



Lead poisoning and its *in vivo* biomarkers in Mallard and Coot from two hunting activity areas in Poland



Łukasz J. Binkowski^{a,*}, Katarzyna Sawicka-Kapusta^b

^a Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Krakow, Poland

^b Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland

HIGHLIGHTS

- Lead poisoning was diagnosed in 8% of birds hunted in southern Poland.
- Lead pellets were found in the gizzard content of 3% of birds.
- Pb content in feathers and excrements were not correlated with the internal one.
- Hb concentration and Ht level were not related to Pb blood concentrations.
- ALA-d activity was significantly correlated with Pb concentrations in blood.

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ABSTRACT

In this study, we aimed to present the status of lead (Pb) poisoning in birds from southern Poland and the evaluation of *in vivo* biomarkers which may be used in the diagnosis without killing animals. This included the activity of δ -aminolevulinic acid dehydratase (ALA-d), hemoglobin (Hb) concentration, hematocrit (Ht) level and concentrations of Pb in blood, feathers and excrements. The significance of this work is the use of hunted birds which allow us to compare the signals of the chosen biomarkers with the internal response. Birds collected in the area of lower hunting activity (the Milicz ponds) revealed statistically lower Pb concentrations than birds from the Zator area. Pb poisoning was diagnosed in almost 8% of birds (including specimens from both areas), but lead pellets were found in 3%. The highest tissue concentration found was noted in kidneys of Mallard from the Zator area ($36.55 \mu\text{g g}^{-1}$ d.w.). Significantly higher concentrations were noted in a few samples of gizzard content (up to $1047 \mu\text{g g}^{-1}$ d.w.) and excrements (up to $82.95 \mu\text{g g}^{-1}$ d.w.). Hb concentration, Ht level, concentrations in feathers and excrements seem not to be efficient biomarkers at noted Pb concentrations in internal tissues (brain, pectoral muscle, kidney, liver, spleen, bone). In contrast, we found a significant negative correlation between Pb concentration in blood and ALA-d activity which confirmed that this parameter can be used successfully as *in vivo* biomarker of lead poisoning also in low environmental pollution.

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1. Introduction

Spent lead (Pb) pellets or fishing sinkers deposited in the habitat of birds may be mistaken by them with little stones which they normally swallow to aid the digestion (Denbow, 2000; Gómez et al., 2004). They are ingested, in the acidic environment of the gizzard they dissolve and get into the bloodstream. Depending on general condition of the bird, food in the gizzard and the

number of ingested pellets, level of lead poisoning may vary (Friend, 1999).

The most common way of diagnosing the lead poisoning among waterfowl is measuring the concentration in various tissues of birds (De Francisco et al., 2003; Martin et al., 2008; Tsipoura et al., 2011). Due to the time consumption, costs and euthanasia requirements of the protocol, other methods are looked for. Among these methods, blood parameters as hemoglobin concentration and hematocrit level are studied. Biomarkers known mostly from occupational risk assessment are also employed as the activity of dehydratase of δ -aminolevulinic acid (ALA-d) – an enzyme of biosynthesis hem pathway (Scheuhammer, 1987a; Nordberg et al., 2007). All of these possible biomarkers are probably negatively

* Corresponding author. Tel.: +48 126626724; fax: +48 12 6626682.

E-mail addresses: ljbinkowski@gmail.com (Ł.J. Binkowski), katarzyna.sawicka-kapusta@uj.edu.pl (K. Sawicka-Kapusta).

related with blood lead concentration, but Hb and Ht relationships were less intensively observed (Dieter and Finley, 1979; Mateo et al., 2000) than ALA-d correlations (Pain, 1989; Gómez-Ramírez et al., 2011).

This work represents studies of lead concentrations in various bird materials and their influence on blood parameters and ALA-d activity in wild living birds (Mallards *Anas platyrhynchos* L. and Coots *Fulica atra* L.) shot in Poland, an area scarcely researched so far in this aspect. All studied specimens were collected by hunters. This seems to be the very cheap and efficient way of obtaining material for ecotoxicological and biomonitoring studies. However, in various studies the scope of which reaches beyond concentrations measurement, the use of hunted animals is merely occasional so a big novelty of this study is the use of the birds in biomarkers analysis.

The main aim of the study was to measure the Pb concentrations in various organs of Mallard and Coot from two important bird areas in Poland and to assess the Pb poisoning from ammunition pellets among waterfowl. The comparisons including the area, species and age were planned to be carried out, as well as, the correlation analysis of lead concentrations in internal tissues and materials which can be taken *in vivo*. In the next step, the relationships among the concentrations, blood parameters and ALA-d were scheduled to verify if these parameters might be useful as *in vivo* biomarkers.

