

Original Research

Dissipation of Phenanthrene and Anthracene from Soil with Increasing Salt Content Amended with Wastewater Sludge

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Abstract

The removal of PAHs was stimulated by wastewater sludge in an alkaline saline soil of the former Lake Texcoco, but not always to the same extent. We investigated how a varying electrolytic conductivity (EC) affected the removal of phenanthrene (PHEN) and anthracene (ANTHR) from wastewater sludge-amended soils. Soil with EC 6, 30, 80, and 146 dS m⁻¹ was contaminated with PHEN and ANTHR and amended with or without wastewater sludge, while emissions of CO₂ and concentrations of ammonium, nitrite, and nitrate were monitored. A decrement on the concentrations of PHEN and ANTHR was observed and was faster in soil with EC 6 dS m⁻¹ than in soil with EC 30 dS m⁻¹ and 80 dS m⁻¹, and was slower than in soil with EC 146 dS m⁻¹. Adding wastewater sludge to soil reduced the concentration of PHEN and ANTHR in soil after 56 days. Spiking soil with PAHs or amending it with wastewater sludge increased the CO₂ emission rate, but decreased at higher EC. The concentration of NO₃⁻ decreased when soil was spiked with PAHs and amended with sludge, except in soil with EC 146 dS m⁻¹. It was found that the removal of PAHs was not inhibited by salt content and a principal component analysis indicated that none of the measured soil characteristics, i.e., pH, EC, particle size distribution, water-holding capacity, or organic C content predicted the removal of ANTHR or PHEN from contaminated soil. The application of wastewater sludge increased the dissipation of PHEN and ANTHR after 56 days.

Keywords: mineral nitrogen, carbon dioxide emission, polycyclic aromatic hydrocarbons, soil degradation, soil organic matter

Introduction

High salt contents affect soil processes in many different ways. Excessive amounts of salts often result in poor soil structure and affect the chemical and biological processes while soil microbial biomass decreases due to its activity is inhibited. Carbon and nitrogen mineralization and soil enzyme activities, which are crucial for the decomposition of organic matter and their release of nutrients required to sustain soil productivity, are often inhibited by high salinity [1]. Consequently, salinity decreases the dissipation of polycyclic aromatic hydrocarbons (PAHs) in soils [2].

PAHs are widespread pollutants that can enter the environment as a result of the incomplete combustion of organic matter (e.g., residential heating, motor vehicles, gas, coal, and wood combustion) or from the spilling of mineral or tar oils [3]. PAHs are monitored because of their possible carcinogenic characteristics and their ubiquity in the environment. Although several hundred PAHs exist, most studies focus on the 16 PAHs listed by the U.S. Environmental Protection Agency and the European Community as pollutants [3]. Seven of them, including phenanthrene and anthracene, both are tricyclic aromatic hydrocarbons and their known effects as irritants of skin and eye, and that might be carcinogenic [4-5].

Sludge originating from wastewater treatment is frequently spread on land to restore degraded soils [6]. Wastewater sludge has a high organic matter content and is rich in nutrients, e.g., mainly nitrogen and phosphorous, stimulating plant growth. The application of wastewater sludge is known to accelerate the removal of PAHs from soil [3, 7]. However, sludge can also contain contaminants such as heavy metals, pathogens, and organic pollutants, so that current regulations require a reduction in pathogen content and periodic monitoring for some metals prior to land application [7].

In a previous experiment that used soil from Texcoco (pH 10.0 and electrolytic conductivity (EC) 12 dS m⁻¹), the application of wastewater sludge to soil accelerated the removal of phenanthrene (PHEN) and anthracene (ANTHR) and nearly halved their final concentrations after 112 days [8]. However, soils with pH 9.3 and EC 7.3 dS m⁻¹ for the application of wastewater sludge had no significant effect on the removal of ANTHR after 112 days [9]. It can be speculated that soil characteristics like salt content or the differences in wastewater sludge affected

the remediation of PAHs from soil. Therefore, as part of a study for removal of PAHs from an alkaline saline soil using organic wastes, soil samples from the former Texcoco Lake at EC 6 dS m⁻¹, 30 dS m⁻¹, 80 dS m⁻¹, or 146 dS m⁻¹ were contaminated with PHEN and ANTHR and amended with the same wastewater sludge. The emissions of CO₂, concentrations of mineral N [ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻)], PHEN, and ANTHR were monitored in an aerobic incubation at 22 ±2°C for 56 days. The objective of this study was to investigate how salinity might affect the removal of PAHs when wastewater sludge was added to soil.

Materials and Methods

Area description and Soil Sampling

The soil samples were taken from soils of the former Texcoco Lake is Texcoco, Mexico, located at N 19°30', W 98°53' at an altitude of 2,250 m above sea level, with annual mean temperature of 16°C and annual mean precipitation of 600 mm. The soil was characterized as alkaline-saline with NaCl and Na₂CO₃ as dominant minerals. The pH ranges were between 8.5 and 10.5, and electrolytic conductivity (EC) by saturation extracts were between 4 and 150 dS m⁻¹ with a large exchangeable sodium percentage (60-80%). Four different sites were selected with different ECs (Table 1). At each of the four sites, three 400 m² plots were defined and the soil was sampled at random by augering the 0-15 cm top-layer 20 times. The soil from each plot was pooled separately so that 12 soil samples were obtained (four soils at different ECs by triplicate).

