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Corresponding author

Juan Manuel Sánchez-Yáñez, Environmental Microbiology Laboratory, Chemical-Biological Research Institute, Ed-B3. Universidad Michoacana de San Nicolás de Hidalgo. Francisco J. Mujica S/N, Col. Felicitas del Rio C.P. 58000, Morelia, Mich, México

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Research Article

# An Sustainable Strategy for Reuse Agriculture Soil Impacted by A Mixture of Hydrocarbons

**Karla Violeta Sánchez-Ortiz, Blanca Celeste Saucedo-Martínez and Juan Manuel Sánchez-Yáñez***

*Environmental Microbiology Laboratory, Chemical-Biological Research Institute, Universidad Michoacana de San Nicolás de Hidalgo, México*

## Abstract

In world agriculture; such as the one implemented in Mexico; the use of fossil fuels in machinery for soil preparation and harvesting of an agricultural crop is common. As a result; waste residual oil (WMO) is generated in that sese agricultural soil impacted by 80;000 ppm of WMO exceeds the limit of 4;400 ppm of hydrocarbons according to Mexican environmental regulation NOM-138-SEMARNAT/SS-2003 (NOM-138). WMO causes loss of fertility and environmental pollution. A sustainable strategy of solution is biostimulation and phytoremediation. The aims of this research were:

i) Biostimulation of an agricultural soil impacted by 80;000 ppm WMO then by

ii) Phytoremediation sowing *H. annuus* inoculated with *B. vietnamiensis* and *P. polymyxa* to reduce WMO at concentration value below the NOM-138 maximum. The variable-response variables of WMO bioelimination from an agricultural soil were: initial and final WMO concentration by Soxhlet; phenology and biomass of *H. annuus* with *B. vietnamiensis* and *P. polymyxa*. Experimental data were validated by ANOVA/Tukey HSD P<0.05 %. The results indicate that biostimulation and phytoremediation sowing *H. annus* with *B. vietnamiensis* and *P. polymyxa* were effective in reducing WMO concentration from 80;000 ppm to 1000 ppm; a value below the maximum limit of NOM-138 an evidence of soil bioremediation. It is concluded that a sustainable strategy to recover the agricultural productivity of a soil impacted by mixture of hydrocarbons It is possible by exploiting the natural microbial heterotrophic aerobic capacity that in combination with plants that tolerate and mineralize hydrocarbon mixtures such as WMO.

