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Impact of moisture dynamic and sun light on anthracene removal from soil

Edgar Vázquez Núñez · Alejandro García Gaytán · M. Luna-Guido · R. Marsch · L. Dendooven

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Abstract In a previous study, remediation of anthracene from soil was faster in the top 0–2 cm layer than in the lower soil layers. It was not clear whether this faster decrease was due to biotic or abiotic processes. Anthracene-contaminated soil columns were covered with black or transparent perforated polyethylene so that aeration occurred but that fluctuations in water content were minimal and light could reach (LIGHT treatment) or not reach the soil surface (DARK treatment), or left uncovered so that soil water content fluctuate and light reached the soil surface (OPEN treatment). The amount of anthracene, microbial biomass C, and microbial activity as reflected by the amount of CO_2 produced within 3 days were determined in the 0–2 cm, 2–8 cm, and 8–15 cm layer after 0, 3, 7, 14, and 28 days. In the 0–2 cm layer of the OPEN treatment, 17% anthracene remained, 48% in the LIGHT treatment and 61% in the DARK treatment after 28 days. In the 2–8 cm and 8–15 cm layer, treatment had no significant effect on the dissipation of anthracene from soil after 14 and 28 days. It was found that light and fluctuations in water content stimulated the removal of anthracene from the top 0–2 cm soil layer, but not from the lower soil layers. It can be speculated that covering contaminated soil or pilling it up will inhibit the dissipation of the contaminant.

Keywords Drying and wetting of soil · Microbial activity · Microbial biomass C

E. Vázquez Núñez · A. García Gaytán · M. Luna-Guido · R. Marsch · L. Dendooven (✉)
Laboratory of Soil Ecology, Department of Biotechnology and Bioengineering, Cinvestav, Mexico D.F C.P. 07360, Mexico
e-mail: dendoove@cinvestav.mx

Introduction

Contamination of soil with hydrocarbons occurs frequently due to accidents during extraction and transport (Wilson and Jones 1993). Different techniques have been applied to accelerate the removal of hydrocarbons from soil (Morgan and Watkinson 1990). If the contamination is severe and the soil is covered with petroleum, then this layer is first physically removed (Riser-Roberts 1998). Surfactants are sometimes used as they render hydrocarbons free for microbial degradation, but chemical removal of hydrocarbons is only sporadically used as it might affect the soil microbial micro-organisms (Volkering and Breure 1997). Organic material or earthworms are added to contaminated soil or plants are cultivated to accelerate removal of hydrocarbons from soil, but autochthonous soil micro-organisms without any interference are very effective in degrading hydrocarbons, even the most recalcitrant ones, such as polycyclic aromatic hydrocarbons (PAHs) (Straube et al. 2003; Grant et al. 2007). For instance phenanthrene and anthracene are rapidly dissipated from soil

and even benzo(a)pyrene is removed from soil (Atagana et al. 2003; Mueller and Shann 2006).

While studying removal of PAHs from an anthracene-contaminated soil, it was found that the decrease in the concentration of anthracene was faster in the 0–2 cm layer compared to the 2–12 and 12–22 cm layer after 7 days (Betancur-Galvis 2005). It was not clear which factors might have caused this difference. Two factors might have interfered; light or fluctuations in water content. Photons are known to accelerate degradation of hydrocarbons, i.e., photodegradation, so the effect of light would have been felt in the upper soil crust but not in the deeper soil layer (Muszkat et al. 1992). Fluctuations in water content will affect microbial activity, aggregates stability, and organic matter availability (Utomo and Dexter 1982). Microbial activity decreases when a soil dries out, but resumes rapidly upon wetting (Lund and Goksøyr 1980). Part of the soil organic matter is physically stabilized within aggregates (Kalbitz et al. 2000). Drying–rewetting will liberate part of that physical stabilized organic material so that it becomes available as C substrate for micro-organisms (Kieft et al. 1987). The same will apply for hydrocarbons so that they will become degradable (Leahy and Colwell 1990).

We wanted to investigate if light and/or fluctuation in the water content of soil accelerated dissipation of anthracene and how this affected microbial biomass. Anthracene-contaminated soil columns were covered with black or transparent perforated polyethylene so that aeration occurred but that fluctuations in water content were minimal and light could reach or not reach the soil surface, or left uncovered so that the effect of light and fluctuations in water content could be studied in a greenhouse for 28 days, while concentration of anthracene, soil microbial biomass, and microbial activity were monitored. The objective of this study was to investigate in depth, how sun-light and dry-wetting cycles affected the removal of anthracene from soil.

