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ECOTOXICOLOGICAL EVALUATION OF BORON, ARSENIC, IODINE AND SELENIUM ON REPRODUCTION, MORTALITY AND AVOIDANCE OF FOLSOMIA CANDIDA (COLLEMBOLAN)

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Abstract: *Soil plays a significant role in the ecosystem. Soil pollutants have increased largely from a wide range of sources in the last decades. Trace elements accumulated in soil because of anthropogenic activities (e.g. industry, agricultural production, and health products) may pose a potential risk to environmental safety. Therefore, it is necessary to evaluate the ecotoxicological effects of trace elements on soil model animals. To evaluate the ecotoxicological impacts of four trace elements (As, B, Se, and I), the 28-days long tests based on the OECD guideline 232 had been executed with Folsomia candida (Collembola). The chosen trace elements were mixed with OECD artificial soil in the following concentrations [mg/kg]: 0, 0.0023, 0.0069, 0.0205, 0.0617, 0.185, 0.555, 1.66, and 5 for selenium; 0, 0.5, 7.5, 11.25, 16.875, 25.312, 37.969, and 56.953 for iodine; 0, 0.0002, 0.001, 0.005, 0.025, 0.125, 0.625, 3.125, and 15.625 for boron; 0, 0.01, 0.03, 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 for arsenic. Meanwhile, 48-hours sublethal test (avoidance tests) had been carried out with Folsomia candida. Survival, reproduction, and avoidance behavior of F. candida were regarded as endpoints with the main ecotoxicological values (NOEC, LOEC, EC_{50}, and LC_{50}). The four trace elements all showed toxic effects on F. candida in different degrees and they had similar toxicity on the survival of F. candida, while boron showed higher toxicity on reproduction than the others. The survival of F. candida was more sensitive than reproduction to each element. Avoidance behaviour of F. candida was the most sensitive to boron, followed by arsenic and iodine, and finally selenium. In this study, boron and arsenic appeared to be potential risks in the natural fields.*

Keywords: *Environmental protection, trace elements, Folsomia candida, soil pollution, ecotoxicological toxicity*

INTRODUCTION

Trace elements are defined as elements occurring at low concentrations (mg/kg or less) in most soils, plants, and living organisms (He et al., 2005). Trace elements can be present in the environment naturally; some of them play influential roles in the growth of plants, animals, and humans as essential nutrients (Hooda, 2010). For example, copper (Cu) and boron (B) are essential for the growth of the plant. Selenium (Se) and iron (Fe) are necessary for the growth and health of animals and humans (He et al., 2005). The presence of trace elements can be natural and anthropogenic (He et al., 2005).

However, the increased amount of trace elements in the natural environment by human activities can generate a series of ecological and health problems (Hooda, 2010). Research by Wang et al. (2020) showed that trace elements were found to be high soil contamination risk (Wang et al., 2020). Their toxicity can affect the organisms' surrounding environment or directly affect the organisms through the food chain (Gupta and Gupta, 1998). It's worth remarking that trace elements appear in different amounts in different regions. For instance, the northeast of China is a typical selenium-deficient region, however, Enshi city in Hubei province which in the southwest of China has a wide range of selenium poisoning due to superabundant selenium in soil and drinking water (Yang et al., 2007).
According to the previous work, *Folsomia candida* is a standard test species to evaluate the ecotoxicological effects of trace elements in soil (Fountain and Hopkin, 2005). *F. candida* belongs to the order Collembolan that is a very common and abundant arthropod living in soil all over the world. *F. candida* is quite easy to maintain in the laboratory. Moreover, several official guidelines are available to this species, ISO guideline 11267 (ISO 11267 1999) and OECD 232 (OECD/OCDE 232, 2009) for the Collembolan reproduction test has been approved successfully for toxicity testing on soil contamination (Krogh et al., 2008). Therefore, evaluating the effects of soil contamination by using ecotoxicological testing on *F. candida* has evidence supporting and scientific values.
Therefore, this study aimed to evaluate the ecotoxicological effects of four trace elements (selenium, iodine, boron, and arsenic) on the survival and reproduction of *F. candida* according to the OECD guideline 232. The aqueous stock solutions containing four trace elements were sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$), potassium iodide (KI), boric acid (H_3BO_3 or $B(OH)_3$), and sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) respectively. Also, the avoidance tests had been carried out to support the OECD tests and evaluate the results filed tolerance. Moreover, the endpoints were survival, reproduction, and avoidance behavior of *F. candida* supported by the main ecotoxicological values (NOEC, LOEC, EC_{50}, and LC_{50}).

MATERIALS AND METHODS

The *F. candida* used in our present study was bred on the laboratory culture mediums. The culture mediums had been contained in 9 cm diameter plastic dishes containing a mixture of plaster of Paris, active charcoal, and tap water in a ratio of 10:1:10. The culture medium in which *F. candida* lives were stored in the thermostat at a temperature 20 ± 0.2°C. The supply of water and food (baker's yeast) was added weekly and the Petri dishes were aerated during the process. To achieve accurate results of reproduction and mortality tests, the organisms had to be synchronized to gain the same age colony which according to the OECD guideline was 9-12 days old at the time of tests (OECD/OCDE 232, 2009).
For OECD reproduction tests, the aim was to evaluate the chronic toxic effects of the four trace elements on *F. candida*. Mortality and reproduction tests were applied based on the guideline OECD 232 (OECD/OCDE 232, 2009). The artificial OECD soil was composed of 5% air-dried and finely grand sphagnum peat, 20% kaolin clay, 74% air-dried industrial sand, and 1% calcium carbonate (OECD/OCDE 232, 2009). Selenium, iodine, boron, and arsenic were added as liquid solutions, which were diluted from their stock solutions respectively. The stock solutions used for preparing the test concentration were $Na_2SeO_4 \cdot 10H_2O$, KI, $B(OH)_3$, and $Na_2HAsO4 \cdot 7H_2O$ for each trace element. The concentration of the mentioned stock solutions was all 1 g/L.
To have a valuable test concentration series: The range finding tests were applied for iodine, boron, and arsenic. It had been done earlier regarded as the range-finding test for selenium; therefore, it was not a part of this study. The range-finding test concentration series for iodine and arsenic were same: 0 mg/kg, 0.005 mg/kg, 0.05 mg/kg, 0.5 mg/kg, 5 mg/kg, and 50 mg/kg. The test concentration series for boron was: 0 mg/kg, 0.005 mg/kg, 0.05 mg/kg, 0.5 mg/kg, 5 mg/kg, and 25 mg/kg. The method for range-finding tests was the same as the reproduction tests based on the OECD guideline 232(OECD/OCDE 232, 2009). In the present study, the test concentrations [mg/kg] are 0, 0.0023, 0.0069, 0.0205, 0.0617, 0.185, 0.555, 1.66, and 5 for selenium; 0, 0.5, 7.5, 11.25, 16.875, 25.312, 37.969, and 56.953 for iodine; 0, 0.0002, 0.001, 0.005, 0.025, 0.125, 0.625, 3.125, and 15.625 for boron; 0, 0.01, 0.03, 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 for arsenic. There were 10 replicates for the control and 5 replicates for each test concentration. About each experimental unit, there was 24.5 g

