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Effects of oil pollution at Kuwait's greater Al-Burgan oil field on polycyclic aromatic hydrocarbon concentrations in the tissues of the desert lizard *Acanthodactylus scutellatus* and their ant prey

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Abstract Using indicator species to monitor the effects of oil pollution was thought to be useful to assess whether local desert reptiles and their insect prey could fulfill such a role in an area damaged in the second Gulf War (1990). Polluted sites with apparently different degrees of contamination (namely tar mat, soot, and clear sites) located at Kuwait's Greater Al-Burgan oil field were compared with control areas outside this region in study conducted in 2002. Five *Acanthodactylus scutellatus* lizards from each study and control site were humanely killed and stored in a freezer at −20°C until analysis. Ants from the same sites were also collected and treated in a similar manner. Lizard and ant whole body tissues were subjected to gas chromatography–mass spectrometry (GC–MS) to determine concentrations of petroleum hydrocarbons (HCs). The study concentrated on sixteen polycyclic aromatic hydrocarbons (PAHs), EPA priority pollutants used as indicators of petrogenic HC contamination. There were significantly different concentrations of total PAHs in lizards and ants among all four study sites. Of the 16 PAHs, phenanthrene, fluoranthene, and benzo[*a*]anthracene were present in both lizard and ant samples from the Greater Al-Burgan oil field sites irrespective of the apparent degree of pollution but were undetectable in materials from the control sites. The range of total PAHs in lizards was 26.5–301 ng g^{-1} and it was 6.7–82.1 ng g^{-1} in ants. Concentrations increased progressively along an expected contamination gradient. Total PAHs were detected in biota even in an area (clear site) that did not appear, virtually, to contain petroleum soil pollution which supports the value of indicator biota species. For all three sites where PAHs were found in biota, the ratio of total PAHs in ants to lizards was consistently 3.3–3.4. These data show that, although 12 years have passed since the Kuwait oil spill catastrophe, all sites are still contaminated with PAHs. Use of lizard and ant materials in monitoring such desert locations seems to be an effective strategy.

Keywords PAHs · *Acanthodactylus scutellatus* · Ants · Oil pollution · GC–MS · Kuwait · Deserts

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Introduction

The Iraqi invasion of Kuwait on 2 August 1990 and the subsequent war activities left many scars on the desert ecosystem (Al-Hassan 1992; Omar et al. 2000). The most severe element in Kuwait's environmental crisis was, however, the burning of oil wells. The oil fires released particles, organic and inorganic gases, hydrocarbons (HCs), and oil droplets (Al-Hassan 1992). Oil spills, aerosol deposits, and seawater use all have had adverse effects on the desert ecosystem. The explosion of oil wells in Burgan and Ahmadi produced enormous volumes of soot and unburned oil in the form of oil-mist that was carried to distant areas of about 49.15 km^2 (Al-Ghunaim 1997). In areas covered by oil-soot, a thin black crust of about 2–5 mm of slightly compacted superficial soil was formed on the surface. Unburned oil escaping from exploded oil

wells accumulated in low-lying areas to form oil-logged soil where oil penetrated 15 cm or more in the soil profile (Omar et al. 2000).

Just as the oil fires were a source of pollution to land, sea, and air, the oil lakes became a source of pollution per se. The formation of the lakes resulted from discharge of oil from damaged wells that acted as gushers and burning wells, whose discharge rate was greater than could be consumed by the flame (such that the spray of oil finally landed back on the ground). The oil subsequently collected on the ground and ran into streams, following slopes and contours of desert topography. Soon there were running streams followed by the formation of lakes (Al-Hassan 1992).

