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Study of the Regeneration Cleaning of Used Mineral Oils – Ecotoxicological Properties and Biodegradation

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The aim of the study was to establish and compare the model of the biodegradability and ecotoxicological properties of oil samples in aqueous environment. The unused new mineral oil Turbinol and used (after 1 year of usage) recovered oil Turbinol purified by the electrostatical method were the tested samples. For the determination of the ecotoxicological properties, the test organisms used were seeds of *Sinapis alba* L. and the small aquatic crustaceans *Daphnia magna*. Preliminary tests were positive and determined the acute toxicity with the values of IC_{50} and EC_{50}. Biodegradability was determined by the manometric method, in tests which lasted 28 days. Tests of toxicity were positive, and the samples were found to be hard to biodegrade. Determination of the oil composition by gas chromatography with mass detection (GC – MS); found that the composition of the electrostatically cleaned oil is comparable to the new oil, which is confirmed by the results obtained with the response inhibition in selected tests. Regeneration extends the oil life, reducing the cost of disposal of waste oils, saving fossil raw materials, thus belonging to the environmentally friendly techniques.

Key words:
mineral oil, electrostatical method, biodegradation, ecotoxicity, gas chromatography

Introduction

Mineral oils are applied in almost every industry. They are substances of petroleum origin formed by mixture of higher hydrocarbons with the addition of special substances. The release of these substances is dangerous for all components of the environment. Moreover, the pollution of air, soil, or water may occur by leakage from equipment or mishandling.[1,2]

From such perspective, the largest contamination caused by mineral oils can be observed in industry and transport. They are used in machines, engines, vehicles, hydraulic and transmission systems, and so on, where they have the secondary function of providing lubrication and cooling.[3,4] Generally, mineral oil is affected by processes of mechanical stress, great changes in temperature and other physical and chemical processes, which may result in changes in their composition and properties. Petroleum is a non-renewable resource and therefore, research and development activities focus on the re-use of previously used oil products, which would otherwise end up in landfills of hazardous waste. Today, we know many effective and relatively inexpensive methods for recycling and regeneration of used petroleum oils. The basic physical and chemical characteristics of mineral oil can be changed to the same level as new oils. Disposal of wastes is potentially hazardous for the environment. The disposal of oil is an expensive process requiring financial resources. The combustion of oils has a negative impact on air quality, and therefore on ecosystems, and it has significant negative impacts on human health.[5] Many components of mineral oils are considered carcinogenic, meaning that is important to know about the determination and the persistence of these substances in nature.[6] In view of the incineration of waste or other substances such as fuels, it is necessary to analyse the issues related to air pollution very carefully.[7]

The acceptable limit concentration of these compounds can be determined using suitably selected bioassays on sensitive organisms. The characteristics of xenobiotics in relation to environment can be used to assess their ecotoxicological properties. Ecotoxicological studies have shown the harmful impact of chemical substances on live organisms and environment.[8] Acute and chronic toxicity tests are the principal means of assessing their activities.

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The effects of specific chemical compounds can be evaluated by using test organisms, such as fish, daphnia, rats, birds, and seeds.[9,10] Given their low cost and good sensitivity, the germination of seeds can be used in important toxicological tests, and is also a practical source of information to assess the impact of toxic substances and organic inhibitors. This issue is addressed in the study by Lopes *et al.*[11], who evaluated the behaviour of different types of automotive lubricating oils on the environment.

Tamada *et al.*[12] investigated the biodegradability of lubricating oils through respirometric tests and toxicological tests by comparing their different levels of toxicity. The tests were performed using earthworms (*Eisenia andrei*), rocket seeds (*Eruca sativa*) and lettuce seeds (*Lactuca sativa*) in mineral, synthetic lubricating oil, investigating different periods of their biodegradation in the soil. Tests of toxicity were used for the indirect measurement of the biodegradation of the pollutants. Mineral and synthetic oils are effectively metabolized in soil, even if their toxicity has not completely disappeared after 180 days.

The behaviour of lubricating oils in the environment is a source for the development of new liquids. There has been a trend to study lubricants with a lower impact on the environment and rapid biodegradation effects, focusing on the development of synthetic and semi-synthetic lubricating oils.[13]

Cecutti and Agius[14] presented the results of their study in which they successfully implemented tests on organisms such as algae, daphnia and fish to determine the ecotoxicological properties of various oils, including new bio-oils, before use and after 1000 hours of use in an aqueous environment. They found that the fish responded well in all samples without distinction and LC_{50} after 48 hours was > 10 000 mg L^{-1}. It was the most sensitive bioassay with algae that allowed the classification and comparison of the samples. The tests with daphnia also provided excellent results. It was found that samples of mineral origin showed acceptable results in the organic products, and it was of interest that the EC_{50} in the samples with the new oil was 5450 mg L^{-1} compared with 2450 mg L^{-1} in used oil.

