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Biological remediation of the engine lubricant oil-contaminated soil with three kinds of earthworms, Eisenia fetida, Eisenia andrei, Dendrobena veneta, and a mixture of microorganisms

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Abstract

The survival of the earthworms in used engine oil-contaminated soil with concentration of 20-100 g/kg was estimated. The investigation was made of the contaminated soil bioremediation efficiency with the use of Eisenia fetida, Eisenia andrei and Dendrobena veneta in presence of the photosynthetic bacteria Thiorhodaceae, Athiorhodaceae and Chlorobacteriaceae, nitrogen-fixing bacteria Azotobacter and Clostridium, yeast cells Saccharomyces, fungi Aspergillus and Penicillium, and Actinomycetales, composing microbiologic product Baykal EM. It is determined that the used oil concentration in the soils with earthworms decreases 4-10 times faster than in contaminated soil without earthworms. High rates of engine oil removal are registered in presence of E. andrei and D. veneta. Oil concentration in soil decreased by 60-90%. With oil concentration of 60-100 g/kg and earthworms (Eisenia fetida) cultivation, the oil concentration decreased 4-6 times, with introduction of Californian earthworms (Eisenia andrei) 12 times and with Dendrobena veneta 12-23 times. At the addition of the microbiologic product along with earthworms, oil content in the soil decreased 58 times and constituted 1.1 g/kg, soil remediation efficiency being 99.9%.

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Keywords: earthworms; Eisenia fetida; Californian earthworm Eisenia Andrei; Dendrobena veneta; bioremediation; removal of petrochemicals; soil contamination

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1. Introduction

Earthworms can accelerate the removal of contaminants from soil. Earthworms change physical and chemical properties of the soil, mixing it with organic matter, they improve aeration and make contaminants available for micro-organisms. Presence of earthworms in contaminated soil indicates their ability to survive in a wide range of different organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and petroleum [1]. The term "vermiremediation" is used to indicate the usage of earthworms for removal of contaminants from soil [2] and remediation of non-recyclable compounds [3]. Positive effect of earthworms on the removal of contaminants such as petroleum, PAHs, and PCBs was mentioned by the authors in [4,5,6,7]. Earthworms burrow through the soil, mixing it in their guts and changing chemical and physical properties of soil [8]. Earthworms increase the contact between a contaminant and soil micro-organisms [9]. Moreover, adding earthworms can increase the removal of a contaminant from soil [10]. Lopes P.R.M., Montagnolli R.N., 2010, investigated the influence of various engine lubricant oils on earthworms. Used oils is highly toxic and carcinogenic for pedobionts. Lubricant oils undergo bioremediation with Eisenia fetida by 40% [11].

Lumbricus terrestris and E. fetida can easily survive at petroleum concentration of 5 g/kg in soil for 15 days [12]. In petroleum-contaminated soil with concentration of 15g/kg, the survival of E. fetida decreased by 40%. On the contrary, other authors reported that petroleum concentration of 9.5 g/kg resulted in high mortality of E. fetida, A. chlorotica and L. terrestris in soils without organic matter after 28 days of colonization [12]. Whitfield Aslund et al., 2013, observed the absence of petroleum-contaminated soil acute toxicity for worms E. fetida and 90% survival at low petroleum concentrations (to 20 g/kg), but failure of the reproduction as well [13]. Eom I.C. et al., 2007, found that survival of E. fetida upon amending soil with petroleum with concentration of 2.6-2.8 g/kg was 18% within 28 days after soil colonization and 8% within 56 days [14].

Addition of microbiologic product and organic matter additional sources increases earthworms survivability and reproduction rates. Ramos et al., 1991, reported the decrease of earthworm mortality by 30-36% compared to 63% upon control after 48 hours of soil remediation with micro-organisms [15]. Contreras-Ramos et al., 2008, concluded that introduction of organic matter and vermicompost accelerated the removal of polycyclic aromatic hydrocarbons from soil. Colonization of earthworms in petroleum-contaminated soil could be an organic way of hydrocarbons removal [5].

