Science of the Total Environment 580 (2017) 26–33

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Oxidative stress responses in relationship to persistent organic pollutant levels in feathers and blood of two predatory bird species from Pakistan

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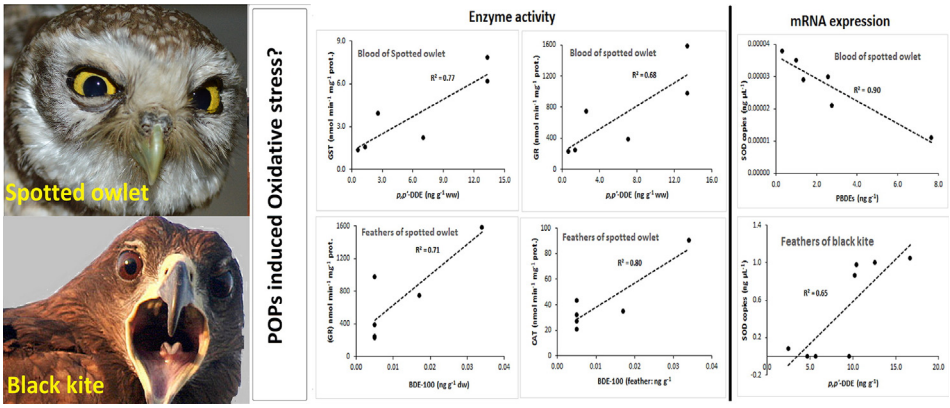
## HIGHLIGHTS

- First report to evaluate POPs induced oxidative stress response in predatory birds
- Various POPs are associated with oxidative stress response in predatory birds.
- Feather concentrations not always reflect the internal body burden of POPs
- Experimental studies are needed to evaluate the potential of POPs to induce oxidative stress.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 17 June 2016
Received in revised form 26 November 2016
Accepted 27 November 2016
Available online xxxx

Editor: D. Barcelo

*Keywords:*
Non-destructive
Black kite
Spotted owlet
Enzyme activity
Gene expression

## ABSTRACT

To date, knowledge of persistent organic pollutant (POP) mediated oxidative stress responses in avian species is rather limited. We therefore investigated whether exposure to polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) in two predatory bird species, namely black kite (*Milvus migrans*) and spotted owlet (*Athene brama*), was associated to activities of antioxidant enzymes, such as glutathione peroxidase (GPx), glutathione *S*-transferase (GST), glutathione reductase (GR) and catalase (CAT), or expression of GPx and superoxide dismutase (SOD) genes. As part of this investigation, we evaluated whether feathers were suitable to reflect internal body burdens and their associated oxidative stress effects. *p,p′*-DDE was unanimously recorded with highest concentrations in feathers and blood of both species. In general, the non-significant associations reflect that feathers are not always a suitable indicator for internal body burdens of POPs, depending on the feather type and the age of the bird. The activity of GST and GR was significantly higher in spotted owlet whereas GPx and CAT was higher (albeit not significant) in spotted owlet and black kite respectively. In comparison, mRNA expression of GPx, SOD and Cu,ZnSOD was significantly higher in black kite. Regression analysis showed that the activity of GST and GR was significantly associated with *p,p′*-DDE in blood of spotted owlet.

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http://dx.doi.org/10.1016/j.scitotenv.2016.11.197

Similarly, activity of CAT and GR was significantly correlated with BDE-100 in feathers of spotted owlet. In comparison, mRNA expression of SOD was found significantly associated with ∑PBDEs in blood of spotted owlet as well as *p,p′*-DDE in feathers of black kite. Significant associations of various POPs with biological responses may suggest that POP exposure may be contributing to oxidative stress in the studied bird of prey species. This first investigation indicates the necessity for further research on cause-effect relationships between POP exposures and changes in general health of free ranging birds.



## 1. Introduction

On a global scale, wildlife populations may be faced with a serious pollution threat due to high levels of persistent organic pollutants (POPs) in the environment (Letcher et al., 2010). After their release from industrial, urban and agricultural sources (Lohmann et al., 2007), POPs persist for a long period of time, disperse in the environment, and consequently bioaccumulate in biota (El-Shahawi et al., 2010). Birds, particularly the predatory species are particularly at risk due to their apex trophic position, as well as their long life span and expanded home ranges, which result in high exposure to POPs (Furness, 1993; Jaspers et al., 2007). Increasing concentrations of POPs in birds of prey have been associated with physiological, neurological, and/or reproductive malfunctioning (Connell et al., 2003), and in recent studies also induction of oxidative stress responses has been reported (Sletten et al., 2016; Hanssen et al., 2013; Nakayama et al., 2006; Kocagoz et al., 2014).

