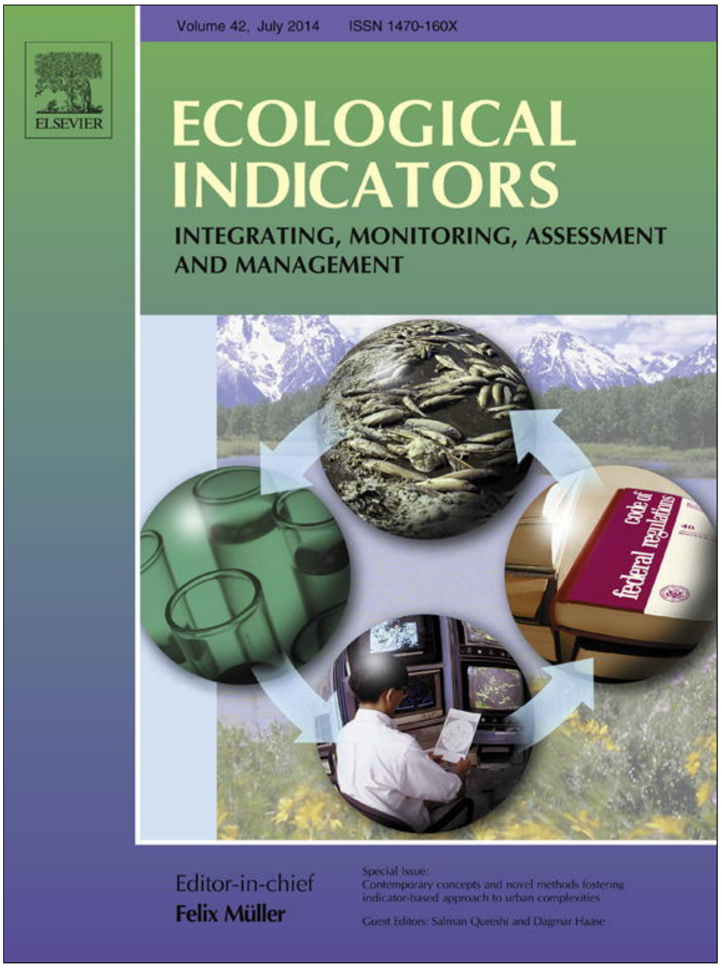
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Oxidative stress of House Sparrow as bioindicator of urban pollution

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ABSTRACT

Air pollution in urban areas constitutes a threat for human health and wildlife. For this reason the effect of these pollutants on living organisms must be monitored accurately. Analysis of oxidative stress generated by exposure to pollutants can be used as a reliable biomarker; and House Sparrow (*Passer domesticus*) may be used as an ecological indicator due to its worldwide distribution, non-migratory status and its association with anthropic areas. In this study, several markers of oxidative stress have been evaluated in blood of House Sparrow using a non-invasive sampling method. Populations from urban and rural areas with differential pollution levels were analyzed. Results showed significant differences in two oxidative stress markers: haemoglobin (Hb) and total antioxidant capacity (TAC), both lower in urban populations. Environment pollution degree may affect oxidative stress status of erythrocytes, therefore these biomarkers could be a useful tool to evaluate the effects of pollutants on living organisms.

1. Introduction

1.1. Urban air pollution

Urban population is continuously uprising. Over half of the worldwide human population live in cities nowadays, and this figure is expected to rise rapidly over the next 25 years (Stagoll et al., 2010). However, cities are far from representing healthy environments: Population growth involves an increase demand for services such as water, electricity, highways, telecommunications, etc. So, urban ecosystems are heavily polluted as a consequence of urbanization and industrial processes (Albayrak and Mor, 2011).

A wide variety of pollutants is released into the atmosphere every day, as a result of human activities (Swaileh and Sansur, 2006), representing an environmental risk for the health of ecosystems, and it could be also considered a potential risk for human health. In fact, it has been reported that exposure to carbon monoxide (CO), sulfur oxides (SO_x), nitrogen oxides (NO_x), and other combustion-derived hydrocarbons gases such as heavy metal particles, is associated with an increase of diverse human diseases like cancer or cardiovascular pathology (Isaksson, 2010).