In the event of contamination of the aquatic environment by petroleum products from anthropogenic activities, which include the above-mentioned mineral oils, activation of the microorganisms present in the water, and their role in the degradation of toxic substances is possible when the concentration of toxic substances in the environment is below a certain level, and when there are suitable physical conditions.[15]

Biodegradability of lubricants was evaluated through respirometric tests, and toxicological tests were carried out to assess their toxicity before and after use. All the lubricants showed toxicity after biodegradation. Used car lubricants are highly toxic because of the presence of a high concentration of polycyclic aromatic hydrocarbons, which are known to be potential carcinogens.[16]

Researchers from the University of Oulu (Finland), Department of Process and Environmental Engineering, have studied biodegradable motor oil in groundwater. They used the manometric respiratory method.[17] The results demonstrated that biodegradation progresses more rapidly in a mineral-rich medium (soil samples were analyzed for the composition of micro and macroelements). The degradation process undoubtedly affects the amount of nitrogen in the soil. In the samples of groundwater, the oil caused a serious environmental risk because of its low biodegradability. From the results and comparison with a standard method, the suitability of the use of the respiratory manometric method has been demonstrated.[18]

Lopes *et al.*[11] examined the biodegradability of control (T1), synthetic (T2), mineral (T3), and used (T4) oil by applying the respirometric method. The results pointed to a downward trend in CO_2 production in Bartha and Pramer respirators in the order T4 > T2 > T3 > T1. This means that the used lubricant oil had the highest biodegradation, followed by the synthetic oil, while mineral oil had the lowest biodegradation. It was also observed that the mineral oil required an extended period of adaptation compared to synthetic oil.

In this study, we deal with the assessment of the composition, ecotoxicological properties, and biodegradability of electrostatically cleaned oil, and compare it with the unused (new) and used (worn) turbine oil Turbinol X-EP 46 in an aquatic environment.

Materials and methods

Preparation of model samples

The assays were carried out in model samples that were prepared with surface water contaminated by oil. The concentration of the oil in the water was 1 g L^{-1}. As the contaminant, the turbine mineral oil X-EP 46 was used, which consists of hydrogenated base oils and specially selected additives, providing very high oxidation stability. The oil is intended for use in turbines and associated equipment.[19] The oils used in the assay were new oil, oil used for one year in operation, and oil regenerated by the electrostatic cleaning method, using the device ELC-R6PSP by the company KLEENTEK Slovakia and the Technical University in Zvolen, Department of Forest Har-

vesting.[20] The surface water used in the assays was from a flowing natural resource and satisfied the quality requirements for surface waters under Government Regulation No. 269/2010[21] Coll. Annex 1 requirements for surface water quality. For evaluation of the results, the program STATISTICA was used. The software STATISTICA 10, ANOVA – Single Factor Dispersion Analysis was used to evaluate the results of the ecotoxicological tests. The graphical presentation of ANOVA was performed using 95 % confidence intervals for average immobilization and inhibition values for individual samples.

Principle of electrostatic cleaning

Electrostatic oil cleaning is completely different from the so far used mechanical methods of filtration, particularly in terms of the impurities from the electrical point of view. It is assumed that only three kinds of dirt exist: positively charged particles, negatively charged particles, and electrically neutral particles. The basic principle of separation of these particles uses electrostatic cleaning equipment for electrophoresis and dielectrophoresis, based on the physical principle of Coulomb's law.[22] When we let the oil flow through the electric field, specially shaped non-conductive collectors (placed between high-voltage electrodes) capture all the particles in the oil, irrespective of whether they are metallic or non-metallic, conductive or non-conductive, organic or inorganic, including water droplets. During this electrostatic cleaning, the oil becomes charged, but this uses the charge of the particles suspended in the oil. According to the particle size, the charge can be amended only to the speed of the separation of oppositely charged electrodes.[20]

Methods for determining acute ecotoxicity

For the determination of ecotoxicological properties, two bioassays were conducted according to STN EN 83 8303[23]: a test of the growth inhibition of the *Sinapis alba* root and a test of acute toxicity on daphnia (*Daphnia magna*).

