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CHANGES IN THE LOCATION OF MAGPIE *PICA PICA* NESTS IN THE AGRICULTURAL LANDSCAPE OF WESTERN POLAND

ABSTRACT

In recent decades, rapid changes have been observed in the species composition of woody and shrubby vegetation of rural areas in western Poland. Deciduous species, including fruit tree and shrub species, are being replaced by coniferous species, changing the potential nesting habitats of many bird species. The aim of this study was to check whether progressive changes in the species composition and age structure of trees and shrubs in agricultural areas affect the abundance of Magpies and their choice of nesting sites. The study was conducted in the agricultural landscape in two study plots near Kożuchów (district K – 100 km²) and Żagań (district Z – 110 km²) in the southern part of Lubuskie Province in the years 1999 and 2016. During field inspections carried out in the 3rd decade of April, occupied Magpie nests were searched for and the species of the tree or shrub and the height of the nest location above the ground were noted. There were no significant differences in the number of nesting pairs between the years in the surveyed plots. In 1999, 53 nests (density = 0.53 p/km²) were found in plot K and 34 nests (density = 0.31 p/km²) in plot Z. In contrast, in 2016 49 (0.49 p/km²) and 37 (0.34 p/km²) nests were recorded, respectively. At the same time, there was a clear change in the type of trees used by Magpies as nesting sites. In 1999, on both plots, Magpies nested exclusively in deciduous trees or shrubs (10 species), while in 2016, the number of species increased to 15, and the proportion of coniferous trees or shrubs to all trees colonized by Magpies was 49.0% on plot K and 29.7% on plot Z. In addition, the height of the location of nests above the ground was significantly higher in 1999 (\bar{x} = 15.4 m) than in 2016 (\bar{x} = 7.5 m). The results of the study indicate changes in the type of nesting habitat of Magpies over two decades as a result of the planting of ornamental vegetation, mainly spruce trees. At the same time, the birds resigned from nesting in tall trees, choosing lower spruce trees, which structure provides more security for nesting than deciduous trees.

Key words: Magpie *Pica pica*, agricultural landscape, Western Poland, nesting sites changes.

INTRODUCTION

In recent decades, changes have been observed within rural areas, related to the modernization of buildings, and transformation of rural areas, including changes in the species composition of woody and shrubby vegetation (Rosin et al. 2016, Switek et al. 2017, Rosin et al. 2020, 2021). In the case of vegetation, the transformations involve the abandonment of deciduous species, including fruit trees and shrubs, in favor of coniferous species. Such changes are not indifferent to rural fauna, including birds. In most cases, the impact is negative (Rosin et al. 2020, 2021), but changes in habitat structure can also be beneficial for rural fauna (e.g., Rosin et al. 2017).

The Magpie as a synanthropic species is a frequent object of study mainly in urban environments and much less frequently in agricultural landscapes. Magpie populations in such areas are strongly associated with human settlements (Bochenski et al. 2001, Orłowski 2005, Przybycin 2005). However, in some regions, such as in central-eastern Poland near Siedlce, the vast majority of nests were located at a considerable distance from buildings (Kasprzykowski & Olton 2008). Nesting of Magpies within rural buildings is associated with the choice of tree species on which their nests are built. The aim of this study was to examine whether changes in the species composition and age structure of trees and shrubs in agricultural areas of western Poland over two decades have affected the abundance and density of magpies and the choice of their nest sites.

STUDY AREA AND METHODS

The study was conducted in an agricultural landscape in the southern part of Lubuskie Voivodeship in the districts of Żagań and Nowa Sól (Fig. 1). Two study plots were selected near Kożuchów (plot K – 100 km²) and Żagań (plot Z – 110 km²). In area K, 10 villages were located in whole or in part. The area was open, and forests and larger wooded areas occupied only 15% of the study area. Within plot Z, 14 localities were located wholly or partially, and forests and larger wooded areas occupied 22% of the area. Most of the largest villages in both plots were characterized by buildings located on both sides of the main roads (“street” type villages).

Fieldwork was conducted in 1999 and 2016. During one-time inspections of the surveyed plots conducted in the 3rd decade of April, active nests of Magpies were searched for. For all occupied nests, the species of tree or shrub and the height of the nest location above the ground were determined.

The percentages and averages presented in the paper are given along with 95% confidence intervals (CL). A generalized linear mixed model (GLMM) with a Gamma distribution and a log link function with year as a fixed factor and study plot as a random

