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Biomonitoring of persistent organic pollutants in Egypt using *Taphozous perforatus* (Chiroptera: Emballonuridae)

Biomonitoring persistentních organických škodlivin v Egyptě s využitím hrobkovce egyptského (*Taphozous perforatus*) (Chiroptera: Emballonuridae)

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Abstract. Organochlorine pesticides (OCP) and polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants (POPs) that have chronic toxicity, tendency to contaminate the environment, and transfer into the food chain. This study was conducted to explore the use of bats as bioindicators to help understanding the time trend of POPs at the present time. Liver and kidney tissues from the Egyptian tomb bat (*Taphozous perforatus*) were subjected to the QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction prior to quantification by LC-MS/MS analyses. DDT (dichlorodiphenyl trichloroethane) metabolites (e.g., *o,p*'-DDT, *p,p*'-DDD, *p,p*'-DDE), PCB congeners (e.g., PCB 118, PCB 138, PCB 180), hexachlorobenzene (HCB), dicofol and sulphur were found in variable concentrations in the tissues of *T. perforatus*. Their concentration levels were affected with the bat sex and the season of sampling. Liver and kidneys were found to contain 0.39 µg/g wet weights of DDTs and 0.11 µg/g wet weights of PCBs. Concentration of the compound dichlorodiphenyl ethane (*p,p*'-DDE) predominated over the other DDT metabolites; giving rise to the DDE/ ∑DDT ratio of 0.82 as an indicative of pronounced decline in new DDT inputs to the environment. Also, concentration of the PCB 138 predominated that of the other congeners. There were correlations between liver and kidney concentrations of OCP and PCBs in both of them. It was concluded that these pollutants are still detectable in the environment; however in low concentration levels and far from lethal toxicity. Nevertheless, these findings may encourage the use of other bat species from urban and rural regions, as well as agricultural and industrial locations, as bioindicators.

Key words. Persistent organic pollutants, dichlorodiphenyl trichloroethane metabolites, polychlorinated biphenyls congeners, tomb bat, biomonitoring, Egypt.

INTRODUCTION

Persistent organic pollutants (POPs) are carbon-based organic compounds that include synthetic compounds of agricultural use, such as organochlorine pesticides (OCP), and industrial applications, such as polychlorinated biphenyls (PCBs). Other substances (e.g., dibenzo-p-dioxins, 'dioxins' and dibenzo-p-furans, 'furans') are by-products generated mainly as results of human and natural activities. OCPs and PCBs are considered an important group of POPs that have become a global concern because of their chronic toxicity and tendency to contaminate the environment. They persist in sediments acting as a non-point source releasing OCPs over many years, after their use is finished. The OCPs may cause adverse effects to organisms and human health through their ubiquitous trophic transfer (Hong et al. 1995).

POPs have been intentionally developed since the first half of the 20th century and used in many products including pesticides, dielectric and hydraulic fluids in industrial machinery, capacitors and transformers. They are unintentionally generated in different processes involving combustion and various industrial activities. POPs are characterized by low water solubility and high fat affinity, they resist breakdown by natural processes, which make them remain in the environment for decades, bio-accumulating exponentially in the food chain. Compounds such as PCBs are long persistent in the environment and may bio-accumulate by factors reaching up to 70,000 fold (UNIDO 2003). Since the early 1960s, the chemical management and safety issues have received great attention and various conventions have been adopted for this purpose. The Stockholm Convention (SC) on POPs signed in May 2001 focused on reducing and eliminating releases of 12 POPs coined the 'Dirty Dozen' by the United Nations Environment Program (UNEP). DDT and PCBs are included in this group (Mansour 2009).

The OCPs, introduced in the 1940s, were used ubiquitously in agriculture for pest control until concerns regarding their persistence and toxicity led to restrictions and bans in the 1970s and 1980s. However, their residues persist in the environment until today, and their use still continues in some developing countries (Van den Berg et al. 2012). PCBs were widely used as dielectric fluids and coolants until they were banned, but their residues still remain in the environment due to incidental and accidental releases (UNEP 2009).

It is a matter of fact that bat populations have been declining in many parts of the world. Indeed, there are many factors responsible for such decline, and the use of OC insecticides in agriculture is demonstrated to be one cause of bat population declines (Kunz et al. 1977), in addition to diseases such as the white-nose syndrome (Kannan et al. 1995). Bayat et al. (2014) reported that organic contaminants (e.g., pesticides and PCBs) are still being detected in bat tissues, many years after their use was banned.

Bats provide many essential ecosystem services that make sustaining of their populations vital for ecosystem health. Except Antarctica, bats dominate in every continent representing the second largest mammal order. They comprise about 20% of mammal species, with the greatest diversity especially in the tropics. They provide a range of essential ecosystem services including pollination, seed dispersal, and insect moderation (Wickramasinghe et al. 2003). Insectivorous bats are common predators on large varieties of insect pests, such as cucumber beetles, June bugs, corn earworm moths, cotton bollworm moths, tobacco budworm moths and Jerusalem crickets, which threaten the yield of important agricultural and forest economic crops. Their voracity possibly contributes to the disruption of population cycles of agricultural pests; giving rise to estimate the value of bats to the agricultural industry in USA at roughly 22.9 billion USD per year; including the reduced costs of pesticide applications that are not needed to suppress the insects consumed by bats (Cleveland et al. 2006). Given the wide distribution and high species richness of bats, these mammals face a noticeable array of threats in the early 21st century. Most of these threats are directly related to human population increase, with the greatest pressure especially in tropical countries (Zukal et al. 2015).