1.2. Use of bioindicators

For environmental health authorities it is being important to ascertain the main pollutants: sources of emissions, physicochemical properties thereof, etc.; to maintain them under control according to the limits set by the laws currently and the World Health Organization (WHO) recommendations (Aránguez et al., 1999). From an ecological standpoint, bioindicator organisms are very useful for environmental monitoring in contaminated ecosystems like cities. Wild birds have been shown to be particularly useful as bioindicators because they are sensitive to pollutants and are important structural components of the ecosystem (Kekkonen et al., 2012; Swaileh and Sansur, 2006).

House Sparrow (*Passer domesticus*) is distributed worldwide. It is sedentary and closely associated with urban environments. These characteristics make them one of the most suitable candidates for urban biomonitoring of atmosphere pollutants (Swaileh and Sansur, 2006). Until now, pollutant quantification with this species has been assessed in biological samples (i.e. feathers, eggs, bones, liver, kidney, brain) to evaluate bioaccumulation of some of these substances (Hoff Brait and Antoniosi Filho, 2010; Kekkonen et al., 2012; Swaileh and Sansur, 2006). Pollutant accumulation by itself, it is a useful tool but not conclusive for establishing particular risks for human and wildlife health status. To evaluate the potential effects on an organism, it is necessary to understand the incidence of such pollutants on physiological functions (Cape et al., 2003).

1.3. Oxidative stress as monitoring tool

Most airborne pollutants as combustion-derived hydrocarbons gases, biocides or heavy metals are very reactive elements. Therefore they are toxic to living organisms when interfering with metabolism and important biochemical reactions. They produce alterations over enzymatic activities and free radical levels (Koivula et al., 2011).

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Under a physiological context, free radicals are by-products of cell metabolism and they are balanced by a variety of antioxidant elements. Atmospheric pollutants may disrupt oxidative-antioxidant balance unleashing oxidative process (Isaksson, 2010). So, response capacity of antioxidant defense plays an important role in the protection of organisms against toxic-induced oxidative stress. Indeed, the maintenance of a high antioxidant capacity in cells may increase tolerance against different types of environmental stress (Koivula and Eeva, 2010).

This antioxidant capacity response against pollution-induced oxidative damage could be used as reliable tool for monitoring potential harmful effect of contamination (Isaksson, 2010). Moreover, this damage could occur in any tissue and antioxidant defense systems have been described in many animal phyla, being one of the more conserved systems through evolution process (Perez-Campo et al., 1993). So, evaluation of oxidative stress status is reported as useful bioindicator of environmental pollution (Koivula and Eeva, 2010).

It is necessary to establish reliable ecological indicators in order to determine not only pollutant levels but their effects over particular organisms. Furthermore, some non-persistent atmospheric pollutants such us derived-combustion hydrocarbon gases cannot be quantified on organisms using traditional techniques but their physiological effects over the oxidative systems may be used as an indirect evaluation of its presence.

2. Material and methods

2.1. Area characterization and bird capture

Samples were collected at four locations with differences in their air pollution levels and land uses: Olmeda de las Fuentes (OF) (40°21′55.91″N; 3°14′35.46″W); El Escorial (EE) (40°35′06.30″N; 4°07′46.26″W); Fuenlabrada (FB) (40°17′01.94″N; 3°48′01.18″W) and centre of Madrid city (MD) (40°26′30.04″N; 3°41′24.54″W) (Fig. 1).

In order to characterize and establish pollution gradient, environmental traits were collected at every study area, including human presence (population), land use (urban and industrial areas) and atmospheric pollutants load: carbon monoxide (CO), nitrogen oxides (NO_x), and sulphur oxides (SO_x). Data referred to human presence and land uses were obtained from the Spanish Statistics Institute while pollutant concentrations were obtained from the station of Air Quality Network of the Province of Madrid assigned to each municipality. These variables were used in a Principal Component Assay (PCA) analysis to obtain a PC value to be used as an indicator of the *degree of pollution* of each study area.

Total numbers of 73 birds were captured between October 2011 and February 2012 to avoid reproduction period and the potential effect of sexual hormones over antioxidant elements. Individuals were trapped in mist nets and individualized using a metal ring with alphanumeric codes. In all the study areas the number of males and females were balanced in order to avoid results bias.

A blood sample of approximately 0.2 ml from each individual was collected by jugular venepuncture using disposable needles (30G) and plastic syringes; blood was stored in heparinized tubes. All samples were kept cool and transported to the laboratory immediately.

