015). It is one of the most significant environmental pollutants, well known for its cancerogenic properties (Nawrot et al., 2006). The main source of this element is waste from non-ferrous metallurgy.

Therefore, it is relevant to conduct experiments modelling the impact of different concentrations of lead and cadmium ions upon laboratory cultures of invertebrates. A significant aspect of such researches is defining the morpho-physiological disorders of an animal organism, with registration of changes related to the concentration of toxic compounds in the environment. This data is necessary for conducting ecological research into anthropogenically transformed ecosystems.

A number of works focuses on the potential impact of heavy metal pollution upon different invertebrates. Among soil micro-Arthopoda, assessment has been made of the effect of heavy metals upon Collembola, which are widely used as biological indicators (Huhta et al., 1979; Hagvar, 1982; Faber and Heijmans, 1997; Van Straalen, 1997). The effect of heavy metal pollution has also been well studied for earthworms (Lumbricidae) (Ireland, 1983; Emmerling et al., 1997; Heikens et al., 2001; Nahmani et al., 2003; Tischer, 2009; Kulbachko et al., 2014), gastropods (Gastropoda) (Nott and Nicolaidou, 1989; Greville and Morgan, 1990; Berger and Dallinger, 1993; Presing et al., 1993; Triebskorn and Köhler, 1996), chironomid larvae (Diptera, Chironomidae) (Mackie, 1989; Westcott and Kalff, 1996), and Drosophilidae (Bahadorany and Hilliker, 2009; Safaee et al., 2014).

Accumulation of heavy metals in an insect organism can affect its development. Heavy metals accumulate in insects' organs during digestion; they are accumulated and taken out in a form of "granules" (Sun et al., 2007). Cell mechanisms protecting the cells and organism in general from stress caused by toxic metals are being intensively studied (Hopkin, 1989; Ratcliffe and Götz, 1990; Luce et al., 1993; Gillespie et al., 1997). The main way Cd ions penetrate insects` cells is through the calcium channels (Braeckman et al., 1999). Cadmium is chemically close to zinc, calcium and iron, which can replace these ions in biochemical reactions as pseudo-activators or, on the contrary, as inhibitors containing metals of enzymes, thus changing the activity of many hormones and ferments (Craig et al., 1999). Due to cadmium's ability to bind with sulfhydryl groups (-SH), it combines with cytoplasmic and nuclear material of the cells, damaging them (Greenwood and Earnshaw, 1997). Ions of lead bind with ferments of cells responsible for metamorphosis of insects, damaging mitochondrial cristae, thus decreasing synthesis of ATP. In addition, a negative effect of lead upon production of growth hormones has been registered (Safaee et al., 2014).

Heavy metals are accumulated in plants and transported through trophic chains, maintained by the soil-adsorption complex (Bessonova et al., 2015). Larvae of many synanthropic Diptera at the stage of puparium dwell in the litter and upper layers of the soil. Therefore, they can be a mediator in the processes of migration and accumulation of pollutants. Besides, blowfly larvae (Calliphoridae) fulfil an important ecological function in the decomposition of animal remains. They are used extensively in forensic entomology, predominantly to establish a minimum time since death, or a minimum post-mortem interval, using the larval parameters as a "biological clock" (Donovan et al., 2006). Thus, study of the impact of heavy metals upon the ontogenesis of these Diptera is of significant interest for ecological control of natural and urban territories. Invertebrate phytophages, saprophages and necrophages are promising objects for the determination of the level of heavy metal pollution in terrestrial ecosystems due to their high capacity to accumulate heavy metals.

The objective of this study is to evaluate the impact of different concentrations (10^{-2}–10^{-9} M) of lead and cadmium nitrates upon the timing of larval development, size and mass of puparia and morphology of the imagines of *Calliphora vicina* Robineau-Desvoidy, 1830 (Diptera: Calliphoridae).

Materials and methods

As an object for research, we chose the Urban Bluebottle Blowfly – *Calliphora vicina* Robineau-Desvoidy, 1830 (Diptera: Calliphoridae), a species common throughout the Hollarctic, a ubiquitous component of the synanthropic Diptera complex in the temperate zone (Vinogradova, 1984). *C. vicina* has long been valued as a convenient object for modelling in a wide range of research due to the ease of its cultivation, large size and the existence of a detailed description of its inner and outer structure at all stages of its development. Our experiments were conducted in September–October of 2013. Larvae of *C. vicina* were grown in the Scientific Research Institute of Oles Honchar Dnipropetrovsk National University (Dnipropetrovsk, Ukraine).

For development (from II stage to the pre-pupal stage or IV stage), *C. vicina* larvae were put into transparent plastic cups (0.3 l). A small amount of tow was put into the cups in order to imitate the insects' natural environment (Fig. 1, a). For each concentration of Cd and Pb (from 10^{-2} to 10^{-9} M) and the control (without addition of metal salts into the substrate) ten larvae of II stage were added to each experimental cup. Each experiment was replicated eight times (n = 8). Therefore, 72 tests were conducted using solution $Cd(NO_3)_2 \cdot 4H_2O$ and 72 tests with solution $Pb(NO_3)_2 \cdot 4H_2O$, i.e. 144 tests with 1,440 *C. vicina* larvae. In the middle stage of experiment, only 50–70% of the initial number of larvae survived in some tests.

