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Oxygen consumption and humoral immunity in *Porcellio scaber* Latr. (*Isopoda*) kept on litter contaminated with sulphuric acid, cadmium chloride and deltamethrin

Zużycie tlenu i odporność humoralna u *Porcellio scaber* Latr. (*Isopoda*) przetrzymywanego na ściółce skażonej kwasem siarkowym, chlorkiem kadmu i deltametryną

SUMMARY

Adult individuals of both sexes of the crustaceans *Porcellio scaber* Latr. were maintained on litter contaminated with H_2SO_4, $CdCl_2$ and deltamethrin (together 600 males and 600 females) as well as on control litter (50 males and 50 females).

The conducted research showed that there are differences between the sexes as far as sensitivity to toxic activity of the tested litter contaminators is concerned. The experimental animals displayed lowered respiratory metabolism because contamination with the above compounds decreased feeding activity. Tested compounds present in the litter may be considered antifeedants by the animals. The contaminants used in this experiment slightly increased the level of the lysozyme type and decreased the level of cecropine-like proteins.

STRESZCZENIE

Materiał doświadczalny stanowiły dorosłe osobniki skorupiaka *Porcellio scaber* Latr. zebrane na rumowiskach okolic Lublina. Zwierzęta kontrolne przetrzymywano na ściółce nieskażonej (50 samców i 50 samic), osobniki grup eksperymentalnych (łącznie 600 samców i 600 samic) na podłożu skażonym kwasem siarkowym, chlorkiem kadmu i deltametryną (Decis 2,5 EC).

Badane skorupiaki okazały się stosunkowo odporne na zakwaszenie środowiska i chlorek kadmu, wykazały natomiast bardzo wysoką wrażliwość na deltametrynę, na co wskazują niskie wartości LC_{50} dla tego związku. Samce były bardziej wrażliwe aniżeli samice.

Wszystkie testowane czynniki wywoływały wyraźny spadek zużycia tlenu już w pierwszej dobie od intoksykacji. W późniejszym okresie doświadczenia zużycie tlenu wzrastało, nie osiągając jednak poziomu wyjściowego, po czym ponownie obniżało się, pozostając na tym poziomie do końca eksperymentu. Obniżenie metabolizmu tlenowego w pierwszych dniach badań związane jest ze zmniejszoną aktywnością pokarmową zwierząt, gdyż stosowane skażenia wykazują charakter antyfidantów.

U badanego gatunku stwierdzono niskie wrodzone miano lizozymu. Obecność tego białka u zwierząt ze skażonej ściółki notowano w różnych dniach eksperymentu, przy czym jego poziom był wówczas wyższy w porównaniu z wartościami notowanymi w hemolimfie natywnej. Zawartość białek cekropinopodobnych stwierdzono w hemolimfie zwierząt kontrolnych, u zwierząt eksperymentalnych ich obecność na poziomie zbliżonym bądź niższym od wrodzonego notowano tylko w okresie zimowym.

Key words: *Porcellio scaber*, contaminants: H_2SO_4, $CdCl_2$, deltamethrin, oxygen consumption, humoral immunity.

INTRODUCTION

Human activity exerts various influences on the environment, which are not always positive. As a result of the rapid progress of chemicalization of life, ecological balance has been affected. It is necessary to recognize and to understand changes in the environment in order to find measures to combat environmental degradation, which endangers many groups of useful animals including land crustaceans.

The question arises then what adaptation mechanisms make it possible for the organism to live in contaminated environment. This paper describes the toxicity of certain chemical stressors according to LC_{50} values as well as variations in respiratory metabolism and humoral immune response in the isopod *Porcellio scaber* Latr. kept on litter contaminated with sulphuric acid, cadmium chloride and deltamethrin.

MATERIAL AND METHODS

Porcellio scaber Latr. is commonly found in gardens and parks as well as in hothorsues and damp homes. It is considered to be a typical debris-living animal. Experimental animals were collected in the Lublin area.

