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Toxicity Assessment of Contaminated Soil Using Seeds as Bioindicators

Jaqueline Matos Cruz

UNESP – Univ Estadual Paulista, Av. 24A, 1515 – Bela Vista

Postal Code: 13506-900, Rio Claro-SP, Brazil

Tel: (55)19-3526-4191. E-mail: jaquemcruz@yahoo.com.br

Paulo Renato Matos Lopes

UNESP – Univ Estadual Paulista, Av. 24A, 1515 – Bela Vista

Postal Code: 13506-900, Rio Claro-SP, Brazil

Tel: (55)19-3526-4191. E-mail: mlopes.paulo@yahoo.com.br

Renato Nallin Montagnolli

UNESP – Univ Estadual Paulista, Av. 24A, 1515 – Bela Vista

Postal Code: 13506-900, Rio Claro-SP, Brazil

Tel: (55)19-3526-4191. E-mail: renatonm3@gmail.com

Ivo Shodji Tamada

UNESP – Univ Estadual Paulista, Av. 24A, 1515 – Bela Vista

Postal Code: 13506-900, Rio Claro-SP, Brazil

Tel: (55)19-3526-4191. E-mail: ist_eco@yahoo.com.br

Natália Maria Maciel Guerra Silva

UENP - Univ Estadual do Norte do Paraná, BR-369, Km 54

Postal Code: 86360-000, Bandeirantes-PR, Brazil

E-mail: natyguerra@uenp.edu.br

Ederio Dino Bidoia (Corresponding author)

UNESP – Univ Estadual Paulista, Av. 24A, 1515 – Bela Vista

Postal Code: 13506-900, Rio Claro-SP, Brazil

Tel: (55)19-3526-4191. E-mail: ederio@rc.unesp.br

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Abstract

The evaluation soil quality after bioremediation processes solely on chemical data does not include the effects of toxic substances in organisms. Thus, ecotoxicological assays with seeds are applied to assess the effect of toxic substances in organisms according to their germination sensitivity. The objective of this study was to evaluate a contaminated soil with diesel, biodiesel and waste lubricat oil in ecotoxicological bioassays using seeds of *Cucumis sativus* (cucumber), *Brassica oleracea* (kale) and *Barbarea verna* (cress) as test organisms. The sample of contaminated soil was buried to allow contact with microorganisms that are naturally present in the soil and can be capable to biodegrade the contaminant. Each soil sample was removed monthly and the potential toxicity of contaminants was evaluated by examining germination rates according to biodegradation time in soil. The results indicate that the species *Barbarea verna* is not a good test organism due to its low germination rate. The study suggest that the contact of waste lubricant oil and diesel with the embryo was hampered by the seed coats and the hydrophobicity these substances, preventing the entry of substances which may be toxic to the embryo. Also, *Cucumis sativus* and *Brassica oleracea* showed that after two months of biodegradation, biodiesel is the most toxic contaminant during seed development.

Keywords: Bioassays, Germination, Lubricating Oil, Biodiesel, Diesel and Ecotoxicity

1. Introduction

Ecotoxicology not only detects dangerous substances, but also biological effects from such substances that may harm the environment (Lynch, 2001). Thus, ecotoxicology integrate the synergistic and antagonistic effects of all contaminants and provide information on the bioavailable fraction of the contaminants only, which would be impossible to evaluate solely with chemical data. Hence, bioassays can serve as a complementary tool in environmental risk assessment of bioremediated places (Plaza et al., 2005; Repetto et al., 2001).

Ecotoxicity tests evaluate the effect of environmental contamination on organisms through assessment of survival, growth, reproduction and behavior. These tests help to determine whether the contaminant concentration at remediated sites is high enough to cause adverse effects on organisms (U. S. EPA, 1996).

Plants depend strongly on soil to germinate and grow, so any alterations in the seed development may reflect the presence of toxic substances in the soil. Germination test in ecotoxicological assays are considered short-term and evaluate acute toxicity effects (Banks & Schultz, 2005). Siddiquii et al. (2001) analyzed grass germination in soil contaminated with diesel oil, demonstrating its high toxicity. Saterbak et al. (1999) recommends various seeds germination as an effective evaluation of site contamination, since such tests present a narrow variability, good sensitivity and applicability to variety of soils.