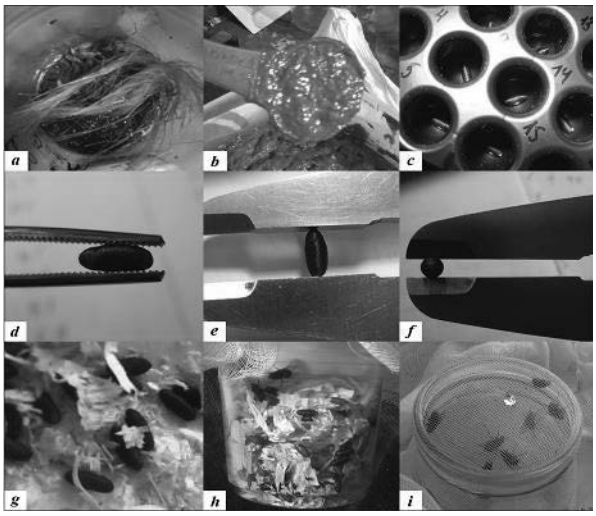

Fig. 1. Methods for laboratory experiments on *C. vicina:* *a* – cups (0.3 l) with food substrate and tow; *b* – minced raw pork liver (measuring spoon 5 ml); *c* – plastic boxes for identifying puparia; *d* – measuring height of puparia (h); *e* – measuring length of puparia (l); *f* – measuring width of puparia(d); *g* – live *C. vicina puparia; h* – container for young hatched flies; *i* – imagines of flies in the container with food for mating.

Initially, the *C. vicina* larvae for the experiment were cultivated in a 30 × 18 × 15 cm general plastic container. For maintaining the larvae, moistened sawdust was used. As a food substrate (source of protein food), we used pork liver. During the process of cultivation, the substrate was occasionally moistened with distilled water. Also occasionally a certain amount of sawdust was added due to the excess of moisture resulting from the feeding of the larvae and decomposition of the substrate, which could have otherwise caused unfavorable conditions which would induce the larvae to try to crawl out of the container.

The larvae were cultivated in the general container at a laboratory air temperature of +12 to 15 °C and atmospheric moisture 45–63% until they reached II stage (72 hours of development after the larvae hatched from the eggs). The food substrate was a uniform mince of raw pork liver. We put 10 g (two portions of 5 g) of mince in each cup (Fig. 1, b). At the beginning of the experiment we put 1 ml of solution of Cd and Pb at the set concentrations (10^{-2}–10^{-9} M) into each cup. The prepared mixture was stirred and left for 3 hours at a temperature of +12…+15 °C and air moisture 45–63%. Then 10 fly larvae of II age were put into each cup. The cup was closed with a mosquito net. The laboratory was kept at a constant temperature and moisture. The larvae developed in conditions of a short day (up to 12 hours of daylight). Each cup was checked every day. If necessary, the substrate was moistened (0.5 ml of distilled H_2O was added). On the 20th day of the experiment we selected puparia to test their water content – 10 individuals from each series of tests (10 puparia from the control 60 from the series of tests with Cd, with a high mortality rate, and 80 – from the series of tests with Pb, where the mortality rate was lower). The puparia were weighed on a torsion balance for determining the live weight of the pupae. Each selection of the puparia was boiled in a bain-marie for 5 minutes. Then they were put into numbered boxes (Fig. 1, c) and dried under a convector heater (temperature of 35 °C, humidity – 30 %). The puparia were weighed 5 times (each 3 days). Then the linear sizes (height, length and width) of the puparia were determined using calipers (Fig. 1, d, e, f). The height was defined as the distance between the highest points of a puparium in a dorsal-ventral direction.

The hatched flies were put into containers for observations (Fig. 1, g). Sawdust was added as a substrate Fig. 1, h). For keeping the imagines active, every day they were given water, honey and slices of rotten liver for protein nutrition of the females (Fig. 1, k).

For comparing the selections, ANOVA (Statistica 8.0, StatSoft Inc., USA) was used. On the diagrams, the small squares show the median, the large rectangles show the 25 and 75% quartiles, the vertical lines show 95% of the variation, the stars and circles show the outliers.

Results

Consumption by *C. vicina* larvae of food rich in lead (Fig. 2) did not cause a statistically significant change in the mass of the puparia ($F = 5.01$, $F_{0.05} = 1.96$; $df_1 = 8$, $df_2 = 351$; $P = 7.0 \cdot 10^{-6}$). Addition of lead at a concentration of 10^{-9} M to the background content of metal (MPC – maximum permissible concentration of chemical elements of meat products 0.5 mg kg^{-1} for Pb and 0.05 mg kg^{-1} for Cd) caused a statistically significant increase in the mass of puparia of 6.7 mg from 45.0 to 51.7 mg ($F = 3.96$, $F_{0.05} = 13.42$; $df_1 = 1$, $df_2 = 78$; $P = 4.5\ 10^{-4}$). Further gradual increase in concentrations of the metal did not cause statistically significant changes in the mass of the puparia ($F = 0.67$, $F_{0.05} = 2.04$; $df_1 = 7$, $df_2 = 312$; $P = 0.699$), and the average mass of the puparia remained between 51.7–53.4 mg.

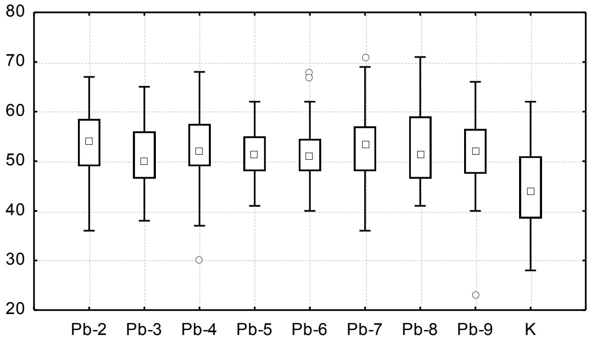

Fig. 2. Changes in mass of *C. vicina* puparia (mg, live weight) in relation to the Pb concentration in food substrate: Pb^{-2}– Pb^{-9} – contents of lead in food substrate correspond to 10^{-2}–10^{-9} M; K – control, without addition of metal (10^{-15} M) into substrate; $n = 40$ for each concentration of metal; Y-axis shows puparia mass (mg).

