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Ecological strategy for the recovery of an agricultural soil polluted by gasoline

Abstract

A soil contaminated by 10,000 ppm of gasoline (GAS), is a concentration higher than the limit of 4,400 ppm established by the Mexican standard NOM-138-SEMARNAT/SSA1-2003. In the soil, it inhibits the mineralization of organic matter causing loss of fertility. Therefore, the aims of this research a) biostimulation of a soil impacted by 10,000 ppm of GAS, and b) phytoremediation through *Zea mays* enhanced with *Azotobacter vinelandii* to decrease the GAS to a value lower than the maximum of the NOM-138-SEMARNAT/SSA1- 2003. In that sense an agricultural soil was impacted by 10,000 ppm of GAS was biostimulated applying a crude fungal extract (CFE)/2 months and vermicompost (VC)/1 month, later it was phytoremediated with *Z. mays* enhanced by *A. vinelandii*/2 months; with the response variables phenology and seedling biomass and flowering: The experimental data were validated by ANOVA/Tukey HSDP<0.05%. Results: showed that the biostimulation of the soil impacted by 10,000 ppm of GAS with 60,000 ppm of CV was sufficient to reduce the concentration of GAS, followed by phytoremediation with *Z. mays/A. vinelandii* at flowering, where 5.79 g of aerial dry weight (ADW) and 2.59 g of root dry weight (RDW) were recorded, numerical values with statistical difference with the 4.49 g ADW and the 2.07 g RDW of *Z. mays* grown in uncontaminated soil by GAS fed with a mineral solution or relative control, with which soil biorecovery was achieved by decreasing GAS from 10,000 to 500 ppm, a value lower than the maximum allowed by NOM-138-SEMARNAT/SSA1-2003. The biorestoration of a soil impacted by GAS through biostimulation and phytoremediation is slow compared with strong oxidizing chemical agents but is ecological and allowed to reuse soil for agricultural production. It's concluded that it is possible to biorecover soil contaminated by GAS due ecological and simple strategy.

Keywords: soil, hydrocarbons, mineralization, gramine, endophytic bacteria, biorestauration

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Introduction

Gasoline (GAS), is a fuel made up of a mixture of aliphatic and aromatic hydrocarbons (HCs), accidentally or deliberately spilled on the soil, causes negative effects on the physicochemical properties, blocks the exchange of O_2 (oxygen) and water, acidifies the pH and inhibits native heterotrophic aerobic microorganism that mineralization of organic matter, including its toxic for domestic crop.[1,2] This environmental damage is caused when the concentration GAS is higher than that indicated by the environmental regulation NOM-138-SEMARNAT/SSA1-2003,[3] which establishes a maximum allowed value of 4,400 ppm of HCs, divided into: 200 ppm of the light fraction; 1,200 ppm of the median and 3,000 ppm of the heavy. According to the literature, a soil impacted by 10,000 ppm of GAS can be remediated by chemical methods, having the disadvantage that they cause negative collateral effects and high economic cost.[4] In contrast, biostimulation followed by phytoremediation is an option for the recovery of soil impacted by 10,000 ppm of GAS,[5] up to a concentration below the maximum permissible limit of NOM-138-SEMARNAT/SSA1-2003. In this sense, Fernández et al.,[6] recovered a soil polluted by 10,000 ppm crude oil by biostimulation with a detergent followed by a mineral solution that reduced crude oil by up to 61%, compared to not biostimulated soil impacted by crude oil or negative control when the GAS concentration did not change. While Zand et al.,[7] reported the phytoremediation of a soil impacted by 40,000 ppm of crude oil sowing *Zea mays* with a decrease in crude oil to 17,920 ppm after 120 days. That is why in this research, an ecological alternative is proposed, the interaction of two biorecovery strategies by biostimulation, first applying crude fungal extract (CFE) which contains an enzyme laccase that partially hydrolyze some aromatics of the GAS,[8] then a biostimulation incorporating vermicompost (VC), a mix of organic and inorganic compounds that enrich the soil and induce the native heterotrophic aerobic microorganisms to coometabolism the GAS;[9–11] finally to conclude by phytoremediation sowing *Z. mays*, a hydrocarbon tolerant gramine that due photooxidation of GAS at root level,[12,13] enhancing by *Azotobacter vinelandii* that has a genetic capacity to hydrolyze aromatics to help for decreasing the concentration of GAS for soil biorecover.[14,15] Therefore the objectives of this research were: i) biostimulation of a soil impacted by 10,000 ppm of GAS b) phytoremediation sowing *Z. mays* enhanced with *A. vinelandii* to decrease the GAS concentration at value lower than that accepted by the NOM-138-SEMARNAT/SSA1-2003.

