International Journal of

Research in Chemistry and Environment

Available online at: www.ijrce.org

ISSN 2248-9649

Research Paper

Hydrocarbons Degradation in Crude-oil Polluted Patagonian Soils with Different Organic Matter Quality: Effect of added Organic amendment

[*]Cecilia E. S. Alvaro[1], Andrea M. Torre[2], Norma S. Nudelman[3]

[1]Facultad de Ingeniería. Universidad Nacional del Comahue, Buenos Aires 1400, Neuquén, ARGENTINA

[2]Facultad Ciencias del Ambiente y la Salud, Universidad Nacional del Comahue, Buenos Aires 1400, Neuquén, ARGENTINA

[3]Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Pab. II, P. 3 Ciudad Universitaria, Buenos Aires, ARGENTINA

(Received 25th February 2015, Accepted 18th March 2015)

Abstract: *Microbiological remediation technologies of oil contaminated soils have proven one of the most efficient techniques, being an environmentally sustainable and cost-effective solution applied at oil contaminated soils. In our country, these processes are commonly performed by adding synthetic fertilizers and control of the environmental conditions process, such as landfarming method. This research study the efficacy of adding biosolids, the effects of soil organic matter (OM) and its association degree with added amendment in bioremediation process of two contrasting quality soils of Comahue region, North Patagonia, Argentine. The obtained results show a statistically significant difference in petroleum total hydrocarbons (TPH) degradation kinetics, where the higher reduction of TPH was obtained with the contribution of biosolids amendment. The heavy hydrocarbon fraction (HFP), considered resistant to biodegradation showed significant reductions, being biosolids an effective treatment. Volatile aromatic hydrocarbons, (BTEX), were eliminated rapidly, due to processes of volatilization and biodegradation. Organic amendment optimize microbial activity producing significant improvement over conventional landfarming treatments. Results presented suggest that the OM soil and amendment markedly interact to determine the degradation rate of hydrocarbons in oil-polluted soil. To date there have been no studies to assess the impact caused by oil spills in high OM content soils of Comahue region, and the behaviour of these soil on degradation oil spills.*

Keywords: bioremediation, biosolids, crude oil degradation, organic carbon fractions, soil microorganisms.

Introduction

Soils play an important role in the environment by controlling the fate and availability of organic compounds. When crude oil is spilled on soil, the environmental behaviour of its components is controlled by a number of processes, such as sorption, dissolution, degradation and evaporation. The sorption of chemical compounds in soil is primarily regulated by organic matter (OM) and minerals. The importance of OM regarding chemical, physical and biological soil quality is now well recognized[1]. Spills effect on soil cause inhibition or reduction of plant cover, changes in fauna and flora population dynamics, microbial contamination by seepage of underground water, as well as the economic and social impacts due to land wastage[2]. It is necessary the application of techniques such as bioremediation, which provides an environmentally safe, effective and economical solution for the remediation of petroleum contaminated soils whose main purpose is accelerate the total or partial degradation of organic pollutants decreasing toxicity, mobility and environmental impact. In this sense, several studies of aged polluted systems along the time have been reported with the aim of evaluating the environmental impact on variables such as the degradation of total petroleum hydrocarbons (TPH) and polynuclear aromatic hydrocarbons (PAHs) concentrations in soils and sediments and the behaviour of oil components in aqueous phase[3,4].

The remediation of soils contaminated with crude oil, drilling muds and derivatives solvents exploitation is a topic of intense research today[2]. Approximately 90% of Patagonia is made up of semi-arid steppe with severe desertification problems, this type of ecosystem is characterized by low OM content, low water and nutrient retention. In turn, the natural content of soil OM, and their concentrations and disposition influences the bioavailability of organic pollutants, because according to the degree of maturity of organic matter, there are different interactions that affect pollutants degradation by microorganisms [5].