In contrast to lead, contamination of the food with cadmium caused a significant death rate at II and III ages of the larvae. On the 7th day of the experiment, during the test with concentrations of Cd 10^{-7} M, 45% of the larvae died, during the tests with Cd 10^{-3} M – 33% and with 10^{-2} M, 50% of the 80 larvae in each variant of the experiment died. The studied range of cadmium concentration covers all values of *C. vicina*'s survivability up to a 98% death rate. *C. vicina*'s consumption of food with extra addition of cadmium salts (Fig. 3) caused a statistically significant change in the mass of the puparia ($F = 12.39$, $F_{0.05} = 2.04$; $df_1 = 7$; $df_2 = 269$; $P = 9.1 \cdot 10^{-8}$). The average mass of the puparia in control (45.0 mg) increased even with a minimal increase in metal concentration 10^{-9} M (59.1 mg). At a concentration of 10^{-3} and 10^{-2} M, more than half the larvae died, and the mass of the remaining puparia decreased to the smallest values compared to the control group (43.5 mg at 10^{-2} M).

As a result of consuming food saturated with lead ions (Fig. 4), the content of dry matter in *C. vicina* puparia did not significantly change ($F = 1.00$, $F_{0.05} = 2.05$; $df_1 = 8$, $df_2 = 81$; $P = 0.439$). The average values for different variants of the experiment fluctuated between 33.6–40.3%. A gradual decrease in water content from control values to 10^{-8} M Pb was registered. Then we observed an insignificant increase in water concentration in the puparia to 10^{-6} M and irregular decrease in concentration of water in puparia to the maximum of the studied concentrations (10^{-2} M).

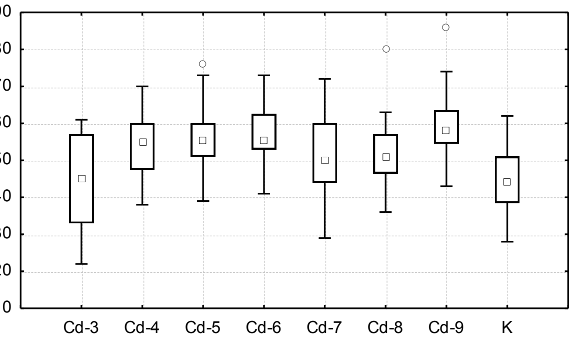

Fig. 3. Changes in mass of *C. vicina* puparia (mg, live weight) in relation to the Cd concentration in food substrate: Cd^{-3}– Cd^{-9} – cadmium contents in food substrate correspond to 10^{-2}–10^{-9} M; K – control, without addition of metal (10^{-15} M) into substrate; $n = 40$ for each concentration of metal; Y-axis shows puparia mass (mg).

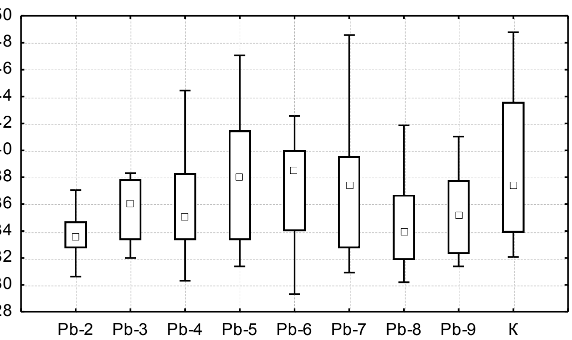

Fig. 4. Changes in dry weight of *C. vicina* R.-D. puparia in relation to Pb concentration in food substrate: Pb^{-2}–Pb^{-9} – lead content in food substrate corresponds to 10^{-2}–10^{-9} M; K – control, without addition of metal (10^{-15} M) into substrate; $n = 10$ for each concentration of metal; Y-axis shows the dry matter content, %.

The influence of cadmium (Fig. 5) caused significant differences in the content of dry matter between the control values and 10^{-6}, and also 10^{-9} M ($F = 5.65$; $F_{0.05} = 3.35$; $df_1 = 2$; $df_2 = 27$; $P = 0.009$). The differences between control values and the remaining variants of the experiment are not significant ($F = 0.58$; $F_{0.05} = 2.43$; $df_1 = 5$; $df_2 = 44$; $P = 0.718$).

The mass of the puparia and their dry matter content showed an inverse relationship (Fig. 6). It is described by the equation of square regression: $A = 0.020\ m^2 - 2.513\ m + 110.3$, where A – content of dry matter (%), m – live weight of puparium (mg). Yet no similar data about other species of Calliphoridae have been obtained.

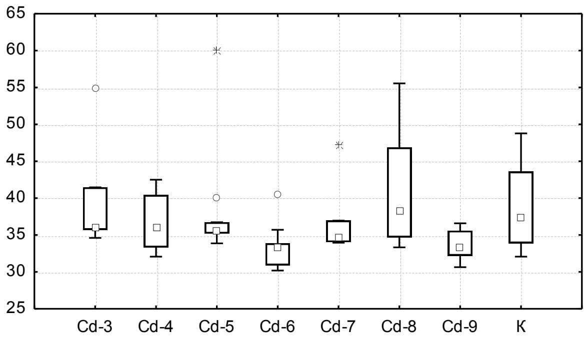

Fig. 5. Changes in dry weight of *C. vicina* puparia in relation to the Cd concentration in food substrate: Cd-3–Cd-9 – cadmium content in food substrate corresponds to 10^{-2}–10^{-9} M; K – control, without addition of metal (10^{-15} M) into substrate; *n* = 10 for each concentration of metal; Y-axis shows the dry matter content (%).

