The effects of trace metals in urbanization-related soil contamination on urban birds

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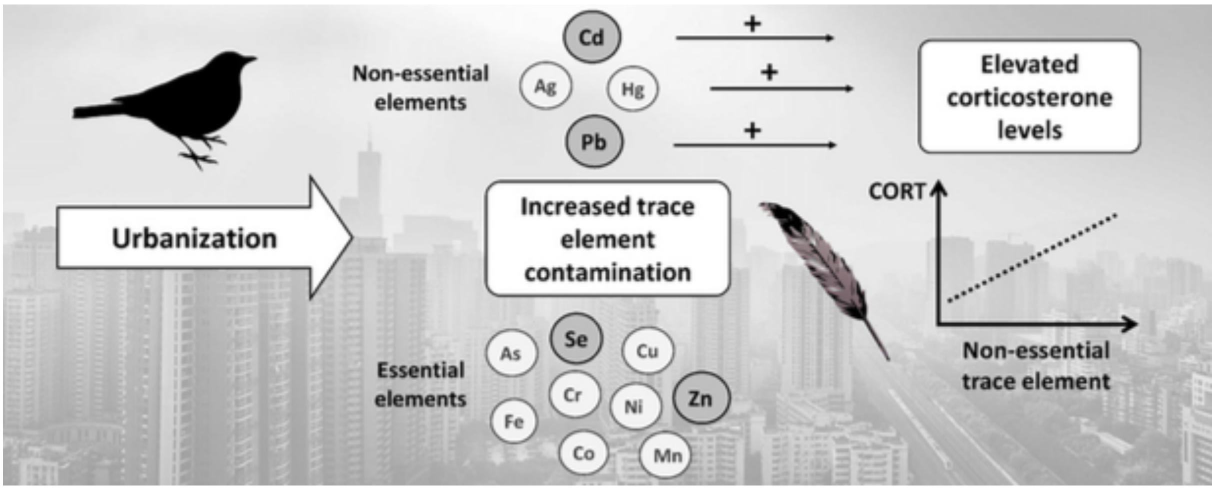

(Source: Meillère et al 2016)

Introduction

With a steep increasing human population comes a great expansion of urban areas in the world. Nowadays mankind lives predominantly in urban areas, and it is predicted that the area of the highly developed world (Europe, North-America) will have reached 86% urbanization halfway in the twenty-first century, as well as a 64% urbanized area in the lower developed world (Africa) (The Economist 2012). Many local bird species will have to adapt through selection, or move out to a more natural environment (McKinney 2002; Møller 2009). For example, the feral pigeon *(Columba livia)* thrives with an urban lifestyle, feeding on human waste and procreating in shafts and niches of buildings (Johnston 1995). Unfortunately most species are not able to take advantage of urbanization like the feral pigeon, and for them life in an urban environment comes at a fitness cost (Møller 2009).

Consequences of urbanization are, among others, chemical changes in the soil and air, increased temperature with smaller fluctuations throughout the seasons, increased industrial as well as city noise (anthropogenic noise) and loss of total darkness during night-time (Ciach and Frölich 2017; Perillo et al 2017; Sol et al 2017). Studies on the influence of these factors on biodiversity showed that only few factors are beneficial for avifauna, while most are harmful (Ciach and Frölich 2017). Czech et al (2000) even stated that urbanization is not only one of the biggest threats for wildlife but also the most significant cause of extinction of species, which is largely due to a lack of open greenery (Ciach and Fröhlich 2017).

Due to urbanization, the increased trace metal elements in the birds' habitat are another major threat to local avifauna. Trace metals such as lead and iron contaminate air, soil and flora, and thus also a bird's diet (Brahmia et al 2013; Kim and Oh 2016) via ingestion of soil or paint chips (Ikemoto et al 2005). Trace metals have negative effects on the endocrine system, reproduction and general well being of birds (Dauwe et al 2004; Espín et al 2014; Stoica et al 2000). Also ingesting excess "essential elements" such as zinc, manganese and copper can be harmful (Franson et al 2012).

Trace metals are found in natural environments, but usually only in low concentrations (as indicated in table 1). Due to anthropogenic activity, trace metal concentrations in the soil and air exceed natural ranges in urban environments (Azimi et al 2005). Typical trace metals found in higher levels in cities of medium to high urbanization-level are lead, iron, zinc, cadmium, copper, calcium and manganese (Azimi et al 2005; Chatelain et al 2016; Chatelain et al 2016; Chen et al 1997; Cuypers et al 2010). Although increased intakes of different metals can induce different physiological effects, they are usually associated with a negative impact on bird survival and reproductive success (including reduced clutch size and fertility) (Chatelain et al 2014). Iron, zinc, copper, calcium and manganese are considered essential elements, as a vertebrate can't survive without low doses of them, whereas lead and cadmium are non-essential (Mellière et al 2016). Lead and zinc are often the most abundant metals in present-day cities (Azimi et al 2005; Chatelain et al 2016; Chen et al 1997), and their effects are also most often studied.