PAHs and Sludge Characteristics

The PHEN (purity >98%) and ANTHR (at >97%) were purchased from Sigma (USA), and acetone (at 99.7%) was obtained from J.T. Baker (USA). Sewage sludge was acquired from Reciclagua Sistema Ecológico de Regeneración de Aguas Residuales Industriales in Lerma in the State of Mexico. Reciclagua is a water treatment plant that treats wastewater from different companies, while 90% of the wastewater is of food industry origin and 10% is from households. The sludge was sampled after the

Table 1. Some characteristics of the sampled soil in the former Lake Texcoco.

	EC ^a	pH	Organic C	WHC ^b	Clay	Silt	Sand	Textural
	(dS m ⁻¹)		(g kg ⁻¹ soil)				classification	
Texcoco A	6	9.0	33	885	50	230	720	Sandy loam
Texcoco B	30	10.6	41	656	90	60	850	Loamy sand
Texcoco C	80	10.5	21	1098	180	310	510	Sandy loam
Texcoco D	146	10.3	14	956	180	230	590	Sandy loam

^aEC: Electrolytic conductivity, ^bWHC: Water-holding capacity

addition of a flocculent was passed through a belt filter to decrease water content. Forty kg of sludge were sampled aseptically in heavy-duty black plastic bags. Some sludge characteristics were pH 8.4, water content 842 g kg⁻¹, organic C content 509 g kg⁻¹ dry sludge, and total N 55.3 g kg⁻¹ dry sludge.

Soil Preparation, Treatments and Experimental Set-up

The soils from the four sites and each plot was sieved using a five mm sieve, and were adjusted to 40% of water-holding capacity (WHC) by adding distilled water (H₂O). After that, the soils were kept at 22±2°C for 10 days in drums containing a beaker with 100 mL 1 M sodium hydroxide (NaOH) that was used to capture the CO₂ produced, and a beaker with 100 mL distilled H₂O to avoid desiccation of the soil.

Forty-eight sub-samples of 5 g soil of each of the 12 soil samples (three plots × four soils) were added to 120 mL glass flasks. Eighteen flasks were contaminated with 2 mL acetone in which PHEN and ANTHR were dissolved and amended with 1,162 mg fresh wastewater sludge kg⁻¹ (Soil + PAHs + sludge), 18 were contaminated with 2 mL acetone where PHEN and ANTHR were dissolved (Soil + PAHs), and 18 were left unamended (Soil). The flasks were placed in a desiccator under vacuum for 20 min, removed, and 15 g soil added to each flask. The soil was then mixed thoroughly. The amount of PAHs added to the 20 g soil was equivalent to 1,200 mg phenanthrene kg⁻¹ and 520 mg anthracene kg⁻¹. To the wastewater sludge we added approximately 150 mg inorganic N kg⁻¹.

Three flasks were chosen at random from each treatment of the three plots and four soils. Ten g soils were extracted for inorganic-N with 100 mL 0.5 M K₂SO₄ solution, and shaken for 30 min after being filtered through Whatman No. 42 filter paper and were analysed, while 1.5 g soil was extracted for PAHs with acetone and analysed on a gas chromatograph (GC). These provided zero-time samples.

The remaining flasks were arranged in 945 mL glass jars containing a vessel with 15 mL distilled H₂O and a vessel with 20 mL 1 M NaOH to capture produced CO₂. All jars were sealed and stored in a dark room at 22±2°C for 56 days. Additionally, 15 jars treated similarly to the other ones were sealed and served as controls to quantify the CO₂ captured from the air. Three jars were selected at random from each treatment after 3, 7, 14, 28, and 56 days, while the soil was analysed for ammonium, nitrite, nitrate, and PAHs as mentioned before. All remaining flasks were opened and aired each of three days for 10 min to avoid anaerobic conditions. After that, they were sealed and incubated again.

Chemical Analysis

pH, EC, WHC, particle size distribution, organic and inorganic C content, and total N and NH₄⁺, NO₂⁻, and

NO₃⁻ were measured in the soil according to Fernández-Luqueño et al. [10].

PAH Analysis

Concentrations of PHEN and ANTHR in the substrate, i.e., soil, sludge, or both soil and sludge were extracted according to Song et al. [11]. The 1.5 g sub-sample of soil was mixed with 3 g of anhydrous sodium sulphate to form a fine powder and placed in a Pyrex tube; 5 mL acetone was added. The tubes were placed in a sonicated bath at 35-40°C 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 centrifuge at 3,000 × rpm for 15 min. This process was repeated three times. The extracts were combined, evaporated in a fume-cupboard at 50°C, and dissolved in 2 mL acetone. From each tube, a 2.0 µL aliquot was immediately analysed for PAHs on a Hewlett-Packard 4890-D GC (USA) fitted with a flame ionization detector. Additional technical specifications regarding PAH analysis and the percentage recovery of PAHs are described in Fernández-Luqueño et al. [10].

Statistical Analyses

Cumulative production of CO₂ and concentrations of ammonium, nitrite, nitrate, PHEN, and ANTHR were subjected to one-way analysis of variance using PROC GLM [12]. Significant differences between treatments for CO₂ emissions were determined using PROC MIXED [12]. Data shown are the mean of nine values ($n = 9$), i.e., three measurements of three plots.

