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Improved bioremediation of a highly soil impacted by waste motor oil, for safe food

Juan Luis Ignacio-De la Cruz[1], Izaney Rodríguez-Díaz[1], Elizabeth Carrillo-Flores[2], Elda María Beltrán-Peña[2] and Juan Manuel Sánchez-Yáñez[1]*

[1]Environmental Microbiology Laboratory,
[2]Signal Transduction Laboratory, Institute of Chemical-Biological Research. Ed-B3, University City. Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mujica S/N, Col Felicitas del Rio, ZP 58000, Morelia, Michoacán, México.

Corresponding author:
*syanez@umich.mx

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ABSTRACT

The use of automobiles generates waste motor oil (WMO), whose inadequate disposal in agricultural soil is acute problem of loss of this natural resource that results in a drastic reduction in agricultural production. The soil bioremediation is complex and slow task when levels of pollution of 95,000 ppm, exceeds the maximum limit of 4,400 ppm, established by the Mexican standard. However, it is possible to reduce the recovery time of this soils, by the combined of process biostimulation and phytoremediation. Therefore, the objectives of this research were: i) biostimulation of a soil contaminated by 100,000 ppm of WMO, ii) phytoremediation by *Phaseolus vulgaris* with *Methylobacterium symbioticum* and *Xanthobacter autotrophicus*, plus a crude extract of carbon nanoparticles. The response variables were: a) initial and final concentration of WMO and b) *P. vulgaris* germination percentage, phenology and biomass at seedling stage. The results indicated that biostimulation of soil contaminated by 100,000 ppm of WMO using detergent, followed it of a crude extract of biodetergents and lipases that emulsified and hydrolyzed the insoluble aliphatic hydrocarbons. After a treatment with a crude extract of extracellular enzymes that degrade lignin and hydrolyzed the aromatic fraction of WMO, and the biostimulation with 50% mineral solution, for efficient mineralization of WMO to reduce pollution from 100,000 to 38,354 ppm in 75 days. Phytoremediation by *P. vulgaris* of remnant of WMO with *M. symbioticum* and *X. autotrophicus*, 45 days after sowing, decreased to 4,100, value lower than that maximum established and statistically different from 85,711 ppm of WMO used as negative control. In that sense this soil could be used for agriculture aims having no risk for producing safe food for humans and animals.

Keywords: biodetergents, biostimulation, bioremediation, extracellular enzymes, hydrocarbons, legumes, lipases, nanoparticles, soil

Introduction

In the world, polluting of agricultural soil by waste motor oil (WMO) is one of the most common serious environmental problems, because it affects the carbon: nitrogen ratio, inhibits microbial life and cause loss of plant fertility which puts the health of humans and animals in a potential risk[1]. This is because WMO, is a mixture of poorly soluble aliphatic, aromatic and polycyclic hydrocarbons that limit water and oxygen exchange[2]. Mexican regulations, classify WMO as a toxic and hazardous waste according to the General Law of Ecological Balance and Environmental Protection[3], so it must be confined. However, when spilled on any environmental, it can easily exceed the maximum allowable limit of Mexican environmental law of 4,400 ppm according to NOM-138-SEMARNAT/SSA1-2012[4]. An alternative ecological solution to a relatively high concentration of WMO, due at its chemical complexity, is bioremediation by biostimulation applying a detergent that emulsified most of the hydrocarbons that compose it; followed by a treatment with a mineral solution that rebalances the C (carbon): N (nitrogen) ratio, caused by the excess of WMO in the soil[5,6]. Simultaneously a treatment with H_2O_2 as an available source of O_2, for the mineralization of WMO and the addition of a crude extract of biodetergents and lipases, to ensure the emulsification and oxidation of aliphatic of WMO. Also, the treatment with a crude fungal extract containing laccase, and other extracellular enzymes to hydrolysis lignin as well as the aromatic fraction of the WMO[7] and a crude extract of carbon nanoparticles, that due to its nanometric size, will enhance the retention and release of the nutrients, that induce to native microorganisms to mineralize WMO. In that sense the WMO concentration, could be decreased enough, to allows phytoremediation by sowing *Phaseolus vulgaris*, a tolerant legume to all types of hydrocarbons and that it could growth in the WMO remains. The final mineralization of WMO by *P. vulgaris* could be enhance by *Methylobacterium symbioticum* and *Xanthobacter autotrophicus*, due are endophytic bacteria able to transform some root metabolite, into phytohormones to enhance and to accelerate WMO´ oxidation. The ability of *M. symbioticum* and *X. autotrophicus* in synergic action with root system of *P. vulgaris*, could be improve by the biostimulation with a crude extract of nanoparticles of carbon, to optimize WMO mineralization[8,9,10]. Therefore, the objectives of this research were: i) biostimulation of a highly soil polluted with 100,000 ppm WMO and ii) phytoremediation by *P. vulgaris* with *M. symbioticum* and *X. autotrophicus* plus a crude extract of carbon nanoparticles of carbon what would allow to achieve a concentration lower than the maximum establishes by the NOM-138--SEMARNAT/SSA1-2012[4].

