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Assessment of ecotoxicological properties of oils in water

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Abstract: The paper deals with the influence of oils on surface water pollution. Two mineral oils of petroleum origin, one synthetic oil and one vegetable oil were tested. Properties of the selected oil types were assessed by ecotoxicological tests. The acute toxicity test on *Daphnia magna* showed that out of the tested oils the most toxic for these aquatic organisms were the petroleum oils. In the phytotoxicity test on *Sinapis alba*, the toxic effect of mineral oils in comparison with synthetic and vegetable oil was more significant. Oils create oil stains visible under the microscope. It was difficult to differentiate them from *Scenedesmus subspicatus*. The calculation of growth rate was not relevant and a significant loss of cells was detected. It follows from the summary of the tests results that vegetable oil is the least aggressive for the aquatic environment and there are no significant differences between synthetic and mineral oils.

Introduction

Petrochemical products are very often the cause surface and groundwater quality deterioration. Pollution can be one--time (crash) or recurrent and the consequences may occur immediately or only after a long time. The change in physical and chemical properties of the water is substantially affected by the petroleum substances which form an oil film on the surface, thereby reducing the transfer of oxygen into water. Another process, in which oxygen in water is depleted, are microbial processes. The result of both processes is a change of dissolved oxygen concentration, which brings about change during chemical reactions in the photosynthesis process. It is very unfavourable to the life of aquatic organisms; algae and other plankton are strongly affected (Hybská et al. 2015, Enujiugha and Nwanna 2004, Rasulov et al. 2017).

Many mineral oil constituents are considered to be carcinogenic. Therefore it is important to know the locus and the persistence of these substances in nature (Shogren et al. 2004). The acceptable concentration limit of these compounds can be determined by bio-tests on suitably selected susceptible organisms. Ecotoxicological studies have highlighted the impact of harmful effects of chemicals on living organisms (Saval 2000) and are one of the main means of assessing the effects of specific chemical compounds on environmental compartments. For acute and chronic toxicity tests, test organisms such as fish, daphnia, rats, birds and seeds are appropriate (Mariano 2007). Due to their low price and good germination, seeds of suitable plants are important toxicological tests to assess the effects of toxic substances or organic inhibitors (Lopes et al. 2010). Tamada et al. (2012) monitored biodegradability using the respirometric tests and compared different toxicity levels of lubricating oils through the toxicological tests. The tests were carried out with *Eisenia andrei, Eruca sativa* seeds and *Lactuca sativa* seeds in mineral, synthetic and used lubricating oils for various periods of their biodegradation in soil. The toxicity tests measured indirectly the biodegradation of the contaminants. The used lubricating oil proved to be the most toxic. Mineral and synthetic oils were effectively metabolised in soil, even though they were toxic after 180 days (Tamada et al. (2012)). In their work, Cecutti and Agius (2008) presented the results of the study in which they successfully applied test organisms such as algae, daphnia and fish to assess ecotoxicological properties of different oils, including bio-oils – new and after 1,000 hours of use – in the aquatic environment. Bordulina et al. (2011) reported the results of the laboratory experiments where they studied the effect of different oil concentrations on *Chlorella vulgaris beijer* and *Daphnia magna* Str. in model samples in the aquatic environment. They found out that with increasing oil concentration the survival of test organisms decreased. Martinez et al. (2005) discovered the acute toxic damage to daffodils after 48 hours of testing due to the contamination of water with petroleum substances.

This article deals with the assessment of the effect of oils as contaminants in water using bioassays. It assesses the impact of the origin (mineral, vegetable and petroleum) oils as contaminants in water on test organisms.

Material and methods

Preparation of experimental samples

The ecotoxicological properties of oils were examined in model samples prepared in the laboratory. The tests were performed using contaminated water samples, which were prepared in the laboratory by contaminating the surface water with selected types of oils: HLP 46 (synthetic hydraulic oil), OT HP 3 (mineral hydraulic oil), BIOPLUS (vegetable oil used to lubricate chainsaws), and TIRMAN (engine mineral oil). All four oils are slightly soluble in water and their selected physical and chemical properties are presented in Tab. 1.

The concentration of oils in individual samples was 1 g of oil/litre of water. The surface water for the model samples was from a flowing natural stream Kováčová, because the level of non-polar extractable substances (NES) in this surface water did not exceed the value of 0.1 mg/L (Regulation of the Government of the Slovak Republic No. 269/2010 Coll.).