artificial soil mixed with a 5.5 ml test solution for every test concentration in one plastic, 100 ml cylinder pot. 10 synchronized *F. candida* juveniles were placed on the surface of mixed soil in each pot. According to the guideline, every test lasted for 28 days. During the experimental period, the temperature condition was 20°C (± 0.2°C) in the thermostat. Counting animals were on day 28 by flooding. Adult Collembolan was recorded as dead if not present in the extraction. The three trace elements tested in the present study were all operated according to the same experimental procedures described above. The method of the OECD reproduction tests was the same as the range-finding tests mentioned above based on the OECD guideline 232 (OECD/OCDE 232, 2009).
The avoidance tests were performed according to the method from Da Luz et al. (2004). The avoidance test lasted for 48 hours. There were 10 replicates for the control group and 5 replicates for each test concentration. The test concentrations were the same as in the OECD reproduction tests. In each experimental pot, there were two sides of artificial soil separately. One side was 24.5g artificial soil mixed with 5.5ml clean lab tap water, while another side was 24.5g artificial soil mixed with 5.5ml test solution. For each pot, 20 adult *F. candida* were randomly placed in the space between two sides of the soil. Food support and moisture control were not needed during the test. Similar to reproduction tests, the experimental temperature condition was 20°C (± 2°C). After 48 hours, the two sides of soil were separated and adults were counted by flooding.

STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

R statistical program 3.6.1 (RStudio Team, 2019) was used to analyze data. The data were analyzed with the general linear model and the first lowest concentration significantly different from control was accepted as the lowest observed effect concentration (LOEC). In some cases, the concentration was transformed to log scale to reach normal distribution, but otherwise, the whole dataset met the requirements of normality according to the diagnostic plots (Residual variances, QQ plot, and Cook distance plot). LC_{50} (50% lethal concentration) and EC_{50} (50% effective concentration) values and confidence intervals were calculated with ToxRat® (ToxRat®Solutions.Gmbh, 2018). The avoidance tests were analyzed with paired t-test.

RESULTS AND DISCUSSION

OECD reproduction tests

As shown in Figure (1), the number of surviving *F. candida* adults was decreased significantly ($P < 0.001$, $t = -11.17$) in a concentration-dependent manner when exposed to arsenic-contaminated soil after 28 days. According to the graph, slightly fluctuation took place from 0 mg/kg to 8.1 mg/kg, the adult number significantly decreased at 0.01 mg/kg ($P < 0.001$, $t = -3.65$) and 0.1 mg/kg ($P < 0.001$, $t = -4.69$) respectively. Then there was no obvious change of adult number between the ranges of 0.3 mg/kg to 8.1 mg/kg. However, a sharp decrease in adult numbers occurred significantly at 24.3 mg/kg ($P < 0.001$, $t = -14.42$) where the adult number decreased to 0. Likewise, the number of juvenile decreased ($P < 0.001$, $t = -3.76$) in a concentration-dependent manner. The significant decrease took place at a concentration of 24.3 mg/kg ($P < 0.001$, $t = -3.8$) where the number of the juvenile was less than 8% of the control. The values of NOEC, LOEC, $LC_{50,}$ and EC_{50} were displayed in Table 1.
From Figure (2), the number of adults ($P < 0.001$, $t = -10.53$) decreased significantly in a concentration-dependent manner when exposed to boron-contaminated soil. The result of the juvenile was also visible in the graph ($P < 0.001$, $t = -3.75$). The number of survival adults was declined to 0 ($P < 0.001$, $t = -10.87$) at a concentration of 15.625 mg/kg, which had a significant difference compared with the control group. About the number of juvenile, it decreased sharply at 0.001 mg/kg ($P < 0.05$, $t = -2.2$). Fluctuation took place from 0.001 to 3.125 mg/kg, and the number of the juvenile at 3.125 mg/kg was lower than it was at 0.001 mg/kg. There were barely juveniles at the highest concentration of 15.625 mg/kg ($P < 0.001$, $t = -3.89$) where the number of the juvenile was only 2.77% of the control group. The values of NOEC, LOEC, $LC_{50,}$ and EC_{50} were displayed in Table (1). Because the difference from the control was not continuous, the NOEC and LOEC of boron were not dependable.

Figure (3) provides the results of the survival and reproduction of *F. candida* exposed to selenium-contaminated soil after 28 days. There were no significant differences ($P > 0.05$, $t = -1.17$) between the contaminated group and the control group in terms of adult mortality, and either there was no significant differences ($P > 0.05$, $t = -1.41$) between contaminated groups and the control group in terms of juvenile number. The values of NOEC, LOEC, $LC_{50,}$ and EC_{50} were displayed in Table 1. Because the difference from the control was not continuous, the NOEC and LOEC of selenium were not dependable.

Figure (4) shows the results of survival and reproduction exposed to iodine-contaminated soil after 28 days. The number of adult ($P < 0.001$, $t = -4.6$) decreased significantly in a concentration-dependent manner. Adult number decreased significantly ($P < 0.001$, $t = -4.71$) at 7.5 mg/kg comparing with the control, and then it decreased slightly at 11.25 mg/kg ($P < 0.001$, $t = -3.93$). Subsequently, the adult number decreased slowly from 11.25 to 37.969 mg/kg, reaching the lowest adult number at 37.969 mg/kg ($P < 0.001$, $t = -6.28$). At the highest concentration of 56.953 mg/kg, the number of adult decreased significantly ($P < 0.001$, $t = -5.24$). In another hand, the number of juveniles decreased significantly in a concentration-dependent manner ($P < 0.001$, $t = -8.28$). The number of juvenile decreased to 1 at the highest concentration of 56.953 mg/kg ($P < 0.001$, $t = -9.53$). The value of NOEC, LOEC, $LC_{50,}$ and EC_{50} can be found in Table 1.