Materials and methods

BIOSTIMULATION OF A POLLUTED SOIL BY WMO

This research was conducted at the greenhouse of Environmental Microbiology Laboratory of the Institute of Chemical-Biological Research UMSNH. The agricultural soil used for cultivation of *Zea mays* was collected from a site located at 19° 39' 27'' north latitude 100° 19' 59'' west longitude with an altitude of 1820 m above sea level in a template climate of Morelia, Mich., Mexico. The soil was sieved with No. 20 mesh and solarized at 70°C/48 h to avoid pests and diseases. After 1.0 kg of soil was weighed and artificially polluted by 100,000 ppm of WMO from a mechanical workshop and emulsified with 1% detergent for first biostimulation. It was then deposited in the upper part of a Leonard jar. In the second biostimulation, the soil was enriched with a 50% mineral solution of the following composition (g/L): NH_4NO_3 10, K_2HPO_4 2.5, KH_2PO_4 2.0, $MgSO_4$ 1.0, NaCl 0.1, $CaCl_2$ 0.1, $FeSO_4$ 001 and 10.0 mL of microelement solution (g/L): H_3BO_3 2.86, $ZnSO_4$*$7H_2O$ 0.22, $MgCl_2$*$7H_2O$ 1.81, pH 6.8. Mineral solution was added at 96 mL/Kg of agricultural soil, every third day throughout the experiment. In the third biostimulation, 20 ppm of crude extract of nanoparticles of carbon suspended in a 0.85%

saline solution and 0.01% detergent was added. Nanoparticles was obtained from *Albizia* sp leaves, disinfected with sodium hypochlorite 5%/5 min and rinsed 6 times, with sterile water. The leaves were cut in 5 cm pieces, dried at 80°C/12 h, 60 g were weighed and suspended in 600 mL of deionized H_2O and heated at 70°C/30 min. The aqueous extract of leaves was filtered and centrifuged at 4000 rpm/10 min. The biostimulation with the crude extract of nanoparticles of carbon was applied at 15 mL/Kg of soil, once a week during the time of experiment. In the fourth biostimulation was applied 15 mL/Kg of soil of 0.5% (v/v) H_2O_2, every third day for one month. In the fifth biostimulation, was utilized a crude extract of biodetergents and lipases produced by *X. autotrophicus* and *Azotobacter vinelandii*. Both bacteria were grown with agitation at 250 rpm/30°C/3 days in a culture media with the following composition (g/L): WMO 10, casein peptone 5.0, yeast extract 2.5, K_2PO_4 1.0, KH_2PO_4 1.0, $MgSO_4$ 1.0, NaCl 0.5, trace element solution 1.0 ml; pH adjusted to 6.7. Then broth was centrifuged at 5,000 rpm/15 min, to separate the bacterial cells and obtained the crude extract of biodetergents and lipases[20,21,22]. While that the crude fungal extracts were obtained from *Penicillium chrysogenum* and *Aspergillus niger* growth in mediums with the following composition (g/L): wheat straw or avocado pit 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, 2.5 mL of 10% (w/v) detergent and 1.0 mL/L of a trace element solution, pH 5.5, in agitation at 120 rpm/30°C/5 days. Then the mycelium of *P. chrysogenum* and *A. niger*, was separated from the broths by centrifugation at 5,000 rpm/15 min and the supernatants were applied at a volume of 40 mL/Kg soil twice a week for 30 days, compared to the same agricultural soil non-polluted by WMO irrigated with water how absolute control (AC); agricultural soil non-polluted by WMO fed with 100% mineral solution how relative control (RC) and agriculture soil polluted by 100, 000 ppm of WMO non biostimulated neither phytoremediated how negative control (NC).