Test of the growth inhibition of the *Sinapis alba* root

The test consists of the cultivation of seeds on pads soaked in solutions of the tested substance in comparison with seeds growing on a pad soaked in diluent water. Mustard is representative of cultural crops and higher plants in the toxicity tests. The test conditions are shown in Table 2.

From solutions No. 1–4 (Table 1), a dose of 10 mL was added to a volumetric flask with a capacity of 1 L and the solution was refilled with distilled (deionised) water up to the mark and used as

Table 1 – *Stock solutions for reconstituted water*

Stock solution	Chemical substance	Concentration of stock solution (g L^{-1})
1	$CaCl_2 \cdot 2H_2O$, p.a.	117.6
2	$MgSO_4 \cdot 7H_2O$, p.a.	49.3
3	$NaHCO_3$, p.a.	25.9
4	KCl, p.a.	2.3

Table 2 – *Conditions of growth inhibition test of Sinapis alba root*

Testing organism	*Sinapis alba,* germination > 90 %, per 30 seeds of *Sinapis alba* L. in Petri dishes
Sample volume	10 mL
Temperature	20 °C ± 1 °C, thermostat TS 606 CZ/2-Var (WTW, Germany).
Control	Reconstituted water (Table 1)
Validity of the test	Germination in control sample = 99.8 % (limit ≥ 90 %)
Reference substance	$K_2Cr_2O_7$, IC_{50}, 72 hours = 23.5 mg L^{-1} (limit 4.1 − 85 mg L^{-1})
Measuring root length	Steel calibrated measuring instrument
Exposure time	72 hours
Response monitored	Growth inhibition of *Sinapis alba* root compared with the control, IC_{50}

a control. The pH of the diluent water should vary around 7.6–8.0.

Inhibition (stimulation) I_i of the roots growth of higher plants is calculated using the equation I_i (%) = $(L_C − L_S/L_C) \cdot 100$ (Eq. 1), where L_S is the average length of the root in the tested concentration of aqueous leachate in cm, and L_C is the average length of the root in the control in cm.

Acute toxicity test on daphnia (*Daphnia magna*)

The test is based on monitoring the immobilization of individuals that were exposed to the samples of water contaminated with oil during 48-hour exposure each, in accordance with the conditions specified in Table 3. The diluting water for the determination of ecotoxicity is prepared by pipetting 2.5 mL from solutions 1 to 4 into a 1-litre volumetric flask and refilling with demineralised water up to the mark. The prepared diluent water must have a pH of 7.8 ± 1.2 and a concentration of dissolved oxygen greater than 7 mg L^{-1}.

Determination of aerobic biodegradation by the manometer respiratory method

According to the law of the Slovak Republic no. 67/2010 Coll. Law on conditions for placing

T a b l e 3 – *Conditions of acute toxicity test on daphnia*

Testing organisms	*Daphnia magna*
Age of organism	Daphnia younger than 24 hours
Control	Reconstituted water (Table 1)
Reference substance	$K_2Cr_2O_7$, EC_{50}, 48 hours = 0.8 mg L^{-1} (limit 0.3 – 1.5 mg L^{-1})
Sample volume	10 mL
Temperature	20 °C ± 2 °C
pH	7.8 ± 0.2
Exposure time	48 hours
Response monitored	% of the immobilized individuals compared to the control EC_{50}, pH, temperature, dissolved O_2

chemical substances and mixtures on the market and on amendments to certain acts[24], the biodegradability of substances in water sparingly soluble, as well as petroleum oil contaminants in water, is to be determined by a suitable respirometric test. Vähäoja *et al.*[25] focus on the biodegradability measurements of tall oil-based wood preservatives and their raw materials in groundwater as determined by the respirometric BOD OxiTop method. Certain substances were also analyzed in standard conditions described by OECD 301 E. The respiratory measurement is based on measuring changes in pressure in a closed sampling bottle. The laboratory equipment OxiTop®Control collects pressure values and allows further processing. The interpretation of the pressure change over time depends on the measured medium, sample preparation, and manipulation during measurement. The laboratory equipment consists of a tray with a stirrer, pressure plugs OxiTop-C, dark glass bottles, and controller OxiTop OC110. The recorded values are processed using the computer software Achat. The biodegrad-

ability of the test substance is determined as the % degradation of the dissolved oxygen in the sample after 28 days.[17]

Determination of the composition of oil samples

Determination of the composition of the oil sample was done by means of gas chromatography (GC–MS) with mass detection using the instrument AGILENT 5975C. Identification of substances consisted of comparing the mass spectra of the samples with a range of substances from the NIST spectral library. The analytical samples were adjusted by multiple extraction with methanol and hexane as the solvent.[26]

Results and discussion

Determination of the composition by GC – MS

The chromatogram in Figure 1 illustrates the individual analytes – samples in the form of chromatographic peaks (zones), where **1** is indicates used oil, **2** is new oil, and **3** is electrostatically regenerated oil. In an ideal regeneration process, undesirable substances are removed from the used mineral oil to the fullest extent possible without altering their hydrocarbon composition[27], as confirmed by this chromatogram.