Ecotoxicological tests

To determine the ecotoxicological properties of the oils as surface water contaminants in the models, the following bio-tests were used:

- terrestrial phytotoxicity test with producer: test of the growth inhibition of the root of *Sinapis alba*;
- aquatic tests:
  - test with consumer: the acute toxicity test on *Daphnia magna*,
  - test with producer: test of the growth inhibition of *Scenedesmus subspicatus*.

All performed tests were static tests. In all ecotoxicological tests the preliminary test was carried out as the first one with the oil concentration 1 g/L in water. After its evaluation, the definitive test with a concentration dilution series was performed. Before their use in the tests, the model samples were mixed at ,.000 rpm for 5 minutes (Laboratory Magnetic Mixer VKH RH-KT/C, IKA Labortechnik, Germany). Conversion of Daphnia, the state of algal culture and suitability of seed plants, as well as meeting the conditions for selected tests, were continuously verified on a reference substance (potassium dichromate).

Growth inhibition test of Sinapis alba root

The effect of the prepared samples on germination of seeds and the growth of roots of *Sinapis alba* in the initial development phases was tested. The test consisted of the cultivation of seeds at the bottom of Petri dish, using filter papers soaked in the solutions of the sample under test and in comparison with the control (seeds growing on a pad soaked in reconstituted water) (STN 83 8303). The reconstituted water was prepared from the solutions, as shown in Tab. 2, namely by pipetting 10 ml of each of solutions 1–4 up to the volume of 1 litre, and was used as a control.

The seeds of Sinapis alba were obtained from the Central Control and Testing Institute in Agriculture. Germination was set by a certified method in the Department of Seeds and Planting Materials – certified laboratory. The determined germination was 96 %.

Inhibition (stimulation) Ii of the growth of root of higher plants should be calculated using the equation:

$$I_i = \frac{L_k - L_v}{L_k}.100,$$

where L_v is the average length of root in the tested concentration of aqueous leachate in cm, L_k is the average length of root in control in cm.

Acute toxicity test on Daphnia magna

The most frequently used test with the primary consumer is the test of acute toxicity – inhibition of movability (immobilisation) of *Daphnia magna*, where the concentration of immobilisation of exposed individuals was determined (Tab. 4). *Daphnia magna* Straus, from at least the third generation, was obtained by acyclic partogenogenesis from laboratory breeding (STN EN ISO 6341, OECD 202).

Growth inhibition test of Scenedesmus subspicatus

Chlorococcal alga *Scenedesmus subspicatus*, which belongs to the class of *Chlorophyceae*, was used as the test organism. The Z-Medium was used as the nutrient solution (Table 5–7). 10 ml

Table 1. Basic physical and chemical properties of oils

		HLP 46	BIOPLUS	OT HP3	TIRMAN
Kinematic viscosity at 40° C	mm²/s	46.0	37.0	31.2	15.2
Density at 15 °C	kg/m	875	920	865	783

Table 2. Stock solution for the preparation of reconstituted water.

Stock solution	Chemical substance	Concentration (g/L)
1	$CaCl_2 \cdot 2H_2O$, p.a.	117.6
2	$MgSO_4 \cdot 7H2O$, p.a.	49.3
3	$NaHCO_3$, p.a.	25.9
4	KCl, p.a.	2.3

of first six stock solutions were added into 900 ml of dH_2O. Then 1 ml of rest solutions was added. The final pH should be 6.6 (Andersen 2005, Zehnder and Staub 1961). It was prepared in accordance with the instructions from its supplier (Culture Collection of Autotrophic Organisms – CCALA, Třeboň, the Czech Republic). The Bürker chamber and a microscope OLYMPUS BX 40 (STN EN ISO 8692, OECD 201) were used to count the number of cells.

Evaluation of results

The software STATISTICA (Version 10, StatSoft Company, Tulsa, USA) was used for processing the results. Averages and their standard deviations (SD) were calculated from the selected data sets in the extent of 6 measurements-repetitions.

Results and discusion

Ecotoxicological tests can be characterised as experimental (usually laboratory) methods for determining toxic effects of stressors (toxic substances) on natural organisms (Fargašová 2009).