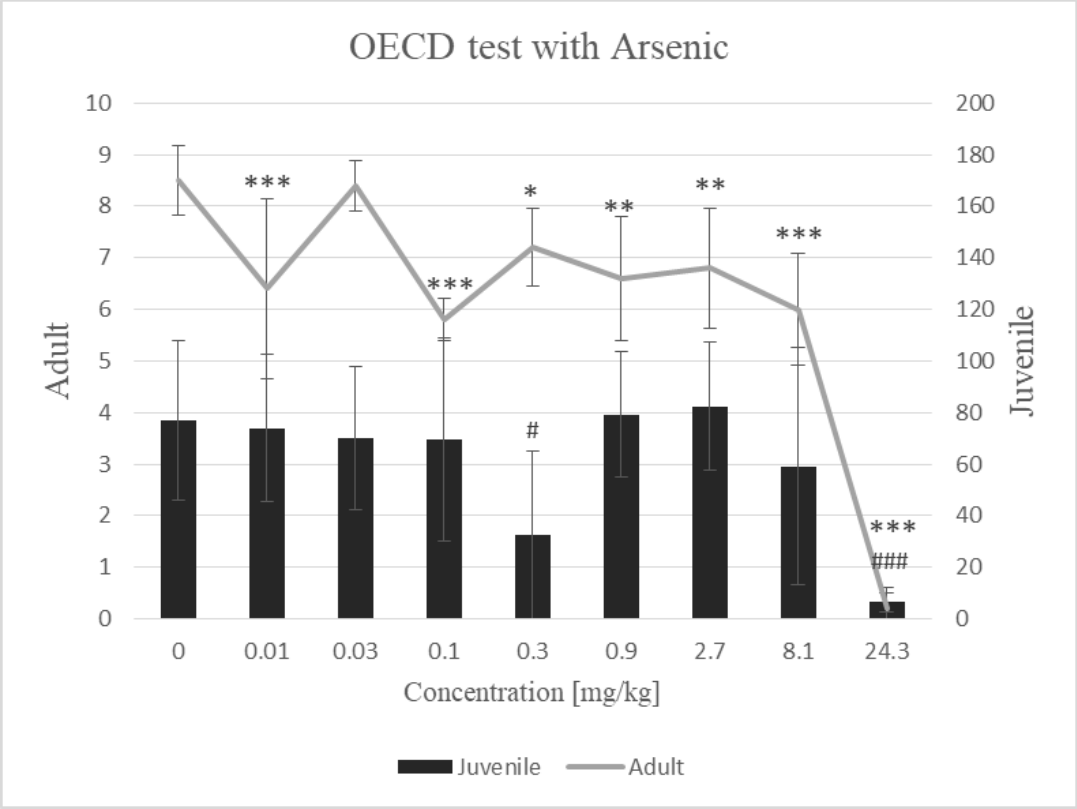


Figure (1): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to arsenic-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$. A pound "#" indicates there was a significant difference in reproduction: "#" $P \leq 0.05$, "##" $P \leq 0.01$; "###" $P \leq 0.001$)

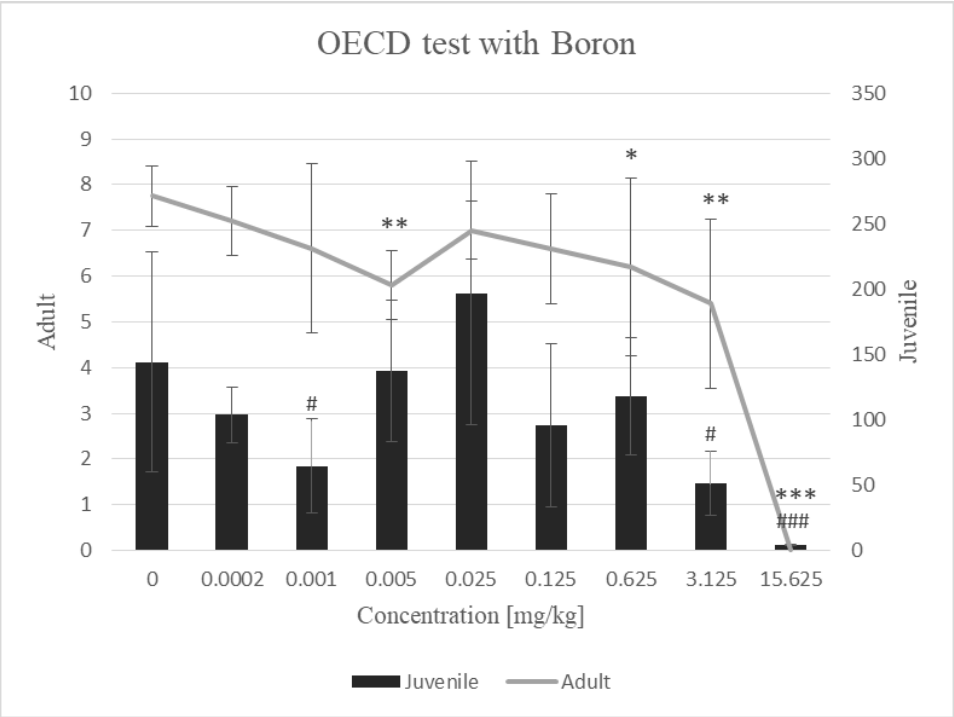


Figure (2): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to boron-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$. A pound "#" indicates there was a significant difference in reproduction: "#" $P \leq 0.05$, "##" $P \leq 0.01$; "###" $P \leq 0.001$).

