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Para o Desenvolvimento Ambiental, Cultural e Socioeconômico

Xosé Somoza Medina
(organizador)

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PRÓLOGO

Este libro presenta una colección de artículos de investigación que bajo distintos ámbitos de conocimiento realizan avances de interés en la ciencia y la tecnología. La sociedad del siglo XXI se distingue de la de épocas pretéritas por su capacidad analítica. A diferencia de lo que ocurría en otras épocas, en nuestro mundo contemporáneo tenemos demasiada información y avanzar en el conocimiento significa realizar una investigación original sobre otros antecedentes previos y analizar una gran cantidad de datos para poder extraer conclusiones que signifiquen un desarrollo, un avance entre la situación anterior y la posterior, aunque sea a pequeña escala en un contexto local y en un ámbito científico muy concreto. La suma de miles de esos pequeños avances y la interconexión mundial sostienen a la ciencia y la tecnología del siglo XXI.

Este es el objetivo de este libro, realizar avances en la ciencia y la tecnología para el desarrollo ambiental, cultural y socioeconómico, desde un posicionamiento académico, comprometido con el rigor científico y el desarrollo del ser humano.

Para ello se han compendiado veinticuatro artículos de investigación en dos apartados, ciencia y tecnología. En el primer conjunto nos encontramos con artículos que desde las ciencias ambientales o las ciencias sociales realizan propuestas de mejora de aspectos concretos sobre hidrología, regeneración de suelo agrícola, cuidado ambiental, recursos humanos, ciudades igualitarias o paisajes culturales.

En el segundo bloque, se agrupan trabajos de ingeniería química, ingeniería industrial o ingeniería forestal que relatan avances en distintas tecnologías, relacionadas con el biogás de los vertederos de residuos, los usos de nuevos materiales sintéticos, la química de determinados productos y su toxicidad, o las características bioestructurales de la madera de roble.

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SUMÁRIO

I CIENCIAS PARA EL DESARROLLO AMBIENTAL, CULTURAL Y SOCIOECONÓMICO

CAPÍTULO 3

BIORESTORATION OF AN AGRICULTURAL SOIL IMPACTED BY WASTE MOTOR OIL

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ABSTRACT: Soil impacted by 50,000 ppm of hydrocarbon mixtures such as waste motor oil (WMO) exceeds the maximum allowable concentration of 4,400 ppm established by the Mexican environmental standard NOM-138-SEMARNAT-2003. Soil impacted by 50,000 ppm of WMO, it causes loss of fertility. The objectives of this work were: a) biostimulation of the soil impacted by 50,000 ppm WMO, with detergent and mineral solution b) phytoremediation by sowing *Phaseolus vulgaris* and *Zea mays* with *Bacillus licheniformis* and *Rhizobium etli* to reduce the concentration of WMO to a value lower than the maximum allowed by NOM-138-SEMARNAT-2003. This agricultural soil contaminated by WMO was biostimulated and phytoremediated, then the initial and final concentration of WMO was determined by Soxhlet experimental data were analyzed with ANOVA/Tukey ($P<0.05$). The results indicated that biostimulation of soil impacted by 50,000 ppm WMO decreased to 30, 378 ppm in 30 days; indicating that mineral solution induced aerobic heterotrophic microorganisms to mineralize the WMO, increase in the microbial density oxidizing the WMO with 13.7 X 10^6 CFU/g dry soil, a statistically different numerical value compared to the population that oxidized the WMO with 13.2 X 10^5 CFU/g dry soil of the soil without biostimulation or negative control. Subsequently, phytoremediation with *P. vulgaris* and *Z. mays* with *B. licheniformis* and *R. etli* at physiological maturity of both plants,

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reduced the concentration of 30,378 ppm of WMO remaining after biostimulation to 700 ppm and 900 ppm of WMO respectively; both values were lower than the maximum allowed by NOM-138-SEMARNAT-2003. It is concluded that biostimulation of soil impacted by WMO followed by phytoremediation is a sustainable strategy for the removal it to a concentration found in soil not impacted by hydrocarbons, due this soil can be reused in agricultural production.
KEYWORDS: Soil. Hydrocarbons. Plants. *Endophytic bacteria*. Mineralization.