Materials and methods

Sampling site, collection, and characterization of soil

Details of the sampling site located near the ex-convent of Acolman in the State of México (N.L. 19°38′ W.L. 98°55′) at an altitude of 2,250 m above sea level and with a mean annual temperature of 14.9°C and average annual precipitation of 624 mm (mainly from June through August) can be found in Betancur-Galvis et al. (2006). The soil was cultivated mainly with maize and that for >20 years, receiving a minimum amount of inorganic fertilizer without being irrigated (http://www.inegi.gob.mx). 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 separately, passed separately through a 5-mm sieve so that three soil samples were obtained and characterized. The size particle distribution in the Acolman soil was 60 g sand kg^{-1}, 270 g silt kg^{-1}, and 670 g clay kg^{-1}, and can thus be classified as a clayey soil with pH 6.3 and electrolytic conductivity (EC) 0.8 dS m^{-1} and had a water holding capacity (WHC) 896 g kg^{-1}, organic C content 19.0 g kg^{-1}, and total nitrogen content 1.4 g kg^{-1}. The bulk density of soil was 1.1 g cm^{-3} characteristic for a clayey soil (Brady and Weil 1999).

Soil treatments, conditioning in the greenhouse and sampling

Thirty-nine sub-samples of 1 kg soil from each plot were amended with 15 ml dichloromethane containing 0.05 M anthracene. As such, the soil was contaminated with 550 mg anthracene kg^{-1} soil. The sub-samples were placed under vacuum in a desiccator for 30 min so that all dichloromethane was removed from the soil and then added to polyvinyl chloride (PVC) tubes (length 25 cm and ∅ 10.5 cm). A 15 cm soil layer was thus obtained. Three sub-samples of soil sample from each plot were analyzed for microbial biomass activity and soil microbial biomass C to provide zero-time samples. Three treatments were applied to the soil columns. Twelve PVC tubes were left open at the top so that the top soil layer could dry out and light could reach the soil (considered the OPEN treatment). The soil was adjusted to 50% WHC by adding tap water every other day. The columns were closed with transparent polyethylene with small perforations to avoid anaerobicity, but so that light could reach the soil and fluctuations in water content were kept to a minimum (considered the LIGHT treatment). Twelve columns were closed with black polyethylene with small perforations to avoid anaerobicity, so that no light could reach the soil and

fluctuations in water content were kept to a minimum (considered the DARK treatment), in the LIGHT and DARK treatments ventilation was allowed by means of small perforations in the plastic. Each other day in the evening, 2 g of soil was taken from each soil column, weighted and dried overnight at 100°C. The next morning, the soil was weighted, the water content calculated and the soil column adjusted to 50% WHC as determined at the onset of the experiment when necessary. No water was added to the soil in the LIGHT and DARK treatments as the loss of water was 2.8% (12 g H_2O kg^{-1} wet soil) in the LIGHT and 5.5% (22 g H_2O kg^{-1} wet soil) in the DARK treatment after 28 days. The OPEN treatment was amended with water every other day as on the average 32% of water was lost within 2 days. The soil columns were placed in the greenhouse. After 3, 7, 14, and 28 days, three PVC tubes were selected at random, the soil was removed from the tube taking care that three soil layers were obtained: the 0–2 cm layer, the 2–8 cm, and the 8–15 cm soil layer. Each of the soil layers from the three soil columns of the three soil samples were analyzed for microbial biomass C, microbial activity, and concentration of anthracene.

