Original Research

Do Application Rates of Wastewater Sewage Sludge Affect the Removal of PAHs from Alkaline Saline Soil?

Fabián Fernández-Luqueño[1]*, Reyna Mendoza-Cristino[1], Luc Dendooven[2]

[1]Sustainability of Natural Resources and Energy Program, Cinvestav-Saltillo, Coahuila. C.P. 25900, Mexico
[2]Laboratory of Soil Ecology, GIP, Cinvestav, México D.F, C.P. 07360 Mexico

Abstract

The application of wastewater sludge to an alkaline saline soil of Texcoco accelerated removal of polycyclic aromatic hydrocarbons (PAHs), but not always. As part of a study into factors that might affect dissipation of PAHs from soil, the effect of different application rates (0, 3, 9, 18, and 36 tons dry sludge ha^{-1}) of wastewater sludge on removal of phenanthrene and anthracene was studied in an alkaline saline soil with pH 9.7 and electrolytic conductivity (EC) of 7.6 dS m^{-1}. The dynamics of phenanthrene, anthracene, ammonium, nitrite, nitrate, and CO_2 were monitored for 56 days. Application of wastewater increased the removal rate of the PAHs independent of the concentration applied, and between 70 and 79% of the phenanthrene and 69 and 79% of the anthracene was removed after 56 days. Spiking soil with PAHs increased emissions of CO_2 while applying sludge further increased it. It was found that the removal of PAHs was initially low as the amount of phenanthrene and anthracene decreased after only 14 days. The addition of wastewater sludge increased the removal of phenanthrene and anthracene, but the effect was independent of the application rate or the PAHs studied. Spiking soil with PAHs increased emissions of CO_2 and reduced the amount of NH_4^+ and NO_3^- in soil.

Keywords: environmental health, mineral nitrogen, PAH degradation, soil pollution, soil sustainability

Introduction

Soil salinity is an environmental problem that threatens croplands in arid regions worldwide while soil pollution grows daily – even in developing countries. Polycyclic aromatic hydrocarbons (PAHs) enter the environment as a result of residential heating; engine, gas, coal, and wood combustion; and oil-spills. Concentrations of PAHs are monitored in the environment because of their possible negative effects on human health [1-2].

Sludge derived from wastewater treatment with its high organic material and nitrogen and phosphorous content is frequently spread on land to restore degraded soils [3]. It is well documented that organic material can accelerate the removal of PAHs when added to soil [4-6]. Wastewater sludge might affect the dissipation of PAHs in soil in different ways. Wastewater sludge contains larger amounts of organic material that will increase microbial activity, which might accelerate the removal of PAHs from soil. It contains nutrients such as

*e-mail: cinves.cp.cha.luqueno@gmail.com

N and P that might increase microbial activity and thus dissipate PAHs from soil, and is rich in microorganisms that might contribute to the removal of PAHs from soil. Additionally, sludge might change soil conditions, such as pH and electrolytic conductivity (EC), thereby stimulating or inhibiting removal of PAHs. The question remains if the amount of wastewater sludge applied to a soil will affect the removal of phenanthrene (Phen) and anthracene (Anthra). As part of a study into the removal of PAHs from an alkaline saline soil using organic wastes, soil of the former lake Texcoco with EC 7.6 dS m^{-1} and pH 9.7 was spiked with Phen and Anthra and amended with 0, 1.5, 4.5, 9, or 18 g dry wastewater sludge kg^{-1}, i.e., 0, 3, 9, 18, and 36 tons dry sludge ha^{-1}, which was equivalent to approximately 0, 50, 150, 300, and 600 kg N ha^{-1} added to the 0-15 cm soil layer with density 1.3 kg dm^3, considering that 40% of the organic N was mineralized. Emissions of CO_2; concentrations of inorganic N (ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-)); and Phen and Anthra were monitored in an aerobic incubation at 22±2ºC for 56 days. The objective of this study was to investigate if the amount of wastewater sludge added to an alkaline-saline soil accelerated the removal of Phen and Anthra.