Maila and Cloete (2002) consider *Lepidium sativum* germination as a potential PAHs bioindicator. On the other hand, Smith et al. (2006) report that none of the treatments with PAHs contaminated soil adversely affected germination. The findings of Lors et al. (2009) suggest a probable relationship between acute toxicity and low-molecular weight PAHs. Banks and Schultz (2005) supported using lettuce in germination toxicity assays in petroleum-contaminated soils. This seed showed sensitivity to contaminant.

Several species have been recommended for phytotoxicity test by U. S. EPA (1996). *C. sativus* germination rate showed excellent reproducibility. Therefore, it is an endpoint suitable for phytotoxicity test (Wang et al., 2001).

The purpose of this study was to evaluate the toxicity of soil contaminated with diesel, biodiesel and lubricating oil used at different times of biodegradation by ecotoxicological assays using seed germination of *Cucumis sativus*, *Brassica oleracea* and *Barbarea verna*.

2. Material and Methods

2.1 Material

The sandy soil was used to prepare the simulated contamination with three different substances: biodiesel (Caramuru®); commercial diesel (B2 - 2% biodiesel) - from Petrobras® and waste lubricating oil, collected in a gas station in the city of Rio Claro, SP.

Pesticide free seeds of *B. verna*, *B. oleracea* and *C. sativus* were obtained from ISLA. It is to be noted that *C sativus* has been previously recommended by the U.S.EPA for phytotoxicity tests (Wang et al., 2001).

2.2 Methods

Contaminated samples were prepared according to a Lopes and Bidoia (2009) adapted methodology (Table 1). About 0.7 kg of sandy soil, 52.5 mL of contaminant, 43.75 mL of distilled water, 1.05 mL of surfactant Tween 80® were placed in a plastic bag and the bag was sealed with adhesive tape. The mixture was then homogenized and kept in a sealed bag. Contaminant volume was replaced by distilled water in control assays.

Table 1. Contaminated soil composition

Assays	Contaminant (mL)	Sand (kg)	Tween 80® (mL)	Distilled water (mL)
Waste lubricant oil	52.5	0.7	1.05	43.75
Diesel	52.5	0.7	1.05	43.75
Biodiesel	52.5	0.7	1.05	43.75
control	-	0.7	1.05	96.25

The sealed bags were perforated to allow contact between samples and microorganisms capable of biodegradation that are naturally present in the soil. The bags were buried in about 30 cm deep, in Sao Paulo State University, Rio Claro (22,3968 S, 47,5454 W).

Biodegradation was measured at five different time periods: time zero (T0), time one (T1=30 days), time two (T2= 60 days), time three (T3= 90 days) and time four (T4=120 days). Test T0 was performed immediately after contamination.

2.3 Ecotoxicity - Seed Germination

Plastic cups containing 50 mL of 50 g of contaminated sand were seeded according to Morales (2004) in three replicates, containing 10 seeds each. To the positive control 2.5 mL of 0.05 mol^{-1} zinc sulfate was added to inhibit germination and evaluate seed sensitivity. Seeds that had at least one protrusion of the radicle were considered germinated.

The percentage of inhibition was related to the negative control. Inhibition values higher than 40% in the test-organisms are considered toxic, while non-toxic inhibition values range is between 0 and of 10% of the test organism. Early toxicity inhibition is between 10 and 40% of non-germinated test-organisms (Lopes et al., 2010).

The toxic effect was calculated using equation 1, which relates the percentage of germination inhibition in the contaminant to negative control, assuming that in control test a maximum number of germinated seeds because there was no addition of contaminants.

$$\%\ of\ inhibition = \frac{number\ of\ germinated\ seeds\ \ in\ contaminated\ soil}{number\ of\ germinated\ seeds\ \ in\ negative\ control} x\,100 \quad \text{(Equation 1)}$$

3. Results and Discussion

The percentage of germination inhibition in different toxicity tests are showed in Figures 1, 2 and 3 for *C. sativus*, *B. oleracea* and *B. verna* seeds, respectively. A negative scale was used to represent no toxicity.