## Introduction

In world agriculture; such as the one implemented in Mexico; the use of fossil fuels in machinery for soil preparation and harvesting of an agricultural crop is common Waste Motor Oil (WMO) is composed of mixtures of aliphatic and aromatic Hydrocarbons (HCs) and is one of the main pollutants in the environment; especially in the soil [1]. In Mexico; (WMO) is classified as hazardous waste by the General Law of Ecological Balance and Environmental Protection (LGEEPA); which establishes that it must be recycled or disposed of to prevent soil contamination. When WMO is accidentally spilled into the soil; it negatively alters the main physicochemical properties of the soil; starting with the formation of a layer that prevents gas exchange with the atmosphere; which inhibits the mineralization capacity of native aerobic heterotrophic aerobic microorganisms for the elimination of WMO; which participate in the biogeochemical cycles that sustain life [2], and the agricultural productivity of the soil is lost [3]. Due that Mexican environmental regulation NOM-138-SEMARNAT/SS-2003 (NOM-138) establishes the maximum HCs concentration recognized as a risk to soil health at 4;400 ppm. In general; when a soil is contaminated by WMO it is remediated with chemical methods with strong oxidizing agents; which have disadvantage of high cost and negative side effects for the environment [4]. An alternative ecological solution is biostimulation; which promoting the metabolic capacity of aerobic heterotrophic microorganisms to eliminate HCs from the soil to mineralize WMO [5], first BIS with a detergent that emulsifies the WMO. This is followed by a BIS with a mineral solution that supplies the soil with N (nitrogen); K (phosphorus); and (K) potassium salts to balance the C: N (carbon: nitrogen) ratio caused by excess WMO [6], where native microorganisms are induced to partially oxidize WMO [7]. Subsequently; a BIS with a vermicompost; simultaneously with the seeding of Vicia sativa or green manure [8]. Biostimulation enrich the soil with organic and inorganic N compounds that induce native soil microorganisms; to mineralize the aliphatic fraction of WMO; to cometabolism of aromatic hydrocarbons WMO mineralization [9]. During BIS; it is necessary to adjust soil moisture to 80% of field capacity; that ensures gaseous and water exchange; to induce the maximum oxidation of WMO [10]. Then WMO bioremoval is concluded with phytoremediation by *Helianthus annuus* [11,12] due phytodegradation at root system level; effectively reduces the concentration of WMO. Phytodegradation is an action of the plant root system for the removal of WMO; that can be enhanced by: endophytic plant growth promoting bacteria as well as: *Burkholderia vietnamiensis and Paenibacillus polymyxa* that accelerate the mineralization of the aromatic fraction of WMO [13], in reducing its concentration value below the maximum value accepted by NOM-138. It is reported in the literature that soil impacted by high concentrations of HCs mixtures such as WMO can treated by biostimulation or phytoremediated individually; but these actions are insufficient to reduce its concentration to a value below the maximum accepted by NOM-138 [14]. The aims of this research were: i) biostimulation of an agricultural soil impacted by 80;000 ppm WMO and ii) phytoremediation sowing *Helianthus annuus* inoculated with *B. vietnamiensis* and *P. polymyxa* to reduce WMO at value below the maximum accepted by NOM-138.

## Material and Methods

This research was carried out in the greenhouse of the Environmental Microbiology Laboratory of the Chemical Biological Research Institute of the UMSNH; Morelia; Mich.; Mexico. In this greenhouse the microclimatic conditions were temperature 23.2°C; luminosity 450 µmol-m-2-s-1 and relative humidity 67%.

### Agricultural soil origin and preparation

An agricultural soil belongs to a site located at 19º 39' 27" north latitude 100º 19' 59" west longitude; with an altitude of 1;820 meters above sea level (masl) temperate climate; in an agricultural place called "La cajita" of Tenencia Zapata in the municipality of Morelia; Mich.; Mexico; on km 5 of the Morelia-Pátzcuaro highway; Mich (Table 1) shows the main physicochemical properties of the soil according to NOM-021-RECNAT 2000. The soil was solarized for 48 h; to reduce the problem of pests and plant diseases; sieved with a No. 20 mesh [15]; then contaminated by 80;000 ppm of WMO collected in an automotive mechanic workshop in the city of Morelia; Michoacán; Mexico; dissolved in 20 mL of Roma® detergent at 1.0 % (w/v) that was the first way to begin with the bioremediation process of the agricultural soil. The initial concentration of WMO was determined by the Soxhlet method [16]. Then 1.0 kg of soil was placed in the upper part of the Leonard jar (Figure 1) with mineral solution (during biostimulation and