Test of acute toxicity on *Daphnia magna*

For testing, we used aquatic organisms *Daphnia magna* Straus, which were laboratory animals from acyclic generations generated by parthenogenesis and bred individuals. The preliminary test of acute toxicity on daphnia was positive (positive in terms of causing death or immobilisation of ≥ 50 % of daphnias during the test when compared to the control), at which time it was necessary to specify the value of EC_{50}. In the basic test, six concentra-
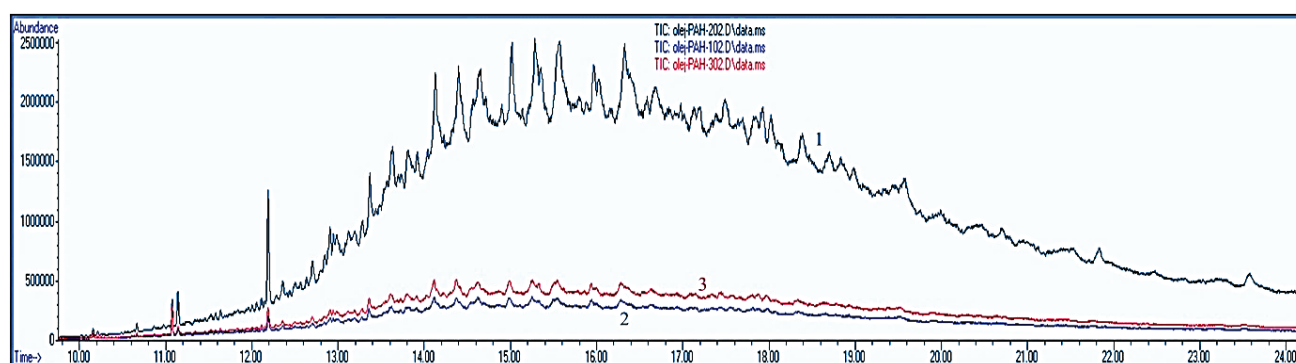

F i g . 1 – *Chromatogram (Note: 1 – used oil, 2 – new oil, 3 – oil after regeneration, axis x – Time, axis y – Abundance)*

tions were tested: 200, 40, 20, 10, 6.7, and 5 mg L^{-1} (mg of mineral oil diluted in 1 L of leachate water). Just as in the experimental samples, a control test
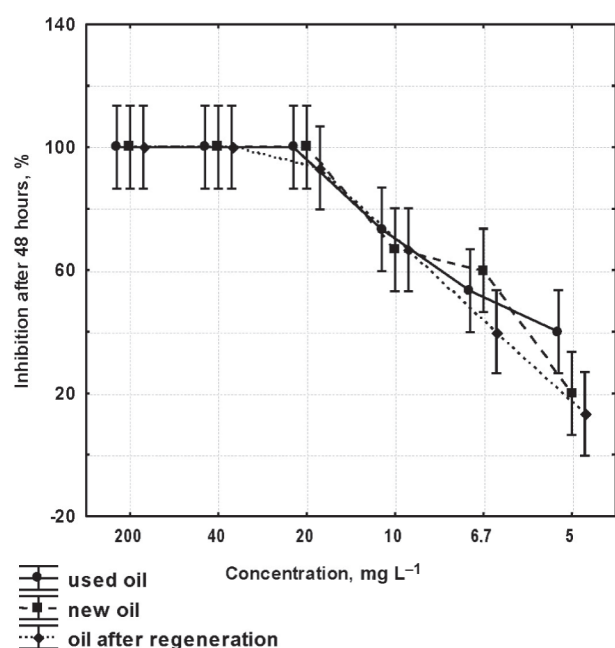

F i g . 2 – *Dependence of concentration and immobilisation after 48 hours – test of acute toxicity on Daphnia magna*

was conducted to validate the results where the test was performed with a reference substance, potassium dichromate, instead of leachate water. After the test, the percentage of immobilisation of daphnia in the samples was calculated. Figure 2 graphically illustrates the % immobilisation depending on the concentration of the samples after the 48-hour test period, and the basic statistical characteristics are shown in Table 4.