PHYTOREMEDIATION BY *PHASEOLUS VULGARIS* WITH *METHYlOBACTERIUM SYMBIOTICUM* AND *XANTHOBACTER AUTOTROPHICUS*

Table 1 shows the experimental design of phytoremediation of an agricultural soil impacted by 38, 354 ppm of WMO remain from biostimulated, where sowed *P. vulgaris* seeds, previously disinfected with sodium hypochlorite 0.6%/2.5 min, rinsed 6 times with sterile water and with alcohol 70%/5 min and rinsed 5 times. Every 10 seeds were inoculated with 1.0 mL of a suspension of *M. symbioticum* and *X. autotropicus* individually and mixed in 1:1 ratio (v/v) with a density equivalent to $1.5X10^8$ CFU/mL obtained by viable plate count on nutrient agar, pH 7.0. In another experiments the seeds were treated with 20 ppm crude extract nanoparticles of carbon suspended in a 0.85% saline solution and 0.01% detergent. The inoculated and treated seeds were then shaken at 200 rpm/28°C/30 min and sown in soil polluted by WMO remaining from biostimulation phase as shown in Table 2. Moisture was controlled throughout the experiment at 80% of field capacity. In the phytoremediation the response variables were: germination percentage; phenology: plant height (PH) and root length (RL); biomass: aerial/radical fresh and dry weight (AFW/RFW)/ (ADW/RDW) at seedling level. Soxhlet determination of WMO concentration and the pH control during all experiment were carried out once every 15 days. To neutralize the soil, 15 mL/Kg of 1% NaOH was added for adjusting the pH between 6.8-7.2. The experimental data obtained were subjected to an analysis of variance (ANOVA) by Tukey HSD ($P<0.05\%$) comparative test of means with the Statgraphics Centurion XVI.II program.

Table 1. Experimental design for phytoremediation of soil impacted by 38,354 ppm of WMO remnant from enhanced biostimulation.

Agricultural soil sown *Phaseolus vulgaris**	Control			Treatment		
	Absolute	Relative	Negative	1	2	3
Methylobacterium symbioticum	-	-	-	+	-	+
Xanthobacter autotrophicus	-	-	-	-	+	+
Water	+	-	+	-	-	-
Mineral solution (%)	-	100%	-	50%	50%	50%
Crude extract of 20 ppm of nanoparticles of carbon	-	-	-	+	+	+
*Number of repetitions (n) = 6; added (+); not added (-)						

Results and discussion

The sextuple biostimulation on an agricultural soil reduced the pollution from 100,000 to 38,354 ppm in 75 days (Table 2). This value, was statistically different to 92,255 ppm of WMO in soil non biostimulated respect to soil polluted by 1000,000 of WMO or the negative control (NC). It was evident that decrease of WMO by a multiple biostimulation, was support by the emulsification of a wide diversity of hydrocarbons of the WMO through detergents[11] and facilitated by the aerobic heterotrophic soil microorganisms could reduce its concentration. These processes enhanced the recovery of soil fertility and in the future it would allow healthy plants to be harvested, without the risk for humans and/or animals that consume them of containing these hydrocarbons. Then enrichment with the mineral solution induced to these aerobic heterotrophic native microorganisms, to mineralize WMO[12]. While the crude extract of carbon nanoparticles facilitated the retention of nitrogen, phosphorus and potassium minerals, necessary for the native aerobic heterotrophic soil microbiota to oxidize aliphatic and aromatic hydrocarbons. Moreover because, simultaneously the biostimulation with H_2O_2 ensured a sufficient O_2 source for a continuous oxidation of WMO[12,13]. Subsequently, biostimulation with crude extract of biodetergents and lipases emulsified and enabled WMO-induced microbial lipases, made it feasible for native soil microorganisms to have the ability to accelerate the removal of WMO hydrocarbons[20,25,28,30]. The following biostimulation with the crude fungi extract that contained extracellular enzymes that attack aromatic hydrocarbons: laccase, lignin peroxidase and manganese peroxidase; which enhanced the hydrolysis of the aromatic fraction of WMO, that accelerated the decrease of WMO. In evident contrast to the soil used as a negative control, impacted by the high WMO concentration, there natural attenuation was sufficient to decrease WMO from 100.00 to 92.255 ppm, due to the C: N imbalance caused by the excess of WMO[25,27,30]. Moreover, in the absence of a sufficient amount of the basic minerals, the native aerobic heterotrophic microorganisms were unable to oxidize the WMO. This implies that in this soil, the plant production is compromised because is impossible to generate healthy and safe plant-based food for humans and

animals, and consequently there is a risk that hydrocarbons from the WMO will leach into aquifers causing a major environmental contamination problem[29,30].