Material and Methods

Area Description and Soil Sampling

The sampling site is located in former Lake Texcoco in the valley of México City (México) (N.L. 19°30'', W. L. 98°53'). Soil was sampled at random by augering the 0-15 cm top-layer of three plots of approximately 0.5 ha. The soil from each plot was pooled so that three soil samples were obtained, with 5 mm sieved and characterized. Characteristics of the soil are given in Table 1.

PAH and Sludge Characteristics

Hydrocarbons were obtained from Sigma (USA) with purity > 98% for Phen and > 97% for Anthra. Acetone was purchased from J. T. Baker (USA) with purity 99.7%. Sludge was obtained from Reciclagua (Sistema Ecológico de Regeneración de Aguas Residuales Ind., S. A. de C. V.) in Lerma, State of México (México). Thirty kg aerobically digested industrial sludge was sampled aseptically in plastic bags. Some characteristics of the sludge can be found in Table 1.

Treatments and Experimental Set-Up

Sub-samples (90) of 5 g dry soil of each plot (*n* = 3) were added to 120 ml glass flasks and contaminated with 2 ml acetone in which PAHs were dissolved. An additional 18 sub-samples were not contaminated and served as control. All the flasks were placed under vacuum in a desiccator for 20 min, removed, and 15 g soil was added to each flask. The soil was then mixed thoroughly. The

Table 1. Characteristics of the Texcoco soil and the wastewater sludge.

	Texcoco soil	Wastewater sludge
pH$_{H_2O}$	9.7	8.4
Water holding capacity (g kg^{-1}) [a]	569	ND [b]
Water content (g kg^{-1})	12	842
Organic carbon (g kg^{-1})	58.2	509
Inorganic carbon (g kg^{-1})	24	ND
Total Kjeldahl nitrogen (g kg^{-1})	0.6	55.3
N-NH$_4^+$ (mg kg^{-1})	13.7	6127
N-NO$_3^-$ (mg kg^{-1})	3.5	48
N-NO$_2^-$ (mg kg^{-1})	1.3	16
Electrolytic conductivity (dS m^{-1})	7.6	14
Clay content (g kg^{-1}l)	24	ND
Silt content (g kg^{-1})	103	ND
Sand content (g kg^{-1})	873	ND
Textural soil classification	Loamy sand	ND
PAHs	NDET [c]	NDET

[a] On a dry base,   [b] Not determined,   [c] Not detected.

amount of PAHs added to the 20 g soil was such that 1,200 mg Phen kg^{-1} and 520 mg Anthra kg^{-1} were applied.

Eighteen of the contaminated soil-samples were amended with 1.5 g dry sludge kg^{-1}, 18 with 4.5 mg dry sludge kg^{-1}, 18 with 9 g dry sludge kg^{-1}, and 18 with 18 g dry sludge kg^{-1}, while 18 were left unamended. Sludge water was removed by air drying. The amount of wastewater added was such that approximately 0, 50, 150, 300, and 600 kg N ha^{-1} was added considering a soil density of 1.3 kg dm^3 for a 15 cm soil layer and 40% of the organic N was mineralized during the experiment. Proportional amounts of distilled water were then added to all treatments so that the same water content (approximately 40% WHC) was obtained in each treatment.

Three flasks were chosen at random from each treatment of the three plots. Ten g soils were extracted for inorganic-N with 100 ml 0.5 M K$_2$SO$_4$ solution, shaken for 30 min, filtered through Whatman no. 42 filter paper and analyzed, while 1.5 g soil was extracted for PAHs with acetone and analyzed on a gas chromatograph (GC). These provided zero-time samples.

The remaining flasks were placed in 945 ml glass jars containing a vessel with 10 ml distilled H$_2$O and a vessel with 20 ml 1 M NaOH to trap evolved CO$_2$. The jars were sealed and stored in the dark at 22±2ºC for 56 days. An additional 15 jars containing a vessel with 10 ml distilled H$_2$O and a vessel with 20 ml 1 M NaOH were sealed and served as controls to account for the CO$_2$ trapped from the atmosphere. After 3, 7, 14, 28, and 56 days, three jars were

selected at random from each treatment and the soil was analyzed for NH_4^+, NO_2^-, NO_3^-, and PAHs as mentioned before. The remaining flasks were opened and aired each of three days for 10 min to avoid anaerobicity, then sealed again and further incubated. Chemical methodologies were carried out according to [4].

