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# Recovery of ostracod with known ages in differently textured sediments and comparison of toxicity of heavily contaminated sediments with ostracod *Heterocypris incongruens* and amphipod *Hyalella azteca*

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**Abstract.** The recovery of 1-, 4-, 6-, and 8-d-old ostracods (*Heterocypris incongruens*) from sediments with different texture has been evaluated. The recovery of ostracods at all ages has been in agreement with the acceptability criterion of 80% of survival for sediment tests. The recovery of ostracods has turned out to be equal to or more than 80% for sand and silt sediments, respectively. The comparison of survival rates between ostracods and amphipods has shown good convergence in the tests of heavily contaminated sediments ( $R^2=0.75$ ,  $p<0.05$ ). The sediment quality criteria (TEC) have been exceeded mostly for total petroleum hydrocarbons (100% samples), Cr (100%), Ni (87%), Cu (87%), Pb (47%), and Cd (53%). The content of acid volatile sulfides (AVS) has been significantly higher than that of simultaneously extracted metals (SEM). The obtained results have indicated that, metals (Cu, Zn, Cd, Ni, and Pb) are non-bioavailable. Only one sample has exceeded TEC for PAHs (dibenz[a,h]anthracene). It was observed that, no significant correlation between the effect of toxicity and the chemical content.

## 1. Introduction

Sediments, due to their capability to accumulate many organic and inorganic compounds, represent a powerful factor influencing self-purification of water bodies. At the same time, they can become a source of secondary pollution. Currently, bioassay tests have been widely used for assessment of water and sediment pollution [1, 2, 3]. There are numerous methods for routine toxicological assessment using freshwater organisms from different trophic levels and characterized by different feeding and habitat requirements. Some of these methods are comparatively less robust, and the protocols for such species (amphibians, oligochaetes, etc.) are considered only as guidelines [4]. Most bioassay species are used for sediment toxicity assessment depending on cultivation conditions, time, personnel labor costs, etc. Among all methods, whole sediment tests with crustaceans are very popular. Whole sediment testing with the amphipod crustacean *Hyalella azteca* has been used especially extensively in North America. It is extremely valuable for detection and quantification of the toxic hazard of contaminated sediments. Nowadays, a new technique called “microbiotest”, which is independent of culturing and maintaining of live stocks of test species, has gained increasing popularity. The choice for a suitable test species has eventually fallen on the ostracod crustacean *Heterocypris incongruens*, whose biological cycle is known to shift to dormant egg (cysts) production under particular



environmental conditions. *H. incongruens* is a cosmopolitan benthic bivalved microcrustacean living in various types of small water bodies within the temperate climate zone [5]. Previously, the researches [5-7] showed that the sensitivity of ostracods is similar to the sensitivity exhibited by the amphipod *H. azteca* and the chironomid *C. riparius*.

The *objective* of this study was to assess the recovery of ostracods with known ages in differently textured sediments, as well as to compare the toxicity of heavily contaminated sediments with ostracods (*Heterocypris incongruens*, microbiotest) and amphipods (*Hyalella azteca*, routine technique).

## 2. Materials and methods

The Admiralteiskii pond is situated in the center of the city of Kazan (Republic of Tatarstan, Russia). It was created during the construction of engineering protection structures of the city against flooding, when the Kuybyshev Reservoir was filled in 1957, and represents a number of reservoirs connected by channels. The pond has total length of about 3.5 km, average width of 30 m, and depth of 0.5-1.5 m. Being cut by two dikes, the pond serves as a pool for collection of rainwater and melted snow with their subsequent transfer to the Kuybyshev Reservoir, in the water protection zone of the water intake area of the Volga River. The pond has been used for a long time as a recipient of wastewaters from the industrial enterprises located along the pond bank.

Sediment samples were collected in June 2015 from 15 sites. Four core samples, with a total volume of 2 L, were used to take the upper 15 cm of sediment using a Van Veen grab to obtain 2 L of sediment. The sediment samples were composited and homogenized by stirring in a stainless steel bowl with a stainless steel spatula. A portion was removed for chemical analysis and toxicity testing. The samples were stored in the dark at 4°C before toxicity testing or chemical analysis.

During the analysis of whole sediment samples, the grain size, total organic carbon (TOC), metals, polycyclic aromatic hydrocarbons (PAHs), and total petroleum hydrocarbons (TPHs) were investigated [10, 11, 12].