1 INTRODUCTION AND BACKGROUND

Modern agriculture is based on the use of fossil fuels with machinery for preparing the soil and harvesting agricultural crops, which causes spills of gasoline and mixtures of aliphatic and aromatic hydrocarbons [1] such as waste motor oil (WMO). Soil contamination by WMO is a serious environmental problem, since it is also a product of the lubrication of automobiles. In Mexico, it is estimated that more than 325 million liters of WMO are generated, most of which are dumped into the soil, cause contamination by preventing gas exchange, water diffusion, and inhibition of aerobic heterotrophic microbiota activity [2], with economic losses due to decreased agricultural production [3]. According to the General Law of Ecological Balance and Environmental Protection [4], WMO is a hazardous waste, while the official Mexican standard NOM-138-SEMARNAT/SSA1-2003 [5] establishes that the maximum concentration of hydrocarbons in permitted soil it is 4,400 ppm divided into 3 light fractions with 200 ppm, the medium with 1,200 ppm and the heavy with 3,000 ppm. One option for the elimination of WMO in an agricultural soil is biostimulation and phytoremediation. Generally, these biological strategies are applied to soils impacted by hydrocarbons such as WMO individually as the main option to solve this problem that negatively affects agricultural production [6]. Consequently, a sustainable option for this soil is to start biostimulation by applying a detergent that rapidly solubilizes the WMO, followed by a mineral solution that equilibrates the C:N ratio and induces a drastic decrease in the WMO concentration and concluding with sowing. of plants that, being tolerant to WMO, eliminate it in a relatively short time, an action known as phytoremediation. The activity of these plants to destroy WMO could be enhanced by genera and species of growth-promoting bacteria such as Bacillus licheniformis and Rhizobium etli are also applied, with the ability to eliminate WMO, it is possible to decrease its concentration to a value below the maximum established by NOM-138-SEMARNAT/SSA1-2003 [5]. Therefore, the objectives of this research were: a) biostimulation of soil impacted with 50,000 ppm WMO, with a detergent and mineral solution, followed by b) phytoremediation by sowing *Phaseolus vulgaris* and *Zea mays* inoculated with *Bacillus*

licheniformis and *Rhizobium etli*, to reduce the WMO to a value below the maximum allowed by NOM-138-SEMARNAT-2003.

2 MATERIALS AND METHODS

2.1 SITE DESCRIPTION

This research was carried out in the greenhouse of the Environmental Microbiology Laboratory with a surface area of 10 X 10 m^2, where the microclimatic conditions were: temperature of 23.2°C, luminosity of 450 $\mu mol\text{-}m^{-2}\text{-}s^{-1}$ and relative humidity of 67%. For the experiment, soil was collected from an agricultural field called "La Cajita" belonging to Tenencia Zapata, municipality of Morelia, Mich., Mexico located at 19° 39' 27" north latitude, 100° 19' 59" west longitude and at an altitude of 1820 m above sea level.