The highest percentage of immobilisation was observed in all samples with the highest tested concentrations. Regression analysis was used to calculate EC$_{50}$ for each of the oil samples. The results are presented in Table 5, showing that 50 % of individuals will be immobilised in a solution of contaminated recovered oil at a concentration of 8.17 mg L^{-1}, which is the most favourable outcome in the test samples, even if they are toxic. The high values of immobilisation are caused by the film that formed on the surface, preventing oxidation of the samples from the outside.

Growth inhibition test of cultivated *Sinapis alba* root

Firstly, a preliminary test of the *Sinapis alba* root inhibition was conducted to model surface wa-

Ta b l e 4 – *Basic statistical characteristics of acute toxicity test results of Daphnia magna*

Sample concentration (mg L^{-1})	Mineral oil Turbinol	Average EC (%)	Confidence intervals		Standard deviation
			− 95.00 %	+ 95.00 %	
200	Used	100.00	–	–	0.00
	New	100.00	–	–	0.00
	After regeneration	100.00	–	–	0.00
40	Used	100.00	–	–	0.00
	New	100.00	–	–	0,00
	After regeneration	100.00	–	–	0.00
20	Used	100.00	–	–	0.00
	New	100.00	–	–	0.00
	After regeneration	93.33	64.65	122.02	11.55
10	Used	73.33	44.65	102.02	11.55
	New	66.67	37.98	95.35	11.55
	After regeneration	66.67	37.98	95.35	11.55
6.7	Used	53.33	−22.56	129.22	30.55
	New	60.00	10.32	109.68	20.00
	After regeneration	40.00	−9.68	89.68	20.00
5	Used	40.00	–	–	0.00
	New	20.00	–	–	0.00
	After regeneration	13.33	−15.35	42.02	11.55

Table 5 – *Test of acute toxicity on Daphnia – calculation of EC_{50}*

Time	Mineral oil Turbinol	Statistical model	A	k	n	EC_{50} (mg L^{-1})
	Used	$y = A\cdot(1\text{-exp}(-(k)\cdot x^{\wedge}(n)))$	100	0.047991	1.45457	6.27
48 hours	New	$y = A\cdot(1\text{-exp}(-(k)\cdot x^{\wedge}(n)))$	100	0.019042	1.83027	7.13
	After regeneration	$y = A\cdot(1\text{-exp}(-(k)\cdot x^{\wedge}(n)))$	100	0.004064	2.44678	8.17

Note: A – inhibition at zero concentration; k – steepness of decline; n – curvature parameter
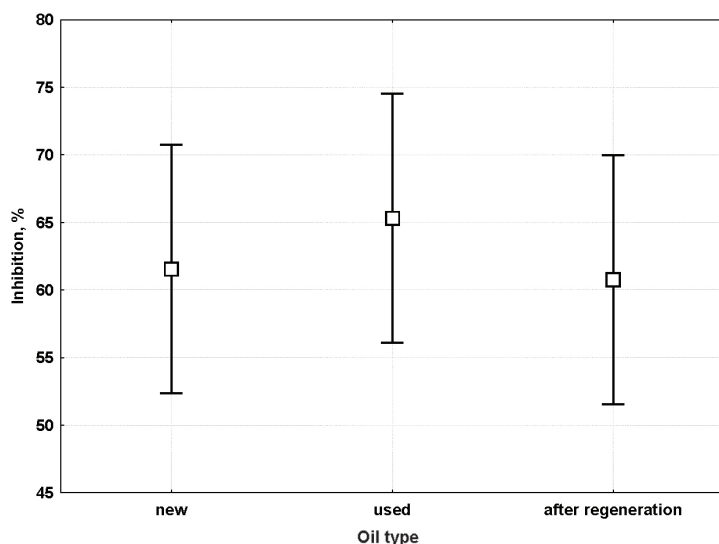