In order to live in an urban environment, individual birds need to detoxify or tolerate the abundance in trace metals. Some species are better able to adapt to this than others. Different bird species tolerate chemicals on different levels; seabirds, for example, can tolerate levels of mercury that are easily lethal to other bird species (Meillère et al 2016). In this paper I focus on what physiological differences an urban-living bird possesses that enables it to better withstand trace metals than a non-urban bird. Which metals are most harmful, and through which factors or mechanisms does an urban bird prevent toxic trace metals from accumulating? Answers are found in pigments as agents of transporting metals, as well as physiological 'buffers' that originate from the immune system. In addition, evolution could have already played a part: species that have consequently lived near urban areas since as early as human civilization could have adapted to higher levels of trace metals.

This study largely focuses on the effects of lead- and zinc-contamination in different bird species. Lead and zinc are sometimes claimed the elements that induce the strongest health effects (both positive and negative) on urban wildlife (Chatelain et al 2016), although recently also estimations of the toxic effects of cadmium seem to be comparable (Meillère et al 2016). The abundance of lead in urban soil is largely explained by anthropogenic activities, such as combustion of lead-containing fossil fuels, mining and other industry. Their fumes and micro particles pollute air and soil upon descending, even though lead-concentrations in fuel have vastly decreased since the seventies (Azimi 2005) and again with new environmental policies in the 2000's (Mellière 2016). The success rate of birds in urban areas is largely driven by their ability to deal with these trace metals (Chatelain et al 2014), but what toxic effects do they have on birds?

***Table 1*. Concentrations of in rural soil: cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), zinc (Zn), iron (Fe) and Manganese (Mn) found in rural Algeria (Brahmia et al 2013).**

Average concentrations in rural soil (in mg kg^{-1})						
Cd	**Cr**	**Cu**	**Pb**	**Zn**	**Fe**	**Mn**
0.01	4.5	4	6.8	6.1	3757	118.3

General immune responses to urban living and metal toxicity

When the environment of a bird changes and trace metal intake increases, the immune system is one of the first lines of defence that will come in contact with the extra trace metals. This results in an increase in immune response in urban birds (Watson et al 2017). In his study on great tits (*Parus major*), urban great tits showed higher expression in genes that are considered 'stressor' genes associated with both biotic and abiotic stressors than rural great tits. Urban tits produced more T and B cells, major histocompatibilitycomplexes (MHC) and inflammation-related molecules such as cytokines and metallothioneins (Cuypers et al 2010; Watson et al 2017). These cells and molecules from the immune system, together with the overexpression of redox genes, support the idea that urban birds live with a higher state of physiological stress and inflammation than a non-urban bird (Watson et al 2017). Meillière et al (2016) contribute to this idea with evidence for increased levels of corticosterone, an indicator of stress, in urban blackbirds (*Turdus merula*). Urban birds apparently have a better innate immunity than non-urban birds (Watson et al 2017), although Snoeijs et al (2004) as well as Meillère et al (2016) contradict this. They found that trace metal accumulation negatively affects immune response in great tits and zebra finches (*Taeniopygia guttata*)(Snoeijs et al 2004), and increase susceptibility to infectious diseases (Meillère et al 2016). It is therefore unclear whether there are differences in effectiveness of the immune responses between urban birds and non-urban birds. Perhaps the truth depends on the age the bird is confronted with high concentrations of trace metals. Watson et al (2017) support this idea: some methylation patterns are dependent on early-life nutrition. Methylation is a form of silencing of genes, prohibiting their transcription and preventing expression. Methylation of the methyltransferase gene leads to increased gene expression related to the immune response, as well as altering the bird's behaviour (analogous to human personality) (Watson et al 2017). Possibly trace metal intake during the early life decreases or alters the urban birds' stress response to metal contamination. It is yet unclear what behavioural differences an urban bird develops as a consequence of diet-induced methylation, as the mechanisms need to be researched in further detail.

Urban tits also showed decreased fatty acid production (Watson et al 2017). This is in accordance with the earlier mentioned inflammation responses in urban birds as fatty acids increase the risk of tissue damage and inflammation, for which the urban bird already has a higher susceptibility (Watson et al 2017). Watson et al (2017) put forth the idea that species with a stronger immune response are more likely to survive in urban environment, even though this increases energetic demand for the immune response.

Concerning the toxicity of specific metals, most toxic effects can be attributed to high lead intake (González et al 2017; Meillière et al 2016; Orlowski 2017), but also cadmium and mercury, which are all non-essential elements (Meillière et al 2016). Lead poisoning seems to be one of the main causes of stress in urban birds. In an investigation of Griffon vultures (*Gyps fulvus*) living in different urban environments, beginning lead intoxication was found in nearly all subjects (González et al 2017). Lead intoxication can already cause symptoms at low levels as it enhances the production of autoantibodies. General symptoms in birds are similar to symptoms seen in humans, including pain and altered cognitive function (CDC 2017, González et al 2017). In birds this results in general weakness, trouble finding food and being more susceptible to predation. Also 'droop-wing syndrome' has been reported in albatross *(Phoebastria)* chicks (Ikemoto 2005). Lead is highly likely to be the cause of urban blue tits (*Cyanistes caeruleus*) remaining smaller in size than rural-lving blue tits (Brahmia et al 2013). Chronic lead exposure can lead to microcytic anaemia as haemoglobin-production decreases (González et al 2017, Puschner and Poppenga 2009). The increased stress-state Meillère et al (2016) found in urban blackbirds (as indicated by their elevated corticosterone-levels) correlated positively with increased blood-lead levels. More evidence for lead-induced stress was found in feral pigeon chicks, when a high heterophil/lymphocyte-ratio was detected, which is considered an indicator for stress (Chatelain et al 2016). Lead-level in urban birds is expected to increase with age due to bioaccumulation, as well as different diet and foraging behaviour in adult birds (Meillère et al 2016). Long-term lead confrontation can increase corticosterone in blackbirds as well as white storks (*Ciconia ciconia*) by affecting the hypothalamic-pituitary-adrenal-axis (HPA in short) (Meillère et al 2016). The same effect is possibly induced by cadmium, but this is not yet fully confirmed (Meillière et al (2016). Not only can lead contamination cause negative effects on the birds' physiology in the short term and at low levels, but the severity of symptoms increases during long-term exposure as lead accumulates in the body.