Bats often coexist with humans in urban, industrial, and agricultural landscapes (Russo & Ancillotto 2015), thereby potentially exposing themselves to different varieties of chemical pollutants such as pesticides and heavy metals. In foraging areas of bats the quality of water is very important to the life of these animals. Wetland habitats are prime foraging areas for insectivorous bats as rivers and lakes support large numbers of insects. Wastewater treatment works (WWTWs) are known to provide profitable foraging areas for insectivorous bats because of their association with high abundance of pollution-tolerant dipterans (Fukui et al. 2006). Ditches and drainage of sewage treatment plants in addition to the El-Mariotteya canal may be considered as a profitable foraging area for the bats inhabiting the Saqqara district of Egypt. These aquatic systems were found to contain residues of OCPs at higher concentrations in the drainage canals than in irrigation ones (El-Kabbany et al. 2000). It has been recently reported that the El-Mariotteya water body at tributaries adjacent to bat roost caves at Saqqara is being subjected to multiple sources of pollution through the dumping of improperly treated organic and inorganic chemical wastes in addition to sewage materials. This caused mass mortalities among the Nile fish, *Oreochromis niloticus* in the El-Mariotteya stream, and was attributed to chemical (e.g., phenols and polycyclic aromatic hydrocarbons) and microbial pollutants (Eissa et al. 2013, Mahmoud et al. 2014). On the other hand, several PCBs were found at measurable concentrations along the stream of the Nile River which feeds the El-Mariotteya canal at El--Haram (Megahed et al. 2015). This leads us to suggest that the El-Mariotteya aquatic ecosystem may represent one of the major sources of pollution for foraging bats in the studied area.

The aim of the present investigation was to detect the presence of POPs and quantify their concentration levels in kidney and liver tissues of an insectivorous bat species, namely the Egyptian tomb bat, *Taphozous perforatus*, and to review the available data on POPs in different aquatic ecosystems that may help to understand the time trend of POPs at the present time. To do this, an explanation for the pollution source(s) of POPs was explored.

MATERIAL AND METHODS

Samples

Within a research of ecological and toxicological issues on some insectivorous bats in Egypt, the Saqqara archaeological area (29° 52’ N, 31° 13’ E) which is located on the west bank of the Nile; app. 17 km south of Giza, and 40 km away from Cairo, was selected for bat sampling. The area is surrounded by many palm groves and cultivated fields irrigated from the El-Mariotteya canal, which is located about 3 km from the bat collecting site. Ditches and drainage of sewage treatment plants are found in the area. The Saqqara archaeological site is characterized by the presence of many desert caves, some of which are used by bats as roosts.

Two neighboring large caves occupied by a relatively high number of bats were selected for collecting the required specimens over a period of two years (January 2012 – December 2013) on a monthly basis. Thanks to a preliminary examination of bats hunted randomly, and based on Dietz (2005), it was possible to identify two colonies of two bat species; namely: the Egyptian tomb bat, *Taphozous perforatus* Geoffroy, 1818, and the lesser mouse-tailed bat, *Rhinopoma cystops* Thomas, 1903. Both bat species were used in ecological studies (Soliman et al. 2015) and monitoring of heavy metals in liver and kidney tissues (Mansour et al. 2016). Specimens of *T. perforatus* were reserved for the present study. The specimens were collected by a mist net at the entrance to the roost cave (Mansour et al. 2016) and were transported alive to the laboratory in the Department of Zoology, Faculty of Science, Ain Shams University, Abbasiya, Cairo, Egypt.

Due to the small weights of liver and kidneys of the studied specimens, and the costs of residue analyses, the monthly collected samples were pooled into 4 portions; each consisted of 3-month collections, so that the pooled samples were designated to winter, spring, summer and autumn seasons. Each combined sample included organs separated from males and females. The tissue samples were then stored at –80 °C for pesticide residue analyses, just after collections. The bats were euthanized by decapitation, as approved by the University of Ain Shams Animal Ethics Committee, and consistent with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (Leary 2013).

Analysis

A total of 24 pooled liver and 24 pooled kidney tissues representing the 4 seasons (i.e., six pooled samples/tissue/season) for the male bats, together with similar numbers for the female bats, were conditioned for extraction after freeze thawing at room temperature. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, originally developed by Anastassiades et al. (2003) was used for extraction of pesticides, using acetonitrile, followed by LC-MS/MS for quantification of pesticides in the analyzed tissues. Based on Afify et al. (2010), the LC-MS/MS was performed on an Agilent 1200 Series HPLC instrument coupled to an API 4000 Qtrap MS/MS from Applied Biosystems with electrospray ionization (ESI) interface. The (ESI) source was used in the positive mode, and N_2 nebulizer, curtain, and other gas settings were optimized according to recommendations made by the manufacturer; source temperature was 400 °C, ion spray potential 5500 V, decluster potential and collision energy were optimized using a Harvard apparatus syringe pump to allow optimization of the MS/MS conditions. Ammonium formate solution (1 mM) was prepared in methanol: water (1:9; pH=4.0) and used as LC-Mobile Phase. A standard mixture of organochlorine (OC) compounds containing HCB, lindane, heptachlor, aldrin, dieldrin, endrin, dicofol, DDT metabolites and PCB congeners (Chemical Service, Inc. , West Chester, PA) was prepared in methanol and kept at –20±2 °C. Intermediate standard solutions of 1 µg/ml were prepared by diluting stock solution in methanol.

Tissue samples (1.0 g wet weight) were mixed with 10 ml acetonitrile and blended for 1 min using Ultra-turrax. The buffer-salt-mixture was added and shacked immediately for 1 min. The samples were centrifuged at 4000 rpm for 5 minutes. Portion of Acetonitrile layer was filtered using acrodisk and directly injected into LC-MS/MS system. The selected parameters for in-house validation were mainly taken from Magnusson & Ornemark (2014).

The limit of detection (LOD) was calculated as the minimum concentration of analyte in the test sample that can be measured with a stated probability that the analyte is present at a concentration above that in the blank sample. Limit of detection (LOD) value was 0.001–0.007 µg/g, and recoveries ranged from 70 to 110%. Results of analyses were calculated in terms of nanogram/gram (ng/g) wet weight tissue.

