Ecotoxicological effects of the leachate from the waste tires on the environment

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Summary

Ecotoxicity is one of the several hazardous properties of waste and presents an acute or delayed danger due to the adverse impact on the environment by its toxic effects on the biotic systems. One of the most important sectors in the waste management are discarded waste tires. The amount of them is arising every year due to the development of automotive industry. The case study is focused on the evaluation of the ekotoxicological impact of waste tires on the environment by using bioassays. Waste tires have been fixed according to the establishment to crushed and ground by sample from which the aqueous extracts have been made. For ecotoxicological testing we used three aquatic tests, namely the test of acute toxicity on Daphnia magna, the growth inhibition test on Lemna minor and on Scenedesmus subspicatus and one terrestrial test with Sinapis alba. Based on the results of the individual preliminary tests have been made the basic tests and the determined value EC_{50} and IC_{50}. The most important toxicity has been shown particularly in an aqueous extract from the ground by sample and the most sensitive testing organism which exhibited the highest toxicity has been Daphnia magna. Results of the terestrical test was negative. It has been found that the preparation of the sample - particle size - has a significant effect on the ecotoxicity. It follows that worn tires pose an environmental risk to the aquatic environment.

***Keywords**: waste tires, aqueous extract, ecotoxicity, tests of toxicity, environmental risk, aquatic environment*

Introduction

The anthropogenic activity originates a lot of the waste, which have negative effects on the environment. The development of the automotive industry closely related with problems of the production of the waste tires. The tires are used in the transport from air to the road. The tires after use are represent as a hardly biodegradable waste[1]. Their accumulation is a global environmental problem and amount is estimated at 1.5 billion annually[2]. Therefore, it is very important to operate with them so that there is no environmental risk. Waste tires are classified in the waste catalog as "other wastes"[3]. They enter the environment during their production already (solid pollutants produced by mixing, homogenization and tire vulcanization; volatile organic compounds; odour in the form hydrogen sulphide). These pollutants degrade air quality. Other environmental risk is mainly related to landfilling of the waste. The tires disturb the stability of the landfill, increase the risk of the fire with release of toxic gases. They are a risk of the toxic leakage and are very hardly biodegradable[4,5]. The studies confirm increased values of the heavy metals such as Cd, Cr, Se, Pb, Zn and Ba in a seepage, especially in acidic environment. The risks of the waste incineration of the tires closely related to the release carbon monoxide and polyaromatic hydrocarbons[6]. An important part of the environmental impact assessment is the determination of the toxic effects of the waste tires using ecotoxicological tests. The tires contain various water-soluble compounds, which can leach into the water and have toxic effects on aquatic organisms. Wik and Dave[7] tested the toxic effect at 12 randomly selected waste tires on *Dafnia magna* in an aqueous extract. The determined value EC_{50} (after 48 hours) was in the range 0.25 – 16 g/l. In the study confirmed clear photo activation with an increase in toxicity > 10 times in four cases by the exposure of the test organisms to UV radiation for 2 hours. According to study, Wik and Dave[8] investigated the toxicity of the abrasive rubber from tires, which they leached into water at 44 °C. They found the toxic effect of the waste tires on *Daphnia magna*, which is caused mainly by non-polar organic compounds. Sediments from the road are likely reservoir of the tire particles in the environment[9]. It was observed the effect of the temperature on the leachability of the toxicants from the sediment. The eluates obtained at the higher temperatures were toxic. In the eluates was confirmed the presence of the mainly zinc and aniline. These are potentially toxic substances with the effect on the organisms *Dafnia magna* and *Pseudokirchneriella subspicata*. The results of the study by Panko et al.[10] pointed to the toxic effect of a mixture of the particles formed from a complex mixture of the rubber from a tire and from roadway (sediment), which represented acute toxicity to aquatic test organisms (green algae and daphnia) at concentration up to 10 000 mg/kg. Wik et al.[11] in an experiment abraded rubber from three different tires and leached them in deionized water, six times in succession. Leach toxicity was tested using standardized tests of the toxicity with *Pseudokirchneriella subcapitata* (72-hour growth inhibition), *Daphnia magna* (24 and 48-hour immobility) and *Ceriodaphnia dubia* (48-hours survival and 9 days reproduction and survival) and *Danio rerio* (48-hours lethality). *Ceriodaphnia dubia* was the most sensitive end organism tested (EC_{50} = 0.013 g/l) until the third leaching of the most toxic tire, which is similar to the expected concentration in the outflows from the road. The evaluation of the toxicity identification indicated that toxicity was caused by zinc and organic compounds[11]. Khan et al.[12] found that the exposure to aqueous extract from waste tires causes short-term and long-term toxicity to the freshwater organism *Hyallela azteca*. The chemical content of the freshwater and marine extracts obtained from waste tires has confirmed adverse effects on the microalgae *Raphidocelis subspicata* (freshwater) and *Skeletonema cistatum* (marine). Chemical analysis confirmed the presence of the organic and metallic compounds in the extracts that inhibited algal growth. The extracts were mild to highly toxic to algae depending on increasing the contamination by organic and inorganic additives. It confirms the necessary for a better understanding of the impact of the plastics-containing chemicals on the aquatic ecosystems[13]. The authors[14] simulated natural conditions and placed a package of the tires in water for 120 days. By analyzing the aqueous extract at specified time intervals, they found changes in the physicochemical parameters of the samples and the presence of the undesirable substances. There are several studies, which are focused on the methods of the recovery of the waste tires. Methods include, for example, the use of the tire pulp in cement brash and asphalt brash. The study[15] presents experience with suitable technologies for the recovery of the waste tires in the construction, but they point out on the toxicity of the used waste products contained in waste tires. They recommend more complex studies to determine the toxic effects of the building materials containing waste tires by testing their leachates at different acidities of the aqueous extract. The study[16] about the microplastic distribution in Charleston Harbor, SC, USA have revealed that a large proportion of the microplastic particles present in the tidal sediments are particles from waste tires. These particles come from the wear of the tires on the roads and are washed into the estuaries during the rains. An autopsy of the fish confirmed that the particles had been absorbed and accumulated in their intestinal tract.