Mercury is labelled the most hazardous metal by some because of the health effects it not only has on direct consumers but also further up the food chain (Burger and Gochfeld 1997), although mercury poisoning is seldom described in the available literature. Exceeding mercury levels are far less common than lead or cadmium, and increased mercury level in the environment has not yet been identified as a consequence of urbanization. (Burger 1994; Mora 2003)

Even though zinc is an abundant trace metal in urban environment, it is an essential mineral that will only be the cause of a wide range of diseases when outside of a healthy range (Azimi 2005; Chatelain et al 2016; Hambidge and Krebs 2007). In all metazoans, zinc is needed for immune responses as it has toxic properties to microorganisms (Chatelain et al 2016). Most toxic effects of zinc (ataxia, lethargy) can be attributed to its ability to function as a transporter in enzymes used for food absorption as well as draining of other trace metals such as copper and lead (via metallothioneins) (Chatelain et al 2016; Chichovska and Anguelov 2006). Blood-zinc level in urban blackbirds was positively correlated with increased urbanization as well as with age (Meillère et al 2016). Zinc can compensate negative effects of other metals (Chichovska and Anguelov 2006) and is therefore believed to be a protector against metal toxicity (Felizola et al 2014). Chatelain et al (2016) found experimentally that zinc protects against lead by inducing a longer and more intense anti-KLH IgY immune response, which lowers as lead intake increases. Zinc also correlates negatively with endoparasite-levels, as opposed to lead, which correlates positively with endoparasite-levels. Other trace metals such as cadmium and copper show no correlation with endoparasites, and it is also because of this that Chatelain et al (2014, 2016) believe lead and zinc are responsible for shaping birds' immune response in urban environments. In high concentrations however, zinc also induces an immune response as more white blood cells are produced (Chatelain et al 2016).

Storage in plumage, different melanin pigments

As consumed trace metals accumulate in the body and increase health risks, the urban birds need to detoxify in order to maintain their health. Bird plumage is known to be an outlet for excess trace metals (Burger 2008). Birds are able to transfer metals via their bloodstream into the feathers (Chatelain et al 2016). Melanin is not only a pigment of the feathers' dark colours, but also the potent agent of binding metal ions due to their composition of negatively charged polymers (Chatelain et al 2014; Liu et al 2004). Melanin binds metals in the blood and then migrates to the feathers. Gaither and Eide (2001) first found no relation between plumage and zinc level, while blood-zinc level in eukaryotes is under strict homeostatic regulation. Later, evidence was found of higher amounts of zinc in feral pigeons that possess high amounts of plumage-melanin, indicating more pigmented pigeons can survive higher trace metal uptake (Chatelain et al 2014). Most recently, increasing amounts of zinc and lead were found in feral pigeons' plumage correlating with their intake, therefore endorsing the theory that plumage is a trace metal-outlet (Chatelain et al 2016). While a direct positive correlation between trace metal supplementation and plumage-metal concentrations was demonstrated, Chatelain et al (2016) do not fully discard Gaither and Eide's (2001) findings, confirming the influence of numerous regulatory mechanisms for blood-metal levels. Furthermore, a positive correlation between plumage-melanin and calcium and manganese was determined as well (Chatelain et al 2016). An indication of lead concentration-ranges in blue tits' blood and plumage from different levels of urbanization can be seen in table 2. From this data it is also apparent that lead in blood and feathers strongly rise with increased urbanisation-level, and higher levels of lead are found in feathers compared to blood-samples. The maximum lead concentration in feathers is found in the rural environment although there is more variation in lead concentrations in more rural environment (UDL to 8.13) as opposed to urban environment (1.24 to 3.28).

Table 2. **Ranges of lead concentrations (in $\mu g\ g^{-1}$) found in blue tits from rural to urban areas in Algeria (Brahmia et al 2013).** Minimum and maximum measured values are shown, as well as the average values. In some cases levels were under detection limit (UDL).