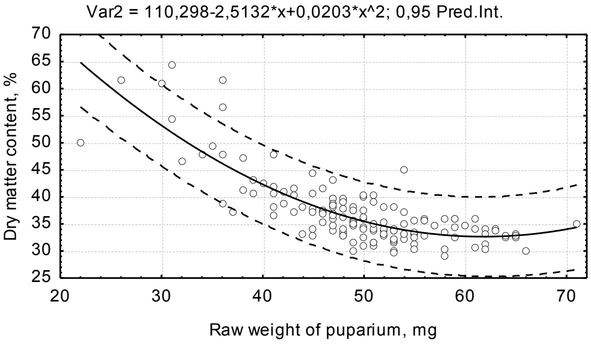

Fig. 6. Relationship between mass of a *C. vicina* puparia and its dry matter content from the control group and experimental samples, described using equation of square regression: A = 0.020 m^2 – 2.513 m + 110.3, where A – content of dry matter (%), m – raw mass of puparia (mg), (*n* = 150).

As we presumed before the experiment, when the mass of a puparium increases, non linear increase in size is observed (Fig. 7): V = 0.013 m^2 – 0.882 m + 57.1, where V – size of puparium (mm^3), m – live weight of puparium (mg). The size of a puparium is connected with the level of the inflation of the larva's breathing system with air just before sclerotization of the puparium. No significant differences in the sizes of puparia between different variants of the experiment were found: when the larvae were affected by excess concentrations of lead, the average size of the puparia ranged between 43.3–50.7 mm^3, and in the control it was at the level of 43.9 mm^3 ($F = 1.93$, $F_{0.05} = 2.06$; $df_1 = 8$, $df_2 = 81$; $P = 0.066$). Also no significant changes for variants of the experiment with cadmium were registered: the size of the puparia ranged between 40.4–48.8 mm^3 ($F = 2.03$, $F_{0.05} = 2.16$; $df_1 = 7$, $df_2 = 62$; $P = 0.065$). When evaluating the variability of linear sizes of the puparia, weakly significant differences for particular variants of the experiment were found.

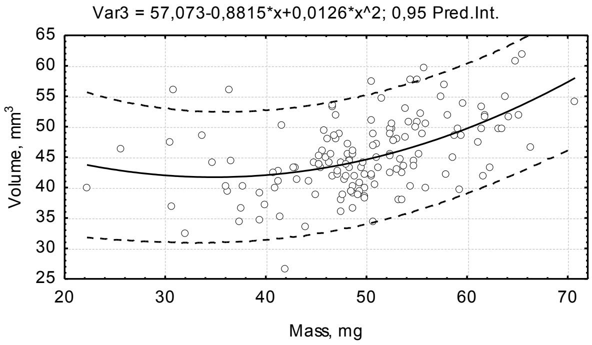

Fig. 7. Relationship between mass of a *C. vicina* puparia (mg) and its size (mm^3) from the control group and experimental samples, (*n* = 150).

In the gradient of lead concentration, the minimum average values for length of puparia (8.30 and 8.34 mm) corresponded to control, and maximum (8.73 and 8.83 mm) – 10^{-4} and 10^{-2} M Pb (Fig. 8). Statistically significant changes in the length of puparia ($F = 2.23$, $F_{0.05} = 2.05$; $df_1 = 8$, $df_2 = 81$; $P = 0.033$) are accompanied, as was mentioned above, by the maintenance of constant size due to decrease in height and width of puparia with increasing Pb concentration.

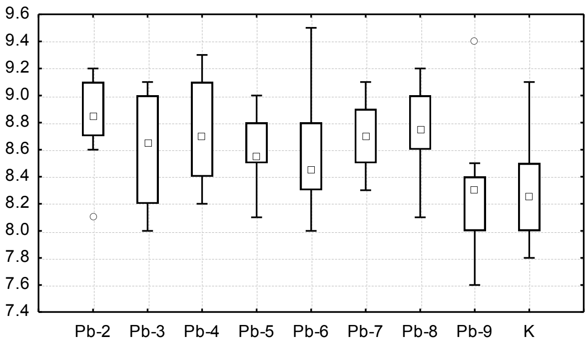

Fig. 8. Changes in parameters of *C. vicina* puparia in relation to concentrations of Pb; on the Y-axis the length of the puparia is shown (mm).

Statistically significant differences in length of puparia length in variants of the experiment with cadmium ($F = 5.74$, $F_{0.05} = 4.41$; $df_1 = 1$, $df_2 = 18$; $P = 0.028$) were registered only in the control group and 10^{-5} M – 8.34 and 8.79 mm, respectively (Fig. 9). For the remaining variants of the experiment, the differences were not statistically significant ($F = 1.76$, $F_{0.05} = 2.28$; $df_1 = 6$, $df_2 = 53$; $P = 0.125$).

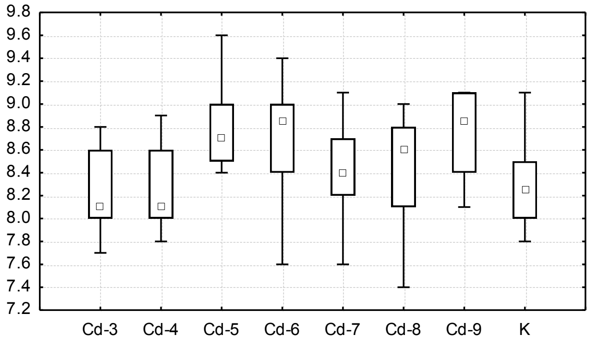

Fig. 9. Changes in parameters of *C. vicina* puparia in relation to Cd concentrations; Y-axis shows length of puparia (mm).