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals formed during the incomplete burning of coal, oil, and gas. They are also found in garbage, creosote, and road and roofing tar. PAHs can be man-made (anthropogenic) or may occur naturally. Indeed, they are ubiquitous environmental pollutants found in air, water, and soil. The US EPA priority PAHs are classified into two major groups. PAHs with 2 to 4 benzene rings may be non-carcinogenic or carcinogenic and include: naphthalene, acenaphthene, anthracene, phenanthrene, acenaphthylene, fluorene, fluoranthene, and pyrene. Carcinogenic PAHs with 4 to 6 benzene rings include: benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno(1,2,3-*c*,*d*)pyrene, dibenzo[*a*,*h*]anthracene, chrysene, and benzo[*g*,*h*,*i*]perylene. Some of these PAHs are probable human carcinogens. Their distribution in the environment and the possible exposure of human beings has been the focus of much research. The total potential dose of carcinogenic PAHs for humans from water, air, sediment, soil, and food has been estimated by Menzie et al. (1992). Many PAHs cause cancers, affecting a variety of tissues. In the PAH class, 16 compounds in potable water and waste waters and 22 compounds in soil and solid wastes are listed as priority pollutants by the EPA. However, benzo[*a*]pyrene is a potent human carcinogen, while benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, chrysene, dibenzo[*a*,*h*]-anthracene and indeno(1,2,3-*c*,*d*)pyrene have shown sufficient evidence of carcinogenicity in animals to be causes of concern (Patnaik 1999).

Spilled petroleum oil can affect organisms by direct physical coating or by altering essential elements of the habitat. Although there are many studies where the impact of oil spills have been looked at in marine locations (IUCN 1983; Fayad et al. 1996; Al-Bakri and Kittaneh 1998; Jones et al. 1998; Al-Hassan et al. 2000) no study has investigated the effects of oil pollution on wildlife in desert locations. This study was initiated to explore whether the PAHs released in the Greater Al-Burgan oil fields could be detected in the tissues of the sand lizard, *Acanthodactylus scutellatus* and a major component of its diet (ants). The basic intention was to determine whether such animals could be employed as indicator species for oil pollution in desert locations.

The Greater Al-Burgan oil field has an area of 349.65 km^2 and lies 20 kilometers to the south of Kuwait City. Types of contaminated soil were categorized in relation to ground observations. The categories used have been designated as "tar mat", "soot", and "clear". The tar mat areas had a soil surface that had been solidified by oil, forming a layer about 1 cm thick that could be peeled off the underlying "clean" soil. The soot areas were found within the upper layer of soil and could be 1–8 mm in depth. Contamination is, however, sometimes continuous and, at other times, discontinuous. The clear sites had no visual evidence of soil pollution. Two sites for each category of contaminated soil were located in the oil field. Two comparable areas well outside the oil field were used as controls. The control area (Sulaibiya) is an Agriculture Research Station at Kabd which was established in 1975. It is a fenced reserve protected from livestock and human interference. People are not allowed to enter without permission. Consequently, the area is highly vegetated especially in spring. The research station covers a total area of 20 km^2, being 4 km (east to west) × 5 km (north to south).

Method

Five lizards (a mixture of adult males and females in the ratio 1:1 in each sample—a total of 40 lizards from polluted and control sites) and samples of the ant populations of the genus *Messor* (500–600 per site—a major prey item of the lizards (Perry and Dmi'el 1994)) were taken from each study site, by using pitfall traps, to investigate the presence and concentrations of PAHs in wildlife tissues in 2002. Lizards and ants were humanely killed, placed in jars that had been washed with ethanol (Fluka Wacker Chemie, Munich, Germany) and stored in a freezer at −20°C until analysis. The method used for the analysis of petroleum HCs in the biota followed techniques used in the Manual of Oceanographic Observations and Pollutant Analysis Methods which have been careful in selecting methods that have been internationally tested and accepted, for example by the Intergovernmental Oceanographic Commission (IOC) and the United Nations Environment Programme (UNEP) (ROPME 1999). Lizards, or other reptiles, have rarely ever been used in toxicity tests, so virtually no information is available on their sensitivity to chemical contaminants such as PAHs. Lambert (1987) was the first to propose using lizards as bioindicators of pesticide contamination. He conducted whole-body residue analyses on

lizards after heavy pesticide spillage (Lambert 1997). For analysis, the samples were defrosted and prepared for solvent extraction. To achieve satisfactory recovery of the petroleum HCs, samples were chopped, freeze-dried and weighed.