Experimental isopods were divided according to sex and further divided into three experimental and a control group. Control animals (50 males and 50 females) were maintained on birch litter in temperature and air moisture conditions similar to those found in nature. Experimental individuals were kept on litter contaminated with sulphuric acid (H_2SO_4), cadmium chloride ($CdCl_2$) and deltamethrin (Decis 2,5 EC) (200 males and 200 females in each group). Other conditions were similar to the controls.

The litter was contaminated with water-based solutions in the following doses:

sulphuric acid: 0.4, 0.6, 0.8, 1.6, 2, 3%
cadmium chloride: 1, 1.5, 2.5, 3, 6, 10 ppm
deltamethrin: 0.002, 0.005, 0.01, 0.02, 0.1 ppm.

LC_{50} values were determined by the Litchfield and Wilcoxon method (11). Significant differences in the sensitivities of the males and females were analyzed by χ^2 test (6). The value $p < 0.05$ was considered statistically significant.

Oxygen consumption was tested in control animals (12 males and 12 females) as well as in animals living on litter contaminated with H_2SO_4 — 0.6%, $CdCl_2$ — 3 ppm, deltamethrin — 0.002 ppm, (12 males and 12 females in each group) which are doses that led to 25% mortality. Measurements were conducted every other day for one month. Oxygen consumption was determined in each individual animal in Drasitch volumetric respirometer modified according to Klekowski (10). The animals were adapted to experimental conditions for 20 minutes. Oxygen consumption was measured for 2.5 hr at 22°C and expressed in terms of percentage changes compared to the control group, in which oxygen use was taken as 100%. Significant differences were analyzed by Student t-test for independent variables (19). The value $p < 0.05$ value was considered statistically significant.

The level of the lysozyme type and cecropin-like proteins was tested in control and experimental groups (12 males and 12 females in each group) by cup-agar diffusion method at pH 6.4 in appropriately prepared agar solution containing a substrate with lysozyme activity (*Micrococcus luteus*) or cecropin-type activity (*Escherichia coli* indicator bacteria). After 48-hour incubation at 28°C the lysis zone around the basin was measured with a tolerance of 0.5 mm. The protein concentration (in μg/ml) was determined according to Mohrig and Messner (17, 18) and Hoffmann et al. (7).

RESULTS AND DISCUSSION

The toxicity of the tested compounds in *Porcellio scaber* varied (Table 1). Crustaceans turned out to be relatively insensitive to acidification. This seems to be related to the fact that animals living in an acidic environment (litter pH 5–6) have developed certain adaptation mechanisms and to the fact that, within limits, they can tolerate an increase in acidity. The acid partly reacts with calcium carbonate saturating the cuticle, it is not possible to bind the sulphuric acid for a longer period of time (24). These authors proved that millipedes use calcium carbonate as a means of defence, which is reflected by the increased amount of calcium in organisms kept in the litter contaminated with sulphuric acid.

The isopods turned out to be relatively resistant to cadmium chloride contamination (Table 1). This is probably associated with the fact that crustaceans have developed a series of defense mechanisms viz. higher heavy metal concentrations in their environment such as mechanism to increase metal excretion and detoxication through binding with metalothioneins as well as sequestration in lysosomes and compartmentalization in endo- and exogenous granules (8, 16). Avoidance is a significant defense mechanism in invertebrates (5, 15). This adaptive behaviour has been noted in those invertebrates that display an ability to differentiate be-

Table 1. LC_{50} values of tested compounds for *Porcellio scaber* Latr.

Tested compounds	Sex	LC_{50} values		p <
		Mean	Range	
H_2SO_4	♂	0.701	0.604–0.814	0,01
[%]	♀	1.099	0.926–1.303	
$CdCl_2$	♂	5.172	4.183–6.394	ns
[ppm]	♀	6.659	5.360–8.273	
Deltamethrin	♂	0.007	0.004–0.011	ns
[ppm]	♀	0.009	0.006–0.015	

tween certain food ingredients through chemoreception. Some invertebrates are able to differentiate between foods that contain optimal levels of zinc and copper. Crustaceans can also limit consumption of feed containing lead salts (25). *Porcellio scaber* also displays the ability to choose food with an optimal concentration of copper (1).

