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APPLICABILITY OF FOLSOMIA CANDIDA TOXICITY TEST FOR URBAN RANGE OF LEAD CONCENTRATIONS

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РЕЗЮМЕ. *У статті розглянуто особливості застосування тесту токсичності ґрунту на Folsomia candida. Розглянуто переваги і недоліки тесту. Запропоновано використовувати в якості тест-об'єкту вид Protaphorura bicampata. Рекомендовані для біотестування параметри: чисельність популяції, смертність, які корелюють із збільшенням концентрацій іонів свинцю негативно. Ще один вагомий параметр — частота линянь, яка збільшується за високих концентрацій свинцю. Кількість відкладених яєць та чисельність ювенільних особин не є інформативними параметрами для визначення токсичності. Значення LC_{50} у тесті токсичності свинцю знаходиться між 40 і 100 мг/кг (від 2 до 5 ГДК). Діапазон концентрацій іонів свинцю, токсичну дію яких тест дозволяє визначити, становить 20 — 100 мг/кг. Види колембол, зібрані в природних умовах, що є зручними для утримання в культурі, також можуть бути використані у тесті токсичності. Загальною тенденцією підвищення надійності тесту токсичності є розширення кола тест-об'єктів.*

Ключові слова: біотестування, токсичність, свинець Collembola, Folsomia candida, Protaphorura bicampata.

РЕЗЮМЕ. *В статье рассмотрены особенности применения теста токсичности почв на Folsomia candida. Предложено использовать в качестве тест-объекта вид Protaphorura bicampata. В биотестировании рекомендовано применять параметры: численность популяции и смертность, значения которых находятся в обратной связи с возрастанием концентраций ионов свинца. Еще одним показательным параметром является частота линек, которая увеличивается при высоких концентрациях свинца. Количество отложенных яиц и численность ювенильных особей не являются информативными параметрами для определения токсичности. Значения LC_{50} в тесте токсичности свинца находятся в диапазоне 40-100 мг кг (от 2 до 5 ПДК). Диапазон токсичности концентраций ионов свинца, измеряемый этим тестом, составляет 20-100 мг/кг. Виды коллембол, собранные в природе и пригодные к содержанию в культуре, могут быть использованы в тесте токсичности. Общей тенденцией увеличения надежности теста токсичности является расширение набора тест-объектов.*

Ключевые слова: биотестирование, токсичность, свинец, Collembola, Folsomia candida, Protaphorura bicampata.

SUMMARY. *Folsomia candida toxicity test is analysed in the present article. Traditional test object F. candida was replaced by soil-dwelling species Protaphorura bicampata. Advantages and disadvantages are characterized. Parameters that are recommended in biotesting are: general population number, mortality. They correlate with toxicity negatively. Moulting occurs more frequent, when toxical concentrations are high. Number of laid eggs, hatched juvenils were not informative parameters for determining toxicity. LC_{50} value in this test was between 40 and 100 mg/kg Pb^{2+} (from 2 to 5 times more, than standard maximal acceptable). Diapason of toxicity that can be measured in test is from 20 to 100 mg/kg. Modified F. candida toxicity test is representative in concentration range of lead from 20 to 100 mg/kg. Collembolan species, that were collected in natural or urban ecosystems, that are good in breeding, may be applied in toxicity test as well. General tendency to improve relevance of toxicity test is enhancing of test species set.*

Key words: biotesting, toxicity, lead, Collembola, Folsomia candida, Protaphorura bicampata.

Two main approaches have been adopted to study the ecotoxicological problems. In the first approach, toxicity experiments involving specific substances are conducted on one of a relatively small number of species. The results obtained are then extrapolated to predict effects of the used chemical in a natural environment and to assess the risks it would entail. The second approach looks at effects of chemicals on natural populations of Collembola. A site is monitored before and after contamination, or contaminated sites are compared with an unaffected, ecologically similar "control" site nearby. Folsomia candida toxicity test, analysed in the present article, has become one of the most simple and widely adopted certified tests following the first approach. It is suitable for predicting pollution effects in terrestrial ecosystems, and is generally a reliable and reproducible method in laboratory ecotoxicology.