More viable larvae of *C. vicina* were found in the experiment with Pb, when most individuals reached pupation. This did not take place in the series of experiments with cadmium: at the concentration Cd 10^{-2} M, not a single larva reached pupation, the death rate was already 25% after 96 hours of the experiment. In the experiments with concentration Cd 10^{-3} M in the food substrate, only 14 individuals out of 40 developed to pupation, with concentration Cd 10^{-7} M – 27 individuals pupated, and with Cd 10^{-9} M – 36 pupated. In the rest of the tests (concentrations Cd 10^{-4}, 10^{-5}, 10^{-6} and 10^{-8}) all larvae formed puparia.

In the series of experiments with the highest concentration of cadmium, activity of larvae was low compared to the experiments with the smallest concentrations and the control. Usually, they concentrated under the sawdust, moved little, and correspondingly, consumed little. But, in the middle stage of the experiment (8–14 days), the remaining viable larvae showed activity in consuming the substrate. It was difficult to find dead larvae in the cups where they died: probably the remaining actively consuming individuals hastened the destruction of the dead *C. vicina* larvae through the action of their decomposing enzymes (carboxypeptidases A and B, leucine aminopeptidases, collagenase, aspartic and serine proteases and metalloproteinases).

At all concentrations of lead, the larvae fed intensely throughout almost the entire duration of the experiment. They were especially active in the experiments with low concentrations of lead ions (10^{-8} M), where the process of decomposition of the food substrate had completely destroyed the sawdust by the end of the experiment. In all gradations of lead, a sort of "foam" was observed – a phenomenon resulting from normal abenteric digestion as a result of vital activity of *C. vicina* larvae, in contrast to the low level of vital activity of fly larvae in the series of experiments with addition of Cd ions, where almost no foam was formed.

It should be mentioned that the larvae pupated faster in the experiments with minimum concentrations of heavy metals: first – with 10^{-9} M, second – with 10^{-8} and 10^{-7} M, third – with 10^{-6}, 10^{-5} and 10^{-4} M, last – with 10^{-3} and 10^{-2} M. The emergence of the imagines was observed in the same sequence. Up to 80% of the young flies were hatched at concentrations of metals 10^{-9} and 10^{-8} M, pathologies in hatching of imagines were observed at concentrations of 10^{-3} and 10^{-2} M (Fig. 10, e, f).

Fig. 10. Imagines of *C. vicina* exposed to influence of cadmium salts in food substrate: 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M: *a, b, c, d, e* – variants of wing malformation; *f, i* – comparison of individuals with wing malformation and individuals with normal wings; *g, h* – comparison of individuals with narrow abdomen (abnormality after Cd effect) and normal abdomen (individuals from control group).

In the control variant of the experiment (without addition of heavy metals salts into the food substrate) after the puparium stage 96.6% of the flies were hatched, 41.4% of them were males (♂) and 58.6 % – females (♀) with total number of puparia – 60. Experiments when salts of Cd and Pb were added showed a high death rate at the larval stage. A similar tendency was also observed at the pupal stages in the experiments with high concentrations of metals salts in the food substrate. For example, in the test with concentration of salt of Cd 10^{-3} M, imagines hatched only in 50% of cases, the rest died. In the test with concentration of salt of Pb 10^{-2} and 10^{-3} M, only 31 and 30 imagines hatched out of 42 and 40 pupae correspondingly (75%). In the first case they included: ♂ – 38.7%, ♀ – 61.3%; in the second: ♂ – 22.5%, ♀ – 52.5%. However, we need to mention that in the series of experiments with lead in the substrate, practically all larvae reached the puparium stage. This may indicate that lead salts are less toxic for *C. vicina* larvae compared to cadmium salts, indeed in the experiments with concentration Cd 10^{-2} M, all larvae died without reaching III age (except for one, which died at the stage of puparium).

In all experiments, the ratio of males to females showed a similar tendency: there were 1.5–2.0 times more females than males. Malformation (abnormality of development) of hatched flies was observed more often among females, too. Deformation of wings was observed 2–3 times more often among females than among males (Fig. 10, a, b, c, e, f, i). In the experiments with salts of Cd 10^{-3} M, such abnormality was found in 6 females out of 24, and only in one male out of 12 (Fig. 10, d, g), and the deformation of the wings was not very significantly manifested.

Deformation of the flies' abdominal parameters was also manifested among individuals (mostly females) from the series of experiments with cadmium (Fig. 10, h; Fig. 11). In the experiments with concentrations of Pb salts at 10^{-2} and 10^{-4}, 10^{-6} and 10^{-7} M, the proportion of wing abnormalities among females came on average to 44.5%, among males – 23.3% (Fig. 12, a, b, c). Nanism of fly imagines was also more strongly manifested among females, especially in the series of experiments with influence of lead salts (Fig. 12, b, c).

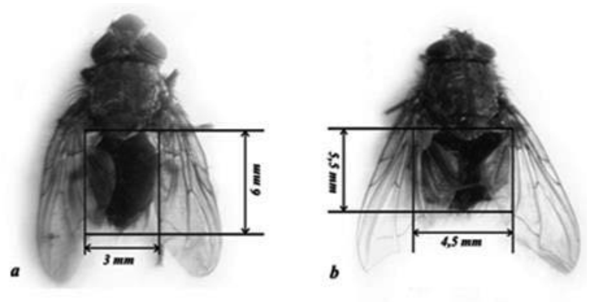

Fig. 11. Parameters of *C. vicina* imago`s abdomen: *a* – after exposure to cadmium salts; *b* – without influence of metal (individual from control group).

Here, some individuals also manifested a "spidery" appearance (small body size and long limbs, the colour was monotonously black). It is interesting that there were no such flies in the series of experiments with cadmium Fig. 12, d).

In the experiments with concentration of Cd salts at 10^{-5} M, nanism was more strongly manifested among females and made up 11.1% of total specimens, unlike males (6.3%). Similar indicators were registered for the experiments with concentrations of lead salts at 10^{-2} and 10^{-4} M, where this malformation was 15.8% (♀) and 15.6% (♂), and also 14.6% (♀) and 14.2% (♂) of total specimens, respectively.