The relationships between the different soil properties (pH, WHC, organic C, inorganic C, clay, silt, sand, the emission of CO₂, and concentration of NO₃⁻ after 56 days) were analyzed by principal component analysis (PCA) using the orthogonal/varimax rotation to achieve either small or large component loading and an eigenvalue of 1 as the lower limit. The matrix of 24 columns (sites) and 9 lines (variables) was used for principal component analysis. All analyses were performed using SAS statistical software [12].

Results

The emissions of CO₂ resembled zero-order kinetics in all treatments of all soils (no data shown). In the unamended and uncontaminated soil (Soil), the CO₂ emission rate was largest in soil with EC 6 dS m⁻¹ and lowest in soil with EC 30 dS m⁻¹ (Table 2). The CO₂ emission rate was significantly higher in soil contaminated with PAHs compared to the unamended soil, but significantly lower than in the contaminated soil amended with wastewater sludge except for soil with EC 146 dS m⁻¹ ($p < 0.05$).

The concentrations of NH₄⁺ decreased in the control treatment with EC 30 and 80 dS m⁻¹ between days 14 and 28, and increased thereafter (Fig. 1a). In soil with EC 6 and 146 dS m⁻¹ the concentrations of NH₄⁺ showed an

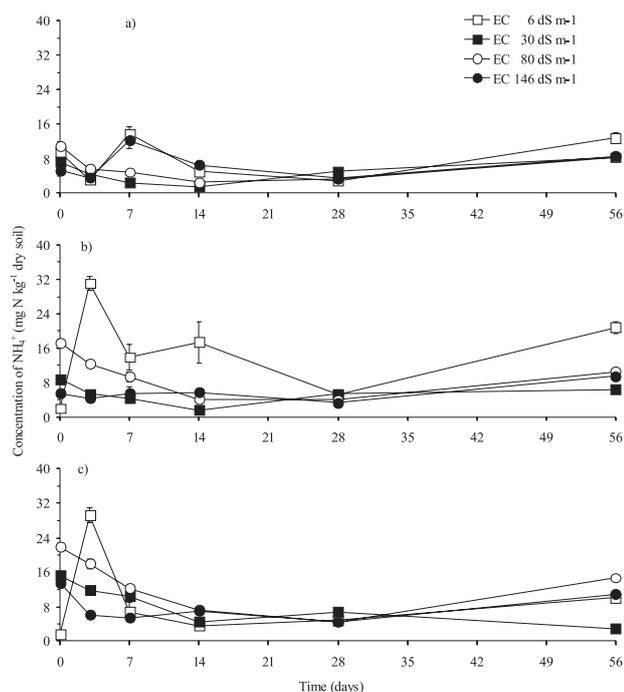


Fig. 1. Concentration of ammonium (mg N kg^{-1}) in a) soil, b) soil spiked with anthracene and phenanthrene, or c) soil spiked with anthracene and phenanthrene and amended with wastewater sludge with electrolytic conductivity (EC) 6 dS m^{-1} (\square), 30 dS m^{-1} (\blacksquare), 80 dS m^{-1} (\circ), or 146 dS m^{-1} (\bullet) incubated aerobically at $22\pm 2^\circ\text{C}$ for 56 days. Bars are \pm one standard deviation.

increase at day 7 and a decrease thereafter (Fig. 1a). The concentrations of NH_4^+ decreased in the unamended soil with EC 30, 80, and 146 dS m^{-1} and amended soil sludge spiked with PAHs at the onset of the experiment and tended to increase toward the end (Fig. 1b). The concentrations of NH_4^+ soil with EC 6 dS m^{-1} amended or not with wastewater sludge and spiked with PAHs showed more fluctuations over time (Figs 1b-c). Mean concentrations of NH_4^+ were larger in the control with EC 6 dS m^{-1} than in soil with EC 30 dS m^{-1} (Table 2). Contaminating the soil with PAHs increased the concentrations of NH_4^+ compared to the uncontaminated soil, except in soil with EC 146 dS m^{-1} (Fig. 1b). Application of sludge to the PAH-contaminated soil increased the amount of NH_4^+ in soil, except in soil with EC 6 dS m^{-1} .

The concentration of NO_2^- in the control treatment and soil spiked with PAHs showed little fluctuation over time except in the uncontaminated soil with EC 6 dS m^{-1} , where it increased until day 28 and decreased again (Figs 2a-b). The concentration of NO_2^- in the soil spiked with PAHs and amended with PAHs showed more fluctuations over time compared to the other treatments. Spiking soil with PAHs increased the concentration of NO_2^- except in soil with EC 80 dS m^{-1} (Table 2). Application of wastewater sludge to the PAH-contaminated soil increased the concentration of NO_2^- in all soils compared to the PAH-contaminated soil.

Concentrations of NO_3^- showed no clear pattern over time in all treatments (Figs 3a-c). The mean concentration of NO_3^- was significantly greater in the soil with EC 80

dS m^{-1} than in soil with EC 6 dS m^{-1} or 30 dS m^{-1} , but significantly lower than in soil with EC 146 dS m^{-1} (Table 2). The application of sludge or contaminating soil with PAHs decreased the mean concentration of NO_3^- , except in soil with EC 146 dS m^{-1} .