Problems in indicator tests. The Folsomia candida toxicity test has a number of limitations and disadvantages. Commonly found in compost dumps, forest and grassland ecosystems, F. candida is characterized by aggressive ecological strategy and may replace another species in a community [1]. A test which does relatively more damage to other species than to F. candida can thus effectively improve the ecological conditions for F. candida at the expense of greater pressure on other species it competes with. Also, different springtail species possess variable and selective tolerance towards different chemicals; for example, F. candida is resistant to metals yet sensitive to organic pesticides [2]. Consequently, results from ecotixicological assessment are not generally universally applicable. Another issue is that, for an adequate ecotoxicological assay, a comprehensive culture of specimens must be collected and maintained in laboratory conditions, which is not always easy or practical, as for example, Parisotoma notabilis and Isotomiella minor, species dominant in forest ecosystems and thus good representatives as test objects, are hardly breeding in laboratory conditions [3].

Our study proposes an ecotoxicological test using soil-dwelling species of Protaphorura bicampata (Gisin, 1956). These species are common in natural conditions, which makes test results more relevant with respect to interactions in natural

ecosystems. Collembola, as well as Isopoda and Diplopoda, are resistant to heavy metal impact. One of the tough reactions is isolating of contaminant inside organism, another is release from cells, containing pollutant. The main intracellular metal storage sites are various types of granules which are widely distributed throughout cell types associated with the digestive system [4]. Collembola utilize at least three mechanisms to reduce metal ion concentration in their bodies. Firstly, a considerable part of metal ions is simply not absorbed and passes out via defecation. Two other mechanisms have been described by Joosse & Buker in 1979 [5] as "fast body burden" and "slow body burden". The first of these is through intestinal exfoliation and moulding, accounting for about 30% of lead contaminant removal. Another 15% is excreted into exuviae and removed with moulting.

Material and methods

Experiment design. The typical schedule of test is outlined below. Experiments are conducted at constant temperature (usually 18 or 20°C) in constant darkness, constant light or regular dark/light cycle. The soil comprises quartz sand, kaolin clay, and Sphagnum peat. The ingredients are mixed on a weight basis so that the dry soil contains 70 % sand, 20 % clay and 10 % peat. A small amount of powdered calcium carbonate is mixed to bring the pH up to 6 and distilled water . The test chemicals are incorporated into soil by adding them in the required concentrations to the water before the soil is hydrated.

Ten F. candida are placed into each cup together with a small amount of dried yeast. These containers are then left for the minimum three weeks by which time the surviving females will have matured and laid eggs. The soil is flooded with distilled water and the adult springtails and their offsprings float to the surface. The total number of adults and offsprings are counted.

The only disadvantage of the test is that reproduction cannot be observed directly, and cannot be separated from juvenile mortality and hatching success [6].

The aim of our study was to evaluate toxical impact of lead on different stages of life cycle of Protaphorura bicampata in laboratory conditions during 40 days. Springtails (20 specimens) were kept in Petri dishes in mixture of charcoal and plaster of Paris (9:1) according to standard methods. Moisture was maintained at 100 %, temperature at 20°C, Petri dishes were placed into climatic camera during all the experiment period. Toxical impact of lead was studied in 5 replacements according to maximum acceptable concentration and real concentration of lead in Kiev city.

Before the experiment started, substrate was moisturised with water solutions of $Pb(NO_3)_2$, in following concentrations calculated concerning Pb^{2+} ions: 9 mg/kg (level of lead in natural soil of Kiev city (Golosiivskiy park), 10 mg/kg (half of maximal accepted level concentration), 20 mg/kg (maximal accepted level concentration), 40 mg/kg (double accepted level concentration), 100 mg/kg (5 times accepted level concentration). Springtails were fed baker yeasts, cultures were ventilated and cleansed regularly, dead animals and rests of food were excluded. The breeding culture originated from forest soil in Kyiv city, and has been kept in the laboratory for two years. P. bicampata occurs in wet meadows, bisexual [7].