Statististics

The data obtained were subjected to statistical analyses using GraphPad Prism 5 Demo (www.graphpad.com/downloads/docs/Prism5Regression.pdf), and expressed as means±SE. The paired samples (t) test was used to compare the basic data for significance at $P<0.05$. The one-way analysis of variance (ANOVA) was performed to test interaction between different variables. Also, the Spearman rank correlation coefficients (*rs*) for each pair of the data set were calculated at 95% confidence intervals.

RESULTS

Intra-specific differences

Analyses of OC compounds in the bat tissues (e.g., liver and kidneys) revealed the presence of eight compounds [i.e., *o*,*p*'-DDT; *p*,*p*'-DDD; *p*,*p*'-DDE; PCB 118; PCB 138; PCB 180; HCB: Hexachlorobenzene and dicofol] at measurable concentration levels, in addition to sulphur (S) in elemental form (Tables 1, 2).

Concentration of pesticide residues in liver with respect to bat's sex is presented in Table 1. In the winter season, the liver of male *T. perforatus* contained 15.0 ng/g tissue of *p*,*p*'-DDD, a value which was significantly ($P<0.05$) higher than that found in females (10.67 ng/g tissue). Similar trend was obtained for the compound PCB 180 (31.33 and 11.67 ng/g, respectively). Concentration of *p*,*p*'-DDE equaled 291.70 ng/g tissue in male livers compared to 210 ng/g tissue in female ones which meant a highly significant difference ($P<0.01$) for the male va-

Table 1. Concentration (ng/g wet weight) of pesticide residues in the liver tissue of adult male and female *Taphozous perforatus* collected from the Saqqara area during the year 2013. * Each season is represented by three-month collections, and the values are means ± standard error. Statistics: In each season, male and female animals, in the same horizontal row marked with "a" are significantly different at $P<0.05$. Those marked with "b" are highly significantly different at $P<0.01$

Tab. 1. Koncentrace (ng/g mokré hmotnosti) residuí pesticidů v tkáních jater dospělých samců a samic hrobkovce egyptského (*Taphozous perforatus*) odchycených v oblasti Sakkary během roku 2013. * Každá roční doba představuje trojměsíční sběry, hodnoty představují průměry ± standardní chyba. Statistická významnost: samci a samice v jedné roční době ve stejném řádku označené "a" jsou statisticky odlišné při $P<0.05$, označené "b" jsou odlišné při $P<0.01$, sulphur = síra

season / doba	winter / zima		spring / jaro		summer / léto		autumn / podzim	
pohlaví / sex	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
o,p'-DDT	13.00±1.16	12.67±0.88	14.33±0.88^{a}	25.00±2.89^{a}	14.00±2.08	12.33±0.88	15.00±1.16^{a}	10.67±0.33^{a}
p,p'-DDD	15.00±1.16^{a}	10.67±0.33^{a}	10.67±0.33^{b}	25.00±2.89^{b}	10.67±0.33	14.67±2.03	14.67±0.88^{a}	10.67±0.33^{a}
p,p'-DDE	291.70±7.27^{b}	210.00±5.77^{b}	209.00±7.81^{a}	256.70±14.53^{a}	296.70±12.02^{a}	224.00±12.49^{a}	173.00±5.69^{b}	95.33±7.42^{b}
PCB 118	10.67±0.33	11.67±0.33	11.67±0.67	12.00±1.53	12.00±0.58^{b}	17.67±0.33^{b}	11.00±0.58	12.00±1.00
PCB 138	44.00±5.03^{b}	11.67±0.33^{b}	11.67±0.67^{a}	34.33±5.36^{a}	11.67±0.88^{b}	32.67±3.71^{b}	22.00±3.06^{a}	11.00±0.58^{a}
PCB 180	31.33±5.81^{a}	11.67±0.88^{a}	11.67±0.33^{b}	41.67±5.55^{b}	11.00±0.58^{b}	21.00±2.08^{b}	20.33±1.45^{b}	11.67±0.88^{b}
HCB	11.00±0.58	11.67±0.67	11.00±0.58	11.00±0.58	11.67±0.67	12.00±1.00	11.00±0.58	11.67±0.88
dicofol	53.67±4.70^{b}	13.33±0.88^{b}	20.67±2.33	32.00±5.29	35.00±2.89	29.33±2.91	38.67±4.49^{b}	11.00±0.58^{b}
sulphur	79.67±2.60^{b}	15.00±1.73^{b}	131.00±6.08	160.30±8.95	30.67±5.21^{b}	90.33±2.60^{b}	72.33±4.49^{b}	29.67±4.33^{b}

Table 2. Concentration (ng/g wet weight) of pesticide residues in the kidney tissue of adult male and female *Taphozous perforatus* collected from the Saqqara area during the year 2013. See Table 1 for explanations

Tab. 2. Koncentrace (ng/g mokré hmotnosti) residuí pesticidů v tkáních ledvin dospělých samců a samic hrobkovce egyptského (*Taphozous perforatus*) odchycených v oblasti Sakkary během roku 2013. Vysvětlivky viz tab. 1

season / doba	winter / zima		spring / jaro		summer / léto		autumn / podzim	
pohlaví / sex	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
o,p'-DDT	14.33±1.86	11.33±0.88	13.67±1.20^{a}	26.00±3.06^{a}	14.33±2.85	14.00±2.08	13.00±0.58	12.00±0.58
p,p'-DDD	10.67±0.33	12.00±0.58	13.33±1.45	11.00±0.58	12.00±0.58	14.00±1.16	14.00±1.16	12.00±1.00
p,p'-DDE	74.67±6.23	93.00±8.89	11.00±0.58^{b}	175.70±12.73^{b}	169.00±6.66^{b}	124.00±3.46^{b}	97.33±9.33	117.00±9.61
PCB 118	12.00±0.58^{b}	24.33±2.33^{b}	20.33±1.45^{a}	14.00±1.16^{a}	13.33±0.88	11.00±0.58	12.33±1.45	12.00±1.16
PCB 138	12.00±1.53^{a}	23.00±2.52^{a}	21.33±0.88	23.67±2.19	19.33±1.20	23.67±2.19	20.33±0.33	21.00±3.79
PCB 180	13.33±1.20^{a}	23.67±2.19^{a}	23.00±2.52^{a}	14.33±0.67^{a}	12.00±1.53^{a}	23.67±2.19^{a}	20.33±0.33	23.00±2.52
HCB	11.67±0.88	13.00±1.16	11.67±0.33	12.67±1.76	12.33±0.67	12.00±1.00	12.00±0.58	11.67±0.88
dicofol	12.33±0.67	12.00±1.16	27.33±1.45	22.67±3.71	16.67±1.76	12.00±1.00	12.33±0.67	14.33±0.67
sulphur	19.67±3.18	13.33±2.40	13.00±0.58	15.67±0.88	17.33±3.71	13.33±1.76	13.67±0.88	14.67±0.88

