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Article

Recovering of soil contaminated by hydrocarbons mixing

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Abstract: In Mexico, an agricultural soil poor in nitrogen (N) contaminated by a hydrocarbon derivative such as automotive residual oil (ARA), with a relatively high concentration of 100,000 ppm, is an environmental problem, but also because it drastically affects soil properties associated with the mineralization of organic matter and loss of fertility, since it exceeds the maximum accepted limit of 4,400 ppm of the Mexican standard called, NOM-138-SEMARNAT-2012 (NOM-138). An alternative solution is to treat it with ecological actions to eliminate the ARA and recover fertility. Therefore, the objectives of this research were: i) bioremediation of soil contaminated by 100000 ppm of ARA ii) phytoremediation using Sorghum vulgare with Aspergillus inger and Penicillium chrysogenum to decrease ARA to a value below 4400 ppm of NOM-138. For this purpose, soil recovery was performed using the variable-response: disappearance of ARA by Soxhlet at the beginning and after bioremediation and at the end of phytoremediation with S. vulgare with phenology and biomass to seedling. All experimental data were validated by ANOVA/Tukey HSD $P < 0.05\%$. The results indicated that bioremediation and phytoremediation of soil contaminated by 100,000 ppm of ARA, decreased it to 3400 ppm, a value lower than the maximum established by NOM-138, sufficient for soil recovery in agricultural production, in 120 days, a relatively short period of time.

Keywords: soil; ARA; biostimulation; NOM-138; S. vulgare; mushrooms.

1. Introduction

Currently, some petroleum derivatives, such as oils used in the lubrication and refrigeration of automobiles, generate products that pollute the environment, such as automotive waste oil (ARA), a combination of aliphatic, aromatic and polycyclic hydrocarbons [1]. In Mexico ARA, according to the General Law of Ecological Balance and Environmental Protection [2] is a toxic environmental waste. In order to determine the damage caused by ARA in the soil, there is a Mexican standard [3] known as NOM-138-SEMARNAT-2012 (NOM-138), which establishes the maximum permissible concentration limit, especially for an agricultural soil of 4400 ppm, an amount that prevents the mineralization of organic matter (OM), prevents gas exchange, which consequently decreases or cancels agricultural production [4], given the phytotoxicity of ARA aromatics. The literature reports that a soil impacted by ARA is reduced by chemical methods, which are fast, of high economic value, but which cause collateral damage by leaving toxic residues for animal and/or plant life [5,6]. An alternative ecological solution is bioremediation (BIO) which, by enriching the soil with basic minerals N (nitrogen), P (phosphorus), K (potassium), rebalances the carbon:nitrogen ratio (C:N) caused by excess carbon: nitrogen (C:N):N) caused by the excess carbon in the ARA, to eliminate it in a relatively long or short time [7], this speed is dependent on the complexity and concentration of the hydrocarbons it

contains, especially when the soil is poor in N, so it is necessary to enrich it with an animal fertilizer, such as vermicompost (LC), which in addition to the high content of essential minerals, incorporates microorganisms that favor the oxidation of ARA, for this type of BIO, it is important to have an adequate demand of O_2 (oxygen) to ensure the constant elimination of ARA, without causing drastic changes in pH, since neutrality accelerates the oxidation of ARA [8].