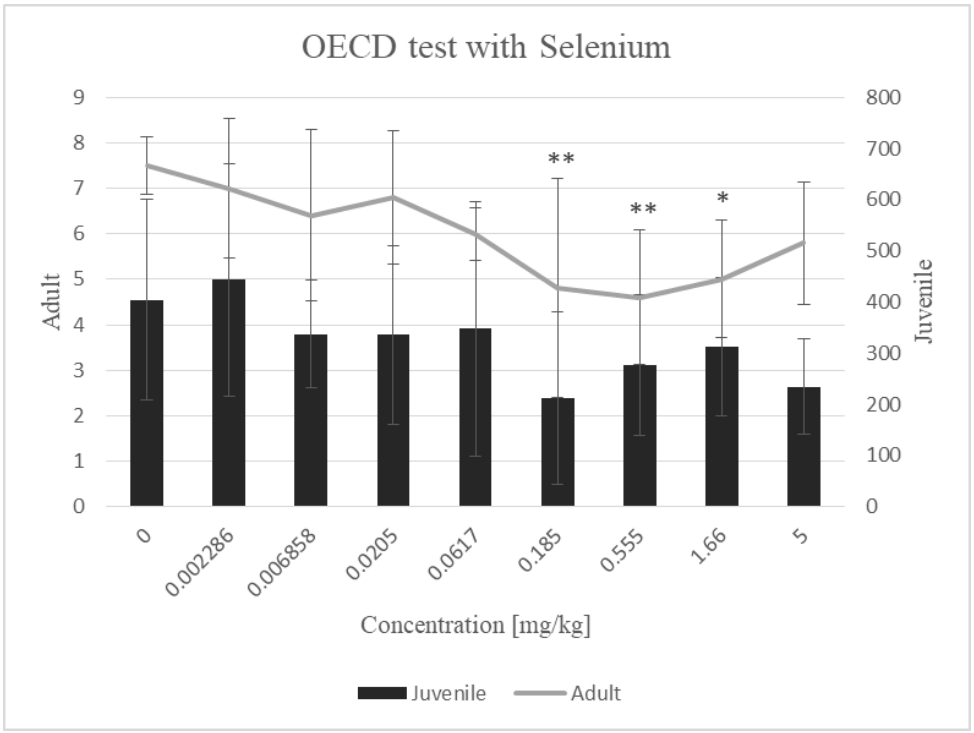


Figure (3): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to selenium-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$. A pound "#" indicates there was a significant difference in reproduction: "#" $P \leq 0.05$, "##" $P \leq 0.01$; "###" $P \leq 0.001$).

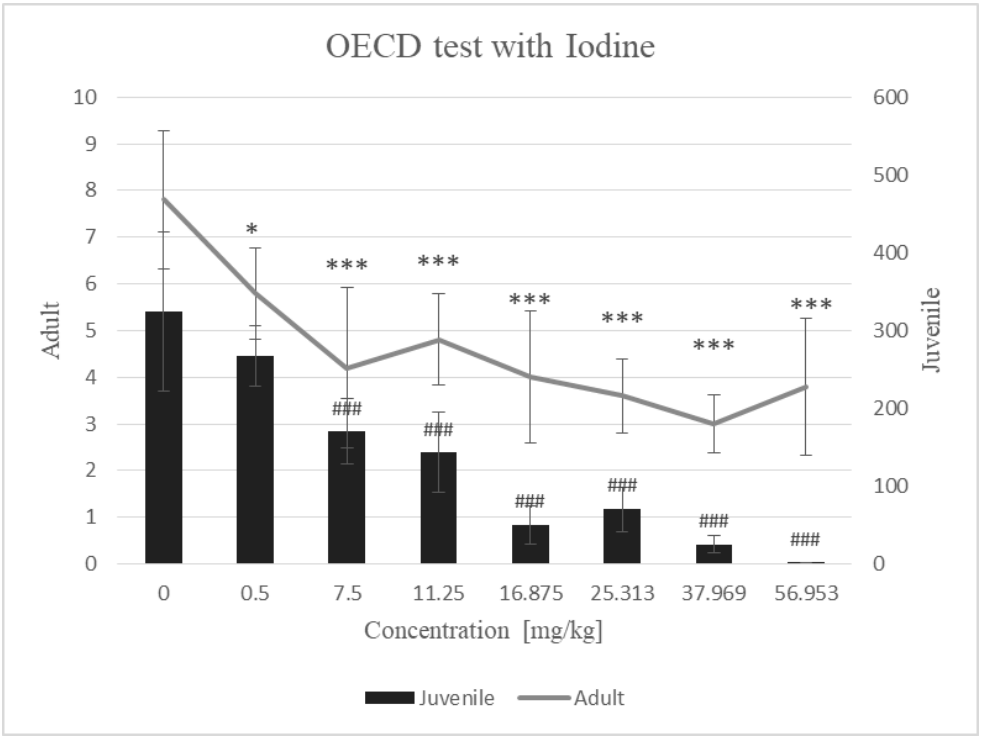


Figure (4): The results (average ± standard deviation) of survival and reproduction of *F. candida* exposure to iodine-contaminated soil after 28 days. (An asterisk "*" indicates there was a significant difference in mortality: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$. A pound "#" indicates there was a significant difference in reproduction: "#" $P \leq 0.05$, "##" $P \leq 0.01$; "###" $P \leq 0.001$).

Table (1) shows the detailed endpoints of OECD reproduction tests of four trace elements. The LC_{50} of arsenic and iodine was much lower than their EC_{50} respectively, which indicated that mortality was a more sensitive parameter than reproduction. It was possible in the case of springtails if the animals lay eggs before they died. On the other hand, the LC_{50} of selenium was marginally higher than its EC_{50}, therefore, the reproduction was more sensitive than survival in this case. About LOEC, the LOEC of arsenic, boron, and iodine on survival were all much lower than on reproduction, while the LOEC of selenium on survival was higher than on reproduction. Except for boron, the values of LC_{50} of arsenic, selenium, and iodine were quite close which were between 2.45 to 2.6 mg/kg. As for the EC_{50}, selenium had the lowest value which was less than 0.001 mg/kg. There was a four magnitude difference between boron and iodine and a five magnitude difference with arsenic. The value of EC_{50} of arsenic was 5 times higher than boron and 1.5 times higher than iodine.

Table (1): The LOEC, LC_{50}, and EC_{50} values with 95%-confidence intervals (CI) of the OECD

	Survival				**Reproduction**			
	NOEC	**LOEC**	**LC_{50}**	**95%-CI**	**NOEC**	**LOEC**	**EC_{50}**	**95%-CI**
As	0.03	0.1	2.595	n.d.	8.1	24.3	13.006	3.698-44.426
B	0.001	0.005	n.d.	n.d.	0.625	3.125	2.562	0.064-86.931
Se	0.0617	0.185	2.493	0.316-n.d	≥5	>5	<0.001	n.d.-n.d.
I	<0.5	≤0.5	2.452	0.045-7.138	0.5	7.5	8.792	2.672-29.612

The concentration was given in mg/kg. "n.d": not determined.