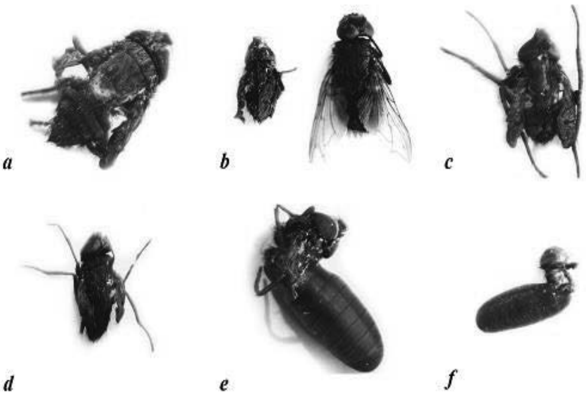

Fig. 12. Imago of *C. vicina* after exposure to influence of lead salts in food substrate: 10^{-2}, 10^{-4} and 10^{-6} M: *a, c, d* – variants of wing malformation; *b* – comparison of individuals with wing malformation, nanism and individuals from control group which were of normal size and without wing pathologies; *d* – individual of black colour and "spidery" appearance (abnormality after influence of Cd); *e, f* – variants of pathologies during processes of adult emergence (inability to hatch out of puparium).

Flies with malformations were placed in separate containers for their behaviour observation and for obtaining the next generation. In ethology of imagines' experimental group a change was identified compared with the flies' control group (Fig. 1, i). Especially this was manifested in the relationship between males and females. Individuals´ attitude to the opposite sex was indifferent or they were unable to mating process because of morphological abnormalities. Attempts to produce a second generation were unsuccessful.

In the experiments with cadmium, with this metal concentration of 10^{-2}, 10^{-3} and 10^{-4} M, we observed a significant dryness of the food substrate in comparison with smaller concentrations of the metal and in comparison with similar indicators from the series of experiments with lead. Perhaps, this was connected with decrease in the motor activity of the larvae as a result of the toxic impact of cadmium in large concentrations. This means that the food substrate was not involved in the moistening process caused by the secretion of proteolytic enzymes when the fly larvae were actively consuming food. The dryness of the food substrate at these high concentrations was also due to the decrease in the amount of bacteria contributing to decomposition of the substrate, which were affected by the same salts of the toxicant. We need also to mention that the food substrate in the tests with Cd 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M had a greenish color, but in the same concentrations with Pb, the substrate had a pinkish tone.

Discussion

Blow flies are among the first insects to discover and colonize animal and human remains (Greenberg, 1991; Donovan et al., 2006). Morphometric parameters and biological features are widely used by forensic entomologists to establish the minimum time elapsed since death (Bharti, 2009; Chen et al., 2011). Thus the information presented in our article is very useful for experts in forensic entomology for the time corpses' decay evaluation (Greenberg, 1991; Zhang and Pu, 2011), especially in industrialized regions of planet, where lead and cadmium are major technogenic pollutants (Bobyliov et al., 2014).

The most interesting fact we discovered during our research was the constant size of the puparia regardless of their mass and the larval death rate from received doses of heavy metals. Factually, when pupating, the low body mass of a larva is compensated by inflation of air pockets in the abdominal region ("air sacs" (Chown and Nicolson, 2004)).

The observation of the experimental group of flies resulted in finding that the influence of heavy metals on the reproductive ability was noticeable. This is in accord with Morgen and Trumble (2010), indicating that high metal concentrations frequently lead to modified ingestion, locomotor and reproductive disturbances (decreased egg laying and reduced fitness of offspring). However, *C. vicina* can be classified as a heavy metal-tolerant insect, with adaptations enabling to tolerate exposure to relatively high concentrations of some heavy metals (Gall et al., 2015). However, in our experiments with concentrations Pb and Cd in 10^{-6}–10^{-8} M the larvae survived, formed puparia and most of them did not show obvious morphological abnormalities.

The small number of larvae which are stunted in growth compared to the rest, is probably connected with endogenous processes (Nesin et al., 1995; Vinogradova and Reznik, 2000), or with individual hypersensitivity to the toxicants.

Conclusions

The investigation on the effect of heavy metals disclosed that the most toxic metal was cadmium – in this series of experiments the death rate of flies at the larval and puparium stages was significantly higher compared to the parallel indicators in the experiments with lead.

Examining the influence of cadmium salts upon the development of larvae of flies, the following malformations were observed: wing deformation, nanism, belly deformation and abnormalities of puparia. The impact of lead salts upon *C. vicina* larvae caused the following developmental abnormalities: wing deformation, nanism, black colour and pathologies in puparia.

There was a delay in the emergence of the flies from the puparia: young flies emerged on average 18 and 24 hours later in the experiments with lead and cadmium, respectively, compared to the parallel indicators in the control group. Pathologies of the wings rendered the flies unable to fly and mate.

Based on our studies, we may conclude that pupae are a convenient developmental stage for ecological and morphological studies in Diptera, as the imagines and larvae are highly dependent on the nutrition degree and on the transition time from one stage to the next.

Thus, in future it seems valuable to explore response to the toxicants impacts in less resistant fly species found in urban ecosystems in lower amounts than *Calliphora vicina*. Another thought provoking proposal may be to study links between the morphological variability of flies populations and the mass and size of pupae.

Experimental studies of the toxicological effects of lead and cadmium show that this issue is a relevant and promising research subject in the context of general problems in area of biological aspects of environmental protection.

References

Ahamed, M., Siddiqui, M.K., 2007. Low level lead exposure and oxidative stress: current opinions. *Clinica Chimica Acta*, 383: 57–64.