Chemical Analysis

Concentrations of Phen and Anthra in the soil were analyzed using an ultrasonic extraction method developed by [5]. The 1.5 g sub-sample of soil was mixed with 3 g anhydrous sodium sulphate to form a fine powder, placed in a Pyrex tube, and 5 ml acetone was added. The tubes were placed in a sonicated bath at 35-40ºC for 20 min, mechanically shaken on a vortex for 15 s, and sonicated again for 20 min. The extracts were separated from the soil by centrifugation at 3,000 × rpm for 15 min. This process was repeated three times. The extracts were combined, evaporated in a fume-cupboard at 50ºC, and dissolved in 2 ml acetone. From each tube, a 2.0 µl aliquot was immediately analyzed for PAHs on a Hewlett-Packard 4890-D GC (USA) fitted with a flame ionization detector. An HP-5 column from Hewlett-Packard (USA) with length of 15 m, inner diameter 0.53 mm, and film thickness 1.5 µm was used to separate the PAHs with He carrier gas flowing at a rate of 7 ml min^{-1}. The oven temperature at 140ºC was increased to 170ºC at a rate of 2ºC min^{-1}, maintained at 170ºC for 5 min, and increased to 280ºC at 30ºC min^{-1} and maintained at 280ºC for 10 min. The temperature of the injector was 280ºC and that of the detector 300ºC [6].

The percentage recovery of the PAHs was tested by adding 1.5 g dry soil of each of the three soil samples (in triplicate) to a Pyrex tube and spiking them with 1,200 mg phenanthrene kg^{-1} dry soil and 520 mg anthracene kg^{-1} dry soil. The PAHs in the soil were then extracted for the added PAHs as described by [4]. The recovery for Anthra and Phen was 96%.

Statistical Analyses

Concentrations of CO_2, NH_4^+, NO_2^-, NO_3^-, Phen, and Anthra were subjected to one-way analysis of variance using PROC GLM [7] to test for significant differences between treatments and the least significance difference (MSD) was then calculated [7].

Results

The emission of CO_2 resembled zero-order kinetics in all treatments (Fig. 1a). Contaminating soil with PAHs increased the CO_2 emission rate > five-fold. Application of wastewater sludge further increased CO_2 emissions. Spiking soil with Anthra and Phen increased CO_2 emissions significantly.

The concentration of Phen decreased in all treatments after 14 days (Fig. 1b), except for the PAH-spiked soil
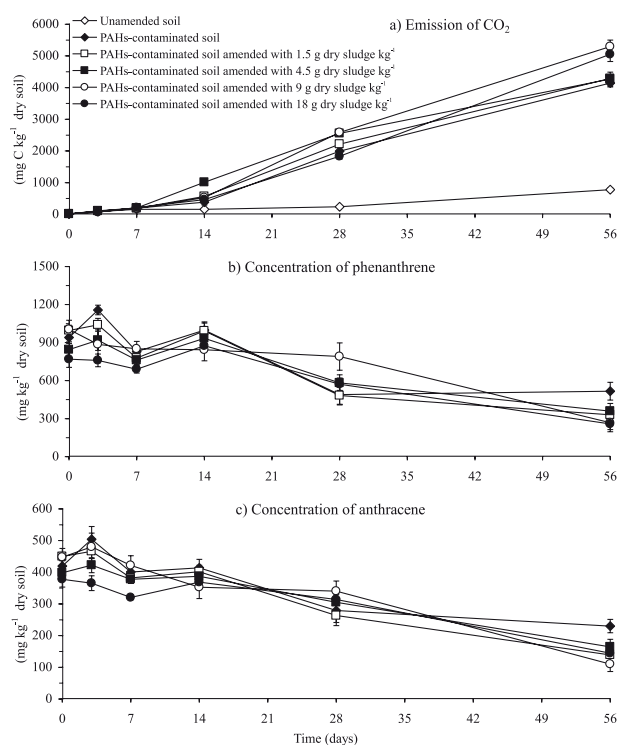

