



# Ecological implications of motor oil pollution: Earthworm survival and soil health



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## ABSTRACT

The nontarget effects of fresh and used motor oil were studied in a soil test system involving such criteria as earthworm survival, response of soil dehydrogenase and urease, and nitrification. When earthworms were exposed to motor oil-contaminated soil for 4 weeks, the observed median lethal concentrations (LC<sub>50</sub>) were 40.33 and 3.88 g kg<sup>-1</sup> soil for fresh and used oil, respectively. Only fresh motor oil application increased earthworms' body weight even at the higher dose of 19 g kg<sup>-1</sup> soil. Gas chromatography/mass spectrometry revealed that used motor oil contained more of aromatic hydrocarbons and heavy metals than fresh oil. This disparity in the chemical composition might be the factor responsible for the significant toxicity of used motor oil towards earthworms. Activities of soil dehydrogenase and urease were significantly enhanced in presence of both the motor oils, while there was a significant inhibition in nitrification by the used motor oil even at a low concentration of 0.2 g kg<sup>-1</sup> soil. This study clearly demonstrated that earthworm survival and nitrification could serve as suitable indices to assess motor oil pollution in soil.

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## 1. Introduction

Lubricants in the form of motor oil or engine oil are consumed largely in automobiles to protect the internal combustion engines. Generally, motor oil consists of 90% petroleum fractions and 10% additives. Different brands of engine oils have different additives that belong to several groups such as antioxidants, detergents, anti-wear agents, dispersants, corrosion inhibitors and viscosity index improvers (Caines and Haycock, 2004). The internal combustion engine acts like an oxidator and the hydrocarbons in the engine oil are partially oxidized when contacted with oxygen at elevated temperatures for long periods. Metals like iron (Fe), copper (Cu), lead (Pb), nickel (Ni), etc. used in the manufacture of the engine are effective oxidation catalysts and increases the rate of oxidation of the engine oil. The oxidation process produces acidic bodies within the motor oil which are corrosive to typical Cu, Pb, and cadmium

(Cd) engine bearings. Furthermore, oxidation products contribute to the formation of sludges within the motor oil and an overall breakdown of viscosity characteristics of the lubricant.

Used motor oil contains minute quantities of gasoline or diesel, additives, nitrogen and sulphur compounds, a broad range of aromatic and aliphatic hydrocarbons with chain lengths ranging from C<sub>15</sub> to C<sub>50</sub>, and metals that arise from normal wear of engine components and from heating and oxidation of lubricating oil (Vazquez-Duhalt, 1989; Dominguez-Rosado, and Pichtel, 2003; Singh et al., 2006). Thus, US EPA (2001) defined used motor oil as "any petroleum-based or synthetic oil that has been used for vehicle lubrication and as a result of normal use, motor oil becomes contaminated with various impurities such as dirt, water, chemicals or metals from vehicle engine". Once degraded motor oil escapes the engine, it has the potential to pollute waterways in the form of runoff and soil. The US Environmental Protection Agency (EPA) reported that 1.0 gallon of used motor oil can contaminate 1.0 million gallons of fresh water. If used motor oil reaches sewage treatment plants, even small concentrations of 50–100 ppm can foul the water treatment process.

One litre of used motor oil can pollute up to 3784 m<sup>2</sup> of soil, making it non-productive for farming or plant growth for up to

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100 yrs (Chin et al., 2012). Petroleum hydrocarbons, the main components of the used oils, spread horizontally on the ground-water surface and partition into water, soil pore, air space, and to the surfaces of soil particles (Plohl et al., 2002). The entry of used oil will alter some of the soil properties resulting in poor aeration, immobilization of nutrients, and lowering of pH which are largely responsible for change in fertility of soil (Shukry et al., 2013). These alterations may lead to mass mortality of animals living in the topmost layers of soil, ultimately disrupting biological equilibrium of soil (Das and Chandran, 2011). Thus, soil becomes less productive when exposed to used motor oil (US EPA, 1996). Used oils are, therefore, considered as one of the most hazardous mainstream categories of environmental pollutants, posing a major threat to the environment and public health.

Soil exposure to the contaminants creates stress or risk, and can have an adverse impact on terrestrial ecosystems. To assess this environmental stress, an integrated approach is required comprising several criteria (Rutgers et al., 2000). The links between chemical, toxicological and ecological information provide the basis for an integrated approach in ecological risk assessment (Breure and Peijnenburg, 2003), and bioassays are used to provide toxicology information in this combined approach (van Straalen, 2002). The biological balance of the soil, affected due to toxicity of petroleum compounds, has been assessed by ecotoxicological assays using earthworms, collembolans, plants and bacteria (Hentati et al., 2013), or by measuring activities of soil enzymes (Wyszkowska and Wyszkowski, 2010). The activity of soil enzymes like dehydrogenases, phosphatases, catalase, urease, etc. has often been monitored in the ecotoxicological studies (Leahy and Colwell, 1990; Margesin et al., 2000; Brohon et al., 2001; Hentati et al., 2013). The toxicity of petroleum hydrocarbons contained in crude oil on soil organisms has been widely studied (Tang et al., 2011; Cermak et al., 2013; Hentati et al., 2013); however, the toxic effects of engine oils, especially used motor oil, on soil health have not been investigated. The present study, therefore, evaluated the toxicity of used oil contamination following earthworm survival and bioaccumulation of hydrocarbons, activities of soil dehydrogenase and urease, and nitrification in soil. Nitrification rate, in particular, as an end point has been considered important in evaluating the risk assessment of motor oil pollution since it is a fairly sensitive toxicity criterion related to the soil ecosystem (Broos et al., 2005).