### 2.1. Whole sediment toxicity testing of ostracods

The whole sediment toxicity tests with ostracods were performed according to the Standard Operational Procedure [8]. Ostracods at the start of exposure were collected 52 h after the onset of cyst incubation, in the standard freshwater at 25°C, and under continuous illumination. Neonates were pre-fed with *Spirulina* microalgae for 4 h before their transfer to the testing cups.

The direct contact test of the whole sediment was performed in six replicates, simultaneously to that of the control sediment (sand from Toxkit) also in six replicates, using six-well microplates. Each well was supplemented with 1 g of the sediment and 2 mL of the water with algae as food. To be precise, 10 neonates were moved into each well.

Exposures were carried out for 6 days without water renewal or additional feeding.

Survival (S) was calculated as in equation (1):

$$S = \frac{n_{end}}{n_{start}} \cdot 100\% \quad (1)$$

where:  $n_{end}$ : the number of ostracods at the end

$n_{start}$ : the number of ostracods at the start

Growth (G) was calculated as in equation (2):

$$G = \left(1 - \frac{\bar{L}_c - \bar{L}_s}{\bar{L}_c}\right) \cdot 100\% \quad (2)$$

where:  $\bar{L}_c$  the mean length increment of ostracods in the control after 6 days from 6 replicates (i.e., their length at the end minus their length at the start,  $\mu\text{m}$ )

$\overline{L}_s$  the mean length increment of ostracods in the sample after 6 days from 6 replicates (i.e., their length at the end minus their length at the start,  $\mu\text{m}$ ).

The length measurement was performed with the help of a special micrometer slip after ostracod fixation with drop of Lugol's solution. The length was not measured if mortality was more than 30% (in one sample only).

## 2.2. Whole sediment toxicity testing of amphipods

The whole sediment toxicity tests with amphipods were carried out according to the Standard Operational Procedure [9].

*H. azteca* of various ages were cultured at 23°C in glass aquaria containing 20 L of water (hardness 220 mg CaCO<sub>3</sub>/L) and with light intensity of about 500 lux. Amphipods were fed with Tetramin<sup>R</sup> fish food and presoaked in maple leaves. Amphipods with known age were obtained from the mixed-aged culture using the sieve (500- $\mu\text{m}$  mesh). Mature amphipods retained in the sieve were pipetted into another sieve (425- $\mu\text{m}$  mesh) placed in a glass pan with water. The mature amphipods were left in the pan overnight to release neonate amphipods. The <24-h-old amphipods were held in the pan for 7 days and fed with maple leaves and Tetramin<sup>R</sup> fish food. The water was changed two times.

On the day before testing, 100 mL of the sediment and 175 mL of overlying water were added to 300-mL beakers. The exposure was performed for 14 days at 23°C and a 16 light: 8 dark photoperiod at the light intensity of about 200 lux. A total of 10 amphipods were exposed in each beaker. Four replicates were tested for each sediment sample. The overlying water was changed three times a week. Amphipods were fed with algae every day. On day 14 of the exposure, the sediments in each beaker were passed through a sieve (300- $\mu\text{m}$  mesh). The survived amphipods were counted.

Survival (S) was calculated as in equation:

$$S = \frac{n_{end}}{n_{start}} \cdot 100\% \quad (3)$$

where:  $n_{end}$ : the number of amphipods at the end

$n_{start}$ : the number of amphipods at the start

Statistical processing was performed using Statistica 8.0® (StatSoft, Tulsa, OK, USA) and the data were expressed as mean  $\pm$  standard deviation for each toxicity endpoint from each treatment, for granulometric and chemical data. Since the data distribution was not normal, the revealed differences between the samples were assessed using the nonparametric Mann–Whitney U-test and Kruskal–Wallis analysis of variance (nonparametric ANOVA). Correlations between the parameters were assessed using Spearman's Rank Order Correlation Coefficient. The differences were considered statistically significant at  $p \leq 0.05$ . Toxicity criteria were used as less than 80% deviation of control for all tests.