In Comahue region, North Patagonia, oil and gas exploration and exploitation are the main economic activities. This proceeding causes, as in other parts of the world, adverse changes in the environment with potential risks to human health and productive activity in the region[6,7]. Fruit and vegetable production is another important economic activity in the Río Negro and Neuquén Valley, North Patagonia, the farm soils have markedly different physicochemical and textural properties than the soil in which oil exploration is performed[7]. Additionally, environmental conditions as adsorption, lixiviation and volatilization, among others, must be considered when evaluating the effects of pollutants in soils.

On the other hand, the use of biochemical capabilities of microorganisms is one of the most environmentally sustainable cleaning techniques acceptable for treatment of contaminated soils, since it presents several advantages over conventional treatments[8]. In addition, microorganisms have a unique ability to interact both physically and chemically on a large number of compounds by changing its structure or bringing them to the total degradation[6],[8]. Hydrocarbons biodegradation has been described both in terms of aerobic and anaerobic conditions, although anaerobic degradation processes are slow and biochemical mechanisms are not yet described, mostly[8].

The OM stock is comprised of labile (actively) and stable (resistant, recalcitrant) pools. The stable OM fraction is slowly altered by microbial activities[9]. Accumulated evidences suggest that certain fractions of OM are sensitive indicators of management processes impact[9]. Changes in OM pools due to different management practices have been studied in a few regions of the wold [10].

This paper describes a new approach for remediation of oil residues on low and high OM content Patagonian soils, based on the use of sewage sludge as organic amendment. To date, there have been no studies to assess the impact caused by oil spills in productive soils of Comahue region and the behaviour of this type of soil on degradation oil spills.

Material and Methods

Soil sampling and physical, chemical and bacteriological analysis

Two soils of contrasting properties were selected: bard soil (Sb), typical Patagonian soil of Aridisol characteristics[6], and farm soil, (Sf) of Entisol characteristics: typical silt loam, granular, slightly hard to soft consistency (dry), friable (humid), slightly plastic, slightly adhesive, lacks carbonates, abundant roots, clear and smooth limit. For sampling and monitoring processes, soil was taken at random in the first thirty inches deep. Samples were air dried and then sieved with mesh of 10 mm. The study was carried out with soils that were contaminated with crude oil in laboratory scale.

The physicochemical and chemical soil properties were determined and subsequently soils were contaminated with oil (10% w/w). Seven days after contamination, biosolids (B) from the local sewage treatment plant wastewater were added at 40 g/kg soil (dry basis) dose. Oil contaminated soil (SC) and contaminated bard and farm soils + biosolids (SbB and SfB) samples were analyzed by triplicate, using standard methods [1]. The determined physico-chemical parameters in soil and amendment (analytical methods) were: pH (by the saturated paste potentiometric method)[1], Electrical conductivity (EC), (Whiston bridge in soil saturation extract)[1], Oxidizable organic carbon (OC) and Organic matter (OM) (modified Walkley-Black method)[9], Total nitrogen, (Kjeldahl), Cation exchange capacity (CEC) and total cations (ammonium acetate saturation method)[1], exchangeable sodium percentage (ESP), (Richards)[1], Sodium (flame photometry)[1], Calcium + Magnesium (complexometry)[1], Water holding capacity (WC) at 0.3 atm and 15 atm (Richards membrane)[1]. The analytical methods carry out to evaluate the remediation process were: Oil Spill Identification (GC/SM), ASTM D-5739/00[11a], Oil aromatic compounds ASTM D 5831/09[11b], TPH, EPA 418.1 [12], extractions with *n*-hexane and subsequent gravimetric determination were performed with the Mexican standard method NMX-AA-134-SCFI/06[13]. Hexane extractables (HEM) and heavy hydrocarbon fraction (HFP) were determined[13]. OC was analyzed by potassium dichromate/sulphuric acid with varying concentrations of acid[9].

The quantification of native microorganisms, total heterotrophic aerobic microorganisms (HAT) and hydrocarbon degraders (DH), was carried out using the most probable number technique (MPN)[14]. The culture media were nutrient broth for HAT and inorganic micronutrient saline and kerosene as carbon source for DH. Using the statistical software InfoStat, 2011e version[15], a nonlinear regression analysis for the replicates of each treatment and an analysis of variance (ANOVA) was performed. The given results are the average of triplicate treatments in each case.