The concentration of PHEN decreased sharply in soil with EC 6 dS m^{-1} in the first 14 days, but did not change significantly thereafter (Fig. 4a). Applying sludge to soil with 6 dS m^{-1} accelerated the removal of PHEN from soil and decreased the concentration after 56 days ($p < 0.05$) (Fig. 4b). The concentration of PHEN decreased significantly in soil with EC 30 and 80 dS m^{-1} , i.e., $3.1 \text{ mg kg}^{-1} \text{ day}^{-1}$ (standard error of the estimate (SEE) 0.7) and $-4.6 \text{ mg kg}^{-1} \text{ day}^{-1}$ (SEE 1.4), respectively. Application of sewage sludge decreased the concentration PHEN and ANTHR in soil with EC 30 and 80 dS m^{-1} after 56 days. The concentration of PHEN decreased sharply in soil with EC 146 dS m^{-1} in the first 14 days, but did not change significantly thereafter. Applying sludge to soil with 146 dS m^{-1} had no significant effect on the concentration of PHEN after 56 days. The mean concentration of PHEN was significantly lower in soil with EC 6 dS m^{-1} than in soil with EC 30 and 80 dS m^{-1} , but higher than in soil with EC 146 dS m^{-1} – independent of the application of wastewater sludge ($p < 0.05$).

The concentration of ANTHR decreased in soil with EC 6 dS m^{-1} between days 7 and 14, but did not change significantly thereafter (Fig. 5a). Applying sludge to soil with 6 dS m^{-1} accelerated the removal and decreased the amount of ANTHR after 56 days (Fig. 5b). The concentration of ANTHR in soil with EC 30 and 80 dS m^{-1} was lower after 56 days than at the onset of the incubation, although decreases were small, i.e., only 5% ANTHR was removed in soil with EC 30 dS m^{-1} and 12% in soil with 80 dS m^{-1} after 56 days. Applying sludge to these two soils decreased the amount of ANTHR significantly compared to the unamended soil after 56 days ($p < 0.05$). The concentration of ANTHR decreased sharply in soil with EC 146 dS m^{-1} between days 7 and 14, but did not change significantly thereafter (Fig. 1a). Applying sludge to the PAHs contaminated soil with 146 dS m^{-1} had no significant effect on the concentration of ANTHR. The mean concentration of ANTHR was significantly lower in soil with EC 6 dS m^{-1} than in soil with EC 30 and 80 dS m^{-1} , but significantly higher than in soil with EC 146 dS m^{-1} ($p < 0.05$). In the sludge-amended soil the concentration of ANTHR was significantly higher in soil with EC 30 and 80 dS m^{-1} than in soil with EC 6 and 146 dS m^{-1} ($p < 0.05$).

Discussion

Contaminating soil with PAHs increased CO_2 emissions. It can be speculated that the acetone used to contaminate the soil that was not removed served as C-substrate, thereby increasing emissions of CO_2 . Microorganisms have been isolated from soil capable of degrading acetone under aerobic conditions [13]. The contribution of PAHs in the emission of CO_2 was presumably low as

Table 2. Concentrations of NH_4^+ , NO_2^- , and NO_3^- (mg N kg^{-1} soil), and CO_2 emission rates (mg C kg^{-1} soil day^{-1}) in soil with different electrolytic conductivities (EC) contaminated with or without anthracene and phenanthrene and amended with or without wastewater sludge incubated aerobically at $25\pm 2^\circ\text{C}$ for 56 days.

EC (dS m^{-1})	CO_2 emission rate (mg C kg^{-1} day^{-1})				P-value
	Soil control	Soil + PAHs	Soil + PAHs + sludge	SEE ^a	
6	17.1 c ^b A ^c	80.6 b A	107.7 a A	3.7	$p < 0.0001$
30	8.6 c C	17.3 b B	74.4 a B	2.6	$p < 0.0001$
80	12.9 c B	16.0 b B	20.9 a C	1.3	$p < 0.0001$
146	13.7 a B	14.0 a B	14.9 a D	2.4	$p < 0.0001$
SEE	2.0	2.4	3.2		
P-value	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$		
Concentration of NH_4^+ (mg N kg^{-1})				MSD ^d	
6	7.9 b A	15.1 a A	9.4 b B	4.4	$p = 0.0003$
30	4.8 b B	5.4 b C	8.6 a B	1.5	$p < 0.0001$
80	6.0 c AB	9.7 b B	13.1 a A	1.6	$p < 0.0001$
146	6.6 a AB	5.7 a C	7.9 a B	2.3	$p = 0.0047$
MSD	1.9	3.4	3.3		
P-value	$p = 0.0009$	$p < 0.0001$	$p < 0.0002$		
Concentration of NO_2^- (mg N kg^{-1})				MSD	
6	1.0 b C	1.5 a D	1.6 a D	0.4	$p = 0.0024$
30	1.4 b C	2.2 b C	5.3 a C	1.2	$p < 0.0001$
80	7.8 b A	5.9 c A	10.0 a B	1.4	$p < 0.0001$
146	4.0 b B	4.1 b B	12.4 a A	1.2	$p < 0.0001$
MSD	0.8	0.5	1.9		
P-value	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$		
EC / dS m^{-1}	Concentration of NO_3^- (mg N kg^{-1})			MSD	P-value
6	22 a C	11 b C	10 b C	6	$p < 0.0001$
30	20 a C	14 b BC	15 b C	3	$p < 0.0001$
80	34 a B	24 b B	27 b B	5	$p < 0.0001$
146	86 a A	86 a A	90 a A	15	$p = 0.7320$
MSD	10	11	9		
P-value	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$		

^aSEE: standard error of the estimate

^bvalues with the same letter are not significantly affected by treatment

^cvalues with the same capital letter are not significantly different between the soil

^dMSD: minimal significant difference

mineralization of PAHs in soil is low or non-existent [14]. In previous experiments, we found that acetone, used as a carrier to spike soil with PAHs, inhibited microbial activity in Texcoco soil with EC 56 dS m^{-1} after 7 days [15]. We speculated that if acetone inhibited microbial activity it would only be temporary [16]. Acetone might kill soil microorganisms so that microbial activity and emissions of CO_2 decrease, but the killed microorganisms will also serve as a C-substrate for the surviving microorganisms,

thereby increasing CO_2 emissions [15]. This was found in this study in soil with EC 146 dS m^{-1} . The emission of CO_2 was lower in contaminated PAHs than in the uncontaminated until day 14, but was larger afterward.