In negative control tests, only *C. sativus* presented germination rate above 90%, a value within the ones indicated by Morales (2004) to guarantee the quality of the bioassay, which indicates that seeds in the present study were viable. The U.S. EPA (1996) index considers a test viable when at least 65% of the seeds have germinated from negative control, so both *C. sativus* and *B. oleracea* are consistent with their recommendations for phytotoxicity. On the other hand, *B. verna* did not fit into any of the indicators since it reached a germination rate below 65% in two times analyzed. The percentage of high inhibition observed in the germination of *B. verna* is not only attributed to the action of the contaminant, but also the combined effect of low germination rates and higher toxicity of the contaminant.

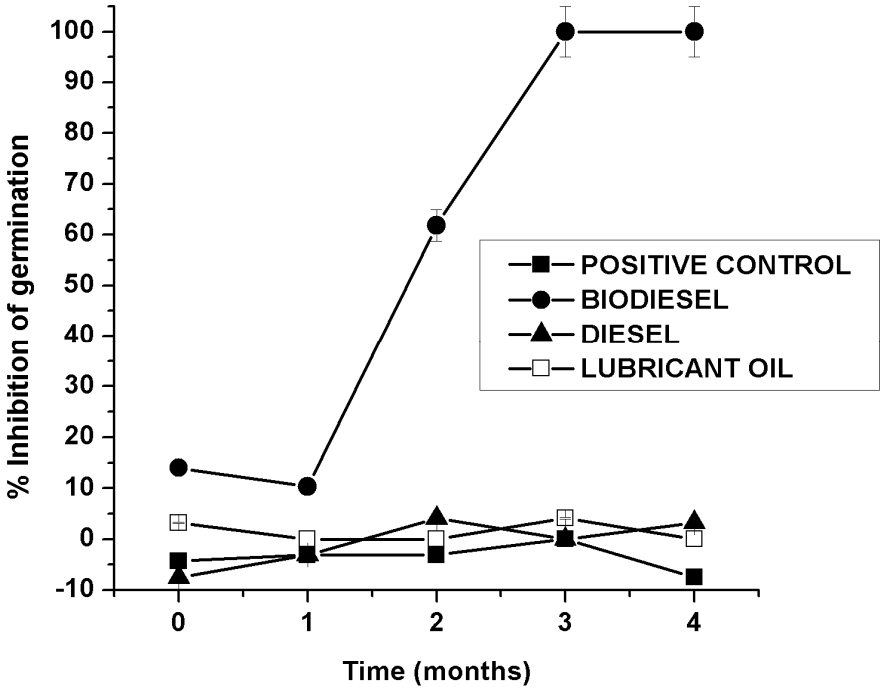

Figure 1. Percentage of inhibition of seeds germination *Cucumis sativus* in soil contaminated with diesel, biodiesel, waste lubricant oil and also positive control, during biodegradation process