Biodegradation experiment design

The laboratory trials were carried out in microcosms that involved three bioremediation treatments with three replicates for each soil sample. The microcosms (six kilograms soil each one) were assembled in glass boxes. The analyzed composition of petroleum hydrocarbons, expressed in %/weight were: total paraffinic: 25.0 % (± 0.4), total isoparaffinic: 31.3% (± 0.6), total naphthenic 37.2% (± 0.6), total aromatics 6.5% (± 0.1). Oil was 40 API degrees (60 ºF/15.6 ºC) and classified as light oil. The humidity was maintained constant at water holding capacity (WC) by adding the amount of water lost by evaporation, determined by weight difference. The soil samples studied during the bioremediation treatments were the contaminated control soils SbC, SfC and the mixture of Sb and Sf with amendment B.

The experiments were carried out at 0, 90, and 180 days in laboratory r. t. (25 ± 4°C). The quantification of HAT and DH microorganisms was carried out at 0 and 180 days in B, SbC, SfC, Sb+B and Sf+B soils. To favour aerobic microbial activity during the 180 days were controlled aeration, soil humidity and temperature twice a week.

Results and Discussion

Physico-chemical characteristics of soils and amendment

Physico-chemical characteristics of bard (Sb) and farm (Sf) soils without oil contamination were analyzed before begin the bioremediation process. Table 1 shows the obtained results for the soils and amendment at zero time. Sb showed typical characteristics of the sampled semiarid region: very low OM (<1%), and high percentages of sand in composition leading to a low cation exchange capacity (CEC) and water holding capacity (WC). Sf corresponds to a production soil with marked differences: appropriate textural composition, higher OM content and consequently a substantial improvement in water holding capacity (WC), however the electrical conductivity (EC) and exchangeable sodium percentage (ESP) show characteristics of sodic saline soils, also common for this area. The water holding capacity (WC) of Sf soil at 0.3 atm and 15 atm, 14.1 % and 11.4 % respectively, and available water, 2.7 %, are expected values for fertile soils[10]. The Sf soil has a pH value close to neutrality, high electrical conductivity (EC) and very high % CEC, while Sb soil is slightly alkaline. In contrast, Sb soil provided an EC of 0.5 mS / cm, a result which classifies as no-saline, a high percentage of sand (80%), very low organic matter (OM <0.5%), low WC and CEC.

B had a high total nitrogen value (4.63 %), the highest percentage corresponds to organic N and the lower values for inorganic nitrogen (N-NH_4^+, NO_3^-). It has a ratio of C/N = 8.3, ratio minor than 20:1. In the same availability is phosphorus (P) with a total value of 0.6%, since the ratio of C/P was < 100: 1. These relationships, C/N and C/P, determine that both nutrients are availble to the microbial metabolism[10].

n-Hexane extractable material and Heavy Fraction Hydrocarbon analysis

First, a pre-treatment was performed in soils to achieve the elimination of interfering substances. Then, soil samples *n*-hexane extractable material (HEM) were quantified gravimetrically. HEM sample were re-dissolved with *n*-hexane, and added silica gel to remove polar compounds to quantify heavy fraction hydrocarbon (HFP) [13].

Volatile aromatics hydrocarbons (BTEX) determination was performed up to the first fifteen days of bioremediation due to its sharp decrease measured during this stage (not shown). These results are consistent with those reported by several authors, who agree that monoaromatic hydrocarbons are volatilized during the processes of soil aeration and dissolution[16].

HEM degradation after hundred eighty days application of biosolids in both types of soils is shown in Figure 1. At zero time the quantification of HEM presented values ranging of 42- 45 x 10^3 mg / kg average in all soils studied. As shown in Figure 1, a similar response in SbC and SfC soils were determined, with a degradation ranged between 12 and 16% at ninety days. The soils with B aggregate shows a marked difference with the control soils, being the soil with higher OM content, SfB, which present the highest HEM degradation. The same degradation pattern was observed to finish the experience, obtaining percentages of HEM degradation of 23%, 45%, 40% and 71% for SbC, SbB, SfC and SfB, respectively.