The difference in CO_2 emissions between the soil spiked with PAHs and the uncontaminated soil decreased with increased salinity. In previous experiments, it was found that mineralization of ^{14}C -labelled glucose and maize also decreased with increased salinity [17].

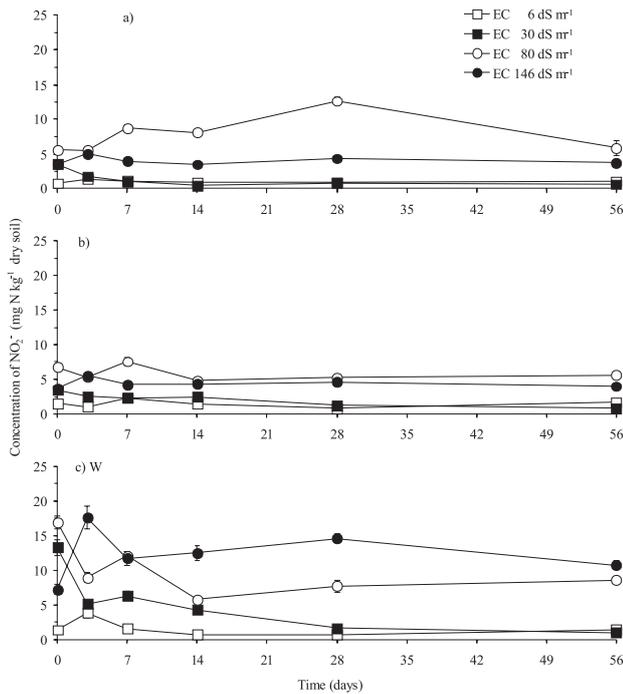


Fig. 2. Concentration of nitrite (mg N kg^{-1}) in a) soil, b) soil spiked with anthracene and phenanthrene, or c) soil spiked with anthracene and phenanthrene and amended with wastewater sludge with electrolytic conductivity (EC) 6 dS m^{-1} (\square), 30 dS m^{-1} (\blacksquare), 80 dS m^{-1} (\circ), or 146 dS m^{-1} (\bullet) incubated aerobically at $22\pm 2^\circ\text{C}$ for 56 days. Bars are \pm one standard deviation.

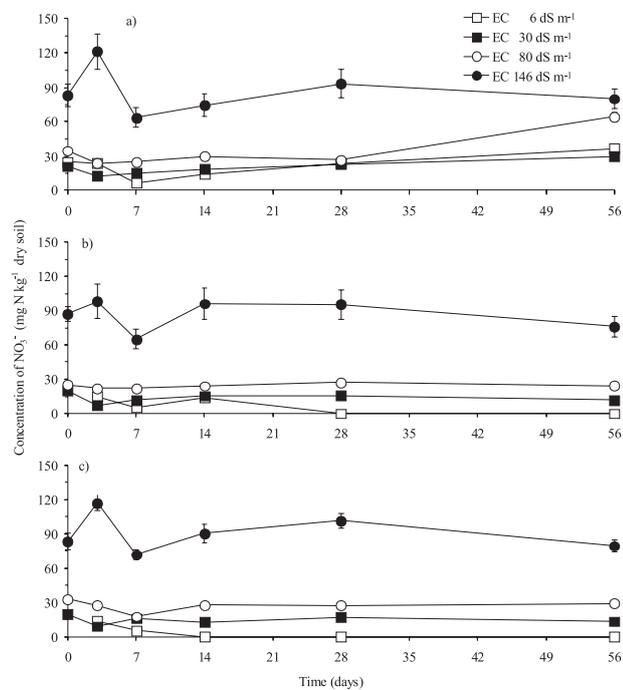


Fig. 3. Concentration of nitrate (mg N kg^{-1}) in a) soil, b) soil spiked with anthracene and phenanthrene, or c) soil spiked with anthracene and phenanthrene and amended with wastewater sludge with electrolytic conductivity (EC) 6 dS m^{-1} (\square), 30 dS m^{-1} (\blacksquare), 80 dS m^{-1} (\circ), or 146 dS m^{-1} (\bullet) incubated aerobically at $22\pm 2^\circ\text{C}$ for 56 days. Bars are \pm one standard deviation.

It has to be remembered, however, that other soil characteristics changed in the studied soils that are known to affect the decomposition of added organic material. Soil characteristics such as pH [18], CEC, soil structure [19], sodicity [20], clay content [21], specific surface area of the clay, and the nature of the clay mineral [22] affect mineralization of organic material.