Study objective lies in estimation of earthworm ability for used engine oil-contaminated soil remediation in presence of photosynthetic bacteria Thiorhodaceae, Athiorhodaceae and Chlorobacteriaceae, nitrogen-fixing bacteria Azotobacter and Clostridium, yeast fungi Saccharomyces, fungi Aspergillus and Penicillium, and Actinomycetales.

Our aim is to estimate the maximum oil concentration in soil for earthworm survival and complete petrochemicals-contaminated soil decontamination time-limits.

2. Study subject

2.1. Earthworm species

Earthworm Eisenia fetida. Worm mean mass was 0.41 - 0.92 gr. Earthworm is cold-resistant, able to digest aggressive substrates, such as poultry manure, cattle dung, and substrate with high concentration of bark and sawdust.

Californian earthworm Eisenia andrei. Red Californian worms can digest any organic matter (cattle dung, kitchen waste, lastyear foliage, paper, etc.), they have high production rates (100 times higher than other species) and live 4 times longer than other worms. Worms mean mass was 0.5 - 0.9 gr.

Worm Dendrobena veneta. They are usually found near human habitats, in gardens, vineyards, forests, and in high mountains. Worm mean mass was 0.9 - 1.42 gr.

2.2. Microbiologic product

As the source of lactic-acid, nitrogen-fixing and photosynthetic bacteria bioproduct Baykal-EM (produced by LLC "NPO EM-Center", Russian Federation, state registration number 226-19.156-1) was used in concentration of 5 ml per 1 kg of substrate with petrochemicals contamination level of 50 g per kilogram of soil. Bioproduct contains large amount of anabiotic micro-organisms living in soil, namely lactic-acid, nitrogen-fixing, nitro bacteria, Actinomycetales, yeast, and fermenting fungi.

3. Methods

3.1. Test substrate

The test substrate for all three tests was sterile grassland soil "Nutrient soil TerraVita Universal". The compost content was 46%, pH was 5.9 - 6.0 and adsorption capacity was 28-40 mg-eq per 100 g of soil; nitrogen (NH_4 + NO_3) 150 mg/l, phosphorous (P_2O_5) 270 mg/ l, potassium (K_2O) – 300 mg/l. During the test the soil was contaminated by used engine oil (initial concentration was 20 g/kg, final concentration was 100 g/kg).

3.2. Analysis techniques for petrochemicals and organic matter content in soil.

Soil samples selection for petrochemicals and organic matter content analysis was conducted due to GOST 28168, GOST 17.4.3.01 and GOST 17.4.4.02. The soils was ground with a pestle. The sample with the mass of 3 - 5 g was selected from the ground soil and then minced till the grains size less than 0.3 mm and sieved using the filter with mesh size 0.25 mm. To estimate the petrochemicals and organic matter content in soil, the technique by Institute of Experimental Metrology was used. The technique is based on petrochemicals extraction from soil with carbon tetrachloride and simultaneous eluate decontamination by aluminum oxide in chromatographic column. Petrochemicals concentration in eluate was determined with IR spectral photometry by petrochemicals analyzer IKN-025 at wavelength of 3.4 mkm.

3.3. Test protocols

The tests were carried out for 4 months. Drainage was put at the bottom of polypropylene containers with volume 2 liters. Then the containers were filled with 15 cm of soil (1 kg). Ten mature adult earthworms each were introduced into the containers and 100 g of distilled water was added once a week. The earthworm were nourished with 5 g of fresh grated potato once a week and the soil was moistured by 100 ml of distilled water twice a week. The earthworms examination was conducted by hand, layer by layer, after 14 days. The earthworms were incubated at the temperature of +15 °C for 4 months. The process was monitored by the following factors: total population, and population of mature adult earthworms. The obtained results were processed with Friedman ANOVA on ranks. The test protocols are demonstrated in Table 1.