Fig. 1. Emissions of CO_2 (mg C kg^{-1} dry soil) and concentrations of phenanthrene and anthracene (mg kg^{-1} dry soil) from unamended soil and soil spiked with PAHs and amended with wastewater sludge at 0, 1.5, 4.5, 9, or 18 g dry sludge kg-1. The soil was incubated aerobically at 22±2ºC for 56 days. Bars are standard errors of the estimates (p<0.05) and each point in the graph is the mean of three plots and three replicates, i.e., n = 9.

Table 2. Removal rates of phenanthrene and anthracene (mg kg^{-1} soil day^{-1}) and CO_2 emission rates (mg C kg^{-1} soil day^{-1}) in soil amended with or without wastewater sludge incubated aerobically at 25±2ºC for 56 days.

Treatment	CO_2 emission rate	Phenan-threne	Anthra-cene
	(mg kg^{-1} day^{-1})		
Unamended soil	12.7 E	ND	ND
Soil spiked with PAHs	70.3 D	-8.7 B	-3.9 B
Soil + PAHs + sludge at 50	74.4 CD	-11.4 AB	-5.3 A
Soil + PAHs + sludge at 150	77.9 BC	-10.6 AB	-4.8 AB
Soil + PAHs + sludge at 300	90.2 A	-10.6 AB	-5.3 A
Soil + PAHs + sludge at 600	81.7 B	-12.5 A	-5.2 A
SEE	3.1	1.6	0.6

[a] SEE: standard error of the estimate (P<0.05),
[b] values with the same letter are not significantly affected by treatment, i.e. within columns (P<0.05).

amended with 9 g dry sludge kg⁻¹. In the unamended soil, i.e., in the untreated soil, 57% of the Phen was removed from soil after 56 days. Applying wastewater sludge increased the removal rate of Phen from soil significantly compared to the unamended soil after 56 days (P<0.05). However, the amount of wastewater sludge applied had no significant effect on the amount of Phen removed after 56 days and between 70 and 79% of the Phen was removed (Table 2; Fig. 1).

The concentration of Anthra decreased in all treatments after 14 days (Fig. 1c), except for the PAH-spiked soil amended with 9 g dry sludge kg⁻¹. In the unamended soil, i.e., in the untreated soil, 56% of the Anthra was removed from soil after 56 days. The application of wastewater sludge increased the removal rate of Anthra from soil significantly compared to the unamended soil after 56 days (P<0.05). However, the amount of wastewater sludge applied had no significant effect on the amount of Anthra removed after 56 days, and between 69 and 79% of the Anthra was removed (Table 2; Fig. 1).

The concentration of NH_4^+ dropped sharply within the first three days in the sludge-amended soil (Fig. 2a). After 14 days, concentrations of NH_4^+ were < 7 mg N kg⁻¹ and not significantly different among the treatments. Concentrations of NO_2^- were < 2 mg N kg⁻¹ and not significantly different among the treatments (Fig. 2b). NO_3^- concentration increased significantly over time in the

uncontaminated soil (Fig. 2c). In soil spiked with PAHs, the concentration of NO_3^- decreased over time and reached a minimum after 28 days. Afterward, the concentration of NO_3^- increased again. The application of wastewater sludge to the PAHs spiked soil had no significant effect on the concentrations of NO_3^- independent of the amount added.

Concentrations of NH_4^+ decreased sharply in the wastewater sludge-amended soil. Decreases in concentrations of NH_4^+ might be due to nitrification, microbial immobilization, NH_4^+ fixation, or NH_3 volatilization. Nitrification did not contribute to the decrease in NH_4^+ as concentrations of NO_3^- did not increase in the wastewater sludge-amended soil (Fig. 2).