The aim of this case study was to determine the ecotoxicity of the waste tires depending on the particle size of the samples using appropriate ecotoxicological bioassays. Based on the obtained results, it is possible to estimate the adverse effects of the waste tires on the aquatic environment.

Experimental part

The waste tires used for the testing were treated by grinding. Subsequently, the aqueous extracts were prepared from them. We determined physicochemical parameters - pH, specific conductivity and COD. The ecotoxicological properties of the extracts were determined by bioassays: Acute toxicity test on *Daphnia magna*, Growth inhibition (stimulation) test of common duckweed (*Lemna minor*) and Growth inhibition test of green algae. At the same time, we also used one terrestrial test, using the seeds of *Sinapis alba*.

The sample preparation

The tire was processed with a retreading device into smaller pieces to achieve the desired fraction. The rest of the tire was cut using an industrial shear mill. The metal cords, as part of the tire, were separated using a magnet. The laboratory samples were ground with a ball mill RETSCH MM 301. One group of the samples was ground under normal laboratory conditions to a particle size > 3 mm. The samples of the tire were cryogenically processed by to achieve a final particle size of 1 mm (by freezing at -200 °C using liquid nitrogen). In this way, a size < 1 mm was achieved.

Preparation of the aqueous extract

The aqueous extracts were prepared from 100 g of the dry matter in 1 liter of the demineralized water by shaking for 6 hours at 175 rpm. The dry matter was determined by drying the sample to constant weight at 105 °C in a laboratory oven. The solid part was separated from the liquid part by centrifugation (MEDITRONIC BL-S) at 4000 rpm for 20 min. The separation by filtration was used as needed. The preparation of the aqueous extracts was performed under normal laboratory conditions. The procedure is based on STN 83 8303.[26]

Determination of physicochemical parameters

pH: device WTW Ino Lab pH Level 3, with combined electrode SenTix 81®, by STN EN ISO 10523.[17]

Specific conductivity: conductivity meter WTW LF 318, by STN EN 27888.[19]

COD: by STN ISO 15705.[22]

Ecotoxicological tests

In the case study, ecotoxicological tests (presented in Table 1 – 4) were used.