Material and methods

This research was carried out in the greenhouse of the Environmental Microbiology Laboratory of the Biological Chemical Research Institute of the UMSNH, Morelia, Mich., Mexico. In this greenhouse, the average microclimatic conditions were: temperature of 23.2°C, luminosity of 450 $\mu mol \bullet m^{-2} \bullet s^{-1}$ and relative humidity of 67%. The soil that was collected was from a site located at 19° 37' 10" north latitude 101° 16' 41.99" west longitude, with an altitude of 2013 meters above sea level, with a temperate climate of an agricultural area called "Uruapilla" del municipality of Morelia, Mich., on the Morelia-Pátzcuaro highway, Mexico, before carrying out the experiment, a physicochemical analysis of the soil was carried out according to NOM-021-SEMARNAT-2000[16] (Table 1). The soil was solarized at 70°C/48 h to minimize the problem of pests and diseases,

later it was sieved with a No. 20 mesh and contaminated with 10,000 ppm of GAS dissolved in commercial detergent La Corona® at 0.1%. Subsequently, 1.0 Kg of soil was placed in the upper part of the Leonard jar (Figure 1) and the water or mineral solution in the lower part of the system depending on the planned experimental design, both parts were connected with a 20 cm long gauge cotton strip to allow the exchange of liquid capilarity.[17]

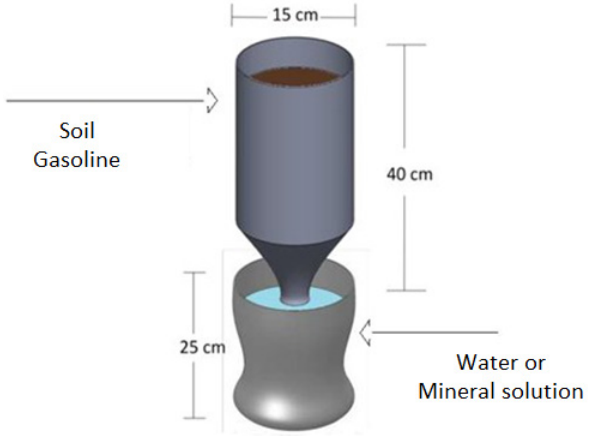

Figure 1 Leonard's jar diagram.