Table 1. Test protocols

	Test1 E. fetida			Test 2 E. andrei			Test 3 Dendrobena veneta		
Sample	Oil concentration	Worm amount	Microbiologic product ml	Oil concentration	Worm amount	Microbiologic product ml	Oil concentrationa	Worm amount	Microbiologic product ml
1	0	10	-	0	10	-	0	10	-
2	0	10	1	0	10	1	0	10	1
3	20	10	-	20	10	-	20	10	-
4	20	10	1	20	10	1	20	10	1
5	40	10	-	40	10	-	40	10	-

6	40	10	1	40	10	1	40	10	1
7	60	10	-	60	10	-	60	10	-
8	60	10	1	60	10	1	60	10	1
9	80	10	-	80	10	-	80	10	-
10	80	10	1	80	10	1	80	10	1
11	100	10	-	100	10	-	100	10	-
12	100	10	1	100	10	1	100	10	1

4. Results

4.1. Test 1.Total population of E. fetida

Test 1 (Fig. 1). 93-100% E. fatida survival and steady earthworm amount increase were registered when exposed to concentration of used oil ranging from 20 to 100g/kg, while survival dropped to 43 - 63% when microbiologic product was added at oil concentration more than 50g/kg.

Earthworm survival was 100% in control sample and in the sample treated with microbiologic product. In samples with oil concentration of 20 - 40g/kg both treated with microbiologic product and untreated, total population increased three-fold. When exposed to concentration of 60g oil/kg soil, total earthworm population increased three-fold as well, while amendment with microbiologic product resulted in 50% mortality of mature adults and the amount equaled to 10 species/container. In oil-contaminated samples with concentration of 80 - 100 g/kg, total population was 10 - 14 species/container, but after adding microbiologic product it dropped to 8 - 10 species/container. Micro-organisms are likely to facilitate the oil adsorption in digestive track and enhance the toxic effect. In control sample the total population was 76 species/container, and addition of microbiologic product provided the increase to 203 species/container (Table 2).

Table 2. Survival, total population, total productivity and individual productivity of earthworms Eisenia fetida under different used oil concentrations in soil

	Test number	Survival %	Total population	Total productivity	Individual productivity
1	control	100	76.66	14	0.9
2	Microbiologic matter	100	203.33	16	0.99
3	oil concentration 20 g/kg	100	33.33	10.66	0.9
4	oil concentration 20 g/kg and microbiologic product	100	30.00	10	1
5	oil concentration 40 g/kg	100	33.67	10	0.9
6	oil concentration 40 g/kg and microbiologic product	100	21.33	5	0.5
7	oil concentration 60 g/kg	100	29.00	7	0.6
8	oil concentration 60 g/kg and microbiologic product	63	10.00	1.66	0.26
9	oil concentration 80 g/kg	93	11,67	2	0.24
10	oil concentration 80 g/kg and microbiologic product	43	8.33	2.3	0.54
11	oil concentration 100 g/kg	93	14.00	3.3	0.33
12	oil concentration 100 g/kg and microbiologic product	43	9.00	2	0.46
	ANOVA on ranks and Kendall's concordance	-	0.708	0.594	0.55