2.2 BIOSTIMULATION OF SOIL IMPACTED BY 50,000 PPM OF WASTE MOTOR OIL WITH DETERGENT AND MINERAL SOLUTION

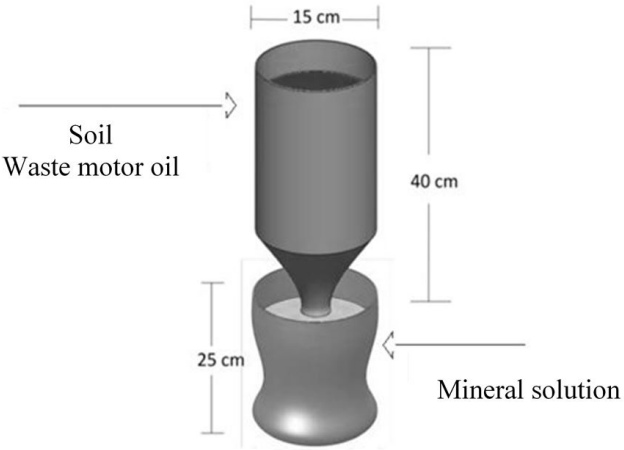
The soil was initially sieved with a No. 20 mesh and then solarized at 70 °C/48 h to eliminate pest and disease problems [7]. The soil was then artificially contaminated with 50,000 ppm WMO obtained from a mechanical workshop in Morelia, Mich, Mexico. Then 1.0 g of the soil with WMO was weighed and placed on top of Leonard jars as shown in Figure 1, where it was biostimulated with commercial detergent at 1% (w/v) and with mineral solution contained in the reservoir at the bottom; both parts were connected with a 20 cm cotton strip to facilitate the movement of water or mineral solution by capillarity to the soil. The research was conducted with an experimental design of 3 treatments and 6 replicates in 2 phases: i) biostimulation of soil impacted by 50,000 ppm WMO with mineral solution with the following chemical composition (g/L): NH_4NO_3 10.0; K_2HPO_4 2.5; KH_2PO_4 2.0; $MgSO_4$ 1.0; NaCl 0.1; $CaCl_2$ 0.1; $FeSO_4$ traces and 10.0 mL/L of oligoelement solution with the following composition (g/L): H_3BO_3 2.86, $ZnSO_4*7H_2O$ 0.22: $MnCl_2*7H_2O$ 1.81; K_2MnO_4 0.09; the solution was adjusted to pH 7.0 [8, 9]. 20 mL of mineral solution was added for 30 days for WMO mineralization. At the end of biostimulation, 15 g of soil was collected and WMO concentration was quantified by Soxhlet.

2.3 PHYTOREMEDIATION OF SOIL IMPACTED BY WASTE MOTOR OIL WITH *PHASEOLUS VULGARIS* AND *ZEA MAYS* PLUS *BACILLUS LICHENIFORMIS* AND *RHIZOBIUM ETLI*

In this phase, a phytoremediation complementary to the biostimulation of the agricultural soil was carried out to reduce the remaining WMO to a concentration below

the maximum accepted by NOM-138-SEMARNAT/SSA1-2003 [5]. To inoculate the seeds of P. vulgaris and *Z. mays*: first, *B. licheniformis* (from the collection of the Environmental Microbiology Laboratory of the Institute of Chemical and Biological Research of the UMSNH) was grown on nutrient agar with the following composition (g/L): casein peptone 5. 0; yeast extract 3.0; 18 (pH 7.0); while *R. etli* was grown on mannitol agar and congo red yeast extract with the following composition (g/L): mannitol 10; K_2HPO_4 0.5; $MgSO_4$ 0.2; congo red solution 1:400 10 mL; NaCl 0.1; yeast extract 10; agar 18; pH adjusted to 6.8. When both *B. licheniformis* and *R. etli* were ready to be used on the seeds of the indicated plants, 1.0 mL of each bacterial genus was taken, equivalent to a cell concentration of $1.5x10^6$ CFU/g of *B. licheniformis* and *R. etli* calculated by viable plate count on yeast mannitol agar and nutrient extract and congo agar respectively. Then, for every 10 seeds of *P. vulgaris* and *Z. mays*, each was inoculated with 1.0 mL of *B. licheniformis* and *R. etli*; 4 seeds of *P. vulgaris* and 4 seeds of *Z. mays* treated with *B. licheniformis* and *R. elti* were immediately taken and sown in agricultural soil with the rest of the WMO. To demonstrate that phytoremediation reduced the remaining WMO concentration in the agricultural soil, the following response variables were used: germination percentage 8 days after planting, as well as phenology and biomass of *P. vulgaris* and *Z. mays* at physiological maturity 60 days after sowing: according to numerical values of plant height (PH) and root length (RL); as well as the values of the fresh weight of the leaf and root part of each plant (FTW): likewise, for the dry weight of leaves and roots of both plants (DTW), they were dried in an oven at 70 °C /24h. The numerical values of *P. vulgaris* and *Z. mays* inoculated with *B. licheniformis* and *R. etli* were compared with the equivalents of *P. vulgaris* and *Z. mays* without inoculation in agricultural soil or WMO irrigated only with water or absolute control (AC), as well as with the same numerical values of *P. vulgaris* and *Z. mays* in soil impacted by WMO, fed with mineral solution, without *B. licheniformis* and *R. etli* considered as negative control (NC). In addition, at the end of phytoremediation, 15 g of soil were taken and the final WMO concentration was determined by the Soxhlet method [10]. All numerical values from this experiment were subjected to analysis of variance (ANOVA) with the Tukey HSD mean test ($P<0.05$) and the statistical programme statgraphics centurion [11].