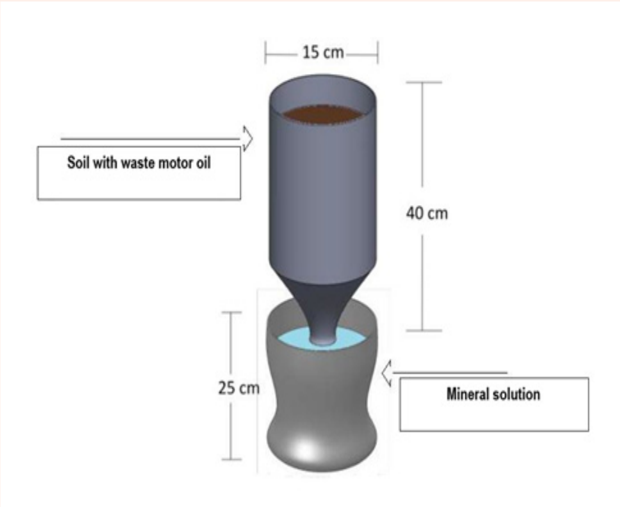


**Figure 1:** Leonard´s jar diagram.

**Table 1:** Physicochemical properties of soil nopolluted by waste motor oil.

| Parameter | Value | Interpretation |
|---|---|---|
| pH | 5.55 | Moderately acid |
| Organic matter | 6.86% | Medium* |
| Cation exchange capacity | 34.00 Cmol(+) $Kg^{-1}$ | High |
| Texture | 18.9 % Ac-19.5 % L-61.6 % Ar | Franco-sandy |
| Real density (Rd) | 2.04 $g/cm^3$ | No interpretation in NOM |
| Apparent density (Ad) | 0.89 $g/cm^3$ | Common in volcanic soil |
| Porosity** | 56.38% | Slightly high + |
| Percentage of moisture saturation | 34.30% | Normal+ |
| Field capacity*** | 15.79% | Normal+ |
| Usable moisture | 7.90% | Normal+ |

Ar: sand, L: silt, Ac: clay, *for soils of volcanic origin, **calculated from Ad and Rd; *** estimated from texture, +reported for sandy loam soils. NOM-021-RECNAT-2000

### Biostimulation of soil impacted by 80;000 ppm of waste motor oil

This research was conducted in two phases; with the experimental design with 3 treatments; 2 controls and 6 replicates:

i. Soil not polluted by WMO; fed with a mineral solution and vermicompost or relative control (CR);

ii. Soil impacted by WMO irrigated with water or negative control (NC).

iii. Soil contaminated by WMO biostimulated first with a mineral solution with the following chemical composition was ($gL^{-1}$): $NH_4NO_3$; 10. 0; $K_2HPO_4$; 2.5; $KH_2PO_4$; 2.0; $MgSO_4$; 0.5; NaCl; 0.1; $CaCl_2$; 0.1; $FeSO_4$; trace and 1.0 mL/L of a microelement solution with the following composition ($gL^{-1}$): $H_3BO_3$; 2.86; $ZnSO_4$*$7H_2O$; 0.22; $MgCl_2$*$7H_2O$; 1.81 and pH adjusted to 6.8. A biostimulation of soil polluted by WMO 18.0 mL of MISO; applied every third day for one month; to maintain moisture at 80 % of field capacity (Wendlassida et al.; 2020) in the second biostimulation it was enriched with a 3 % vermicompost for 2 months. For the third biostimulation; Vicia sativa was sown in pots with agricultural soil not pollutes by WMO; it was allowed to grow until seedling; one month later it was fallowed and incorporated to enrich the soil impacted by WMO as green manure for 2 months [8], at the end of this phase the WMO concentration was quantified by the Soxhlet method [16].

### Phytoremediation of soil contaminated by waste motor oil; sowing *Helianthus annuus* inoculated with *Burkholderia vietnamiensis and Paenibacillus polymyxa*

In this step; *B. vietnamiensis* and *P. polymyxa* I endophytic plant growth promoting bacteria isolated from roots of Zea mays var mexicana or teosinte (ancient maize) in that sense: *B. vietnamiensis* was reproduced on Pseudomonas cepacia azelaic acid and tryptamine agar (PCAATA) with the following composition ($gL^{-1}$): MgSO4 0. 1; azelaic acid 2.0; tryptamine 0.4; $K_2HPO_4$ 4.0; $KH_2PO_4$ 4.0; yeast extract 0.02; agar 18.0; pH was adjusted to 6.8; PCAATA was incubated at 30 °C/24 h. While *P. polymyxa* was grown on nutrient agar (NA) with the following composition ($gL^{-1}$): meat extract 3.0; meat peptone 5.0; agar 18.0; pH adjusted to 7.0; which incubated at 30°C/72 h; then *H. annuus* seeds were disinfected with Clorox®/2.5 min and 70 % alcohol/2. 5 min; washed with sterile water four times; every 20 seeds were inoculated with 0.5 mL of *B. vietnamiensis* and/or *P. polymyxa* with a bacterial density of each equivalent to 1 x106 CFU/ml per viable plate count in PCAATA and NA; respectively; which were sown in soil not polluted by WMO; there *H. annuus* germinated; after that it was transplanted into soil not polluted by WMO. *H. annuus* germinated; it was transplanted to soil polluted by WMO remaining from the biostimulation; according to the experimental design with 5 treatments; 2 controls and 6 replicates:

i. Soil not polluted by WMO; fed with a mineral solution or RC; there *H. annuus* was transplanted without *B. vietnamiensis* and/or *P. polymyxa*;

ii. Soil impacted by WMO without biostimulation or NC; there *H. annuus* was transplanted without *B. vietnamiensis* and/or *P. polymyxa* and iii) soil contaminated by 37;600 ppm WMO remaining from the BIS; there *H. annuus* inoculated with *B. vietnamiensis* and/or *P. polymyxa* was transplanted fed with mineral solution. The variable-response during Phytoremediation of the soil impacted by WMO sowing *H. annuus* inoculated with *B. vietnamiensis* and/or *P. polymyxa* at pre-flowering were phenology: plant height (PH); Root Length (RL); biomass: Aerial and Root Fresh Weight (AFW)/(RFW) and Aerial and Root Dry Weight (ADW)/(RDW). At the end of this phase; the concentration of WMO remaining in the soil was determined by Soxhlet [16].

### Statistical analysis

The experimental data were validated by ANOVA/Tukey HSD P<0.05 % with the statistical program Statgraphics Centurion XVI.I [17].

## Results

(Table 2) shows the biostimulation of soil contaminated by 80;000 ppm WMO; was reduced to 37;600 ppm in 5 months; this value was statistically different compared to 79;200 ppm WMO in soil polluted by WMO not biostimulated used as NC.

**Table 2:** Biostimulation of soil impacted by 80,000 ppm of waste motor oil.

| * Soil with waste motor oil | Waste motor oil (ppm) | |
|---|---|---|
| | Initial | Final |
| Irrigated with water or negative control (NC) | 80,000[a] | 79,200b*** |
| **Biostimulated | 80,000[a] | 37, 600[a] |

*n=6; ** Sequential biostimulation with mineral solution, vermicompost and Vicia sativa as green manure *** Different letters with statistical difference at 0.05 % according to Tukey.

(Table 3) shown the phenology and biomass of *H. annuus* with *P. polymyxa* at pre-flowering during phytoremediation of agricultural soil impacted by WMO: with 35.71 cm PH and 7.67 cm RL registered; numerical values statistically different to 23.38 cm PH and 4.38 cm RL of *H. annuus* with *B. vietnamiensis* biostimulated. While in terms of biomass of *H. annuus* with *B. vietnamiensis* and/or *P. polymyxa* in soil impacted by WMO reached 2.04 g AFW and 0.45 g RFW both numerical values had no statistical difference compared to 2.45 g AFW and 0.41 g RFW of *H. annuus* without *B. vietnamiensis* or *P. polymyxa* in soil not contaminated by WMO or RC. While *H. annuus* with *B. vietnamiensis* and *P. polymyxa* in agricultural soil impacted by WMO 0.80 g ADW and 0.06 g RDW; these numerical values had no statistical difference compared to the 0. 29 g ADW and 0.04 g RDW of *H. annuus* with *P. polymyxa* in soil polluted by WMO; with 0.62 g ADW and 0.03 g RDW of *H. annuus* uninoculated with *B. vietnamiensis* and/or *P. polymyxa* used as RC.