About 5 g of the freeze-dried sample (whole body residue) were extracted with a Soxhlet extractor with 250 mL of methanol in a flask. 20 mL of 0.7 mol L^{-1} reagent-grade potassium hydroxide (Riedel de Haan, Seelze, Germany) and 30 mL of distilled water were added to the flask to saponify the lipids overnight. The content of the extraction flask was transferred into a separation funnel and extracted with 90 mL of analytical grade hexane (BDH Laboratory Supplies, Poole, UK) and re-extracted twice with 50 mL of hexane. Subsequently, all hexane extracts were combined, filtered through glass wool, and dried with reagent grade anhydrous sodium sulfate (JT Baker, Phillipsburg, USA) until a volume of 5 mL was reached, after which the material was transferred to a clean-up column.

Silica gel–aluminium oxide and glass wool columns were prepared by being cleaned for 8 h with methanol and then for 8 h with hexane. Silica gel–aluminium oxide and glass wool were dried in an oven at 60°C for 45 min to remove the solvent and subsequently at 150°C overnight. The above items were kept separate in amber glass bottles. Before use, they were activated at 200°C for 4 h and partially deactivated with 5% water per 10 mL silica–aluminium oxide. A chromatography column was prepared using a 50 mL burette in which a piece of glass wool was added near the stopcock to support the packing material. Silica-gel (10 mL) was transferred into the column, then 10 mL of aluminium oxide, and finally 1 g of sodium sulfate in order to reduce disturbance of the first layer when solvents were poured into the column.

The sample was applied on top of the column, then fraction 1 was obtained by eluting the sample with 20 mL of hexane. As this fraction contained saturated aliphatics, it was discarded. The second fraction containing the aromatic HCs, was obtained by eluting the sample with 30 mL of a 90:10 mixture of hexane and analytical-grade dichloromethane (Sharlau Chemie, Barcelona, Spain). This sample was then reduced in volume using a rotary evaporator (Brinkmann Instruments, New York, USA) until a volume of 5 mL was reached and the sample was transferred into a concentrator (eBay, Los Angeles, USA) where nitrogen gas was used to reduce it to a final volume of 1 mL. This sample was then ready for gas chromatography–mass spectrometry (GC–MS) analysis of PAHs. GC–MS (Perkin–Elmer, Boston, MA, USA) operated in MS-SIM. The sample was injected splitless on a capillary column coated with SE-54. The detection limits for total PAHs were 5 ng g^{-1}. Phenanthrene, fluoranthene, and benzo[*a*]anthracene were detected and measured in lizard and ant tissues. The concentrations of these compounds were summed to give total PAH concentration. Data analysis was performed using the one-way analysis of Variance (ANOVA) parametric test to test whether there were differences among mean total PAH concentrations at the four study sites. Parametric post hoc Scheffe tests were used to compare between pairs of study sites; PAH concentration was the dependent variable and the sites were the independent variables.

Results

The results of PAH estimates in ant and lizard tissues are shown in Table 1. The ANOVA test showed a highly significant variance in the mean total PAH concentrations in ants ($F_{3,4} = 572$, $P < 0.0001$) over the four study sites. Post hoc Scheffe tests showed that the total PAH concentrations in the control sites differed significantly from the clear, soot, and the tar mat sites (all $P < 0.0001$). The total PAH concentrations in ants at the clear sites did not differ from values at the soot sites ($t = 3.87$, $P = 0.07$), but they significantly differed from the tar mat sites ($t = -12.8$, $P < 0.001$). The PAH concentrations of ants in the soot sites differed significantly from samples from the tar mat sites ($t = -16.6$, $P < 0.0001$).