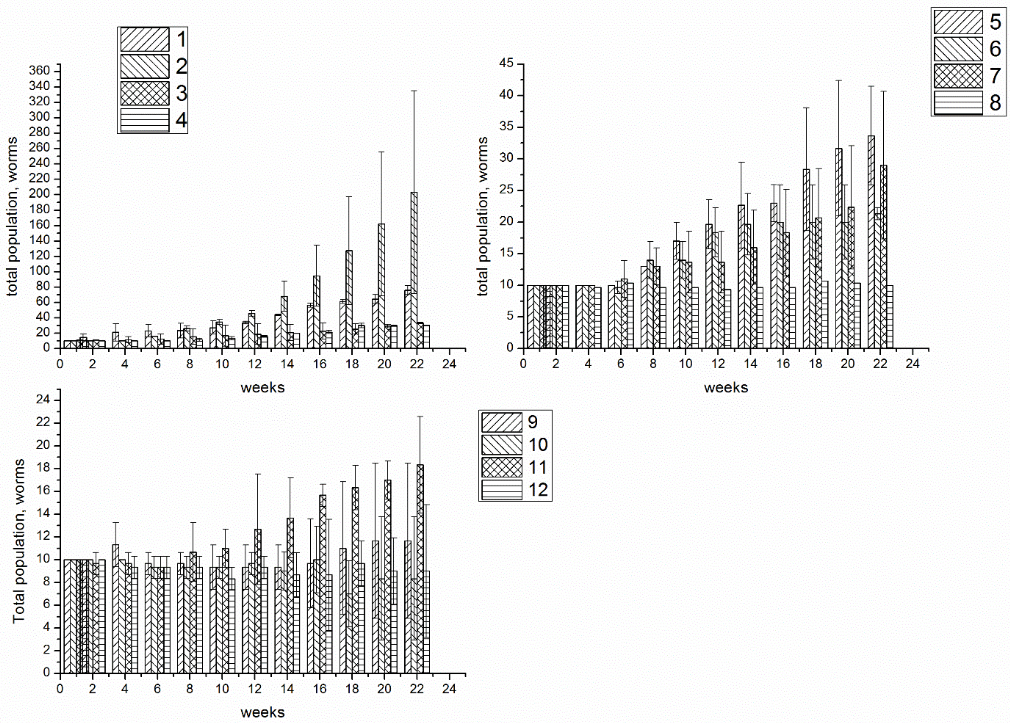

Fig. 1. E. fetida total population change in used engine oil-contaminated soils (p<0.05) (absolute uncertainty value is provided with 95% certainty). Figures from 1 to 12 correspond to test 1 samples from table 2.

4.2. Test 2. Total population of E. andrei

Test 2 (Fig. 2). Californian earthworm demonstrated high resistance towards engine oil contamination of soil. 100% survival of E. andrei was registered when exposed to concentration of used oil ranging from 20 to 60 g/kg. Amendment with microbiologic product at oil concentration more than 50g/kg resulted in Californian worm survival decrease till 46 - 70%. In control sample total population was 78 species/container, and after addition of microbiologic product it rose till 275 species/container. In the sample with oil concentration of 20g/kg, the total population increased 6.7 times, and 3.8 times at microbiologic product amendment. At oil concentration of 40g/kg total population increased three-fold both in microbiologic product treated soil and in untreated one. Under concentration of 60 - 100g oil/kg soil, total population rose 1.5 - 3 times, both with microbiologic product and without it (table 3).

Table 3. Survival, total population, total productivity and individual productivity of earthworms Eisenia andrei under different used oil concentrations in soil

	Test number	Survival %	Total population	Total productivity	Individual productivity
1	control	100	78	17.1	1.6
2	Microbiologic matter	100	275.6	16.66	1.7
3	oil concentration 20 g/kg	100	67.33	18.33	1.61
4	oil concentration 20 g/kg and	100	38.33	16.66	0.49

	microbiologic product				
5	oil concentration 40 g/kg	100	31.66	15.00	1.36
6	oil concentration 40 g/kg and microbiologic product	100	38.66	17.33	1.62
7	oil concentration 60 g/kg	90	18.00	7.00	1.05
8	oil concentration 60 g/kg and microbiologic product	66	17.33	5.33	0.61
9	oil concentration 80 g/kg	90	29.66	13.33	0.35
10	oil concentration 80 g/kg and microbiologic product	46	27.33	11.66	0.23
11	oil concentration 100 g/kg	70	15.00	7.00	1.4
12	oil concentration 100 g/kg and microbiologic product	70	20.66	8.00	1.09
	ANOVA on ranks and Kendall's concordance	-	0.81	0.73	0.67