Laid eggs and offsprings were registered after experiment finished. Petri dishes were filled with water, survived and died specimens were collected, adults and juveniles were counted separately. Parameters of surviving and life cycle are shown in table 1.

Results

Inverse relationship was observed between lead concentration and population size(total number of animals). Number of specimens did not correspond directly with lead concentration. Population size in control did not differ significantly from population size in the most contaminated sample (Fig. 1). We suggest, that total population number is not a representative parameter in F. candida biotest. It decreases noticeably in our experiment only when concentrations of lead are very high. Another problem is that total calculation of all springtails may be misinterpreted, as when an increasing of population size may be caused by intensity of reproductive processes.

The number of dead animals increased with lead concentration. Maximal observed mortality was 125 animals at concentration 50 mg/kg Pb^{2+} (5 times maximal acceptable concentration). Deviation from general tendency of increasing was obtained in dishes, when lead concentration was 10 and 20 mg/kg. General tendency in change of this parameter is: increasing mortality with lead concentration increases (Fig. 2). It means direct response to Pb^{2+} concentration. Amount of dead animals a is simple and reliable characteristic of toxicity in this test, which has been approved by our study. It correlates with lead concentration in all studied cases.

Mortality (rate of live animals to total) was variable in gradient of lead concentration, in some cases of lead impact mortality was less than in control dishes. Nevertheless, in the highest toxicity impact, mortality was 41 %. It can be explained, that mechanisms of compensations did not work, when toxical concentration exceed critical level.

Moulting increases with lead concentration. This is consistent with results from studies where Collembola peel internal cuticle and deliver from metal, incorporated in cells during moulting [8, 9]. Maximal number of offsprings was found in dishes with lead concentration 20 mg/kg and 100 mg/kg (Fig. 3). General tendency in dynamics of this parameter was increasing moulting with concentration.

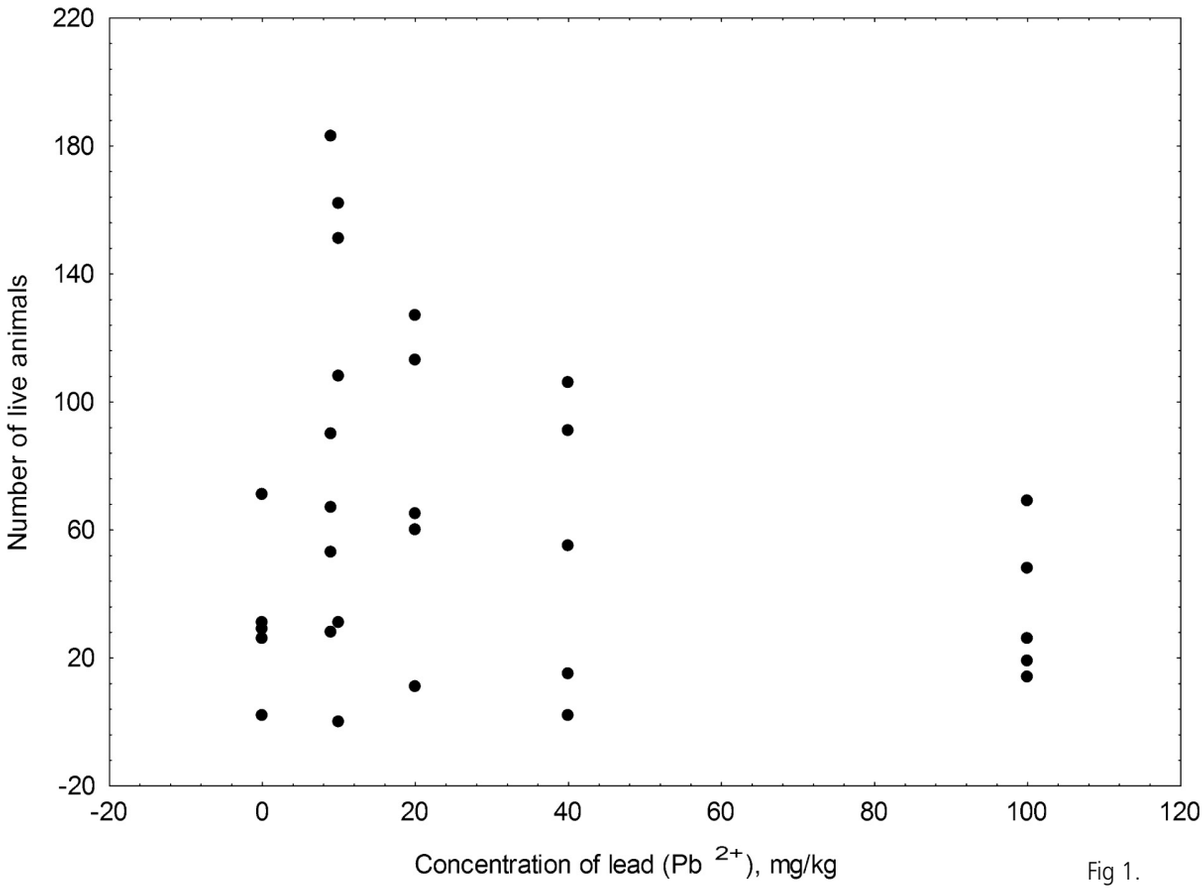
Number of live animals
220
180
140
100
60
20
-20
-20
0
20
40
60
80
100
120
Concentration of lead (Pb 2+), mg/kg