Table 2. Concentration of waste motor oil (WMO) in agricultural soil remaining from biostimulation for 75 days.

Agricultural soil polluted by 100,000 ppm WMO*	WMO final concentration
Negative control	92,255ppm[a] **
Enhanced biostimulation by 1% commercial detergent + 50% mineral solution + crude extract of carbon nanoparticles + 0.5% H_2O_2 + crude extract of biodetergents and lipases + crude fungal extract.	38,354 ppm[b]
*n = 6; **different letters indicate statistical difference according to ANOVA/Tukey (P<0.05%).	

The germination percentage of *P. vulgaris* was improved by *M. symbioticum* or *X. autotrophicus,* when the seeds were sown in soil contaminated by 38,354 ppm of WMO, remaining from first multiple biostimulation (Table 3). There was 66.81% and 66.54% germination at 13 days after sowing, values not statistical difference respect to 70% germination of *P. vulgaris* in soil non-polluted by WMO and fed with the 100% mineral solution or respect to relative control (RC). The differences in the percentage of germination of the seed of *P. vulgaris* could be because they were inoculated with amino acids generated at the beginning of the germination and also by the elimination of phytotoxic hydrocarbons of WMO. Since in the soil impacted by the hydrocarbons, the phytotoxicity prevented the germination of *P. vulgaris* seeds. This shows the importance of inoculating *X. autotrophicus* and *M. symbioticum* followed by biostimulation to recover soil impacted by WMO[20,25,31]. The enhanced of germination of *P. vulgaris* seeds inoculated with *M. symbioticum* it is due at auxins and gibberellins, which are associated with accelerating of the emergence of stem and root primordium; while biostimulation using crude extract of carbon nanoparticles accelerated and increased seed germination[14]. In addition, the inoculation with *M. symbioticum* does not represent without any risk of affecting the health of humans and animals by consuming them and at the same time they prevent that WMO hydrocarbons being mobilized and negatively impact surface water or aquifers. Therefore the integration of biostimulation with phytoremediation, is a useful ecological strategy for the recovery of water and soil impacted by hydrocarbon mixtures[28,30,31]

Table 3. Germination of *P. vulgaris* with *M. symbioticum* and/or *X. autotrophicus* in soil polluted by 38,354 ppm of WMO remaining from enhanced biostimulation.

Phaseolus vulgaris sown in soil*	Germination percentage (%)
Absolute control Non-polluted by WMO irrigated water	60.55[b**]
Relative control Non polluted by WMO fed 100% mineral solution	70.00[a]
Negative control Polluted by 100,000 ppm of WMO irrigated water	40.02[c]
Treatment 1 Polluted by 38,354 ppm of WMO + *M. symbioticum*	66.81[a]
Treatment 2 Polluted by 38,354 ppm of WMO + *X. autotrophicus*	66.54[a]
Treatment 3 Polluted by 38,354 ppm of WMO + *M. symbioticum* and *X. autotrophicus*	63.33[b]
*n = 6; **different letters indicate statistical difference according to ANOVA/Tukey (P<0.05%).	

Table 4 shows the phenology and biomass at the seedling level of *P. vulgaris* from seeds sown in the phytoremediation polluted soil by 38,354 ppm of WMO, remaining from the enhanced biostimulation and inoculated with *X. autotrophicus*. There was registered 20.56 cm of plant height (PH) and 12.62 root length (RL), both values were statistically different compared to 16.60 cm of PH and 9.35 cm of RL of *P. vulgaris* sown in soil of relative control. The biomass of *P. vulgaris* enhanced with inoculation of *X. autotrophicus* registered 1.85 g of aerial fresh weight (AFW) and 0.32 g of radical fresh weight (RFW), as well as 0.19 g of ADW and 0.04 g of RDW, values statistical difference respect to these registered in *P. vulgaris* sown in soil of relative control. (Table 4). The numerical values of phenology and biomass of *P. vulgaris* allow us to conclude that the removal of WMO hydrocarbons were enhanced with *M. symbioticum* and *X. autotrophicus* which are reported to have the biochemical capacity16,17,27 to oxidize the two main hydrocarbons: aliphatic and aromatic and thus decreasing the concentration of WMO. This consequently enhanced the radical activity of *P. vulgaris* to maintain constant mineralization of both types of hydrocarbon. Therefore, the synergistic action of phytohormone synthesis by *M. symbioticum* and *X. autotrophicus* favored a denser and more WMO-tolerant root[26,27]. Cleanup of agriculture soil contaminated with WMO, now allow it user that soil for a healthy and safe agricultural production for humans and animals.