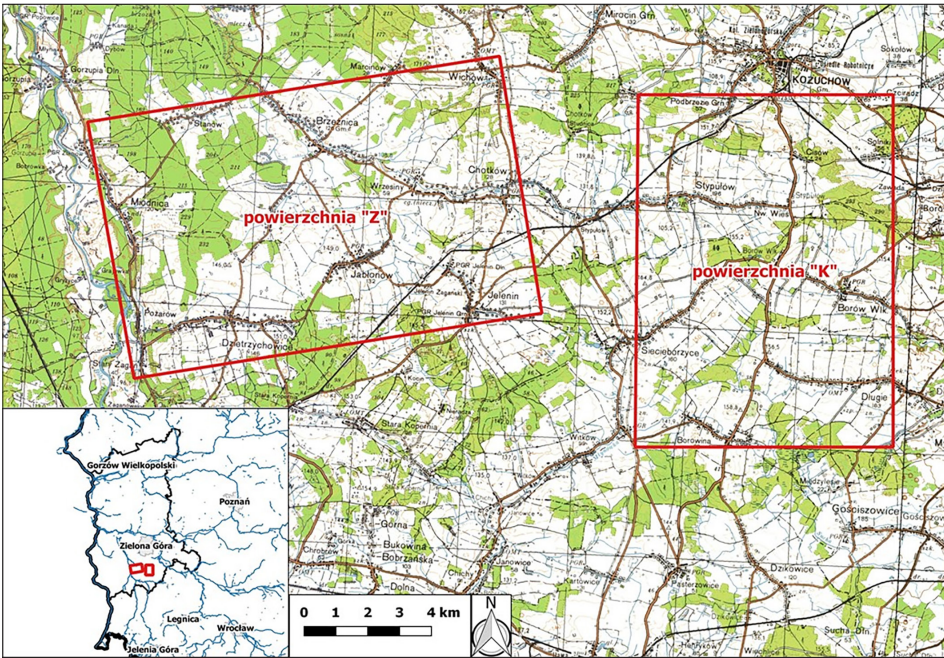


Fig. 1. Two study plots

effect was used to analyze differences in the height of nest location above the ground between the years. All calculations were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp. 2020).

RESULTS

In 1999, 53 nests (density 0.53 p/km^2) in the area K and 35 nests (0.32 p/km^2) in the area Z were found. In contrast, in 2016, it was 49 (0.49 p/km^2) and 37 nests (0.34 p/km^2), respectively. There were no significant differences in the number of breeding pairs between the years in the study plots (chi-square = 0.19, $df = 1$, $p = 0.663$). Over the years there was a clear change in the type of trees used by Magpies as nesting sites (chi-square = 44.83, $df = 1$, $p < 0.001$). In 1999, all nests ($n = 88$, 100%) were located in deciduous trees, while in 2016 only 59.3% ($n = 51$) were already located in this type of tree, of which 51.0% ($n = 25$) in the K plot and 70.3% ($n = 26$) in the Z plot. In 1999, in both plots, Magpies nested exclusively on 10 species of deciduous trees or shrubs, while in 2016 the number of species increased to 15 and also coniferous trees and shrubs were used by Magpies (Table 1). Among coniferous species, spruce *Picea* sp. was the most frequently used by Magpies (85.7% of coniferous species cases, $n = 30$; Table 1). Simultaneously, nests were located higher above the ground in 2009 than in 2016 (GLMM, $F_{1,172} = 159.27$, $p < 0.001$, Fig. 2).

Table 1. Summary of types and species of trees and shrubs used by Magpies as nesting sites in the agricultural landscape of western Poland in 1999 and 2016. K – area of Koźuchów, Z – area of Żagań; coniferous trees – bold font

Species	K 1999	K 2016	Total (%)	Z 1999	Z 2016	Total (%)
<i>Alnus glutinosa</i>	9	2	11 (10.8)	6	8	14 (19.4)
Fruit tree	2	4	6 (5.9)	0	11	11 (15.3)
<i>Fraxinus excelsior</i>	0	1	1 (1.0)	0	0	0 (0.0)
<i>Juglans regia</i>	0	1	1 (1.0)	0	0	0 (0.0)
Larix sp.	0	1	1 (1.0)	0	0	0 (0.0)
Unmarked	1	1	2 (2.0)	2	0	2 (2.8)
Picea sp.	0	21	21 (20.6)	0	9	9 (12.5)
Pinus sylvestris	0	1	1 (1.0)	0	2	2 (2.8)
<i>Populus sp.</i>	17	2	19 (18.6)	13	4	17 (23.6)
<i>Prunus spinosa</i>	1	1	2 (2.0)	0	0	0 (0.0)
Pseudotsuga sp.	0	1	1 (1.0)	0	0	0 (0.0)
<i>Qercus sp.</i>	5	7	12 (11.8)	6	0	6 (8.3)
<i>Rosa sp.</i>	1	1	2 (2.0)	0	0	0 (0.0)
<i>Salix sp.</i>	12	5	17 (16.7)	6	2	8 (11.1)
<i>Tilia sp.</i>	5	0	5 (4.9)	2	1	3 (4.2)
Total	53	49	102 (100.0)	35	37	72 (100.0)