This research was divided into two phases, described in Table 2, where 2 controls, 3 treatments and six repetitions are shown: i) soil not contaminated by GAS, fed with a mineral solution or relative control, ii) soil impacted by 10,000 ppm of GAS and VC or plant control and iii) soil impacted by biostimulated GAS with a CFE, prepared from: *Aspergillus fumigatus*, *A. tubingensis*, *Fusarium thapsinum* and *Penicillum chrysogenum* in the Environmental Microbiology Laboratory of the UMSNH; these fungi were mixed, seeded a 500 mL flask, with 250 mL of liquid with residual lignin from wheat straw (RLWS) whose content was (g/L^{-1}): RLWS 10.0, casein peptone 5.0, yeast extract 1.3 , K_2HPO_4 0.17, KH_2PO_4 2.61, $MgSO_4$ 1.5, NaCl 0.9, $CuSO_4$ 0.05.0, 2.5 mL of 10% (p/v) la Corona® detergent, and 1.0 mL/L of a solution of trace elements, adjusted to pH 5.5 which sterilized at 121°C/20 min; The flask with *A. fumigatus*, *A. tubingensis*, *F. thapsinum* and *P. chrysogenum* was incubated at 30°C/18 days at 150 rpm, then the culture medium with the mixture of fungi was filtered and centrifuged to eliminate the fungi, subsequently, 100 mL of the CFE/Kg of soil impacted by GAS were used every week for two months.[18] Then it was biostimulated with 30,000 and 60,000 ppm of VC for a month, to enrich the soil with organic nutrients based on nitrogen, phosphorus and potassium. In the second phase, *A. vinelandii*, from the UMSNH Environmental Microbiology Laboratory, was activated. Subsequently, the *Z. mays* seeds were disinfected with Clorox®/2.5 min and 70% alcohol/2.5 min, washed with sterile water 4 times; every 20 seeds were inoculated with 0.5 mL of *A. vinelandii* planted in soil impacted by the GAS remaining from biostimulation, fed with a mineral solution with the following chemical composition (g/L^{-1}): NH_4NO_3, 10.0; K_2HPO_4, 2.5; KH_2PO_4, 2.0; MgSO4, 0.5; NaCl, 0.1; $CaCl_2$, 0.1; $FeSO_4$, traces and 1.0 mL/L of a microelement solution with the following composition (g/L^{-1}): H_3BO_3, 2.86; $ZnSO_4$•$7H_2O$, 0.22; $MgCl_2$•$7H_2O$, 1.81 and pH adjusted to 6.8. Biostimulation with the mineral solution was applied every third day for 2 months at a volume of 180 mL/kg of soil to maintain humidity at 80% of field capacity. The response variables of the phytoremediation of the remaining GAS through *Z. mays* were: the percentage of germination at 11 days; with phenology: plant height (PH) and root length (RL); with the biomass: aerial and radical fresh weight (AFW)/(RFW) with the aerial and radical dry weight (ADW)/(RDW) at seedling and flowering.[17] The experimental data was validated by ANOVA/Tukey HSD P<0.05% with the statistical program Statgraphics Centurion XVII.[19]

To determine the final concentration of GAS in the soil after phytoremediation, the extraction of hydrocarbons was used according to methods 3500B and 3540C of the USA-EPA, 1996,[20,21] and that reported by Schawb et al.,[22] and Zeneli et al.,[23] with modifications in relation to the speed of agitation and volumes of solvent used; with 1 g of dry GAS impacted soil, anhydrous sodium sulfate (Na_2SO_4) was added as a dehydrating agent and dichloromethane (CH_2Cl_2) as a solvent in a Falcon tube; then it was shaken in a vortex/1 min so that the solvent was incorporated into the soil; subsequently the mixture was centrifuged for 20 min; the supernatant was removed with a Pasteur pipette; the solid residue extracted was washed until obtaining approximately 15 mL of supernatant of the organic extract from the rotary evaporator, where the solvent (dichloromethane) was evaporated from the hydrocarbons soluble in dichloromethane The extracts were then measured by direct injection into a Varian chromatograph (model 37-D; Instrument Scientific CG Ltd) equipped with a flame ionization detector (FID) and a 25 m x 0.25 mm (diameter) fused-silica capillary column, with an immobilized (OV-101) phase. The hydrogen carrier flow rate was 30 mL/min and the sample size was 1 µl. The injection port temperature and the flame ionization detector were 170ºC. The temperature program was:initial temperature 25ºC, held for 3 min, programming rate 12ºC/min to 150ºC. The components searched for were toluene, ethylbenzene, n-nonane (n-C_9), n-undecane (n-C_{11}) and n- tridecane (n-C_{13}). Peak areas and retention times were compared to reference standards. The injections were done in triplicate.

