EVALUATION OF THE PHYTOTOXIC EFFECT, THROUGH TESTS WITH *LACTUCA SATIVA, OF SOILS CONTAMINATED WITH EXTRA HEAVY CRUDE TREATED WITH A BIOLOGICAL COUPLING*

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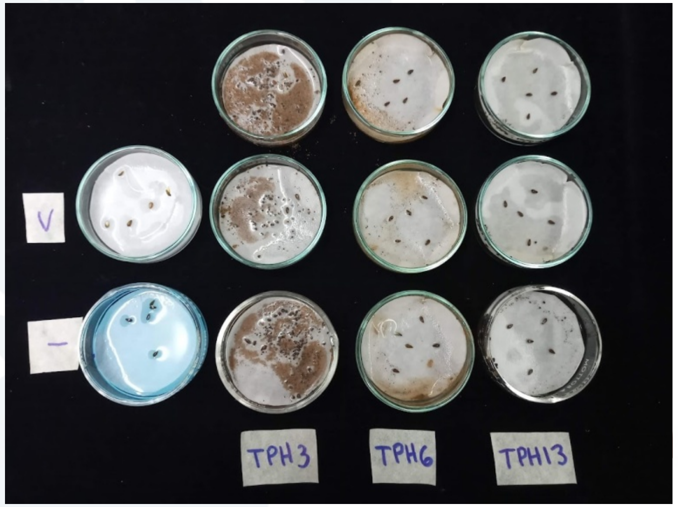
INTRODUCTION

Petroleum and its derivatives are the main source of energy for industry and daily life, as well as being the raw material in numerous processes in the chemical industry. This dependence on fossil fuels has resulted in serious environmental problems, including soil contamination. Venezuela is the country with the largest oil reserves (OPEC, 2021), which makes it highly vulnerable to environmental degradation due to the activities of the oil industry; therefore, the aim of this work is to evaluate the phytotoxic effect, through tests with *Lactuca sativa, of soils contaminated with extra-heavy crude biotreated with a biological coupling.*

MATERIALS AND METHODS

Samples of soils impacted with extra-heavy crude oil from the Orinoco Oil Belt, Venezuela, were used. The soil nomenclature is as follows: Soil 1 (TPH3), Soil 2 (TPH6) and Soil 3 (TPH13). The bioremediation treatment of these was carried out in triplicate in microcosms of 250 gr for each concentration of TPH, using a biological coupling with a grass (*Chrysophogon zizanioides) and the mycorrhizal fungus Rhizophagos manihotis. The phytotoxicity test was carried out using Lactuca sativa seeds at the initial time and final time of the bioremediation test (Pernía et al., 2018; Aguirre et al., 2022).*



MATERIALS AND METHODS

- A soil sample free of hydrocarbons was used as a control to determine the Integral Phytotoxicity Index (IPI). Seeds of Lactuca sativa Variety Great Lakes 659 were used. The seeds were washed with distilled water and distributed at a rate of 5 seeds per Petri dish, which contained a sterile Whatman number 2 filter paper, 5 grams of soil and 5 ml of distilled water. Distilled water was used as a positive control for seed viability and a copper sulfate solution as a negative control. The plates were wrapped in aluminum foil, stored in plastic bags to maintain a humid environment, and left in the dark at a temperature of 25 ºC for 144 hours.

MATERIALS AND METHODS

- The Integral Phytotoxicity Index (IPI) proposed by Pernía et al. (2018) was used, since it incorporates both measures of the seedlings (length of the radicles and of the hypocotyls). The formula is presented below:
- $IIF = 100 - \left(\frac{SGM}{SGC}\left(\frac{\frac{LRM}{LRC}+\frac{LHM}{LHC}}{2}\right)100\right)$

RESULTS

The percentage of seed germination was affected by the contaminant in the three impacted soils, decreasing between 80 and 100% before the soils were biotreated. After treatment, it reached values close to those of clean soil. The growth of the structures of the *L. sativa* seedlings was also affected by the contaminant. In soils 1 and 2 there was a significant difference in the lengths of radicles and hypocotyls before and after treatment; in soil 3, the difference was observed only in the radicles. The IIF at the initial time indicated that the soils had extreme toxicity (with values between 89 and 100). After 90 days of treatment, the samples decreased their toxicity to the low toxicity condition, with values between 10.2 and 25.3.