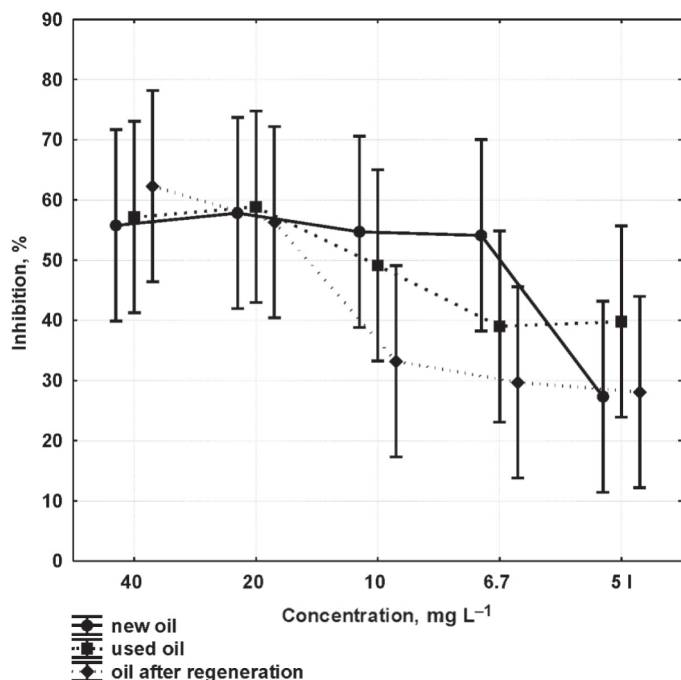F i g . 3 – *Results of the preliminary growth inhibition test of Sinapis alba root*

F i g . 4 – *Dependence of % inhibition and concentration for each type of oil*

ter contaminated with mineral oil Turbinol X-EP with a concentration of 1 g L^{-1}. The *Sinapis alba* seeds were germinated 99.8 %. After 72 hours of incubation under the conditions in Table 2, the length of the roots was measured, from which the inhibition of *Sinapis alba* root growth was calculated (Eq. 1). The results are presented in Figure 3.

The preliminary test for growth inhibition of *Sinapis alba* roots compared to the control was positive in all samples. The percentage of inhibition was greater than 30 % (65.30 % in the water sample with used oil, 61.55 % in the sample contaminated with new oil, and 60.75 % in the sample with recovered oil).

In all samples, the inhibition was greater than 50 %, and therefore, a baseline test was conducted to determine IC_{50}.[23] The basic test used the same dilution series of model samples as in the case of the acute toxicity tests with Daphnia without 200 mg L^{-1}. The test was conducted under the same conditions as the preliminary test. At the same time, a control was tested, where the sample used leachate water instead of dilution water.

The results of the evaluation are shown in Figure 4, and the basic statistical characteristics are shown in Table 6.

From Figure 4, it is apparent that there is a significant relation between the % inhibition and the quantity of contaminant (oil) in the sample. We found that a gentle film of oil formed on the surface of the *Sinapis alba* seeds in the samples. The mineral oil is almost insoluble in water and is less dense than water, which implies that this caused a significant inhibitory effect.

Regression analysis of the measured values was used to calculate the IC_{50}, from which it followed that in this test also, the most favourable outcome was observed in the sample of the regenerated oils, while the used and new oils were not significantly different and are more toxic than the regenerated oil. A graph of the analysis is shown in Figure 5.

Aerobic biodegradation with the manometric respiratory method

The biodegradability test lasted 28 days with the same concentration of oil (1 g / 1 L water) as in the case of the ecotoxicological tests. The measuring head during the entire duration of the test recorded the amount of biodegradation of the organic pollution with mineral oils. After the test, these data

Table 6 – *Basic statistical characteristics of test results of growth inhibition of Sinapis alba root*

Sample concentration (mg L^{-1})	Mineral oil Turbinol	Average inhibition (%)	Standard error	Confidence intervals	
				– 95.00 %	+ 95.00 %
40	New	55.79	8.10	39.90	71.68
	Used	57.16	8.10	41.27	73.06
	After regeneration	62.30	8.10	46.41	78.19
20	New	57.85	8.10	41.96	73.74
	Used	58.86	8.10	42.97	74.75
	After regeneration	56.32	8.10	40.43	72.21
10	New	54.71	8.10	38.82	70.60
	Used	49.12	8.10	33.23	65.01
	After regeneration	33.21	8.10	17.32	49.10
6.7	New	54.12	8.10	38.23	70.01
	Used	39.00	8.10	23.11	54.89
	After regeneration	29.71	8.10	13.82	45.60
5	New	27.31	8.10	11.42	43.20
	Used	39.80	8.10	23.90	55.69
	After regeneration	28.09	8.10	12.20	43.98

Table 7 – *Statistical model of the calculation of IC$_{50}$ for the growth inhibition of Sinapis alba root*

Mineral oil Turbinol	Statistical model	A	k	IC$_{50}$ (mg L^{-1})
Used	$y = A \cdot \exp(k \cdot x)$	40.4096	0.0106	20.09
New	$y = A \cdot \exp(k \cdot x)$	40.7186	0.0104	19.74
After regeneration	$y = A \cdot \exp(k \cdot x)$	26.8737	0.0237	26.20

Note: A – inhibition at zero concentration; k – steepness of decline

were readings from the measuring heads using the controller OxiTop OC110. By using the program Achat, the data were transmitted into the computer and evaluated. Plots of the waveforms of the samples of oil contaminants in water are given in Figure 6.