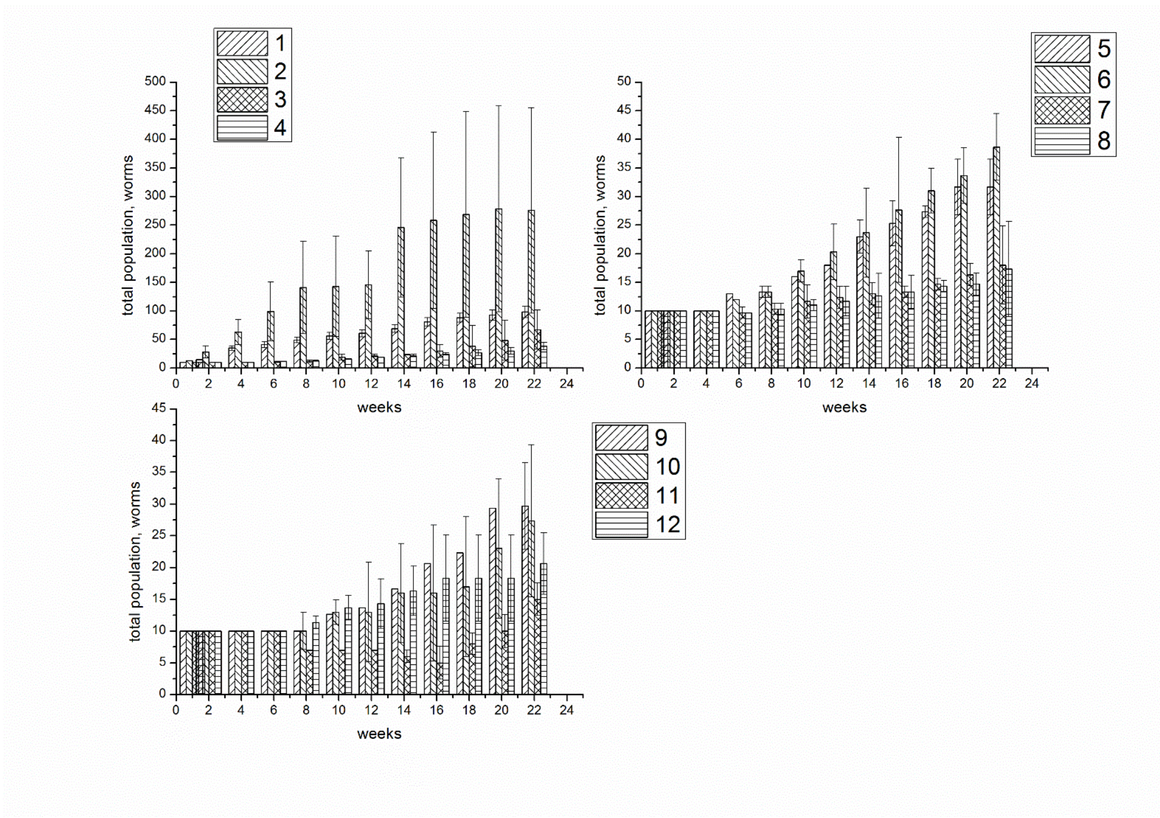

Fig. 2. E. andrei total population change in used engine oil-contaminated soils ($p<0.05$) (absolute uncertainty value is provided with 95% certainty). Figures from 1 to 12 correspond to test 1 samples from table 3.

4.3. Test 3. Total population of Dendrobena veneta

Test 3 (Fig. 3). High survival of Dendrobena veneta (86 - 100%) is recorded in control sample, in sample with microbiologic product and with low oil concentration of 20 - 40 g/kg. The increase in oil concentration till 60 - 100% resulted in decreased survivability of 53 - 66%. Total population was 45 species/container in control sample, and 94 species/container at amendment with microbiologic product. In the sample with oil contamination of 20 g/kg, total population increased 6-fold and was 60 species/container, after microbiologic product addition the total population rose 2.7 times and was 27 species/container. At oil concentration of 40g/kg, total population was 2.6 times higher, and 1.5 times higher after microbiologic product amendment. Upon exposure to oil concentration of 60 g/kg, total population multiplied 1.5 times, but remained unchanged after treatment with microbiologic product. In the samples with oil-concentration of 80 - 100 g/kg worm survival and total population decrease was recorded (Table 4).

Table 4. Survival, total population, total productivity and individual productivity of earthworms Dendrobena veneta under different used oil concentrations in soil.

	Test number	Survival %	Total population	Total productivity	Individual productivity
1	control	100	45.00	18.33	1.5
2	Microbiologic matter	100	94.00	20.66	1.3
3	oil concentration 20 g/kg	90	60.33	18.33	2.14
4	oil concentration 20 g/kg and microbiologic product	96	27.00	10.66	1.25
5	oil concentration 40 g/kg	80	26.66	7.33	0.84
6	oil concentration 40 g/kg and microbiologic product	86	15.66	5.33	0.66
7	oil concentration 60 g/kg	80	15.00	2.33	0.61
8	oil concentration 60 g/kg and microbiologic product	66	10.00	1	0.35
9	oil concentration 80 g/kg	70	5.00	5	0.23
10	oil concentration 80 g/kg and microbiologic product	66	13.00	0.00	0.75
11	oil concentration 100 g/kg	60	6.00	0	0
12	oil concentration 100 g/kg and microbiologic product	53	11.00	3.66	0.68
	ANOVA on ranks and Kendall's concordance	-	0.67	0.72	0.56