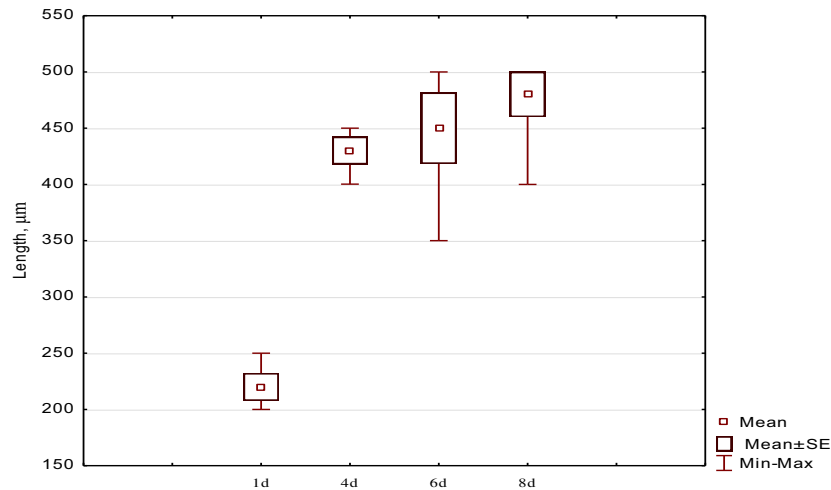
## 3. Results

### 3.1. Recovery of the ostracod *Heterocypris incongruens* with known age from differently textured sediments

The largest difficulty of testing procedures involving small organisms (400-800  $\mu\text{m}$ ), such as ostracods, is their recovery at the end of experiments. The standard methods for performing sediment toxicity tests with *H. incongruens* require the minimum control survival of 80% at the end of the 6-d exposure [8]. However, the low "survival" of ostracods at the end of the sediment test may be a result of the low recovery of smaller organisms from sediments.

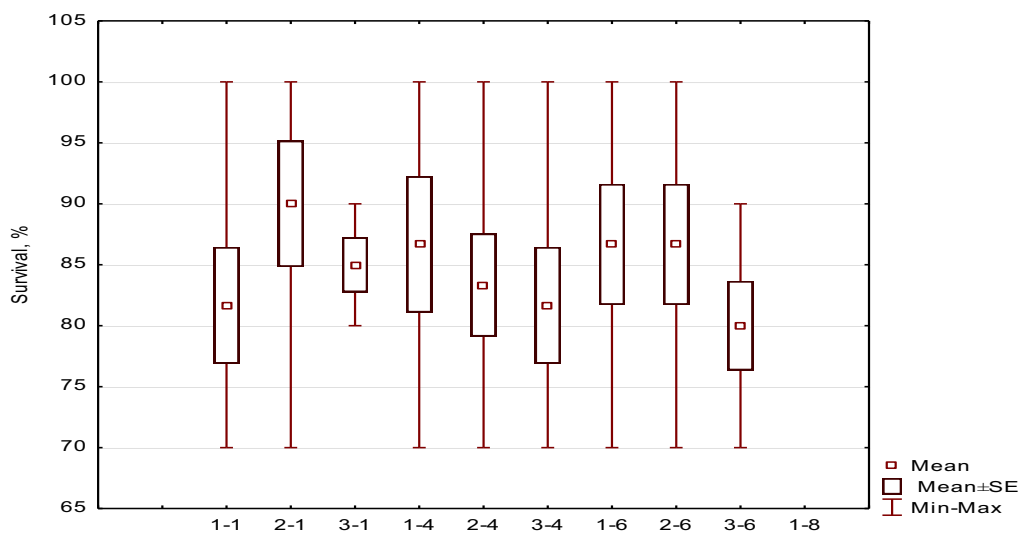
The sediment tests with *H. incongruens* were started with 1-d-old ostracods. The small size of *H. incongruens* can make it difficult to isolate organisms at the end of the sediment test. The length of 1-d-old ostracods was about 220 $\pm$   $\mu\text{m}$ , whereas the length of ostracods after 4 days was 442 $\pm$   $\mu\text{m}$ . The

most rapid growth was observed between the first and fourth days of testing and did not change considerably in the following four days (figure 1).



**Figure 1.** Changes in the length of ostracods during sediment toxicity test.

A special investigation was carried out to evaluate the recovery of ostracods with known age (1, 4, 6, and 8-d-old) in three differently textured clean sediments used as control (1 – sand from the Mariy Chodra National Park (Mari El Republic, Russia), 2 – sand from Ostracodtoxkit, 3 – silty sediment from the Spring River (bioassay practice in the US Geological Survey, Columbia Environmental Research Center).