	Blood			Feather		
	Min	Max	**Avg**	Min	Max	**Avg**
Urban	0.02	0.67	**0.12**	1.24	3.28	**2.26**
Intermediate	UDL	0.25	**0.045**	0.29	6.35	**1.24**
Rural	UDL	0.2	**0.025**	UDL	8.13	**0.97**

Since melanin seems to be a key molecule for an urban bird, it is logical to think that the birds that are best able to withstand trace metal contamination are the ones with high amounts of melanin. In molluscs, darker pigmentation has already been positively correlated with increased trace metal content, but an analogous relationship in birds has not yet been confirmed (Orlowski 2017). Chatelain et al (2014) confirmed a correlation between urbanization-level and melanin-based coloration in the feral pigeon. In barn owls (*Tyto alba*) it has been found that black pigment positively correlates with resistance to oxidative stress (Chatelain et al 2016). Oxidative stress is when high amounts of reactive oxygen species are released into the body as a result of inflammation. In addition to resistance to oxidative stress, having more melanin has also been linked to a stronger T-cell mediated response, as well as lower endoparasite intensity as a secondary effect of melanin synthesis through the proopiomelanocortin (POMC) gene (Jacquin et al 2011).

With all these positive effects being attributed to melanin, why are not all birds living in urban environments black of colour? This is not yet clear, but it is possible that there has not been enough selection pressure through trace metals. Also, an important

factor seems to be the difference between different melanins. Two basic melanins are as of yet distinguished: pheomelanin, which is responsible for all red shades, and eumelanin, which is responsible for black and all diluted shades (Roulin 2004).
Eumelanin and pheomelanin have different properties apart from the plumage colour that they induce. In relation to detoxification, early experimental research suggests eumelanin is better capable of transporting zinc than pheomelanin (Chatelain et al 2016), although these differences have as of yet been poorly studied.

Although these novel experimental results look promising, there have not yet been interspecies-comparisons of trace metal-levels versus amount of melanin they store. Comparing species is difficult, but even within a species it can be tricky to compare trace metal-concentrations, as trace metal-levels can fluctuate between different feather types (Meillère et al 2016). The importance of the role of melanin in urban birds' must be underlined though, as Chatelain et al (2016) for example state that it possibly 'shapes' the birds' tolerance to trace metals.

Moult frequency

As the maximum capacity for melanin uptake is used in a feather, a limit is reached in storage of metals. Detoxification does not end there, as birds are able to replace their feathers in moult and eliminate all excess metals leaving room to bind newly consumed metals (Burger 1993). The primary goal for moulting is to replace worn feathers, which are dead structures at maturity. An adult bird moults on average two times per year. Since replacing feathers creates the opportunity for more metal be stored via melanin-transport, does this mean moulting duration determines the ability to detoxify?

Chatelain et al (2014) state that feral pigeons store metals most effectively during moult. So not only more frequent moults but also its intensity contribute to effective detoxification via plumage. Moulting intensity is determined by the growth rate of new feathers and the amount of feathers that are regrown simultaneously. The feral pigeon has an extended moulting period (Johnston 1995). Although there have been different selection pressures in the evolution of the feral pigeon that have resulted in their moulting behaviour, their extended moult could well be a factor that explains why feral pigeons are such successful city dwellers (Chatelain et al 2016). Burger (2008) however, presumes birds in general store metals in (breast) feathers from circulating blood and local tissue during the few weeks of feather formation, when the feather is directly exposed to local tissue. The metal-concentrations being are therefore a representation of recent and local exposure. Although it is a generally accepted idea that moulting increases metal storage ability, it is still subject of debate when the storage efficiency peak is. It is known that the peak differs between species (Burger 2008).

Storage in eggshells

Detoxification in urban birds may differ between sexes. Apart from different trace metal uptake thanks to differences in diet (Meillère et al 2016), it is apparent that females are capable of producing eggs. It is known that for many birds, including rosaete sterns (*Sterna dougallii*), herring gulls (*Larus argentatus*) as well as common eiders (*Somateria mollisima*) that females are able to store excess metals in the shell as well as contents of their eggs (Burger 1994, 2008). The correlation between trace metal uptake and egg-metals was confirmed for all metals but mercury, and significantly higher concentrations in the egg contents than in the shell (Burger 2008). Burger therefore concluded that rosaete sterns and herring gulls detoxify via egg production, which is later confirmed by Dauwe et al (1999). In blue tits however, no differences were found in lead-concentration in eggshells between different urbanisation levels, whereas liver-lead level in adults increased with urbanisation level (Brahmia et al 2013). Maybe female detoxification via eggshell-storage is not as apparent in some species, perhaps because other detoxifying mechanisms suffice for them. Overall the evidence for birds' trace metal storage in eggshells is paramount.

Spotted patterns in eggshells have always been thought of as camouflage for potential predators. It is still unclear however, what causes the different patterns found in eggshells and what the evolutionary reason for this variability is (Orlowski et al 2017). Also it has long been unknown what the chemical contents of spots (pigmented area) and unpigmented areas are, although there is increasing novel research (Burger 2008; Orlowski et al 2017). Orlowski et al (2017) shows that the pigments protoporphyrin and biliverdin, which are respectively responsible for red/brown and blue/green colour in Japanese quail (*Coturnix coturnix japonica*) eggshells, bind trace metal ions in a similar fashion as melanin does in bird plumage. Maculated eggshells are also not chemically homogenous: protoporphyrin attracts mainly calcium, magnesium, copper and cadmium, whereas lead is mainly found in presence of biliverdin or in unpigmented area. Orlowski et al (2017) distinguish between bright or dark eggs due to coloration (see figure 1).

Figure 1. Examples of different spotted (pigmented) patterns on bright and dark quail eggs (Orlowski et al 2017).

