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Associations of urban environmental pollution with health-related physiological traits in a free-living bird species

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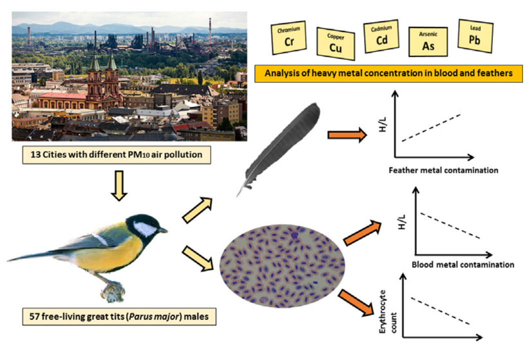
HIGHLIGHTS

- Cities varying in air pollution can vary in organism heavy metal contamination.
- Across regions heavy metal contamination in the great tits was linked to health.
- Higher blood contamination was associated with lower H/L in blood.
- Increased blood contamination was related to decreased erythrocyte counts.
- Urban pollution may affect physiology of synanthropic free-living organisms.

GRAPHICAL ABSTRACT



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ABSTRACT

Urban environmental pollution results in contamination of the tissues of synanthropic organisms by toxic trace elements with potential impacts on human health. Passerine birds may serve as convenient indicators of such contamination. In this study we investigated the effect of blood and plumage contamination with heavy metals (lead Pb, cadmium Cd, copper Cu, chromium Cr) and arsenic metalloid (As) on condition, health and ornamental colour in free-living great tit (*Parus major*) males from 13 cities across the Czech Republic (EU), mist netted during the early breading season (April–May). Our results showed a significant association of heavy metal tissue contamination with immune function, namely leukocyte composition in the avian blood circulation. High heavy metal contamination in bird feathers was linked to a high heterophil/lymphocyte (H/L) ratio, indicating long-term stress in individuals inhabiting heavily polluted environments. In contrast, males with higher concentrations of heavy metals in blood had a lower H/L ratio, assumingly due to the direct toxicity of heavy metals in certain cell types. This is also supported by traits indicative of anaemia-like haemolytic conditions (decreased absolute erythrocyte count) and increased haematopoiesis (a tendency for increased frequencies of immature erythrocytes). We did not find any association of heavy metal contamination with the bacteriolytic activity of plasma complement, feather growth or ornamentation (black breast stripe area and yellow colouration). There was no significant relationship between heavy metal contamination in blood or feathers and PM_{10} pollution at the study sites. Our correlational study is the first to show on a large geographic scale that despite strict European air pollution regulations and regular monitoring that have allowed general improvements in

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atmospheric contamination, non-degradable heavy metals persistently contaminate animal blood and feathers in anthropogenic environments at levels that may have subclinical yet physiological effects with varied influence on health.

1. Introduction

Metals are released into urban environments through atmospheric particulate matter pollution (including PM_{10} < 10 μm particles) and waste water, both commonly related to industrial production. Given the impacts of pollution on health of living organisms (Jarup, 2003; WHO, 2007) the potential environmental risks associated with industrial development must be considered. Heavy metals and metalloids (for simplicity considered as heavy metals in this article) are especially dangerous due to their persistence, high mobility and ability to accumulate in human and animal tissues (Mora, 2003; Walker et al., 2012). The world-wide understanding of the severity of this issue (WHO, 2000) has led most countries to establish measures reducing particulate matter and heavy metal emissions (WHO, 2006a; WHO, 2006b). In the Czech Republic the Clean Air Act No. 201/2012 Coll. (reflecting present EU directives) sets hygienic limits for PM_{10} air pollution and some metals (e.g., arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb); also EEA, 2015). For other metals (e.g. chromium (Cr), copper (Cu) and mercury (Hg)) no legal measures have yet been set, even though their ecological importance in the environment is significant.

It has been shown that simple contamination of the environment with heavy metals may not reliably reflect their absorption rates and physiological effects in living organisms (Scheifler et al., 2006). Therefore, biological indicators of such a relationship are needed to monitor environmental quality (Burger and Gochfeld, 2001). Avian tissue contamination has been frequently considered a valuable and cost-effective bioindicator of environmental pollution (Burger and Gochfeld, 2001). Unlike most other tissues such as lungs, kidneys or liver (Cui et al., 2016), feathers (Bianchi et al., 2008; Dauwe et al., 2002a; Dauwe et al., 2000; Markowski et al., 2013) and avian blood (Carneiro et al., 2015; Coeurdassier et al., 2012; Costa et al., 2014; Geens et al., 2010) may serve as non-destructive and easy-to-obtain biological materials for such monitoring. Heavy metal contamination in feathers is assumed to be representative of intra-annual exposure (Dauwe et al., 2002a). It has been documented that heavy metals deposited into tail feathers (rectrices) reflect the levels in key internal tissues resulting from long-term accumulation (Dauwe et al., 2002b). In contrast, blood is a transport medium and accumulation of heavy metals there is unlikely. Thus, blood contamination reflects immediate exposure (Geens et al., 2010; Scheifler et al., 2006), and there is typically no correlation between blood and feather heavy metal contents (Dauwe et al., 2005).

