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MONOGRAPH

Comparison between aluminum bioaccumulation in samples of the Muscovy duck *Cairina moschata* (Linnaeus, 1758) (Aves Anatidae) from the city (Palermo) and the country (Monreale) (Italy)

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ABSTRACT *Cairina moschata* (Linnaeus, 1758) is an anatid originating from South America, easily adapted to the European climate. In this work, feathers and blood were used as samples from living individuals to evaluate the bioaccumulation of aluminum. The determination of Al accumulation was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analysis was conducted in the "Istituto Zooprofilattico Sperimentale della Sicilia" (Palermo, Italy) with validated methods (accredited by ACCREDIA) for biodiversity monitoring and analysis of fauna samples. Samples were collected from a total of twenty individuals of *C. moschata*, ten samples coming from a park in the city center of Palermo (Southern Italy, Parco d'Orleans), and ten from the field of Monreale (Palermo). Blood from city samples showed a higher level of aluminum than city samples; feathers have had an opposite trend. Al median value determined in blood was ±4,27259 mg/Kg and ±2,61815 mg/Kg respectively for the city (Palermo) and Monreale. In feathers, the median value was ± 402,24218 for samples collected in city and ±1260,75603 for samples collected in Monreale. The concentration levels of Al in feathers were higher in Monreale samples than in Parco D'Orleans, probably because the individuals that live in nature attend the reservoirs where pollutants are poured.

KEY WORDS Anatid; aluminum; bioaccumulation; *Cairina moschata.*

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INTRODUCTION

According to the European Environment Agency (E.E.A), environmental monitoring is the "*measurement, assessment and determination of environmental parameters and pollution levels in order to prevent negative and harmful effects on the environment*". A natural disturbance event usually has a biological consequence that tends to strengthen the stability of the ecosystem: a possible natural fire leads to the loss of some species, leaving a void (patch) that will be filled over time by one or more species with different ecological roles, restoring the environment in a steady-state following the disturbance

(Connell, 1978). Quite different consequences, on the other hand, cause an external disturbance, which is therefore not natural. The anthropic perturbations are not tied to spatial and temporal scales. Therefore, they cause a temporary or permanent alteration of the natural functionalities (Odum et al., 2007).

The biological or biomonitoring approach measures structural and functional parameters on different scales, taking into account alterations that affect each level of the ecosystem (Boswell & Bayne 1986). Bioaccumulative organisms, biotic indicators at the organism level, can assimilate measurable quantities of chemical and xenobiotic elements, returning information on the presence of contaminants in the environment. A good bioaccumulator tends to accumulate in its body concentrations of contaminants higher than those of the environmental compartment in which it is located. The storage capacity of these organisms is directly proportional to the time spent in the polluted environment. In top predators, it is then possible to detect several pollutants due both to their longevity (accumulation during the life cycle) and to their feeding (accumulation by the prey ingested, in turn, contaminated). The need to verify possible strategies of biomonitoring led us to use sentinel species as subjects of a species of aquatic birdlife, in particular Muscovy duck (*Cairina moschata*). In many cases the birds have proved more sensitive to environmental contaminants than other vertebrate species (Furness & Camphuysen, 1997). Specifically, water birds can serve as sentinel species both in natural environments and in environments reproduced by humans. Many species of birds, in fact, live in close contact with human activities and are therefore exposed to xenobiotics whose effects they can reveal (Malik & Zeb, 2009). Thanks to the easy manipulation of the ducks and to carry out a biomonitoring analysis using non-deterrent biomarkers, we performed sampling on subjects of different sex of *C. moschata.*

MATERIAL AND METHODS

Sampling method

A total of 40 samples were collected divided into 20 blood samples taken from the brachial vein, 20 samples of feathers taken from the ventral, and wingspan of each subject. Once collected, all biological samples were stored in their sampling packages and transferred to the laboratory in refrigerated containers, duly labeled and stored in the refrigerator, and then treated for analysis within a week of their arrival. Each feather was washed with 5% of nitric acid. The samples were taken from 10 different individuals, mixed between male and female individuals, located in one of the aviaries at Parco d'Orleans (Palermo, Italy) and ten other individuals, equally mixed in sex, in the Monreale area (Palermo) between June and November 2018. The two sampling areas were chosen according to the central and peripheral location of the province of Palermo, considering therefore the area of Parco d'Orleans (in Fig. 1 marked by the letter A) more subject to exposure of environmental contaminants than to the peripheral area of Monreale (in Fig. 1 marked by the letter B), in which car traffic and human activities are lower.

Instrumentation

The analysis was conducted by an ICP-MS (7700x series, Agilent Technologies, Santa Monica CA, USA) with ASX-500 series (Agilent Technologies, Santa Monica (CA), USA) as autosampler. Microwave-assisted system Multiwave 3000 (Anton-Paar, Graz, Austria) was used for digestion; it was equipped with a rotor for eight MF100 PTFE-TFM (poly-tetrafluoroethylene-tetrafluoroethylene) vessels.