Table 5 shows the concentration of 38,354 ppm in soil after biostimulation and the initiation of phytoremediation with *P. vulgaris* plus *M. symbioticum, X. autotrophicus* and a crude extract of nanoparticles at the seedling level, where were reached WMO values lower than 4,400 ppm in 45 days. This result supports that a biostimulation with the crude extracts of biodetergents and lipases[20,21,22], extracellular enzymes that lignin degradation and so attack aromatic fraction of mineral solution, were fundamental for the reduction of the WMO from 100,000 to 38,354. Such reduction allowed at *P. vulgaris* to be tolerant to the toxicity of WMO, which combined with the endophytic capacity of *X. autotrophicus* and *M. symbioticum* reduced its value below of established by NOM 138-

SEMARNAT/SSA1-2012[4]. The values of 4,300, 4,200 and 4,100 ppm of WMO were statistically different, respect to the 85,711 ppm WMO in soil used as a negative control, where it was evident that the actions of natural attenuation, were insufficient to detoxify the soil with excess of WMO. This reinforces why it is necessary after a mechanical action to eliminate excess WMO through of biostimulation and phytoremediation how a viable ecological option for the recovery of soil highly polluted[12,24].

Table 4. Phenology and biomass of *P. vulgaris* with *M. symbioticum* and *X. autotrophicus* at seedling level during phytoremediation polluted soil by WMO remaining from biostimulation.

Phaseolus vulgaris sown in agricultural soil*	Plant height (cm)	Root length (cm)	Fresh weight (g)		Dry weight (g)	
			Aerial	Radical	Aerial	Radical
Absolute control Non-polluted by WMO irrigated water	15.84^{b**}	9.93^{b}	1.64^{b}	0.69^{a}	0.18^{a}	0.08^{a}
Relative control Non-polluted by WMO fed 100% mineral solution	16.60^{b}	9.35^{b}	1.45^{b}	0.65^{a}	0.15^{a}	0.06^{ab}
Negative control 100,00 ppm of WMO irrigated water	14.43^{c}	3.46^{d}	0.63^{d}	0.23^{c}	0.08^{b}	0.02^{c}
Treatment 1 38,354 ppm of WMO + *M. symbioticum* 50% mineral solution	16.78^{b}	7.69^{c}	0.82^{c}	0.32^{b}	0.09^{b}	0.04^{b}
Treatment 2 38,354 ppm of WMO + *X. autotrophicus* 50% mineral solution	20.56^{a}	12.62^{a}	1.85^{a}	0.32^{b}	0.19^{a}	0.04^{b}
Treatment 3 38,354 ppm of WMO + *M. symbioticum and X. autotrophicus* 50% mineral solution	13.83^{c}	7.65^{c}	0.80^{c}	0.36^{b}	0.09^{b}	0.04^{b}

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

Table 5. Concentration of WMO in agricultural soil remaining from phytoremediation with seedling 45 days after sowing.

Phaseolus vulgaris sown in soil*	WMO final concentration
Absolute control: Uninoculated and irrigated water in soil unpolluted by WMO	0 ppm^{e**}
Relative control: Uninoculated and fed with 100% mineral solution in soil non-polluted by WMO	0 ppm^{e}
Negative control: Uninoculated and soil polluted by 100,000 ppm WMO	85,711 ppm^{d}
Treatment 1: *M. symbioticum* and fed with 50% mineral solution and a crude extract of carbon nanoparticles in soil pollute by WMO remaining from biostimulation	4,300 ppm^{b}
Treatment 2: *X. autotrophicus* and fed with 50% mineral solution and a crude extract of carbon nanoparticles in soil polluted by WMO remaining from biostimulation	4,100 ppm^{a}
Treatment 3: *M. symbioticum* and *X. autotrophicus* with biostimulated by 50% mineral solution and a crude extract of carbon nanoparticles in soil polluted by WMO remaining from biostimulation	4,200 ppm^{c}
*n = 6; **Different letters indicate statistical difference according to ANOVA/Tukey (P<0.05%).	