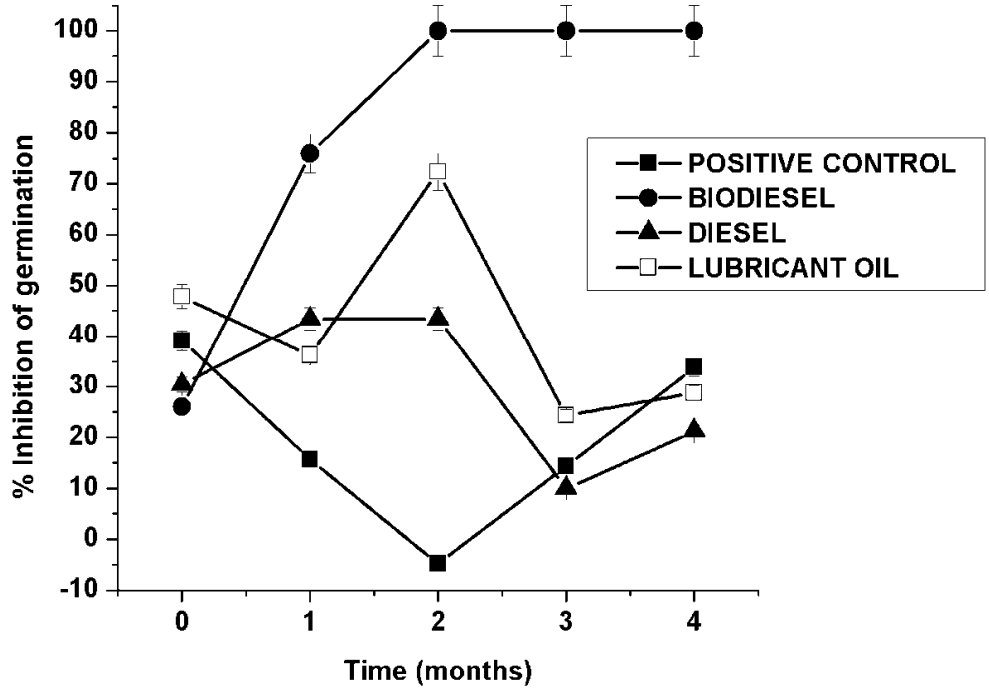

Figure 2. Percentage of inhibition of seeds germination *Brassica oleracea* in soil contaminated with diesel, biodiesel, waste lubricant oil and also positive control, during biodegradation process

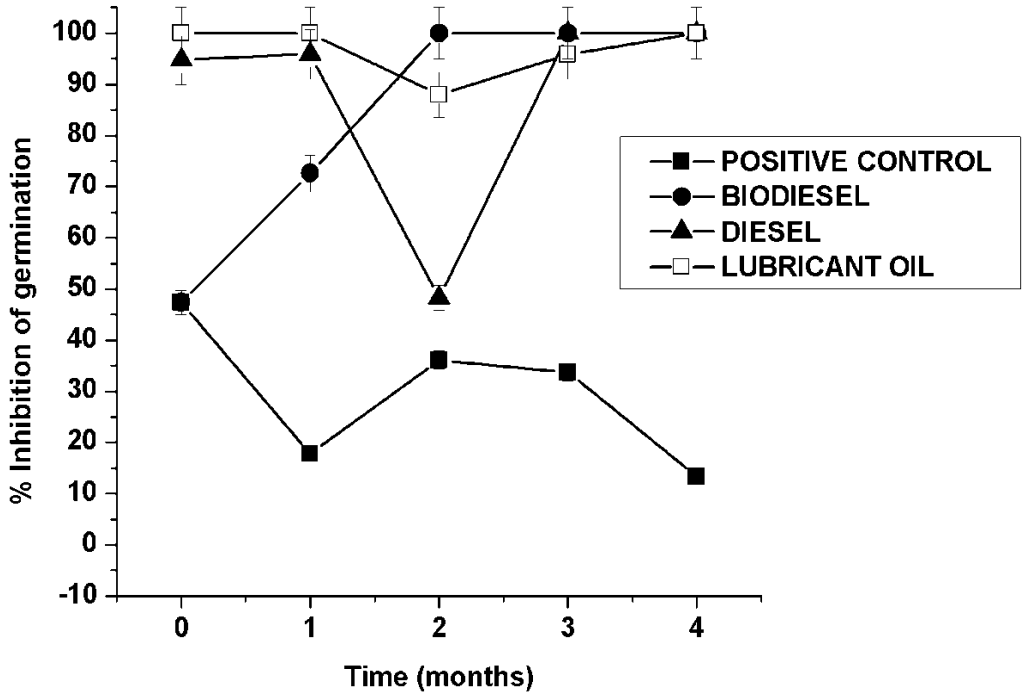

Figure 3. Percentage of inhibition of seeds germination *Barbarea verna* in soil contaminated with diesel, biodiesel, waste lubricant oil and also positive control during biodegradation process

C. sativus seeds showed no toxicity in diesel contaminated soil; however, seeds of *B. verna* and *B. oleracea* were sensitive to the same contaminant. Such inhibitory effect on germination might be attributed to volatile fractions of diesel oil and their property of repelling water by forming a film around the seed (Adam & Duncan, 2002). Thus, the ability to germinate depends on species sensitivity to the contaminant.