Assessment of the growth inhibition test of Sinapis alba root

After completion of the preliminary test with 1 g of oil L^{-1} in water (after 72 hours of incubation and 4 repetitions), the length of all grown roots was measured and the growth inhibition (IC) was calculated in comparison with the control. It was found from the preliminary test that the highest inhibition occurred in the sample of surface water contaminated with the synthetic hydraulic oil HLP 46 and mineral oil TIRMAN. There was no significant difference between the set values in the samples with oil BIOPLUS and OT HP3. From the results of the preliminary test (inhibition of root growth ≥ 30% and < 50% inhibition), (STN 83 8303) further testing was not necessarily required, but due to the physical and chemical properties of the oils (water insolubility, density and viscosity) and creating oil film on the surface, the definitive test was also conducted. In the definitive test, dilutions of samples were set in the range from 0.1 to 1 g of oil in 1 litre of water. The results from the definitive test are shown in Tab. 6. Averages and their standard deviations (SD) were calculated from the selected data sets in the extent of 6 measurements-repetitions.

Table 3. Conditions for the test of *Sinapis alba* growth inhibition

Test organism	*Sinapis alba*, ochre yellow, size 1.5–2 mm, 30 seeds in Petri dish, 10 ml sample, 72 hours – exposure period
Temperature	20°C ± 1°C, incubator TS 606 CZ/2-Var (WTW, Germany)
Control sample	reconstituted water
Validity of the test	germination in control sample = 97.8 % (limit ≥ 90%)
Reference substance	$K_2Cr_2O_7$, $IC_{50,\ 72hours}$ = 32.50 mg/L (limit 4.1–85 mg/L)
Measuring root length	Steel calibrated measuring instrument
Preliminary test	30 seeds on the filter paper soaked in 10 ml of the undiluted sample/under the same control (reconstituted water) conditions
Definitive test	30 seeds on filter paper soaked in 10 ml of ascending concentration sample/under the same control (reconstituted water) conditions
Monitored response	root growth inhibition compared to control, IC

Table 4. Acute toxicity test conditions for *Daphnia magna*

Test organism	*Daphnia magna* Straus (more than the third generation obtained by acyclic parthenogenesis under the conditions of healthy breeding), individuals younger than 24 hours since birth (no feeding).
Incubation temperature	20°C ± 2°C
Control sample	reconstituted water
Number of replications	6
Reference substance	$K_2Cr_2O_7$, $EC_{50,\ 48hours}$ = 0.62 mg/L (limit 0.3–1.5 mg/L)
Preliminary test	10 ml – sample volume, 20 daphnia in an undiluted sample and simultaneously under control (reconstituted water) conditions
Definitive test	20 daphnia in an ascending concentration sample and simultaneously under control (reconstituted water) conditions
Test duration	48 h
Validity of the test	% of immobilised individuals in control = 5% (limit ≤ 10 %)
Monitored response	% of immobilised* individuals compared to the control, inhibition of immobilisation (EC) compared to the control

Note: * macroscopically observed disability of individual spatial mobility of Daphnia within the period of 15 seconds after stirring the sample; immobilised individuals are the ones which move using the feelers of the second pair, but are not able to perform this movement.

In all model samples IC_{50} was > 1g of oil/ L in water (Tab. 9). The effect of oil concentration was significant only in the samples with vegetable oil BIOPLUS and mineral oil TIRMAN. This emerges from the IC values at the concentration of 0.1 g oil L^{-1} in water. There is no significant effect of concentration of mineral oil OT HP3 and synthetic oil HLP 46. The results in this test and in the concentration range are influenced only partially by the origin of the oil.

Assessment of the acute toxicity test on Daphnia magna

The result of the preliminary test for vegetable oil BIOPLUS as water pollutant was negative (STN EN ISO 6341, OECD 202).The value of immobilisation was 10% (Tab. 10) and further testing was not needed. Other samples were positive and during the test there was a death rate or immobilisation ≥ 50% of *Daphnia magna* in comparison with the control (STN EN ISO 6341, OECD 202) and therefore the definitive test was performed. From counted individuals with unchanged vital functions (48 hours after the start of the test), percentage of immobilisation was calculated in individual water samples.

The results of *Daphnia magna* immobilisation (EC) are shown in Tab. 10.