Table 1: Test conditions for Daphnia magna[18, 26]

Test organism	*Daphnia magna* Straus *(Cladocera, Crustacea),* more than the third generation obtained by acyclic parthenogenesis under the conditions of healthy breeding, individuals younger than 24 hours since birth
Biotest conditions	21 ± 2°C; 7.8 ± 0.2; laboratory conditions
Control sample	diluting water prepared from the solutions of $CaCl_2.2H_2O$ (1), p.a., $MgSO_4.7H_2O$ (2), p.a., $NaHCO_3$ (3), p.a., KCl (4), p.a. by the addition of solutions (1)-(4) per 10 ml and adding demineralized water into a volume of 1 liter
Reference substance	$K_2Cr_2O_7$, EC_{50} = 0.82 mg/l (limit 0.3 – 1.5 mg/l)
Amount of the sample	min. 5 ml per individual, maintaining the height of the solution column min. 3 cm
Test duration	24, 48 hours
Basic test	20 daphnia/undiluted sample (10 ml), same conditions for a control, more different concentrations and parallel tests
Validity of the test	immobilisation ≤ 10 %, change of concentration of dissolved oxygen O_2 ≤ 2 mg/l
Monitored response	% of immobilised individuals, dissolved oxygen, pH and temperature, EC_{50} (EC_X – the concentration of the toxicant, which causes the death of X % of the test organisms, or causes their immobilisation)

Table 2: Conditions for the test of growth inhibition of common duckweed[20,21, 26]

Test organism	*Lemna minor*
Biotest conditions	Incubation temperature 25 °C ± 2 °C, simulation of day and night; lighting continually, min. intensity of 6 500 lux
Control sample	Z-medium (was used as the nutrient solution and prepared in accordance with the instructions from its supplier - Culture Collection of Autotrophic Organisms - CCALA, Třeboň, the Czech Republic.
Reference substance	3,5-dichlorophenol, EC_{50} = 2.75 mg/l (limit 2.2 – 3.8 mg/l)
Amount of the sample	50 ml
Exposure	7 days
Basic test	12 –15 leaves at the beginning, more different concentrations
Criterion of validity	average number of leaves in the control after the termination of the test > than octuple at the beginning of the test, pH at the end of the test < than 1.5 in comparison with initial pH
Monitored parameters	the number of leaves is counted and the appearance of the leaves is evaluated (chlorosis, necrosis) at least three times during the test; growth inhibition (IC) in %; inhibition biomass in %, IC_{50} (IC_X - refers to X % inhibition of the growth of the monitored plant or growth rate at a given time compared to control)

Table 3: Conditions for the test for green algae[23,24]

Test organism	*Scenedesmus subspicatus*
Biotest conditions	25 ± 2 °C; lighting continually, min. intensity of 5 000 lux
Control sample	Z-medium (10%) and deionized water (90%)
Reference substance	$K_2Cr_2O_7$, $IC_{50,\ 72h}$ = 1.1 mg/l (limit 0.5 – 2.0 mg/l)
Amount of the sample	min. 5 ml per individual, maintaining the height of the solution column min. 3 cm
Exposure	min. 72 hours
Basic test	50 – 70 ml of undiluted sample with the addition of inoculum, initial algal concentration: min. 10^3 cells in 1 ml of the sample, gradually increasing the concentration of the sample with the addition of inoculum
Monitored parameters	cell growth (counting) every 24-hours (microscope NIKON ECLIPSE 50i, Bürker chamber), growth rate Iμ, growth inhibition IC_{50}

Table 4: Conditions of test of inhibition of growth of root of Sinapis alba[26]

Test organism	*Sinapis alba*, germination > 90%, per 30 seeds of Sinapis alba L. in Petri dishes
Biotest conditions	20 °C ± 1 °C, thermostat TS 606 CZ/2-Var (WTW, Germany)
Control sample	Reconstituted water
Reference substance	$K_2Cr_2O_7$, IC_{50}, 72hours = 31.5 mg l^{-1} (limit 4.1 – 85 mg L-1)
Amount of the sample	10 ml
Exposure	72 hours
Basic test	30 seeds in Petri dishes, more different concentrations
Monitored parameters	inhibition/stimulation of growth of root of *Sinapis alba* compared with the control (IC), preliminary test

Results in graphs and tables are processed in the programme Microsoft Office - Excel and STATISTICA 12, ANOVA, one-factor analysis of variance (using 95 % intervals of reliability for average values of individual samples).