Avoidance tests

Figure (5) shows the result of avoidance test with boron. The y-axis presented the mean number of adult found in uncontaminated soil side. In some cases, the used concentrations caused mortality; therefore, the total number of animals could deviate from the originally introduced one. From the results, there was a significant avoidance behavior at 0.0002 mg/kg ($P < 0.01$, $t = 4.27$). Then, the

avoidance behavior of *F. candida* adult was significant at 0.005 mg/kg ($P < 0.001$, $t = 10.63$), 0.025 mg/kg ($P < 0.001$, $t = 5.43$), 0.125 mg/kg ($P < 0.001$, $t = 7.09$), 0.625 mg/kg ($P < 0.001$, $t = 5.4$), and 3.125 mg/kg ($P < 0.05$, $t = 3.24$). The highest adult number at uncontaminated side was recorded at the concentration of 0.625 mg/kg.

As shown in the Figure (6), *F. candida* at contaminated side showed significant avoidance behaviors only at the concentrations of 1.66 mg/kg ($P < 0.001$, $t = 5.74$) and 5 mg/kg ($P < 0.05$, $t = 3.2$). The number of the adult at the uncontaminated side was the highest at 1.66 mg/kg, and then at 5 mg/kg.

According to the results in Figure (7), there were significant differences of avoidance behaviors at 0.5 mg/kg ($P < 0.01$, $t = 5.74$) and 56.953 mg/kg ($P < 0.05$, $t = 2.63$). And the number of adult at uncontaminated side at 0.5 mg/kg was more than it was at 56.953 mg/kg.

Figure (8) shows the results of avoidance test with arsenic. Avoidance behaviors were significant at 0.3 mg/kg ($P < 0.01$, $t = 4.12$), 2.7 mg/kg ($P < 0.05$, $t = 2.49$) and the highest concentration 8.1 mg/kg ($P < 0.01$, $t = 3.49$) respectively. Meanwhile, the results showed the highest adult number at the uncontaminated side was at the concentration of 8.1mg/kg, and then followed by 0.3 mg/kg, finally the lowest adult number was at 2.7 mg/kg.

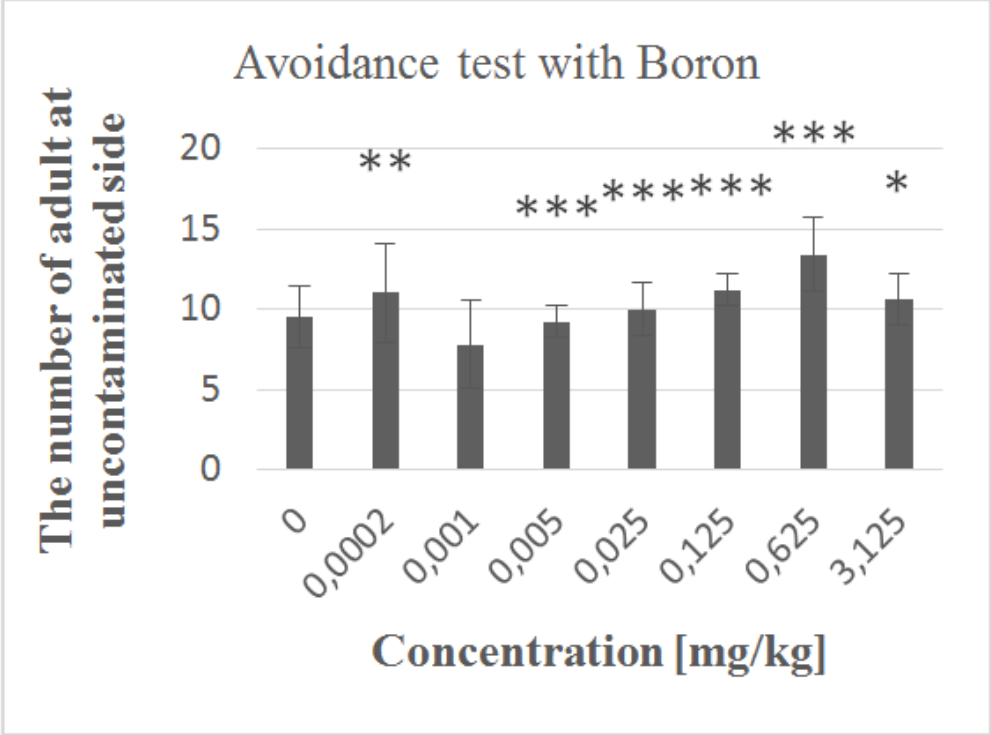


Figure (5): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and boron-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

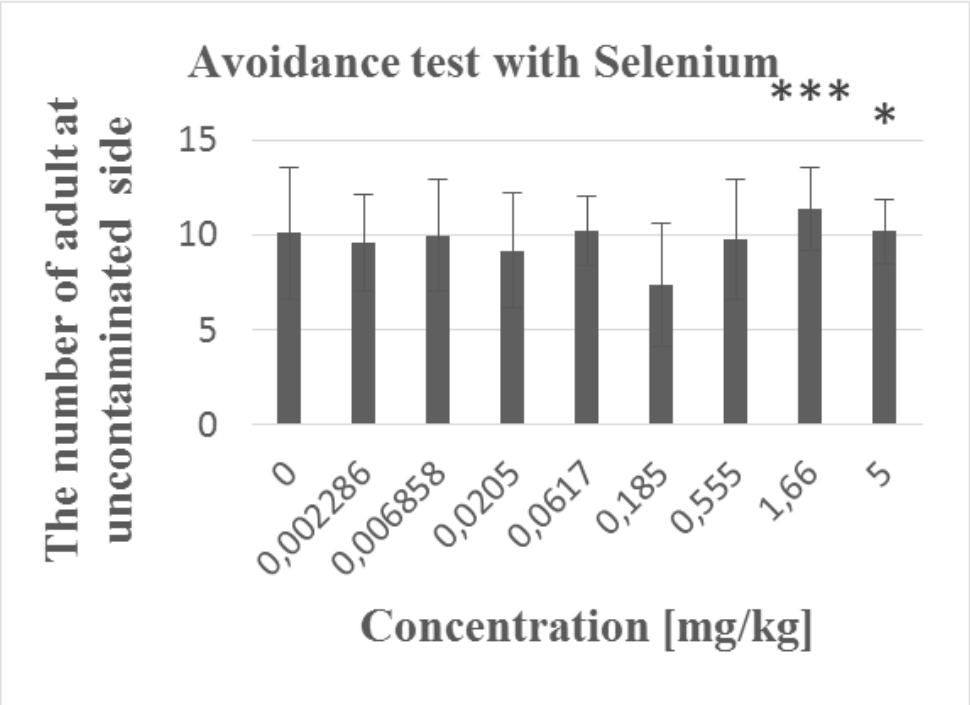


Figure (6): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and selenium-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