Fig 1.

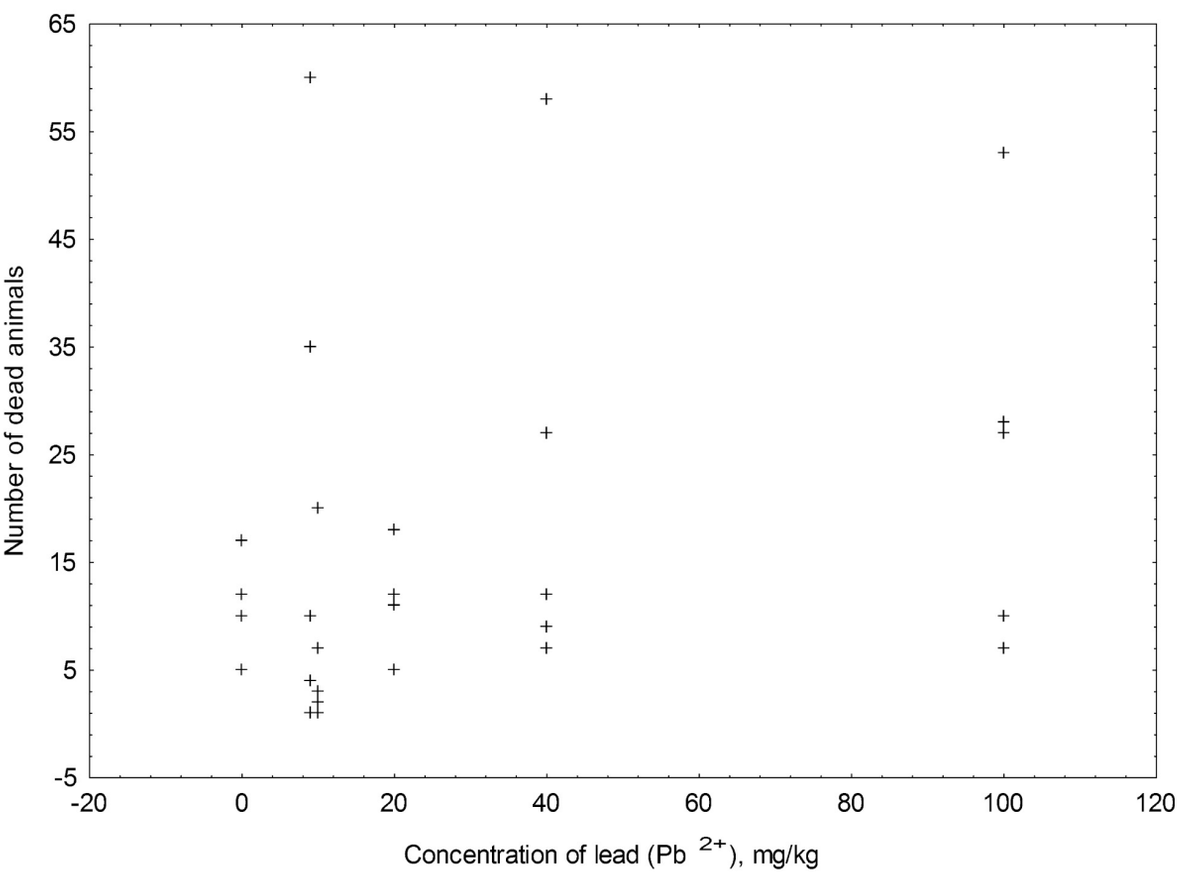
Number of dead animals
65
55
45
35
25
15
5
-5
-20
0
20
40
60
80
100
120
Concentration of lead (Pb 2+), mg/kg