**Table 3:** Phenology and biomass of *Helianthus annuus* inoculated with *Burkholderia vietnamiensis and Paenibacillus polymyxa* after phytoremediation of soil impacted by 37,600 ppm of waste motor oil remaining from biostimulation.

| **Helianthus Annuus* in Soil | Plant Height (cm) | Root Length (cm) | Fresh Weight (g) | | Dry weight(g) | |
|---|---|---|---|---|---|---|
| | | | Aerial | Root | Aerial | Root |
| 79,200 ppm of WMO + water (negative control) | 0 ± 0c | 0 ± 0c | 0 ± 0c | 0 ± 0c | 0 ± 0c | 0 ± 0c |
| Not polluted by WMO + MISO (relative control) | 39.0 ± 6.63[a]** | 5.8 ± 0.83ab | 2.45 ± 0.71[a] | 0.41 ± 0.30[a] | 0.62 ± 0.41[ab] | 0.03 ± 0.017[ab] |
| 37,600 ppm of WMO + *H. annuus* + *B. vietnamiensis* + MISO | 23.38 ± 2.28b | 4.83 ±0.28b | 1.14 ± 0.12b | 0.06 ± 0.04b | 0.07 ± 0.0b | 0.02 ± 0.016b |
| 37,600 ppm of WMO + *H. annuus* + *P. polymyxa* + MISO | 35.71 ± 8.71[a] | 7.67 ± 1.75[a] | 1.23± 0.16[ab] | 0.20 ± 0.11[ab] | 0.29 ± 0.36[ab] | 0.04 ± 0.027[ab] |
| 37,600 ppm of WMO + *H. annuus* + *B. vietnamiensis/P. polymyxa* + MISO | 34.38 ± 4.46ab | 6.08 ±1.43ab | 2.21 ± 1.04ab | 0.45 ± 0.18a | 0.8 ± 0.67a | 0.06 ± 0.029a |

(Table 4) shown the biostimulation of the soil impacted by 80;000 ppm of WMO; that decreased to 37;600 ppm; followed in the final stage of phytoremediation; the agricultural soil impacted by 37;600 ppm of WMO when H. annus was inoculated with *B. vietnamiensis*; the WMO was reduced to 1500 ppm; while H. annus was inoculated with *P. polymyxa*; the WMO was reduced to 2500 ppm. Finally; phytoremediation was applied sowing *H. annuus* inoculated with *B. vietnamiensis* and *P. polymyxa*; there the maximum decrease in WMO concentration registered an reduction to 1000 ppm in 2 months; this numerical value was lower than the maximum limit established by NOM-138; this numerical value was statistically different compared to soil polluted by 76;824 ppm of WMO not biostimulated or phytoremediated used as NC in the same period of time.

**Table 4:** Concentration of waste motor oil remaining from phytoremediation sowing *Helianthus annuus* inoculated with *Burkholderia vietnamiensis and Paenibacillus polymyxa*.

| *Soil with Waste Motor Oil | Waste Motor Oil (ppm) | |
|---|---|---|
| | Final Biostimulation | Final Phytoremediation |
| Irrigated with water or negative control (NC) | 79,200b*** | 76,824d |
| ***Helianthus annuus* with Burkholderia vietnamiensis | 37, 600[a] | 1,500b |
| ** *H. annuus* with Paenibacillus polymyxa | 37, 600[a] | 2.500c |
| ***H. annuus* with *B. vietnamiensis* and *P. polymyxa* | 37, 600[a] | 1,000[a] |

*n=6; ** Sequential biostimulation with mineral solution, vermicompost and *Vicia sativa* as green manure. ***Different letters with statistical difference at 0.05 % according to Tukey.