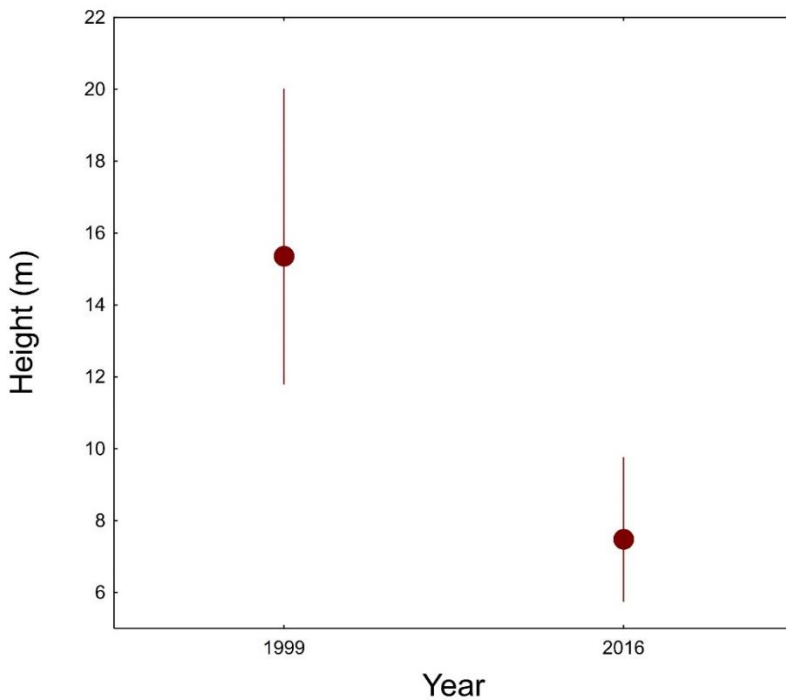


Figure 2. The height of the nests above the ground in the years 1999 and 2016

DISCUSSION

Over the past decades, an increase in Magpie abundance has been reported in urban areas, with a decrease within agroecosystems (Gregory & Marchant 1995, Górski 1997, Siriwardena et al. 1998, Chiron et al. 2008, Tomiałołć 2009, Gedeon et al. 2014). This phenomenon may be related to changes in environmental quality resulting from increased intensity of agricultural land use (Orlowski 2005). However, such a trend has not occurred everywhere, e.g., no changes have been recorded in central and eastern Poland over 30 years (Dombrowski 1997, Kasprzykowski & Olton 2008). In the case of the present study, there were no changes in the density of nesting pairs in the two study plots over the two decades. Most of the nests in the compared periods were located exclusively within human settlements, and only a few pairs of Magpies were found away from them. The recorded densities are comparable to those obtained in Poland's agricultural landscape, but they are closer to the lower values. The breeding densities of Magpies in Poland's agricultural landscape range from 0.2-1.0 p/km² near Siedlce (Dombrowski 1997), through 0.3-0.7 p/km² in the Lubuska region (Jerzak 1995, Dombrowski 1997), 0.8-1.0 p/km² near Szczecin and 0.9-1.1 p/km² near Białystok (Dombrowski 1997) to 2.1-3.0 p/km² near Tarnowskie Góry (Dombrowski 1997). These differences are due to the characteristics of the studied areas, i.e., the type of biotope (e.g., arable land, river valleys), forest cover, and buildings (Dombrowski 1997, Jerzak 2002, Jerzak 2005, Przybycin 2005, Kasprzykowski & Olton 2008). Analyses of breeding densities in agricultural landscapes have shown that in western Poland this parameter is higher than in eastern Poland (on average 1.2 p/km² and 0.6 p/km² respectively; Dombrowski 1997).

During the first survey period, all nests were located on deciduous trees. This result was in line with trends in the selection of nests in the surveyed plots in Poland from the last decades of the 20th century. Many studies describing the location of Magpie nests in different types of habitats indicate that in most cases the species chose deciduous trees and in urban environments preferred poplars, where the proportion of which could be up to 65% (Jerzak 2005). Research in recent years shows that in some urban and suburban zones, the share of coniferous species as nest trees is increasing (Lesinski 1998, Dulisz 2005, Zbyryt & Banach 2014, Jokimäki et al. 2017, Ciebiera et al. 2021). Some symptoms of this phenomenon were noticeable at the turn of the 20th and 21st centuries, also in plots in the agricultural landscape, when Magpie nests were found to appear on coniferous trees (Przybycin 2005, Wojciechowski et al. 2005, Kasprzykowski & Olton 2008). In the region of České Budějovice (southern Czech Republic) at the end of the second decade of the 21st century, it was shown that 10.1% of Magpie nests were built on coniferous trees, and the same tree species were found to be used with similar frequency in urban and agricultural areas (Šálek et al. 2020). Previously, nesting of Magpies in coniferous trees was rarely recorded (Jerzak 1988). The results of the present study indicate a progressive preference for coniferous tree choices, related

to the prevailing mode for this type of trees, followed by their increasing availability because they are fast-growing species. The increase in the proportion of conifers as nesting sites for Magpies is explained by several factors: evergreen tree species with an unchanging crown (they do not lose their leaves in winter), which makes nests less visible in early spring and thus less accessible to predators, and provides an opportunity for earlier initiation of breeding (Antonov & Antanasova 2022, Jokimäki et al. 2017, Ciebiera et al. 2021).

The height of nest placement above the ground in human-inhabited areas may depend on the human and other mammals on Magpies pressure (Lesiński 1998, Jerzak 2002). However, differences in the height of nest locations above the ground in both urban and rural habitats may also be due to differences in the species composition of trees growing in the areas in question. Such conclusions were made based on a study near Siedlce (Kasprzykowski & Olton 2008). This factor is also confirmed by the present study, which noted a decrease in the height of nest locations above ground due to a change in the choice of nest trees, resulting from the planting of coniferous species. Magpies in the second study period were more likely to choose coniferous trees than in the first period, at the expense of nest placement height. Dense, inaccessible coniferous trees, although lower than the deciduous species chosen earlier, provided a safer nesting location. Within a dozen years or so, coniferous trees planted near buildings reached sufficient height to allow Magpies to build nests in them. With dense, safe trees, the height of the nest location no longer matters. Confirmation of this phenomenon is provided by the results of a study conducted in central and eastern Poland (Kasprzykowski & Olton 2008) showing a large proportion of nests located on willows that form osiers. The authors describe such sites as a specific habitat dominated by low and widely branched shrubs. There, Magpies built their nests deep within, rather than at the top of, clumps of willows, i.e. in places that were difficult to access. Such observations indicate that Magpies are highly plastic in choosing safe nesting sites.

In conclusion, the study did not show any changes in the density of nesting pairs of Magpies in the study plots, while there were clear changes in the type of nesting habitat of the studied species over the two decades, resulting from the planting of ornamental vegetation, mainly in the form of spruce trees. At the same time, birds gave up nesting in tall trees, choosing lower spruce trees, whose structure provides greater safety for nesting and greater protection against predators and weather conditions than deciduous trees.