2.2. Nutritional state and oxidative stress

Using fresh blood, we determined haemoglobin (Hb) concentration by the cyanmethaemoglobin method. After this evaluation, blood was centrifuged to obtain erythrocytes pellet, and plasma to determine albumin and cholesterol concentrations using commercial kits from Spinreact based on the Rodkey (1956) and Meiattini et al. (1978) protocols respectively. Remaining plasma and erythrocytes pellet was stored separately at −80 °C until use.

Lipid peroxidation was estimated in erythrocytes pellet by thiobarbituric acid reaction (TBA) with malondialdehyde (MDA), a product of the peroxidation of membrane lipids according to the method of Ohkawa et al. (1979). Carbonyl groups formed from oxidation with 2,4-dinitrophenyl hydrazine (DNPH) were estimated in pellet using the methods by Reznick and Packer (1994). Estimation of derivatives of 2,4-dinitrophenyl hydrazones may be used as a pattern of oxidative modification of proteins during oxidative stress in cells. Regarding the antioxidant enzymatic defense, superoxide dismutase (SOD) activity was measured by pyrogallol oxidation method (Marklund and Marklund, 1974), and catalase (CAT) activity was evaluated using the hydrogen peroxide breakdown method (Cohen et al., 1970); both enzymatic activities were measured in erythrocytes pellet. Glutathione ratio (GSH/GSSG) and levels of total antioxidant activity (TAC) in plasma were estimated using commercial kits from Arbour Assays and Nanjing Jiancheng Bioengineering Institute respectively.

2.3. Statistical analyses

Principal components analysis (PCA) was performed to summarize environmental parameters related to urbanization process (population, land uses and air pollutants) into independent factors in order to establish an urbanization value for each study area, based on which were classified into rural and urban locations.

In order to evaluate sexual differences and urban/rural effects, sex and categorized study areas were used as fixed factors in ANOVA tests while haemoglobin concentration, amount of albumin and cholesterol, level of lipid peroxidation and protein oxidation, catalase and SOD activities, GSH/GSSG ratio and total antioxidant capacity (TAC) were tested as response variables.

Normal distribution of data was assessed using the Kolmogorov–Smirnoff test. All analysis were performed using STATISTICA© software.

3. Results and discussion

3.1. Area characterization and the use of birds as bioindicator

Classification of study areas was established running a PCA using the environmental traits previously mentioned. The first principal component (PC 1) explained 55.20% of the variability of the environmental data. To simplify the study and according to this rank the localities were separated into rural or unpolluted areas: OF, EE; and urban or polluted areas: FB, MD (Table 1).

Urban areas are polluted with potentially toxic chemicals, like heavy metal particles, gases and other substances; mostly released into the atmosphere as a result of incomplete combustion of fossil fuels associated with motor vehicles, industrial activities and electricity generation (Georgiadis and Kyrtopoulos, 1999).

Monitoring of environmental quality is usually based on the quantification of contaminants bioaccumulated by birds. However, in some cases these procedures show disadvantages such as: do not highlight the complexity of the interactions that take place between air pollutants and cellular systems, non-accumulative pollutants cannot be detected, and quantification procedures in some cases involved the death of birds because large tissue volumes are needed (Swaileh and Sansur, 2006).

Conversely, analysis of urban environmental quality from an ecophysiological standpoint presents fewer legal and ethical problems. Almost any tissue, including blood, can be used for assaying oxidative stress; as they are all susceptible to oxidative damage and