Figure 1. Design diagram of Leonard jar.



3 RESULTS AND DISCUSSION

Table 1 shows the WMO-mineralizing bacterial population in the soil biostimulated by detergent and mineral solution. The results indicate that in the soil uncontaminated with WMO, irrigated only with water or AC, the native microbial population recorded a value of 97.31 X 10^4 CFU/g dry soil in response to the addition of water and the absence of WMO [12]. On the contrary, in the soil impacted by 50,000 ppm WMO biostimulated with detergent and mineral solution, the bacterial density recorded 13.7 X 10^6 CFU/g dry soil, indicating that the initial biostimulation with detergent solubilized part of the WMO, while the mineral solution enriched the soil with nutrients such as N, P and K, which induced a higher density of the autochthonous heterotrophic aerobic hydrocarbon oxidizing, and therefore, the WMO concentration was decreased [13]. The 97.31 X 10^4 CFU/g dry soil recorded in the AC soil and the 13.7 X 10^6 CFU/g dry soil recorded in the soil impacted with WMO biostimulated with detergent and mineral solution, had a statistical difference compared to the value of the WMO oxidizing bacterial population of 13.2 X 10^5 CFU/g dry soil, recorded in the soil impacted by WMO without biostimulation or NC, indicating that the phytotoxic compounds of WMO inhibited the activity of the aerobic heterotrophic native bacterial population, which also lacked the essential minerals of biostimulation to decrease the concentration of WMO [14].

Table 1. Bacterial population able to oxidize 50,000 ppm waste motor oil in soil biostimulated with detergent and mineral solution.

Soil*	CFU/g dry soil
Absolute control: water (AC)	97.3 X 10^{4c**}
Negative control: waste motor oil (NC)	13.2 X 10^{5b}
Treatment: waste motor oil + detergent + mineral solution	13.7 X 10^{6a}

*Number of replicates (n) =3; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05).

Table 2 shows the biostimulation of soil impacted by 50,000 ppm WMO with detergent and mineral solution, where the concentration of WMO after 30 days was reduced to 30,378 ppm by the action of biostimulation with the detergent that solubilized some carbon hydrocarbons from the WMO and made them available for aerobic heterotrophic microorganisms to initiate partial mineralization of the WMO. Then the application of the mineral solution with the main macroelements of the type NH_4NO_3 and K_2HPO_4/KH_2PO_4 enriched and adjusted the soil imbalance, and induced the native hydrocarbon oxidizing microbiota to decrease WMO by up to 39.29% [15, 16]. In contrast, in soil without biostimulation or NC, natural attenuation did not reduce WMO, as the excess of this hydrocarbon mixture inhibited aerobic heterotrophic soil bacterial activity to remove WMO [17].

Table 2. Concentration of waste motor oil remaining after 30 days of biostimulation with detergent and mineral solution.

Soil*	Initial concentration WMO (ppm)	Final concentration WMO (ppm)	Percentage (%) of mineralization WMO	Remaining WMO (%)
Absolute control: water (AC)	0^{d**}	0^{d}	0^{d}	0^{d}
Negative control: waste motor oil (NC)	$50{,}000^{a}$	$40{,}000^{a}$	20^{b}	80^{a}
Treatment: waste motor oil + detergent + mineral solution	$50{,}000^{a}$	$30{,}378^{b}$	39.29	60.75^{b}

*n =4; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05). WMO=Waste motor oil.