In Orlowski et al's (2017) search for trace metals in eggshells they mention calcium and magnesium as 'staple elements' of the eggshell. Copper, manganese, iron, cobalt and cadmium are called 'trace elements', and lead is rendered toxic (Orlowski et al 2017). Table 3 is an overview of these elements and where they are mostly located in eggshells, binding to either or both pigments. Some elements (Mn, Fe and Co) had similar concentrations with either pigments and varied much overall. Lead (Pb) was found in markedly higher concentrations (over 9 times higher) in unpigmented areas than in speckled area. Between pigments, lead seems to be located in deeper shell layers and have a higher affinity with biliverdin.

Orlowski et al (2017) theorize speckling in eggshells functions in bird eggs to distribute resources (micronutrients, trace elements, pigments) for the embryo to absorb from the shell, although it is unclear how this contributes to embryo-development. All essential elements are, as expected, usually in significantly higher concentration in the egg content than in the shell, and an embryo's development is therefore not dependant of the eggshell-stored trace metals (Burger 1994; Mora 2003). Female birds further use protoporphyrin to increase eggshell thickness and trace metals probably get distributed afterwards (Orlowski et al 2016).

***Table 3*. Overview of most trace elements and where they are predominantly found in quail eggshells.** Calcium and magnesium are considered 'staple components' of eggshells. Based on data from Orlowski et al (2017).

Element	Type	Closely bound to	Pigmented/unpigmented	Bright/dark eggs
Calcium	Staple	Protoporphyrin	Pigmented	Either
Magnesium	Staple	Protoporphyrin	Pigmented	Either
Copper	Trace	Protoporphyrin	Pigmented	Dark eggs
Manganese	Trace	Varies	Pigmented	Bright eggs
Iron	Trace	Varies	Pigmented	Bright eggs
Cobalt	Trace	Varies	Varies	Bright eggs
Cadmium	Trace	Protoporphyrin	Pigmented	Dark eggs
Lead	Toxic	Biliverdin	Unpigmented	Dark eggs

Despite the evidence for main mechanisms of trace metal storage in either plumage or eggshell, several other factors also contribute to the accumulation of metals in these locations. For example, concentrations in which metals are stored are probably highly dependant on the physiology of the tissues surrounding the storage-location. This could explain for example why mercury is mainly found in feathers, and zinc is rarely found in eggs (Burger 2008). Burger further states that differences in trace metal-concentrations between species in the same area can also be related to their trophic level and age, as they are positively correlated. This is why terns and gulls generally have higher cadmium and lead-concentrations as opposed to eiders, as the former eat fish and other organisms (Burger 2008). Also metal accumulation increases with older age in birds (Ikemoto 2005). As for egg maculation pattern and intensity, these may vary within an individual female over time, depending on her body condition. Female quails with poor body condition due to oxidative stress may lay darker and more spotted eggs (Orlowski et al 2017). Also, with age female albatrosses turn out to lay larger eggs, as well as transfer increasing amounts of elements such as mercury (Ikemoto et al 2005). Ikemoto et al (2005) however also state that mercury-levels are known to remain quite stable over the lifetime of adult birds thanks to existence of HgSe compounds in the liver, with which they seem to contradict themselves. Perhaps both the HgSe compounds as well as increased mercury-storage in eggshells over time serve the purpose of maintaining low mercury levels throughout a birds' life.

Hatching success and early chick development

There are concerns about the toxic effect of trace metals in the shell and egg content on hatching success and chick health. Romanoff (1967) speculated that there is potential for inorganic elements from the eggshell to become mobilized in the latter half of incubation, when considerable transport of minerals occurs, and affect the late stage embryo. However, trace metals are not yet known to have effects on bird embryos. In a study on passerine birds in Arizona, USA, no differences in hatching success were found between flocks of birds with different metal intake (Mora 2003). The correlation between metal intake and eggshell metal-concentration was confirmed in the same study, although egg content metal-concentrations could not be monitored as this influences hatching success. In a similar study on blue tits no differences in breeding success, laying date, incubation duration, clutch size or predation of eggs were found in birds from different urbanization levels (Brahmia et al 2013). Instead Brahmia et al (2013) states that breeding phenology is largely determined by food availability, which then depends more on local vegetation. For now it seems no link has been found between urban trace metal concentration in egg contents and reproductive success. Increased mercury has been reported to influence hatching success and nesting behaviour in albatrosses, but increased mercury has not yet been linked to urbanisation (Ikemoto et al 2005). Mora (2003) further speculates that only in high concentrations, that are found in the most severe urbanized areas, trace metals in eggshells can influence hatching success. Furthermore, it's hypothesized that strontium (Sr) could indirectly induce lower hatching rate, based on a survey on Tonto Creek where 80% hatching rate relative to normal was found as well as some of the highest Sr concentrations in birds (Mora 2003). In contrast, strontium was found to positively correlate with calcium-levels and physiologically behaving similar to calcium (Ikemoto et al 2005). They suggest strontium might also contribute to the formation of bone, just as calcium does. High concentrations of strontium were found in eggshells, in contrast to low concentrations (<1ug/g dry weight) in adult albatrosses (Ikemoto et al 2005).

