Toxicity Assessment with Earthworm Test Systems for Petroleum-Contaminated Soil from Kazakhstan Oilfield

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***Abstract*:** *To evaluate soil quality and risk assessment, earthworm toxicity tests can be useful tools to measure the potential toxicity of contaminants. In this study, the toxicity of petroleum-contaminated soil on earthworms was assessed. The soil samples were collected from an oilfield in Kulsary, Atyrau province, Kazakhstan. All tests were performed according to ISO guidelines and natural uncontaminated soil was used as control. Earthworms were exposed to contaminated soils and evaluated their response after a certain exposure period. The LD_{50} of petroleum-contaminated soil for earthworms was 2,3 ml of petroleum/kg of soil, with 73 % of the earthworms dying after 48 h. Concentration of 6,2 ml/kg soil sample caused high toxic effects (100% lethality), but at lower test concentrations (0,9 ml/kg), only slight toxic effects were observed. Petroleum had adverse effect on growth and fecundity in earthworm exposed to 5,0 ml/kg contaminated soil. The avoidance response tests showed similar results when compared with other test systems. Significant repellent effects at concentration were between 0,9 and 6,2 ml/kg petroleum. The obtained results show that these test methods can be regarded as a valuable tool in the screening evaluation of soil contamination.*

Keywords: *petroleum-contaminated soil, biotest systems, toxicity, earthworm*

1. Introduction

Soil contamination with hydrocarbons causes extensive damage of local and global ecosystem. For safe solution of bioremediation of oil pollution, the following approaches, including introduction of oxidizing microorganisms in contaminated sites and optimization of life of indigenous hydrocarbon-oxidizing microflora are used. In implementing these approaches or their combination thereof having some problems.

The level of pollution by petroleum and oil products are different. Low levels of soil contamination, does not require any bioremediation activities. In moderate pollution, it is enough to apply nitrogen source to stimulate spontaneous microflora. With the increased level of environmental pollution by petroleum and oil products requires the use of microbial based biopreparations or compositions of monocultures of oil-oxidizing microorganisms.

In modern conditions the ever increasing anthropogenic pressure on the environment test analysis techniques occupy an intermediate niche between the indicator and quantitative methods for the determination of toxicants, allowing to instant assess potentially dangerous levels of contamination for humans and the biosphere as a whole. Thus, the test method will fill the gap that has emerged between the need for information and the possibility of its actual receipt due to the ever-growing list of monitored components and tightening of requirements to their minimum determines the content.

Among the special advantages of biological methods to be noted that they are sensitive to a large range of chemicals and allow fixing adverse changes in the environment at low concentrations of contaminants. Also, the main positive aspect of the use of biological indicators is that the reaction of a living organism to evaluate human impact on the environment in terms of having a biological sense and giving an integrated picture of the nature of the changes in ecosystems. It should be noted that the soil contamination monitoring studies of natural and man-made ecosystems, biological oil, and, in particular, indicating microbiological methods and test underutilized. At the same time, it allows them to use the most accurate assessment of the current state of ecosystems and predict the dynamics of possible changes in the situations of powerful human impacts such as pollution by oil.

In this regard, the selection of test systems based on biological test objects quickly and efficiently assess the cumulative toxicity of the soil is important. In addition, the test system will not only evaluate the toxicity of the soil, but also can be used for observation of the process of bioremediation [1-2].

The aim of this research was the development of rapid test methods based on earthworms for determining integrated soil toxicity contaminated with petroleum and oil-products.

2. Methods

2.1. Soil samples

The soil samples were collected from an oilfield in Kulsary, Atyrau province, Kazakhstan according to a standardized procedure [3]. The climate of the area is generally warm and arid. Predominant soil in the area is alkali-saline soil contaminated with different concentrations of petroleum and oil products.

2.2. Earthworms experiment

Three types of bioassay based on earthworm have been used including acute (screening), behavior (avoidance) and chronic (prolonged) tests. These all tests were conducted with the earthworm Eisenia fetida.

Acute tests

The desired amount of oil contaminated soils were thoroughly mixed and placed into glass boxes. Then ten adult worms were placed in every boxes, to prevent the worms from escaping, test containers were covered with a gauze sheet. After 2 days of incubation, surviving worms were computed. Three replicates were applied for this test.

Avoidance tests

At the beginning, 500 ml of soil samples are added to the boxes and control soil with no contamination was included. Five bio-indicators are added to each box and this time was recorded at the beginning of the test (t=0 min). Three replicates were applied for the avoidance test.