In the biodegradation model of the sample contaminated with new oil, the degradation reached a level of 5.3 % in the 14-day test, and by the end of the 28-day test, this value did not change. The microorganisms in the surface water were forced to adapt quickly (without a lag phase), and this is not shown in the statistical model during the degradation of the sample (Figure 6).

The growth phase (degradation phase) lasted 13 days before passing into stationary phase, which represents the maximum level of degradation.

The maximum level of degradation was that of the used oil in the aquatic medium, at 9 %, and the

degradation process (phase of accelerated growth) stopped on the 17th day of the test and passed smoothly to stationary phase, which lasted until the end of the test.

In the samples contaminated with the regenerated oils, the degradation phase stopped on the 21st day and the maximum level of degradation of the stationary phase was 7.2 % (Figure 7).

Boiko[29] presented the absence of contaminants as an elementary quality requirement for turbine oil. Impurities may consist of water and organic compounds. Aging of mineral oils is caused by microbiological processes that occur in the oils because of the presence of free water. The free water allows the growth of microorganisms, which feed on the oil and reproduce as colonies over the surface of the free water.

The products of their vital activity are acids, which reduce the pH of the water and oil. The reaction in the acid medium of the metal facilitates the formation of bioemulsions and their precipitation in the form of sludge.

Conclusion

Regeneration of waste oils is an environmentally friendly solution to the renewal of their basic properties, which also helps reduce the volume of the hazardous waste they produce. Our research aimed at the determination and comparison of the
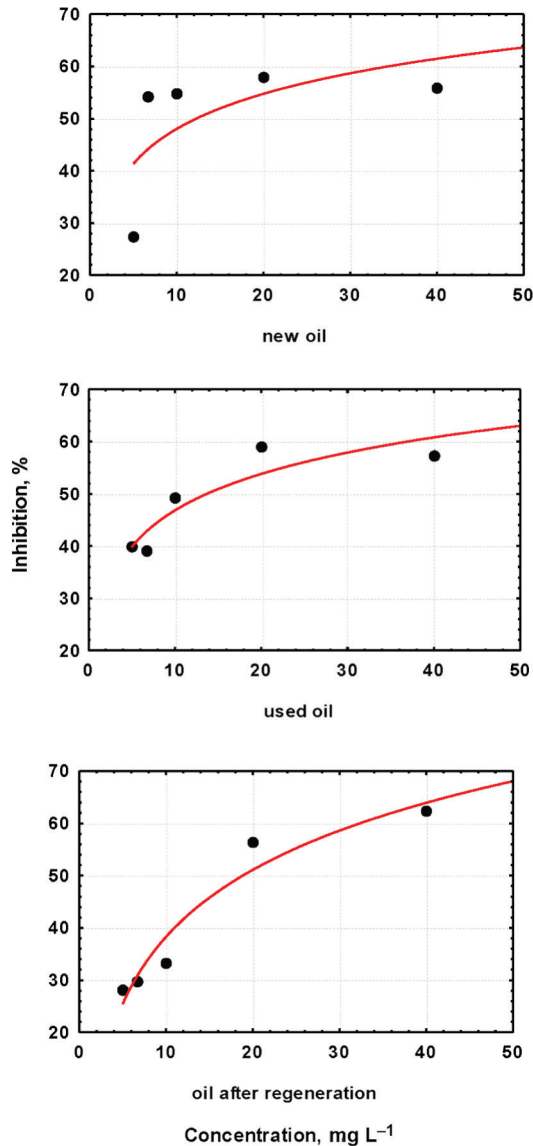
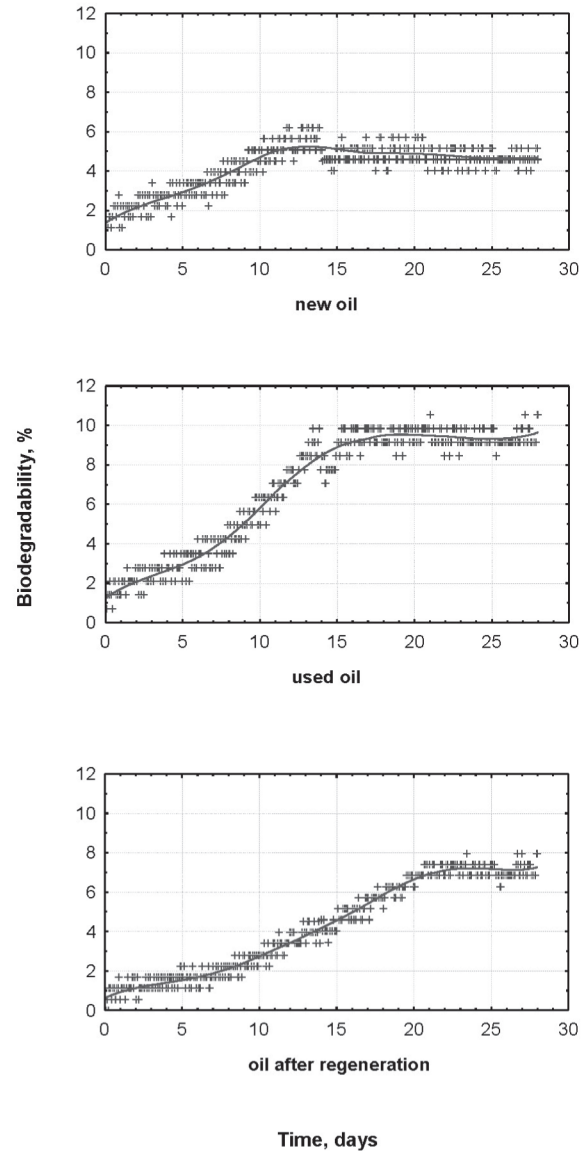Fig. 5 – *Regression analysis of growth inhibition of Sinapis alba root*