Soil microbial biomass, anthracene concentration, and microbial activity

Concentrations of anthracene in the soil were analyzed using an ultrasonic extraction method developed by Song et al. (1995). The 1.5 g sub-sample of soil was mixed with 3 g of anhydrous sodium sulfate to form a fine powder, placed in a Pyrex™ tube and 12 ml acetone was added. The tubes were placed in a sonicating 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 centrifugation at 3,500 rpm for 15 min. This process was repeated three times. The extracts were combined, evaporated, and dissolved in 1 ml acetone. From each tube, a 2.0-µl aliquot was immediately analyzed for anthracene on a Hewlett-Packard 4890-D GC (USA) fitted with a flame ionization detector. A HP-5 column from Hewlett-Packard (USA) with length 15 m, inner diameter 0.53 mm, and film thickness 1.5 µm was used to separate the anthracene with carrier gas He flowing at a rate of 7 ml min^{-1}. The oven temperature at 140°C was increased to 170°C at a rate of 2°C min^{-1}, maintained at 170°C for 5 min and increased to 280°C at 30°C min^{-1} and maintained at 280°C for 10 min. The temperature of the injector was 280°C and that of the detector 300°C.

The percentage recovery of the anthracene was tested by adding 1.5 g dry soil of each of the six soil samples (in triplicate) to a Pyrex™ tube and spiking them with 520 mg anthracene kg^{-1} dry soil. The anthracene in the soil was then extracted for the added anthracene as described above. The percentage of recovery was 95%.

The CO_2 emitted after 3 days was used as an indicator of the microbial activity in soil. Sub-samples of 20 g soil of each of the three layers (0–2 cm layer, the 2–8 cm and the 8–15 cm soil layer), three treatments (OPEN, LIGHT, and DARK), and the three plots were adjusted to 50% WHC and added to 120 ml glass flask. The flasks were placed in 945 ml glass jars containing 10 ml distilled H_2O and a flask with 20 ml 1 M NaOH. An additional 12 flasks were incubated without soil. The CO_2 in flasks incubated without soil accounted for the CO_2 in the atmosphere. The jars were sealed and stored in the dark for 3 days 22 ± 2°C. After 3 days the jars were opened, the flask with 1 M NaOH removed and the CO_2 trapped determined. The CO_2 trapped in the sodium hydroxide was titrated with hydrochloric acid 0.1 N through to pH 4 (Amato 1983).

The soil microbial biomass was measured with the substrate induced respiration technique (SIR) (Anderson and Domsch 1978). Three sub-samples of 20 g oven dry soil were added to 60 ml bottles and amended with 1 g glucose kg^{-1} soil. The bottles were closed with plastic screw caps fitted with a septum and placed on an incubator at 22°C for 150 min (Anderson and Domsch 1978). After 30 and 150 min, the headspace of each bottle was sampled and analyzed for CO_2 with a GC. The CO_2 emitted between 30 and 150 min was used to calculate microbial biomass C defined as (Anderson and Domsch 1978):

$$\text{SIR-biomass C } (\mu g\, g^{-1}\, \text{soil}) = (\mu l\, CO_2\, g^{-1} \text{soil}\, h^{-1}) \times 40.04 + 0.37$$

Chemical and microbiological analyses

Soil pH was measured in 1:2.5 (W/V) soil–H_2O suspension using a glass electrode (Thomas 1996). The total C in soil was determined by oxidation with potassium dichromate and trapping the evolved CO_2

in NaOH and then titrate it with 0.1 M HCl (Amato 1983). Inorganic C in soil was determined by adding 20 ml 1 M HCl solution to 1 g air-dried soil and trapping CO_2 evolved in 20 ml 1 M NaOH and then titrate it with 0.1 M HCl to determine the CO_2. The organic C was defined as the difference between total and inorganic C. Total N was measured by the Kjeldhal method using concentrated H_2SO_4, K_2SO_4, and HgO to digest the sample (Bremner 1996) and soil particle size distribution by the hydrometer method as described by Gee and Bauder (1986). The WHC was measured on soil samples water-saturated in a funnel and left to stand overnight.

The CO_2 in the headspace of the bottles used to measure the soil microbial C was measured on an Agilent 4890D GC (USA) fitted with a thermal conductivity detector (TCD) at 250°C. The HP-Plot Q column (30 m × 0.32 mm) from Hewlett-Packard, used to separate CO_2 from the other gases with the carrier gas of He flowing at a rate of 5 ml min^{-1} was maintained at 32°C.

Statistical analyses

Significant difference for the treatment on concentrations of microbial biomass C, CO_2 production within 3 days and anthracene concentration was determined by analysis of variance (ANOVA) and based on the least significance difference using the General Linear Model procedure (PROC GLM) (SAS Institute 1989). This procedure can be used for an analysis of variance (ANOVA) for unbalanced data, i.e., when some data are missing. Data presented were the mean of three soil samples done in triplicate, i.e., $n = 9$.