In a soil contaminated by 100,000 ppm of ARA, BIO is insufficient to reduce it to a value lower than 4,400 ppm, which allows agricultural recovery in accordance with NOM-138 [3], consequently, phytoremediation (FITO) is indispensable, with plants whose root system tolerates phyto-toxicity to hydrocarbons and facilitates the oxidation of ARA [9–12]. As it is reported for other petroleum products, mainly because these plants can improve the elimination capacity of these hydrocarbons, mainly when inoculated with microorganisms that naturally hydrolyze aromatics. In soil impacted by a relatively high concentration of hydrocarbons, so that, by oxidizing them, it is possible to recover fertility for agricultural production, according to some environmental regulation in force [3]. In relation to improving the capacity of a plant to mineralize soil hydrocarbons, it is reported that genera and species of fungi such as Aspergillus niger and Penicillium chrysogenum not only stimulate the growth of the plant root system [1,9,13], but also metabolize hydrocarbons similar to those detected in the ARA and consume them until they are reduced to a value that facilitates the recovery of useful soil for agricultural production [14–17]. Based on this information, the objectives of this research were: i) bioremediation of soil contaminated by 100,000 ppm of ARA, ii) phytoremediation with Sorghum vulgare inoculated with A. niger and P. chrysogenum to reduce ARA to a value below the maximum of NOM-138 as evidence of its recovery.

2. Materials and methods

This research was conducted in the greenhouse of the Environmental Microbiology laboratory of the Instituto de Investigaciones Químico Biológicas (IIQB) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH). Under the following microclimatic conditions: temperature of 23.2 °C, luminosity of 450 μmol m^{-2} s^{-1}, relative humidity of 67%. It was used, an agricultural soil collected in an area at 19° 37' 10″ north latitude and 101° 16' 41.00″ west longitude, with an altitude of 2013 masl, with a temperate climate of a place called “Uruapilla” of the municipality of Morelia, Michoacán, Mexico, on the Morelia-Pátzcuaro highway, Michoacán. The ARA was collected from an oil change shop in Morelia, Michoacán, Mexico.

Table 1. Physicochemical properties of agricultural soil not artificially contaminated by automotive waste oil.

Parameter	Interpretation value
pH (1:20)	6.02 acid
Total nitrogen (%)	0.11 poor
Organic matter (%)	0.58 poor
Ion exchange capacity ($Cmol^{(+)}$ kg^{-1})	26.64 saline
Texture (%) [+]	50 (Ac) low aeration, 7(L), 43 (Ar)
True density or RD (g/cm^3)	2.22
Bulk density or DA (g/cm^3)	1.05
Porosity (%)	47.5
Field capacity (%)	54 low humidity

[+]Ar: sand, L: silt, AC: clay, *for soils of volcanic origin, **calculated from DA and DR.
**estimated texture, +for clayey soil according to Mexican standard: NOM-021-RECNAT-2000.

Table 1, details the physicochemical properties of the agricultural soil uncontaminated by ARA [18], with a pH of 6.02 slightly acidic, with low MO content 0.58%, a poor concentration of total N of 0.11%, a high ion exchange capacity of 26.64 $Cmol^{(+)}$ kg^{-1} or saline, with a texture composition: clay 50 %, silt 7% and sand 43%, so the soil was classified as clayey, this soil was sieved with a No. 20 mesh, solarized for 48 h and reduced the problem of pests and diseases, then contaminated with ARA, and began the BIO of the soil by dissolving the 100000 ppm of ARA in the commercial detergent "La Corona" at 0.5 % (w/v). Then 1.0 kg of this soil contaminated with ARA was placed in the upper part of the Leonard jar, in the lower part water or mineral solution (SM) was deposited, both parts were connected by a cotton strip, for the movement of the liquid by capillarity (**Figure 1**).

