Using of *Tenebrio molitor* larvae to study the toxicity of heavy metals

Oleg Zhurlov[1*]

[1]Center of Shared Scientific Equipment «Persistence of microorganisms», Institute for Cellular and Intracellular Symbiosis, Orenburg Federal Research Center of the Ural Branch of the RAS, Orenburg, Russia

Abstract. Studies of environmental pollution by heavy metals associated with the outpouring of mine waters onto the soil surface and the flushing of heavy metal solutions into reservoirs from the dumps of copper ores are relevant for the mining regions of the Urals. The use of *Tenebrio molitor* larvae to study the "chronic" toxicity of water contaminated with heavy metals will contribute to the study of a longer-term temporary effect of exposure to heavy metals. Contamination of wheat bran contaminated with heavy metals and microscopic fungi contributed to a significant increase in larval biomass and a reduction in the metamorphosis periods of *Tenebrio molitor* beetle. Standardization of methods for biotesting the "chronic" toxicity of high concentrations of heavy metals in water and soil, using of *Tenebrio molitor* beetle, with a full cycle of transformation, is a promising area of research.

1 Introduction

The relevance of studying the content of heavy metals in the water of man-made lakes formed at the site of quarries of copper-crusted deposits is associated not only with their toxic effect on the natural ecosystem, but also from the standpoint of the emerging modern technical possibilities of their recovery [1].

The Levikhinskaya group of copper pyrite ore deposits is a large ore-bearing area and consists of 14 deposits. Mining at the Levikhinsky field has been discontinued since 2003, and since 2004 it has been in wet conservation. However, mine waters rich in heavy metals continue to be released onto the soil surface, which poses a threat of their ingress into the water of the Tagil River. To assess the impact of mine waters and dumps, spent deposits, on the natural ecosystem, various methods of biotesting the level of their contamination with heavy metals are used. They make it possible to determine "acute" and "chronic" toxicity. Biotesting of "acute" and toxicity is carried out on two test objects from different biological groups.

The effectiveness of exposure to high concentrations of heavy metals on biological test objects is assessed by bacteriolytic effect (*Photobacterium phosphoreum (Cohn) Ford*), a decrease in the growth of the infusorians population (*Tetrahymena pyriformis (Ehrenberg) Schewiakoff*), a decrease in the growth rate of unicellular green protococcal algae

* Corresponding author: zhos-rus@mail.ru

(*Scenedesmus quadricauda (Tiger) Breb.*), and the death of crustaceans (*Daphnia magna Straus (Cladocera, Crustacea)*), on the death of fish (*Poecillia reticulate Peters*). To determine the "acute" lethal toxicity of marine and salt waters, crustaceans *Artemia salina L.* are used as a test object. In the literature available to me, I found only one method for determining the "chronic" toxicity of crustacean survival and fertility (*Ceriodaphnia affinis Lilljeborg (Cladocera, Crustacea)*). However, the study of the effect of heavy metals on biological objects with prolonged exposure ("chronic" toxicity) will allow us to study the consequences of such exposure on the next generations of a biological object.

Tenebrio molitor (*Linnaeus, 1758*), a mealworm, belongs to the order *Coleoptera* and *Tenebrionidae* family. Insect development occurs with complete metamorphosis (egg, larva, pupa and imago). At the end of the larval period (the larva of age 17) turns into a pupa [2]. Representatives of *Tenebrionidae* family are involved in the destruction of organic matter on the soil surface and thus play an important role in carbon sequestration in natural biocenoses. Therefore, the purpose of our study was to work out the procedures for selecting, synchronizing and analyzing the stages of development of *Tenebrio molitor* beetle larvae when feeding them wheat bran contaminated with heavy metals.

2 Problem statement

In most biotesting techniques, "acute" toxicity is assessed, and only a few methods allow a conditional assessment of "chronic" toxicity. Insects with complete transformation (egg, larva, pupa, and imago) have a number of advantages that allow us to assess the duration of the toxic effect and its effect on morphological and physiological properties during various periods of growth. In addition, the presence of an innate immune system makes it possible to predict the immune response to prolonged administration of toxic substances.