Inhibitions (EC_{50}), assessed by the regression analysis, are shown in Tab. 11. The synthetic oil HLP 46 was the most toxic to the test organisms: only 0.03 g of oil/L in water was

Table 5. Z – Medium

Composition	Stock solution (g/l dH_2O)	Used volume (to 1 litre)
$NaNO_3$	46.7	10 ml
$Ca(NO_3)_2.4H_2O$	5.9	10 ml
K_2HPO_4	3.1	10 ml
$MgSO_4.7H_2O$	2.5	10 ml
Na_2CO_3	2.1	10 ml
Fe-EDTA solution	Tab. 6	0.2 ml
Trace metals solution	Tab. 7	0.08 ml

Table 6. Fe-EDTA solution

Composition	Used volumes
HCl (35%)	2.2 ml
dH_2O	250 ml
$FeCl_3.6H_2O$	4.5 g
Na_2EDTA	4.65 g

Table 7. Trace metals solution

Composition	Used volumes (to 100 ml)
H_3BO_3	0.31 g
$MnSO_4.4H_2O$	0.223 g
$Na_2WO_4.2H_2O$	0.003 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0088 g
KBr	0.0119 g
KI	0.0083 g
$ZnSO_4.7H_2O$	0.0287 g
$Cd(NO_3)_2.4H_2O$	0.0154 g
$Co(NO_3)_2.6H_2O$	0.0146 g
$CuSO_4.5H_2O$	0.0125 g
$NiSO_4(NH_4)_2SO_4.6H_2O$	0.0198 g
$Cr(NO_3)_2.7H_2O$	0.0037 g
$V_2O_4(SO_4)_3.16H_2O$	0.0035 g
$Al_2(SO_4)_3K_2SO_4.24\ H_2O$	0.0474 g

Table 8. Conditions for the *Scenedesmus subspicatus* growth inhibition test

Test organism	*Scenedesmus subspicatus*
Sample volume	50–70 ml
Incubation temperature	25°C ± 2°C, thermostatic cabinets ST FOT (Poland)
Lighting and exposure	continually, min. intensity of 5 000 lux, min. 72 hours
Initial concentration of algal culture	min. 10^3 cells in 1 ml
Control sample	Z – medium (10%) and deionized water (90%)
Reference substance	$K_2Cr_2O_7$, $IC_{50,\ 72hours}$ = 1.3 mg/L (limit 0.5–2.0 mg/L)
Preliminary test	undiluted sample with the addition of inoculum (aliquot nutrient suspension and simultaneously under control conditions)
Definitive test	gradually increasing the concentration of the sample with the addition of the inoculum and simultaneously under control conditions
Monitored parameters	the growth of algal culture (cell counting) is done every 24 hours, growth rate (μ), growth inhibition (IC)
Other conditions	no aeration, mixing of algae suspension at least 3 times a day, maintenance, adaptation and cultivation of algal culture

enough to cause immobilisation of 50% of individuals (EC_{50}). Mineral oil TIRMAN with an EC_{50} value of 0.31 g oil/L in water also had a significant toxic effect on *Daphnia magna*. This corresponds to the findings published by Cecutti and Agius (2008), who found that toxicity levels of bio-oils (such as vegetable oil) were not higher in comparison with the liquid contaminated by the oil – based sample (EC_{50} in samples with mineral oil was 5.450 g/L and in bio-oil < 10 g/L).

Bordulina et al. (2011) confirmed that with increasing concentration of oil in water, the survival rate of *Daphnia magna* decreased. They found out that at the concentration of 0.2 mg of oil/L, irrespective of its origin (mineral and bio--oil – sunflower oil), there was no difference in the number of immobilised individuals.

Oil affects *Daphnia magna* to a certain extent by the presence of toxic substances dissolved in water but mainly by forming a film on water surface. The organisms are coated with a layer of the oil and this prevents them from respiring. This is confirmed by Bejarano et al. (2014) in their article, where they dealt with the size of the dispersed droplets of oil in water and observed their effect on *Daphnia magna*. The effect of oil droplets on the toxicity of the aquatic environment has not been examined to a large extent, as reported by Redman et al. (2017). They performed acute toxicity tests on *Daphnia magna* and the results indicated that oil droplets in the aquatic environment do not contribute significantly to the toxicity.

Assessment of the growth inhibition test on Scenedesmus subspicatus

Results of the growth inhibition of *Scenedesmus subspicatus* in the preliminary test were positive (growth inhibition of the algal culture was ≥ 30 % in comparison with the control), (STN EN ISO 8692, OECD 201) and based on this, the definitive test was performed. All the results showed high inhibition irrespective of the origin of the contaminant (Tab. 12).