Results and discussion

Determination of physicochemical parameters

The aqueous extracts from the waste tires have increased pH value - alkaline, compared to the demineralized water used to prepare the extracts. Determined specific conductivity indicates a low rate of the concentration of the ions of the dissolved substances in water (demineralized water had a value of 0.1 mS/m). We determined the organic substances dissolved in aqueous extracts by chemical oxygen demand (Table 5). In the aqueous extract of the smaller particles, the parameters were higher.

Table 5: Physicochemical parameters of the aqueous extracts

Parameter	pH	specific conductivity (mS/m)	COD (mg/l)
> 3 mm	7.85	3.62	45.26
< 1 mm	8.21	10.51	62.84

Ecotoxicological tests

The bioassays were performed as preliminary tests and after their evaluation, the basic tests were performed to determine IC_{50} and EC_{50}. Each test was at least 6 repetition.

Acute toxicity test on *Daphnia magna*

The immobilisation of *Daphnia magna* was determined by a preliminary test after 24 and 48 hours. Physicochemical parameters of the water leachates of the experimental samples are shown in Table 6. The results of the acute toxicity test on *Daphnia magna* are shown in Table 7. Results of the preliminary acute toxicity test with used the test organisms of *Daphnia magna* are:

- assessed as negative, if the immobilisation or the death of test organisms is < 50 % in comparison with the control - in this case, a verification test must be performed.
- assessed as positive, if the immobilisation or the death of test organisms is ≥ 50 % in comparison with the control - in this case, indicative and basic test must be performed and value EC_{50} needs to be determined[26].

The preliminary test for the aqueous extract for the sample 1 (particle size > 3 mm) is negative (Table 7) and therefore no further testing is required. The tire sample with a particle size < 1 mm was immobilised on average 76 % of the individuals. For the basic test, the concentration range was selected the dilution of the aqueous extract.

Table 6: Physicochemical parameters of the water leachates of the experimental samples

	pH		Dissolved oxygen [mg/l]		Temperature [°C]	
	0 hours	**48 hours**	**0 hours**	**48 hours**	**0 hours**	**48 hours**
Control	8.89	8.85	10.2	9.8	21.5	21.7
> 3 mm	8.47	8.45	10.5	9.8	21.5	21.7
< 1 mm	8.59	8.44	10.2	9.8	21.6	21.5

Based on the data in Table 6, we can state compliance with the monitored parameters.

The determined value EC_{50} = 8.53 g/l (immobilisation of the test organisms *Daphnia magna*) after 48 hours was calculated according to the regression equation: Immobilisation (%) = 62.2816 – 1.0479*x (Figure 1). Wik and Dave[11] found in a series of the extracts from the waste tires EC_{50} in the range of 0.55 – 5 g/l, after 48 hours. Marwood et al.[9] report the value EC_{50} = 5 g/l in the extract, which was prepared at 44 °C. The authors studied the influence of the conditions on the preparation of the extracts (mainly temperature). The study shows the toxic effect of the waste tires on the aquatic environment, which was also confirmed by our experiments. We have shown a significant effect of the particle size, from which the extract was prepared. The particle size affects the solubility of the substances contained in the samples of the waste tire in demineralized water. It causes increasing the immobilisation of *Daphnia magna* in comparison with the samples, which had a particle size > 3 mm.

Table 7: Basic statistical characteristics of test acute toxicity for Daphnia magna – preliminary test

Sample	Immobilisation, after 48-hours (%)			
	Average	Standard error	95 % Confidence intervals	
			lower limit	upper limit
> 3 mm	23	1.25	19.77	27.73
< 1 mm	76	2.39	68.63	83.87