It seems trace metals accumulated in the shell of bird eggs generally do not have any negative effect on the embryo. As a matter of fact, a variety of trace metals that are indispensable for developing avian embryos (micronutriënts calcium and magnesium, and apparently strontium) are believed to get mechanically supplemented to the embryo via storage in the eggshell and taken up by the embryo/chick before hatching (Orlowski et al 2017). Increased maculation (thus trace metal supplementation) has also been determined to strengthen the eggshell and have a positive effect on hatchability as well as lowering embryonic mortality (Orlowski et al 2017). Can the eggshell become too strong, and complicate the hatching process? This is not yet known, as no evidence for it has been found.

Discussion

This paper is based on novel research with in some cases incomplete data, but most importantly with facts based on different species from in and around urban areas. Comparing these species and drawing conclusions from them is not always appropriate, but it can shed light on the overall picture on how birds detoxify.

Although effects of elevated trace metal concentrations caused by urbanization have diverse effects in birds, it must be underlined that urbanization is a source of stress for urban living birds. Trace metal contamination is at least a contributing stress factor herein (Meillère et al 2016). Despite repeated revising of the environmental pollution laws high levels of trace metals remain in urban soil and in birds living in urban areas. It seems that more time must pass and/or more severe measures must be taken to reduce trace metal contamination.

More research is needed to be able to properly chart the degree of trace metal contamination in urban areas, the concentrations of each metal in the soil and the toxic effects they cause in birds. The effects they bring about in birds are increasingly studied, and there is a lot to discover as many physiological mechanisms cannot yet be fully explained. As for now, it seems lead is the main source of stress for urban birds (Chatelain et al 2014, 2016), while cadmium may well be determined equally toxic for birds in the future (Meillère et al 2016). Lead is positively correlated with genes and molecules that are inflammation-related. The effects of lead poisoning may be counteracted to a certain degree by zinc. To what level that could work is not clear.

Increased zinc uptake is expected to have detrimental effects once the physiological boundary is exceeded, although in experimental work that seems to have rarely happened. One general conclusion is that species with a stronger immune response are more likely to survive in urban environment, even though this increases energetic demand for the immune response (Watson et al 2017).

Increased trace metal consumption will lead to accumulation in the body if a bird does not detoxify. Some metals, such as iron, accumulate in the body despite detoxification but have not yet been linked to reduced bird health. Urban birds are to a certain extent able to store excess metals in their plumage and their eggs. The leading agents that facilitate this are the coloration pigments, and this could explain the relatively high amount of melanic birds in cities. It is not yet known why not all city birds are dark of colour, but it is possible that the selection pressure is not yet high enough, although it is more likely that other mechanisms contribute to the pigmentation of city birds. Different pigments can transport metals with differing efficiency, and this could, among other factors, contribute to where a certain metal is stored. The storing of metals does not happen randomly, as concentrations of metals in plumage seem to differ between feather types. This seems to depend on nearby tissues determining which metals are released into the blood. Storage in plumage is not a single event, as moulting facilitates the opportunity to store trace metals to the bird, and the moult time and frequency are both described as factors that increase storing efficiency. This has as of yet mainly been described in feral pigeons as they thrive in urban environments, and could be one explanation as to how they are able to cope with trace metal contamination so well. A possible way of determining specialization in feral pigeons would be to compare moult behaviour to their ancestors, the homing pigeon (*Columba livia domestica*) and the rock pigeon (*Columba livia*). In general the feral pigeon and the rock pigeon still share the same moult behaviour, replacing feathers gradually throughout the year and each feather taking three to five weeks to grow (Mallet-Rodrigues 2012). Differences in moult behaviour might be found in the details, which are as of yet unclear: how long do different pigeon species moult per year, and where do they store trace metals when they are not moulting? It is unlikely that the feral pigeon has evolved to moult long and frequently due to increased urbanisation, instead it is more probable that different selection pressures have shaped their moulting behaviour (Chatelain et al 2014, 2016).

In eggs, metals are stored in either the egg contents or in the shell, serving to a certain function, and the location depending on the tissues that surround it. To the embryo, several trace metals are nutritious, while some metals increase the strength to the egg when accumulated in the shell. The speckles that arise on the shell can serve a double function, being also a camouflage for predators.

As a mother bird deals with the effects of trace metal contamination, she might simultaneously prepare her unborn chick for an urban life. Via transportation of metals from the shell towards the embryo, the young bird is confronted with the excess in metals and can create a tolerance towards them through mechanisms that are mostly unknown. It is in accordance with the hypothesis that a chicks' diet can induce methylation of genes that regulate the immune response but also the birds' behaviour. As opposed to the idea that trace metal contamination is detrimental to bird embryos, it seems an embryo is unaffected and might even benefit from the early confrontation. The statement that strontium lowers hatching rate (Mora 2003) seems contradictory to research results from Ikemoto et al (2005) that strontium is beneficial to bird health as it increases bone strength in chicks. Since no other experiments have determined strontium to influence hatching rates and Mora (2003) found these lower rates by accident, it is more likely that the low hatching rates found by Mora have a different, unregistered cause.

