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Toxicity and Biodegradation in Sandy Soil Contaminated by Lubricant Oils

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Abstract The objective of this study was to evaluate the environmental behavior of different types of automotive lubricant oils. Based on respirometry assays the biodegradability was monitored, and toxicological tests were executed to assess the lubricants toxicity before and after microbial activity. Used oil was the most biodegradable, however, it was the most toxic. Also, all lubricants presented toxicity even after biodegradation due to 40% *Eruca sativa* germination inhibition and a low LC50 to *Eisenia foetida* (0.50–0.25 mL). Moreover, used automotive lubricants have a high toxicity because of polycyclic aromatic hydrocarbons concentration that establishes them as a potential carcinogen.

Keywords Toxicology · Bioremediation · Hydrocarbon · LC50

Significant amounts of lubricants are lost in the environment, particularly in environmentally sensitive applications such as forestry and mining, or through engine losses (Battersby 2000). Consequently, considerable attention has been given to lubricant biodegradability and persistence in the environment. Microbiological decontamination of petroleum hydrocarbons in polluted environments is claimed to be an efficient, economic, and versatile alternative, or complement, to physico-chemical treatments (Menendez-Vega et al. 2007). Thus, microbial biodegradation is the major route by which these oil products are removed from soil and water compartments (Atlas and Cerniglia 1995).

The large variation in the composition of used oil relates to its crude oil source, chemical additives, fuel type, and combustion and mechanical properties of the engine (Delistraty and Stone 2007). And, due to its chemical composition, global distribution, and potentially toxic effects, used motor oil can be a serious environmental problem (Vazquez-Duhalt 1989). The environmental pollution by hydrocarbons can cause enormous damage to biota. The evaluation of long term impact and risk of oil spill is a complex process involving chemical analyses and development of the ecosystem-based toxicology. Bolognesi et al. (2006) monitored the genotoxic impact of the Exxon Valdez accident with mussels and oysters. Their results revealed that even many years after the disaster, significant genotoxic damage still exist in species living in the impacted area. Hence, the purpose of this study was to evaluate biodegradation and toxicity of different lubricant oils.

Materials and Methods

In biodegradation and toxicity tests a sandy soil was used as it is present in most riversides and benthic zones of lakes and rivers. The mineral and semi-synthetic lubricant oil were, respectively, F1 Super Plus – SAE 25W/50 (API SJ) from Ipiranga® and Magnatec – SAE 10W/40 (API SL/CF) from Castrol®, and the used oil was obtained in a lubrication center. This is a mixture of mineral, semi-synthetic and synthetic oil lubricants that had already been used in ethanol and gasoline automotive engines. *Eruca sativa*'s seeds (Brassicaceae) from Feltrin® and *Eisenia foetida* (Oligochaeta) were used as test organisms in toxicity assays.

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The inoculum used in biodegradation assays was prepared following Lopes and Bidoia (2009). Microorganisms that are able to biodegrade lubricant oils were obtained through a soil contamination simulation in a plastic bag filled with 3 kg of sandy soil, 50 mL of a lubricant oil mixture, 1 mL of Tween 80® and 100 mL of distilled water. The plastic bag was homogenized and perforated with small holes of approximately 1 mm diameter and spaced at 1 cm from each other. It was then buried at 15 cm depth to allow microorganism exchange between the soil containing oil and the substrate from outside. After 30 days a previous selection of microorganisms acclimatized to the environment with oil, 500 g of this inoculum was added to 500 mL of distilled water. This solution was homogenized and the supernatant removed, which led to the base liquid.

Addition of nitrogen and phosphorus salts was held as the Technical Standard L6.350 issued by Cetesb (1990). It is essential to use reagents that do not interfere in soil pH, hence the nutrient solution was composed of a 1:1 ratio of $(NH_4)_2SO_4$ and KH_2PO_4, 4.25% and 3% w/v, respectively.

Respirometry was used to monitor the lubricant oils biodegradation. The methodology also followed Technical Standard L6.350 (Cetesb 1990) which recommends the Bartha and Pramer's respirometric method. The composition of each system (S) is described in Table 1.

Experiments were conducted in triplicates for each assay. The Bartha and Pramer's technique consists of a closed system, featuring two connected chambers, one where biodegradation occurs and the other with an alkaline solution that was able to quantify the CO_2 produced by microbial respiration (Fig. 1).

Microorganisms and the oil were located in the respirometric chamber. When this hydrocarbon was metabolized by the microbial community, carbon dioxide was released into the flask. Because it is a closed system, a KOH solution captured the CO_2 in the other chamber.

The CO_2 evaluated in respirometers was quantified by titrating the residual KOH with an HCl solution, after the addition of $BaCl_2$ to precipitate the carbonate ions. After each CO_2 quantification, the respirometers were incubated at 28°C.