Reagent and gases

All solutions were prepared with ultra-pure grade analytical reagent. Ultrapure deionized water was obtained by Milli-Q® Integral water purification system with Q-pod (Millipore, Bedford, MA, USA). Ultrapure nitric acid was purchased from Merck KgaA (Darmstadt, Germany). The different Al solutions (0.05-10 µg/L) were prepared from a solution of 1000 mg/L ICP-MS grade standard, traceable to the National Institute of Standards and Technology (NIST) purchased from Merck KgaA (Darmstadt, Germany).

Chemical analysis

The Al concentration was conducted according to the protocol of Lo Dico et al. (2018). About 1 g

Figure 1. Area of sampling: Palermo Parco d'Orleans (A) and Monreale (B).

Type of samples	Orleans (PO)	Monreale (MO)	Statistical significance (p=value) Wilcoxon test	% differences between habitat
Blood	±4.27259 (2.91974-11.5398)	±2.61815 (0.97769-10.23846)	0.08098	+38% (PO)
Feathers	±402.24218 (79.6690-1472.2538)	±1260.75603 (550.709-5668.875)	1.97E-5	+ 89% (MO)

Table1. The median of Al content is expressed as mg/Kg. The values in brackets are minimum and maximum. The p-value is referred to the Wilcoxon signed-rank test.

of the samples were transferred into a decontaminated PTFE (poly-tetrafluoroethylene-tetrafluoroethylene) vessels with 3 ml of Ultrapure nitric acid 60% (V/V) and 5 ml of water. The samples digestion was carried out using a microwave digester Multiwave 3000 (Anton-Paar, Graz, Austria).

Statistical analysis

Statistical analyses were conducted with R (3.2.4). A Shapiro-Wilk test was carried out in order to verify the normality of distribution. A Wilcoxon signed-rank test was carried out in order to verify significant differences between Parco d'Orleans (PO) and Monreale (MO) samples and between feathers and blood from the same samples.

RESULTS

Statistical analysis

Shapiro-Wilk normality test showed a non-normal distribution for all samples with a p-value < 0.05 for blood MO (p-value = 0.003003), blood PO (p-value = 0.003127), feathers MO (p-value = 0.0003087) and feathers PO (p-value = 0.01122). Significative differences were found between Al content of feathers collected in MO and PO (Wilcoxon signed-rank test p-value< 1,97E-5) and between Al content in feathers and blood collected from the same samples in the same place (MO Wilcoxon p-value = 1.451e-11, PO Wilcoxon p-value = 1.451e-11). No significative differences were found between blood from MO and PO sam-

ples (Wilcoxon p-value = 0.08098). Results of statistical analysis are showed in Table 1.

Aluminum content in the blood

The median of the aluminum content in the blood was ±4.27259 mg/Kg and ±2.61815 PO and MO, respectively, with a higher-min value of ±2.91974 - ±11.15398 mg/Kg for PO samples and ±0.97769 - ±10.23846 mg/Kg for MO samples (Table 1). The median of PO was higher (+38,72%) than the median of MO samples.

Aluminum content in feathers

All feathers samples have a higher Al value than blood samples. In PO feathers were found a median of ±402.24218 mg/Kg in a range of ±79.6690 mg/Kg as min and a maximum of ±1472.2538 mg/Kg. Unlike blood, the aluminum content was higher in the MO samples (+68%) with a median of ±1260.75603 mg/Kg and a range of ±550.709-5668.875 mg/Kg (Table 1).

DISCUSSION

Different species of aquatic birds were already used to determine the concentration of metallic elements in their habitat. However, most of these studies have used tissue samples taken during necropsies (Binkowski et al., 2013; Braune & Scheuhammer, 2008; Di Giulio & Scanlon, 1984). Due to the ease with which we took and analyzed non-destructive *C. moschata* matrices, the use of this species proved to apply to biomonitoring investigations. In the studies of biomonitoring, it is fundamental the close connection of the animal with the analyzed habitat (Walker et al., 2012); the accumulation trend was opposite for blood (higher in PO) and feathers (higher in MO), probably this is due to a higher concentration of aluminum in the food of PO specimens compared to those of MO and to a higher concentration of aluminum in the MO environment compared to that of PO.

From the results emerged, it is evident that the matrix of the feathers results from having a higher capacity of bioaccumulation (Abdullah et al., 2015), probably because the presence of metalloproteins in the blood can sequester the circulating metal elements (Church et al., 1993).

Furthermore, the concentration of Al in feathers reflects endogenous and exogenous exposure, and it is quite useful when it is impossible to take other tissues without resorting to animal sacrifice (Mikoni et al., 2017). However, the matrix "blood" remains an excellent tool for assessing low bioaccumulative concentrations during rather short periods (Berglund, 2018), before the detoxification mechanisms are "chelated" entirely to the metals present.

CONCLUSIONS

This study reveals that non-destructive matrices of easy availability such as blood and feathers of *C. moschata* could be valid instruments that can be used in biomonitoring the exposure of Al as pollutants. The levels measured in the feathers make it possible to make a prediction of the concentrations of Al in the internal tissues and in the area. The comparison of these matrices over time also makes it possible to observe the fluctuations of the contaminants in environments where there are no air quality detection stations. However, it is necessary to deepen the presence of aluminum in the food of the two areas, in the aquifers and the presence of the other trace elements in feathers and blood to have a better biomonitoring framework.

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