A quite central question that remains unanswered is how a male urban bird compensates for the fact that it cannot lay eggs. Based on the evidence, a female should be far better at detoxifying. Especially a female feral pigeon has an advance in detoxifying, as she can produce eggs all year long (Johnston 1995). Maybe the simplest answer is that there is no compensation, and that, as mentioned before, the selection pressure is not high enough and male feral pigeons detoxify sufficiently. Alternatively, a male bird may store more metal in their plumage, may tolerate accumulation better or express a different, not yet discovered, detoxifying mechanism.

In some urban species, such as the blackbird, it can be hard to determine whether individuals are sedentary or migratory. Trace metals therefore do not necessarily have to have accumulated in the area where the experiment was conducted on the bird (Meillère et al 2016). As for measuring stress in birds, most methods of measuring immunologic 'stress' responses can be considered a 'snapshot' of the physiological state of a bird, but fail to determine stress-levels in the long term. A hormone such as corticosterone is known to fluctuate daily, but the average can also vary seasonally (Meillère et al 2016). Experimental results would be more solid when measurements are taken during longer periods of time. Experimental results could be most convincing when the most heavily contaminated urban areas in the world would be studied. In many studies even the most contaminated study site still does not qualify as 'heavily contaminated' (Brahmia et al 2013), which can explain why often no physiological effects of contamination are found. Health effects can be better determined in birds that have been severely contaminated, preferably with a single metal. It is therefore still unclear whether there are differences in effectiveness of the immune responses between urban birds and non-urban birds, as the research subjects are apparently not far different than 'non-urban birds'.

Further research should be done to exclude other influencing factors that have not been taken into account while this paper was written. Overall it is safe to say that trace metal detoxification is an important factor for survival in urban areas, but that it consists of several mechanisms that work together to maintain health in a bird. Differences in detoxification between successful urban-living birds and unsuccessful urban-living birds as well differences between male and female detoxification are largely to be discovered. For now, it seems that ideally confrontation with trace metals occurs in an embryo before the egg is even laid, after which storage in plumage and egg in combination with a strong immune response decide the birds' survival success.

References

Andrews GK. 2000. *"Regulation of metallothionein gene expression by oxidative stress and metal ions"*. Biochemical Pharmacology, Vol 59: 95-104

Azimi S, Rocher V, Muller M, Moilleron R, Thevenot D. 2005. *"Sources, distribution and variability of hydrocarbons and metals in atmospheric deposition in an urban area"*. Science of the Total Environment, Vol 337: 223-239

Brahmia Z, Scheifler R, Crini N, Maas S, Giraudoux P, Benyacoub S. 2013. *"Breeding performance of blue tits (Cyanistes cæruleus ultramarinus) in relation to lead pollution and nest failure rates in rural, intermediate, and urban sites in Algeria"*. Elsevier Environmental Pollution, Vol 174: 171-178

Burger J. 1993. *"Metals in avian feathers: bioindicators of environmental pollution"*. Reviews of Environmental Contamination and Toxicology, Vol 5: 203-311

Burger J. 1994. *"Heavy-metals in avian eggshells – Another excretion method"*. Journal of Toxicology and Environmental Health, Vol 2: 207-220

Burger J. 2008. *"Assessment of metals in down feathers of female common eiders and their eggs from the Aleutians: arsenic, cadmium, chromium, lead, manganese, mercury and selenium"*. Environmental Monitoring and Assessment, Vol 41: 207-220.

Centers for Disease Control and Prevention: www.cdc.gov/niosh/topics/lead/health. Retrieved 3-8-2017.

Chatelain M, Gasparini J, Jacquin L, Frantz A. 2014. *"The adaptive function of melanin-based plumage coloration to trace metals"*. Biology Letters, Vol 10: 20140164

Chatelain M, Gasparini J, Frantz A. 2016. *"Do trace metals select for darker birds in urban areas? An experimental exposure to lead and zinc"*. Global Change Biology, Vol 22: 2380-2391

Chen TB, Wong JWC, Zhou HY, Wong MH. 1997. *"Assessment of trace metal distribution and contamination in surface soils of Hong Kong"*. Environmental Pollution, Vol 96: 61-68

Chichovska M, Anguelov A. 2006. *"Study on the influence of L-lysine and zinc administration during exposure to lead and ethanol in rats"*. Veterinarski arhiv, Vol 76: 65-73

Ciach M, Fröhlich A. 2017. "*Habitat type, food resources, noise and light pollution explain the species composition, abundance and stability of a winter bird assemblage in an urban environment*". Urban Ecosystems, Vol 20: 547-559

Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ, Nawrot T, Vangronsveld J, Smeets K. 2010. *"Cadmium stress: an oxidative challenge"*. Biometals, Vol 5: 927-940

Czech B, Krausman PR, Devers PK. 2000. "*Economic associations among causes of species endangerment in the United States*". Bioscience, Vol 50: 593-601

Dauwe T, Bervoetz L, Blust R, Pinxten R, Eens M. 1999. *"Are eggshells and egg contents of great and blue tits suitable as indicators of heavy metal pollution?"*. Belgian Journal of Zoology, Vol 129: 439-447

Dauwe T, Janssens E, Kempenaers B, Eens M. 2004. *"The effect of heavy metal exposure on egg size, eggshell thickness and the number of spermatozoa in blue tit Parus Caerulens eggs.* Elsevier Environmental Pollution, Vol 129: 125-129