lue. Similar trend was achieved for the compounds PCB 138 and dicofol, as well as sulphur. No significant differences were obtained for the other detected compounds (i.e., *o,p*'-DDT, PCB 118 and HCB). In the spring season, the female livers contained concentration levels of *o,p*'-DDT (25 ng/g), *p,p*'-DDE (256.7 ng/g) and PCB 138 (34.33 ng/g), which were significantly ($P<0.05$) higher than those found in the males. Concentrations of *p,p*'-DDD (25 ng/g) and PCB 180 (41.67 ng/g) were significantly ($P<0.01$) higher than those found in the males. The other measured contaminants (e.g., PCB 118, HCB, dicofol and sulphur) were found in both female and male bats but without any significant differences. In the summer season, the residue of *p,p*'-DDE (296.70 ng/g) in the male livers was found significantly ($P<0.05$) higher than that recorded for the female ones (224.0 ng/g). But the female livers contained residues of PCB 118 (17.67 ng/g), PCB 138 (32.67 ng/g), PCB 180 (21 ng/g) and sulphur (90.33 ng/g) much higher ($P<0.01$) than the male ones. No statistical differences were observed for the other estimated compounds (i.e., *o,p*'-DDT, *p,p*'-DDD, HCB and dicofol). In the autumn (fall) season, the residues of the analyzed contaminants in male livers were generally higher than those in female ones. The differences were significant at $P<0.05$ in the case of *o,p*'-DDT, *p,p*'-DDD and PCB 138, but highly significant ($P<0.01$) for residues of *p,p*'-DDE, PCB 180, dicofol and sulphur. On the other hand, the residues of PCB 118 and HCB were found in both sexes without significant differences (Table 1).

Concentration of pesticide residues in kidney with respect to bat's sex is presented in Table 2. In the winter season, the kidneys of female *T. perforatus* contained 24.33 ng/g of PCB 118, a value which was significantly higher ($P<0.01$) than that found in the males (12 ng/g). Similar trend, but with statistical difference at $P<0.05$, was obtained for the compounds PCB 138 and PCB 180 (23.00 and 23.67 ng/g, respectively). No significant differences were obtained for the other detected compounds (i.e., *o,p*'-DDT, *p,p*'-DDD, *p,p*'-DDE, HCB, dicofol) and sulphur. In the spring season, the female kidneys contained *o,p*'-DDT residue levels of 26 ng/g, compared with 13.67 ng/g for the males, and 175.7 ng/g tissue of *p,p*'-DDE compared with 11 ng/g tissue for the males; achieving significant differences at $P<0.05$ and $P<0.01$, respectively. An opposite trend was obtained for PCB 118 and PCB 180 where concentration values of these compounds were significantly higher in the males ($P<0.05$). The other measured contaminants (i.e., *p,p*'-DDD, PCB 138, HCB, dicofol and sulphur) were found in both female and male bats but without any significant differences. In the summer season, the residue of *p,p*'-DDE in male kidneys (169 ng/g) was found significantly ($P<0.01$) higher than that recorded for female ones (124 ng/g tissue). But the female kidneys contained 23.67 ng/g from PCB 180, compared with 12 ng/g for the male kidneys; a difference statistically significant at $P<0.05$ level. No statistical differences were observed for values of the other estimated compounds (i.e., *o,p*'-DDT, *p,p*'-DDD, PCB 118, PCB 138, HCB, dicofol and sulphur). In the autumn (fall) season, the analyzed kidney specimens showed to contain different residue levels of the analyzed contaminants, but without any significant differences with respect to the sex of the studied bat species (Table 2).

Inter-organic differences

Liver

The compound o,p-DDT showed its highest concentration in female bats collected during spring (25 ng/g tissue). Similar results were found in the case of the metabolite p,p-DDD (25 ng/g). These values were significantly ($P\leq0.05$) higher than those recorded for male animals (14.30 &

10.70 ng/g, respectively). Concentration of the metabolite p,p-DDE showed the lowest value in autumn female samples (95.3 ng/g); there was a highly significant difference ($P \leq 0.05$) from the samples from the other seasons. Except the summer female samples, the concentration of PCB 118 exhibited insignificant values for the female and male samples collected in the other seasons.

Concentrations of PCB 138 showed no sex differences attributed to bats of winter (female), spring (male), summer (male) and autumn (female). Similar trend was obtained for the compound PCB 180. Whatever the sex or season, all the samples analyzed for the pesticide HCB contained insignificantly different values (ca. 11–12 ng/g). It seemed that the pesticide Dicofol was affected by sex and season, its concentration in winter male samples showed the highest value (53.70 ng/g). There was an obvious interaction between the concentration of sulphur in liver samples with respect to the bat sex and the season of sampling.

