INFLUENCE OF THE BREEDING GROUND LOCATION ON THE FLUORIDE ION LEVEL IN EGG SHELLS OF THE EUROPEAN BLACKBIRD (*TURDUS MERULA*)

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ABSTRACT: The common blackbird is a model species in toxicological studies in different environments and its egg shells are very often used as a material to determine the content of different elements, including fluorine, which might be environment pollutants. Considering the above, we decided to determine the factors affecting the fluoride ion (F^-) content in the egg shells of the common blackbird living at different distances from the chemical plant Grupa Azoty Zakłady "Police" SA, one of the major producers of phosphate fertilizers in Poland. Determination of the F^- concentration was performed using a potentiometric ion-selective electrode. The lowest average F^- content was observed in samples from Wełtyń (a non-contaminated area 36 km from the chemical plant) and the highest was in samples from Bartoszewo (a contaminated area 8 km from the chemical plant). Statistically significant differences in F^- content were observed in samples taken from Szczecin (16 km from the chemical plant) (p=0.0001) and Wełtyń (p=0.002) compared to the results obtained for the Bartoszewo samples. The month of egg laying had a significant impact on the content of F^- in the egg shells in the high F^- contaminated area (Bartoszewo) but not in the low F^- non-contaminated area (Wełtyń). In the Szczecin area, there were no differences in the F^- content of egg shells related to the year, the brood number, and the age of the females.

Keywords: Common blackbird; Egg shells; Fluoride; Impact of environment.

INTRODUCTION

A change in the chemical composition of the environment caused by the anthropogenic release of various elements affects the homeostasis of ecosystems and may threaten their very existence.[1] One of the significant pollutants is the element fluorine which occurs in inorganic and organic forms with varying degrees of solubility and assimilability by plants, animals, and humans, and may cause various levels of toxicity.[2] The affinity of the fluoride ion (F^-) to metals, in particular divalent ones (Ca, Mg, Mn, Fe, Cu, and Zn), results in the formation of complexes with undesirable physiological effects. In addition, precipitated magnesium and calcium fluorides accumulate in the body, mainly in hard tissues.[1] When ingested in large quantities, fluoride is harmful to humans and animals, particularly given the very narrow margin of safety between the tolerated and toxic doses.[3,4] Although environmental contamination in areas around industrial plants has long been known to adversely affect the functioning of plants and animals, it is now known that more distant areas can also be affected. The range of contamination depends not only on the size of the industrial emissions, but also on the direction and the power of the winds, as well as general climatic conditions.[5]

The common blackbird is a model species in toxicological studies in different environments and its egg shells are very often used as a material to determine the

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content of different elements, including fluorine, which might be environment pollutants.[6] It was assumed that fluoride ion levels should increase in egg shells with the proximity to the source of emission. In addition, given that fluoride accumulates in the female's body, older individuals should lay eggs with higher fluoride levels. Moreover, the shells of the first brood should have higher fluoride levels than the shells of the second and subsequent broods. Considering the above, we decided to determine factors affecting the fluoride content in the egg shells of the common blackbird. Analysis concerned the effect of distance of breeding grounds from the chemical plant Grupa Azoty Zakłady "Police" SA, one of the major producers of phosphate fertilizers in Poland.[7] We also analyzed the effect of the age of females and the time of hatching on the fluoride levels in egg shells.

MATERIAL AND METHODS

Studied area: The blackbird egg shells were collected in three areas: Bartoszewo (8 km SW from the chemical plant Grupa Azoty Zakłady "Police" SA), Szczecin (16 km S from the chemical plant), and Wełtyń (36 km S from chemical plant).

Material: The studied material consisted of 83 blackbird egg shells obtained in the area of Szczecin in 2006–2015 (72 shells from 41 broods), the area of Bartoszewo (a contaminated area) in 2010–2011 (7 eggs from 4 broods) and the area of Wełtyń (a non-contaminated area) in 2007 (4 eggs from 3 broods). The eggs or egg shells were collected when unhatched, after abandonment of the brood, or after predation. In the case of individually marked populations (from 2000 over 90% of the breeding birds had a unique combination of color rings) from Żeromski Park in Szczecin, the exact age of the females and the brood number in the breeding season was known (69 shells from 41 broods). In 2004 Wysocki presented detailed information on the biology of this population.[8]