Table 3 shows 41.66% germination of *P. vulgaris* in soil not contaminated by WMO, irrigated with water used as AC; this numerical value was statistically different compared to 79.16% germination of *P. vulgaris* enhanced with *B. licheniformis* and *R. etli* in soil impacted by 30,378 ppm WMO. This indicates that the remaining WMO after biostimulation did not inhibit the germination of *P. vulgaris*, nor the beneficial activity of *B. licheniformis* and *R. etli*, as both bacteria converted seed exudates into phytohormones that made seed germination possible [18, 19]. The 41.66% germination recorded in *P. vulgaris* in AC soil was statistically different in relation to the 66.6% germination of *P. vulgaris* in a soil impacted by 50,000 ppm WMO without biostimulation used as NC, indicating that the percentage decrease in germination of *P. vulgaris* was due to excess WMO [20].

Table 3. Percent germination of *P. vulgaris* with *B. licheniformis* and *R. etli* during phytoremediation of soil impacted by 30,378 ppm of waste motor oil.

P. vulgaris* in soil	**Germination percentage (%)**
Absolute control: water (AC)	41.66^{c**}
Negative control: 40,000 ppm waste motor oil (NC)	66.6^{b}
Treatment 1: 30,378 ppm waste motor oil + detergent + mineral solution + seed inoculation with *B. licheniformis* and *R. etli*	79.16^{a}

*n =6; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05).

Table 4 shows that *Z. mays* seeds with *B. licheniformis* and *R. etli* in soil impacted by 30,378 ppm WMO, recorded 87.5% germination after 8 days, suggesting that both *B. licheniformis* and *R. etli* converted seed exudates into phytohormones for a higher germination percentage of *Z. mays*. Furthermore, this result shows that biostimulation using the detergent was sufficient to solubilize WMO and avoid a toxic effect on seeds, while biostimulation with mineral solution was useful for *Z. mays* germination as it adjusted the C:N imbalance in the soil impacted by WMO [9]. The numerical value of *Z. mays* with *B. licheniformis* and *R. etli* at this stage was statistically different compared to that for *Z. mays* with 54.16% germination in the soil used as AC, indicating that irrigation was not sufficient to break embryo dormancy and induce better germination of *Z. mays* [21], while 70.83% germination of *Z. mays* in soil impacted by 50,000 ppm WMO or NC supports that the hydrophobic properties of WMO partially reduced both water and nutrient exchange indispensable for germination [22].

Table 4. Percent germination of *Z. mays* with *B. licheniformis* and *R. etli* during phytoremediation of soil impacted by 30,378 ppm of waste motor oil remaining after biostimulation.

Z. mays* in soil	**Percentage of germination (%)**
Absolute control: water (AC)	54.16^{c**}
Negative control: 40,000 ppm waste motor oil (NC)	70.83^{b}
Treatment 2: 30,378 ppm waste motor oil + detergent + mineral solution + seed inoculation with *B. licheniformis* and *R. etli*	87.5^{a}

*n =6; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05).

Table 5 shows the phenology of *P. vulgaris* at physiological maturity enhanced with *B. licheniformis* and *R. etli* in soil impacted with WMO biostimulated with detergent and mineral solution; there it recorded 23.25 cm plant height (PH) and 29.5 cm root length (RL); in *P. vulgaris* planted in soil without WMO irrigated with water or AC, it recorded 23.37 cm PH and 28.87 cm RL. These numerical values showed statistical difference when