Espín S, Martínez-López E, Jiménez P, María-Mojica P, García-Fernández AJ. 2014. *"Effects of heavy metals on biomarkers for oxidative stress in Griffon Vulture (Gyps fulvus).* Environmental Research, Vol 129: 59-68

Franson JC, Lahner LL, Meteyer CU, Rattner BA. 2012. *"Copper pellets simulating oral exposure to copper ammunition: absence of toxicity in American Kestrels (Falco sparverius)"*. Archives of Environmental Contamination and Toxicology, Vol 62: 145-153

Felizola SJ, Nakamura Y, Arata Y, Ise K, Satoh F, Rainey WE, Midorikawa S, Suzuki S, Sasano H. 2014. *"Metallothionein-3 (MT-3) in the human adrenal cortex and its disorders"*. Endocrine Pathology, Vol 25(3): 229-235

Gaiter LA, Eide DJ. 2001. *"Eukaryotic zinc transporters and their regulation"*. Zinc Biochemistry, Physiology and Homeostasis: 65-84

González F, López I, Suarez L, Moraleda V, Rodríguez C. 2017. *"Levels of blood lead in Griffon vultures from a Wildlife Rehabilitation Center in Spain"*. Ecotoxicology and Environmental Safety, Vol 143: 143-150

Hambidge KM, Krebs NF. 2007. *"Zinc deficiency: a special challenge"*. Journal of Nutrition, Vol 137(4): 1101-5

Ikemoto T, Kunito T, Tanabe S, Tsurimi M, Sato F, Oka N. 2005. *"Non-destructive monitoring of* *trace element levels in short-tailed albatrosses (Phoebastria albatrus) and black-footed albatrosses (Phoebastria nigripes) from Torishima Island, Japan using eggs and blood".* Marine Pollution Bulletin, Vol 51: 889-895.

Jacquin L, Lenouvel P, Haussy C. 2011. *"Melanin-based coloration is related to parasite intensity and cellular immune response in an urban free living bird: the feral pigeon Columba livia".* Journal of Avian Biology, Vol 42: 11-15

Johnston RF. 1995. *Feral Pigeons*: 1-167

Liu Y, Hong L, Kempf V, Wakamatsu K, Ito S, Simon J. 2004. *"Ion-exchange and adsorption of Fe(III) by Sepia melanin"*. Pigment Cell Research, Vol 17: 262-269

Kim J, Oh JM. 2016. *"Assessment of Trace Element Concentrations in Birds of Prey in Korea"*. Archives of Environmental Contamination and Toxicology, Vol 71: 26-34

Mallet-Rodrigues F. 2012. *"Replacement and growth of primary feathers in captive rock pigeons, Columba livia (Aves: Columbidae)"*. Zoologia, Vol 29: 121-125

McKinney ML. 2002. *"Urbanization, biodiversity and conservation"*. Bioscience, Vol 52: 883-890

Meillère A, Brischoux F, Bustamante P, Michaud B, Parenteau C, Marciau C, Angelier F. 2016. *"Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (Turdus merula)".* Science of the Total Environment, Vol 566-567: 93-101

Møller AP. 2009. "*Successful city dwellers: a comparative study of the ecological characteristics of urban birds in the Western Palearctic*". Oecologia, Vol 159: 849-858

Mora MA. 2003. *"Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona"*. Environmental Pollution, Vol 125: 393-400

Orlowski G, Halupka L, Pokorny P, Klimczuk E, Sztwiertnia H, Dobicki W. 2016. *"The effect of embryonic development on metal and calcium content in eggs and eggshells in a small passerine"*. Ibis, Vol 158: 144-154

Orlowski G, Pokorny P, Dobicki W, Lukaszewicz E, Kowalczyk A. 2017. *"Speckled and plain regions of avian eggshells differ in maternal deposistion of calcium and metals: A hitherto overlooked chemical aspect of egg maculation"*. The Auk, Vol 134: 721-731

Perillo A, Mazzoni LG, Passos LF, Goulart VDLR, Duca C, Young RJ. 2017. *"Anthropogenic noise reduces bird species richness and diversity in urban parks"*. IBIS, Vol 159: 638-646

Puschner B, Poppenga RB. 2009. *"Lead and zinc intoxication in companion birds."* Compendium of Continuing Education for the Practicing Veterinarian, Vol 31: E1-E12

Romanoff AL. 1967. *Biochemistry of the Avian Embryo"*. John Wiley & Sons, NY.

Roulin A. 2004. *"The evolution, maintenance and adaptive function of genetic colour polymorphism in birds".* Biological Reviews, Vol 79: 815-848

Snoeijs T, Dauwe T, Pinxten R, Vandesande F, Eens M. 2004. *"Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (Parus major)"*. Archives of Environmental Contamination and Toxicology, Vol 46: 399-404

Sol D, Bartomeus I, González-Lagos C, Pavoine S. 2017. *"Urbanisation and the loss of phylogenetic diversity in birds"*. Ecology Letters, Vol 20: 721-729

The Economist. 2012. "*Urban life: Open-air computers*". 27 oct. 2012, retrieved 8-7-2017

Watson H, Videvall E, Andersson MN, Isaksson C. 2017. *"Transcriptome analysis of a wild bird reveals physiological responses to the urban environment".* Scientific Reports, Vol 7: 44180