Kidney

The compound o,p-DDT showed its highest concentration in female bats collected during spring (26 ng/g tissue). The estimated concentrations of this metabolite in the other analyzed kidney tissues were significantly ($P \leq 0.05$) lower than the above mentioned value. Residues of the metabolite p,p-DDD seemed not to be affected by sex and season. Its concentrations ranged between ca. 11.00 and 14.00 ng/g without significant differences. Concentration of the metabolite p,p-DDE showed the lowest value in spring male samples (11 ng/g); there was a highly significant difference ($P \leq 0.05$) from the samples from the other seasons. Except the winter female and spring male samples, the concentration of PCB 118 exhibited insignificant values for the female and male samples collected in the other seasons.

Concentration of PCB 138 in winter male samples was 12 ng/g; a value which was significantly ($P \leq 0.05$) lower than the estimated values for the other specimens. Over the different sampling seasons, the residues of PCB 180 recorded lower concentration in male samples compared with the female ones at $P \leq 0.05$. Whatever the sex or season, all the samples analyzed for the pesticide HCB contained insignificantly different values (ca. 12–13 ng/g). The spring male and female specimens showed higher dicofol residues than the other analyzed samples. There was an obvious interaction between the concentration of sulphur in kidney samples with respect to the bat sex and the season of sampling. Except the winter and summer males, the concentration of sulphur showed insignificant values for the female and male samples collected in the other seasons.

Relation between pesticide residues in liver and kidney: The Pearson rank correlation coefficient (r) for the mean values of the analyzed compounds, either in liver or kidneys (without sex or seasonal differentiation) was performed. The obtained data are presented in Table 3 in terms of r and P values for each pair of the data set at 95% confidence intervals. The statistical analyses considered three routes of associations:

(i) *Correlation liver vs kidney concerning the same compound*. The correlation between liver and kidney with respect to the compound *o,p*'-DDT was positive and strong (r=0.983), as well as highly significant at $P<0.01$ level. Strong negative correlations were obtained for *p,p*'-DDD ($r=-0.865$; $P=0.067$), and PCB 118 ($r=-0.749$; $P=0.126$). Correlation coefficient for HCB was very weak ($r=0.035$). The other tested contaminants showed intermediate values and there was a negative correlation in most of them.

Table 3. Pearson correlation analysis (*r*) between pesticide residues in liver *vs* kidney tissues in *Taphozous perforatus* adults collected from the Saqqara area throughout the year 2013 (without sex differentiation). Pearson rank correlation coefficients (*r*) for each pair of the data set at 95% confidence intervals. Number of pairs = 4; each data set of a compound was represented by mean values of the four seasons after combining male and female data (refer to Tables 1 & 2), then subjected to correlation analysis. *P* values marked with (*) mean a significant correlation for the two compared values at $P<0.05$, and those marked with (**) mean a highly significant correlation at $P<0.01$
Tab. 3. Pearsonova korelační analysa (*r*) koncentrací residuí v tkáních jater *versus* ledvin u dospělých hrobkovců egyptských (*Taphozous perforatus*) sebraných v oblasti Sakkary během roku 2013 (bez rozlišení pohlaví). Koeficienty Pearsonovy korelace (*r*) pro každý pár hodnot při 95% konfidenčním intervalu. Počet párů = 4; každá sada analysovaných proměnných obsahuje průměrné hodnoty pro čtyři roční doby ze zkombinovaných samčích a samičích údajů (viz tab. 1 a 2). Hodnoty *P* označené (*) představují významnou korelaci dvou porovnávaných hodnot při $P<0.05$ a hodnoty označené (**) vysoce významnou korelaci při $P<0.01$

code, compound kód, složka	correlation korelace (*r*)	*P*	difference rozdíl liver / játra	correlation korelace (*r*)	*P*	difference rozdíl kidney / ledvina	correlation korelace (*r*)	*P*
1 *o,p*'-DDT	0.98	**0.01	1 *vs* 8	–0.41	0.30	1 *vs* 8	0.99	**0.04
2 *p,p*'-DDD	–0.87	0.07	2 *vs* 8	–0.29	0.35	2 *vs* 8	–0.05	0.48
3 *p,p*'-DDE	0.14	0.43	3 *vs* 8	0.72	0.14	3 *vs* 8	–0.20	0.40
4 PCB 118	–0.75	0.13	1 *vs* 2	0.63	0.18	1 *vs* 2	–0.05	0.48
5 PCB 138	–0.54	0.23	1 *vs* 3	0.18	0.41	1 *vs* 3	–0.12	0.44
6 PCB 180	–0.40	0.30	2 *vs* 3	0.43	0.29	2 *vs* 3	0.79	0.11
7 HCB	0.04	0.48	4 *vs* 5	–0.90	*0.05	4 *vs* 5	–0.88	0.06
8 dicofol	–0.39	0.31	4 *vs* 6	–0.96	*0.02	4 *vs* 6	–0.31	0.35
9 sulphur / síra	–0.48	0.26	5 *vs* 6	0.76	0.12	5 *vs* 6	0.01	0.50

(ii) *Correlation between different compounds in the liver* (N.B.: the name of compounds could be depicted from the code given in Table 3). A significant negative correlation ($P<0.05$) was obtained for PCB 118 *vs* PCB 138 ($r=-0.904$) and PCB 118 *vs* PCB 180 ($r=-0.957$). Strong positive correlations were obtained for *p,p*'-DDE *vs* dicofol ($r=0.723$), PCB 138 *vs* PCB 180 ($r=0.756$), and *o,p*'-DDT *vs* p,p'-DDD ($r=0.632$). The other tested contaminants showed intermediate values and there was a positive correlation in most of them.

(iii) *Correlation between different compounds in the kidneys* (N.B.: the name of compounds could be depicted from the code given in Table 3). A high significant correlation ($P<0.01$) was obtained for *o,p*'-DDT *vs* dicofol ($r=0.992$; $P=0.0039$). Strong correlations were obtained for PCB 118 *vs* PCB 138 ($r=-0.875$) and *p,p*'-DDD *vs* *p,p*'-DDE ($r=0.790$). Very weak correlations were obtained for PCB 138 *vs* PCB 180 ($r=0.005$), *o,p*'-DDT *vs* *p,p*'-DDD ($r=-0.046$), and *p,p*'-DDD *vs* dicofol ($r=-0.051$).