Fig 2.

Table 1

Parameters of Collembolan test cultures population under lead impact

Mean ± standard error of mean	Control	Pb^{2+}, mg/kg				
		9	10	20	40	100
Total population number	31,8±11,1	84,2±26,6*	90,4±32,3*	75,2±20,7*	53,8±20,4*	35,2±10,3
Died (total)	11,2±1,93	22±11,22*	6,6±3,5*	10,2±2,4	22,6±9,5*	25±8,2*
Mortality, %	26	20	7	12	8	41
Number of live adults	5,8±2,4	5±3,6	3,4±2	0	1,4±0,9	3,4±1,2
Eggs	39,6±5,7	32,6 ±10,6	21±6,1	23,4±2,5	43,6±12,2*	38,6±12,1
Hatched juveniles	26±9,3	79,2±27	87±31,9	75,2±20,7	53,4±20,6	31,8±9,7
Offsprings (exuviae)	34,6±3,04	38±4,1*	34±3,2*	56,4±10,9*	27,2±5,1*	46,6±11,2*

**Significantly different from control at 5% level of significance (Van-der-Varden's test).*

Another parameters did not correspond with toxicity. Number of laid eggs was maximal in case of toxicity was 2 times more, than acceptable level. In dishes, where concentration was 5 times higher, number of eggs was slightly lower, than in control dish. Minimal number of eggs was in dish, when concentration was half of maximal acceptable. We could not observe any tendency of these parameter: neither increasing, nor decreasing. Hatching was not easy to register and did not reflect toxicity changes.