Discussion

Scelza et al. [8] found similar results and reported a rapid increase in soil respiration and microbial biomass in a soil added with Phen, which suggested that microbial growth and activity had increased. However, Yang et al. [9] reported that the soil microbial activity was inhibited in a soil contaminated with phenanthrene. Acetone used as carrier to spike the soil with Anthra and Phen can be used as C substrate by soil microorganisms, such as *Rhodococcus* sp EC1, so that emission of CO_2 increases [10]. Acetone can also kill soil microorganisms so that microbial activity and thus emissions of CO_2 are inhibited, but the killed microorganisms might also serve as a C-substrate for the surviving microorganisms, thereby increasing emissions of CO_2 [11].

Autochthonous microorganisms can remove PAHs from soil [12], even in the presence of salt [13] and at pH>10 [14]. This was confirmed in this experiment as approximately 55% of the Phen and Anthra was removed from soil after 56 days. Both the physico-chemical characteristics of contaminants as well as the physical, chemical, and biological properties of soils affect the degradation of PAHs [4]. However, some of the results indicated that the removal of the PAHs in this experiment was low. First, the removal of PAHs is normally most accentuated in the first days after spiking the soil and further decreases are normally small. Betancur-Galvis et al. [15] found that 33% of added Phen was removed from an agricultural soil in seven days and an additional 15% in the next 105 days. The effect was even more outspoken in Texcoco soil with EC 12 dS m⁻¹ and pH 10, as 25% of Phen was removed within seven days and only 4% in the next 105 days. In the experiment reported here, a significant decrease in the concentration of Phen was only observed after 14 days. Second, the percentage of removal of Anthra and Phen was similar in this experiment, although the removal of Phen is normally higher than that of Anthra. Betancur-Galvis et al. [15] stated that only 16% of added Anthra was removed from an agricultural soil, and 33% of Phen in seven days. The slower degradation of Anthra can be attributed to its low solubility in aqueous systems
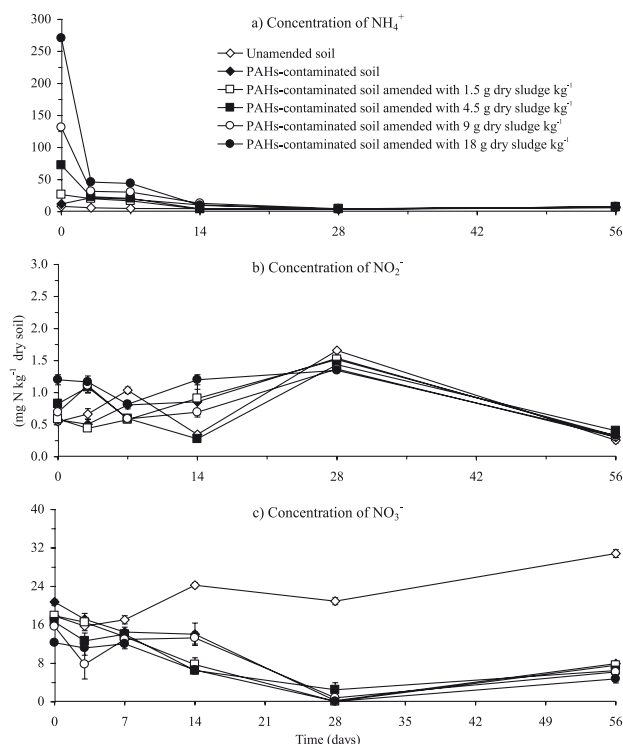

Fig. 2. Concentrations of NH_4^+, NO_2^-, and NO_3^- (mg N kg⁻¹ dry soil) from unamended soil and soil spiked with PAHs, and amended with wastewater sludge at 0, 1.5, 4.5, 9, or 18 g dry sludge kg-1. The soil was incubated aerobically at 22±2ºC for 56 days. Bars are standard errors of the estimates (p<0.05) and each point in the graph is the mean of three plots and three replicates, i.e., n = 9.

(0.07 mg l^{-1}) compared to that of Phen (1.29 mg l^{-1}), which renders it only slowly available for microbial degradation. It appears that in our experiment, the bio-accessibility of both PAHs was similar so that the difference in solubility did not affect their degradation.

