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Reproductive effects in hybrid sparrow from a polluted area in Tunisia: Oxidative damage and altered testicular histomorphology

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ABSTRACT

Air pollution is a threat for human health and wildlife. The aim of this study is to assess the pathophysiological changes and the oxidative–antioxidative status in testicular tissues of 40 Hybrid sparrows collected from four areas in Gabès city, one of the most polluted areas in Tunisia. The testis histopathological analysis revealed alterations in birds from Ghannouche, the polluted area. The thiobarbituric acid reactive substance (TBARS) levels were higher in testis of birds from the contaminated site compared to less polluted areas indicating oxidative damage to membrane lipids. Antioxidant enzyme activities (superoxide dismutase and catalase) were lower in testis sparrows from the polluted site compared with the reference site, suggesting deficiency of the antioxidant system to compensate for oxidative stress. Overall, our results suggest that the hybrid sparrow offers a suitable model for biomonitoring programs of atmosphere pollutants and the selected biomarkers could be useful tool to evaluate pollution impacts in living organisms.

1. Introduction

Rapid progress in the industrial sector during the last century has resulted in the production of wide range of industrial effluents which can lead to various deleterious effects not only to the living organisms but also to the ecological equilibrium of the biosphere. In fact, it has been shown that exposure to carbon monoxide (CO), sulfur oxides (SOx), nitrogen oxides (NOx), heavy metal particles and other combustion-derived hydrocarbons gases, is associated with numerous harmful effects (Isaksson, 2010).

It has been also reported that the exposure to pollutants can decrease measures of reproductive performance (Marettová et al., 2015). Therefore, their toxic effects on male reproduction system have become a major health concern in the globe (Chowdhury, 2009; Sharma and Garu, 2011).

Wild birds are often used as environmental sentinels for industrial contamination due to their high metabolic rates and sensitivity to xenobiotics (Belskii et al., 2005; Eeva and Lehikoinen, 1996; Morrison, 1986). Particularly, air pollutants, such as heavy metal pollution, are shown to affect different phases of the avian life cycle, from egg development to adult reproduction (Scheuhammer, 1987; Eeva and Lehikoinen, 2000; Janssens et al., 2003a,2003b).

Hybrid sparrow (*Passer domesticus* × *Passer hispaniolensis*) is distributed worldwide. It is sedentary and closely associated with urban environments. These characteristics make it one of the most suitable candidates for urban biomonitoring of industrial contamination in terrestrial ecosystems of atmosphere pollutants (Swaileh and Sansur, 2006).

In recent years, there has been growing concern about the deleterious effects of pollutants on developing male reproductive system in terrestrial free-living birds (Tsipoura et al., 2008; Sánchez-Virosta et al., 2015). The impact of pollutants on reproduction need to be more studied to understand the real ecological impact of contaminants and to complete the evaluation of their toxicological profile.

To evaluate the impact of xenobiotic substances on reproduction performance of birds, numerous ecotoxicological biomarkers

Abbreviations: CAT, catalase; Cd, cadmium; CO, carbon monoxide; Cr, chromium; Cu, copper; GSI, gonadosomatic index; IT, interstitial tissue; MDA, malondialdehyde; NOx, nitric oxides; Pb, lead; ROS, reactive oxygen species; SOD, superoxide dismutase; Sox, sulfuric Oxides; ST, seminiferous tubules; TAS, total antioxidant status; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; VA, vitamin A; VE, vitamin E; Zn, zinc

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have been employed in the last decades. Some authors propose the use of ecological indexes like gonadosomatic indexes to evaluate the influence of biotic processes or as an additional tool in biomonitoring approaches (Adams and Ryon, 1994). Histopathological changes in gonads have been widely used as biomarkers in the evaluation of the health of animals exposed to contaminants (Badraoui et al., 2010; Marettová et al., 2010; Atef, 2011; Marettová et al., 2015).

Many pollutants may exert toxicity through oxidative stress, disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species (ROS) or by the depletion of antioxidant molecules (Ercal et al., 2001; Stohs and Bagchi, 1993). An excess of ROS may cause oxidative damage to membrane lipids, DNA and proteins, and their oxidation can lead to cellular dysfunction and tissue injury (Hoffman et al., 1998; Valavanidis et al., 2006). Therefore, tissue levels of lipid peroxidation (LPO) are proven to be an indicator of oxidative stress (Tandon et al., 2003; Alvarez et al., 2004). A common biomarker for measuring lipid peroxidation is thiobarbituric acid reactive substances (TBARS) level (Oakes and Van Der Kraak, 2003; Almroth et al., 2005).

Antioxidants are a major resource of most living organisms for protection against diverse free radicals and other oxidative stressors (Cross et al., 1987; Griffith, 1999). Because several antioxidants are needed to protect against ROS and antioxidant defense may respond differently depending on species, previous studies have shown that the measure of the levels of antioxidant molecules could be interesting biomarkers of pollutant exposure (Berglund et al., 2007; Koivula and Eeva, 2010). It has also been shown that oxidative stress toxicity caused by xenobiotics intoxication affected fertility (Aruldhas et al., 2005).