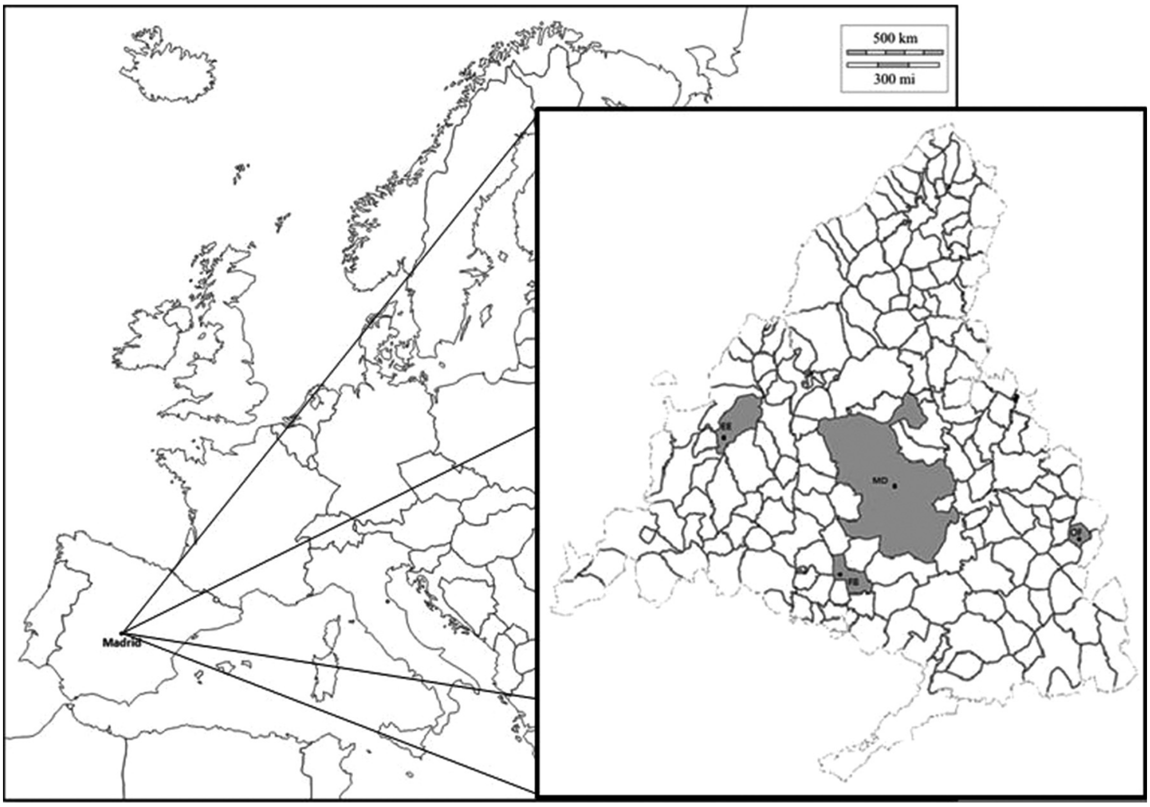

Fig. 1. Location of the sampling sites (in grey): Madrid (MD), Fuenlabrada (FB), El Escorial (EE), Olmeda de las Fuentes (OF).

all, to a greater or lesser extent, express some antioxidant defense system. Red blood cells, which are in close contact with toxics, are susceptible to suffer oxidation and sampling can be performed without harming the bird (Costantini, 2008; Geens et al., 2010).

This should be always taken into account, especially in case of threatened species. Although House Sparrow may be a good bioindicator of urban environment, their populations are declining in many European cities, so the accomplishment of studies involving the death of birds is not feasible (De Laet and Summers-Smith, 2007).

3.2. Nutritional state and oxidative stress

In relation with human health, epidemiological studies have consistently found direct relation between air pollution and respiratory and cardiovascular disease and possible increased cancer risk (Seaton et al., 1995; Wolterbeek, 2002). Many of these pathologies have in common uncontrolled production of free radicals that exceeds capacity of antioxidant defense systems (Georgiadis and Kyrtopoulos, 1999; Romieu et al., 2008). In fact, oxidative stress is common factor mechanism of toxicity of anthropogenic pollution like heavy metals and combustion-derived hydrocarbons gases, inter alia (Isaksson, 2010). This approach is consistent with other authors who agree to consider the analysis of oxidative stress status in urban birds as a good biomarker of environmental quality (Isaksson, 2010; Kaminski et al., 2007; Koivula and Eeva, 2010). However, discrepancies arise when determining what parameters related to oxidative stress are the most representative.

In our case, significant difference parameters between polluted and unpolluted areas were haemoglobin concentration and total antioxidant capacity.

Haemoglobin (Hb) as well as total antioxidant capacity (TAC) were significantly lower in urban areas, ($F = 6.04$; $p \leq 0.05$) and ($F = 4.01$; $p \leq 0.05$) respectively. However no differences were found in the rest of nutritional state and oxidative stress parameters (Table 2). In addition, ANOVA test confirmed no difference between sexes for analyzed variables.

Oxidizing molecules can lead to methaemoglobin (HbM) formation if present at concentrations exceeding those of the natural antioxidant systems. Atmosphere air pollutants, like CO, NO_x, SO_x and other fuel combustion products, induce methaemoglobinemia in Pidgeon (*Columba livia*) (Sicolo et al., 2009). In the same way, presence of heavy metal particles as lead (Pb) and cadmium (Cd) seemed to have a negative effect on the haemoglobin concentration of Great Tits (*Parus major*) (Geens et al., 2010). So, haemoglobin value significant decrease found in House Sparrow urban population could be explained by urban air pollution detected in cities.

