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Environmental and biological monitoring of persistent organic pollutants in waterbirds by non-invasive versus invasive sampling

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HIGHLIGHTS

- POP concentrations represent a decreasing trend over time.
- Venous blood represents promising biomonitor for internal PCB concentrations.
- Enzyme activities correlate with the liver concentrations of several OCPs.
- Egg DDE levels are below the threshold for the risk of hatch and reproductive success.

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ABSTRACT

Three main groups of persistent organic pollutants (POPs); namely organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) were quantified in water and sediment samples, as well as in various invasive and non-invasive samples from waterbirds in the Büyük Menderes River (BMR). Liver and muscle tissues, blood, and preen gland oil samples of yellow-legged gull (*Larus michahellis*) and Euroasian coot (*Fulica atra*) were collected both from the origin (Işıklı Lake) and the estuary (Söke) of the river, blood and preen gland oil samples of grey heron (*Ardea cinerea*) and pelican (*Pelecanus crispus*) were collected from the estuary only. In addition, non-hatched eggs from several above species and Mediterranean gull (*Larus melanocephalus*), in either station were collected. In all samples, POP contamination was measured and the potential usefulness of those invasive and non-invasive sampling for biomonitoring was evaluated. Activities of antioxidant enzymes were measured as potential indicators of POP exposure and of changes in the cellular defence. Venous blood proved to be a promising biomonitor for the concentrations in liver and muscle, especially for PCBs. Activities of antioxidant enzymes were correlated with the liver concentrations of several OCP congeners. The measured egg DDE concentrations were below the established threshold concentrations for the risk of hatch and reproductive success.

1. Introduction

Environmental and biological monitoring represent the first and the most important step of ecological, as well as human risk assessments for the environmental pollutants that pose a health risk to humans and ecological species. Waterbirds have been proved suitable for monitoring environmental pollution because they are long-lived and highly mobile, thus they integrate pollutants over a broad area (Furness, 1993), and they are at the top of the food chain (Fossi et al., 1999). There have been studies to establish POP levels in birds by both invasive and non-invasive ways (Albanis et al., 2003; Chen et al., 2009; Custer et al., 2010a,b; Goutner et al., 2001, 2011; Rajaei et al., 2011; Colabuono et al., 2012).

In addition to represent a source of human exposure via edible seafood (fish, shellfish, waterbirds etc.), environmental pollution threats also the existence of those species, especially the ones under the risk of extinction. In this sense, ecological species can be utilized in several ways; estimating the dimensions of the pollution in a certain area, establishing the levels for human exposure, and analysing the adverse effects to the species itself due to internal dose of the pollutants. The ideal approach for such purposes would be measuring the concentration of the pollutant in the respective tissue of the species; in the edible parts for estimating human exposure, in the best representing tissue for environmental concentrations, and in the target tissue for determining adverse effects of the pollutant. In most cases, the life of ecological species should

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be terminated for those purposes. However, this is undesirable both from ethical and ecological perspectives. As to the ecological perspective, several species are facing with the risk of extinction. Although various aquatic species are already consumed as food by humans, using them for the purpose of monitoring the environmental pollution brings an additional risk for their existence. These facts prompted researchers in the area of life sciences to seek alternatives to those destructive or invasive approaches. One promising non-invasive approach is utilizing preen oil from bird species (Johnston, 1976; Van den Brink, 1997; Yamashita et al., 2007). Preen oil is secreted from uropygial gland located at the base of the tail feathers. This gland produces lipids which may protect the feathers from wear, aid in waterproofing and protect against dermatophytes (Soini et al., 2007). It is especially relevant for the pollutants to be accumulated which are lipophilic such as OCPs, PCBs, and PBDEs. Yamashita and co-workers collected preen gland oil samples from 30 seabirds at North Pacific Ocean and determined PCB concentrations (Yamashita et al., 2007). They established a weak but significant correlation between the total PCB levels in the preen gland oil and adipose tissue, which was improved when metabolic losses were taken into account. They proposed that the oil values can be used to estimate the abdominal adipose tissue burden (internal dose) of seabirds within one order of magnitude (Yamashita et al., 2007). On the other hand, correlations between non-invasive samples and various tissues should be evaluated for the purposes above; whether the non-invasive approach is relevant to reflect the chemical concentration in the tissue of interest. From this point of view, correlation to muscle tissue for estimating human exposure, and correlations to liver tissue for evaluating toxicity to the ecological species should be analysed. This is what we considered in the present study. The other alternative samplings are focusing on blood (Finkelstein et al., 2006), droppings (Sun et al., 2006), feathers (Jaspers et al., 2006), and non-hatched eggs (Goutner et al., 2001; Albanis et al., 2003; Antoniadou et al., 2007; Wang et al., 2011). As Yamashita et al. (2007) commented, blood sampling requires trained technical skill and dropping sampling requires cooling during preservation and transportation which is a potential problem in remote areas. Collecting feathers is a non-invasive approach; however, it may not accurately reflect the body burden of the pollutant as they are exposed to physical environment and are open to outer effects.