Fig. 6 – *Plot of biodegradability with time in the oil samples*

composition of the mineral oil Turbinol X-EP 46: unused, used for one year, and electrostatically regenerated. From an overall assessment of the impact of wear and tear on the properties of the tested mineral oil Turbinol X-EP 46, it appears that the regeneration process of electrostatic precipitation has a positive impact on the ecotoxicological and biodegradation properties of oils. By eliminating the many products resulting from the use of the oil, the composition of the regenerated oil is similar to the composition of new oil, and we also assume that the process causes no significant changes to the required properties (viscosity, pour point, and other) in terms of the use of the oil in practice. From comparison of the inhibition of said products in the ecotoxicological tests of the model samples, we conclude that, in ecotoxicological terms, the used
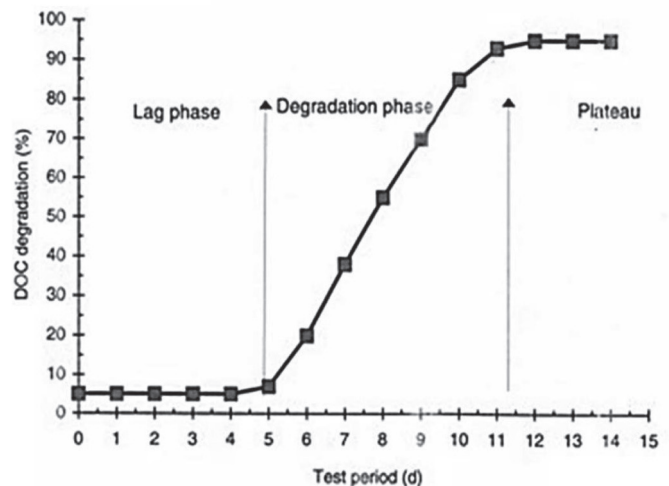

Fig. 7 – *Degradation curve[28]*

mineral oil possesses the worst characteristics and the regenerated oil has the best, although they are always toxic, given their petroleum origin. Providing that the ecotoxicological effects of the recovered oil are comparable to or more favorable than those of the new oil, oil regeneration is found to be an effective method. This is important in terms of saving raw material sources, but also in terms of reducing the environmental burden from the disposal of hazardous wastes, which include waste mineral oils.[30] From the determination of biodegradability using the aerobic manometric respiratory method, where only natural microflora of the surface water participate was sed in the process of biodegradation, the result showed that individual samples are hard to biodegrade. Thus, for water as a component of the environment, this poses environmental risks.

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