RESULTS

Effects of extra-heavy crude oil on the Germination Percentage PG

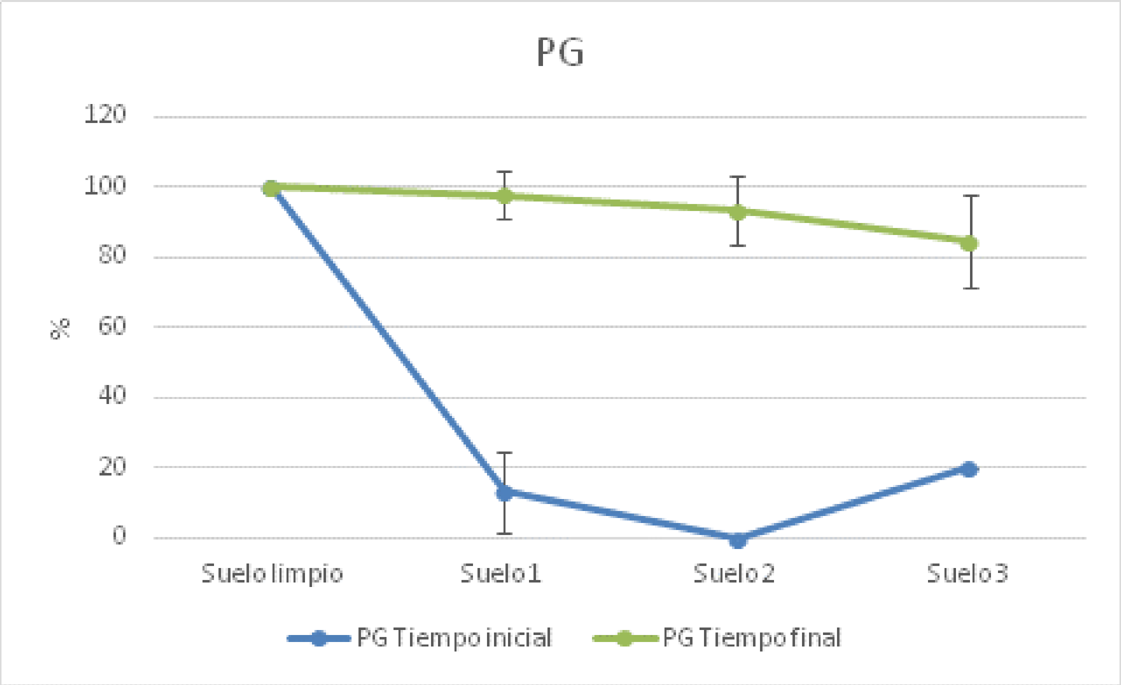


Figure 1. Percentage of germination for each of the studied soils, before and after the bioremediation test. Results are shown as means ± standard deviation (n = 45).

RESULTS

Effects of extra-heavy crude oil on the elongation of radicles and hypocotyls

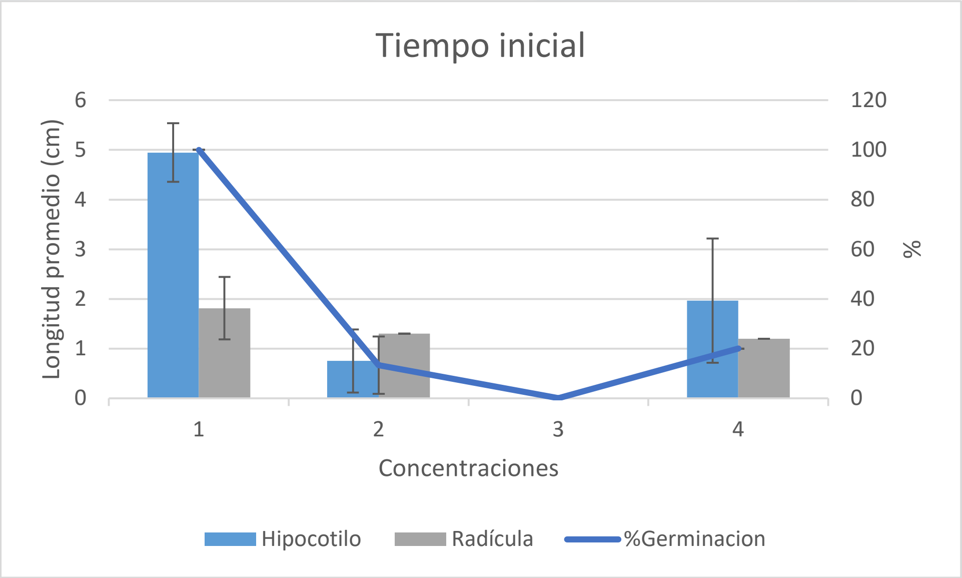


Figure 2. Percentage of germination and lengths (cm) of hypocotyls and radicles of *Lactuca sativa* seedlings at the initial time. Results are shown as means ± standard deviation (n = 45).