Oils are almost insoluble in water and create oil stains visible under the microscope. It was difficult to differentiate them from unicellular organisms (*Scenedesmus subspicatus*) or their colonies and it was complicated to count the exact number of cells in the Bürker chamber under the microscope. The calculation of growth rate was not relevant and a significant loss of cells was detected. It is assumed that low growth rate of algae cells is probably caused by adhesion of oil to algae cells, bringing about low growth rate and preventing photosynthesis, as presented by Borodulina et al. (2011). Ramadass et al. (2015) examined the toxicity of motor oil on the growth and antioxidant enzyme microalga, *Pseudokirchneriella subcapitata*, and found that contamination of water systems with oil could potentially affect the health of ecosystems by disrupting primary producers at the base of the food chain.

Forth et al. (2017) tested the toxicity of petroleum (mineral) oil in water samples using the standard aquatic toxicity tests and observed changes in the size of oil droplets. The average

Table 9. Results of the definitive test of the root growth and inhibition (IC) of the root of *Sinapis alba*

Conc. [g/L]	HLP 46		OT HP3		BIOPLUS		TIRMAN	
	Root growth [cm]	ICAverage [%]±SD	Root growth [cm]	ICAverage [%]±SD	Root growth [cm]	ICAverage [%]±SD	Root growth [cm]	ICAverage [%]±SD
0.1	3.6	30.8±0.82	3.9	25.0±1.05	4.3	17.3±1.30	4.2	19.9±2.24
0.2	3.6	30.8±1.00	3.8	26.9±1.72	4.2	19.2±2.47	3.7	28.9±3.49
0.5	3.5	32.7±0.31	3.6	30.8±3.27	3.6	30.8±3.37	3.5	32.7±4.37
0.8	3.3	36.5±0.27	3.6	30.8±3.75	3.6	30.8±3.42	3.4	34.6±3.90
1	3.1	40.4±1.40	3.5	32.7±0.38	3.5	32.7±0.67	3.3	36.5±2.82
Control	5.2							

Table 10. Results of the preliminary (*) and definitive tests of acute toxicity (EC) on *Daphnia magna*

	Control	HLP 46					OT HP 3					BIOPLUS	TIRMAN				
Conc. [g/L]	–	0.01	0.05	0.1	0.2	1*	0.1	0.2	0.5	0.8	1*	1*	0.1	0.2	0.5	0.8	1*
Immobil. individuals Number	1	7	13	14	19	20	3	5	6	6	8	2	6	9	14	17	18
EC [%]	5	35	65	70	95	100	15	25	30	30	40	10	30	45	70	85	90

Table 11. Calculated values for EC_{50}

	HLP 46	OT HP 3	BIOPLUS	TIRMAN
Conc. [g/L]	0.03	> 1	> 1	0.31

Table 12. Data recorded from the growth inhibition test on *Scenedesmus subspicatus*

Sample	Concentration [g/L]	Number of cells in 1 ml × 103 Start of the test	Number of cells in 1 ml × 103 End of the test	ICAverage [%]±SD
HLP 46	1.0	10.00	5	70.15±4.2
	0.8	9.50	7.5	55.22±1.4
	0.5	9.75	9.5	43.28±4.6
OT HP 3	1.0	10.75	3.75	77.61±4.1
	0.8	10.50	6.25	62.69±1.1
	0.5	10.25	7.25	56.72±1.4
BIOPLUS	1.0	10,00	6	64.18±1.2
	0.8	9.50	7.25	56.72±3.8
	0.5	10.50	7.75	53.73±1.5
TIRMAN	1.0	10.50	3.5	79.10±5.1
	0.8	10.50	4.75	71.64±4.4
	0.5	10.25	8.25	49.75±2.1
Control	–	10.25	16.75	–

droplet size at the beginning of the test was 5–10 µm (a few were > 30 µm) and after 96 hours of testing, that size was reduced to 3–5 µm. This data provides significant information for measurements, which were important for determining the effects of oils on the aquatic organisms that were used in these aquatic tests.

Conclusion

Results for the ecotoxicological properties of these oils obtained here help with the completion of this missing data and at the same time verify the safety of these oils, for example during economic activities in protected areas. The use of an ecotoxicological approach has added value to the assessment of risks owing to contamination of surface water by anthropogenic activities. This tool can be advantageously used as a part of environmental management. Ecotoxicological tests identify danger and can be used for ecological risks assessment. The results in safety data sheets for tested oils are absent.

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