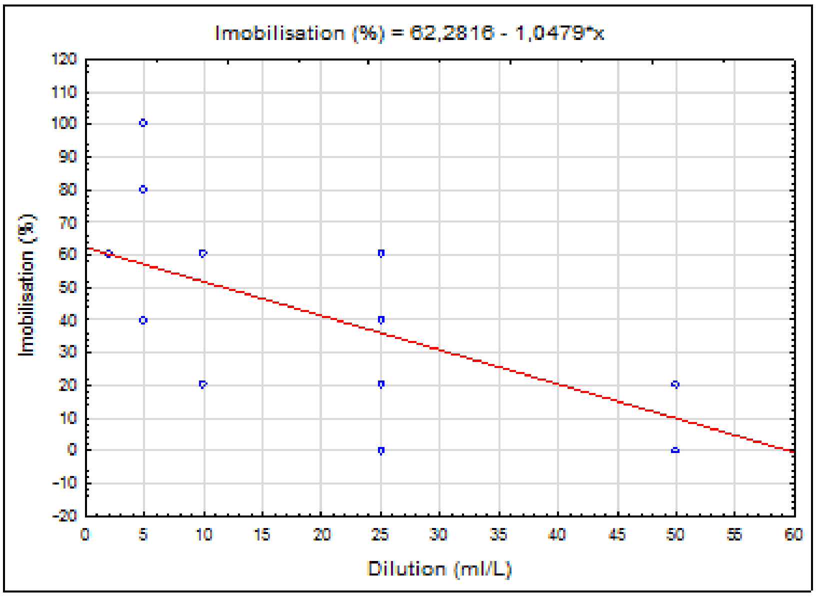

Figure 1: Immobilisation of Daphnia magna in the aqueous extract of the ground tire sample

Test of inhibition (stimulation) of the growth of *Lemna minor*

During the seven-day exposure, the number of the leaves in the samples was counted every 24-hours (including start and end of the test). At the same time, the appearance of the test organisms was observed. The death of the leaf, necrosis and chlorosis were not recorded. The growth inhibition (Iμi; %) is shown in Table 8.

Table 8: Growth inhibition (Iμi; %) of Lemna minor

Sample	Growth inhibition (Iμi; %)			
	Average	Standard error	95 % Confidence intervals	
			lower limit	upper limit
> 3 mm	27.11	0.40	25.85	28.37
< 1 mm	45.06	0.66	42.98	47.15

The test is negative if the growth inhibition is < 30 % or stimulation is < 75%, compared to the control[21]. The results showed that a both groups of the samples were negative. The leachate of the samples with a particle content < 1 mm caused 17.84 % greater inhibition of the growth inhibition compared to the samples with a particle content > 3 mm. The test organism *Lemna minor* has been used successfully to evaluate the toxic effects of the chemicals for several years[20]. Based on the experience[23], we have included this test in the environmental risk assessment of the samples. The toxicity test with *Lemna minor* is important in the whole set of the different toxicity tests.

Growth inhibition test of green algae

During the seven-day exposure to *Scenedesmus subspicatus*, we recorded the numbers of the cells in the samples each day (including start and end of the test). At the same time, the appearance of the colonies was observed. Based on the results of the growth rate inhibition Iµ (Table 9) the test is negative. The test is negative if the inhibition of the algal culture growth is < 50 % compared to the control - in this case no further testing is performed.

Table 9: Growth inhibition (Iµi; %) of green algae

Sample	Growth inhibition (Iµi; %)			
	Average	Standard error	95 % Confidence intervals	
			lower limit	upper limit
> 3 mm	27.17	0.65	25.11	29.24
< 1 mm	44.56	1.39	40.12	48.99

The test results are negative for both fractions. The inhibition of the aqueous extract of the fraction > 3 mm is 17.39 % lower compared to the inhibition of the aqueous extract of the fraction < 1 mm. Gaultieri et al.[25] confirmed the inhibition of the growth rate of the freshwater green algae *Rahidocelis subcapita* after 72 hours in leachate with 50 g of the waste tires in one liter of water. The study[13] evaluated the effect of the chemical content of freshwater and marine extracts made from the car waste tires on the microalgae *Raphidocelis subcapitata* (freshwater) and *Skeletonema costatum* (marine). Current additives such as benzothiazole, cobalt, zinc have been identified in high concentrations. The extracts with the value EC_{50} ranging from 0.5 % to 64 % of the total concentration (100 g/l) inhibited algal growth. The study provides evidence of a relationship between the chemical composition and toxicity of the plastic extracts/rubber extracts. With the increasing contamination of the organic and inorganic additives, the extracts ranged from mild to highly toxic to algae. It emphasizes need better understanding of the overall impact chemicals associated with the plastics to the aquatic ecosystems. The ingredients are considerably diluted in the natural environment however, the value EC_{50} determined in this study on algae showed that they can pose a danger in closed ecosystems (bays, lagoons or lakes) or in oceanic "hotspots" where plastics accumulate.