Generally, results of correlation analysis between the tested compounds in liver seemed to be more pronounced than in kidney (Table 3).

Table 4. Total pesticide residues in the liver and kidney tissues from *Taphozous perforatus* in different seasons (unsexed). Data refer to Tables 1 & 2

Tab. 4. Úhrn residuí pesticidů v játrech a ledvinách hrobkovců egyptských (*Taphozous perforatus*) v různých ročních dobách (bez ohledu na pohlaví). Údaje se vztahují k tab. 1 & 2; mean±SE = průměr ± standardní chyba

season / doba	winter / zima		spring / jaro		summer / léto		autumn / podzim		mean±SE		total
residues / residua	liver játra	kidney ledvina	liver játra	kidney ledvina	liver játra	kidney ledvina	liver játra	kidney ledvina	liver játra	kidney ledvina	úhrnem
o,p-DDT	12.83	12.33	19.70	19.83	13.20	14.16	12.81	12.50	14.64±1.69	14.71±1.76	29.35
p,p-DDD	12.81	11.35	12.83	12.16	12.67	13.00	12.67	13.00	12.75 ± 0.04	12.38±0.40	25.13
p,p-DDE	250.85	83.33	232.85	93.35	260.35	146.50	134.10	107.16	219.50±29.04	107.60±13.86	327.10
PCB 118	11.17	18.16	11.83	13.16	14.83	12.17	16.50	12.16	13.58±1.26	13.91±1.44	27.49
PCB 138	27.83	17.50	23.00	22.50	22.17	21.50	16.50	20.67	22.38±2.32	20.54±1.08	42.92
PCB 180	21.50	18.50	21.50	18.65	16.00	17.83	16.00	21.67	18.75±1.59	19.16±0.85	37.91
HCB	11.33	12.33	11.00	12.17	11.83	12.17	11.33	11.83	11.37±0.17	12.13±0.11	23.50
dicofol	33.50	12.16	26.33	25.00	42.16	14.33	24.83	13.33	31.71±3.97	16.21±2.97	37.92
sulphur / síra	47.33	16.50	145.65	14.33	60.50	15.16	51.00	14.17	76.12±23.34	15.04±0.53	91.16

DISCUSSION

During the period of the 1960s and 1970s when the use of organochlorine pesticides was widespread, evidence of bats being affected by these pesticides was discovered. Field and laboratory studies carried out in North America and Europe pointed out to some of these pesticides as responsible for the mortality of several bat species (Jefferies 1972, Gelusco et al. 1976). In Egypt, OCPs were used as early as 1950 up to 1980 when both DDT and lindane were officially prohibited in agricultural use, followed by banning of other OCPs (El-Sebae & Soliman 1982). PCBs have been used in a wide variety of manufacturing processes in Egypt, especially as plasticizers and insulators, and are widely distributed in the environment (Mansour 2009). They were banned worldwide in the 1990s because of their high toxicity (Li 1999). Until the present investigation, no data regarding the effect of pesticides on bats in Egypt were available.

In the present study, we found some DDT metabolites, some PCB congeners, HCB, dicofol and sulphur in liver and kidney tissues, both in male or female *T. perforatus* (Tables 1, 2). Concentrations of these pollutants showed variations between sexes and sampling seasons. Such variations have been long attributed to the foraging activity of both sexes with respect to seasonal reproductive cycles, as well as annual periods of relative inactivity in winter (hibernation or dry season torpor) (Kunz et al. 1996).

The literature offers much information on the occurrence of OCP compounds in different bat species from a vast variety of locations other than Egypt. However, our results may be compared with others to evaluate the present status of POPs with respect to what we have biomonitored in the employed bat species. To do this, we have to take into consideration that extremely high heterogeneity in the published data prevents statistical analysis and thus comparison could be based only on general variability and trends (Bayat et al. 2014). To facilitate comparisons, the total pesticide residues in liver and kidney as well as the total in both organs, without sex differentiation, are computed in Table 4.

Carcasses of two bat species caught at four ecologically diverse locations in Spain were found to contain heptachlor epoxide, dieldrin, some DDT metabolites, some PCB congeners, HCH isomers and dichlorobenzophenone. Concentrations of *p*,*p*'-DDE, *p*,*p*'-DDT and PCBs ranged between 0.65–8.33, <0.01–0.11, and 0.42–1.05 µg/g wet weight in carcasses, respectively (Hernandez et al. 1993). If we compute levels of DDTs and PCBs in the liver of *T. perforatus* (Table 4), it will reach 0.25 µg/g for DDTs and 0.055 µg/g for PCBs. Similarly, the kidneys (Table 4) will contain 0.14 µg/g for DDTs, and 0.054 µg/g for PCBs on wet weight tissue. Therefore, liver and kidneys contained 0.39 µg/g wet weights of DDTs and 0.11 µ/g wet weights of PCBs. Of course, concentration levels of DDTs and PCBs in our study were lower than those found by Hernandez et al. (1993), but we have to note that carcasses include other organs besides liver and kidneys. However, our findings coincide with the results of the above mentioned investigators regarding to the superiority of *p*,*p*'-DDE concentration levels as compared with the other detected DDTs levels, either in liver or in kidneys (Table 4). Similarly, in six bat species collected from the desert parts of North America, DDE concentration (4.3 ppm median wet weight) predominated the concentration of DDT (0.2 ppm median wet weight); according to Reidinger (1976). In the same context, Streit et al. (1995) reported *p*,*p*'-DDE concentration between 4.2 and 18.0 µg/g lipid; PCB 180 1.7–18.6 µg/g, and PCB 138 4.2–22.0 µg/g in five bat species. Also, Kannan et al. (2010) reported significant concentrations in fat tissues of males and females of *Myotis lucifugus* (e.g., DDT: 4.0–5.3 ppm; chlordanes: 0.35–1.27 ppm; and hexachlorobenzene (HCB): 0.07–0.12 ppm). Having reviewed the literature data, Bayat et al. (2014) concluded that a considerable decline in PCB concentrations seemed evident between the periods 1970–1980 and 1981–1999, and 2000–2013; again demonstrating the continuing exposure to these persistent compounds worldwide.