Preparation of material for determination of fluoride: The collected egg shells were cleaned of any remnants of internal membrane, and the samples were then dried to a constant weight in an oven at 105ºC. The dried samples were ground in an agate mortar and then samples weighing ~10 mg were mixed with 1 mL of perchloric acid (Sigma Aldrich, Poznań, Poland) and shaken at 90ºC for 1 hr. After cooling, 0.5 mL of the sample was transferred to a plastic tube and 2 mL of sodium citrate (Sigma Aldrich, Poznań, Poland) solution and 2.5 mL of TISAB II (Thermo Scientific, USA) were then added. After mixing, the potential difference of each sample was measured for 10 minutes: 5 minutes before the addition of the appropriate standard, and 5 minutes after the addition. The F^- content in the sample was calculated based on the potential difference measured in each sample, the sample weight, and the concentration of the added standard. Determination of the F^- concentration was performed using a potentiometric ion-selective electrode (Thermo Scientific Orion, USA) according to the works of Gutowska et al.[9-11] The reagent solutions were prepared with highly purified water (Sigma Aldrich, Poznań, Poland).

Statistical analysis: Statistical analysis was performed using Statistica 10.0 (StatSoft, Poland). The arithmetic means (AM) and standard deviations of the AM

(SD) were calculated for each study group. The distribution of results for the individual variables was obtained with the Shapiro-Wilk W test. As most of the distributions deviated from the normal Gaussian distribution, non-parametric tests were used for further analyses. Correlations between the changes of the parameters were examined with the Spearman's rank correlation coefficient. To assess the differences between the study groups, the non-parametric Mann-Whitney test was used. The level of significance was p=0.05. The General Linear Model (GLM) was used to identify the most important factors affecting fluoride contents in the Żeromski Park. In the model, the fluoride content was the dependent variable and the fixed factors included the explanatory variables (year, the brood number in the breeding season, and the female's age). Because the sample size was restricted, we combined the data into three categories. For the year, we pooled the data from 2006 and 2007 (N=20), 2009–2011 (N=17), and 2014 and 2015 (N=32). For the brood number, the first (N=34) and second broods (N=17) were treated as separate categories but the third and subsequent broods were pooled together (N=18). For the female's age the categories used were: young—in their second calendar year of life (N=20), middle age—between 3 and 5 years of life (N=33), and old—older than 5 years (N=16).

RESULTS

The mean contents of the fluoride in the eggshells of blackbirds are presented in Figures 1–3. The lowest average fluoride content was observed in samples from Wełtyń, and the highest from Bartoszewo (Figure 1). Statistically significant differences in the fluoride content were observed in samples taken from Szczecin (p=0.0001) and Wełtyń (p=0.002) compared to the results obtained for the Bartoszewo samples (Figure 1).

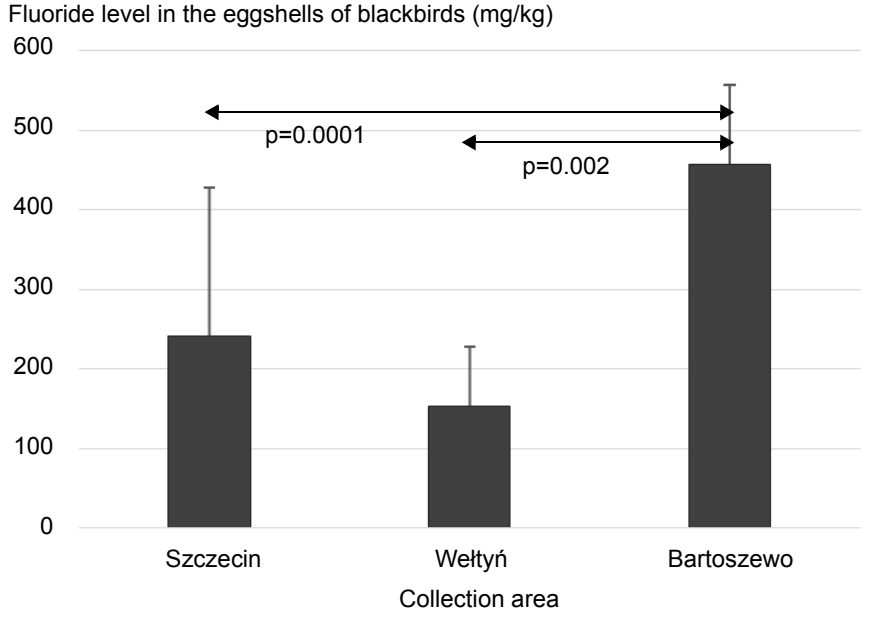

Figure 1. Fluoride level in the eggshells of blackbirds according to the collection area.