In wild animals, toxic metals may have direct measurable negative physiological effects even in low sublethal concentrations (Geens et al., 2010; Hawley et al., 2009; Janssens et al., 2003). Effects on bird physiology (Aggarwal et al., 2008), reproduction (Belskii et al., 2005; Eeva et al., 2009; Eeva and Lehikoinen, 2015), diet composition (Eeva et al., 1998; Geens et al., 2009) and nutritional condition (Blanco et al., 2004; Eeva et al., 1998) have been reported. Pb, Cd and Cu in particular have also been reported to directly alter antioxidant capacity (Geens et al., 2009), immunity and health in free-living birds (Blanco et al., 2004; Fair and Myers, 2002; Geens et al., 2010; Snoeijs et al., 2004). Heavy-metal-induced changes in haematological parameters associated with erythrocytes (haematocrit, erythrocyte count and haemoglobin content) reflect anaemia resulting from intoxication (Geens et al., 2010; Papanikolaou et al., 2005). Since health, antioxidant capacity as well as immune function can be related to avian ornament expression (Vinkler and Albrecht, 2010), several authors (e.g. Dauwe and Eens, 2008 and Eeva et al., 1998) have suggested the possibility of decreased carotenoid-based ornamentation in birds at heavily polluted sites. In contrast, melanin-based ornamentation was found to be increased in adult great tits from highly-polluted sites (Dauwe and Eens, 2008).

The heavy metal burden in birds is higher in cities than in the countryside (Hargitai et al., 2016), as has been confirmed in blackbirds (*Turdus merula*; Meillere et al., 2016; Scheifler et al., 2006), and great (*Parus major*) and blue tits (*Cyanistes caeruleus*; Eens et al., 1999). However, even cities themselves may differ markedly in their levels of heavy metal contamination (CHMI, 2009). It has been previously shown that amounts of heavy metals in great and blue tit tissues decrease with the distance from the source of pollution (Dauwe et al., 2002b). We may predict, therefore, that urban habitats differing in sources of pollution will also differ in their levels of contamination. However, no comparison of avian tissue heavy-metal contamination across a large-scale geographic area has yet been done to show the effects of varying pollution levels on animal health-related physiological traits.

In this study, we hypothesised that differences in avian heavy metal contamination in urban free-living populations would manifest in variation in their health-related traits. We predicted that birds from more polluted cities would show impaired condition and health compared to birds from cleaner cities. To test this hypothesis we compared heavy metal (As, Cu, Cd, Pb, and Cr) concentrations in the blood and feathers of 57 great tit males originating from 13 different city regions varying in air pollution. Contrary to most other studies dealing with this issue, we did not focus on any particular candidate source of environmental contamination, but rather performed a large-area survey, with study sites distributed all over the area of interest, in our case the territory of the Czech Republic, EU. Study localities were chosen according to their annual average concentrations of PM_{10} (ranging from 27 μg/m^3, to 65 μg/m^3, Fig. 1, (CHMI, 2010), for closer details see Table 1). We used principal component analysis (PCA) of heavy metal contamination to reveal general effects of all heavy metals on individual health- and condition-related traits, namely fat deposition index, ptilochronologically-measured feather growth rate, leukocyte and erythrocyte levels in blood circulation, differential leukocyte count, immature erythrocyte frequencies, complement activity in plasma, and carotenoid- and melanin-based ornamentation.

2. Methods and materials

2.1. Field procedures

During the early breeding season (April–May) 2010 great tit adult males ($N = 57$) attracted by a song record were mist netted during the morning hours at 13 different urban areas in the Czech Republic, EU (map, Fig. 1 and Supplement 2). These areas were selected based on their PM_{10} air pollution levels in the previous year (the annual average of year 2009; data from the Czech Hydrometeorological Institute, CHMI, 2010), comprising putatively highly polluted sites ($PM_{10} \geq$ 40 μg/m^3, $n = 5$) as well as putatively lowly polluted sites (PM_{10} < 40 μg/m^3, $n = 8$), and reflecting the distribution of the most important urban centres in the Czech Republic (see Table 1 for additional details). The mist-netting sites were always located within a radius of 2 km from a CHMI automatic air pollution monitoring station (see Table 1 and Supplement 2 for a detailed description), always in the same habitat type (deciduous forest with no major roads or industrial buildings in their vicinity). In most locations the sample sizes of