Crustaceans display a very high sensitivity to deltamethrin (Table 1). This accords with data on other invertebrates which show high sensitivities to pesticides (3, 4).

High pyrethroid toxicity in the case of arthropods is associated with their easy passage through body coverings, trachea and alimentary canal as well as specific mechanisms. Basically pyrethroids react upon ion channels in the membranes of nervous cells, specifically with nicotine/acetylcholine receptors. Pyrethroids probably react with γ-aminobutyric acid receptors (GABA) blocking calcium ion conduction. The ultimate effect of the action of pyrethroids with receptors is the blocking of ionic conduction, which makes impulse transmission in the nervous system impossible (23).

P. scaber males turned out to be more sensitive than females to all three types of contamination. The differences were statistically significant for sulphuric acid only ($p < 0.01$) (Table 1).

A similar relationship of sensibilities to cypermethrin according to sex was described by Gromysz-Kałkowska et al. (3) in *Diplopoda* and acidification by Tracz et al. (24) and cadmium in crickets by Migula (14).

The sensitivity of animals to pesticides depends on variation activity of enzymes in detoxication systems. The activities of these systems probably depend on sex. Other defense and immune mechanisms seem to be strongly developed in females which is important in species survival. These mechanisms are mainly due to fat body cells, which while in males these cells store reserve materials (14).

All the tested compounds caused a marked decrease in oxygen consumption on the first day of intoxication compared to controls: sulphuric acid about 25%

in males and 22% in females, cadmium chloride about 22% in males and 27% in females and deltamethrin about 17% in males and 10% in females (Fig.1). During the experiment, oxygen consumption increased approaching initial value. In the presence of CdCl2, H2SO4 and deltamethrin the increase of oxygen consumption was observed on 5th, 15th, and 9th and 21st days respectively. The remaining measurements in all cases showed that oxygen consumption was lower than the initial value (Fig. 1).

The above variations are in accordance with results reported by Pytasz et al. (20), who noted that industrial emissions lowered metabolism in soil invertebrates.

Lowered respiratory metabolism of tested animals is associated with lower feeding activity of the animals during the first days of the experiment. Tested compounds present in the litter may be considered antifeedants by the animals and food products coated by them may be taken as inedible. As was mentioned above Crustaceans are able to differentiate between certain food ingredients by way of chemoreception (15).

The fact that digestion, assimilation and utilization of food in energy metabolism are handicapped due to destruction of microflora of the alimentary canal and because of decreased digestive enzyme activity due to the toxic effects of the above food contaminants must be taken into account.

Decreased oxygen consumption toward the end of the experimental period may be a result of changes in respiratory enzyme activity located in mitochondria. Richter and Klausnitzer (21, 22) determined the activity of malate dehydrogenase in certain species of plant lice and observed that decrease activities of NAD related dehydrogenases is a result of non-competitive enzyme blockage associated with the production of enzyme/NAD/SO_4^{-2} complexes.

Migula (12) reported similar results in experiments testing the influence of SO_2 air pollution on plant lice (*Acyrthosiphan pisum*). High SO_2 concentrations blocked the activities of a series of enzymes associated with NAD and FAD cofactors as well as cytochrome activities. However, the same author maintained that certain *Lepidoptera* react to SO_2 in an opposite manner than *Homoptera*. Variation in the quality of concentrations of certain gases and dust particles caused proportional increase in the activities of a series of enzymes, except for PEK and GTP. Butterflies exhibited the ability to take advantage of a series of glycolytic enzymes and the tricarboxylic acid cycle (TCA) associated with improvement detoxication mechanisms of sulphur and heavy metals (13).

Humoral immunity in invertebrates is dependent upon the appearance in the hemolymph soluble substances, which destroy biotic or abiotic substances foreign to the organism. This is a natural (congenital) immunity of lysozyme-type as well as an acquired immunity (induced) of cecropine-type (2).

