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House sparrow biomarkers as lead pollution bioindicators. Evaluation of dose and exposition length on hematological and oxidative stress parameters

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ABSTRACT

House sparrows (*Passer domesticus*) have been proposed as a key ecological indicator of urban pollution. Remarkably, we lack knowledge about the physiological effects of lead on this bird species. Therefore, this study was aimed to evaluate the effect of Pb on several physiological parameters in house sparrows exposed to environmental Pb concentrations. In a first experiment, birds were exposed to Pb sub-lethal doses (from 1.3 to 14.0 µg of Pb/g animal/day) during 5 days, which resulted in a dose response increase of blood Pb levels and decrease of blood ALAD activity. However, at the higher doses tested (> 7 µg of Pb/g animal/day) the blood ALAD activity inhibition (~82%) remained constant. Hematocrit and hemoglobin were significantly reduced only at the highest-doses, and the stress indicator, heterophils to lymphocyte (H/L) ratio, did not show apparent changes.

In a second experiment, house sparrows were exposed to Pb in drinking water (12.3 ppm) during either 15 or 30 days. Pb concentration used in this study was enough to produce blood lead levels equivalents to those found recently in house sparrows inhabiting urban areas, reduced blood ALAD activity and inversion of the H/L ratio. Decreasing blood ALAD activities were correlated with increasing blood Pb levels. In addition, Pb exposure produced modification in the levels of hepatic antioxidant enzymes, increased GST activity and decreased CAT activity, without lipid peroxidation.

In conclusion, our results suggest that blood ALAD activity is a reliable and sensitive biomarker for environmental Pb exposure in house sparrows, additionally chronic exposure produce physiological stress (H/L inversion) and small changes in antioxidant enzyme activity. Finally, this specie could be considered a bioindicator for monitoring the urban Pb contamination.

1. Introduction

Lead (Pb) is a toxic element that occurs naturally and is found in small amounts in the earth's crust, but industrial activities and urbanization led to its redistribution in the environment (Levin et al., 2008). Even though, in recent times the Pb released to environments have been significantly reduced, they may remain contaminated for very long time periods because of high past emissions and the persistence of this heavy metal. Industrial and urban soils are frequently enriched in Pb (Kabata-Pendias, 2010) with levels of micrograms per gram in urban areas, while background concentrations in unpolluted soils do not exceed the tenths of micrograms per gram (dry soil) (Kabata-Pendias, 2010). In line with this observation, wildlife that live in industrialized, urbanized and intensive agricultural areas has been reported having augmented trace metal concentrations. For example, urban populations of house sparrows, starlings, and pigeons displayed higher heavy metals concentration than rural populations (Bichet et al., 2013; Kekkonen et al., 2012; Millaku et al., 2015; Nam and Lee, 2006; Swaileh and Sansur, 2006).

Bird biomarkers are useful bioindicators of pollution and very

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frequently used as such (Burger, 1995). Some of the reasons behind this choice are the broad diversity of bird species found in most biomes and the different trophic levels they may occupy according to their position within food webs, which can provide useful information about bioavailability, magnification and bio-transference of pollutants (Cid et al., 2009; Swaileh and Sansur, 2006). Birds accumulate high levels of pollutants in their tissues and are particularly susceptible to display physiological effects. Recently, certain urban birds species were suggested as bioindicators for urban heavy metals contamination (Bichet et al., 2013; Nam and Lee, 2006; Swaileh and Sansur, 2006).

The analysis of bioaccumulation of contaminants in the biotic components of ecosystems is an important and useful tool for understanding persistence, movement and allocation of these compounds. However, ecosystem and species conservation and management require the evaluation of the health risks of organisms as well, which in turn primarily compels to assess the effects of contaminants on organism physiology (Herrera-Dueñas et al., 2014). The adverse effects of Pb have been well documented in birds and other animals (Eisler, 1988). The fastest toxic effect reported after Pb exposure is an inhibition of the δ-aminolevulinic acid dehydratase (ALAD) (Finley et al., 1976), an enzyme involved in heme biosynthetic pathway. Pb exposition also can produce a decrease of blood hemoglobin, hematocrit and increased blood porphyrin levels (Eisler, 1988). Changes in ALAD enzyme activity combined with hematocrit and hemoglobin levels have been extensively used as a proxy for Pb exposure. Nevertheless, ALAD inhibition varies between species, and is influenced by the level and time of Pb exposition (Eisler, 1988). Thus, in spite of the broad and routine use of ALAD assays in numerous wildlife species, its use, especially in comparative studies, must be cautiously interpreted.