Apostoli, P., Catalani, S., 2011. Metal ions affecting reproduction and development. *Metal Ions in Life Sciences*, 8: 263–303.

Bahadorany, S., Hilliker, A.J., 2009. Biological and behavioral effects of heavy metals in Drosophila melanogaster adults and larvae. *Journal of Insect Behavior*, 22: 399–411.

Berger, B., Dallinger, R., 1993. Terrestrial snails as quantitative indicators of environmental metal pollution. *Environmental Monitoring and Assessment*, 25: 65–84.

Bessonova, V.P., Ivanchenko, O.E., Ponomaryova, E.A., 2015. Combined impact of heavy metals (Pb^{2+} and Cd^{2+}) and salinity on the condition of Lolium perenne long-term assimilation apparatus]. *Visnyk of Dnipropetrovsk University. Biology, Ecology*, 23: 15–20.

Bharti, M., 2009. Studies on life cycles of forensically important flies, Calliphora vicina and Musca domestica nebula at different temperatures. *Journal of Entomological Research*, 33: 273–275.

Bobyliov, Y.P., Brygadyrenko, V.V., Bulakhov, V.L., Gaichenko, V.A., Gasso, V.Y., Didukh, Y.P., Ivashov, A.V., Kucheriavyi, V.P., Maliovanyi, M.S., Mytsyk, L.P., Pakhomov, O.Y., Tsaryk, I.V., Shabanov, D.A., 2014. *Ekologija* [Ecology]. Kharkiv: Folio. 672 p.

Braeckman, B., Smugghe, G., Brutsaert, N., Cornelis, R., Raes, H., 1999. Cadmium uptake and defense mechanism in insect cells. *Environmental Research*, 80: 231–243.

Brygadyrenko, V.V., Ivanyshin, V.M., 2014. Impact of ferric salt on body weight of Megaphyllum kievense (Diplopoda, Julidae) and litter granulometric composition in the laboratory experiment. *Visnyk of Dnipropetrovsk University. Biology, Ecology*, 22: 83–87.

Brygadyrenko, V.V., Ivanyshyn, V.M., 2015. Changes in the body mass of Megaphyllum kievense (Diplopoda, Julidae) and the granulometric composition of leaf litter subject to different concentrations of copper. *Journal of Forest Science*, 61: 369–376.

Chen, C.D., Nazni, W.A., Ramli, R., Chia, K.H.M., 2011. First study on the larval growth parameter and growth rate of a forensically important blow fly, Hypopygiopsis violacea (Macquart, 1835) (Diptera; Calliphoridae). In Yang, D. (ed.). *Biomedical engineering and technology: selected, peer reviewed papers from the 2011 International conference on biomedical engineering and technology (ICBET 2011). June 17-19, 2011, Kuala Lumpur, Malaysia*. International proceedings of chemical, biological & environmental engineering, vol. 11. Singapore: IACSIT Press, p. 159–163.

Chown, S.L., Nicolson, S.W., 2004. *Insect physiological ecology mechanisms and patterns*. Oxford: Oxford University Press, 153 p.

Craig, A., Hare, L., Tessier, A., 1999. Experimental evidence for cadmium uptake via calcium channels in the aquatic insect Chironomus staegeri. *Aquatic Toxicology*, 44: 255–262.

Donovan, S.E., Hall, M.J.R., Turner, B.D., Moncrieff, C.B., 2006. Larval growth rates of the blowfly, Calliphora vicina, over a range of temperatures. *Medical and Veterinary Entomology*, 20: 106–114.

Emmerling, C., Krause, K., Schrader, D., 1997. The use of earthworms monitoring soil pollution by heavy metals. *Zeitschrift für Pflanzenernährung und Bodenkunde*, 160: 33–39.

Faber, J., Heijmans, G., 1997. Polycyclic aromatic hydrocarbons in soil detritivores. In Van Straalen, N.M., Krivolutsky, D.M. (eds). *Bioindicator systems for soil pollution*. NATO ASI Series. Dordrecht: Kluwer Academic Publishers, 16: 31–42.

Florea, A.M., Busselberg, D., 2006. Occurrence, use and potential toxic effects of metals and metal compounds. *Biometals*, 19: 419–427.

Fu, F., Wang, Q., 2011. Removal of heavy metal ions from wastewaters. *Journal of Environmental Management*, 92: 407–418.

Gall, J.E., Boyd, R.S., Rajakaruna, N., 2015. Transfer of heavy metals through terrestrial food webs: a review. *Environmental Monitoring and Assessment*, 187 (4): 1–21.

Gillespie, J.P., Kanost, M.R., Trenszek, T., 1997. Biological mediators of insect immunity. *Annual Review of Entomology*, 42: 611–643.

Greenberg, B., 1991. Flies as forensic indicators. *Journal of Medical Entomology*, 28: 565−577.

Greenwood, N.N., Earnshaw, A., 1997. *Chemistry of the elements*. Oxford: Elsevier Butterworth-Heinemann, 1305 p.

Greville, R.W., Morgan, A.J., 1990. The influence of size on the accumulated amounts of metals (Cu, Pb, Cd, Zn and Ca) in six species of slug sampled from a contaminated woodland site. *Journal of Molluscan Studies*, 56: 355–362.

Hagvar, S., 1982. Collembola in Norwegian coniferous forest soils. I: Relations to plant communities and soil fertility. *Pedobiologia*, 22: 255–296.

Heikens, A., Peijnenburg, W.J., Hendriks, A.J., 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. *Environmental Pollution*, 113: 385–393.

Hopkin, S.P., 1989. *Ecophysiology of metals in terrestrial invertebrates*. London: Elsevier Applied Science Publishers Ltd. 366 p.