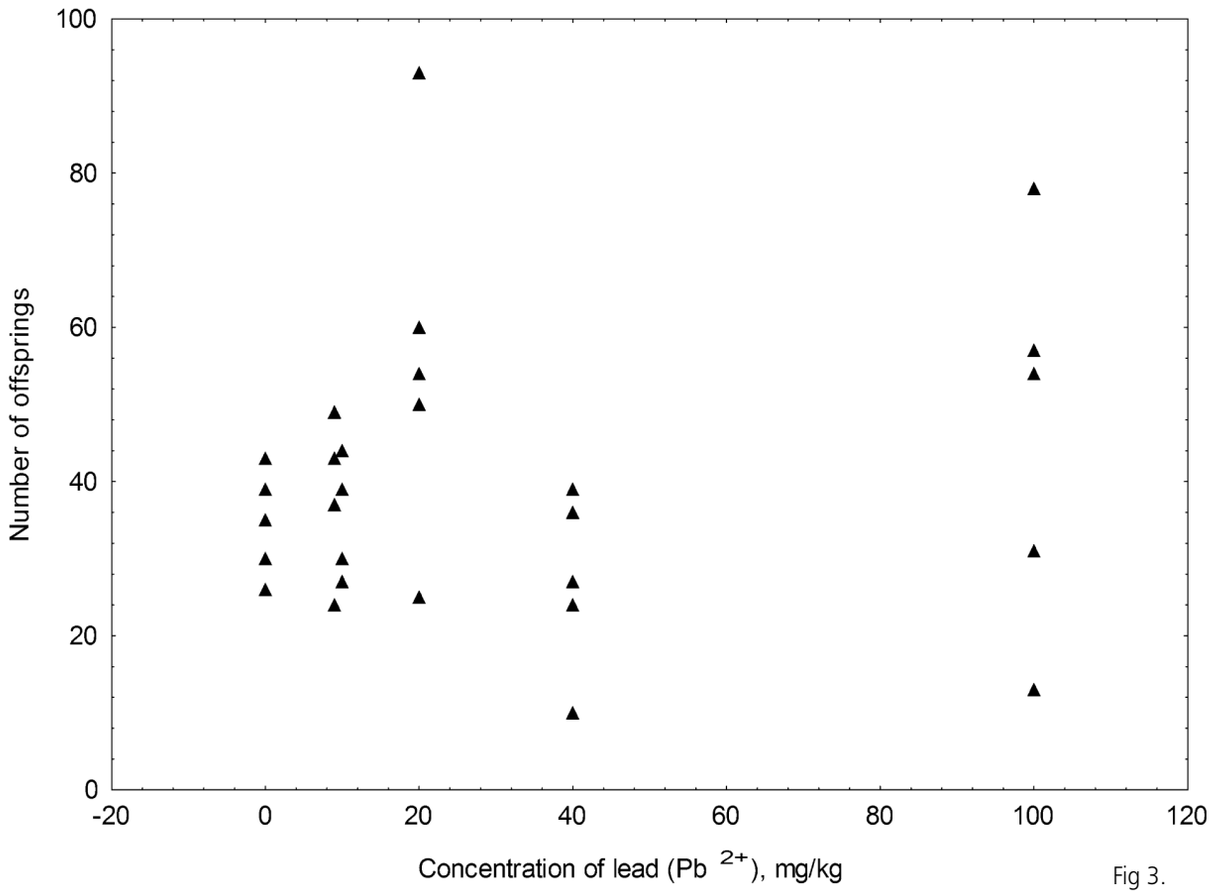

Fig 3.