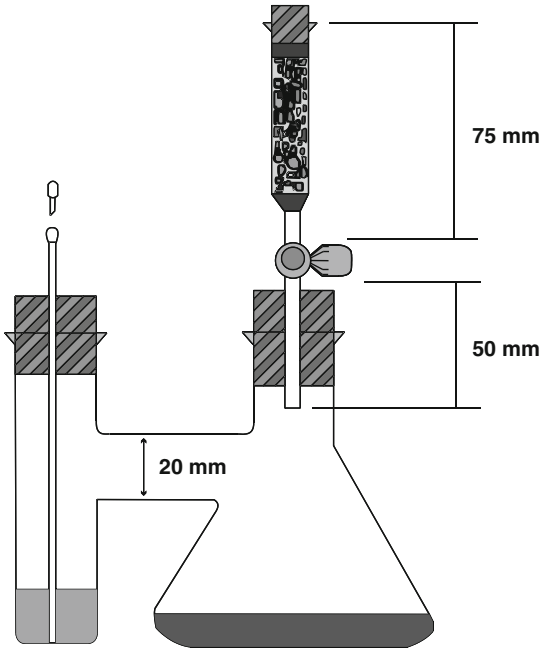

Fig. 1 Respirometer flask based on Bartha and Pramer's respirometric method used in biodegradation assays

Therefore, the carbon dioxide accumulated can be calculated and represented as a function of incubation time (Balba et al. 1998). The incorporated carbon dioxide permitted the evaluation of lubricant oils biodegradation.

Initially, the toxicity tests consisted of evaluating the toxicity of soil contaminated by the different lubricant oils before and after a 90 days period of biodegradation. Test organisms were *Eruca sativa* and *Eisenia foetida*.

The method employed in *Eruca sativa*'s seeds used plastic cups of 50 mL with 50 g of soil from each respirometry system. The experiment was conducted with five replicates each of these containing five seeds added equidistantly and at a 1 cm depth with 2 mL of Milli-Q water. The cups were covered with PVC film and kept for 72 h at 21°C ± 2. After this period, germinated and non-germinated *E. sativa*'s seeds were counted. Also, two control tests were executed (positive and negative). In the positive control, 2 mL of zinc sulphate 0.05 M were added to the soil to cause the inhibition of seed germination. The

Table 1 Respirometric assays composition

Systems	Composition
S0	100 g of sandy soil + 5 mL of base liquid + 1 mL of nutrient solution + 1 mL of distilled water
S1	100 g of inoculum + 5 mL of base liquid + 1 mL of nutrient solution + 1 mL of distilled water
S2	100 g of inoculum + 5 mL of base liquid + 1 mL of nutrient solution + 1 mL of mineral oil
S3	100 g of inoculum + 5 mL of base liquid + 1 mL of nutrient solution + 1 mL of semi-synthetic oil
S4	100 g of inoculum + 5 mL of base liquid + 1 mL of nutrient solution + 1 mL of used oil

negative control was formed by the S0 soil, i.e., without contamination by any automotive lubricating oil.

The other toxicological test employed earthworms (*Eisenia foetida*) as test organisms following a manual of tests for evaluation the ecotoxicity of chemical agents (Brazil 1988), with adjustments. The specimens were obtained at the Bioscience Institute of the State University of Sao Paulo – campus Rio Claro, Brazil. Ten earthworms were selected for each test. These organisms were initially washed and weighed. Thus, only those that weighed between 300 and 600 mg were selected. The recipients for the tests were 1.500 mL plastic bags, each composed of ten earthworms, 300 g of the substrate contaminated by oil (before and after microbiological action), 500 g of glass balls with a diameter between 1.5 and 2.0 cm and 50 mL of distilled water. Finally, these bags were incubated for 7 days at 20°C ± 2. After a week, alive and dead organisms were counted.

Results and Discussion

The respirometry results are shown in Fig. 2. The used lubricant (S4) showed a greater CO_2 production, followed by the semi-synthetic oil (S3) and the mineral oil (S2), which is less biodegradable. Finally, the assay containing only the inoculum (S1) and the control (S0) showed the least CO_2 production, respectively.

The semi-synthetic oil biodegradation curve initially followed the used oil one. Both curves moved far apart from the mineral oil curve after the 12th day, and the used oil curve began to differentiate from the semi-synthetic oil on the 26th day.

In toxicological tests, the compound was considered: toxic, when it caused mortality/inhibition at least to 40% of the test organisms; non-toxic, when the mortality/inhibition was between 0 and 10% of the organisms; and with signs of toxicity, when the mortality/inhibition was between 10 and 40%.