Results and discussion

Table 1 shows the physicochemical properties of the soil prior to contamination by GAS, which was classified as silt-clayey with a texture: clay 31.8%, silt 26.92% and sand 42.0%; with a modern acidic pH of 5.67 that limits the solubility of PO_4^{-3}, with a high content of organic matter of 10.44% and an average content of total nitrogen with 0.32%, high phosphorus and potassium, sufficient to stimulate the activity of autochthonous aerobic heterotrophic microorganisms that oxidize GAS.[17]

Table 3 shows the percentage of germination of *Z. mays* seeds without *A. vinelandii* in the phytoremediation of soil impacted by 10,000 ppm of GAS with the CFE/60,000 ppm of VC at 11 days after sowing, where 100% germination was registered, indicating the partial elimination of the GAS due to biostimulation applying a CFE containing enzymes that hydrolyzed part of the aromatic fraction of the GAS,[8] while biostimulation using the VC enriched organic carbon and nitrogen compounds that induced the microbial activity for the partial oxidation of the GAS.[24,25] The maximum germination of *Z. mays* seeds was observed, the same as that registered in the soil without GAS fed with a mineral solution or relative control; soil enriched by 60,000 ppm VC or plant control. This percentage of germination was statistically different compared to 75% of *Z. mays* without *A. vinelandii* in soil impacted by the GAS biostimulated applying CFE followed with 30,000 ppm of VC; at 50% of *Z. mays* enhanced using *A. vinelandii* in soil impacted by 10,000 ppm of GAS that was biostimulated applying 60,000 ppm of VC. It was evident that the remaining GAS in the soil after biostimulation caused a negative effect on the germination of *Z. mays*, due to the type of hydrocarbons that blocked gaseous exchange and water interaction, avoiding the seed to die or delay germination.[26] The GAS in the soil was due to inhibition of the emergence of *Z. mays* reported by Grifoni et al.,[27] indicating that soil contamination by fossil fuel of the type of GAS was phytotoxic for the germination and growth of *Z. mays*.

Table 1 Physicochemical properties of soil uncontaminated by 10,000 ppm gasoline

Parameters	Value	Interpretation
pH	5.67	Moderately acidic
Organic matter (%)	10.44	Very high
Texture (%)	31.8 (clay), 26.92 (sand), 42.0 (silt)	Loamy-clay
Total, nitrogen (%)	0.32	Medium
Phosphorus (ppm)	219.34	Very high
Sodium (Na^+) ppm	153.38	High
Potassium (K^+) ppm	168.61	High
Microelements (ppm):		
Iron (Fe^{2+})	13.91	High
Zinc (ZN^{2+})	0.37	Low
Copper (Cu^{2+})	0.54	Low
Manganese (Mn^{2+})	4.62	Low

Physicochemical characteristics of the soil under study according to the criteria established by NOM-021-SEMARNAT-2000.[16]

Table 2 Experimental design to evaluate biostimulation of soil contaminated by 10,000 ppm gasoline and subsequent phytoremediation with *Zea mays* enhanced by *Azotobacter vinelandii*

*Soil	Crude fungal extract	Vermicompost	*Zea mays*	*Azotobacter vinelandii*	Mineral solution
Without gasoline (relative control)	-	-	+	-	100%
Without gasoline (plant control)	-	60,000 ppm	+	-	-
With gasoline (Biostimulation 1)	+	60,000 ppm	+	-	-
With gasoline (Biostimulation 2)	-	60,000 ppm	+	+	-
With gasoline (Biostimulation 3)	+	30,000 ppm	+	-	-

*Number of repetitions (n) =6; added (+); not added (-).

Table 3 Percentage of germination of *Zea mays* seeds enhanced by *Azotobacter vinelandii* during phytoremediation of soil impacted by 10,000 ppm of gasoline

*Zea mays	Germination percentage (%)
Relative control = soil + mineral solution	100 a**
Plant control = soil + 60,000 ppm of vermicompost	100 a
Biostimulation 1 = soil + gasoline + crude fungal extract + 60,000 ppm of vermicompost	100a
Biostimulation 2 = soil + gasoline + 60,000 ppm of vermicompost + *Azotobacter vinelandii*	50c
Biostimulation 3 = soil + gasoline + crude fungal extract + 30,000 ppm of vermicompost	75b

*n =6; **Different letters indicate statistical difference at 0.05% according to Tukey.