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BIOMARKERS OF LIPID AND PROTEIN OXIDATION IN THE BRAIN OF PIGEONS *COLUMBA LIVIA F. URBANA* INHABITING AREAS WITH VARIOUS ANTHROPOPRESSURE (NORTHERN POLAND)

ABSTRACT

The mechanisms of functioning of organisms in anthropogenically modified habitats are an important issue of ecotoxicology and ecophysiology. Birds are sensitive indicators of the impact of various factors, as evidenced by the levels of pollutants such as heavy metals in their bodies, especially in their feathers. The aim of the study was to analyze the level of oxidative stress biomarkers using substances reactive to 2-thio-barbituric acid (TBARS) as biomarkers of lipid peroxidation and ketone and aldehyde derivatives as biomarkers of oxidatively modified proteins in the brain tissue of pigeons *Columba livia f. urbana* inhabited two sites of different exposure to lead in Pomerania (N Poland). We studied birds from two environments (Słupsk and Szpęgawa), which differ in the content of metals in the soil. Statistically significant higher levels of chemical elements were found in the area of Szpęgawa compared to the results obtained from Słupsk soils. Thus we classified this area as polluted, compared to the unpolluted area of Słupsk. Pigeons from polluted areas were characterized by statistically significant higher levels of Si and Pb in feathers. Data showed that pigeons accustomed to lead-contaminated areas (Szpęgawa) had statistically higher levels of TBARS and carbonyl derivatives of oxidatively modified proteins in brain tissue than pigeons from unpolluted region (Słupsk). Environmental lead pollution related to anthropogenic pressure caused a significant increase in the oxidation of free radicals and modification of protein structures in the brain of birds, which may lead to dysfunction of appropriate physiological processes in organisms.

Key words: anthropogenically changed environments, lead, oxidative stress, biomonitoring, feathers, brain, birds

INTRODUCTION

The extensive development of human activity has led to disturbances in the ecological conditions of natural ecosystems (Bassi *et al.* 2021). The majority of heavy metals in the ecosystems are derived from their content in soil-forming rocks. These elements are also replenished by human activities. Pollution of soils near industrial centres is mainly due to emissions of harmful compounds from industrial plants and transport. Cd, Pb, Zn, Hg, and Cu are widely used in industry and are active environmental pollutants (Hou *et al.* 2019; Walter 2023). Combustion of coal, oil shale, and oil is the dominant and more intensive source of pollution than metallurgical production. Up to 5 billion tonnes of fuels are burned each year. Almost all metals can be found in coal and oil ash, sometimes in concentrations that economically justify their extraction from the ash. The scale of fossil fuel use is such that the combustion of coal, oil shale, and oil is the dominant and more intensive source of pollution than metallurgical production (Pain *et al.* 2019).

The mechanisms of functioning of organisms in anthropogenically modified habitats are an important issue of ecotoxicology and ecophysiology. Birds are sensitive indicators of the impact of various factors, as evidenced by the levels of contaminants (especially heavy metals) in their bodies, particularly in feathers (Nam and Lee 2006; Kozák *et al.* 2022). The attachment of pigeons *Columba livia f. urbana* to their nesting sites creates conditions for long-term monitoring of the state of ecology of their habitats (Janiga and Zemberyová 1998).

The effects of lead exposure on two pigeon populations from N Poland have been investigated previously, e.g. Kurhaluk *et al.* (2021) and Tkachenko *et al.* (2021). We investigated the effects of lead accumulated in soils and in feathers of pigeons on the biomarkers of oxidative stress and antioxidant defense in various tissues. In this study, we look at the effects of lead exposure on one of the most sensitive system of organism, i.e. the brain tissue. It is known that the negative effects of lead on the nervous system are largely related to the disturbances in the formation of myelin (Monje 2018). Myelin surrounds the processes of nerve cells, isolating them from external influences. This is important for more reliable and faster transmission of signals through the nervous system. It is necessary to note the negative effect of lead on the synthesis of neurotransmitters as chemical messengers of signals between neurons and from neurons to the effector cells, which enable the association of individual neurons in the brain and ensure the successful fulfilment of all its numerous and vital functions (Monje 2018).

Lead has been shown to affect lipid metabolism in animals. It is associated with impaired cholesterol kinetics, which can have serious implications for energy status, and synthesis of steroidal adrenal and sex hormones, ultimately leading to impaired maturation of sex products and reduced survival of individuals (Pain *et al.* 2019). In animals, lead exposure disrupts the ratio of serum glycoprotein and lipoprotein fractions, causing an increase in triglycerides, total cholesterol, and low-density lipoprotein

cholesterol and a decrease in high-density lipoprotein cholesterol. This leads to atherosclerosis of the blood vessels (Zhao *et al.* 2023).

Lead induces oxidative stress, which is associated with a sharp increase in the reactive oxygen species (ROS) in animal cells (Raine *et al.* 2015). As a result, the intensity of lipid peroxidation increases, the levels of damaged proteins also increase, and membrane structure and function are altered (Kurhaluk *et al.* 2022). At the same time, the damage to the mitochondrial membranes, e.g. disrupts energy budget of cell metabolism (Koivula *et al.* 2011). It needs to be highlighted that lead may bind to the sulfhydryl groups of proteins, which leads to adverse effects related to the change and/or disruption of their functional role, including the incorrect functioning of enzymes, transcription factors, stress proteins, etc. (Walter 2023).