It is well known that the application of wastewater sludge increases CO_2 emissions from soil. Wastewater sludge contains large amounts of easily decomposable organic material that is rapidly mineralized when added to soil [9, 23]. For instance, Zhang et al. [24] reported that approximately 40% of the organic material in sludge mineralized within the first 50 days. The difference in CO_2 emissions between the PAH-contaminated soil amended with sludge and the soil treatment decreased with increased salinity. This appears to indicate again that increased salinity decreased mineralization of added organic material.

Spiking soil with PAHs had no clear effect on the concentration of NO_2^- , but the addition of wastewater sludge increased it significantly with the largest increase found in the soil with the highest EC. The NH_4^+ or the mineralized organic material of the wastewater sludge is oxidized to NO_2^- and NO_3^- in soil. It is well known that nitrifiers are affected by elevated salt content and the oxidation of NO_2^- is more sensitive to increased soil salt content than the oxidation of NH_4^+ [25]. As such, the concentration of NO_2^- increased with the largest increase found in soil with the largest EC. However, oxidation of

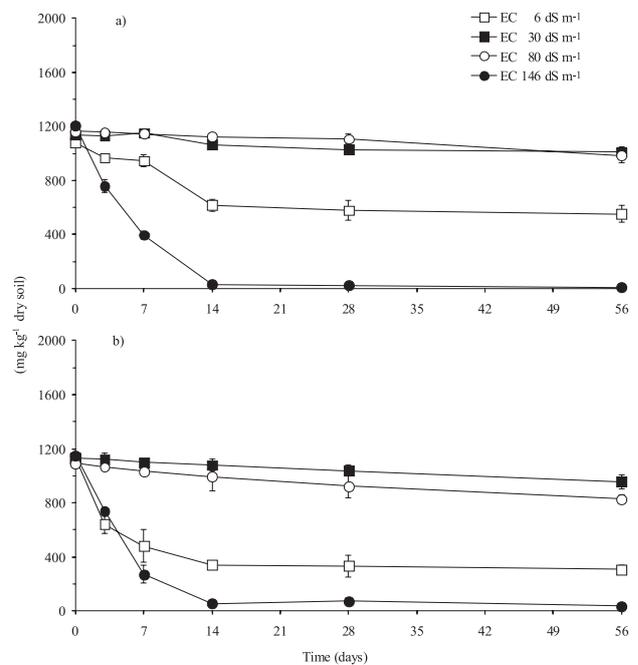


Fig. 4. Concentration of phenanthrene (mg kg^{-1}) in a) unamended soil or b) soil amended with wastewater sludge with electrolytic conductivity (EC) 6 dS m^{-1} (\square), 30 dS m^{-1} (\blacksquare), 80 dS m^{-1} (\circ), or 146 dS m^{-1} (\bullet) incubated aerobically at $22\pm 2^\circ\text{C}$ for 56 days. Bars are \pm one standard deviation.

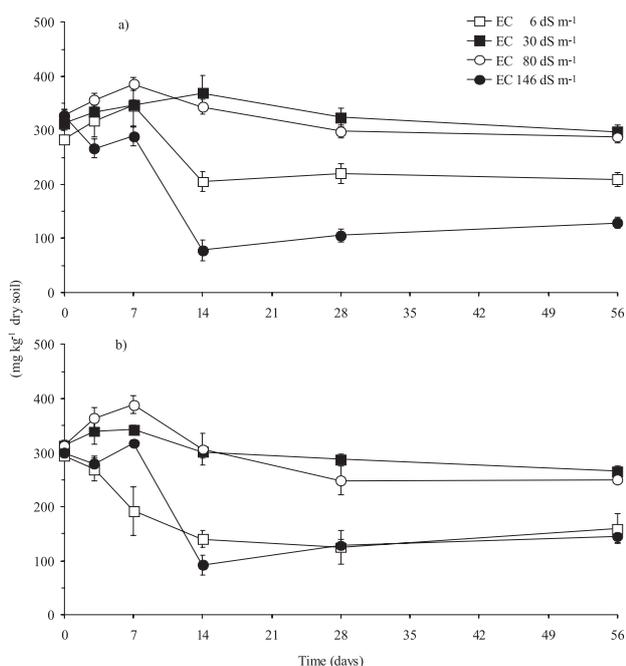


Fig. 5. Concentration of anthracene (mg kg^{-1}) in a) unamended soil or b) soil amended with wastewater sludge with electrolytic conductivity (EC) 6 dS m^{-1} (\square), 30 dS m^{-1} (\blacksquare), 80 dS m^{-1} (\circ), or 146 dS m^{-1} (\bullet) incubated aerobically at $22 \pm 2^\circ\text{C}$ for 56 days. Bars are \pm one standard deviation.

NO_2^- occurred even in soil with 146 dS m^{-1} , as large amounts of NO_3^- were detected.