The ANOVA test showed highly significant variance in the mean of total PAH concentrations in lizard whole body tissues ($F_{3,4} = 1416.88$, $P < 0.0001$) from the different study sites. Post hoc Scheffe tests showed a significant difference between the PAH concentrations of lizard tissues at the control and the clear ($t = -42.5$, $P < 0.0001$), the soot ($t = -37.7$, $P < 0.0001$), and the tar mat ($t = -63.9$, $P < 0.0001$) sites. The PAH concentrations in lizards at the clear sites were also significantly different from the soot ($t = 4.8$, $P < 0.03$) and tar mat ($t = -21.4$, $P < 0.0001$) sites. These concentrations in lizard tissues significantly differed at the tar mat and soot sites ($t = -26.2$, $P < 0.0001$).

Three of 16 PAHs (phenanthrene, fluoranthene and benzo[*a*]anthracene) were detected in lizard and ant tissues (Tables 2 and 3). They were proved to be present in

Table 1 Mean ± s.d of total PAH concentrations (ng g^{-1}) in ant and lizard tissues from the different study sites

Location ($N = 10$)	Ants	Lizards
Control	Undetectable	Undetectable
Clear	27.43 ± 2.5	91.6 ± 22.5
Soot	23.92 ± 2.0	80.7 ± 21.3
Tar mat	39.3 ± 3.2	136.5 ± 37.5

Table 2 Mean ± s.d of the three PAH concentrations (ng g^{-1}) that were identified of the 16 PAHs measured in lizard tissues from the different study sites ($N = 10$)

PAHs	Control	Clear	Soot	Tar mat
Phenanthrene	Undetectable	32.9 ± 2.0	26.5 ± 3.3	57.4 ± 4.3
Fluoranthene	Undetectable	190.6 ± 21.5	182.1 ± 23.1	301.7 ± 34.2
Benzo[*a*]anthracene	Undetectable	51.4 ± 3.5	33.6 ± 2.2	50.3 ± 3.4

Table 3 Mean ± s.d of the three PAH concentrations (ng g^{-1}) that were identified of the 16 PAHs measured in ant tissues from the different study sites ($N = 10$)

PAHs	Control	Clear	Soot	Tar mat
Phenanthrene	Undetectable	14.8 ± 0.8	16.4 ± 1.4	20.6 ± 1.8
Fluoranthene	Undetectable	53.5 ± 3.0	48.7 ± 3.3	82.1 ± 6.5
Benzo[*a*]anthracene	Undetectable	14.0 ± 0.6	6.67 ± 0.2	15.2 ± 1.2

samples from the tar mat, soot and clear sites but they were not detected in the reference (control) sites.

Discussion

We found no other studies that determined concentrations of PAHs from oil pollution in reptiles or ants. Our results suggest that the levels of PAHs found in lizards and ants living in the Kuwait desert could be high enough to affect vital organs (such as the liver in the former animals). Very low levels of PAHs might be toxic to lizards. The results also suggest that these species can tolerate and concentrate potentially harmful PAH concentrations.

Lizards are vulnerable to habitat changes through their limited powers of migration and dispersal but this vulnerability makes them potentially excellent indicators of local contamination of terrestrial habitats (Lambert 1993). Culley and Applegate (1967a) sampled wildlife from cotton fields, desert, and desert periphery within a 30-mile radius of Presidio, Texas. Dichlorodiphenyldichloroethylene (DDE) residues as high as 7.0 μg g^{-1} were found in the tail muscle of Whiptail lizards (*Cnemidophorus* spp.) collected from cotton fields and desert periphery sites (op. cit.). Pesticide concentrations decreased in samples gathered at greater distances from the cotton fields. Gravid females had an average of 16.4 μg g^{-1} DDE in their eggs but only 3.4 μg g^{-1} DDE in their muscle tissue (op. cit.). In a subsequent study, DDE residues of up to 49.9 μg g^{-1} were measured in the liver of Whiptail lizards from cotton-field sites (Culley and Applegate 1967b). High levels of DDE were still found in the Rio Grande and Pecos River drainages of New Mexico and Texas when White and Krynitsky (1986) extensively sampled vertebrates in these areas in 1982 and 1983. A Whiptail lizard from Pecos, Texas, had the highest DDE concentration (104 μg g^{-1}, whole body) of all animals (birds, bats and lizards) examined in this study. The high concentrations found in such lizards provided strong support for the view that the source of contamination was local.