compared with the 16.5 cm PH and 23.25 cm RL of *P. vulgaris* grown in soil impacted with WMO without biostimulant. This suggests that, *B. licheniformis* and *R. etli* transformed root exudates into plant growth-promoting compounds that induced *P. vulgaris* development and root proliferation, which improved its tolerance to phytotoxicity [23]. In relation to fresh biomass, *P. vulgaris* in soil not contaminated by WMO or AC recorded 1.41 g total fresh weight (TFW), while *P. vulgaris* in soil contaminated with 40,000 ppm of WMO without biostimulant used as NC, recorded 1.36 g TFW; both values had statistical difference in comparison with the 1.12 g TFW of *P. vulgaris* inoculated with *B. licheniformis* and *R. etli* planted in soil contaminated by 30,387 ppm of biostimulated WMO. While in dry biomass, *P. vulgaris* in soil used as AC recorded 0.17 g total dry weight (TDW); *P. vulgaris* in soil with 40,000 ppm WMO used as NC recorded 0.13 g TDW. These values showed statistical difference compared with the 0.2 g TFW of *P. vulgaris* enhanced with *B. licheniformis*/*R. etli* planted in soil contaminated by 30,387 ppm of biostimulated WMO. This indicates that the 30,378-ppm concentration of WMO remaining after biostimulation did not affect the healthy growth of *P. vulgaris* [24]; furthermore, when inoculated with *B. licheniformis* and *R. etli*, these bacteria were able to convert the radical exudates into phytohormones. Simultaneously *B. licheniformis*/*R. etli* were able to mineralize WMO aromatics at the rhizosphere level, as has been reported by Lopez-Ortiz *et al*., 2012 [9] who, in agricultural soils impacted by naphthalene and phenanthrene, applied phytoremediation using *Leucaena leucocephala* inoculated with *Rhizobium tropici*, where a higher biomass was recorded despite the high concentration of hydrocarbons, than when *L. leucocephala* was not inoculated with *R. tropici.*

Table 5. Phenology and biomass of *P. vulgaris* with *B. licheniformis* and *R. etli* during phytoremediation of soil impacted by 30,387 ppm of waste motor oil remaining after biostimulation.

P. vulgaris* in soil	**Plant height (cm)**	**Root length (cm)**	**Total fresh weight (g)**	**Total dry weight (g)**
Absolute control: water (AC)	23.37^{a**}	28.87^{a}	1.41^{a*}	0.17^{b}
Negative control: 40,000 ppm waste motor oil (NC)	16.5^{b*}	23.25^{b}	1.36^{b}	0.13^{c}
Treatment 1: 30,387 ppm waste motor oil + detergent + mineral solution + inoculation of seeds with *B. licheniformis* and *R. etli.*	23.25^{a}	29.5^{a}	1.12^{c}	0.2^{a}

*n =6; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05).

Table 6 shows the phenology of *Z. mays* at physiological maturity enhanced with *B. licheniformis* and *R. etli* planted in soil impacted by 30,387 ppm of WMO biostimulated with detergent and mineral solution, where a PH of 29.5 cm was recorded. This numerical

value presented a statistical difference in comparison with the 13.5 cm PH of *Z. mays* cultivated in soil not contaminated by WMO and irrigated with water used as AC, as well as with the 35.7 cm PH of *Z. mays* planted in soil impacted by 40,000 ppm WMO without biostimulation or NC. Numerical phenology data when *Z. mays* were enhanced with *B. licheniformis/R. etli* suggest that the bacteria transformed seed and root exudates into phytohormones that enhanced the tolerance of *Z. mays* to the phytotoxic effect of WMO [25]. On the other hand, *Z. mays* with *B. licheniformis* and *R. etli* in soil with 30,387 ppm biostimulated WMO, recorded 31 cm RL, a statistically different value in relation to the 29.7 cm RL of *Z. mays* grown in uncontaminated soil with WMO used as AC, and to the 25 cm RL of *Z. mays* in soil impacted by 40,000 ppm WMO used as NC. In fresh biomass, *Z. mays* with *B. licheniformis/R. etli* in soil impacted by 30,378 ppm biostimulated WMO recorded 0.70 g TFW, while *Z. mays* in soil uncontaminated by WMO used as AC recorded 0.41 g TFW. Both numerical values presented statistical differences compared to the 1.44 G TFW of *Z. Mays* in soil impacted by 40,000 ppm WMO not biostimulated referred to as NC. In dry biomass, *Z. mays* with *B. licheniformis* and *R. etli* in soil impacted by 30,378 ppm of biostimulated WMO recorded 0.15 g TFW, a value similar to that recorded in *Z. mays* grown in soil with WMO without biostimulant. The above showed that the biomass of *Z. mays* despite being inoculated with *B. licheniformis/R. etli* was negatively affected due to the remaining concentration of WMO after biostimulation. This is possible because the fraction of WMO limited water uptake and gas exchange by the formation of a hydrophobic layer, which caused inhibition of root growth [24].