Table 4 shows the seedling stage phenology of *Z. mays* without *A. vinelandii* during the phytoremediation of soil impacted by 10,000 ppm of GAS biostimulated applying 60,000 of VC. There, 50.45 cm of PH and 34.60 cm of RL were registered, numerical values with statistical difference compared to the 35.50 cm of PH and the 40.85 cm of RL of *Z. mays* without *A. vinelandii* in soil polluted by GAS biostimulated using the CFE/30,000 ppm CV; with the 65.45 cm of PH and the 38.10 cm of RL of *Z. mays* in soil without GAS fed with a mineral solution or relative control. This indicates that the biostimulation applying the CFE and using 60,000 ppm of the VC was not sufficient for the mineralization of the GAS, since during the phytoremediation sowing *Z. mays/A. vinelandii* in soil impacted by GAS, the remaining concentration was toxic for growth of *Z. mays*, a result similar to that reported by Grifoni et al.,[27] who reported the phytotoxicity of residual hydrocarbons on the phenology and biomass of *Z. mays* in polluted soil by oil spills. Depending on the biomass, *Z. mays* plus *A. vinelandii* in soil impacted by 10,000 ppm of biostimulated GAS with 60,000 ppm of VC, 7.82 g of AFW and 4.11 g of RFW were registered, as well as 0.77 g of ADW and 0.45 g of RDW. All these numerical values were statistically different compared to the 4.85 g of AFW and 2.27 g of RFW, the 0.52 g of ADW and the 0.30 g of RDW of *Z. mays* without *A. vinelandii* in the soil polluted by GAS, biostimulated applying CFE and 60,000 ppm of the VC; and with the 6.06 g of AFW and the 2.19 g of RFW, as well as with the 0.72 g of ADW and the 0.34 g of RDW of *Z. mays* without *A. vinelandii* in soil without polluting by GAS fed with mineral solution or relative control. This variable-response shows that biostimulation with 60,000 ppm of VC accelerated the mineralization of GAS due to sufficient N, P and K generation for the nutritional demand of *Z. mays*, similar to what was registered in *Z. mays* in soil without polluted by GAS fed with the mineral solution or relative control.[28] The foregoing supports that when *Z. mays* was enhanced with *A. vinelandii*, in the rhizosphere it transformed the exudates from the roots of *Z. mays* into phytohormones, to induce a denser radical system that optimized the minerals necessary for a healthy growth, simulately it was possible the phytodegradation of the GAS consequently by decreasing its hydrocarbons at level equal to any natural never polluted by fossil fuel.[29]

Table 5 shows the phenology of *Z. mays* at flowering enhanced with *A. vinelandii* during the phytoremediation of soil impacted by 10,000 ppm of GAS, biostimulated with 60,000 ppm of VC, where 113.00 cm of PH and 77.65 cm of RL, both numerical values had statistical difference in relation to the 52.20 cm of PH and the 39.50 cm of RL of *Z. mays* without *A. vinelandii* in the soil impacted by GAS biostimulated with CFE and 60,000 ppm of VC; in comparison with the 45.05 cm of AP and the 42.00 cm of LR of *Z. mays* in soil impacted by GAS biostimulated with the CFE and the 30,000 ppm of VC; compared to 67.00 cm PH and 72.10 cm LR of *Z. mays* in soil without GAS enriched with 60,000 ppm VC or relative control. The healthy growth of *Z. mays* enhanced with *A. vinelandii* in soil impacted by the GAS biostimulated with the VC stimulated the native microbiota activity of the soil that oxidizes the remaining