The highest content of fluoride was recorded in egg shells from the area of Bartoszewo where determinations were made in 2010 and 2011. There was significant increase (p=0.002) in the egg shell fluoride in 2011 compared to 2010 (Figure 2).

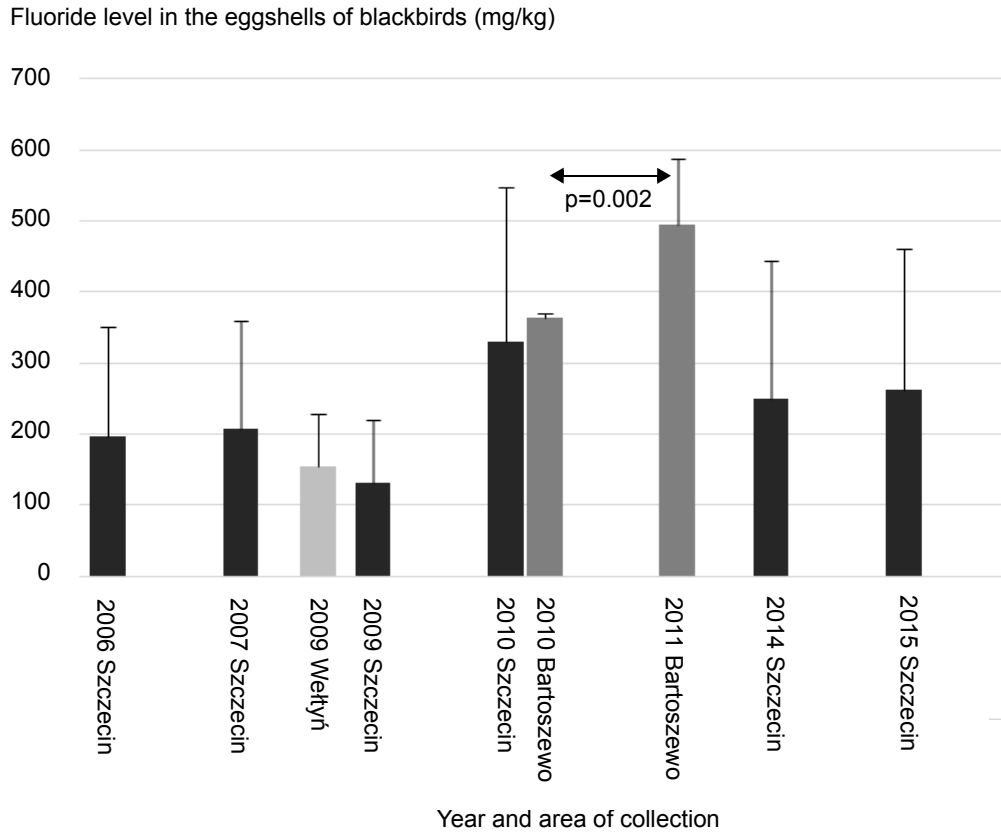

Figure 2. Fluoride level in the eggshells of blackbirds according to the year and the area of collection.

The analysis of the fluoride content in the samples from the green areas of the city of Szczecin also showed an upward trend in the concentration of this element in the egg shells, but it was not statistically significant. The samples came from the years 2006–2015 (Figures 2 and 3).

The month of egg laying also had a significant impact on the content of fluoride in the egg shell. Statistically significant differences were present between the months for the eggs from Bartoszewo, i.e., the area closest to chemical plant Grupa Azoty Zakłady "Police" SA (Figure 3). The later the eggs were laid, the higher were the fluoride levels found in the shell (p=0.00056, R=0.8).