Table 6. Phenology and biomass of *Z. mays* with *B. licheniformis* and *R. etli* during phytoremediation of soil impacted by 30,387 ppm of waste motor oil remaining after biostimulation.

Z. mays* in soil	**Plant height (cm)**	**Root length (cm)**	**Total fresh weight (g)**	**Total dry weight (g)**
Absolute control: water (AC)	13.5^{c**}	29.7^{b}	0.41^{c*}	0.11^{b}
Negative control: 40,000 ppm waste motor oil (NC)	35.7^{a}	25^{c}	1.44^{a}	0.15^{a}
Treatment 2: 30,387 ppm waste motor oil + detergent + mineral solution + inoculation of seeds with *B. licheniformis* and *R. etli.*	29.5^{b}	31^{a}	0.70^{b}	0.15^{a}

*n =6; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05).

Table 7 shows the WMO concentration at the end of agricultural soil biostimulation and phytoremediation; when P. vulgaris was sowed with B. licheniformis and R. etli at physiological maturity level, the maximum decrease of WMO from 30,378 ppm to 700

ppm in 60 days was detected; while Z. mays with B. licheniformis and R. etli reached a decrease from 30,370 ppm to 900 ppm WMO in 60 days. Both numerical values of WMO concentration were lower than the maximum level accepted by NOM-138-SEMARNAT/SSA1-2003 [5]. This fact shows that both B. licheniformis and R. etli increased the mineral uptake capacity of P. vulgaris and Z. mays roots, and at the same time B. licheniformis and R. etli were able to mineralize WMO aromatic used as a carbon and energy source; this increased the phytodegradation capacity of P. vulgaris and Z. mays roots to decrease the concentration value of remaining WMO in agricultural soil to a concentration value below the maximum accepted by NOM-138-SEMARNAT/SSA1-2003 which ensured that this soil could be reused for agricultural production [5, 26].

Table 7. Concentration of waste motor oil, after biostimulation with detergent and mineral solution, and phytoremediation by *P. vulgaris* and *Z. mays*.

Soil*	Initial concentration WMO (ppm)	Final concentration WMO (ppm)	Percentage (%) of mineralization WMO	Remaining WMO (%)
Absolute control: water (AC)	0^{d**}	0^{d}	0^{d}	0^{d}
Negative control: 40,000 ppm waste motor oil (NC)	$50{,}000^{a}$	$40{,}000^{a}$	20^{c}	80^{a}
Treatment 1: 30,387 ppm waste motor oil + detergent + mineral solution + *P. vulgaris* with *B. licheniformis* and *R. etli*	$50{,}000^{a}$	700^{c}	98.6^{a}	1.4^{c}
Treatment 2: 30,387 ppm waste motor oil + detergent + mineral solution + *Z. mays* with *B. licheniformis* and *R. etli*	$50{,}000^{a}$	900^{c}	98.2^{a}	1.8^{b}

*n =4; ** values with different letters indicate statistical difference according to ANOVA/Tukey (P<0.05). Waste motor oil (WMO).

4 CONCLUSION

The use of fossil fuels in agriculture has caused the contamination of agricultural soil by WMO that contributes to climate change due the loss of plant productivity. This demands an alternative sustainable solution such as biostimulation and phytoremediation that allows the reuse of soil without risk to human, animal and plant health.

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6 CONFLICT OF INTEREST STATEMENT

The participants in this research assure that there is no conflict of interests related to the planning, execution and reporting of this research that compromises the value of the results obtained or their consequences in scientific, technical, or any other type of terms.

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SOBRE O ORGANIZADOR

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