Results

The removal of anthracene from soil was fastest within the first 3 days of incubation (Fig. 1). On the average 26% of anthracene was dissipated from soil within 3 days. After 28 days, the concentration of anthracene in soil was significantly larger in the LIGHT treatment of the 0–2 cm layer than in the DARK treatment, but lower than in the OPEN treatment ($P < 0.05$) (Fig. 1a). After 14 and 28 days, treatment had no significant effect on the dissipation of anthracene in the 2–8 cm and 8–15 cm layer (Fig. 1b, c). The layer had no significant effect on the

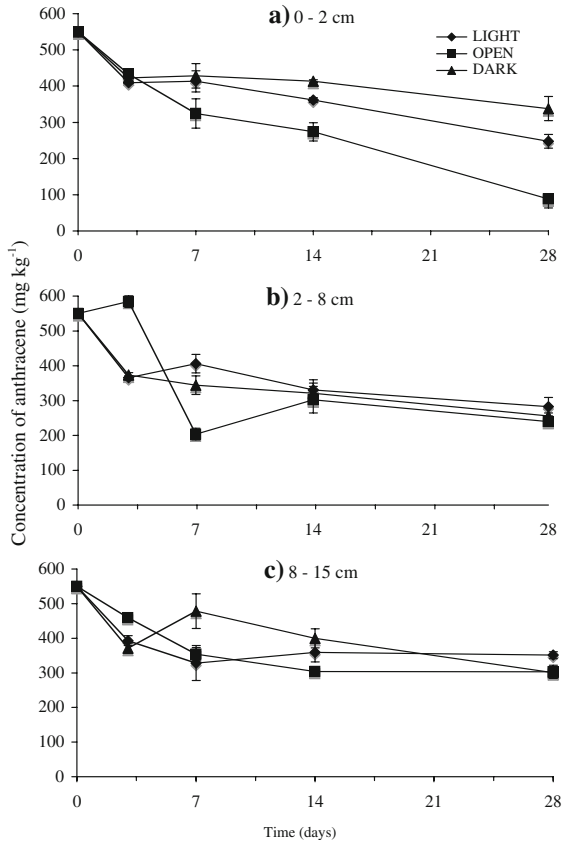

Fig. 1 Concentration of anthracene (mg kg^{-1} dry soil) in the (**a**) 0–2 cm, (**b**) 2–8 cm, and (**c**) 8–15 cm layer of soil where water loss was limited, but sun-light could reach it (LIGHT treatment) (◆), where water loss was limited and soil was kept in the dark (DARK treatment) (■), and where water content fluctuated and sun-light could reach the soil (▲) incubated aerobically 22 ± 2°C. Bars are standard deviations ($n = 9$)

removal of anthracene from soil except in the OPEN treatment where the dissipation of the PAHs was significantly larger from the 0–2 cm layer than from the other two layers ($P < 0.05$). The amount of anthracene decreased significantly over time depending on treatment and layer ($P < 0.05$).

The microbial biomass was 493 mg C kg^{-1} soil at the onset of the incubation. The microbial biomass, i.e., mean of all treatments, was significantly 1.3 times larger after 3 days compared to the value found at the onset of the incubation, significantly 1.1 times larger after 7 and 14 days and similar after 28 days ($P < 0.05$) (Table 1). The mean microbial biomass C was 665 mg C kg^{-1} soil in the 2–8 cm layer and significantly 1.1 times larger than in the other two layers ($P < 0.05$). The mean microbial biomass was

Table 1 Microbial biomass C (mg C kg^{-1} soil) in Acolman soil where light could penetrate and water content fluctuate (OPEN treatment), where light could penetrate but water content not fluctuate (LIGHT treatment), and where light could not penetrate and water content not fluctuate (DARK treatment) incubated for 28 days