hydrocarbons of the GAS,[30] at the same time it provided the necessary nutrients for the healthy *Z mays* at the level of physiological maturity, collaterally, it is suggested that *A. vinelandii* transformed the exudates of *Z. mays* into phytohormons that not only increased tolerance to GAS, but also optimized the root uptake of salts essential for healthy plant growth even with values of the phenology and biomass of *Z. mays* in unpolluted agricultural soil used as plant control.[31] In relation to the biomass of *Z. mays* enhanced by *A. vinelandii* in soil impacted by 10,000 ppm of biostimulated GAS 60,000 ppm of VC, 40.81 g of AFW and 19.22 g of RFW were recorded, as well as 5.79 g of ADW and 2.59 g of RDW; statistically different numerical values at 7.43 g of AFW and 6.38 g of RFW, as well as with 0.96 g of ADW and 1.85 g of RDW of *Z. mays* without *A. vinelandii* in soil impacted by GAS biostimulated with the CFC/60,000 ppm from VC; and with 6.40 g of AFW and 6.54 g of RFW, as well as with 0.84 g of ADW and RDW of *Z. mays* without *A. vinelandii* in soil impacted by GAS biostimulated with CFE and the 30,000 ppm of VC; compared to: 39.25 g AFW, 18.73 g RFW, 4.49 g ADW and 2.07 g RDW from *Z. mays* in soil without GAS fed mineral solution or relative control. The fresh and dry weight of *Z. mays* enhanced with *A. vinelandii* in soil impacted by GAS, biostimulated with 60,000 ppm VC, where a decrease in GAS concentration was recorded and observed the healthy growth of *Z. mays*,[28] indicating that *A. vinelandii* al convert root exudates into phytohormones,[32] simultaneously with the hydrolysis of some aromatics of the GAS, which was evidenced by the decrease in the concentration detected at the end of the phytoremediation from 10,000 ppm to 500 ppm of GAS, to decrease the concentration to a value lower than the maximum accepted by NOM-138-SEMARNAT/SSA1-2003,[3] for soil biorecovery, in contrast to what was observed in *Z. mays* cultivated in soil impacted by GAS without *A. vinelandii* in where the toxicity of hydrocarbons from the gas caused an abnormal growth of *Z. mays*.

The chromatography analysis of GAS concentration in the biostimulated[5,33] and phytoremediated soil[7,34] showed that the GAS concentration decreased from 10,000 ppm to 500 ppm, value below the maximum concentration of the mexican regulation NOM-138-SEMARNAT/SSA1-2003[3] which supports that the soil can be used for agricultural production with not risk for human or animal consume. In contrast to the soil without biostimulate or phytoremediated where the GAS only decreased from 10,000 ppm to 8,500 ppm due to the action of natural attenuation.[6]

In Table 6, the density of the GAS oxidizing aerobic heterotrophic microbial population induced by biostimulation by 60,000 ppm VC reached $200X10^4$ CFU/g of dry soil, due to enriched it with organic compounds and inorganic compounds of nitrogen (N), phosphorus (P) and potassium (K) that increased density of the GAS oxidizing bacterial population, since due to its genetical diversity they were able to use this hydrocarbons as a source of carbon (C) and energy.[35,36] In opposite way to increase the native fungal density of the soil.[37] The numerical values of bacterial density were statistically compared to the $100.40X10^4$ and $120.60X10^4$ CFU/g dry soil of soil impacted by GAS biostimulated applying a CFE/VC combination, in that sense there was an increase in bacterial density by biostimulation using a CFE, which hydrolyzed the aromatics of the GAS, without inducing the growth of the fungi; physiological condition could happened during coometabolism of GAS consequently, a low fungal density of $2.00X10^2$ and $13.00X10^2$ CFU/g of dry soil was registered.[38,39]

Table 4 Phenology and biomass of *Zea mays* to seedling stage enhancing by *Azotobacter vinelandii* during phytoremediation of soil impacted by 10,000 ppm of gasoline