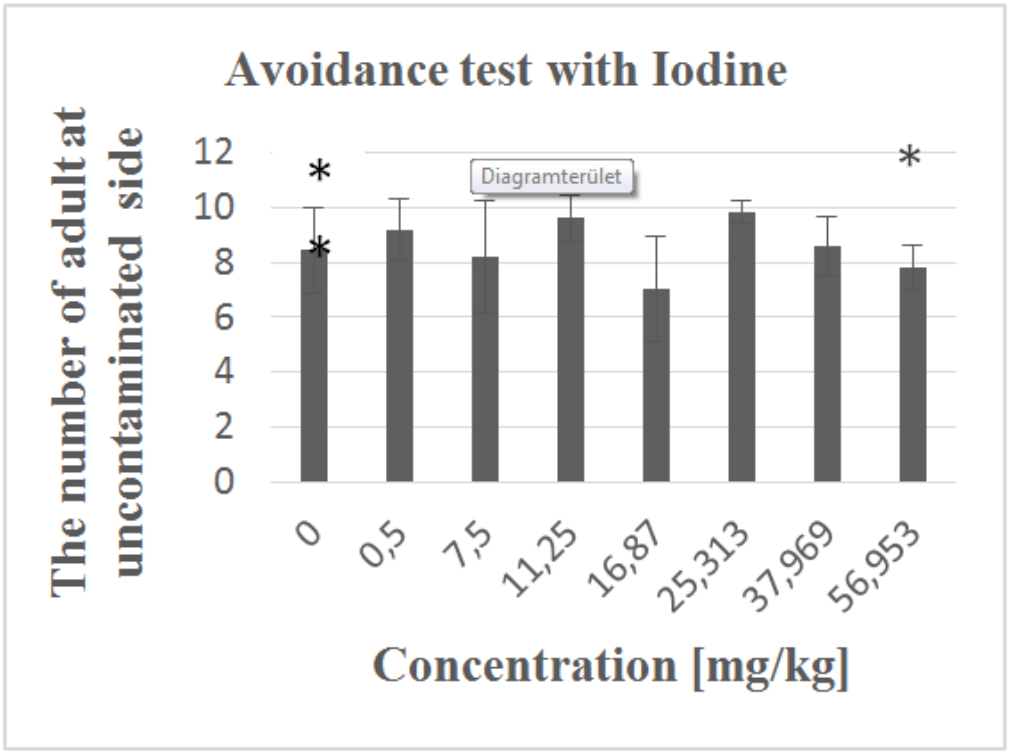


Figure (7): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and iodine-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

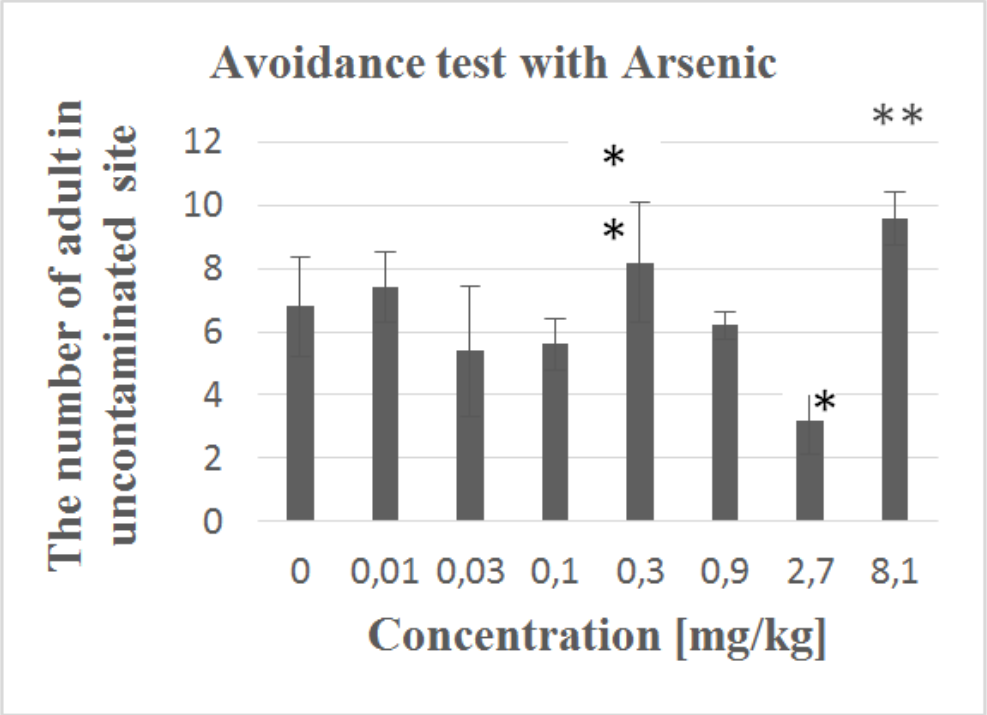


Figure (8): The avoidance test results (average ± standard deviation) of *F. candida* adult exposure to paired uncontaminated soil and arsenic-contaminated soil after 48 hours. (An asterisk "*" indicates there was a significant difference: "*" $P \leq 0.05$, "**" $P \leq 0.01$; "***" $P \leq 0.001$)

In the present study, the toxic effects of four trace elements were evaluated on *F. candida*, including two metalloid elements: arsenic and boron, and two non-metal elements: selenium and iodine. It had been well known that the reproduction and survival of *F. candida* had been the most frequently used endpoints to evaluate the toxicity of metal elements (Lin et al., 2019). The two-day long avoidance tests can indicate soil contamination as an early warning and a preliminary test to provide a reference for the OECD reproduction test and to trigger other tests (Bao et al., 2017; Da Luz et al., 2004; Lin et al., 2017). Besides, it could reflect on the environmental relevance of the data of OECD tests. In our study, arsenic, boron, selenium, and iodine all showed toxic effects on the survival and reproduction of *F. candida* in different degrees.