The aim of the present study was to analyze the levels of biomarkers of oxidative stress using 2-thiobarbituric acid reactive substances (TBARS) as biomarkers of lipid peroxidation, and ketonic and aldehydic derivatives as biomarkers of oxidatively modified proteins in the brain tissue of pigeons *Columba livia f. urbana* habituated in two sites of different lead exposure in the Pomeranian region (N Poland).

MATERIAL AND METHODS

Study area and characteristics of groups

We studied pigeons *Columba livia f. urbana* from two localities (Słupsk and Szpęgawa). The first is Szpęgawa (N 54°05'44.4", E 18°43'15.7"), a village in the Pomeranian Voivodeship near the Voivodeship road No. 224 in Northern Poland. Szpęgawa is situated about 120 km East of Słupsk (Photo 1). Słupsk (N 54°27'57.681", E 17°1'50.366") is a city of 90 thousand inhabitants located in the central part of Pomerania. The pigeon colony from Słupsk, numbering over 300-400 individuals for many years, was analyzed in 2007-2008 in order to conduct a series of ecophysiological studies (Hetmański 2011).

The Stanisławie junction of the A1 motorway is located near Szpęgawa. Its importance as the A1 motorway in Poland is due to the fact that it is part of the international route E75 of the Trans-European Transport Corridor. It is the only Polish highway



Photo 1. Birds from two localities – Szpęgawa (A) and Słupsk (B). Photo by Tomasz Hetmański

with a southern route. The A1 motorway is characterized by heavy traffic. Currently, it connects Gdańsk, Gdynia and Sopot, a large urban center, with the Czech border (D1 motorway). One of the problems with such highways is that they can be accessed from narrow, single-lane roads. Nearby areas, such as the village of Szpęgawa and the Stanisławie road junction, are strongly affected by emissions of pollutants into the atmosphere. This is also related to the increase in the volume of freight traffic and the popularity of car tourism, which is associated with the emission of so-called aerosol mixture of fuels and combustion products into the atmosphere. In the village there are farms located on agricultural land.

For the study of suburban pigeons, a farm was selected in the area with coordinates of N 54°05'44.4", E 18°43'15.7". This old farm was built after World War II. In the 1980s, loose slag (sludge), waste from metallurgical production, was used to harden the soil on the farm. The farm is also a place for breeding city pigeons from the population of Słupsk pigeons.

Ethics Statement

The experiments were conducted by the Guidelines of the European Union Council and the current laws in Poland. Adult pigeons, at least 1 year old, were used in the study. Sexual maturation in these birds begins at the age of 3 months (Hetmański 2007; Hetmański 2011). The study was conducted with the approval of the Bioethics Committee (licence number 44/2012).

Tissue samples

Brain tissue from 17 pigeons living in Słupsk and 14 pigeons living in Szpęgawa were used for the study. The brains of birds were removed after decapitation. One pigeon was used for each preparation. Briefly, the brain tissues were excised, weighed and washed in ice-cold buffer. The minced tissue was rinsed with cold isolation buffer to remove blood and homogenized in a homogenizer H500 (POL-EKO, Poland). Brain homogenates were centrifuged at 3,000 g for 15 minutes at 4°C. The isolation buffer contained 180 mM KCl, 10 mM HEPES, 10 mM EGTA and 0.5% of bovine serum albumin; the pH was adjusted to 7.3 with KOH. The suspension was then used for analysis. The Bradford method (1976) with bovine serum albumin as standard was used for protein quantification. The absorbance was recorded at 595 nm.

Chemical elements concentration

Concentration of chemical elements from which pigeons collect gastroliths was determined in surface soil samples taken from both sites. In Słupsk, 4 soil samples were taken (each sample was analyzed in triplicate) from the Old Market Square, where the largest flock of pigeons is located. In Szpęgawa, 4 soil samples were collected (the analysis of each sample was carried out in three repetitions) from the area between

the farm buildings, where the birds receive food and water. Feather samples were also collected from 5 adult birds (at least 1 year old) at each site. Contour feathers were taken from the birds' backs.

Soil samples were taken from a depth of 1-3 cm. Samples were then aggregated and air-dried before storage and analysis. Each soil and feather sample was analyzed in three series. Between different readings, the soil sample was thoroughly mixed in the same bag. The results of three readings were averaged. The analysis of the concentration of chemical elements in feather and soil samples was carried out using an X-ray fluorescence analyzer (XRF) at the Faculty of Physics of the Pomeranian University in Słupsk. An XRF analyzer model Sci Sps X-200 from Sci Sps, Inc. was used to determine the concentration of chemical elements in samples. The analyzer is designed to test elements in various samples such as soil, alloys, precious metals and some others.

An XRF (X-ray fluorescence) analyzer generates an X-ray beam that can be used to irradiate a sample. The interaction of X-ray quanta with the analyzed sample causes characteristic X-ray emission from chemical elements contained in the sample. Analysis were performed with a Rh target (50 kV, 600 μ A) and poly-capillary optics providing a 25 μ m spot size. The X-ray fluorescence signal was collected using two XFlash[®] silicon drift detectors. They provide a high spectral resolution of 135 eV measured at full width at half maximum, FWHM, with a K- α line of 5.95 Mn. Detectors record X-ray fluorescence spectra, i.e. X-ray fluorescence, containing information about the presence of chemical elements and their concentrations. K and L series X-ray fluorescence are commonly used to identify chemical elements because they provide the best results. The detectors have an active area of 30 mm² and are placed at an angle of 45° to the X-ray beam. Analysis were performed in vacuum (20 mbar) using a sampling step of 20 μ m and a dwell time of 10 ms. The device is factory calibrated with 37 standard elements, including all measurable pathfinders. X-ray fluorescence hyperspectral data were processed using PyMca 5.1.3 (Solé *et al.* 2007) and Datamuncher (Alfeld and Janssens 2015). The device software uses either standard methods, such as the basic parameters of the spectra of given elements (this is the method we used in our measurements), or user-generated empirical calibration curves to relate X-ray spectrum to elemental concentrations.