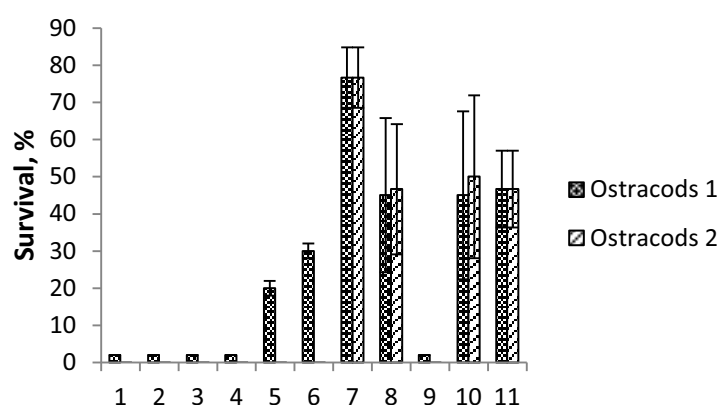


**Figure 2.** The recovery of ostracods with known age in different types of sediments (1-1 – sand (Mariy Chodra National Park, Russia) and 1-d age; 2-1 – sand (Ostracodtoxkit) and 1-d age; 3-1 – silt (Spring River, USA) and 1-d age; 1-4 – sand (Mariy Chodra National Park, Russia) and 4-d age; 2-4 – sand (Ostracodtoxkit) and 4-d age; 3-4 – silt (Spring River, USA) and 4-d age; 1-6 – sand (Mariy Chodra National Park, Russia) and 6-d age; 2-6 – sand (Ostracodtoxkit) and 6-d age; 3-6 – silt (Spring River, USA) and 6-d age; 1-8 – sand (Mariy Chodra National Park, Russia) and 8-d age; 2-8 – sand (Ostracodtoxkit) and 8-d age; 3-8 – silt (Spring River, USA) and 8-d age).

The recovery of ostracods was equal or higher than 80% in all variants (figure 2). These results indicated that neither ostracod age nor sediment texture influenced the recovery of ostracods.

### 3.2. Repeatability of results of toxicity measurements of sediments from the Admiralteiskii Pond with *H. incongruens*

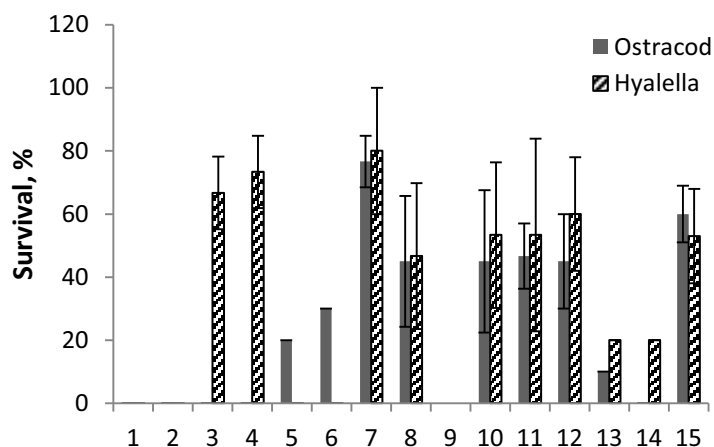
The results of toxicity testing with ostracods were repeated twice with an interval of two months (June and August, 2015) on the sediments from the Admiralteiskii Pond. The repeatability of ostracods survival was very similar in nine samples (figure 3). Only two samples demonstrated difference in ostracods survival in the range of 0-20% and 0-30%, respectively.



**Figure 3.** The survival of ostracods exposed to the same sediments with the difference of eight weeks.

### 3.3. Comparison of toxicity using *H. incongruens* and *H. azteca* exposed to sediments from the Admiralteiskii Pond

The comparison of the results of whole sediment toxicity tests with ostracods and amphipods showed good convergence, based on survival, and growth inhibition criteria. The survival of ostracods and amphipods in all samples (except sample no. 7) was less than 80%, thereby characterizing the latter as toxic. The total mortality was observed in samples nos. 1, 2, and 9 (figure 4).



**Figure 4.** The toxicity of sediments with ostracods and amphipods based on the criterion of “survival”.

It was shown that values in 82% of tests results were either identical or very similar. Differences in the survival rate of two species were observed between samples nos. 3-4 where the survival rate of amphipod varied from 20 to 30% and the total mortality of ostracods was observed.