In terms of the reproduction test with arsenic, the LC_{50} (2.595 mg/kg) was one order of magnitude less than the EC_{50} (13.006 mg/kg). It indicated survival of *F. candida* was more sensitive than reproduction to arsenic. While Alves et al. (2018) found the EC_{50} of arsenic was 26.1 mg/kg on *F. candida* in tropical artificial soil (TAS), which was higher than the EC_{50} of arsenic in this study (Alves et al., 2018). Besides, Lin et al. (2019) found the LC_{50} of arsenic on *F. candida* was in the range of 320 to 1280 mg/kg in 10 different natural soils that were spiked from different regions in China (Lin et al., 2019). Arsenic-induced avoidance behaviors in *F. candida* occurred mostly at high concentrations, while avoidance behavior also occurred at the concentration of 0.3 mg/kg, which even lower than the LC_{50} (2.595 mg/kg) of arsenic. Thus, Collembolan could probably avoid slightly polluted soil in a natural field. Generally, the concentration of arsenic in uncontaminated soil is in the range of 0.2 - 40 mg/kg. A high level of 100 – 2500 mg/kg was found in the ambient soil around some copper smelters. Additionally, the concentration of arsenic had been found at a higher level (200-2500 mg/kg) than above in the soil of orchards because of the numerous arsenical pesticides applications (World Health Organization, 1996). So it would appear that arsenic might be a potential toxic risk in natural field, especially in contaminated regions.

About the reproduction test with boron, the LC_{50} value and its confidence interval were not calculated, the EC_{50} was 2.562 mg/kg with a wide range of confidence intervals (0.064-896.931). Therefore, we preferred to compare the sensitivity of survival and reproduction with their NOEC and LOEC values. The NOEC and LOEC of survival were three orders of magnitude less than NOEC and LOEC of reproduction respectively, which revealed survival was much more sensitive than reproduction to

boron. Besides, boron showed higher toxicity on reproduction than arsenic and iodine in this study. Research by Krogh et al. (2008) found the EC_{50} of boric acid was 90 mg/kg on *F. candida* in the OECD artificial soil (Krogh et al., 2008). Also about boric acid, Amorim et al. (2012) found the EC_{50} was 54.5 mg/kg on *F. candida* in another type of soil (LUFA 2.2). The main differences between OECD soil and LUFA 2.2 soil are organic matter and clay content. Besides, Amorim et al. (2012) did not found avoidance behavior in boron-contaminated soil. Worthy be noticing, *F. candida* avoided boron-contaminated soil at almost all concentrations in this study, even when the concentration was lower than the LOEC (0.005 mg/kg) of survival, which could mitigate the toxic effects of boron. Because the *F. candida* could leave the contaminated area before toxic amounts of boron were uptaken. In nature, boron had been found to occur from 10 to 300 mg/kg with an average of 30 mg/kg in uncontaminated soils. While the level of concentration depends on the type of soil, amount of organic matter, and the amount of rainfall (World Health Organization, 1998). Based on the above description, our results showed boron had a potential toxic risk in nature.

Concerning the reproduction test with selenium, there were no toxic effects on the reproduction of *F. candida*. On the one hand, the EC_{50} value (< 0.001mg/kg) was not trustworthy, because the confidence interval was not determined by the program, and the NOEC (≥ 5 mg/kg) and the LOEC (> 5 mg/kg) were no less than the highest test concentration of selenium. What can be believed was that the EC_{50} of selenium might out of the range of test concentrations in this study. On the other hand, the toxic effect of selenium on mortality was a sensitive parameter from the results, and the LC_{50} was 2.493 mg/kg. While Kuperman et al. (2018) found in the natural soil Sassafras sandy loam (SSL), the LC_{50} of selenium was 12.7 mg/kg on *F. candida*, and 6.2 mg/kg on *Enchytraeus crypticus* (Kuperman et al., 2018). Speak about avoidance test with selenium; *F. candida* avoided only high concentrations. Generally, the concentration of selenium in soils is in the range of 0.005-1200 mg/kg, and the most common range is between 0.1-10 mg/kg. A high content of Se had been found in volcanic rocks (120 mg/kg) (Alexander, 2015). The available limitation analytical data of selenium was typically found in some foods in a range of 0.1-1.5 mg/kg, and < 0.1 mg/kg in most fruits and vegetables (World Health Organization, 1986). Although our results showed there existed toxic effects of selenium on *F. candida*, the concentration of selenium in nature seems to be a low potential toxic risk.

As for the reproduction test with iodine, the LC_{50} (2.452 mg/kg) was smaller than EC_{50} (8.792 mg/kg), which indicated reproduction was less sensitive than survival. The toxic evaluations of iodine on *F. candida* were rare. Irvine and Sikdar (1998) found the EC_{50} of iodine which from waste brine was less than 0.1 mg/kg on *Ceriodaphnia dubia.* The different EC_{50} values may be connected with inhabitations and individual variation (Irvine and Sikdar, 1998). In addition, Ilin and Nersesyan (2013) found the LC_{50} of I on *Eisenia fetida* was greater than 1000 mg/kg in dry soil and 740 mg/kg in wet soil (Ilin and Nersesyan, 2013). As for the avoidance behavior of *F. candida* to iodine, there were only two concentrations that induced the avoidance behaviors, which were at the lowest and the highest concentration respectively. With regard to the iodine in nature, the concentration of iodine had a huge variability from media to media and also from place to place on earth. Normal levels of iodine were in the range of 0.3-10 ppm. 0.5 ppm could be regarded as an average concentration in most parent materials. A concentration of 5 ppm had been adopted mostly in organic soils (Johanson, 2000). Based on the above data, our results showed that there exists a low potential toxic risk of iodine.

As described above, the survival of *F. candida* was more sensitive than its reproduction to each element. It implied individual *F. candida* was affected by contaminations firstly, and then followed by its reproduction ability under environmental stress. It was possible that the *F. candida* could lay eggs before they die because of the exposure. This symptom may be related to the physiological characteristics of Collembolan (Fountain and Hopkin, 2005). Likewise, Linares et al. (2007) found in some marine invertebrates, their survival was more sensitive than reproduction in undersea environmental stress (Linares et al., 2007). Besides, The LC_{50} value of arsenic, selenium, and iodine was extremely close, which expressed their toxicities were similar. However, their EC_{50} values were different. Various toxicity on reproduction may be related to the different properties of trace elements, as well as the dissimilar metabolic process in collembolans (Bao et al., 2017). Additionally, the variability of EC_{50} value and LC_{50} value of a specific trace element on *F. candida* might be related to the strong influence of soil properties, which contribute to a different bioavailability of each element in soil (Alves et al., 2018; Lin et al., 2019).