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

From the measured root lengths of Sinapis alba, we calculated the inhibition of the root growth Ii (%). The test is negative, if the inhibition of the root growth compared to the control is < 30% or the stimulation < 75%, then no further testing is required, by STN 83 8303. The calculated values of Sinapis alba root growth inhibition is shown in Table 10. The aqueous extract obtained from waste tires shall not have a phytotoxic effect on the seed species of the higher cultivated plant used.

Table 10: Inhibition of Sinapis alba root growth compared to the control

Sample	Ii (%)
> 3 mm	40,88
< 1 mm	42,23

Conclusion

The ecotoxicity is one of the several hazardous properties of the waste. The ecotoxicity is an acute or delayed hazard due to adverse effects on the environment, bioaccumulation or toxic effects on biotic systems. The aim of the case study was to evaluate the toxicity of the aqueous extracts prepared by grinding whole the waste tires. Only metal parts were removed. Experimental samples prepared by cryogenic milling to a particle size < 1 mm caused higher inhibition. Contamination of the aqueous leachate in the water with dissolved organic substances from the waste was demonstrated by COD parameter. All extracts were alkaline.

Aquatic tests with the using test organisms *Lemna minor, Scenedesmus subspicatus* and *Daphnia magna* were positive, however, terrestrial test was negative. *Lemna minor* is one of the frequently occurring organisms in the aquatic environment that's why we used them[27, 28].

We confirmed the sensibility of the use of test organisms *Lemna minor* for the toxicity assessment also of this type of the samples. The significant impact of the homogenization of the samples by grinding (particle size) was confirmed. The presence of inorganic and organic components in the tire causes the toxicity of the aqueous extracts. However, most toxicological studies were focused on the samples prepared from the tire tread. The results of this study are useful in practice in the recovery of the waste tires as waste in various applications.

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Ekotoxikologické účinky výluhu z použitých pneumatík na životné prostredie

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Souhrn

Ekotoxicita patrí k jednej z viacerých nebezpečných vlastností odpadov a v dôsledku nepriaznivého pôsobenia na životné prostredie svojimi toxickými účinkami na biotické systémy, predstavuje akútne či oneskorené nebezpečenstvo. Dôležitým odvetvím v odpadovom hospodárstve sú vyradené odpadové pneumatiky, ktorých množstvo vplyvom rozvoja automobilového priemyslu každým rokom rastie.

Prípadová štúdia je zameraná na zhodnotenie environmentálnych vplyvov odpadových pneumatík na akvatické prostredie ako zložku životného prostredia použitím biotestov. Odpadové pneumatiky boli pre stanovenie upravené na drvenú a mletú vzorku, z ktorých sa pripravili vodné výluhy. Na ekotoxikologické skúšanie boli použité tri akvatické ekotoxikologické testy, a to test akútnej toxicity na Daphnia magna, test inhibície rastu Lemna minor a test inhibície rastu Scenedesmus subspicatus, a jeden terestrický test s použitím semien vyššej kultúrnej rastliny Sinapis alba. Na základne pozitívnych výsledkov z jednotlivých predbežných testov boli uskutočnené základné testy a stanovené hodnoty EC_{50} a IC_{50}. Najvýznamnejšia toxicita bola preukázaná vo vodnom výluhu zo vzorky mletej pneumatiky, pričom najcitlivejším testovacím organizmom, ktorý vykazoval najvyššiu toxicitu boli perloočky Daphnia magna. Zistilo sa, že na ekotoxicitu má výrazný vplyv príprava vzorky – veľkosť častíc. Z uvedeného vyplýva, že opotrebované pneumatiky predstavujú pre vodné prostredie environmentálne riziko.

Klíčová slova: *odpadové pneumatiky, vodné prostredie, toxicita, ekotoxikologické testy, environmentálne riziko*