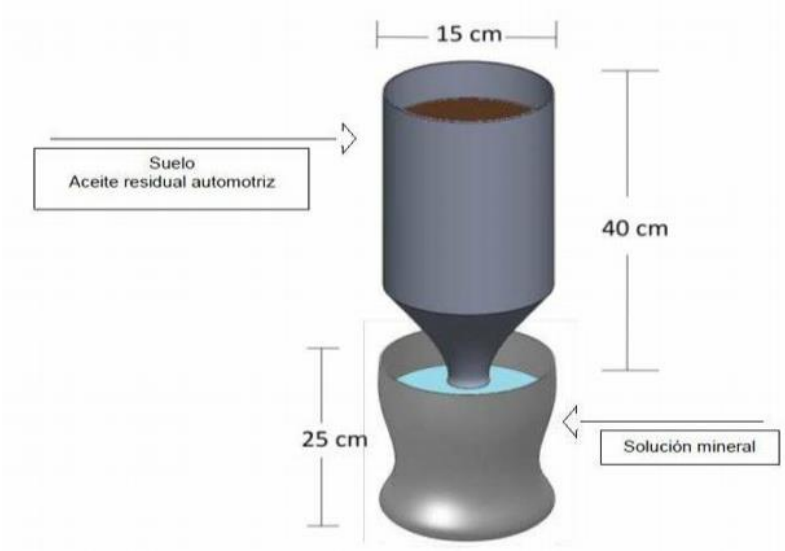

Figure 1. Leonard pitcher diagram [13].

The experiment was divided into i) soil BIO by 100,000 ppm ARA, according to **Table 2**, ii) soil PHYTO by S. vulgare with A. niger and/or P. chrysogenum, according to **Table 3**. With a randomized block experimental design of six treatments and six replicates: soil without ARA irrigated only with water or absolute control

(AC), soil fed with a mineral solution referred to as relative control (RC), soil with ARA without biostimulation or FITO or negative control (NC), and soil with ARA, biostimulated and phytoremediated.

Table 2. Experimental design of bioremediation of agricultural soil contaminated by 100,000 ppm of residual automotive oil.

Agricultural land	**100000 ppm of ARA**	**Detergent at 0.5% and LC at 3%.**	**SM at 100% and H_2O_2 at 0.05%.**
Absolute control (AC)	-	-	-
Relative control (RC)	-	-	+
Negative control (NC)	+	-	-
Bioremediation	+	+	+

*n = 6; aggregate (+); not aggregate (-). LC vermicompost, SM mineral solution.

In the first phase, the soil with 100000 ppm of ARA was dissolved in the commercial detergent "La Corona" at 0.5% and a LC at 3% for 30 days, then biostimulated with a SM with the following composition (gL^{-1}): NH_4NO_3 10.0, K2HPO4 2.5, KH_2PO_4 2.0, $MgSO_4$ 0.5, NaCl 0.1, CaCl2 0.1, $FeSO_4$ 001 and 1.0 mL/L of a microelement solution ($g{\cdot}L^{-1}$): H3BO3 2.86, ZnSO4 $7H_2O$ 0.22, $MgCl_2$. $7H_2O$ 1.8, adjusted to pH 6.8 [19], was biostimulated with the SM, and simultaneously with H_2O_2 at 0.05% for 72 h for one month, while to facilitate gas exchange the moisture was adjusted to 80 % of the field capacity of the agricultural soil. The variable-response of soil recovery was ARA detected by Soxhlet at the beginning and end of BIO and FITO [15].

Table 3. Experimental design for phytoremediation of a soil impacted by automotive waste oil remaining from biostimulation.

Sorghum vulgare*.	**ARA**	**A. niger**	**P. chrysogenum**
Irrigated with water (absolute control)	-	-	-
Fed with mineral solution (relative control)	-	-	+
	+	+	-
Biostimulation	+	-	+
	+	+	+

*n = 6; added (+); not added (-), ARA automotive waste oil.

In the second phase, soil contaminated by 100,000 ppm ARA after biostimulation was phytoremediated according to **Table 3** by planting *S*. vulgare obtained from the Secretaria de Agricultura Ganadería *y* Desarrollo Rural Pesca *y* Alimentación of the Mexican government. S. vulgare was treated with A. niger and/or P. chrysogenum, both fungi were isolated from decaying wood for their ability to degrade lignin, and molecularly identified as the species indicated 20, to inoculate on S. vulgare seeds. They were replicated on avocado pit agar with the following composition (g L^{-1}): avocado pit 10, casein peptone 5, yeast extract 1.3, K_2HPO_4 0.17, KH_2PO_4 2.61, $MgSO_4$ 1.5, NaCl 0.9, $CuSO_4$ 0.05, bromothymol blue 10 ppm, 2.5 mL 10% deter- people solution 1.0 mL trace element solution, agar 18.0 g, pH adjusted to 5.5. S. vulgare seeds were disinfected with 0.2 % NaOCl/5 min, washed 6 times with sterile distilled water, then for every 10 S. vulgare seeds were