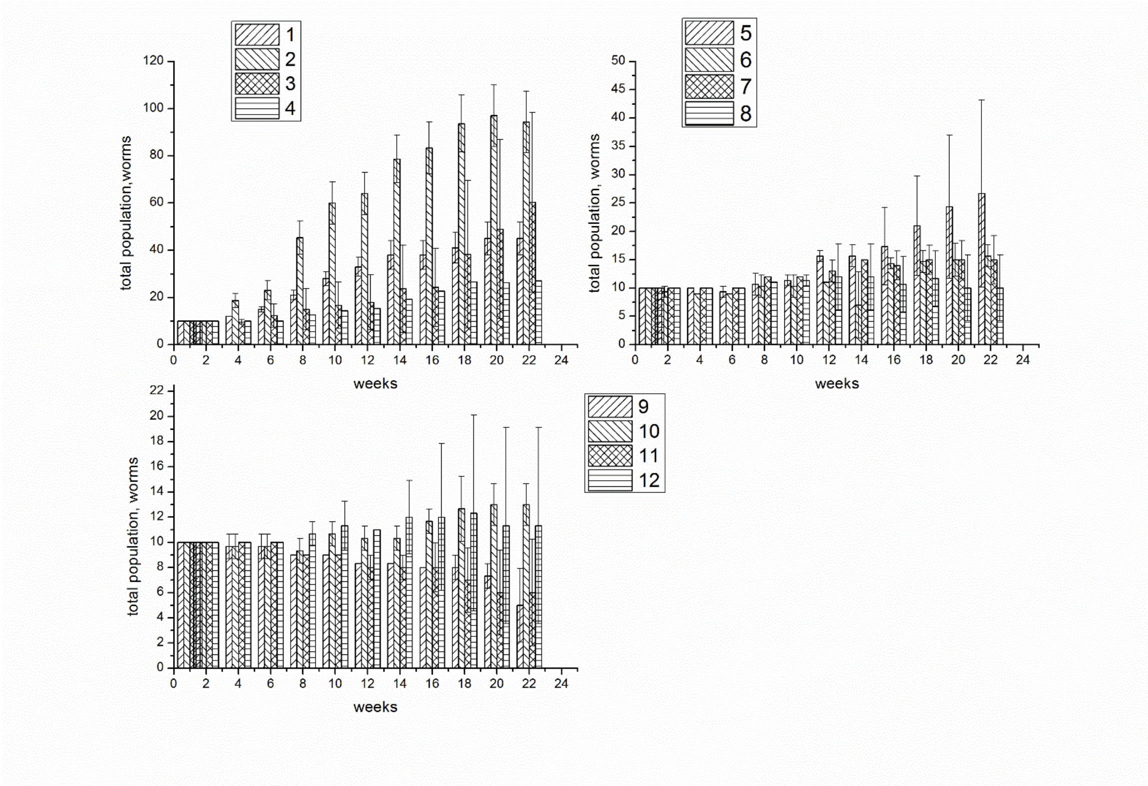

Fig. 3. D. veneta total population change in used engine oil-contaminated soils ($p<0.05$) (absolute uncertainty value is provided with 95% certainty). Figures from 1 to 12 correspond to test 1 samples from table 4.

5. Engine oil petrochemicals remediation

Fig. 4a-d show the dependence of oil content changing in contaminated-soil samples with earthworms E. ferida and bioproduct Baykal-EM since the incubation time at the temperature of 15 - 17 °C. Maximum incubation period was 4 months for all samples. Hydrocarbons content was determined with colorimetric method monthly. To analyze oil content change during incubation period due to light hydrocarbons natural evaporation within 4 months, control samples of contaminated soils with hydrocarbons amount of 20, 60, 80, and 100 g/kg (without worms) were prepared and investigated. Four control samples with oil content of 20 - 100 g/kg and bioproduct Baykal-EM content of 1ml/kg (without worms) were prepared as well.