In the present study, hexachlorobenzene (HCB) was found in very low concentrations (e.g. 0.011 µg/g in liver; and or 0.012 µg/g wet weight in kidneys; Table 4); giving rise to a sum of 0.023 µg/g wet weight of both organs. Such value could be compared with other reported values in different bat species, 0.10–0.49 µg/g lipid in five bat species (Streit et al. 1995); 0.0001 in whole body of *Pteropus marianus* (Senthilkumar et al. 2001); 0.01 in liver of *Eptesicus serotinus* (Luftl et al. 2005); 0.005 and 0.004 in carcasses of *Miniopterus* cf. *bassanii*, respectively (Allinson et al. 2006); and 0.035 in fat tissue-lipid weight in *Myotis lucifugus* (Kannan et al. 2010). Such variations may refer to many factors including bat species, analyzed part of body, location and timing of sample collections, and so on.

In addition to DDTs and PCBs compounds analyzed in the present study, we found dicofol and sulphur in the bat tissues. Dicofol is an OCP that is very effective against mites such as the red spider mite. This pesticide was used in Egypt several years after the ban on DDT. Dicofol is structurally similar to DDT and it differs from DDT by the replacement of the hydrogen (H) on C-1 by a hydroxyl (OH) functional group. Dicofol is usually synthesized from technical DDT. During the synthesis, DDT is first chlorinated to an intermediate, Cl-DDT, followed by hydrolyzing to dicofol. At the end of the synthesis reaction, DDT and Cl-DDT may remain in the dicofol product as impurities. It was reported that dicofol is considered as one of DDTs releasing source in the environment (Yang et al. 2008). The latter investigators reported that application of dicofol had resulted in serious DDT pollution in cotton fields in China. It was shown that dicofol-type DDT accounted for up to 80% of the DDTs residues. The authors added that their work provided implications for reasons why there was no apparent decrease of DDT level in China (Yang et al. 2008). In Turkey, different dicofol formulations were analyzed for DDT and DDT-related compounds by Turgut et al. (2009). They found total DDT content between 0.3% and 14.3% in the formulated dicofol. Concentrations of 167–1,042 mg/kg; *p,p*'-DDE, 32–183 mg/kg; *p,p*'-DDT, and 2–34 mg/kg; *o,p*'-DDT were found in the analyzed samples of dicofol formulations.Sulphur has been extensively used in Egypt for many decades. It is used in mite and fungi control in field crops, soil improvement and fertilizers. Therefore, the detection of dicofol and sulphur in the analyzed bat tissues seems to be explicable. However, the extensive survey conducted by Bayat et al. (2014) on OCPs and PCBs in bats did not refer to dicofol presence in any publications reviewed.

Also, our findings reveal the association between liver and kidney in the studied bat species with respect to OCPs and PCBs in these organs (Table 3). The strong positive relationship between a pollutant in liver and kidney or between different pollutants in the same organ suggests that individuals exposed to high levels of this pollutant are likewise to accumulate it in high levels in both of the two concerned organs. It seems likely that transfer rates (and possibly pathways) differ not only between organ types but also between toxicants (Walker et al. 2007).

According to Bayat et al. (2014), the OCP concentrations reported in bat tissues have declined significantly since the late 1970s, presumably as a result of restrictions in use. The DDT metabolite, DDE was reported most often to be present at highest concentrations in the tissues analyzed. For example, mean concentrations of DDE over time periods 1970–1980, 1981–1999 and 2000–2013 ranged between 2.6–6.2, 0.05–2.31, 0.08–0.19 ppm wet weight, respectively. Exposure, however, still occurs from the remaining residues, many years after the use of the compounds was banned. On the other hand, the predominance of DDE in the DDT compounds is frequently reported in organism samples and is considered to indicate DDT conversion in tissues and also preferential DDE storage (Kelce et al. 1995). The DDE/ ∑DDT ratio can be used as an index to understand DDT inputs to the environment (Borrell & Aguilar 1987). The latter authors suggested that higher values of this index in recent years can be an indicator of no new inputs of this pesticide to the environment. Bayat et al. (2014) calculated the DDE/ ∑DDT ratio from the studies on bats they reviewed using mean wet weight values. The mean ratio was 0.76 over 1970–1990, while it increased to 0.96 over 1991–2006. This is an obvious increase supporting the significant decline in new DDT inputs to the environment over these time periods. As mentioned above, our estimations revealed occurrence of 0.39 µg/g wet weight of DDTs in liver and kidneys, of which DDE will equal 0.33 µg/g wet weight; giving rise to DDE/ ∑DDT ratio of 0.82, suggesting an indication to pronounced decline in new DDT inputs to the environment in light of results of the present investigation. A ratio of 0.97 was estimated by Bayat et al. (2014) based on OC residues reported by Allinson et al. (2006) in carcasses of *Miniopterus* cf. *bassanii* in southeastern Australia. In a recent study, Stechert et al. (2014) determined different OC pesticides in aerial hawking molossid bats (*Chaerephon pumilus* and *Mops condylurus*) foraging on cotton fields in northern Benin, West Africa. The mean concentrations of *p*,*p*'-DDE and ΣDDT were 377 and 842 µg/kg wet weight, respectively; giving rise to a *p*,*p*'-DDE/ ΣDDT ratio of 0.44. The authors suggested that DDT had probably been recently used in the study region, and larger scale use would pose an increased risk for bat populations due to the high biomagnification of DDT.