inoculated with 1.0 mL of A. niger and/or P. chrysogenum equivalent to 1 × 10^6 Propule-forming units (PFU)/1 mL [19] to be sown in agricultural soil artificially contaminated by ARA, 60 days later, phenology: plant height (PA) and root length (RL), and biomass: aerial and root fresh weight (AFW/RFW) and aerial and root dry weight (AFW/RDW) were measured [19]. Experimental data were validated with ANOVA/Tukey HSD $P < 0.05$ % with the statistical program Statgraphics Centurion [20,21].

3. Results

In **Table 4**, BIO reduced ARA from 100,000 to 37620 ppm ARA in 60 days, a statistically different numerical value relative to 90,000 ppm ARA in soil without bioremediation or CN.

Table 5 shows the phenology of *S.* vulgare boosted with A. niger and P. chrysogenum at 60 days in soil with 37620 ppm of ARA, 29.2 cm PA, 17.0 cm LR were recorded, both numerical values with statistical difference compared to 20.0 cm PA and 8.5 cm LR of S. vulgare without inoculation irrigated only with water in soil without ARA referred to as CA. While S. vulgare with A. niger and P. chrysogenum in soil with ARA, recorded 10.0 g PFA and 5.0 g PFR, both statistically different numerical values compared to 4.77 g PFA and 2.89 g PFR of S. vulgare fed with a SM, in soil without ARA used as CR. Regarding the biomass of S. vulgare with A. niger and P. chrysogenum in soil with ARA, 2.2 g of PSA and 1.6 g of PSR were recorded, these numerical values were statistically different compared to the 1.14 g of PSA and 1.31 g of PSR of S. vulgare fed with the SM or CR.

Table 4. In soil concentration of residual automotive oil remaining from bioremediation for 60 days.

*Agricultural soil artificially polluted by 100,000 ppm of ARA	Final concentration
Irrigated with water or CN	90000b***
**Multiple Bioremediation	37620a

*B ioremediation: detergent at 0.5%, vermicompost at 3%. Mineral solution at 100% and H_2O_2 at 0.05%. ***Different letters indicate that they are statistically different according to ANOVA/Tukey at 0.05%.

Table 5. Phenology and biomass of Sorghum vulgare with A. niger and P. chrysogenum after soil phytoremediation with 37,620 ppm of residual automotive oil, after 60 days.

Sorghum vulgare*.	AP (cm)	LR (cm)	PF (g)		ps (g)	
			Aerial	Radical	Aerial	Radical
Irrigated with water (absolute control)	20.0^{b} **	8.5^{d}	5.20^{c}	2.90^{c}	1.15^{c}	1.20^{c}
Fed with mining solution to the	$2\ 5.0^{a}$	16.0^{b}	4.77^{c}	2.89^{c}	1.14^{c}	1.31^{b}
100% (relative control) with Aspergillus niger	18.0^{c}	14.0^{bc}	7.27^{b}	3.71^{b}	1.84^{a}	1.28^{b}
with Penicillium chrysogenum	22.0^{b}	15.0^{b}	8.93^{ab}	5.73^{a}	1.50^{b}	0.99^{d}
with A. niger and P. chrysogenum	29.2^{a}	17.0^{a}	10.0^{a}	5.0^{a}	2.2^{a}	1.60^{a}

*n = 6. **Different letters indicate statistical difference according to ANOVA/Tukey at 0.05%. AP plant height, LR root length, PF fresh weight, PS dry weight, PS dry weight.