Fig. 4a shows that at engine oil concentration of 20 g/kg hydrocarbons content in control petroleum-contaminated samples changes only slightly throughout the whole incubation period. In samples with oil concentration of 20 g/kg with worms, soil bioremediation proceeds more actively. Petroleum hydrocarbons concentration in soil samples with worms (Fig. 4a) decreased by 97 - 99% after 4 months. Introduction of bioproduct Baykal-EM in samples with 20 g/kg concentration had no effect on soil bioremediation process. In oil-contaminated samples with concentration of 20 g/kg, the most effective remediation proved to be by Dendrobena veneta with 99.9%, followed by 96% of E. fetida and 72% of E. andrei.

As Fig. 4a shows, in concentration of 60 g engine oil/kg soil, hydrocarbons content in control contaminated samples differs slightly during all incubation period. More intensive remediation of hydrocarbons results from introduction of bioproduct Baykal-EM into the samples. In control sample with 60g/kg concentration (Fig. 4b) and product Baykal-EM, oil content decreased almost 1.5 times after 4 months of incubation and equaled to 42 g/kg. With oil concentration of 60 g/kg, the most effective remediation proved to be by Dendrobena veneta with 95%, followed by 92% of E. fetida and 70 % of E. andrei. With microbiologic product amendment, the best remediation results were with the use of E. fetida - 99%.

Fig. 4c demonstrates that at engine oil concentrations of 80g/kg hydrocarbons content in the control contaminated samples differs slightly during the whole incubation period. Amendment with bioproduct Baykal-EM results in more intensive remediation of hydrocarbons (by 30%), oil content decreasing almost 1.5 times and being 54 g/kg. With oil concentration of 80 g/kg, the most efficient remediation was recorded to be of Dendrobena veneta with 94%, followed by 77% of E. fetida and 77 % of E. andrei. Amendment with microbiologic product leads to the best remediation results from E. fetida, 94%.

As Fig. 4d demonstrates, at engine oil concentrations of 100g/kg, hydrocarbons content in the control contaminated samples differs slightly during the whole incubation period. Introduction of bioproduct Baykal-EM into the samples results in more intensive remediation of hydrocarbons (by 20%) and equals to 80 g/kg. With oil concentration of 100 g/kg, the most effective remediation proved to be by E. andrei with 94%, followed by 90% of E. fetida and 80 % of Dendrobena veneta. With microbiologic product amendment, the best hydrocarbons remediation results were with the use of Dendrobena veneta, 90%.