Due to their recalcitrance, HFP quantification is especially important since allow assess with more certainty the influence of B addition for bioremediation of oil contaminated soils. At zero time, it was observed that the HFP values in all cases were approximately 22 x 10^3 mg / kg (2.2%), *i.e*, almost 50% less than the values obtained in the HEM quantification. As shown in Figure 2, HFP showed similar degradation behaviour to the HEM registered in this study, obtaining the greatest HFP reductions in soil that has higher OM content. Degradation HFP percentages obtained at 180 days was 28%, 54%, 46% and 83% for SbC, SbB, SfC and SfB, respectively.

The degradation kinetics showed statistically significant differences ($p > 0.05$) for the treatments with B aggregate, being HFP decay highly significant in soils with higher OM content. It is worthwhile to note that due to the characteristic of Patagonian soils, associated with the desert climate of the region, the bioremediation by B amendment is markedly more effective and sustainable than the conventional processes such as moisture management [17] or phytoremediation[18] methods.

These results proved to be satisfactory and encourage applying this type of treatments in field trials to evaluate the sensitivity of the model to variations in the main factors involved. The observed reductions in the HFP contents of oil polluted Patagonian soils confirm these assumptions.

Table 1: Physico-chemical properties of soils and amendment before bioremediation (zero time)

Physico-chemical parameters	**Control bard Soil (SbC)**	**Control farm Soil (SfC)**	**Biosolid (B)**
pH (± 0.2)	8.5	7.6	5.5
EC (mS/cm,± 0.03)	0.5	4.5	1.8
CEC (meq/100g soil ± 0.2)	1.2	24.5	----
OM (% ± 0.03)	0.18	4.2	38.3
OC (% ± 0.05)	0.11	1.8	4.2
N (%± 0.03)	0.7	2.1	4.63
N-NO_2^- + NO_3^- (mg/kg ± 1.2)	----	----	723
N-NH_4^+ (mg/kg ± 0.7)	----	----	180
Na^+ (meq/l ± 0.4)	4.57	23.0	----
K^+ (meq/l ± 0.4)	1.20	3.26	0.11
Ca^{++} (meq/l ± 0.4)	9.37	20.0	1.33
Mg^{++} (meq/l ± 0.3)	6.72	9.37	0.27
P (meq/l ± 0.05)	0.36	1.6	0.6
P Olsen (mg/kg ± 2.4)	----	----	440
ESP % (± 0.2)	5.6	15.0	----
WC % (0.3 atm, ± 0.1)	3.3	14.1	----
WC % (15 atm, ± 0.1)	2.52	11.4	----
Available water (% ± 0.1)	0.78	2.7	----
Texture (%)	Af	fA	----
Clay	9.7	7.3	----
Silt	9.7	29.1	----
Sand	80.6	63.6	----

Note: Table abbreviations are cited in the text.

Organic carbon fractions

The importance of organic carbon on the physical, chemical, and biological features of soil quality is well recognized[9, 10]. Contaminants in soil can be sequestered in ground microspores and even reacting with organic matter or incorporated in humic substances of soil. Labile fractions of soil OC are classified as very labile C (f1), labile C (f2), less labile C (f3), non-labile C (f4) [10].

To analyze the effects of the quality of organic matter and the degree of association with the remediation of soils studied, the percentage of oxidizable OC in soils and amendment used were determined. Contrary than expected, biosolids showed more percentage of less labile OC fraction (f3). This result is consistent with the pH value obtained for B, wich indicates that has not been exposed to stabilization, because after this process pH values are near neutrality.

In contaminated soils, at zero time OC provided by oil showed a differential distribution. As indicate Table 2, Sb has the highest value of intermediate fraction (f2), while Sf has higher percentage of less labile fraction (f3), *i.e.* the same dose and quality of oil pollution hydrocarbon contributions were distributed into different fractions, probably due to variation in OC content of the untreated soils. When bioremediation treatments end up, values OC fractions are reversed and Sb shows an increase of f3 and Sf of intermediate lability fraction. In SbB and SfB, at 180 days of bioremediation the fractions analyzed showed a marked reduction. These results are consistent with the increase of microorganisms population as well as the evident hydrocarbons degradation determined at the end of treatments.