Biochemical assays

2-Thiobarbituric acid reactive substances (TBARS) assay

The degree of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reactive substances (TBARS) using the Kamyshnikov method (2004). This method is based on the reaction of the lipid peroxidation degradation product, malondialdehyde (MDA), with 2-thiobarbituric acid (TBA) at high temperature and acidity, producing a colored adduct that is measured spectrophotometrically.

The nmol of MDA per mg of protein was calculated using an extinction coefficient of $1.56 \cdot 10^5 \text{ mM}^{-1} \text{ cm}^{-1}$.

Protein carbonyl derivative assay

The determination of oxidative modification of carbonyl derivatives of proteins (OMP) was based on spectrophotometric measurement of the level of aldehyde (AD) and ketone derivatives (KD) in tissue samples. The rate of oxidative degradation of proteins was estimated from the reaction of the resulting carbonyl derivatives of amino acid reactions with 2,4-dinitrophenylhydrazine (DNFH), as described by Levine *et al.* (1990) with some modifications. DNFH was used to determine the carbonyl content of soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP₃₇₀) and 430 nm (ketonic derivatives, OMP₄₃₀).

Statistical analysis

Results were expressed as arithmetic means \pm S.D. Significant differences between means were measured using the multiple range test, where at least $p < 0.05$. Normally distributed data were log-transformed. Tests for basic statistical analysis (significance of regression slope, analysis of variance for significance between locations) were performed using STATISTICA 13.3 (TIBCO Software Inc., USA) with 95% confidence intervals ($\alpha = 0.05$) to determine the significance of differences between types of regions and enzyme activity in the brain tissue of birds from various regions (Stanisz 2006, 2007).

RESULTS

Chemical elements concentration

In the case of some chemical elements, their concentrations in the soils of both regions were statistically significant, as we have already written about in our previous publications (Kurhaluk *et al.* 2021; Tkachenko *et al.* 2021). We would like to emphasize this point because it allowed us to determine our model of the relative predominance of lead contaminants. The metal content in the soils of the studied areas differed significantly, i.e. in Szpęgawa, a statistically significantly higher level of metals was observed, compared to the results obtained from the Słupsk soil. This allowed the area to be classified as polluted (polluted area), as the content of metals except Si, Ni and Cu was statistically higher compared to the data from the Słupsk area. In soil samples from Szpęgawa, the content of Al was 121%, Ti – 23%, Mn – 242%, Fe – 15.5% and Pb – 543.5% more than in Słupsk (unpolluted area). The lead content in the soil from Szpęgawa was five times higher. The content of metals such as Zn, Zr and Si was much higher in Słupsk than in Szpęgawa. The content of elements in pigeon feathers was ambiguous. Pigeons from polluted areas were characterized by statistically significantly higher contents of

Si and Pb in feathers and lower contents of Fe, Cu and Zn, compared to pigeons from the Słupsk area.

Lipid peroxidation and oxidatively modified proteins

The first stage of our biochemical research was to determine the level of lipid peroxidation in the brain tissue of pigeons living in the regions with different levels of lead exposure. These important biomarkers that are end products reacting with 2-thiobarbituric acid, namely malondialdehyde levels, are shown in Figure 1A. The data show that pigeons tamed in areas contaminated with lead (Szpeęgawa) were characterized by a statistically higher level of TBARS (by 150.3%, $p < 0.05$) in the brain tissue than the pigeons from unpolluted areas.

In the second stage of the study, the oxidative modification of proteins was examined, assessed on the basis of the content of aldehyde derivatives (OMP AD) and ketone derivatives (OMP KD) in the brain tissue of pigeons from two habitats. We showed that lead-contaminated bird habitat was associated with statistically significant increases in AD OMP and KD OMP levels in this tissue (Figure 1B).

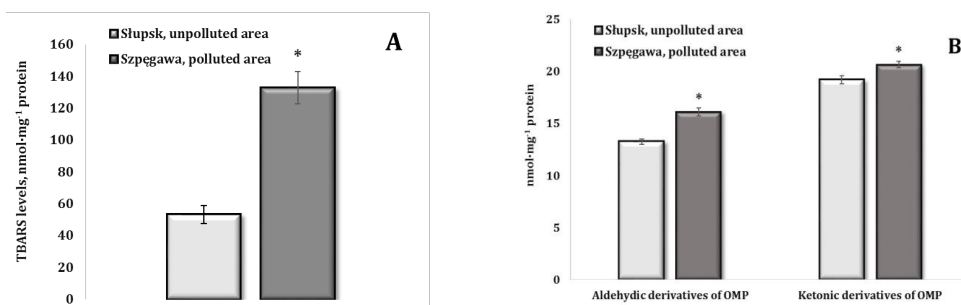


Figure 1. TBARS levels (A, $\text{nmol}\cdot\text{mg}^{-1}$ of protein), aldehydic and ketonic levels of oxidatively modified proteins (B, $\text{nmol}\cdot\text{mg}^{-1}$ of protein) in the brain tissue of pigeons *Columba livia f. urbana* living in the areas with different levels of pollution (Pomeranian Voivodeship, Northern Poland).