The pollution of living environment in Gabès, one of the most remarkable pollution hotspots in North Africa and the Mediterranean (Azri et al., 2002a, 2002b), presents an ecological problem because of the installation in the early 1970s of intense phosphate treatment industries for acid and fertilizer production in the Gabès–Ghannouche factory complex. It has been proposed that the Gabès–Ghannouche factory complex releases 10,000 to 12,000 t of phosphogypsum, containing heavy metals mainly, cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and chromium (Cr), per day in the sea (Béjaoui et al., 2004; Ayadi et al., 2015). In addition, the industrial process of phosphate treatment ejects huge quantities of toxic gases, especially SOx and NOx into the air (Azri et al., 2002a, 2002b). The industrial activities in the region may have contributed to the degradation of the biodiversity of the ecosystem. A previous study showed a decreased breeding performance of passerines living near the factory complex (Alaya-Ltifi et al., 2012). However, to our knowledge, no studies have assessed histopathologic alteration and oxidative stress biomarkers in Hybrid sparrow testes captured from Gabès which could of considerable interest.

In this regard, the present study is designed to investigate the impact of pollutants on reproduction performance in Hybrid sparrow using many biomarkers including histological alterations, levels of TBARS, antioxidant enzymes activities of superoxide dismutase (SOD) and catalase (CAT) in testes and plasmatic levels of vitamins E and A (VE and VA respectively) and total antioxidant status (TAS) in the oasis habitat close to Gabès city in south-eastern Tunisia. Our approach was based on the comparison between one oasis situated about five hundred meters from the factory complex, and hence exposed to a high pollution level, (polluted oasis), one oasis situated eight kilometers, one oasis situated eleven kilometers and one oasis situated twenty kilometers apart and less exposed to pollution (control oasis).

2. Material and methods

2.1. Study area and species

Samples used in this work were collected in four locations in the gulf of Gabès, in the south-east of Tunisia;

(i) Ghannouche oasis (33°56′N–10°04′E), which is situated close to the Gabès–Ghannouche factory complex, (ii) Mettouia oasis (33°58′N–10°0′E), iii) Ouedref (33°59′N–9°58′E) and (iv) Kettena oasis (33°45′N–10°13′E), which is situated 20 km to the Southeast in one industry free area. Kettana was chosen to serve as reference site not only because it's the furthest from the factory complex, but also because it's located on the opposite side of the global direction of the wind in Gabès (west) (Elamouri and Ben Amar, 2007) (Fig. 1).

The hybrid sparrow, in Tunisia, is a result of hybridization of Spanish sparrow (*P. hispaniolensis*) and the House sparrow (*P. domesticus*) (Johnston, 1969). Our specie has been suggested to be of hybrid origin because the plumage of male individuals which is intermediate to males of the Spanish sparrow and the House sparrow, but it's not the same as the Italian sparrow (*Passer italiae*). Birds, chosen for this study, are residents and often nesting and feeding in urban areas close to humans constructions (Selmi, 2000). The selected specie is primarily granivorous during the breeding season (Alaya-Ltifi and Selmi, 2014). It is a sexually dimorphic bird (Summers-Smith, 1988). There were also some subtle differences between yearlings and male adults. In particular, adults showed new rufous feathers, while yearlings had a mixture of male and female-type plumage (Hammouda et al., 2015). In this study, no phenotypic change was detected between the four populations of sparrows sampled. The identification of the specie has been confirmed by an ornithologist (M.A.C.).

A total number of 40 male sparrows were captured between March 2014 and April 2014, to coincide with the reproduction period. Individuals were trapped in mist nets. Birds were captured and brought alive to the laboratory immediately. Upon arrival at the laboratory, each sparrow was weighed. A 500 µl blood sample was taken from the jugular vein, using a sterile syringe. Then, the sparrow was sacrificed under anesthesia by an intraperitoneal injection of 8% chloral hydrate (400 mg/100 g BW) and the testes were removed and weighed. One testis was fixed in formaldehyde solution for histological examination and the other was strored at −20 °C until analyze for oxidative damage biomarkers. The blood was centrifuged (10 min at 5000 g) and the resulting plasma

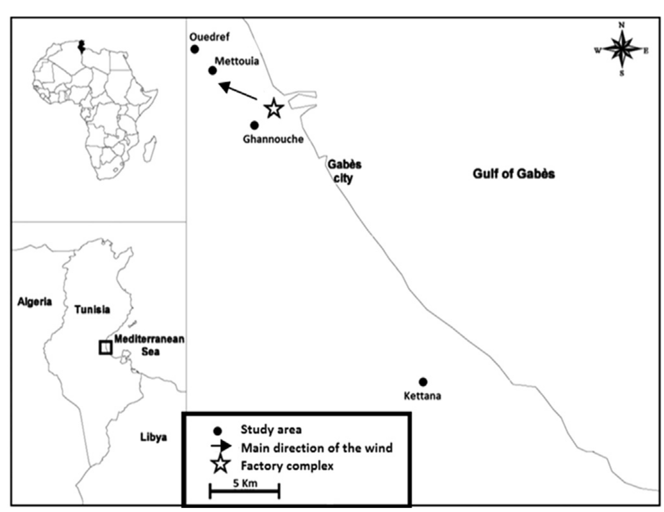

Fig. 1. Map of the gulf of Gabès in south-eastern Tunisia showing the locations of Gabès–Ghannouche factory complex and the four studied areas: Ghannouche, Mettouia, Ouedref and Kettana.