These results suggest that the quality and quantity of soil OM and the OC added with amendment interact strongly to determine the rate of remediation in petroleum hydrocarbon contaminated soils.

Microbiological analysis

Biological treatment is a fast growing promising remediation technique increasingly being studied and applied in practical use for pollutant clean-up[19]. Biodegradation of organic compounds is based on biological oxidation thereof by the action of microorganisms. The spontaneous degradation is due to the activity of indigenous microorganisms and occurs generally by the competition of mixed microbial populations, which operate under symbiotic regulations. Mixed populations are often very efficient in use complex substrates such as oil residues[8,20]. A large number of enzymes from bacteria, fungi, and plants have been reported to be involved in the biodegradation of toxic organic pollutants. The research activity in this area would contribute towards developing advanced bioprocess technology to reduce the toxicity of the pollutants and also to obtain novel useful substances[21].

Table 3 shows the fluctuations of hydrocarbon degrader microorganisms (DH) and total heterotrophic aerobic microorganisms (HAT) populations in B, uncontaminated soils and SbC, SfC, SbB and SfB soils at the end of treatments. It can be noticed that HAT and DH microorganisms, expressed as MPN/g soil, showed high values of both populations in B respect uncontaminated soils. The soil pollution by oil increases the MPN of microorganisms, except control farm soil in which a retraction is evident in DH population, but this is recovered with the addition of B in the bioremediation treatment.

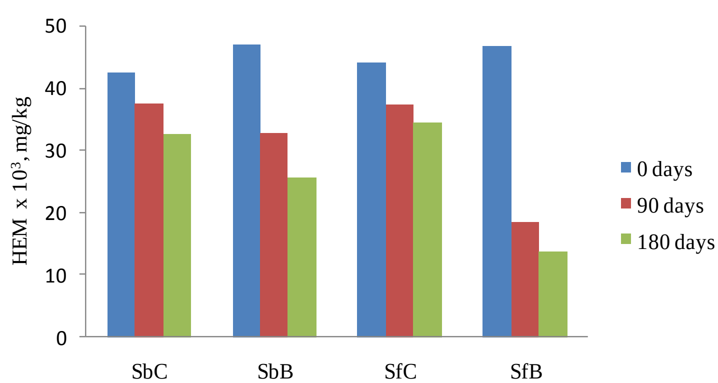

Figure 1: HEM degradation in control contaminated soils (SbC, SfC) and biosolids amended (SbB, SfB) at 0, 90 and 180 days

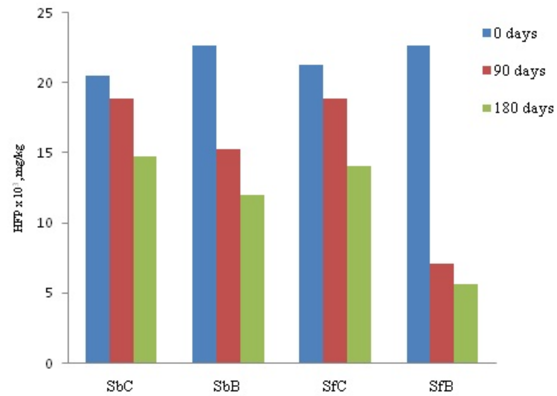

Figure 2: HFP degradation in control contaminated soils (SbC, SfC) and biosolids amended (SbB, SfB) at 0, 90 and 180 days

Table 2: Fractionation of organic carbon from biosolids, untreated and treated soils after 180 days using a modified Walkley-Black method with varying concentrations of sulphuric acid