Table 6 shows the decrease in soil ARA from 37,620 ppm to 3400 ppm by the activity of S. vulgare with A niger and P. chrysogenum, this last value was statistically different compared to the 80000 ppm ARA of the soil without bioremediation and phytoremediation used as CN.

Table 6. In soil concentration of residual automotive oil after phytoremediation with Sorghum vulgare enhanced with Aspergillus niger and Penicillium chrysogenum at 120 days.

***Agricultural soil contaminated by ARA**	**ARA+ (ppm)**	
	Initial	Final
Soil without phytoremediation or negative control	100000a**	80000b
Phytoremediated soil, S. vulgare + A. niger + P. chrysogenum	37620a	3400b

**n* = 6; ***Different letters are statistically different at 0.05% according to Tukey, + ARA automotive residual oil

4. Discussion

In **Table 4**, the agricultural soil with 100,000 ppm of ARA, started the BIO with the detergent, which solubilized it, to facilitate the native heterotrophic aerobic microorganisms to carry out a partial reduction of the concentration [4,5,7,8], while the BIO with the LC by enriching the soil with urea allowed the equilibrium of the ratio C:N ratio, so that the native microorganisms could partially oxidize the ARA [22,23], in the same way that the SM with salts NH_4^+ , NO_3^- and PO_4^{-3} accelerated the mineralization of the ARA [24,25] , simultaneously the BIO with H_2 O_2 supplied the demand O_2 to optimize the oxidation of ARA [14,26], therefore the field capacity of the soil was controlled at 80%, which allowed the exchange of gases and the decrease of the concentration of ARA [17,27] to a sufficient level for the sowing of S. vulgare inoculated with A. niger and P. chrysogenum, in the soil recovery route according to NOM-138.

In comparison with the soil with 100,000 ppm of ARA, used as CN, in which natural attenuation was insufficient to eliminate it, due to the excess of C in the ARA that formed a hydrophobic film of the ARA and prevented the exchange of gases such as O_2 while the lack of minerals essential for the oxidation of ARA prevented that concentration from being reduced [25,26,28].

Table 5 shows the phenology and biomass of S. vulgare with A. niger and P. chrysogenum, planted in soil when the ARA concentration was decreased to 37620 ppm. Where indirectly a decrease in ARA was recorded due to the healthy growth of S. vulgare partly because it is naturally tolerant to phytotoxic stress of ARA, and due to the positive effect of A. niger and P. chrysoge- num in the rhizosphere of the plant, where these fungi can generate plant growth-promoting substances [25,29], to enhance the root mineral uptake capacity and decrease the concentration of ARA leading to the recovery of soil health and allowing it to be used in agricultural production [10], in stark contrast to S. vulgare uninoculated with A. niger and P. chrysogenum planted in soil contaminated by 86,000 ppm ARA without bioremediation, where the relatively high concentration caused inhibition of healthy S. vulgare growth [11].

Table 6 shows the concentration of soil ARA impacted by 37,620 ppm of ARA

remaining from BIO, then by FITO with S. vulgare enhanced with A. niger and P. chrysogenum that stimulated healthy root growth. chrysogenum that stimulated healthy root growth, suggests the conversion of root exudates into phytohor monas [11,17,30] that increased the amount of root hairs, to expand the area of exploration and mineral uptake, and thereby increased the tolerance of S. vulgaris to ARA [10]. In addition, there is evidence that both A. niger and P. chrysogenum have the capacity to degrade aromatics of ARA [12,14,30,31], and facilitated the elimination of ARA up to a concentration of 3400 ppm, a value lower than the maximum established by NOM-138, thus achieving soil recovery for reuse in agricultural production.

Ethical approval: The approval of the research by the Ethics Committee of the Universidad Michoacana de San Nicolás de Hidalgo—Mexico, followed the guidelines established for this committee.

Conflict of interest: The author declares no conflict of interest.

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