Results are expressed as arithmetic means \pm S.D.

* changes were statistically significant at $p < 0.05$ compared to the birds living in the unpolluted areas.

Therefore, anthropopressure-related environmental pollution with lead is associated with the induction of a significant intensification of free radical oxidation processes in the brain tissue of birds and a significant modification of protein structures in this tissue, which may cause functional disorders of physiological processes.

DISCUSSION

Many studies have shown the adverse effect of lead on the functioning of the redox system in various tissues. Our studies showed a high correlation between oxidative stress biomarkers, assessed by TBARS levels, and increased levels of aldehyde and ketone

derivatives of oxidatively modified proteins in the brain tissue of pigeons accustomed to polluted, high-traffic areas. These data also confirm the relationships demonstrated by other authors that lead is one of the most dangerous chemical elements for all living organisms and has a polytropic, negative effect on them (Skalny *et al.* 2021). At the same time, the risk of lead is increased by the large number of possible sources of its entry into the living organism, as well as its ability to accumulate in tissues and organs and to maintain toxic properties for a long time, which has also been convincingly demonstrated in the works of many authors (Hou *et al.* 2018; Andrew *et al.* 2019).

Relationship between the content of heavy metals in pigeon feathers and the color of their plumage is considered by scientists to be an element of the adaptive response (Chatelain *et al.* 2014). For example, it is believed that pigeons have developed an adaptive response to the abundance of heavy metals by partially excreting them through their own feathers. This is because researchers have found that dark pigeons – those with more melanin pigment in their feathers – are more common in large cities than in suburbs and villages (Chatelain *et al.* 2016a, b). Scientists have proposed that melanin binds metal atoms, thanks to which pigeons with darker plumage have a better life in cities: they remove heavy metals from the body more effectively by simply transferring them to the feathers (Chatelain *et al.* 2016a, b). Similarly, birds can remove zinc, lead, or both, and their dark plumage gives them an evolutionary advantage in environments polluted with heavy metals. In reality, however, this problem is still not fully understood. It is uncertain whether urban pigeons' plumage is becoming darker due to the cleansing properties of melanin-rich feathers, as the genes that produce melanin are also linked to the regulation of stress hormones and the immune system. Thus, pigeon plumage color and the level of heavy metals in their environment may not be directly related. This may be part of a more complex adaptation because the stress levels and immune systems of urban animals differ from those of their wild counterparts (Mundy 2005).

Such numerous and multidirectional effects of lead on cells lead to disruption of neurovegetative and digestive processes in animals, progression of vegetative dystonia and anemia, increase in the incidence of cardiovascular diseases, and reduced immunity and resistance of the body to stress factors (Burger and Gochfeld 2000, 2005; Kanstrup *et al.* 2019; Wang *et al.* 2022). Generally speaking, when lead accumulates in large amounts in the body of animals, it has a strong negative impact on various aspects of their life activity, reduces their functional adaptation capabilities, and in some cases even leads to their death (Kanstrup *et al.* 2019). Since animals are one of the intermediaries of the food chain, high lead concentrations in the body of wild birds reflect the direction of physiological regulatory processes during their poisoning and are of interest as bioindicators of the level of environmental pollution (Williams *et al.* 2018; Monclús *et al.* 2020).

High concentrations of lead inhibit mitochondrial respiration, disrupt protein synthesis processes and negatively affect the composition of microelements. Moreover,

lead causes oxidative stress in cells, associated with a sharp increase in the amount of ROS, which is an important mechanism of the toxic effect of the metal (Patra *et al.* 2011; Lopes *et al.* 2016). Due to its variable valency, lead can initiate free radical processes, the intensity of which increases in the context of a slowdown in the activity of antioxidant enzymes (Adonaylo and Oteiza 1999; Patil *et al.* 2006). The observed activation of lipid peroxidation leads to disruption of the structure and integrity of cell membranes (Ayala *et al.* 2014). As a result, the metabolism is disturbed and many irregularities and changes in the functioning of the body occur. For example, damage to mitochondrial membranes disrupts tissue respiration and inhibits ATP synthesis. Damage to lysosomal membranes is associated with the release of hydrolytic enzymes and excessive intensification of hydrolytic processes. We have already demonstrated these dependencies in the course of oxidative processes (Kurhaluk *et al.* 2021; Tkachenko *et al.* 2021).

It is well known that the toxic effect of heavy metals in general, and lead in particular, is that they can mechanically clog the body, i.e. deposit on the walls of blood vessels, renal and hepatic ducts, reducing the filtration capacity of these organs. This leads to the accumulation of toxins and metabolic products from the body's cells, i.e. to self-poisoning of the body, because the liver is responsible for processing toxic substances that enter the body, and the kidneys are responsible for their removal from the body. Additionally, the accumulation of excessive amounts of lactic acid and ketoacids in cells due to disruption of redox processes leads to the development of acidosis, i.e. a shift of the acid-base balance towards increased acidity, which in turn leads to even greater acidification of the cell membrane permeability. The toxic effect of lead on the animal's body is also associated with its competitive relationship to certain elements, since it can replace mono- and divalent cations (sodium, calcium, magnesium, iron) in biologically important molecules, thereby disrupting various biological processes (Flora *et al.* 2012; Wani *et al.* 2015).