Samples	Time (days)	Organic carbon fractions (%)		
		Fraction 1	Fraction 2	Fraction 3
Biosolids (B)	-	6.30 ± 0.12	0.80 ± 0.07	30.9 ± 0.16
SbC	0 180	0.50 ± 0.03 0.40 ± 0.02	2.50 ± 0.07 1.40 ± 0.08	1.00 ± 0.04 2.40 ± 0.10
SbC + B	0 180	1.38 ± 0.09 0.52 ± 0.04	2.84 ± 0.11 0.32 ± 0.02	0.47 ± 0.01 0.60 ± 0.03
SfC	0 180	1.50 ± 0.08 0.70 ± 0.03	1.70 ± 0.04 5.40 ± 0.16	2.50 ± 0.06 0.70 ± 0.04
SfC + B	0 180	0.51 ± 0.02 ---	4.76 ± 0.08 3.47 ± 0.05	0.47 ± 0.02 0.44 ± 0.02

Note: Fraction 1 (f1): 12*N* H_2SO_4, Fraction 2 (f2): 18*N*–12*N* H H_2SO_4, Fraction 3 (f3): 24*N*–18*N* H H_2SO_4

Table 3: Most Probable Number (MPN) of DH and HAT microorganisms in uncontaminated, oil contaminated and bioremediated soils

Sample	Microorganisms (MPN/g soil)	
	HAT	DH
Biosolids (B)	4.9×10^6	2.71×10^3
Sf (without oil)	4.6×10^5	1.53×10^2
SfC	2.00×10^6	4.78×10^3
SfC + B	2.20×10^7	1.00×10^6
Sb (without oil)	1.17×10^3	0.90×10^2
SbC	3.12×10^4	1.19×10^3
SbC + B	4.90×10^6	4.90×10^5

Conclusion

Bioremediation of semi-arid and productive Patagonian soils with biosolids presented a higher degradation rate of HEM and HFP petroleum hydrocarbon fractions during the first period of remediation, while control contaminated soils doubled or tripled its maximum hydrocarbon reduction at the end of the treatment. In all cases, it was observed that the microbial population increase is inversely proportional to the HEM and HFP degradation rate. In conclusion, the determined data indicate that petroleum hydrocarbon biodegradation by microbial metabolism constitute a very effective formulation to remediate oil contaminated soils with different OM contents. Contaminated unamended soils achieved an important reduction HEM and HFP in all cases at the end of bioremediation, highlighting the efficacy of adding organic amendment to improve the degradation of petroleum hydrocarbons. Counting microorganisms expressed as MPN/g soil showed that the natural content of soils increased with hydrocarbon and was higher with the addition of amendment.

According to a recently reported study[19], our results indicate that soils with higher amounts of natural OM showed better degradative conditions performing the bioremediation in less time. In this sense, the bioremediation experiments allowed concluding that the quality and quantity of OM present in biosolids markedly interact with the OM soils content to determine the rate remediation of petroleum hydrocarbon contaminated soils. Comparison with other traditional treatments[17], Trindade et al. 2005 [22] shows that bioremediation method here reported is a sustainable and effective technique also for this type of soils.

Acknowledgment
Authors gratefully acknowledges National University of Comahue, Project I183, for the financial support.

References

1. Sparks D.L., Environmental Soil Chemistry, (Hardcover) 2nd ed., San Diego, California USA, Elsevier Science, **(2003)**

2. Erdogan E., and Karaca A., Bioremediation of Crude Oil Pollutted Soils, *Asian J. of Biotechnology*, **3 (3**), 206-213 **(2011)**

3. Ríos S.M., Nudelman N.S. and Katusich O., Effects of solution and soil chemistry on the distribution of oil residual in Patagonian soil, *Latin Am. Appl. Res. J.*, **34**, 149–153 **(2004)**

4. Ríos S.M. and Nudelman N.S., Natural attenuation of oil spills in Patagonian Soils, characterization by ^{1}H-NMR spectroscopy, *Environ. Technol*., **29 (1)**, 23-33 **(2008)**

5. Ríos S.M. and Nudelman N.S., Multilayer sorption model for the interactions between crude oil and clay in Patagonian soils, *J. Disp. Sci. Technol., **26 (1)**,* 19-25 **(2005)**