The application of wastewater to Texcoco soil sometimes accelerates removal of Phen from soil, but not always. Fernández-Luqueño et al. [6] reported that in soil of Texcoco with EC 30 dS m^{-1} or 80 dS m^{-1}, removal of Anthra was not affected by the application of wastewater sludge. However, Betancur-Galvis et al. [15] found that the removal of Phen in wastewater sludge amended soil with EC 12 dS m^{-1}, and pH 10 was 54% compared to only 25% in the unamended soil after seven days and 71% and 29%, respectively, after 112 days. In the experiment reported here the wastewater sludge had less effect on the removal of Phen and Anthra than as reported by Betancur-Galvis et al. [15], but more than found by Fernández-Luqueño et al. [6]. This would indicate that the application of the wastewater sludge from the same treatment plant did not always have the same effect on the removal of Anthra and Phen from soil. It can be speculated that the combination of soil and sludge characteristics will determine the removal of contaminants from soil.

The pH of the Texcoco soil was 9.7, which will favour NH$_3$ volatilization. In a previous experiment approximately 50 mg NH$_4^+$-N was lost through NH$_3$ volatilization and a similar amount of NH$_4^+$ was fixed on the soil matrix. Additionally, the soil of Texcoco is N-depleted, so it can be speculated that some of the NH$_4^+$ was immobilized into the microbial biomass considering the large amounts of CO$_2$ emitted. Deni and Penninckx [16] found that the addition of hydrocarbons at a rate of 4 g C kg^{-1} soil stimulated immobilization of N. It is known that the addition of organic material, especially that with large C-to-N ratio, such as acetone and wastewater sludge, will induce N immobilization [17]. Acetone can be used as C substrate by soil microorganisms, e.g., *Methylobacterium*, *Rhodococcus,* and *Arthrobacter* [11], but it is N-free so upon decomposition microbial N immobilization can be induced. In addition, it has to be remembered that wastewater sludge contains nanoparticles and polyacrylamide – two factors that might have increased PAH dissipation and modified the soil conditions [18]. However, the application rate of wastewater sludge did not significantly affect PAH dissipation.

Conclusions

It was found that the removal of PAHs in the studied soil was low at the onset of the experiment as the amount of Phen and Anthra decreased after 14 days, but after 56 days the removal > 55%. Normally, the removal of PAHs is most accentuated in the first days, with subsequent decreases being very low. The removal of Phen and Anthra was similar in our study although the dissipation of the latter is normally much lower than that of the first. The addition of wastewater sludge increased PAH removal, but the effect was independent of the application rate. Spiking soil with PAHs increased CO$_2$ emissions and reduced the amount of NH$_4^+$ and NO$_3^-$ in soil.