3 Solution method

In my research, I suggest using of *Tenebrio molitor* larvae to study the "chronic" toxicity of water containing heavy metals.

4 Object of research

The object of the study is the larvae, pupae and imago of *Tenebrio molitor* beetle.

5 Methods and materials

5.1 The origin of water containing heavy metals

Water containing heavy metals was taken from man-made reservoirs of the Levikhinsky deposit of copper ores: a lake at the site of a flooded quarry (57°35'03.24"N 59°54'35.94"E), then into a lake under a dump of waste rock (57°34'45.95"N 59°54'47.99"E), and into the stream after the neutralization station (57°34'52.99"N 59°55'15.75"E), the storage pond (57°34'55.94"N 59°56'44.30"E). The exact codes obtained as a result of using the Google Earth Pro program (kh. google.com).

5.2 Method of determination of heavy metals in water

The concentration of heavy metals in water was determined using the Quantum 2A atomic

absorption spectrometer.

5.3 The method of spraying wheat bran

Wheat bran was purchased in a supermarket and sterilized in an autoclave at 120°C, 30 min. An amount of 1 gram was added to the experimental Petri dishes. Water contaminated with heavy metals in a volume of 1 ml was injected into wheat bran. Petri dishes with wheat bran were dried in a dry oven at 70°C, 30 min.

5.4 Statistical analyses

The obtained results were analyzed using statistical methods, including the calculation of the arithmetic mean (M), average error of the mean (m), and standard deviation (σ).

6 Results

For experimental work, 150 of *Tenebrio molitor* larvae were selected, which were synchronized according to the growth stage (17 larval instars) or the last molt before the pupal stage. The larvae were standardized by weight and body length (25.3±0.3 mm; 140.2±4.1 mg) (Figure 1).

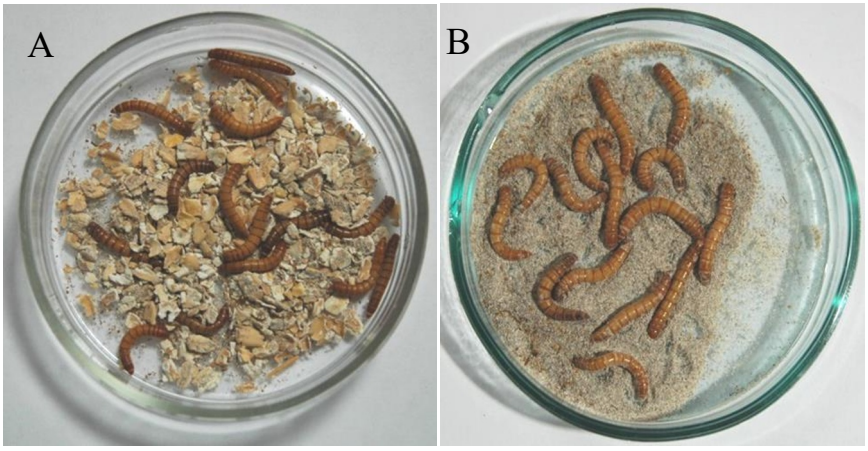

Fig. 1. Synchronized *Tenebrio molitor* larvae selected for the experiment in non-contaminated (A) and contaminated (B) with microscopic fungi bran.

At the beginning of the experimental work, all *Tenebrio molitor* larvae were divided into 2 groups: group 1 (75 larvae) – sterile wheat bran contaminated with heavy metals from technical reservoirs of the Levikhinsky copper-pyrite ore deposit; group 2 (75 larvae) – wheat bran was contaminated with microscopic fungi (unidentified) pathogenic to cereals. Each group consisted of five experimental Petri dishes, according to the number of samples (Control, water from a lake at the site of a flooded quarry, water from a lake under a dump of waste rock, water from a stream after a neutralization station, water from a storage pond). Each experimental Petri dish contained 15 *Tenebrio molitor* larvae (Figure 2).