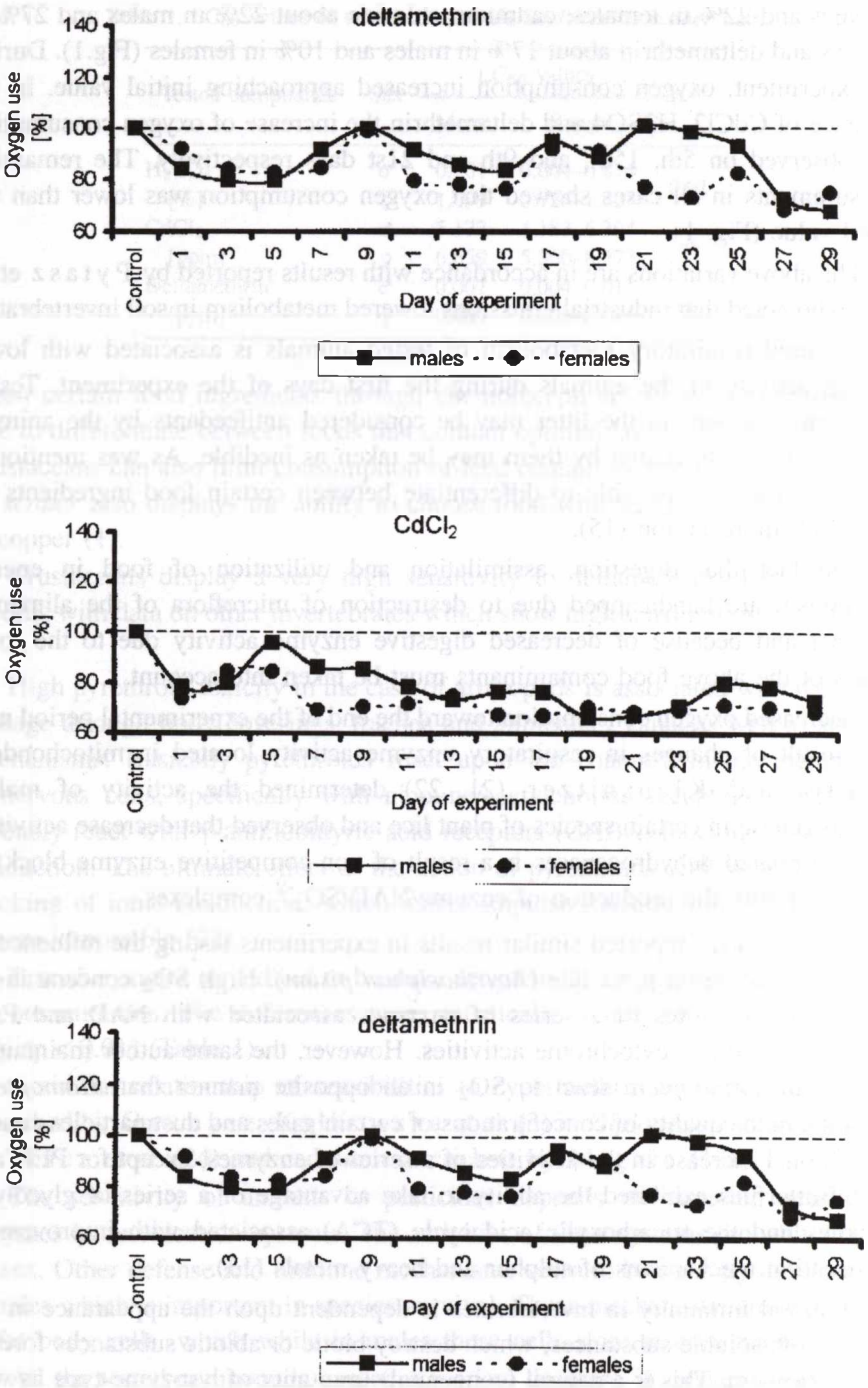

Fig. 1. Oxygen consumption in *Porcellio scaber* Latr. kept in contaminated litter

Tested crustaceans exhibited low congenital lysozyme protein titres (Table 2). Small amounts of lysozyme have been noted in the millipedes *Rhapidostreptus virgator* (26) and in *Ommatoiulus sabulosus* (9) as well as in chilopodes *Lithobius forficatus* and *Scolopendra sp.* (26).