First, the toxicological test was performed with *Eruca sativa*. Figure 3 shows the inhibition percentage before and after 90 days biodegradation. This percentage is defined as the inhibition of seed germination.

All oils were toxic because there was an inhibition percentage over 40%. Even after 90 days biodegradation, all oils also inhibited over 40% of the seeds germination. However, the semi-synthetic lubricant had a greater toxicity drop after the biological treatment and the used oil showed only a small reduction. The inoculum is located in a 10% and 40% range of inhibition that ranks it as showing signs of toxicity.

Despite the used oil being the most biodegradable, it is also the most toxic in relation to the others lubricants. The used oil toxicity can be explained by Chaîneau et al. (1997) who demonstrate that the phytotoxicity was highly correlated with the presence of aromatic hydrocarbons.

The toxicological test with *Eisenia foetida* was conducted in two stages: before biodegradation and after 90 days of treatment with microorganisms (Fig. 4).

Oil toxicity was evaluated for the Lethal Concentration 50 (LC50). This analysis is defined as concentration of a pollutant or effluent at which 50% of the test organisms die. So, it is a common measure of acute toxicity.

Thus, the test was performed with different volumes of oil per 100 g of soil (mL oil:100 g soil): 0.25, 0.50, 0.75, and 1.00 mL. The LC50 refers to 50% of dead earthworms in Fig. 4.

Toxicity tests with the contaminated soils before biodegradation are shown in Fig. 4a. Before the microbial action, the used oil (S4) was the most toxic by presenting