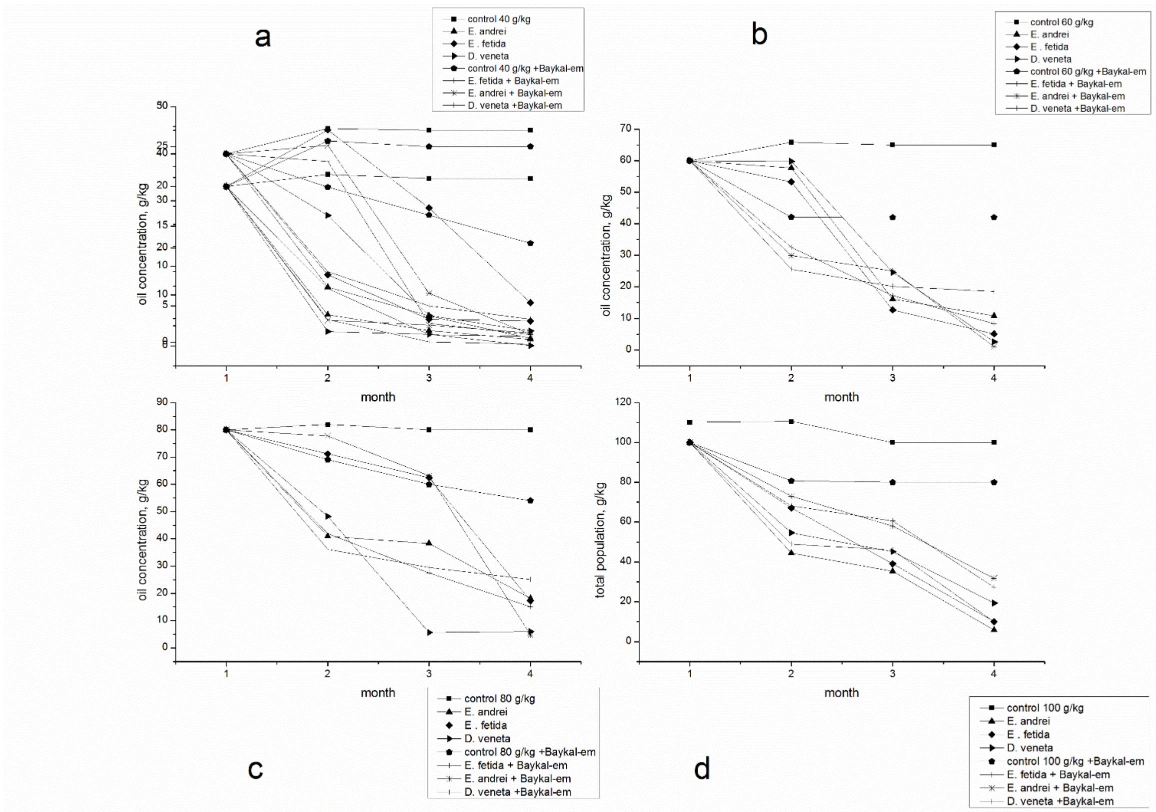

Fig. 4. Oil concentration changes during vermiremediation

6. Results and discussion

Micro-organisms can accelerate the hydrocarbons bioremediation rate [16,17]. Some authors demonstrated that PAHs removal was accelerated by the presence of E. fetida and L. rubellus [18, 19]. Nevertheless, PAHs bioremediation rates differ greatly and depend not only on the polycyclic aromatic hydrocarbons structure but also on the soil structure influencing bioavailability and soil microbial communities activity [20]. Our research recorded high survival of earthworms at high oil concentrations to 100 g/kg (50 - 70%), probably resulting from soil substrate composition, high nitrogen content 150 mg/l, phosphorous 270 mg/l, potassium 300 mg/l. Microbiologic product introduction affected survival and reproductive potential of Eisenia fetida; Eisenia andrei, and Dendrobena veneta significally, resulting in decreased survival and reproductive potential of earthworms.

Our findings indicated high survival of earthworms (to 100%) at used oil low concentrations (to 50 g/kg). Low oil concentrations of 20-40 g/kg have enhancing effect on all earthworm species increasing the hatching of cocoons. Microbiologic product introduction decreased survival and reproductive potential of E. fetida to 50%, E. andrei to 70% and D. veneta to 53%.

The most resistant to engine oil contamination appeared to be E. andrei. Three-fold increase of population was recorded upon exposure to different oil concentrations. With oil concentration from 20 to 80 g/kg E. andrei survival was 100% and earthworm population growth was steady. At oil concentration of 100g/kg survival equaled to 70%.

When exposed to concentration of used oil ranging from 20 to 100g/kg, E. fetida survival was 80 - 100% and earthworm population growth was steady, while survival dropped to 50 - 60% when microbiologic product was added at high oil concentration more than 50g/kg. Upon exposure to oil concentration of 60-100 g/kg total population increased three-fold, and microbiologic product introduction resulted in 50% mortality of mature adult worms. Micro-organisms were likely to facilitate the oil adsorption in digestive track and enhance the toxic effect.

With oil concentration from 20 to 60 g/kg, 80 - 90 % survival of Dendrobena veneta and steady population growth were reported, while at 80 - 100 g oil/kg soil the survival equaled to 40 - 50%. Oil concentration increase to 60 - 100% resulted in survival decrease till 53 - 66%.

7. Conclusion

Oil remediation efficiency and rate depended on its concentration in soil. With low used oil concentrations of 20 - 40 g/kg remediation process took 4 months, with hydrocarbons content decreasing by 97 - 99%, as a result. Microbiologic product introduction had a significant effect on oil remediation process. During vermiremediation oil content decreased by 60 - 90%. The most resistant to soil contamination with used engine oil proved to be E. andrei. Different oil concentrations resulted in significant population growth (3-fold). The best oil hydrocarbons bioremediation results were in tests with Dendrobena veneta and Eisenia fetida.

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