Layer	Treatment	Days				LSD ($P < 0.05$)
		3	7	14	28	
0–2 cm	OPEN	729 A^a	532 B^b	429 C^c	156 D^b	91
	LIGHT	669 A^b	584 A^b	633 A^b	593 A^a	113
	DARK	741 A^a	712 A^a	746 A^a	602 B^a	86
	LSD ($P < 0.05$)	64	129	56	126	
2–8 cm	OPEN	792 A^a	577 B^b	564 B^b	599 B^c	65
	LIGHT	654 A^b	722 A^a	597 A^b	712 A^a	125
	DARK	754 A^{ab}	658 AB^{ab}	741 A^a	626 B^b	109
	LSD ($P < 0.05$)	136	112	116	33	
8–15 cm	OPEN	734 A^a	534 B^b	620 AB^a	604 AB^a	136
	LIGHT	672 A^{ab}	675 A^a	548 B^a	442 C^b	67
	DARK	579 B^b	618 AB^a	622 AB^a	667 A^a	61
	LSD ($P < 0.05$)	97	71	96	116	

Soil was contaminated with 550 mg anthracene kg^{-1} soil and the initial microbial biomass C was 493 mg C kg^{-1} soil

[a] LSD: Least significant difference ($P < 0.05$)

[b] Values with a different letter in the row are values significantly different over time ($P < 0.05$)

[c] Values with a different capital letter in the column are treatments significantly different from each other ($P < 0.05$)

572 mg C kg^{-1} soil in the OPEN treatment and significantly 1.2 times lower than in the DARK treatment and 1.1 times than in the LIGHT treatment ($P < 0.05$).

The emission of CO_2 was similar for the different layers of the different treatments (Table 2). On the average, the CO_2 production was 72 mg C kg^{-1} soil after 3 days and significantly 1.1 times larger than found after 7 and 14 days and 1.4 times larger than observed after 28 days ($P < 0.05$). Treatment and layer had no significant effect on emission of CO_2 over the 28-day period.

Discussion

Micro-organisms are known to remove anthracene from soil (Mahmood and Rao 1993). The fastest decrease in the amount of the contaminant in soil normally occurs within the 1st days of contamination (Betancur-Galvis et al. 2006). Kästner et al. (1999) for instance reported that 20% of anthracene added to soil was dissipated within 30 days and only 24% in the next 146 days. In the experiment reported here, on the average 26% of anthracene was removed from soil within 3 days and only a further 22% in the next 25 days. It can be speculated that most of the anthracene was bioavailable in the 1st days of the incubation and could thus be degraded by soil micro-organisms. Reichenberg and Mayer (2006) suggested that the bioavailability of contaminants depended on two factors, its degradability and/or bio-accessibility. On the one hand, dry-wetting liberates the contaminant initially, i.e., increase bio-accessibility. On the other hand, anthracene gets fixed on the soil matrix, i.e., organic matter, between aggregates and on soil minerals, so that its dissipation decreased, i.e., its bio-accessibility is reduced in the long run (Nam and Alexander 2001). In the relative short incubation, drying and wetting of soil released contaminants that were not available for degradation otherwise. This effect was only observed in the top 0–2 cm soil layer where the drying–wetting effect was the most extreme. As such, 83% of the anthracene was removed from the 0–2 cm top soil layer after 28 days. Additionally, in the OPEN treatment UV might have contributed to the degradation of anthracene. Photodegradation of organic components in soil has not often been reported before, but it has been reported in water. Bertilsson and Widenfalk (2004)

Table 2 Emission of CO_2 (mg C kg^{-1} soil) in Acolman soil where light could penetrate and water content fluctuate (OPEN treatment), where light could penetrate but water content not fluctuate (LIGHT treatment), and where light could not penetrate and water content not fluctuate (DARK treatment) incubated for 28 days

Layer	Treatment	Days				LSD ($P < 0.05$)
		3	7	14	28	
0–2 cm	OPEN	21.4 C^{a}	18.7 A^{ab}	12.4 B^{b}	22.3 A^{a}	8.2
	LIGHT	28.4 B^{a}	21.6 A^{ab}	17.2 AB^{b}	12.4 B^{b}	11.1
	DARK	38.1 A^{a}	15.2 A^{c}	23.0 A^{b}	27.4 A^{b}	5.7
	LSD ($P < 0.05$)	5.6	13.0	9.5	8.2	
2–8 cm	OPEN	16.1 B^{a}	19.3 A^{a}	16.3 B^{a}	28.0 A^{a}	12.2
	LIGHT	30.4 A^{a}	18.0 A^{bc}	11.7 B^{c}	22.2 AB^{b}	6.5
	DARK	28.7 A^{a}	19.1 A^{b}	25.3 A^{a}	16.4 B^{b}	5.7
	LSD ($P < 0.05$)	5.6	13.0	9.1	11.0	
8–15 cm	OPEN	37.1 A^{a}	18.0 A^{bc}	8.7 A^{c}	24.2 A^{b}	12.6
	LIGHT	40.5 A^{a}	21.1 A^{b}	16.4 A^{bc}	10.6 B^{c}	7.2
	DARK	23.2 B^{a}	19.6 A^{ab}	14.5 A^{ab}	13.4 AB^{b}	9.7
	LSD ($P < 0.05$)	14.0	6.1	8.5	10.9	