Waste lubricant oil had low toxicity to cucumber seeds compared to negative control. In this case, the seed coating can constitute a barrier against compounds that inhibit the germination, such as PAHs and metals in lubricant oil (Adam & Duncan, 2002; Araújo & Monteiro, 2005; Di Salvatore et al., 2008) The results obtained by Wierzbicka and Obidzinska (1998) showed that the cucumber seed coat is selectively permeable to metals such as lead. Therefore, the cucumber seed coat effectively protects its embryo.

The *B. oleracea* (kale) seeds results demonstrated the highest toxicity in initial times of soil contamination, which decreased after 90 days of biodegradation. Inhibition of *B. verna* (cress) seed germination remained high (up to 87%) throughout the biodegradation period. Lubricating oil toxicity can be related to its volatile fractions and PAHs. These compounds are known to inhibit germination (Henner et al., 1999; Henry, 1998; Nkereuwem et al., 2010), as corroborated by Plaza et al. (2005) in which some plants were very sensitive about the presence of polycyclic aromatic hydrocarbons – PAHs during toxicity tests. Also, Abioye et al. (2012) observed decrease of the germination in contaminated soil tested with 5% of waste lubricating oil (used lubricant oil).

Considering biodiesel contamination assays, seeds of *B. oleracea* and *B. verna* did not germinate from T2, i.e., 100% toxicity after 60 days of biodegradation. This effect was also observed for cucumber seeds in T3, where intermediate compounds produced by the biodegradation may be more toxic to seed germination than the original substance and also, may have contributed to combined toxicity of different compounds through synergisitc or antagonistic effects (Lapinskiene et al., 2006; Plaza et al., 2005). Result corroborates the findings of Tamada et al. (2012), who reported increase in the inhibition percentage of the germination on soil contaminated with biodiesel, after 180 days of biodegradation by autochthonous microorganisms. Also, biodegradation of biodiesel increases its acidity due to the breakdown of fatty acid methyl ester molecules being broken down during degradation and the fatty acid chains generate acidity that might have affected the process of germination of the seed used in the study (Leung et al., 2006). Therefore, we suggest that acidic substances that caused soil acidity with biodiesel were more bioavailable than the hydrocarbons that are difficult to degrade and insoluble being difficult to penetrate within the seed, in other words, the interference to germination is due physical barrier.

Although the ability to germinate in contaminated soil depends on each species, the three species submitted to assays showed toxicity increase in contaminated soil with biodiesel after 60 days of biodegradation. However, when the seeds were placed in soil contaminated with hydrocarbons each specie presented different results.

The interspecific differences observed for germination can be explained by the different seeds sizes, due to the fact that larger seeds have less surface area relative to volume, so they may

offer greater protection from exposure to contaminated soil (Saterbak, 1999; Wierzbicka & Obidzinska, 1998). Thus, the results corroborate this assertion, because the size of seed used was in the following order *B. verna* (cress) (1mm), *B. oleracea* (kale) (2 mm) and *C. sativus* (cucumber) (9 mm), and the sensitivity to contaminated samples inversely followed this sequence, i.e., the cucumber seeds had smaller sensitivity to contaminants. Thus the seed coat protected the endosperm from contamination until it can develop and become self-sufficient (Bewley, 1997).

4. Conclusion

The low germination percentage presented by *B. verna* did not guarantee the bioassay quality, since germination inhibition rates cannot be attributed solely to the contaminant effect. However, according to the results obtained in *C. sativus* and *B. oleracea*, biodiesel becomes toxic to the developing seeds from 60 to 90 days of biodegradation.

These results are importantly related to the agricultural scenario, where the replacement of diesel with biodiesel consumes a large share of biofuel production. It is necessary to evaluate toxicity in order to assess the degree of contamination, since this could affect the viability of seeds. Further studies on the toxicity of biodiesel are recommended.

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