Spiking soil with PAHs decreased the concentration of NO_3^- independent of the application of wastewater sludge, except in soil with 146 dS m^{-1} . Both acetone, used to spike the soil with ANTHR, and PHEN and the PAHs added to soil do not contain N, so their decomposition will induce the immobilization of mineral N, except in soil with 146 dS m^{-1} , where their mineralization was low. The immobilization of NO_3^- , however, is normally inhibited by even small amounts of NH_4^+ . Yang et al. [26] stated that assimilatory reduction of NO_3^- is not expected to be important in soil unless there are NH_4^+ -free microsites in the soil. NH_4^+ was always present in the soils so it did not inhibit the immobilization of NO_3^- in Texcoco soil. In previous experiments, it was found that the addition of an easily decomposable C-substrate to soil decreased the concentration of NO_3^- and increased the concentration of NO_2^- independent of the concentration of NH_4^+ in soil [27]. We speculated that the aerobic respiration NO_3^- by Archaea might explain part of the decrease in the concentration of NO_3^- [28]. Additionally, the concentration of NO_3^- might have decreased as a result of anaerobic reduction or denitrification, aerobic respiration, assimilatory reduction, or a combination of these processes. In addition, it has to be remembered that the soil of the former lake Texcoco is an extreme soil that is highly saline and alkaline and that has Na^+ , Cl^- , HCO_3^- , and CO_3^{2-} as predominant ions. Valenzuela-Encinas et al. [29] showed that the archaeal diversity in an alkaline-saline soil was low with yet uncharacterized and unclassified species, but some of

the clones identified were capable of reducing NO_3^- aerobically to NO_2^- , such as *Halorubrum alkaliphilum*, *Natronococcus amylolyticus*, *Natrinema pallidum*, and *Natrinema pellirubrum*. Dendooven et al. [30] stated that the soil of the former Lake Texcoco can be categorized as a unique ecosystem. Actually, this environment affects the dynamics of C and N differently than in a typical soil. Additionally, microorganisms immobilize $\text{NH}_4^+\text{-N}$ in excess of what was required for metabolic activity. Moreover, large amounts of NO_3^- are reduced under aerobic conditions to NO_2^- , and smaller amounts of NH_4^+ when an easily decomposable C-substrate is added to soil in excess of what is required for normal metabolic activity and it is not inhibited by NH_4^+ , L-glutamine, acetylene, or O_2 . According to Dendooven et al. [30], the metabolic mechanisms involved in some processes of the soil from Texcoco are still not fully understood and need to be unravelled.

The concentrations of PHEN and ANTHR decreased in soil with 6 dS m^{-1} until day 14 and remained constant thereafter. Approximately 49% of the PHEN and 26% of ANTHR added to soil was removed from within 56 days. It is well known that autochthonous microorganisms can remove PAHs from soil [31] – even in the presence of salt [32]. In a previous experiment, 99% PHEN and 85% ANTHR were removed from an agricultural soil [23]. The removal of PHEN and ANTHR was much lower from soil with 30 dS m^{-1} (i.e., 11% and 5%, respectively), or with 60 dS m^{-1} (i.e., 15% and 12%, respectively). It was hypothesized that the high pH and EC in Texcoco soil inhibited the removal of PAHs [8]. However, nearly all the ANTHR and PHEN was removed from soil with 146 dS m^{-1} after 56 days, i.e., $> 95\%$. This made it clear that other factors affected the removal of PAHs from soil, and salt content and pH were not determinant factors [33–34].

APCA analysis of the soil factors indicated that none of the soil characteristics measured (i.e., EC, WHC, particle size distribution, and organic and inorganic C or pH) determined the removal of PHEN or ANTHR from soil (Fig. 6). The four soils studied were clearly separated from each other, but there was no clear relationship between soil characteristics and the amount of PAHs removed from the soils. For instance, the soils with 80 dS m^{-1} had a positive PC1, i.e., high WHC and silt content, while the soil with 30 dS m^{-1} had a negative PC1, i.e., high sand and organic C content, but similar amounts of PAHs were removed. Additionally, the removal of PAHs was larger in soil with 146 dS m^{-1} than in soil with EC of 6 dS m^{-1} , so that salt content was not the determinant factor. Even the microbial activity as evidenced by the emission of CO_2 was not related to the removal of PAHs from soil.

The production of CO_2 was similar in soil with 80 dS m^{-1} and 146 dS m^{-1} , but the removal of PHEN and Antra was much larger in the latter than in the first. As such, the EC and the other measured soil characteristics did not determine removal of PAHs from soil and another unmeasured factor or factors intervened. These factors, such as specific surface area of the clays and/or the nature of the clay mineral [21–22], might have