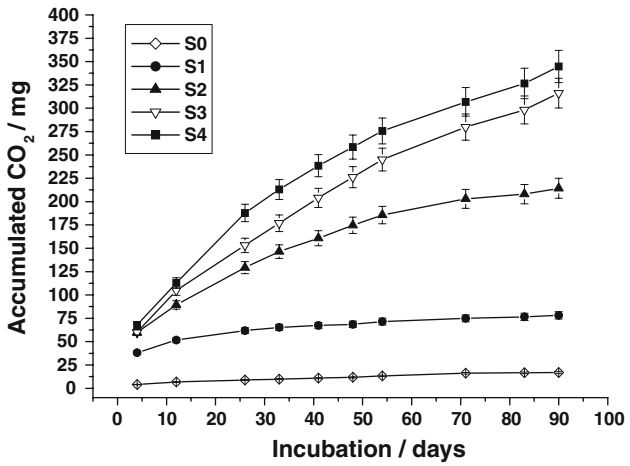

Fig. 2 Accumulated CO_2 production during 90 days of incubation

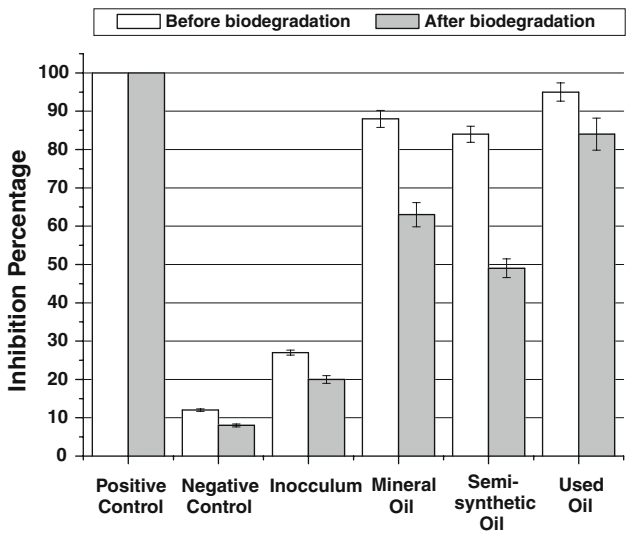

Fig. 3 Germination inhibition of *Eruca sativa*'s seeds

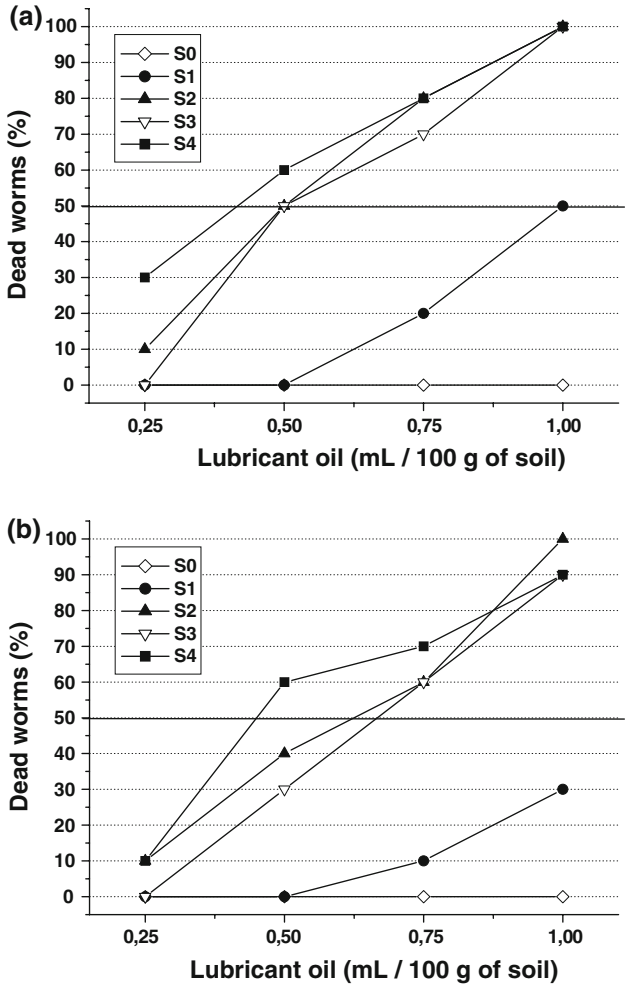

Fig. 4 Death of *Eisenia foetida* in each lubricant assay before (**a**) and after (**b**) biodegradation

LC50 between 0.50 and 0.25 mL. The mineral (S2) and semi-synthetic (S3) lubricant oils have their LC50 at 0.50 mL. The inoculum (S1) is lethal to 50% of the earthworms in 1.00 mL:100 g concentration and the sandy soil (S0) did not cause any death.

After the respirometry assays with 90 days biodegradation, the test was repeated with the same contaminated soils by the lubricants (Fig. 4b).

Microorganisms increased the LC50 in mineral and semi-synthetic oils to the range of 0.75–0.50 mL: 100 g soil. Also, there was a LC50 increase in the inoculum assay and in the control system no organisms had died.

Regarding used oil, even after the biological treatment, its LC50 remained high (between 0.50 and 0.25 mL). These results indicate that the lubricants already used in automotive engines are highly toxic to biota when discarded in nature.

According to the respirometry results, used oil was the most biodegradable lubricant followed by the semi-synthetic oil. The CO_2 production in the mineral oil system indicates that this oil was less biodegradable than the other lubricants analyzed.

In corroboration to these results, synthetic esters are regarded as environmental-friendly base oils due to the high biodegradability (Lea 2007). Reuschenbacha et al. (2003) demonstrated that mineral oil-based lubricants are more difficult to biodegrade, the biodegradability of which is 20%–60%. And Yang et al. (2008) determined a mineral oil-based lubricant's biodegradability as 41%, and the biodegradability of the binary-acid ester as up to 93%.

Supporting the present work, lubricant oils biodegradation in liquid medium was studied in respirometry assays by Lopes and Bidoia (2009). They concluded that the used oil is the most biodegradable and the semi-synthetic oil is the second in accumulated CO_2 by the microbial metabolism. Mineral lubricant presented less biodegradability because of its bigger adaptation period by microorganisms.

As for the toxicological studies with the soil contaminated by lubricant oils, both tests (*E. sativa* and *E. foetida*) established that although the used oil is the most biodegradable, it was more toxic for test organisms.

In accordance with the results, used oils present a more complex toxicological picture (Henry 1998). Their hazards are harder to quantify since used oils may have a wide range of compositions, reflecting the potential range of service conditions and possibilities of contamination. Henry (1998) established that polycyclic aromatic hydrocarbons (PAHs) concentration increases in used oils. These compounds derive from fuel combustion and lubricant decomposition. Because of PAH formation in service, used engine oil is recognized as a potential carcinogen. Therefore, the use in automotive engines contributes to a high toxicity in used lubricant oils.

According to the toxicity results obtained, when the oil concentration decreased, the earthworms mortality was reduced to levels similar to that of the control experiment. Chaîneau et al. (2003) suggested that as biodegradation proceeds, the potential toxicity is reduced, but a substantial toxicity remained as hydrocarbons persist in the soil. These authors concluded that, even at low rates, the residual hydrocarbons may cause serious perturbations to the cellular metabolisms of various living organisms.

Thus, automotive lubricant oils are hydrocarbons that cause severe damage to the environment when they are discarded in wastewater. The oil pollution affects riversides and benthic zones of lakes and rivers hence, harming the aquatic and soil biota in the contaminated environment. Despite the fact that the used oil was the most biodegradable in the respirometry assays, it also has a high toxic potential both for seeds and for earthworms. Even after the lubricants were submitted to the biological treatment, all of them were considered toxic. Finally, it is important for environmental managers and lubricant manufacturers to

understand the environmental performance of alternative products under the conditions of intended use. This information is critical in the continuing challenge to develop more environmentally friendly products such as vegetable based lubricants.

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