Since a large proportion of OC compounds are known to biomagnify in the egg yolk (Kleinow et al., 1999), contaminant levels in waterbird eggs serve as an important tool for monitoring changes in the environmental quality. In addition, collection of eggs is a relatively non-invasive technique that has minimal adverse effects on the bird community (Connell et al., 2003). Hence, collecting non-hatched eggs prevents the destructive effect on next generations. To the best of our knowledge, contaminant concentrations in both preen gland oil and non-hatched eggs have not been reported for the same waterbird species yet.

Toxic effects of OCPs, PCBs and PBDEs in aquatic life forms include endocrine disruption (such as reduction of testosterone levels in the blood) (Tanabe, 2002; Brouwer et al., 1989; Reijnders, 1990; Boon et al., 1992), thinning the eggshell (causes death of embryo before hatch) (Ratcliffe, 1967; Hickey and Anderson, 1968) and induction of oxidative stress (Bainy et al., 1993; Padmini and Vijaya Geetha, 2007; Glauert et al., 2008; Gurer-Orhan et al., 2006). Therefore, OCPs have played an important role in the decline of waterbird populations (Fox et al., 1991). Measuring the activity of antioxidant defence enzymes such as SOD, CAT, GPx and GST is utilized as a marker of POP pollution (Regoli et al., 2002; Ozmen et al., 2008; Richardson et al., 2008), alteration in the activity was proposed as early signals of environmental disturbance (Santos et al., 2004). Oxidative stress induced by OCPs was reported in rat (Bainy et al., 1993) and in *Mugil cephalus* (Padmini and Vijaya Geetha, 2007). As a consequence of oxidative stress, peroxidation of membrane lipids may result in degradation of membrane integrity and subsequently cell damage. POPs have been shown to induce membrane lipid peroxidation in CHO cell culture *in vitro* (Gurer-Orhan et al., 2006).

In the present study, we aimed at evaluating the potential applicability of both preen gland oil and blood, as well as egg samples of waterbird species in estimating the internal dose of the POPs; liver and muscle concentrations. Beside, any possible correlation between POP concentrations of liver and muscle to non-invasive samples was also evaluated. In order to achieve this, the destructive liver and muscle tissues were obtained from Euroasian coot and yellow-legged gull, which are not under the risk of extinction. From grey heron and pelican, however, only non-destructive preen gland oil and blood samples, as well as non-hatched egg samples were collected. Egg samples were also collected from Euroasian coot and Mediterranean gull. Such a monitoring study for OCPs, PCBs and PBDEs in both blood and preen gland oil of endangered species such as grey heron and pelican, and in liver, muscle, egg, blood and preen gland oil samples of Euroasian coot and yellow-legged gull has been conducted for the first time. In addition, the present study enabled us to compare any possible difference between the origin and the estuary of BMR, and also between various biosamples of different species in terms of POP pollution, as the latter was reported for concentrations in egg samples from different species (Dong et al., 2004; Luo et al., 2009). Antioxidant enzyme activities were evaluated as potential early sign of chemically-induced oxidative stress.

2. Material and methods

2.1. Chemicals

Acetone pestanal and *n*-hexane pestanal were purchased from Fluka (USA). Dichloromethane (DCM) suprasolv and silica gel 60 were obtained from Merck (Germany). Organochlorine pesticide mix 3, PCB mix, PCB103 and PCB198 were purchased from Dr. Ehrenstorfer (USA). PBDE mix was from Wellington (USA). All other reagents were of analytical grade.

2.2. Sampling

Biotic and abiotic samples were collected from two stations (Işıklı and Söke) in the BMR. Işıklı is the origin and there is relatively fewer agricultural activity around the lake compared to Söke station. Söke is the estuary of the river and one of the two most intensive agricultural plateaus in Turkey. Surface water samples were collected and filtered from 55 micron pore width plankton-filter into 2.5 L borosilicate bottles. After closing tightly, they were delivered to the laboratory and were kept at 4 °C until analysis. Surface sediment samples were collected into 1 L of borosilicate jar, closed tightly, and kept at 4 °C until analysis. After drying in the oven at 75 °C, sediment samples were sieved through vibrating stainless steel sieves with mesh size of 50 μm. Sampling period for both abiotic and biotic samples was between May and July 2009. Grey heron and pelican were chosen as they are the species under the risk of extinction. Euroasian coot and gull species (yellow-legged, and Mediterranean gull) were chosen as they integrate pollutants over a broad area of the river estuary, and represent an ideal biomonitor. The required official permissions from the Ministry of Environment for systematic sampling were provided prior the study. Upon catching the waterbird; blood, liver and muscle as invasive samples were obtained from yellow-legged gull and Euroasian coot, and only blood samples were obtained from grey heron and pelican due to the risk of extinction. Preen gland oil and non-hatched eggs as non-invasive samples were obtained from all