6. Alvaro C.E.S., Olave A.J., Schmid P.M. and Nudelman N.S., Microbial Remediation Performance of Environmental Liabilities from Oil Spills in Patagonian Soils: A Laboratory – Scale. *Int. J. Res. Chem. Environ.,* **4 (3)**, 119-126 **(2014)**

7. Alvaro C. E. S., Laos F., Arocena L., Olave A. J., Garrido N., and Nudelman N. S. Biorremediación de Suelos Afectados por la Producción Hidrocarburífera en la Región del Comahue. *Revista Petroquímica, Petróleo, Gas & Química.* Edit. PGQ. Ed. **278**, 236-239 **(2012)**

8. Lladó S., Solanas A.M., Lapuente J. de, Borras M. and Viñas M., A diversified approach to evaluate biostimulation and bioaugmentation strategies for heavy-oil contaminated soil, *Sci. of the Total Environ.*, **435-436**, 262-269 **(2012)**

9. Chan, K.Y., Bowman A., Oates A., Oxidizible Organic Carbon fractions and soil quality changes in an oxic paleustalf under different pasture leys. *Soil Science*, **166 (1)**, 61-67, **(2001)**

10. Majumder B., Mandal B., and Bandyopadhyay, P. K., Soil Organic carbon pools and productivity in relationt nutrient management in a 20-year-old rice berseem agroecosystem, *Biol. Fertil Soils*, **44**, 451-461, **(2008)**

11. a) American Society for Testing and Materials. Standard Practice for Oil Spill Identification by Gas Chromatography and Positive Ion Electron Impact Mass Spectrometry, W. Conshohocken PA, **(2000)**

b) American Society for Testing and Materials. Standard Test Method for Screening Fuel aromatic compounds in Soils. ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States, **(2009)**

12. USEPA (U.S. Enviromental Protection Agency), Total Petroleum Hydrocarbons in Analytical Methods and Laboratories, Petrochemicals-Oils, Washington DC, **(1983)**

13. NMX-AA-134-SCFI/06. Suelos-Hidrocarburos de fracción pesada por extracción y gravimetria. Secretaría de Economía de Mexico, 23, **(2006)**

14. Woomer P.L., Most Probable Number Counts, SSSA Book Series, **5**, 59-79 **(1994)**

15. Di Renzo J.A., Casanoves F., Balzarini M.G., González L., Tablado M. and Robledo C.W., InfoStat versión 2011e. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentine **(2011)**

16. Eweis J.B., Ergas S.J., Chang D.P.Y. and Schroedes E.D., Principios de Biorrecuperación: Tratamientos para la descontaminación y regeneración de suelos y aguas subterráneas mediante procesos biológicos y fisicoquímicos, (Hardcover) Madrid, España Mc Graw Hill/Interamericana de España **(1999)**

17. Adams Schroeder R.H., Comparison of moisture management methods for the bioremediation of hydrocarbon contaminated soil, *African J. of Biotechnol.*, **10(3)**, 394-404 **(2011)**

18. Lee J.H., An overview of phytoremediation as a potentially promising technology for environmental pollution control, *Biotechnology and Bioprocess Engineering*, **18 (3)**, 431-439 **(2013)**

19. Juwarkar A.A., Singh S.K. and Mudhoo A., A comprehensive overview of elements in bioremediation, *Rev. Environ. Sci. Biotechnol.*, **9**, 215–288 **(2010)**

20. Alves Soares A., Albergaria J. S., Fernandes Domingues V., Alvim-Ferraz M.C.M. and Delerue-Matos C., Remediation of soils combining soil vapor extraction and bioremediation: Benzene, *Chemosphere*, **80**, 823–828 **(2010)**

21. Karigar C.S., and Rao S.S., Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review, *Enzyme Research*, **2011**, Article ID 805187, 11 pages, 2011 doi:10.4061/2011/805187 **(2011)**

22. Trindade P.V.O., Sobral L.G., Rizzo A.C.L., Leite S.G.F. and Soriano A.U., Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: a comparison study, *Chemosphere*, **58**, 515-522 **(2005)**.