2. Material and methods

2.1. Study area

Research was carried out on two separate areas in Poland (Fig. 1): fishponds around Zator (birds' $n = 110$) and fishponds near Milicz (birds' $n = 70$). In main research area (Zator) two fish farms (Spytkowice and Przeręb) were studied where around 3200 waterbirds are hunted annually (ZO PZŁ, 2010). This sampling site is included in the Nature 2000 area (the Lower Skawa River Valley PLB 120005) and in the Important Bird Areas by BirdLife (PL

125). The only type of ammunition which has been and still is used in the area is lead shot. Because of this activity, the possibility of lead poisoning occurs among waterfowl in that area (Binkowski et al., 2013). However, concentrations of lead in pond water from the area are low (Binkowski and Rzonca, 2014). The concentration in deposits runs up to $100\text{--}200\ \mu\text{g g}^{-1}$ (Lis and Pasiieczna, 1995). The load of Pb which is being deposited annually with precipitation in the area, was evaluated at the level of $0.0147\text{--}0.0228\ \text{kg ha}^{-1}$ (Toczko, 2011).

The comparative area – fishponds near Milicz, is characterized by very low hunting activity. Most of those ponds are included in the nature reserve of “Stawy Milickie” (Witkowski and Orłowska, 2010) which was founded in 1973 and is acknowledged by Ramsar Convention (Site number 758), BirdLife IBA (PL 092) and Nature 2000 system (Barycz River Valley PLB 020001). The annual load of precipitation Pb is fitted there between 0.0033 and $0.0093\ \text{kg ha}^{-1}$ (Toczko, 2011) and deposits concentrations are low – between 25 and $50\ \mu\text{g g}^{-1}$ (Lis and Pasiieczna, 1995).

2.2. Sampling

Mallards ($n = 102$) and Coots ($n = 78$) used in the research were shot between 2006 and 2009 during commercial or individual hunts generally between 15th August and 15th September. The collection of Mallards was done each year just before the migration season (when we observed the significant migratory moves we finished the sampling). During that time the main food of the birds was cereals available (of the local origin) in the ponds (feeding by hunters) and in the nearby farming area.

Just after the shot two blood samples of c.a. 1 mL each were taken from the ventricle of the heart. One sample was placed in a tube with anticoagulant agent K_2EDTA , for blood analysis. The second sample was placed into a clean tube for metal analyses. Next specimens were aged (two groups – immature up to one year and adult; Mallard on the basis of the shape and stain size of the sixth grater covert, Coot thanks to the color of the pupil Baker, 1993). Carcasses were transported, in $4\ ^\circ\text{C}$, to the laboratory

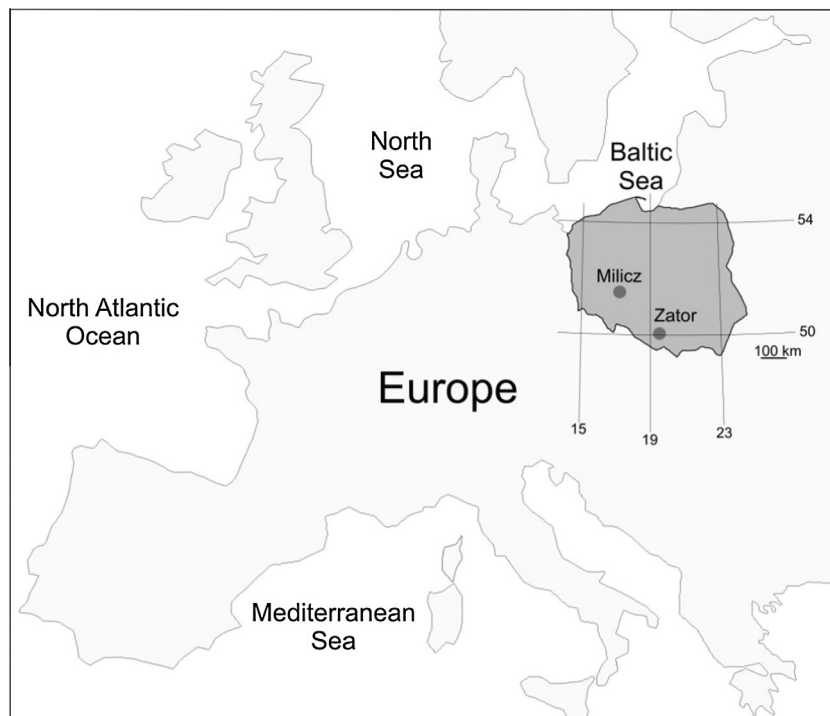


Fig. 1. Sampling areas (dark dots) in Poland – fishponds around Zator (southern Poland) and fishpond near Milicz (western Poland) in Western Europe (dark grey).

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