## 2. Materials and methods

### 2.1. Soil used in the study

Soil with undetectable petroleum hydrocarbon content was collected from the upper 15 cm layer of a site (34.8140° S, 138.6120° E) located in South Australia, Australia. The soil was air-dried and passed through a 2 mm sieve (Khan et al., 2012). The soil physico-chemical properties were determined in triplicate samples following standard analytical procedures. The pH and electrical conductivity were measured in 1:5 (soil:water; w/v) slurry using Smart CHEM-Lab (TPS Pty Ltd, Brisbane, Australia). Soil particle size distribution was determined using the micropipette method (Miller and Miller, 1987), and the soil organic carbon (OC) content by the wet oxidation method (Walkley and Black, 1934). The soil used in this study was a silty loam (170 g clay kg<sup>-1</sup>, 550 g silt kg<sup>-1</sup> and 280 g sand kg<sup>-1</sup>) with 49% water-holding capacity (WHC), pH of 7.1, and an OC content of 23 g kg<sup>-1</sup> soil.

### 2.2. Motor oils

Used engine oil of the grade SAE 15W-50 (semi-synthetic grade from Shell Helix plus), changed from a four-cylinder engine car

after travelling 5133 km (with an odometer reading of 198,230 km), was collected from an automobile service station at Adelaide, South Australia. The Society of Automotive Engineers (SAE) defines a numerical system for grading motor oils according to viscosity, and the suffixes (0, 5, 10, 15, 25 and 50) followed by the letter W designate the engine oil's "winter" grade. Hence, the same brand of fresh engine oil was used for control experiments. Volatile and semi-volatile hydrocarbons including Benzene, Toluene, Ethylene and Xylene (BTEX), total petroleum hydrocarbons (TPHs), and PAHs were analysed in different extracts of fresh and used oils.

### 2.3. Soil spiking

Soil spiking was done following the stainless steel spoon method (Doick et al., 2003). Briefly, air-dried soil samples (500 g), contained in a large glass mixing jars, were mixed with motor oils at various concentrations. Acetone was used as a carrier solvent at the concentration of 30 mL kg<sup>-1</sup> soil. Based on the range-finding tests, the soil samples were spiked with motor oils at nine different concentrations between 0 and 0.75% (w/w) for used motor oil, and 0–6% for the fresh motor oil. The jars were closed for 3 h to let the solvent disperse, and thereafter acetone was allowed to evaporate in a fume hood for 16 h. Double-distilled water was added to each jar to maintain 60% WHC. All the treatments including controls (oil and solvent control) were maintained in triplicates.

### 2.4. Earthworm toxicity assay

The OECD guideline 207 (1984), the most common earthworm toxicity testing, was used in this study. The earthworm (*Eisenia fetida*) was purchased from Bunnings, South Australia. Before exposing to oil-contaminated soil samples, the worms were acclimatized for 2 weeks in the fresh soil at 25 °C under a controlled light (500 lux)—dark cycle of 8:16 h (Cáceres et al., 2011). Only fully-matured worms, weighing between 0.31 and 0.39 g, with well-developed clitellum were selected for the experiment. The worms were cleaned with deionized water and softly blotted on an absorbent paper to remove excess water, and were depurated overnight on moist blotting paper in Petri-dishes. After cleaning and blotting again, ten worms were weighed and introduced into each glass jar containing oil-treated and control soils. The mouth of the jars was wrapped with aluminium foil punched with holes to allow proper aeration. The test jars were incubated for 28 days at a constant room temperature of 20 ± 2 °C under controlled light (Cáceres et al., 2011), and the live worms from all the treatments, in triplicates, were recorded to estimate the per cent mortality. The weight of the live worms was also recorded after depuration, cleaning and blotting. In order to determine the influence of earthworms on oil degradation, another set of soil samples similar to the worm experiment but without worms was included.

### 2.5. Analysis of motor oils

#### 2.5.1. TPH analysis

Aliquots (0.1 mL) of engine oils or extracts from soil samples and earthworm tissues, dissolved separately in 10 mL hexane, were injected into a gas chromatograph fitted with a flame ionization detector (GC-FID Agilent model 6890). Chromatography was performed on a fused-silica capillary column BPX-5 from SGE (15 m × 0.32 mm internal diameter) coated with HP-5 (0.10-μm film thickness). Helium was used as the carrier gas at 2.5 mL min<sup>-1</sup>, and the FID detector temperature was kept at 300 °C. Splitless injection with a sample volume of 1 μL was applied. The oven temperature was increased from 50 to 300 °C at a gradient of

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