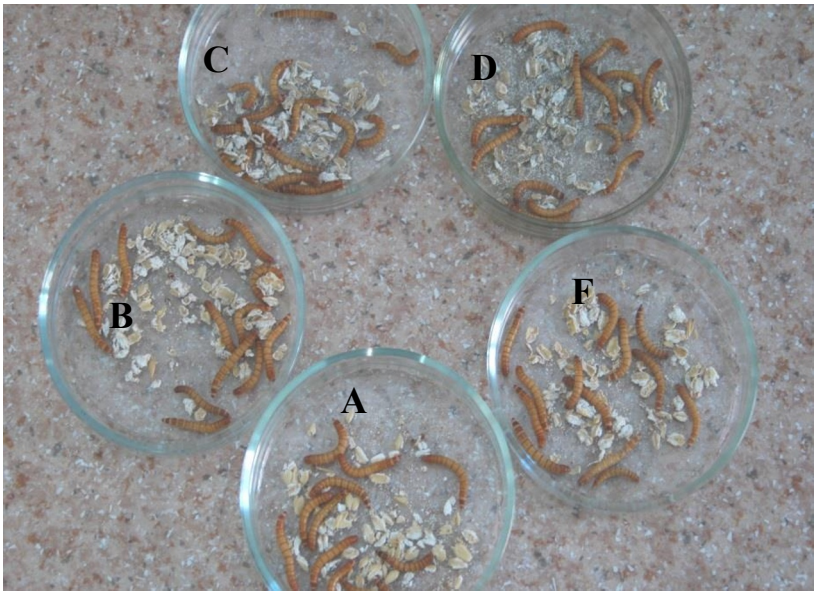

Fig. 2. Experimental Petri dishes with sprayed water containing heavy metals wheat bran and *Tenebrio molitor* larvae: A – control; B – water from the lake at the site of the flooded quarry; C – water from the lake under the waste rock dump; D – water from the stream after the neutralization station; F – water from the storage pond.

Interestingly, over 10 days of experiment increase in larval biomass (n=50) in wheat bran contaminated with microscopic fungi was significantly higher than in sterile wheat bran (173.2±3.8 mg and 140.2±4.1 mg, respectively), despite the indistinguishable average larval sizes (25.4±0.3 and 25.3±0.3 mm; accordingly). The concentration of heavy metals in water of man-made reservoirs of the Levikhinsky copper-pyrite deposit differed significantly (Table 1).

Table 1. Concentration of heavy metals in man-made reservoirs of the Levikhinsky copper-pyrite deposit.

Heavy metals (mg/l)	Water samples			
	A lake on the site of a flooded quarry	Lake under waste rock dumps	The stream after the neutralization station	Storage pond
Cd	-	-	7.1	-
Cu	8.6	43.1	361.0	2.0
Fe	142.0	1272.0	5520.0	2.8
Mg	97.2	250.0	1302.0	250.0
Mn	10.8	15.5	93.8	17.4
Pb	-	2.3	15.0	-
Zn	28.1	24.2	273.0	26.8

The analyzed metals iron and magnesium ions had the highest concentration in the water of man-made reservoirs. Cadmium and lead were present to a lesser extent, and they were not present in all samples.

The content of sulfur oxide (SO_4) in the water of the formed man-made lakes differed significantly. The highest values of SO_4 content were noted in the stream, after the

neutralization station (12366.7 mg/l) and the reservoir formed under the waste rock dumps (9454.9 mg/l). Lower concentrations of sulfur oxide (SO_4) were found in the water of the flooded quarry and in the storage pond (5780.2 and 5788.6 mg/l; respectively).

The effect of heavy metals on growth and development of *Tenebrio molitor* larvae was assessed by the duration of larval stage and the pupal stage. The relative average values of the growth intensity index were calculated using the formula (Growth intensity index = **a**/15×100%; **a -** where is the number of pupae or imagos over a certain time interval). The growth intensity index was evaluated within 5, 10 and 20 days after the appearance of the first pupa (larval stage) and imago (pupal stage). There was a general tendency to decrease the growth intensity index (Table 2) in all samples of water containing heavy metals, compared with the control at the larval stage of development.