### 3.4. Physical-chemical characterization of sediments

The level of toxicity effect hardly depends on the content of sediments (table 1). One of the most important roles is played by the factors of sorption and accumulation of pollutants. Most samples were silt mixed with sand in the range of 9.9-55.6%. Only samples nos. 7 and 11 were sandy (71.1-79.5%). The content of clay varied from 0.1 to 2.3%. The content of organic substances was 1.9-42.8%.

Due to the discharge of heavily contaminated wastewaters into the pond during the previous period, sediments accumulated numerous pollutants, especially metals and total petroleum hydrocarbons (TPHs) (table 1). Threshold effect concentrations [13] and TPHs criteria [14] were used for assessment of sediment pollution. The sediment quality criteria were mostly exceeded for TPHs (100%), Cr (100%), Ni (87%), Cu (87%), Zn (73%), Pb (47%), and Cd (34%). Only one sample contained dibenz[a,h]anthracene at the level of 1.9, which is above TEC [13].

## 4. Discussion

As it was shown in the research with the amphipod *Hyalella 6zteca* [15], the age and, therefore, size of an animal at the start can be essential for its recovery at the end of testing. The recovery of ostracods was 80% or higher in the tests with known age ostracods (1-, 4-, 6- and, 8-d-old). It is well known that the sensitivity increases the smaller the age of test organisms is. If the size (age) of ostracods does not influence their recovery, it is better to use 1-d-old ostracods for testing as recommended by the procedure [8].

It should be also emphasized based on the use of control sediments with different texture that recovery is not dependent on sediment structure (granular size): the obtained recovery was in agreement with the procedure requirements – not less than 80%. The mean loss of organisms in all types of sediments was 15%.

Significant correlation was observed in this study between the survival rate of *Heterocypris incongruens* and *Hyalella 6zteca* ( $R^2=0.78$ ,  $p<0.05$ ). Chial and Persoone [6] reported about the correlation between the mortality of ostracods in microbiotests and *H. 6zteca* in whole sediment tests based on 26 samples from various watercourses in Flanders, Belgium. The results of this comparative study revealed that the intensity of the toxic effect varied from “nearly identical” to “substantially different” between the amphipod and the ostracod tests. In this regard, it is interesting that some sediments were only slightly toxic to ostracods and very toxic to amphipods, whereas for other samples opposite trends were noted [6]. The pairwise comparison of toxicity data between the two crustacean assays revealed a significant correlation ( $r = 0.71$ ).

In the comparison of two bioassays (ostracods and the amphipod *H. 6zteca*), De Cooman et al [5] noted that, the mean percentage mortalities for the two bioassays in samples collected immediately after the addition of oil were all extremely toxic to both crustaceans (75-100% mortality). After six weeks, the toxicity of sediments from some plots decreased substantially for the amphipod (mortality around 30%), whereas the ostracod mortality was still between 80% and 90%. The results of the present study concerning the repeatability of ostracod tests (Figure 3) confirm the assumption about retention of ostracod test sensitivity of after eight weeks of sediment store. On the contrary, the survival of ostracods was higher than that of amphipods in two sediment samples (Figure 4).

No significant correlations were observed between the toxicity with both animals and the chemical content of sediments. Despite the high level of sediment pollution by metals (Table 2), no correlation was found between sediment content and toxicity. Based on the chemical interactions between simultaneously extracted metals (SEM) and acid volatile sulfides (AVS), the concentrations of these two components were used to assess the potential toxicity for sediment-dwelling organisms. The content of acid volatile sulfides (AVS) was significantly higher than that of simultaneously extracted metals (SEM). Thus, sulfides were available to bind with metals, thereby rendering them non-bioavailable.