Acknowledgements

References

1. KAMAL A., CINCINELLI A., MARTELLINI T., MALIK R.R. A review of PAH exposure from the combustion of biomass fuel and their less surveyed effect on the blood parameters. Environ. Sci. Pollut. R. **22** (6), 4076, **2015**.
2. PAWELCZAK M., DAWIDOWSKA-MARYNOWICZ B., OSZYWA B., KOSZALKOWSKA M., KRECIDLO L., KRZYSKO-LUPICKA T. Influence of bioremediation stimulators in soil on development of oat seedlings (*Avena sativa*) and their aminopeptidase activity. Arch. Environ. Prot. **41** (1), 24, **2015**.
3. ANTONIADIS V., KOUTROUBAS S.D., FOTIADIS S. Nitrogen, phosphorus, and potassium availability in manure- and sewage sludge-applied soil. Commun. Soil Sci. Plan. **46** (10), 393, **2015.**
4. FERNÁNDEZ-LUQUEÑO F., LÓPEZ-VALDEZ F., DENDOOVEN L., LUNA-SUAREZ S., CEBALLOS-RAMIREZ J.M. Why wastewater sludge stimulates and accelerates removal of PAHs in polluted soils? Appl. Soil Ecol. **101**, 1, **2016**.
5. SONG Y.F., OU Z.Q., SUN T.H., TEDILER A., LORINCI G., KETTRUP A. Analytical method for polycyclic aromatic hydrocarbons (PAHs) in soil and plants samples. Chin. J. Appl. Ecol. **6**, 92, **1995**.
6. FERNÁNDEZ-LUQUEÑO F., THALASSO F., LUNA-GUIDO M.L. CEBALLOS-RAMÍREZ J.M., ORDOÑEZ-RUIZ I.M., DENDOOVEN L. Flocculant in wastewayter affects dynamics of inorganic N and accelerates removal of phenanthrene and anthracene in soil. J. Environ. Manage. **90** (8), 2813, **2009**.
7. SAS Institute. Statistic guide for personal computers. Version 6.04, Edn. SAS Institute, Cary. **1989**.
8. SCELZA R., RAO M.A., GIANFREDA L. Effects of compost and of bacterial cells on the decontamination and the chemical and biological properties of an agricultural soil artificially contaminated with phenanthrene. Soil Biol. Biochem. **39** (6), 1303, **2007**.
9. YANG H., SU Y.H., ZHU Y.G., CHEN M.M., CHEN D.B., LIU Y.X. Influences of polycyclic **aromatic** hydrocarbons (PAHs) on soil microbial community composition with or without Vegetation. J. Environ. Sci Heal. A, **42** (1), 65, **2007**.
10. LEE E.H., CHO K.S. 2008. Characterization of cyclohexane and hexane degradation by Rhodococcus sp EC1. Chemosphere **71** (9), 1738, **2008**.
11. LEE P.H., CHAO K.P. ONG S.K. Solvent-water extraction method for the evaluation of polycyclic aromatic hydrocarbons bioavailability in coal-tar-contaminated soils, Int. J. Environ. Sci. Te. **11** (7), 1999, **2014**.
12. NIKOLOPOULOU M., EICKENBUSCH P., PASADAKIS N., VENIERI D., KALOGERAKIS N. Microcosm

evaluation of autochthonous bioaugmentation to combat marine oil spills. New Biotechnol. **30** (6), 734, **2013**.

13. CASTILLO-CARVAJAL L.C., SANZ-MARTIN J.L., BAR-RAGAN-HUERTA B.E. Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms: a review. Environ. Sci. Pollut. R. **21** (16), 9578, **2014**.

14. WANG J.B., WANG C., HUANG Q.Y., DING F., HE X.W. Adsorption of PAHs on the sediments from the yellow river delta as a function of particle size and salinity. Soil Sediment Contam. **24** (2), 103, **2015**.

15. BETANCUR-GALVIS L. A., ALVAREZ-BERNAL D., RA-MOS-VALDIVIA A.C., DENDOOVEN L. Bioremediation of polycyclic aromatic hydrocarbon-contaminated saline-alkaline soils of the former Lake Texcoco. Chemosphere **62** (11), 1749, **2006**.

16. DENI J., PENNINCKX M.J. Nitrification and autotrophic nitrifying bacteria in a hydrocarbon-polluted soil. Appl. Environ. Microb. **65**(9), 4008-13, **1999**.

17. BUSBY R.R., TORBERT H.A, GEBHART D.L. Carbon and nitrogen mineralization of non-composted and composted municipal solid waste in sandy soils. Soil Biol. Biochem. **39** (6), 1277, **2007**.

18. FERNANDEZ-LUQUEÑO F., LOPEZ-VALDEZ F., VALE-RIO-RODRÍGUEZ M.F., PARIONA N., HERNÁNDEZ-LOPEZ J.L., GARCIA-ORTIZ I., LOPEZ-BALTAZAR J., VEGA-SANCHEZ M.C., ESPINOSA-ZAPATA R., ACOSTA-GALLEGOS J.A. Effects of nanofertilizers on plant growth and development, and their interrelationship with the environmental. In: Fertilizers: components, uses in agriculture and environmental impact, LOPEZ-VALDEZ F., FERNANDEZ-LUQUEÑO F. (Eds.), NOVA Science. New York, USA, 211-24, **2014**.