Table 2. Changes in the relative growth intensity index in the larval stage and pupal stage of *Tenebrio molitor* beetle (on the 10th day, after the appearance of the first pupa).

Water samples	Stages of development of *Tenebrio molitor* beetle			
	Larval stage		Pupal stage	
	Without contamination *	Contamination	Without contamination	Contamination
Control	19.7±0.9	60.0±1.2	39.7±0.9	80.3±0.9
A lake on the site of a flooded quarry	11.8±1.0	26.2±0.6	40.3±0.9	53.4±0.9
Lake under waste rock dumps	5.9±0.9	46.2±1.2	39.3±1.2	79.7±1.5
The stream after the neutralization station	12.1±0.7	52.8±1.5	25.9±1.5	86.6±0.9
Storage pond	12.4±1.2	25.9±0.9	45.9±0.9	33.1±0.6

*** %**

Contamination of wheat bran by microscopic fungi contributed more to an increase in the growth rate in the control and to a lesser extent in water containing heavy metals. At the stage of pupal transformation into imago, the relative growth rate did not differ between the water samples and the control sample, with the exception of the water sample taken from the stream after the neutralization station and containing the highest concentrations of heavy metals. It is possible that feeding the larvae with bran containing high values of heavy metals reduces the rate of transformation of pupa into an imago. Larvae feeding on bran contaminated with microscopic fungi have a significantly increased growth rate, which reduces the time of pupal transformation into an imago.

The pupae, which at the larval stage fed on bran contaminated microscopic fungi with a high content of heavy metals, significantly shortened the period of transformation of the pupa into an imago.

7 Discussion

Insects have recently become in-demand biological objects for research in pharmacology and medicine. Low financial costs for feeding and care, high fertility, are often the determining criteria for choosing insects for research in various fields of science [3, 4]. For the first time, we investigated the possibility of using *Tenebrio molitor* beetle larvae as a biological test object to determine the toxicity of water containing heavy metals. As an additional factor affecting the duration of the larval and pupal stages, we used pathogenic microscopic fungi, which very often infect cereals and bakery products. The results of our work show that contamination of wheat bran with heavy metals from the water of man-made reservoirs of the Levikhinsky copper-and-titanium deposit did not lead to the death, morphological changes of larvae, pupae and imago of *Tenebrio molitor* beetle. This indicates a safe level of heavy metal contamination of the water of man-made reservoirs of the Levikhinsky deposit. In addition, we have shown the role of microscopic fungi in the possible fermentation of organic compounds and bioleaching of metals, which possibly stimulates the growth and development of larvae of *Tenebrio molitor* beetle.

8 Conclusion

Our results indicate that spraying wheat bran with water containing heavy metals reduces the growth rate of *Tenebrio molitor* beetle at the larval stage. However, contamination of bran by pathogenic microscopic fungi contributes to an increase in the growth rate of larvae and pupae of *Tenebrio molitor* beetle. Today, there are many methods for assessing "acute" toxicity, but the changes that occur in biological objects with prolonged exposure to a toxic factor at the genotypic level cannot be assessed by existing methods for assessing "acute" and "chronic" toxicity.

The research was funded by the Ministry of Science and Higher Education in accordance with the state assignment of science for Ural State Mining University №075-03-2024-132 dated 17.01.2023 «Development and environmental and economic substantiation of new technology for recovery of technogenic mineral formations under the circumstances of circular economy and the need of industry in mineral raw materials».

References

1. B. B. Zobnin et al., Patent RU 2739259 C1 (2020)
2. J. B. Park et al., International Journal of Industrial Entomology **28**, 1 (2014)
3. G. Cotter, S. Doyle, K. Kavanagh, FEMS Immunol Med Microbiol **27**, 6 (2020)
4. J. M. Loh et al., Virulence **4**, 9 (2013)