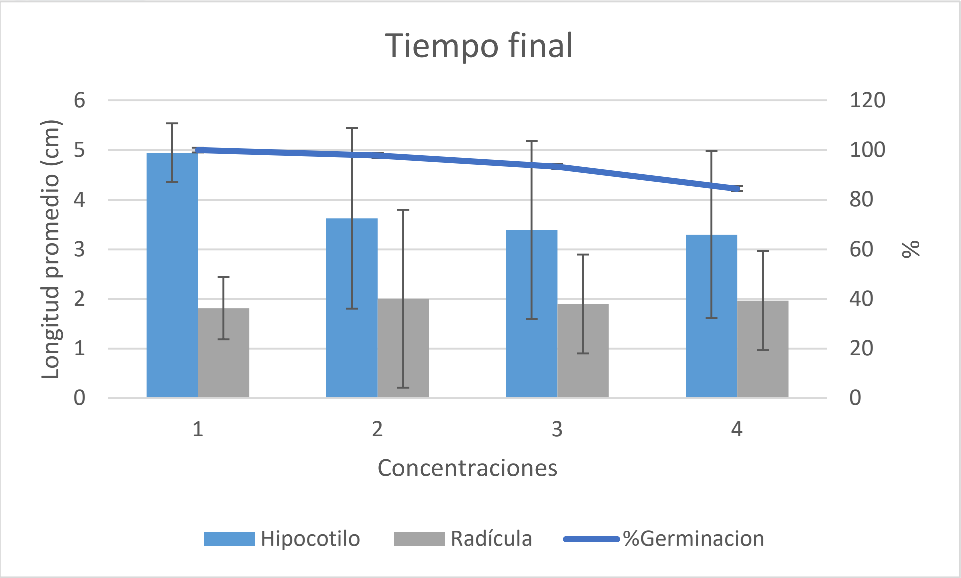


Figure. 3. Percentage of germination and lengths (cm) of hypocotyls and radicles of *Lactuca sativa* seedlings at the final time. Results are shown as means ± standard deviation (n = 45).

RESULTS

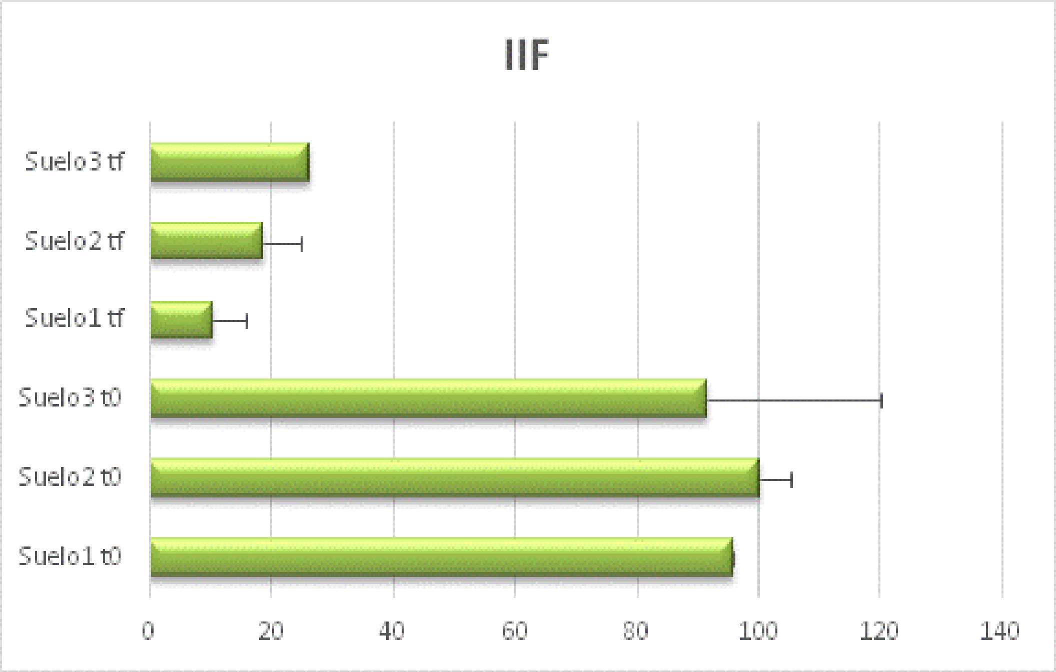


Figure 4. Integral Phytotoxicity Index for the three impacted soils, before and after the phytoremediation treatment. Results are shown as means ± standard deviation (n = 45).

DISCUSSION

The negative effect of the presence of extra-heavy crude oil on seed germination and on the growth of *L. sativa* structures was evidenced, even inhibiting the germination process. The results obtained do not show a direct relationship with the concentration of TPH and its effect on germination, since the toxicity of a hydrocarbon also depends on the complex and variable interaction between the characteristics of the crude and the soil. After the bioremediation treatment, the germination percentage was similar to that of a clean soil. *The soils went from an extreme toxicity condition to a low toxicity condition after treatment. The results* infer that increasing the time in the methodology used could lead to better results in terms of toxicity.

CONCLUSIONS

The biological coupling used showed tolerance to extra-heavy crude oil concentrations in terms of TPH and was an appropriate technology for reducing the toxicity of impacted soils.

ACKNOWLEDGMENTS

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Thank you !