Heavy metals are considered as environmental stressors that could produce physiological stress in animals. In this sense, several studies demonstrated that stress increases the heterophils/lymphocyte (H/L) ratio, supporting the use of this hematological parameter as proxy of stress status (Davis et al., 2008). For this reason, H/L index is becoming widely reported as a complementary method for understanding the physiological stress of heavy metals exposure in birds (Grasman and Scanlon, 1995; Plautz et al., 2011). Additionally, the accumulation of δ-aminolevulinic acid (ALA) in cells produced by the inhibition of ALAD activity along with the Pb activity itself, induce the generation of reactive oxygen species (ROS) and produce cellular oxidative stress (Martinez-Haro et al., 2011). Congruously, the assessment of oxidative stress parameters is increasingly used as biomarkers of heavy metal exposure in free-living birds (Berglund et al., 2007; Espin et al., 2014; Martinez-Haro et al., 2011). However, the differences between bird species still quite poorly known (Koivula and Eeva, 2010). To our knowledge, there are not studies on the effects of each heavy metal, particularly Pb, on oxidative stress biomarkers on house sparrows and passerine birds under controlled experimental conditions.

House sparrow (*Passer domesticus*) has been proposed as a suitable bioindicator to evaluate and compare Pb pollution within and between urban zones, because it is sedentary, strongly related to urban environments and has a worldwide distribution (Swaileh and Sansur, 2006). This species have been used to evaluate bioaccumulation of heavy metals by measuring the concentrations of these metals in different biological samples (i.e., bone, liver, kidney, brain, feathers, eggs, etc.) (Bichet et al., 2013; Kekkonen et al., 2012; Millaku et al., 2015; Swaileh and Sansur, 2006). However, strikingly, information on the Pb effects on most functional traits of house sparrows is not available. Therefore, the main objective of this work was to evaluate the effect of different doses and different time of Pb exposure, on the blood lead levels, the activity of ALAD, the hematocrit, the concentration of hemoglobin, the heterophil/lymphocyte (H/L) index and oxidative stress parameters [e.g., glutathione-S-transferase (GST), catalase (CAT) and glutathione reductase (GR) activities, and thiobarbituric acid–reactive substance (TBARS)] in house sparrows.

2. Materials and methods

2.1. Animal care and housing

Adult house sparrows were live-trapped (mist net) near the Universidad Nacional de San Luis Campus (San Luis, Argentina). The birds were housed individually in cages (40 × 25 × 25 cm) in a room maintained at constant environmental conditions, temperature 23 ± 1 °C, relative humidity 40 ± 10%, photoperiod of 14:10 h light-dark cycles, and provided with water and food ad libitum (seeds supplied with vitamins and minerals). Animals were acclimated to laboratory conditions at least for eight weeks before using them in experiments.

All animal experiments and procedures were approved by the Animal Care and Use Committee (CICUA UNSL permit number B86/11) of the Universidad Nacional de San Luis, Argentina and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council U.S.).

2.2. Experimental design

2.2.1. Experiment 1: Exposure to different doses of Pb

After the acclimation period, thirty house sparrows weighing 22–27 g were selected and randomly divided in six groups of five animals each. One of these groups was randomly chosen as control group and the other five, as experimental groups. The body masses of animals were not statistically different at the beginning and end of the experiment (data not shown). Additionally, values for hematocrit, hemoglobin, ALAD enzyme activity, and H/L index before treatments were similar between groups (data not shown).

Each individual of each of the five experimental groups received a single daily dose of Pb acetate solution for five days; dose for each group was 1.3, 3.5, 5.5, 7.0, 14.0 µg of Pb/g animal/day, respectively. Pb acetate has been widely used to evaluate Pb toxicity, because it is a form of lead that is highly bioavailable (Eisler, 1988). All the administered doses were lower than those considered as sublethal for other bird species (Eisler, 1988). Control animals received an equivalent sodium acetate solution. A gavage of 270µl Pb or sodium acetate solution was administered in less than 30 s by carefully inserting a blunt-edge cannula deeply through the esophagus of the bird without anesthesia. All gavages were performed between 9:00 and 10:00 a.m. Twenty-four hours after the last administration, animals were weighed, and blood (< 10% total blood volume) was sampled by brachial vein puncture and collected in heparinized capillary tubes.

2.2.2. Experiment 2: Different time Pb exposure

In this experiment, house sparrows were Pb-exposed via drinking water supply for 15 or 30 days. Thirty-three acclimated house sparrows weighing between 22 and 26 g were randomly divided in three groups of eleven individuals each, six males and five females in each group. Negative control (without Pb) and experimental groups were setup to compare the effects of Pb exposure for 15 days (EP15) or 30 days (EP30). Drinking water of experimental groups was prepared with Pb acetate to obtain a concentration of Pb of 12.3 ppm, this concentration produced similar blood Pb levels as those reported recently in urban sparrows (see below). Drinking water of all groups (with and without Pb) and food was daily and freshly supplied ad libitum. Water intake was daily measured every morning between 08:00 and 09:00 a.m., and animals were weighed at the beginning and end of the experiment. After treatments, between 08:00 and 09:00 A.M., birds were blood sampled [< 10% of total blood volume] by brachial wing vein puncture with heparinized capillary tubes, eighteen blood samples were stored in acid-cleaned vials for Pb determination. Then, animals were anesthetized with isoflurane, the abdominal cavity was opened and liver was removed. Eighteen livers were immediately frozen in liquid nitrogen for oxidative stress determinations and fifteen livers were