## Discussion

(Table 3) shows the sequential BIS of the soil impacted by 80;000 ppm WMO which decreased to 37;600 ppm in 5 months that shown that it is essential to start biostimulation with detergent for emulsification of hydrocarbons of WMO to ensure its mineralization. Followed by biostimulation with mineral solution to enrich soil with $NH_4NO_3$ to balance the C:N ratio necessary for WMO mineralization; in that sense $K_2HPO_4$ and $KH_2PO_4$ salts accelerated a WMO removal [18]. In the third biostimulation with vermicompost; nitrogenous organic compounds (N) such as urea and nucleotides; as well as organic carbon compounds (C) such as cellobiose and glucose; were added to induce heterotrophic aerobic microorganisms to co-metabolize WMO hydrocarbons and decrease their concentration [19,13, 20] While BIS in sequence with V. sativa or green manure enriched the soil with organic N compounds: peptides and amino acids; to maintain the balance of the C:N ratio; as well as with simple organic C compounds to maintain the cometabolism of WMO hydrocarbons to decrease the concentration of WMO [8]. During soil biostimulation; moisture was retained at 80% of field capacity that facilitated water and gas exchange that accelerated WMO bioremoval [10]; compared to soil not biostimulated or NC; there the concentration of the hydrocarbons mixture of WMO did not vary due to the excess of C; that inhibited the capacity of WMO mineralization by native heterotrophic aerobic soil microorganisms; in addition to the deficit of inorganic and organic N that complicates the possibility of bioremoval of WMO removal [21,22].

(Table 4) shown the sequential biostimulation of soil impacted by 80;000 ppm WMO; that reduced to 37;600 ppm; followed by phytoremediation sowing *H. annuus* since it has been reported that in the roots of *H. annuus* there are extracellular enzymes of lignin synthesis metabolism; associated with protection to chemical and/or biological agents; that are suggested to partially degradation some aromatics of WMO [23]. In that sense phytoremediation of soil polluted by WMO sowing H.annus enhanced with *B. vietnamiensis* and *P. polymyxa* was achieved the maximun reduction in WMO concentration to 1;000 ppm registered in 2 months; at the same time *H. annuus* without WMO phytotoxicity stress; supported that *B. vietnamiensis* and *P. polymyxa* converted *H. annuus* seed exudates and organic compounds derived from root metabolism into phytohormones ; which increased the root uptake capacity of the minerals N; PO4-3 (phosphates) and K (potassium); that increases the tolerance of *H. annuus* to WMO [25]. While it is supported that *B. vietnamiensis* and *P. polymyxa* at the root level of *H. annuus* had a positive effect on the partial hydrolysis of some WMO aromatics and effective mineralization that reduced their concentration; to concentration value lower than the highest value accepted by NOM-138 [24,25]. On the opposite way; *H. annuus* without *B. vietnamiensis* or *P. polymyxa* in the non-biostimulated or NC soil; there the excess of WMO forms a hydrophobic film that adheres to the *H. annuus* seeds and prevented germination and caused their death. This shown that WMO not only negatively affects the soil; but also prevents the cultivation of domestic plants; if WMO is mineralized; then its concentration decreases; so, without phytotoxicity it is feasible to reuse the soil in the production of agricultural crops safe for consumption. human and animal [26,12]. While in the soil not biostimulated the excess WMO was phytotoxic and killed *H. annuus*; that prevents plant growth for sustainable agricultural production [27], so the combination of biostimulation and phytoremediation are necessary for the bioremediation of agricultural soil impacted by HCs mixtures as well as WMO [28-34].

## Conclusion

For the contamination of an agricultural soil impacted by mixture of hydrocarbons of WMO; it is possible to use a sustainable ecological solution that allows the reduction of the WMO concentration for the *H. annuus* planting; whose WMO mineralization capacity can be accelerated and increased by the inoculation of *B. vietnamiensis* and *P. polymyxa* for a bioremoval of the WMO that allows the reuse of the soil in a safe agricultural production for human and animal consumption.

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## Conflicts of Interest

The authors declare that there is no type of conflict of interest in its planning; execution and writing with the institutions involved; as well as those that financially supported this research.

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