Soil was contaminated with 550 mg anthracene kg^{-1} soil

[a] LSD: Least significant difference ($P < 0.05$)

[b] Values with a different letter in the row are values significantly different over time ($P < 0.05$)

[c] Values with a different capital letter in the column are treatments significantly different from each other ($P < 0.05$)

found that anthracene and other polycyclic hydrocarbons are degraded in aqueous media by solar radiation.

In the experiment reported here, 83% of the anthracene was removed from the 0–2 cm top soil layer after 28 days. In the soil kept in the dark, only 39% of the anthracene was removed from soil within 28 days. As such, in soil where light could penetrate and the water content fluctuated, changes in water content contributed to 31% of the dissipation of anthracene from soil and light for 13% assuming that the type of plastic did not affect fluctuations in soil water content. Margesin et al. (2000) reported that light increased microbial activity. It might be assumed that increased microbial activity increased removal of anthracene.

Although the soil in the columns was kept under aerobic conditions, i.e., the water content <50% WHC, anaerobic microsites cannot be excluded. It is well known that anaerobiosis reduces removal of PAHs from soil compared to aerobic conditions (Johnsen et al. 2004). Drying of the soil will decrease water content, anaerobic conditions and thus accelerate removal of anthracene from soil. Decreases in water content were absent in the other two treatments, so removal of anthracene might have been delayed in anaerobic micro-sites. However, lack of water in soil is also known to decrease microbial activity and that might have inhibited removal of anthracene in the OPEN treatment.

The effect of changes in water content and light appeared to be mainly physical as the microbial biomass C did not increase in the 0–2 cm layer of the OPEN treatment. Contrarily, the biomass C decreased in the 0–2 cm layer of the OPEN treatment over time. The faster removal of anthracene from the 0–2 cm layer of the OPEN treatment was neither related to microbial activity. The emission of CO_2 within 3 days which reflected microbial activity was on the average not larger in the 0–2 cm layer of the OPEN treatment compared to the LIGHT or DARK treatment.

It has been reported that degradation of PAHs decreases under anaerobic conditions. For instance, Mcnally et al. (1998) reported that the rates of degradation of anthracene by pseudomonad strains were generally slower under anaerobic conditions compared to those under aerobic conditions. It appears that the soil was sufficiently aerated and no anaerobic conditions existed in soil as the removal of anthracene was similar in the 8–15 cm soil layer of

the OPEN treatment as in the 0–2 cm soil layer of the DARK treatment. As such, anaerobicity did not affect the removal of anthracene from soil in the experiment reported here. No abiotic treatment was included in this study so that possible changes in extraction efficiency of anthracene could not be excluded. It can be speculated, however, that those changes, considering the technique used, were small.

It is well known that treating a soil affects soil microbial biomass (Jenkinson and Powlson 1976). Soil sieving, drying and wetting, and freezing and thawing increases microbial biomass (Winding et al. 1994; Feng et al. 2007; Ford et al. 2007). The microbial activity as witnessed by the emission of CO_2 decreased over time. Soil organic matter is mineralized so the available substrate decreases over time (Van Veen and Kuikman 1990).

Conclusion

It was found that removal of anthracene from the top 0–2 cm soil layer was faster than from the deeper soil layers, i.e., 2–8 cm and 8–15 cm, when the top layer was dried and rewetted and sun-light could reach it. Photodegradation, and adsorption and desorption due to drying and wetting of the soil accelerated the removal of anthracene from the 0–2 cm top layer. The effect of dry-wetting on removal of anthracene, however, was more important than that of sun-light.

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