In living cells, production of pro-oxidant and reactive oxygen species (ROS) is balanced through antioxidant defense mechanisms (Espín et al., 2014a), including the production of low molecular weight proteins such as glutathione and metallothionine (MT), vitamins A and E, and antioxidant enzymes (Cheung et al., 2004). Glutathione (GSH) is one of the most important antioxidants because of its capability to directly bind with ROS (Kocagoz et al., 2014). The antioxidant enzyme glutathione peroxidase (GPx) oxidizes GSH to glutathione disulfide (GSSG), thus facilitate the conversion of ROS into less reactive species, while glutathione reductase (GR) metabolizes ROS by reducing GSSG back into GSH (Koivula and Eeva, 2010). Other antioxidant enzymes such as glutathione *S*-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) also catalyze and convert ROS to less reactive compounds (Gibson et al., 2014). However, POP exposure may cause an unnatural high imbalance between ROS and antioxidant enzymes occurs leading to oxidative stress conditions (Espín et al., 2014a, 2014b; Gibson et al., 2014; Koivula and Eeva, 2010).

Responses of antioxidant enzyme activities and related gene expressions have been established as biomarkers of exposure to POPs and other pollutants (Gibson et al., 2014; Quirós et al., 2007; Cheung et al., 2004; Zhang et al., 2004). It has been reported that responses of antioxidant enzymes and their associated mRNA expressions are usually greater for many aquatic and terrestrial organisms living in polluted environments, compared to reference sites (Adeogun et al., 2016; Cheung et al., 2001, 2004). Recently, the use of antioxidant enzymes as biomarkers of contaminant exposure has been extensively studied in invertebrates, fish and mammals (Adeogun et al., 2016; Valavanidis et al., 2006; Cheung et al., 2004; Zhang et al., 2004). In addition to enzyme activity analyses, mRNA expressions have also been successfully utilized as effective molecular biomarkers for contaminant exposure (Christiansen et al., 2014; Piña et al., 2007). In birds of prey, oxidative stress induced by metal exposure has been well studied and threshold levels have been suggested (Espín et al., 2014a, 2014b; Koivula and Eeva, 2010). On the other hand, POPs induced oxidative stress is still poorly understood in birds (Sletten et al., 2016; Kocagoz et al., 2014).

Because of their lipophilic nature, POPs preferably accumulate in lipid-rich tissues, such as adipose, muscle, liver and kidney, compared to lipid-deficient tissues, such as feathers and blood (Abbasi et al., 2016a; Voorspoels et al., 2006). Nevertheless, blood and feathers are valuable matrices in non-destructive toxicological investigations and can provide information about dietary and historical exposure that would otherwise be difficult to obtain (Eulaers et al., 2014a). Feathers are connected to the blood circulation during their growth and therefore receive compounds proportional to their blood concentration, before they are disconnected from the blood supply and become inert upon growth completion (García-Fernández et al., 2013). In birds of prey, a fully-grown feather has the potential to serve as a sentinel for exposure during feather growth (Jaspers et al., 2007) although external deposition of POPs on the feather surface can disturb the original relationship to internal concentrations (Jaspers et al., 2007, 2008). On the other hand, blood is considered the standard surrogate tissue for internal body burdens, although it only provides information about recent exposure (Eulaers et al., 2014a). Despite efforts investigating blood-feather relationships for POP concentrations in free-ranging birds of prey (Jaspers et al., 2008, 2013; Eulaers et al., 2011a,b, 2014a,b), studies in this regard is missing particularly from Asian continent (Abbasi et al., 2016a). In addition, there is general scarcity of knowledge on POP-induced oxidative stress in bird of prey species. Abbasi et al. (2016a) indicated a serious scarcity of such information for top predatory bird species from South Asia. Therefore, the objectives of this study were (1) to assess the relationship between blood and feathers POP concentrations and the expression of antioxidant enzymes, both at transcriptional and functional (activity) levels, and (2) to evaluate whether feathers are a valuable matrix to investigate internal POP body burdens and their associated oxidative stress impacts.

## 2. Materials and methods

### 2.1. Sample collection

Sample collection was carried out around the outskirts of Rawalpindi city (33° 37′ 33.80″ N; 73° 4′ 17.19″ E) of Pakistan between March–September 2014. Two commonly occurring predatory bird species, namely black kite (*Milvus migrans*) and spotted owlet (*Athene brama*) were sampled for blood and feathers. The black kite is a large sized bird living in and around human settlement areas where it consumes human refusals, as well as invertebrates, rats, snakes, and other species from open agricultural areas. Because of its omnivorous dietary habits, the black kite is usually considered as a versatile omnivorous bird (Barón et al., 2014), while spotted owlet is a small to medium sized nocturnal predator living around the outskirts of cities (mostly close to human activity), but feeding on more specialized prey species which include rats, mouse, lizards, beetles etc. (Nadeem et al., 2012). A total of 14 adult birds, comprising of 8 black kites and 6 spotted owlets were captured with the help of local hunters, using large mesh nets, and sampled for a tail feathers and blood prior to release. Approximately 3–5 mL of blood was taken from the brachial vein of each individual in a heparin-coated 5 mL syringe fitted with a 25 G needle. About 1–1.5 mL of blood was stored at −80 °C and later used for mRNA and oxidative stress enzyme activity analysis, while the remaining blood was transferred to acid-cleaned polyethylene vials and used for POP quantification. Both feathers and blood for POP analysis were stored at −20 °C prior to analysis. The ethics committee of Quaid-i-Azam University, Islamabad, Pakistan, approved the study and sampling protocol.