In brief, the results showed the avoidance behavior in *F. candida* was most sensitive to boron, followed by arsenic and iodine, and finally selenium. Previous researches by others, for example, Lin et al., (2017) found the *F. candida* avoided antimony (Sb) even at a concentration much lower than its LC_{50} (Lin et al., 2017). Bao et al., (2017) found *F. candida* only had avoidance behaviors at high concentrations of cadmium (Cd) which were much higher than its EC_{50} (Bao et al., 2017). Li et al., (2011) found *F. candida* avoided copper (Cu) at almost all concentrations (Li et al., 2011). These different avoidance behaviors of *F. candida* induced by different elements indicated that *F. candida* indeed can sense the presence of soil contaminations and had great variabilities in the avoidance behavior to different contaminations. Diverse avoidance behaviors might due to *F. candida* has different abilities of sense to different elements.

CONCLUSIONS AND RECOMMENDATIONS

To conclude, this paper achieved the aim of the study. The toxicity of each element on survival was similar, while boron showed higher toxicity on reproduction than the other three elements, besides, boron made *F. candida* showed the most avoidance behaviors among the four trace elements. Combined with official data, boron and arsenic had shown potential risks in natural field conditions. As a parameter in the ecotoxicological tests, survival appeared more sensitive than the reproduction of *F. candida* to every element in OECD reproduction tests. Ultimately, the whole four trace elements showed higher toxicities on the *F. candida* comparing with the previous studies by others. Many researchers have focused on this problem. The above conclusions further confirmed the importance of ecotoxicological evaluations of trace elements and gave an early warning of soil pollution caused by trace elements. This paper had a positive effect on the protection of the soil environment and it can provide help for further ecotoxicological researches. However, deeper researches should be continued on the toxicity of various elements to see long-term effects on different life-history parameters of *F. candida*. Also, the molecular mechanism of the toxicity should be revealed.

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REFERENCES

[1] Alexander, Jan. “Selenium.” *Handbook on the Toxicology of Metals: Fourth Edition*, vol. 1, Academic Press, 2015, pp. 1175–208, doi:10.1016/B978-0-444-59453-2.00052-4.

[2] Alves, Paulo Roger Lopes, et al. “Ecotoxicological Impact of Arsenic on Earthworms and Collembolans as Affected by Attributes of a Highly Weathered Tropical Soil.” *Environmental Science and Pollution Research*, vol. 25, no. 14, Environmental Science and Pollution Research, 2018, pp. 13217–25, doi:10.1007/s11356-016-6839-2.

[3] Bao, Qiongli, et al. “Ecotoxicity of Soil Cd-Pollution to Collembolan Folsomia Candida.” *Asian J. Ecotoxicology*, vol. 2, no. 2, 2017, https://baijiahao.baidu.com/s?id=1602214129364118470.

[4] Da Luz, Tiago Natal, et al. “Avoidance Tests with Collembola and Earthworms as Early Screening Tools for Site-Specific Assessment of Polluted Soils.” *Environmental Toxicology and Chemistry*, vol. 23, no. 9, 2004, pp. 2188–93, doi:10.1897/03-445.

[5] Fountain, Michelle T., and Steve P. Hopkin. “Folsomia Candida (Collembola): A ‘Standard’ Soil Arthropod.” *Annual Review of Entomology*, vol. 50, no. 1, 2005, pp. 201–22, doi:10.1146/annurev.ento.50.071803.130331.

[6] Ilin, Alexander, and Armen Nersesyan. “Toxicology of Iodine: A Mini Review.” *Archive of Oncology*, vol. 21, no. 2, 2013, pp. 65–71, doi:10.2298/AOO1302065I.

[7] Irvine, R. L., and S. K. Sikdar. *Bioremediation Technologies: Principles and Practice*. Taylor & Francis, 1998, https://books.google.hu/books?id=oLNtgk_VKXsC.
[8] Johanson, Karl Johan. "Iodine in Soil Iodine in Soil." *Enzyme*, 2000.
[9] Krogh, P. H., et al. "Toxicity Testing with the Collembolans Folsomia Fimetaria and Folsomia Candida and the Results of a Ringtest." *Ecology*, vol. 23, no. August, 2008, pp. 1–44, http://www.oecd.org/dataoecd/3/63/41389036.pdf.
[10] Kuperman, Roman G., et al. "Selenium Toxicity to Survival and Reproduction of Collembola and Enchytraeids in a Sandy Loam Soil." *Environmental Toxicology and Chemistry*, 2018, doi:10.1002/etc.4017.
[11] Li, Xiaoyong, et al. "Acute Toxicity of Copper Pollution to Folsomia Candida(Collembolan)in Soil." *Acta Pedologica Sinica*, vol. 48, no. 1, 2011, pp. 197–201.
[12] Lin, Xianglong, Zaijin Sun, Long Zhao, et al. "The Toxicity of Exogenous Arsenic to Soil-Dwelling Springtail Folsomia Candida in Relation to Soil Properties and Aging Time." *Ecotoxicology and Environmental Safety*, vol. 171, no. January, Elsevier Inc., 2019, pp. 530–38, doi:10.1016/j.ecoenv.2018.12.059.
[13] Lin, Xianglong, Zaijin Sun, Weiyu Chen, et al. "Toxicity Effect of Antimony to Soil-Dwelling Springtail (Folsomia Candida)." *Research of Environmental Sciences*, vol. 30, no. 7, 2017, pp. 1089–97, http://www.hjkxyj.org.cn/html/2017/7/20170712.htm#top.
[14] Linares, Cristina, et al. "Life History and Viability of a Long-Lived Marine Invertebrate: The Octocoral Paramuricea Clavata." *Ecology*, 2007, doi:10.1890/05-1931.
[15] OECD/OCDE 232. "Collembolan Reproduction Test in Soil." *OECD Guidelines for the Testing of Chemicals*, no. September, 2009, pp. 1–19.
[16] RStudio Team. *RStudio.Version 3.6.1*. 2019, https://rstudio.com/.
[17] ToxRat®Solutions.Gmbh. *ToxRat Professionals 3.3.0.* 2018, https://www.toxrat.com/home-56.html.
[18] World Health Organization. "Chapter 6.1 Arsenic." *Comprehensive Analytical Chemistry*, vol. 30, no. 4, 1996, pp. 303–27, doi:10.1016/S0166-526X(96)80018-3.
[19] *International Programme on Chemical Safety-Boron*. 1998, https://apps.who.int/iris/bitstream/handle/10665/42046/9241572043_eng.pdf;jsessionid=CE4F066E7BC3E27AFEA623534B3134E3?sequence=1.
[20] *International Programme on Chemical Safety-Selenium*. 1986, http://www.inchem.org/documents/ehc/ehc/ehc58.htm.