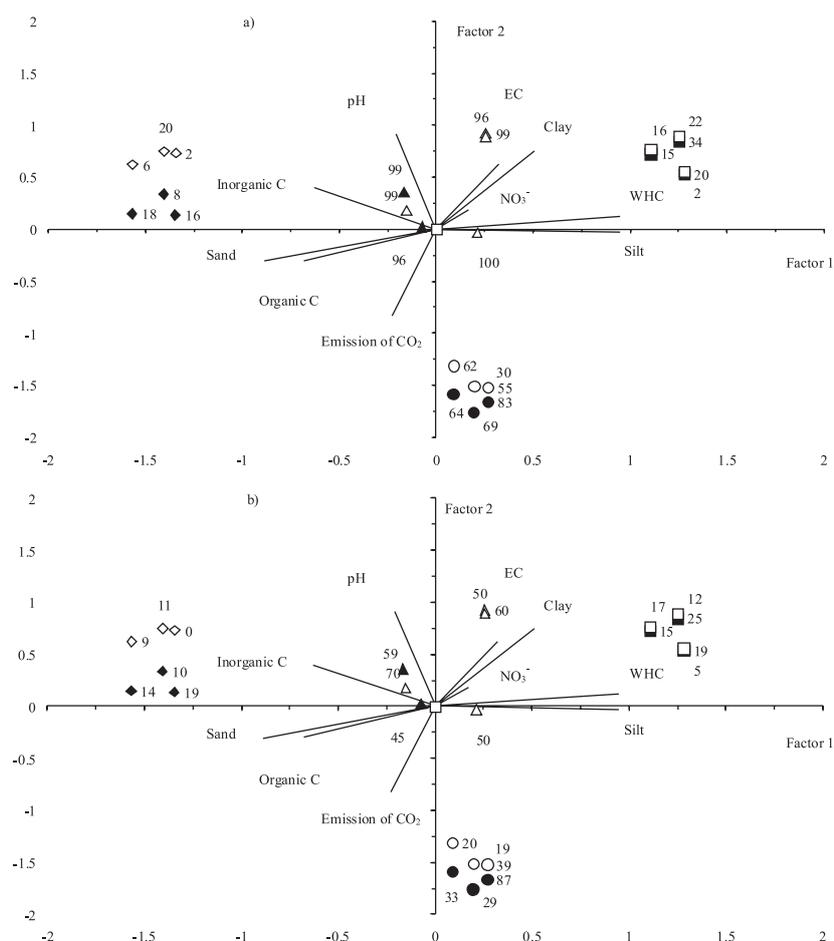


Fig. 6. Principal component analysis (PCA) performed on soil properties data after a varimax rotation with emission of CO₂ and concentration of NO₃⁻ after 56 days, organic C, inorganic C, WHC (water holding capacity), pH, EC (electrolytic conductivity), clay, loam, and sand content. The first factor (PC1) loaded by sand, silt and clay content, WHC, and inorganic and organic C explained 58% of the variation, while the second factor (PC2) loaded by pH, EC, clay content, inorganic C, and emission of CO₂ explained 23% of the variation. Unamended soil ($n = 3$) with EC 6 dS m⁻¹ (○), 30 dS m⁻¹ (◇), 80 dS m⁻¹ (□), or 146 dS m⁻¹ (△) soil or with EC 6 dS m⁻¹ (●), 30 dS m⁻¹ (◆), 80 dS m⁻¹ (■), or 146 dS m⁻¹ (▲) amended with wastewater sludge incubated aerobically at 22±2°C for 56 days. Values besides the symbols are the percentages (mean of $n = 3$) of a) phenanthrene or b) anthracene removed from that soil after 56 days.

affected availability and thus the removal of PAHs from soil. It can be speculated that the 'availability' or 'bio-accessibility' of the studied PAHs might have been another factor that determined the removal of ANTHR and PHEN from soil [35]. In a previous experiment, it was found that the removal of ANTHR was faster from the top 0-2 cm layer than from the 2-8 or 8-15 cm layers [8]. Further investigation revealed that fluctuating water contents increased anthracene availability, contributing to increased removal [15]. It can be speculated that the large salt content in soil with EC 146 dS m⁻¹ dispersed the soil particles in such a way that the PAHs were not physically protected and became available for degradation [36]. Consequently, the removal of PAHs was not inhibited by salts in soil with EC 146 dS m⁻¹.

It is worth noticing that the percentages of ANTHR and PHEN removed from soil with EC 30 and 80 dS m⁻¹ were similar after 56 days, but in soil with EC 6 and 146 dS m⁻¹ the dissipation of ANTHR was lower than that of PHEN. The removal of ANTHR is normally slower from soil than that of PHEN. This can be attributed to the low

solubility of ANTHR in aqueous systems (0.07 mg L⁻¹) compared to that of PHEN (1.29 mg L⁻¹), which renders it only slowly available for microbial degradation [2]. This would indicate in soil with EC 30 and 80 dS m⁻¹, the availability or 'bio-accessibility' of PHEN and ANTHR was low, so that their removal was low and similar.

It is well known that the application of organic material accelerates the removal of PAHs from soil [35, 37]. Adding sludge to soil can affect the removal of PAHs in different ways. First, the sludge contains larger amounts of C-substrate that will stimulate microbial activity, thereby accelerating the removal of PAHs from soil. Second, the sludge contains nutrients that will further stimulate microbial activity. Third, sludge contains large amounts of microorganisms that can contribute to the removal of PAHs from soil. Fourth, sludge can change soil conditions, such as pH and EC, thereby stimulating or inhibiting the removal of PAHs. Fifth, organic matter might conduce absorption and/or adsorption processes on the dissipation of PAH concentrations. Wastewater sludge reduced the concentrations of PHEN and ANTHR

in soil after 56 days compared to the amount extracted at the beginning of the experiment, although the effect was much smaller in soils with EC 30 and 80 dS m⁻¹ than in soil with EC 6 dS m⁻¹. In soil with EC 146 dS m⁻¹ the removal rate of PHEN and ANTHR was so high that sludge had no effect on their dissipation rate. This would indicate that a positive effect of wastewater sludge on the removal of PHEN and ANTHR will only be observed when the PAHs are available and not shielded from microbial degradation.

Conclusions

It was concluded that CO₂ emissions increased when soil was spiked with PAHs and further increased when amended with wastewater sludge, but the increase decreased with increased soil salt content. Spiking soil with ANTHR and PHEN decreased the concentration of NO₃⁻. The removal of PAHs was high in soil with 6 or 146 dS m⁻¹, but low in soils with EC 30 or 80 dS m⁻¹. It appears that 'bio-accessibility' of ANTHR and PHEN in soil was a more important determinant in their removal than soil characteristics such as EC, pH, particle size distribution, organic matter content, or microbial activity. Wastewater sludge had a positive effect on the removal of PHEN and ANTHR from soil, but the degree of its effect appeared to be related to the availability of the contaminant.

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