Chronic tests

This test conformed to the acute test described above, but the test endpoints were cocoon production after 30 days.

3. Results and discussion

The screening toxicity test was applied to measure the short-term acute effects of contaminated soil samples on earthworm survival. Their survival was indicated following 48 hours of exposure to the oil-contaminated soil.

Analysis of the experimental results shown in Table I, reveals that the petroleum in the concentration of 2,3 ml/kg has a negative impact on earthworms compared with the control. Acute toxicity of petroleum was observed at the concentrations between 6, 2 and 8, 3 ml/kg.

TABLE I: Petroleum toxicity in relation to the earthworms

Petroleum concentration, ml/kg	Number of earthworms	Survival rate, %
Control	10	100
0,1	10	100
0,9	10	100
2,3	8	80
3,1	8	80
4,2	3	30
5,6	2	20
6,2	0	0
7,1	0	0
8,3	0	0

An environmental monitoring based on earthworm behavior test-system are used as a potential method to assess contaminated soils. It was an avoidance-response bioassay with 5-hour duration, and has been shown to be sensitive of the results of the prolonged (chronic) tests.

An indicator of behavioral test was the worm burrowing speed into the substrate. The toxicity criterion was the lack of burrowing earthworms in the test soil, active crawling on the ground and attempt to crawl out the box.

The samples tested were site-specific contaminated soils with different concentrations of petroleum and collected from the field.

TABLE II: The worm burrowing speed into the substrate

#	Petroleum concentration, ml/kg	Burrowing speed, min
1	Control	9
2	0,1	9
3	0,9	11
4	2,3	15
5	3,1	26
6	4,2	38
7	5,6	40
8	6,2	-
9	7,1	-
10	8,3	-

In this test, the toxic effect of petroleum was observed already at 0,9 ml/kg. It was noted the aggressive behavior of worms and slowing them burrowing into the soil (11 min).

However, by observing the earthworms it was recorded not only changes in the rate of burrowing, but also an alteration the appearance of worms that disrupted the cuticle and the epidermis surface of the worms.

Germination of earthworms was significantly reduced with increasing petroleum concentration in chronic biotests. Germination was highest at the concentration of 0, 1 ml/kg and was decreased started from the sample at the concentration of 0, 9 ml/kg.

The germination rate, calculated 30 days after sowing, varied between 80 and 93 % in low contaminated samples and less than 30 % in highly contaminated soils.

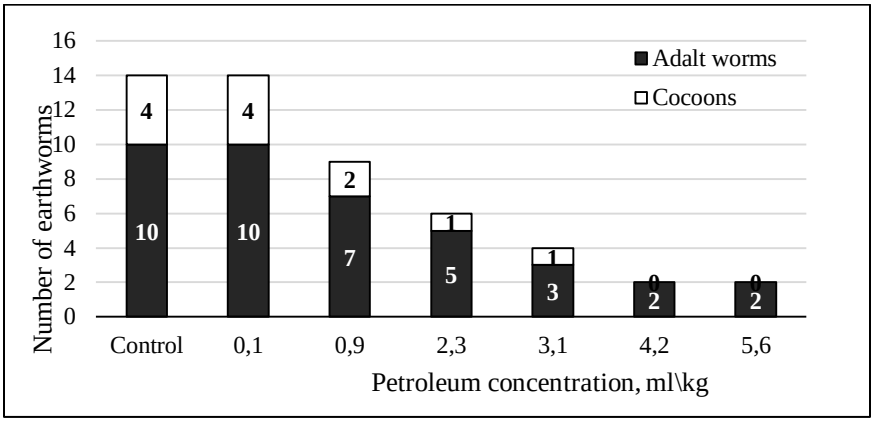

Fig. 2: The survival and fertility of earthworms

In this study, for the earthworms, effects were observed at oil concentrations exceeding 0,9 ml petroleum/kg dry soil. Their reproduction (cocoon production) was already affected at the concentration of 0,9 ml/kg. *Eisenia fetida* seems to be more sensitive to petroleum and oil contaminants than other biotest systems, including plants or microorganisms [4-5].

4. Conclusion

As in the research, results of various toxicity tests demonstrated that petroleum and oil products have adverse impact on behaviour, growth and reproduction in earthworms, but this is largely dependent on pollutant concentration and exposure period.

The use of these test-systems for contaminated sites evaluation would bring quick data for future decisions on the evaluation procedure.

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