Lambert (1993) monitored the impact of ground spraying with DDE on lizards in Mopane woodlands and gritstone outcrops of northwestern Zimbabwe in 1989 and 1990. In the woodland-dwelling lizard *Mabuya striata wahlbergi*, whole-body total dichlorodiphenyltrichloroethane (DDT) levels increased significantly with the number of annual treatments. Highest DDT and DDE concentrations found in these lizards were 15.38 μg g^{-1} (dry weight) and 6.00 μg g^{-1} (dry weight), respectively over the treatment period. Lizards from the outcrops (*Mabuya quinquetaeniata margaritifer* and *Agama kirkii*) showed some accumulation of residues, but these were significantly lower than in the woodland species.

Heavy spillage from a pesticide store that was bombed near Hargesia, Somaliland caused contamination of 3700 m^2 of soil (Lambert 1997). Lizards and frogs were used as bioindicators of contamination in the area. In lizards, the highest levels of dieldrin and total DDT were found in *Chalcides ragazzi* and *Mabuya striata striata* from three sites in Hargesia that were 4.1–9.0 km downstream of the spillage site (op. cit.). Other lizards (*Hemidactylus parkeri*) from the spillage vicinity and an area 350 m downstream, had the highest concentrations of beta-hexachlorocyclohexane residues.

In this present study, PAHs that are strong indicators of petrogenic HC contamination were detected in lizard and ant samples. The analyses of *A. scutellatus* and ant samples collected from sites with apparently different levels of oil pollution from Burgan oil fields produced evidence of contamination with oil HCs at all locations. This study

confirmed the presence of highly significant concentrations of phenanthrene, fluoranthene, and benzo[*a*]anthracene in lizards and ants from the oil polluted sites (tar mat, soot, clear), while these PAHs were undetectable in the control sites. These three PAH compounds were dominant in both lizard and ant samples, confirming that one of the sources of PAH contamination in lizards is by ingesting contaminated food. Ants are a major food source for *A. scutellatus*.

The clear sites generated higher PAH concentrations than the soot sites in both ant and lizard samples. This was an interesting result because the clear sites have generally been thought to be clear of oil pollution. The present study proves that the clear sites are polluted perhaps to a greater extent than the supposedly more polluted soot sites.

Few other studies were located that concerned PAH levels in lizards especially those dealing with dietary contamination. An exception is Wikelske et al. (2002) who suggested that the high mortality of Galapagos marine iguanas (*Amblyrhynchus cristatus*) in the aftermath of an oil spill was due to the oil either having a direct toxic effect on the iguanas per se or by contaminating the algae they consume. The iguanas may have been endangered because they decline to eat their fouled food or because their hindgut becomes poisoned and they no longer can digest the food they eat.

The natural background levels of PAHs in lizards in the present study are still largely unknown but the results suggest that higher total PAHs in the tar mat sites for both lizards and their prey may be responsible for reducing the lizard's survival, and may be responsible for histopathological features that link tissue damage to prey contamination (Al-Hashem 2006). In some areas of the Kuwait desert natural weathering has converted the spilled crude oil to a hard residue containing high levels of toxic PAHs. Because of their low metabolic rates and relatively simple enzyme systems, lizards may not be able to detoxify complex chemical compounds that they inhale or ingest with contaminated invertebrate prey as quickly as do endotherms (Walker and Ronis 1989).

The major conclusion that can be drawn from this present study is that, although 12 years have passed on Kuwait oil spill at the Burgan oil fields, the terrestrial environment is still contaminated with PAHs (including some carcinogenic compounds). The sand lizard *A. scutellatus* is confirmed to be a suitable bioindicator species for studies on the bioaccumulation of PAH compounds in biota in such desert locations. Further, this study suggests that the clear sites are, in fact, contaminated with PAHs rather than being free of these materials as has been generally assumed from the lack of physical signs of contamination. Perhaps the major difference between the contaminated sites is in their physical properties, with the tar mat being obviously very different from the clear site in forming a protective crust over the underlying soil.

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