CONCLUSIONS

The study analyzed the level of biomarkers of lipid peroxidation and oxidatively modified proteins in the brain tissue of birds inhabiting areas with various degrees of anthropopressure. Environmental lead pollution related to anthropogenic pressure is associated with a significant increase in oxidative stress in the brain tissue of birds and a significant modification of lipid and protein structures in this tissue, which may lead to functional disorders of physiological processes.

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SHORT NOTE

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**WHAT TYPE OF HOUSE SPARROW (*PASSER DOMESTICUS*)
PLUMAGE IS THIS? COLOUR ABERRANT OR NORMAL –
A CASE REPORT**

The plumage is a distinctive colouration of a species that serves essential roles in bird communications (Hill and McGraw 2006). Birds with aberrations in plumage colourations have been reported and their types and causes were explained by many (Harrison 1963; McCormac 2001; Guay et al. 2012; Zbyryt et al. 2021). Colour aberrations concerning House Sparrows [*Passer domesticus* (Linnaeus, 1758)] have been described precisely (van Grouw 2012). Such records provide us with information on spatial distribution and frequency.

On June 16th, 2019, I was observing House Sparrows at Kolvihire, Purandar Taluka, Pune District, Maharashtra, India (18°16'02.7"N 74°12'15.6"E). During my observation, I spotted a male House Sparrow with noticeable white portions on the tip of the upper greater coverts on both sides (Figure 1). At first glance, it appeared like a second white wing-bar otherwise observed in Tree Sparrows (*Passer montanus*) (Long 1964). Since the literature describes the presence of a conspicuous white shoulder patch (Ali and Ripley 2001), also referred to as a wing-bar (Long 1964; Anderson 2006) only on the upper middle (median) coverts of House Sparrows (Lowther and Cink 2020); and nobody described the upper greater coverts tipped with white portions or a second wing bar. Also, such a partial lack of melanins in the feathers can be seen in the case of disturbed melanin synthesis as a result of either tyrosine-deficient nutrition or the inefficiency of a bird in extracting tyrosine (van Grouw 2013). So, the question arises: What type of House Sparrow plumage is this? Further studies are required to fill in the missing description and confirm whether such white portions on the tip of the upper greater coverts are considered as a colour aberration or a normal plumage.

Additionally, I observed a white uppertail covert indicating colour aberration (Figure 1). The eyes, feet, and bill were normally coloured, though the bill was not dark black. The white plumage, complete or partial, can be a result of leucism, partial leucism, Progressive Greying, or dietary deficiency. Due to uncertainty, I did not attempt to name the aberration, to avoid misinterpretation of the record (van Grouw et al 2016).



Figure 1: Photographs of the male House Sparrow with noticeable white portions on the tip of the upper greater coverts on both sides (A, B) and a white uppertail covert (©Rushikesh Sankpal)

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SHORT NOTE

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**ERYTHRISM IN HOUSE SPARROW (*PASSER DOMESTICUS*):
A RECORD FROM RAJASTHAN, INDIA**

Plumage colouration helps birds in various behavioural and physiological processes (Hill et al. 1999, Senar 2006 & Barragán-Farías et al. 2019). Sometimes, unusual plumage colourations are caused by an abnormal distribution of pigments (Van Grouw 2013). The major reasons for such unusual plumages can be changes in the microstructure of feathers (Laczi et al. 2019), environmental factors (Dorst 1971, Zbyryt et al. 2020), dietary factors (Gonçalves Jr. et al. 2008), chemical changes in pigments and genetic mutations (Venizelos and Benetti 1999 & Mills and Patterson 2009).

The House Sparrow (*Passer domesticus*) is one of the bird species that are commonly found in most places of the world (Ali & Ripley 2001) and Various kind of unusual plumages and colourations are described in house sparrows (Van Grouw 2012). Plant materials like grains, fruit, seeds etc., forms the major portion of House Sparrow's diet and rest consist of animal matter like worms and insects (Gavett and Wakeley 1986 & Fitzwater 1994).

On 20th March 2023, we observed a female House Sparrow with erythristic plumage (Figure 1) feeding in a group under *Lantana camara* an invasive plant in Ajmer, Rajasthan, India (26°30'32.0"N 74°40'59.0"E). The observed individual showed reddish colouration (erythrism) on coverts and flank regions. The bird was seen repeatedly feeding and roosting on *Lantana camara* is native to tropical America (Holm et al. 1977) and is described as one of the worst alien species (Cronk & Fuller 1995). Its berries attract frugivorous birds and mammals that help to disperse its seeds widely (Day et al. 2003). Bright orange, yellow and red colours in bird plumages are mostly because of carotenoids (Brush 1981, Stradi 1998, McGraw 2006) and carotenoids can only be absorbed by the feather cells during feather growth, birds are able to store an excess of them in their livers where they are readily available for colouration of their feathers whenever required (Van Grouw 2012). Erythrism is usually defined as an unusually increased amount of red pigmentation (carotenoids) on an animal's body surface. Cases of erythrism are reported in many species associated with invasive plant species



Figure 1: Erythristm in House Sparrow (*Passer domesticus*) observed at Ajmer, Rajasthan, India

(Mulvihill et al. 1992, Hudon et al. 2013, Hudon and Mulvihill 2017). β -Carotene gives the red colour to berries and flowers of *Lantana camara* (Zutshi and Madiyappa 2020). According to Van Grouw (2012), carotenoids are responsible for pale yellow to scarlet red colour in birds and are acquired from food sources. This record described here is the first on erythristm found in House Sparrows in India.

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