As mentioned above, the DDE/ ∑DDT ratio can be used as an index to understand DDT inputs to the environment (Borrell & Aguilar 1987). In addition to the pesticide use pressure in a surveyed location, there are, however, many other factors which have to be considered when estimating such index (e.g., the biomonitored bat organism with respect to species, age, sex, nutritional status and food source, season and location of sampling, population size, analyzed organs, and so on). It is worth mentioning that the dominance of DDE over the other DDT metabolites had been reported in all samples analyzed in different bat species from several countries.

Nevertheless, PCB levels were found lower than those of DDE and residues of both pollutants in different bat species were in general very far from the reported lethal levels (Clark & Stafford 1981). In this respect, it may be useful to mention that *p*,*p*'-DDE has little ability to bind the oestrogen receptor, but inhibits androgen binding to the androgen receptor, thus it is considered as a potent androgen receptor antagonist (Kelce et al. 1995). PCBs may reduce metabolic rate in bats at high environmental levels of these compounds (Clark & Stafford 1981).

The literature offers much information about many features of bat life history and biology that make these organisms perfect species for monitoring of environmental contaminants including pesticides and heavy metals. Therefore, bats have been extensively used as ecological indicators of habitat quality (Park 2015). Bats, similarly as human beings, may be exposed to pesticide residues through three main pathways including direct ingestion, inhalation through mouth and nose, and dermal absorption through skin exposures. Variability in the levels of pollutants found in bat bodies is certainly influenced by their background environmental levels, which reflect the amounts accumulated. It is well documented that dietary accumulation and metabolic capacity increases in organisms at higher positions in the food chain, and thus insectivorous bats, such as *T. perforatus*, are likely to be excellent indicators for monitoring and assessment of environmental contaminants and disturbances. As mentioned above, irrigation and drainage canals in the El-Mariotteya region were reported to contain residues of OC pesticides and PCBs (El-Kabbany et al. 2000, Eissa et al. 2013, Mahmoud et al. 2014, Megahed et al. 2015). So, the water bodies associated with the El-Mariotteya stream, which is considered the nearest foraging place to the bats of the Saqqara caves, are likely to act as a probable source of pesticide contamination to the studied bats.

CONCLUSIONS

This study is the first evaluation of toxic OC and PCB concentrations in bats in Egypt. Our results suggest that the accumulation of these POPs in individuals is confirmed by detection and quantification of some DDT metabolites, some PCB congeners, HCB and dicofol, as well as sulphur in liver and kidneys of *T. perforatus*. The time-trend of POPs in Egypt, represented by DDT metabolites and PCB congeners, revealed that these pollutants are still detectable in the environment, however in low concentration levels and far from lethal toxicity. The DDT metabolite (*p*,*p*'-DDE) predominated concentrations of the other detected compounds. The dominance of *p*,*p*'-DDE metabolite with DDE/ ∑DDT ratio of 0.82 is indicative of a pronounced decline in new DDT inputs to the environment. One of the major influx of DDT and other OCs may refer to inputs from other Nile-Basin African Countries (NBACs) which still use DDT and other OC pesticides (Mansour 2009). The problem is that wild bats are frequently exposed to multiple anthropogenic stressors at the same time, which may show both antagonistic or, more frequently, combined or synergic effects. Such stressors may include natural toxins, anthropogenic pollutants such as pesticides, heavy metals, and infectious agents; however the combined effects of such stressors remain practically unexplored (Bayat et al. 2014). The present study shed light on feasibility of the use of bats as bioindicators of environmental pollutants. So, continued monitoring of the long-term trends in POP accumulation and associated health status in bats, together with exploration of the extent to which contamination may be greater in individuals from different regions, is merited. The findings may also encourage the use of other bat species from urban and rural regions, as well as agricultural and industrial locations. On the other hand, the study urges the need of education programmes for the public and school children about bat conservation to be established, and the laws on bat protection to be enacted and enforced.

SOUHRN

Organochlorinové pesticidy (OCP) a polychlorované bifenyly (PCB) jsou skupinou persistentních organických škodlivin (POP), které vykazují dlouhodobou toxicitu, tendenci kontaminovat životní prostředí a vstupovat do potravního řetězce. Tato studie se pokusila zhodnotit možnost využití netopýrů jako bioindikatorů napomáhajících porozumění časovým trendům POP v současné době. Z tohoto důvodu byly tkáně jater a ledvin hrobkovce egyptského (*Taphozous perforatus*), sebrané v oblasti Sakkary v severním Egyptě, podrobeny extrakci QuEChERS (rychlá, jednoduchá, levná, účinná, silná a bezpečná) a po té analyse LC-MS/MS. V těchto tkáních byly nalezeny různé koncentrace metabolitů DDT (dichlor-difenyl trichlorethan; např. *o*,*p*'-DDT, *p*,*p*'-DDD, *p*,*p*'-DDE), některých polychlorovaných bifenylů (např. PCB 118, PCB 138, PCB 180), hexachlrobenzenu (HCB), dicofolu a síry. Úrovně těchto koncentrací byly ovlivněny pohlavím netopýra a roční dobou sběru. V játrech a ledvinách bylo nalezeno 0.39 µg/g mokré hmotnosti DDT a 0.11 µg/g mokré hmotnosti PCB. Úrovně koncentrací dichlor-difenyl ethanu (*p*,*p*'--DDE) převyšovaly koncentrace ostatních metabolitů DDT; poměr DDE ke všem metabolitům DDT byl 0.82, což se ukázalo být indikací nového užívání DDT a jeho trvajícího dopadu na životní prostředí. Stejně tak úroveň koncentrace PCB 138 převyšovala koncentrace ostatních PCB. Byla nalezena korelace mezi koncentracemi OCP a PCB v jádtrech a ledvinách. Na základě výsledků naší analysy jsme konstatovali, že tyto škodliviny jsou stále zaznamenatelné v životním prostředí, avšak v nízkých koncentracích a zcela

mimo úroveň smrtelné toxicity. Tyto údaje však ukazují na použitelnost také dalších druhů netopýrů jako bioindikátorů v urbánních a venkovských oblastech, včetně zemědělských a průmyslových center.

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