Table 6 shows the pH dynamics in the soil polluted by WMO, biostimulated and phytoremediated, where values between 6.61 and 4.20 were registered. That values indicating that the aerobic heterotrophic microbiota oxidized the WMO, via *β*- oxidation and generated organic acids[19], which cause the release of H+ ions in the soil solution and the pH decrease. That acidity inhibited microbial oxidation of WMO[20]. To maintain the oxidation of the WMO, it was necessary to neutralize at a range of pH values between 6.8 and 7.2 with NaOH. In this range the majority of nitrogen, magnesium, potassium minerals and especially minerals of PO4-3 of the soil limit the growth of heterotrophic native aerobic microorganisms that efficiently mineralize aliphatic elements of WMO and partially the aromatic ones, especially when there were biostimulation and phytoremediation actions that facilitate the decrease of WMO[25].The pH values of the soil polluted by WMO, that was biostimulated and phytoremediated, show pH 6.7-6.9 values that were statistically different, compared to soil polluted by WMO used as a negative control, where the excess of WMO inhibiting its mineralization, due at insufficient natural attenuation of the soil. In this sense, pH control is part of both strategies: the biostimulation phase and of phytoremediation[26] for controlled and accelerated the mineralization of aliphatic and aromatic hydrocarbons, which it allowed reuse the agricultural land for the generation of healthy and safe plants for consumption by humans and animals[27].

Table 6. pH dynamics of soil polluted by WMO during the biostimulation and the phytoremediation by *P. vulgaris* plus *M. symbioticum and X. autotrophicus*

pH control in agricultural soil*	Days								
	0	15	30	45	60	75	90	105	120
Absolute control non-polluted by WMO biostimulation or phytoremediation, irrigated only with water	6.90^{d**}	6.85^{c}	6.85^{d}	6.90^{c}	6.90^{c}	6.95^{d}	6.75^{c}	6.89^{d}	6.88^{d}
Relative control non-polluted by WMO, fed with 100% mineral solution and uninoculated *Phaseolus vulgaris*	6.83^{d}	6.79^{c}	6.73^{d}	6.70^{c}	6.80^{c}	6.75^{d}	6.75^{c}	6.79^{d}	6.78^{d}
Negative control non-polluted by WMO, non biostimulated or phytoremediated	6.61^{c}	5.59^{b}	5.89^{c}	5.27^{b}	5.66^{b}	5.96^{c}	6.49^{c}	6.43^{c}	6.41^{c}
Treatment 1 Polluted by WMO, biostimulated and phytoremediated plus *P. vulgaris* and *M. symbioticum* fed 50% mineral solution and a crude extract of carbon nanoparticles	6.65^{c}	4.62^{a}	4.73^{a}	4.17^{a}	5.16^{a}	5.66^{b}	5.58^{b}	6.34^{c}	5.36^{a}
Treatment 2 Polluted by WMO biostimulated and phytoremediated by *P. vulgaris* plus *X. autotrophicus* fed 50% mineral solution a crude extract of carbon nanoparticles	5.89^{a}	4.60^{a}	5.30^{b}	4.20^{a}	5.10^{a}	5.22^{a}	5.11^{a}	5.12^{a}	5.44^{a}

pH control in agricultural soil*	Days								
	0	15	30	45	60	75	90	105	120
Treatment 3 WMO, biostimulated and phytoremediated by *P. vulgaris* plus *M. symbioticum* and *X. autotrophicus* fed 50% mineral solution a crude extract of carbon nanoparticles	6.20^{b}	5.33^{b}	5.45^{b}	5.30^{b}	5.55^{b}	5.87^{c}	5.36^{b}	5.44^{b}	5.82^{b}
*n = 6; **Different letters indicate statistical difference according to ANOVA/Tukey (P<0.05%).									

Conclusion

The biostimulation of soil polluted followed by the phytoremediation with *P. vulgaris, M. symbioticum* and *X. autotrophicus,* demonstrated the existence of microorganisms survives. At a relatively high concentration of 100,000 ppm WMO the soil has the capacity to respond to biostimulation with crude extracts of biodetergents and lipases that it combined with a enzymes mixture that hydrolyze the aromatic fraction of the WMO. These processes accelerates by the crude extract of carbon nanoparticles facilitates the phytoremediation of the WMO remaining from of biostimulation soil, with *P. vulgaris* plus *M. symbioticum* and *X. autotrophicus* and the crude extract of nanoparticles that accelerated the degrading action of the legume root and that of *M. symbioticum* with *X.autotrophicus* to lower the concentration of WMO, to a value below the maximum of the NOM-138-SEMARNAT/SSA1-2012, what allowed the effective recovery of the agriculture soil for the production of safe food for humans and animals.

Conflicts of Interest:

The authors declare no conflicts of interest.

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