**Table 1.** The physical-chemical characterization of sediments from the Admiralteiskii Pond (TECQ – quotient of pollution content to sediment quality criteria (TEC) [13], \*Russian criteria for TPHs [14]).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Number of sample															
Sand, %	37.3	9.9	54.8	39.7	53.5	45.1	79.6	55.6	53.6	23.0	77.1	31.7	24.5	38.8	14.8
Silt, %	62.2	87.8	45.1	59.6	46.2	53.6	20.4	44.2	45.4	75.5	22.9	67.1	74.6	60.5	84.7
Clay, %	0.5	2.3	0.1	0.7	0.3	1.3	<0.1	0.2	1.0	1.5	<0.1	1.2	0.9	0.7	0.5
Org, %	21.0	33.2	42.8	42.5	41.9	6.9	1.9	8.0	36.6	17.8	5.5	18.1	15.8	31.2	35.8
TECQ Cd	0.5	2.3	11.1	106.1	55.6	0.8	0.6	0.7	0.5	0.7	0.4	5.3	8.2	5.9	2.7
TECQ Cu	22.2	19.0	25.3	45.9	20.6	5.2	3.26	0.9	0.3	2.1	2.0	12.9	28.6	3.8	10.6
TECQ Ni	15.4	20.9	39.7	59.5	39.7	18.7	11.01	2.4	0.9	4.4	0.7	5.9	12.3	25.1	3.8
TECQ Pb	1.1	2.0	1.5	2.7	1.5	0.6	0.4	0.0	0.0	0.0	0.0	1.8	0.5	0.2	1.1
TECQ Zn	5.4	3.5	4.6	12.4	4.3	1.4	0.98	0.6	0.2	1.2	0.8	4.0	2.8	1.9	3.5
TECQ Cr	184.3	83.0	110.6	216.6	80.7	62.2	1.61	43.8	1.2	9.2	3.5	2.1	1.9	1.3	1.1
$\Sigma$ SEM-AVS, mmol kg <sup>-1</sup>	-74.2	-69.7	-80.9	-49.6	-87.4	-28.4	-44.9	-2.3	0.05	-25.7	-26.7	-52.9	-19.6	-72.3	-53.8
TPHQ*	119.7	117.1	13.9	13.7	13.7	71.2	72.8	53.2	48.7	65.0	73.9	102.5	95.8	23.7	10.4
TECQ Naphthalene	ND <sup>a</sup>	ND	ND	ND	ND	0.04	0.02	0.02	0	0.02	0.02	0.06	0.04	0.02	0.02
TECQ Phenanthrene	ND	ND	ND	ND	ND	0.29	0.17	0.02	0	0	0	0.10	0.06	0	0.01
TECQ Anthracene	ND	ND	ND	ND	ND	0.01	0.01	0.01	0	0	0.02	0.02	0.01	0.02	0.02
TECQ Chrysene	ND	ND	ND	ND	ND	0	0	0	0	0	0	0	0.01	0	0
TECQ Benzo(a)pyrene	ND	ND	ND	ND	ND	0.30	0.07	0.08	0	0.03	0.09	0	0.05	0.07	0.04
TECQ Dibenz(a,h)anthracene	ND	ND	ND	ND	ND	1.88	0.41	0	0	0	0	0.81	0.15	0.20	0

<sup>a</sup>ND – no data



The compliance with the criteria for PAHs indicated fresh oil pollution. The reason for sediments toxicity to ostracods and amphipods was not revealed. No significant correlation between the toxicity effect and chemical content was observed. It seems that toxicity may be related to combined effects of the pollutants or non-detected toxicants.

## 5. Conclusions

Laboratories should evaluate the ability of their personnel to recover *Heterocypris incongruens* from control and test sediments. Results of ostracod recovery evaluation can be used to separate effects on survival from the inability to recover small organisms from sediment. There were no significant differences between the recovery of ostracods with known age (1, 4, 6, and 8-d-old). The same results were obtained with the exposure of ostracods to sediments with different texture (sand and silt). Comparison of the survival rates of two crustacean ostracods and amphipods showed good convergence in the testing of heavy contaminated sediments. The sensitivity of ostracods did not change after two month of sediment storage.

Thus, it is possible to conclude that both methods demonstrated sensitivity to sediment pollution, as well as are characterized by good convergence and reproducibility of results. However, the bioassay with *Heterocypris incongruens* ostracods has a number of advantages compared to the routine test with *Hyalella azteca*: no need to maintain laboratory culture of ostracods; shorter time of exposure (6 days against 14 days with amphipods); smaller volume of sample (1 g against 100 g with amphipods). Along with the sensitivity comparable to other toxicity tests, the above-mentioned benefits create favorable conditions for the wide usage of microbiotests with *Heterocypris incongruens* ostracods for toxicity assessment of sediments.

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