Likewise, atmospheric pollutants could be responsible for significant decrease in total antioxidant capacity found in urban populations. Major atmospheric pollutants have been described as

Table 1
Urbanization scores are the PC 1 values from a principal components analysis conducted on scores of the six habitat variables. PC 1 is statistically significant ($p \leq 0.05$) and it explains 55.20% of variation. Population (total inhabitants), industrial land (%), urban land (%), CO (mg/m^3), NO_x ($\mu g/m^3$).

Site	Population	Industrial land	Urban land	CO	NO_x	SO_x	PC 1	Classification
OF	338	0	15.86	0.30	5	3	−3.14	Rural
EE	15,161	0	92.51	0.50	45	4	−0.26	
FB	198,132	7.81	100	0.60	51	4	1.04	Urban
MD	3,233,527	9.78	100	0.45	55	6	2.36	

Table 2
Mean values of each population of nutritional state, oxidative damage and antioxidant defence. All values are expressed in mean ± SD; sample size (n).

		Urban	Rural
Nutritional status	Hb (g/dL)	15.11 ± 3.70* (36)	16.99 ± 2.72 (36)
	Albumin (g/dL)	1.17 ± 0.21 (36)	1.23 ± 0.28 (35)
	Cholesterol (mg/dL)	152.53 ± 37.66 (36)	162.33 ± 42.73 (35)
Oxidative damage	TBARS (nmol MDA/mL)	103.06 ± 17.55 (36)	109.99 ± 23.13 (36)
	Carbonile groups (μM)	24.11 ± 3.84 (36)	22.40 ± 5.50 (35)
Antioxidant defense	CAT activity (UK/mL)	1.71 ± 1.74 (33)	1.77 ± 1.38 (30)
	SOD activity (UID/mL)	3.21 ± 1.27 (36)	3.59 ± 1.27 (35)
	GSH/GSSG	32.28 ± 28.30 (34)	32.12 ± 26.30 (36)
	TAC (U/mL)	37.50 ± 10.87* (34)	43.61 ± 13.38 (29)

* Significant statistical differences ($p \leq 0.05$) are indicated.

pro-oxidant elements (Isaksson, 2010). Total antioxidant capacity (TAC) evaluates balance between degree of oxidation (peroxidation, protein oxidation and DNA); and response of antioxidant defense systems: enzymatic (SOD, catalase), non-enzymatic (GSH, metallothionein) and exogenous (vitamins and minerals from diet) (Costantini, 2008). Previous studies in White Stork (*Ciconia ciconia*) concluded that activity of antioxidant enzymes like SOD and catalase as well as biomarkers of oxidative damage like content of TBARS, are determined by toxic heavy metals present in atmosphere (Kaminski et al., 2007). Kurhalyuk et al. (2009), reported similar results; in their study in urban Pigeons (*C. livia*), presence of lead (Pb) and cadmium (Cd) was related with an increase of oxidative damage in lipid and protein. Thus, disruption of this balance in urban areas may be due to an increased degree of oxidation together with a lack of response of antioxidant defense systems. However this parameter is not enough to exactly determine what oxidative stress marker is affected (Costantini and Verhulst, 2009).

Conversely with these results, Isaksson (2010) in a meta-analyze described that reduced form of glutathione (GSH) and its associated enzymes are the most reliable biomarkers, although it is reported that responses are species-specific. Therefore, response of each species should be analyzed and characterized independently in order to use as a reliable biomarker.

Not only environmental pollutants could increase oxidative stress. Sex, age, diet, physical condition or a pathological situation could also influence on cell oxidative balance (Costantini, 2008).

In this way, the next step to follow is increase number of species, sample size and analyzed localities to determine which oxidative stress markers are the most reliable. Moreover, improved quantification of environmental pollutants and the characterization of habitat could confirm our hypothesis about the influence of air quality on oxidative stress of urban species.

4. Conclusions

House Sparrow oxidative damage and consequent antioxidant response to pollution of cities seem to be a reliable biomarker and an essential complement for monitoring urban environmental quality. Furthermore, analysis of the parameters in the blood is a simple and non-invasive method for birds.

However, an increase of birds and localities analyzed and an improved characterization of environmental pollution, are necessary to obtain more conclusive results.

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