***Zea mays* in soil**	**Plant height (cm)**	**Root length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			Aerial	**Radical**	**Aerial**	**Radical**
Relative control = soil + mineral solution	65.45[a**]	38.10[c]	6.06[b]	2.19[cd]	0.72[ab]	0.34[b]
Plant control = soil + 60,000 ppm of vermicompost	60.00[b]	47.00[a]	6.07[b]	2.87[b]	0.62[b]	0.33[b]
Biostimulation 1 = soil + gasoline + crude fungal extract + 60,000 ppm of vermicompost	50.45[c]	34.60[d]	4.85[c]	2.27[c]	0.52[c]	0.30[b]
Biostimulation 2 = soil + gasoline + 60,000 ppm of vermicompost + *Azotobacter vinelandii*	41.30[d]	34.20[d]	7.82[a]	4.11[a]	0.77[a]	0.45[a]
Biostimulation 3 = soil + gasoline + crude fungal extract + 30,000 ppm of vermicompost	35.50[e]	40.85[b]	3.01[d]	2.24[c]	0.30[d]	0.23[c]

*n=6; **Different letters indicate statistical difference at 0.05% according to Tukey.

Table 5 Phenology and biomass of *Zea mays* to flowering stage enhancing with *Azotobacter vinelandii* during phytoremediation of soil impacted by 10,000 ppm of gasoline

***Zea mays* in soil**	**Plant height (cm)**	**Root length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			Aerial	**Radical**	**Aéreo**	**Radical**
Relative control = soil + mineral solution	113.55[a**]	46.20[c]	39.25[b]	18.73[b]	4.49[b]	2.07[b]
Plant control = soil + 60,000 ppm of vermicompost	67.00[b]	72.10[b]	17.51[c]	14.98[c]	1.92[c]	1.85[c]
Biostimulation 1 = soil + gasoline + crude fungal extract + 60,000 ppm of vermicompost	52.20[c]	39.50[e]	7.43[d]	6.38[d]	0.96[d]	1.85[c]
Biostimulation 2 = soil + gasoline + 60,000 ppm of vermicompost + *Azotobacter vinelandii*	113.00[a]	77.65[a]	40.81[a]	19.22[a]	5.79[a]	2.59[a]
Biostimulation 3 = soil + gasoline + crude fungal extract + 30,000 ppm of vermicompost	45.05[d]	42.00[d]	6.40[e]	6.54[d]	0.84[e]	0.84[d]

*n=6; **Different letters indicate statistical difference at 0.05% according to Tukey.

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Table 6 Soil microbial population able to mineralize gasoline due to biostimulation of soil polluted by 6,000 of gasoline applying crude fungal extract and/or vermicompost before phytoremediation

***Zea mays* in soil**	**Bacteria Colony forming units by 10^4/g of dry soil**	**Fungi Propagule forming units by 10^2/g of dry soil**
Relative control = soil + mineral solution	5.22[c**]	0[c]
Biostimulation 1 = soil + gasoline + crude fungal extract + 60,000 ppm of vermicompost	100.40[a]	2.00[b]
Biostimulation 2 = soil + gasoline + 60,000 ppm vermicompost + 60,000 ppm of vermicompost	200.00[a]	0[c]
Biostimulation 3 = soil + gasoline + crude fungal extract + 30,000 ppm vermicompost + 30,000 ppm vermicompost	120.60[a]	13.0[a]

*n=6; **Different letters indicate statistical difference at 0.05% according to Tukey

Conclusion

The biostimulation of agricultural soil impacted by GAS applying VC induced the native microbial oxidation of the aliphatic fraction. In that sense phytoremediation sowing *Z. mays* enhanced with *A. vinelandii*, allowed reduced the concentration of GAS to a concentration equivalent to that detected in a natural soil that will allow its agricultural exploitation without risk to human or animal health.

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Conflicts of interest

The authors declared no have conflict interest for the study.

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