The results presented in this paper are in accordance with the above noted results since lysozyme values measured for *Porcellio scaber* are small both in summer and winter.

In the case of animals kept on contaminated litter the level of lysozyme type protein was diversified; it depended on the kind of contamination, the duration of contact with the contaminated litter as well as on the season and sex. In the majority of cases the level of this protein was higher than in the controls (Table 2).

Table 2. The level of lysozyme type protein in *Porcellio scaber* Latr. kept in contaminated litter

Tested compounds	Season	Sex	Initial value	Concentration of lisozyme type protein [μg/ml]			
				Experimental days			
				1–3	5–9	13–17	23–25
H_2SO_4 0.6%	summer	♂	0.36	0	0.36	0	0
		♀	0.36	0	0.49	0	0
	winter	♂	trace	0.8	0	4.8	0
		♀	0	0.8	0	4.5	0
$CdCl_2$ 3 ppm	summer	♂	0.36	0	0.36	trace	0.68
		♀	0.36	0	2.43	0.42	1.29
	winter	♂	trace	0	1.9	0.8	0.8
		♀	0	0	0.8	0.8	0.8
Deltamethrin 0.002 ppm	summer	♂	trace	0.8	0	1.9	0
		♀	trace	0.8	0	0.8	0
	winter	♂	trace	0.8	2.9	0	0.8
		♀	0	0.8	4.5	0	1.9

Cecropine-like proteins were found in the native hemolymph both in summer and in winter. In experimental animals the presence of cecropine-like proteins was occasionally noted in winter at the level similar or lower than the congenital one (Table 3).

The obtained data point to the fact that the animals' reaction to the examined contamination is an insignificant increase of the lysozyme-type immunity as well as a considerable decrease of the immunity connected with the cecropine-like protein.

To sum up the obtained results, it should be stated that litter contamination with sulphuric acid, cadmium chloride and deltamethrin influence life processes of

Table 3. The level of cecropine-like protein in *Porcellio scaber* Latr. kept in contaminated litter

Tested compounds	Season	Sex	Initial value	Concentration of lisozyme type protein [μg/ml] Experimental days			
				1–3	5–9	13–17	23–25
H_2SO_4 0.6%	summer	♂	5.26	0	0	0	0
		♀	5.26	0	0	0	0
	winter	♂	25	0	0	0	5.3
		♀	11.6	0	5.3	0	0
$CdCl_2$ 3 ppm	summer	♂	5.26	0	0	0	0
		♀	5.26	0	0	0	0
	winter	♂	25	17	0	0	5.3
		♀	11.6	0	5.3	0	0
Deltamethrin 0.002 ppm	summer	♂	5.26	0	0	0	0
		♀	5.26	0	0	0	0
	winter	♂	25	0	0	11.6	11.6
		♀	11.6	0	0	11.6	11.6

a tested species of isopods in a negative way. The influence of these contaminators can be seen in the animals' increased mortality rate, changes of the respiratory metabolism level and changes of the humoral immunity.

The conducted research showed that there exist differences between sexes as far as the sensitivity to toxic activity of the tested litter contaminators. Males were more sensitive than females. Lowered respiratory metabolism in experimental animals may be a result of stress associated with being moved to new toxic environments or decreased taste value of food. The humoral immunity, dependent on the anti-bacterial anti-*M. luteus* and anti-*E. coli* protein presence in hemolymph, should be treated as an additional mechanism, enhancing immune reactions of the cellular type. The presence of anti-*E. coli* proteins in native hemolymph points to their non-inductive character. Therefore these substances should not be classified as cecropine-like proteins despite their effect on *E. coli*, indicative bacterium used to detect humoral immunity proteins of the cecropine type in insect's hemolymph.

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