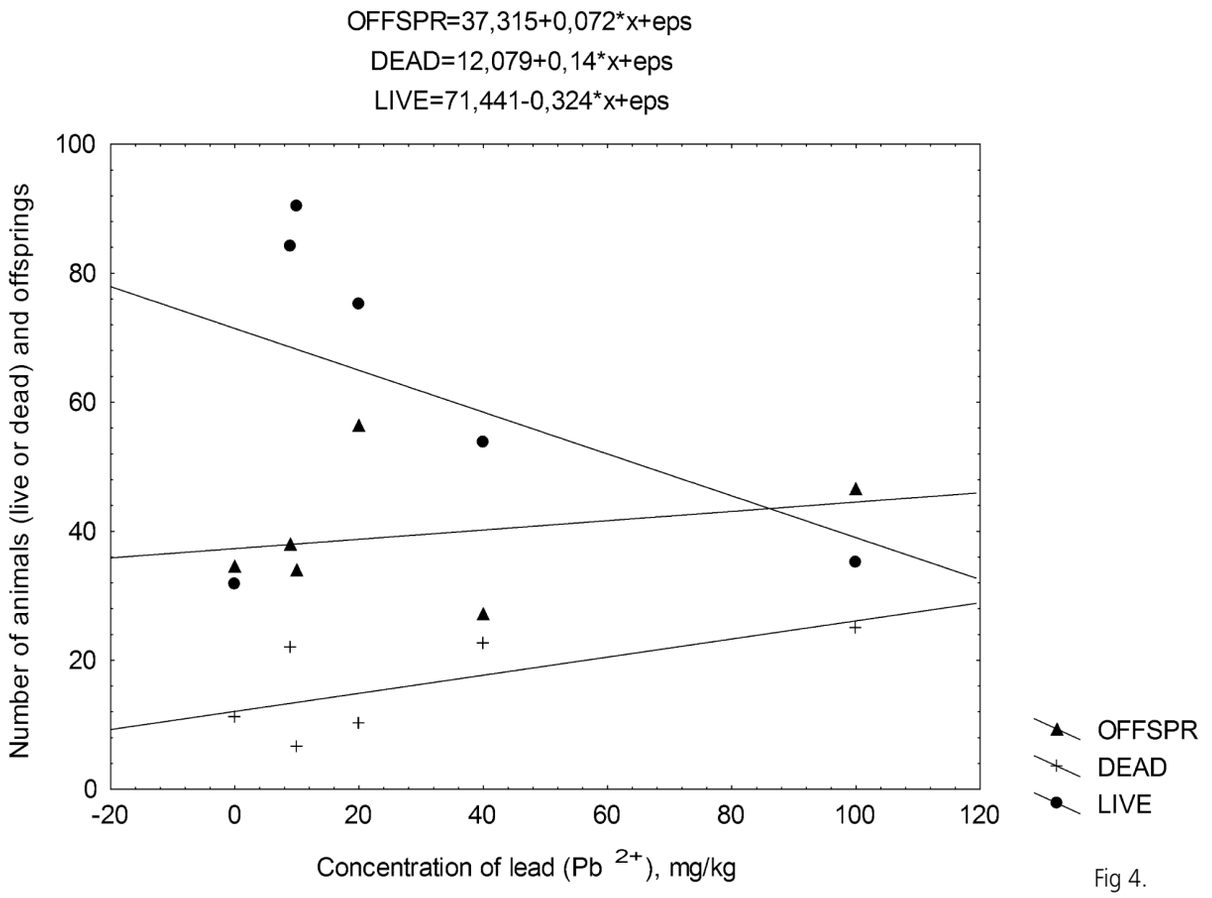

Fig 4.

Discussion

F. candida toxicity test was conducted according to standard procedure and slightly changed. Protaphorura bicampata was used as a test object. The lead concentrations chosen in our experiment are multiples of the maximal accepted level (C_{max}) as follows: 0,5; 1; 2; 5 and lead content in urban soils. Parameters of reproduction differed from each other and control measurement, differences were significant according to van-der-Varden test. Two parameters which are recommended in biotest process, are: number of died animals, mortality. They correlate with toxicity negatively. Moulting occurs more frequent, when toxical concentrations are high (Fig. 4). Number of laid eggs, hatched juvenils were not informative parameters for diagnostic toxicity. LC50 value in this test was between 40 and 100 mg/kg Pb^{2+}. In case of concentration lead, that was equal to maximal accepted, surviving parameters did not changed, otherwise physiological parameters Diapason of toxicity, that can be measured in test with Protaphorura bicampata, is from 20 to 100 mg/kg. LC_{50} concentration of polluted soil proves unacceptable high lead concentration (from 2 to 5 times more, than standard maximal acceptable).

Exactly value of maximal acceptable concentration was reflected in physiological parameter of moulting. Animals try to release their body burden from pollutant. This parameter is able to register before lethal effect of toxical impact. Using physiological parameters is additional modified feature in standard F. candida toxicity test procedure. Moulting throughout all life-cycle is peculiarity of Collembola group, and may not to be applied to another test species.

We recommend to provide F. candida toxicity test, which is proved to be reliable tool for toxicity evaluation. As was shown in outlined study, P. bicampata is good test object. Another collembolan species that were collected in natural or urban ecosystems and are good in breeding may be used in toxicity test as well. One way to improve relevance of toxicity test is by carefully selecting the species used. Our studies Test is representative in concentration range of lead from 20 to 100 mg/kg.

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