Huhta, V., Ikonen, E., Vilkamaa, P., 1979. Succession of invertebrate populations in artificial soil made of the sewage sludge and crushed bark. *Annales Zoologici Fennici*, 16: 223–270.

Ireland, M.P., 1983. Heavy metal uptake and tissue distribution in earthworms. In Satchell JE. (ed.). *Earthworm ecology – from Darwin to vermiculture*. London: Chapman and Hall, p. 247–265.

Janssens, T.K.S., Roelofs, D., Van Straalen, N.M., 2009. Molecular mechanisms of heavy metal tolerance and evolution in invertebrates. *Insect Science*, 16: 3–18.

Kulbachko, Y.L., Didur, O.O., Loza, I.M., Pakhomov, O.Y., Bezrodnova, O.V., 2015. Environmental aspects of the effect of earthworm (Lumbricidae, Oligochaeta) tropho-metabolic activity on the pH buffering capacity of remediated soil (Steppe zone, Ukraine). *Biology Bulletin*, 42: 899–904.

Kulbachko, Y.L., Didur, O.O., Pakhomov, O.Y., Loza, I.M., 2014. Trophic-metabolic activity of earthworms (Lumbricidae) as a zoogenic factor of maintaining reclaimed soils' resistance to copper contamination. *Visnyk of Dnipropetrovsk University. Biology, Ecology*, 22: 99–104.

Li, G., Zhao, Z., Liu, J., Jiang, G., 2011. Effective heavy metal removal from aqueous systems by thiol functionalized magnetic mesoporous silica. *Journal of Hazardous Materials*, 192: 277–283.

Luce, M.C., Schyberg, J.P., Bunn, C.L., 1993. Metallothionein expression and stress response in aging human diploid fibroblasts. *Experimental Gerontology*, 28: 17–38.

Mackie, G.L., 1989. Tolerances of five bentic invertebrates to hydrogen ions and metals (Cd, Pb, Al). *Archives of Environmental Contamination and Toxicology*, 18: 215–223.

Morgen, C., Trumble, J., 2010. The impact of metals and metalloids on insect behaviour. *Entomologia Experimentalis et Applicata*, 135: 1−17.

Nahmani, J., Lavelle, P., Lapied, E., Van Oort, F., 2003. Effects of heavy metal pollution on earthworm communities in the north of France. *Pedobiologia*, 47: 663–669.

Nawrot, T., Plusquin, M., Hogervorst, J., Roels, H., Celis, H., Thijs, L., Vangronsveld, J., Van Hecke, E., Staessen, J., 2006. Environmental exposure to cadmium and risk of cancer: A prospective population-based study. *Lancet*, 7: 119–126.

Nesin, A.P., Simonenko, N.P., Humata, H., Chernysh, S.I., 1995. Effects of photoperiod and parental age on the maternal induction of larval diapause in the blowfly, Calliphora vicina R.-D. (Diptera: Calliphoridae). *Applied Entomology and Zoology*, 30: 351–356.

Nott, J.A., Nicolaidou, A., 1989. The cytology of heavy metal accumulation in the digestive glands of three marine gastropods. *Proceedings of the Royal Society B: Biological Sciences*, 237: 347–362.

Presing, M., Balogh, K.V., Salinki, J., 1993. Cadmium uptake and depuration in different organs of Lymnaea stagnalis L. and the effect of cadmium on the natural zinc level. *Archives of Environmental Contamination and Toxicology*, 24: 28–34.

Ratcliffe, N.A., Götz, P., 1990. Functional studies on insect haemocytes, including non-self recognition. *Research in Immunology*, 141: 919–923.

Safaee, S., Fereidoni, M., Mahdavi-Shanri, N., Haddad, F., Mirshamsi, O., 2014. Effects of lead on the development of Drosophila melanogaster. *Periodicum Biologorum*, 116: 259–265.

Sharma, R.K., Agrawal, M., 2005. Biological effects of heavy metals: an overview. *Journal of Environmental Biology*, 26: 301–313.

Sun, H.-X., Liu, Y., Zhang, G.-R., 2007. Effects of heavy metal pollution on insects. *Acta Entomologica Sinica*, 50: 178–185.

Tischer, S., 2009. Earthworms (Lumbricidae) as bioindicators: the relationship between in-soil and in-tissue heavy metal content. *Polish Journal of Ecology*, 57: 513–523.

Triebskorn, R., Köhler, H.R., 1996. The impact of heavy metals on the grey garden slug, Deroceras reticulatum (Müller): metal storage, cellular effects and semi-quantitative evaluation of metal toxicity. *Environmental Pollution*, 93: 321–343.

Van Straalen, N.M., 1997. Community structure of soil arthropods as bioindicator of soil health. In Pankhurst, C., Doube, B.M., Gupta, V.V.S.R. (eds). *Biological indicators of soil health*. Wallingford, New York: CAB International, p. 235–264.

Vinogradova, E.B., 1984. The blowfly Calliphora vicina as a model object for the ecological and physiological studies. *Trudy Zoologičeskogo Instituta = Proceedings of the Zoological Institute of the Russian Academy of Sciences*, 118: 1–272 .

Vinogradova, E.B., Reznik, S.Y., 2000. Endogenous changes of the tendency to larval diapause in laboratory generations sequences of the blowfly, Calliphora vicina R.-D. (Diptera, Calliphoridae). *International Journal of Dipterological Research*, 11: 3–8.

Westcott, K., Kalff, J., 1996. Enviromental factors affecting methyl mercury accumulation in zooplankton. *Canadian Journal of Fisheries and Aquatic Sciences*, 53: 2221–2228.

Zhang, M., Pu, J., 2011. Mineral materials as feasible amendments to stabilize heavy metals in polluted urban soils. *Journal of Environmental Sciences (China)*, 23: 607–615.

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