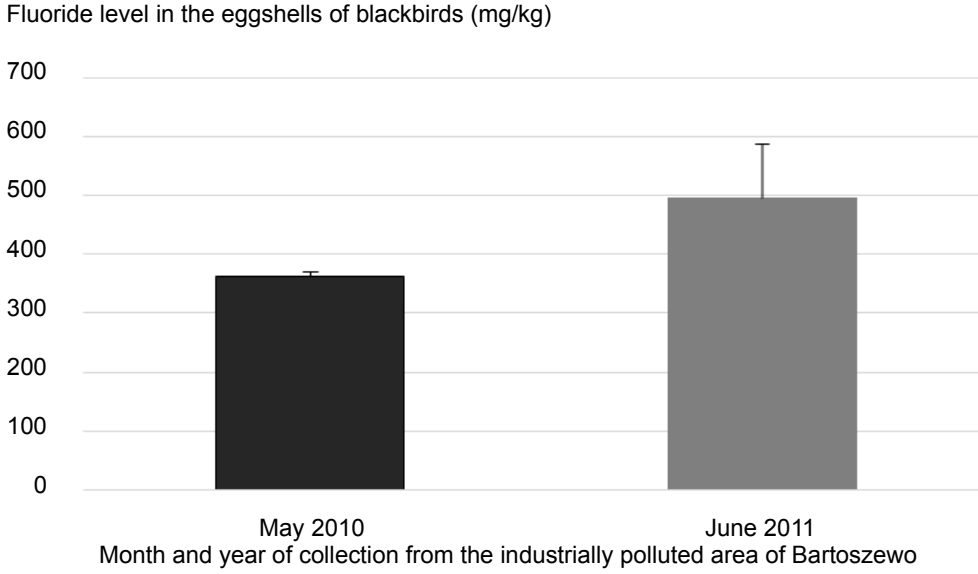

Figure 3. Fluoride level in the eggshells of blackbirds from the industrially polluted area of Bartoszewo according to the month and year of collection.

Samples from the park areas of the city of Szczecin in 2006, 2010, 2014, and 2015 showed a statistically insignificant upward trend in egg shell fluoride, related to the time of egg laying with higher levels in each subsequent month. An opposite trend, also not statistically significant, was observed for the years 2007 and 2009 (Figure 4).

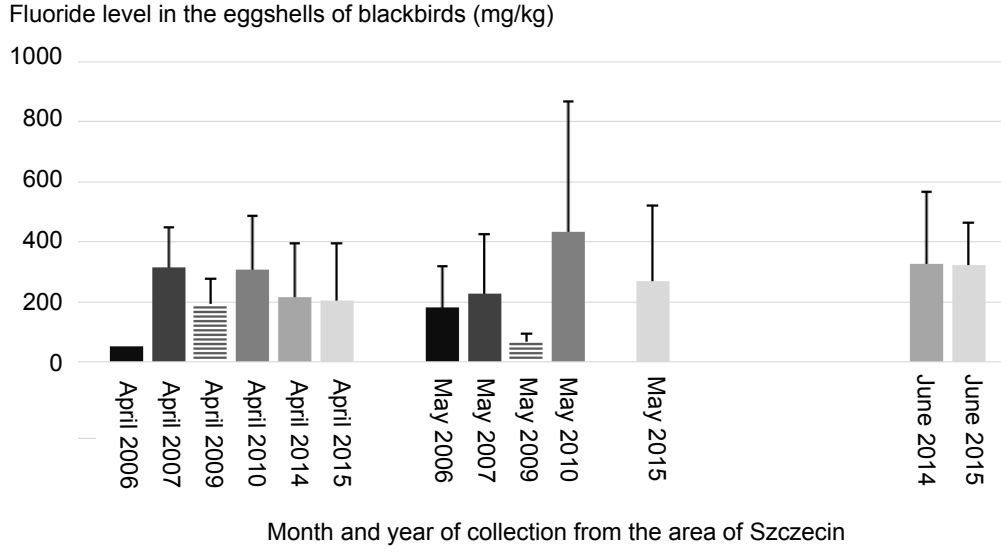

Figure 4. Fluoride level in the eggshells of blackbirds from the Szczecin area according to the month and year of collection.

Because of the small sample size from Bartoszewo and Wełtyń, the detailed analysis for year, brood number, and female age, was only conducted on egg shells from Żeromski Park, Szczecin. No significant differences were found in the egg shell fluoride content related to the year, the brood number, and age of the female birds (Table).

Table. Dependency of fluoride contents of blackbird egg shells on analyzed factors in Żeromski Park, Szczecin (GLM analysis)

Factor	F	df	p
Free factor	106.7	1	0.0000
Year	1.19	2	0.31
Brood number	0.64	2	0.53
Age of females	1.12	2	0.33

DISCUSSION

Acute fluoride ion poisoning affects virtually all body organs of animals and humans, and may even be fatal due to the blockage of cell metabolism. The fluoride ion is capable of inhibiting or even stopping enzymatic processes, especially those involving metalloenzymes. It can disrupt the essential functions of the body which are regulated by calcium, although other metal ions may also be bound by fluorides resulting in the blockage of certain biochemical reactions.[12]

The uncontrolled consumption of fluorine compounds is an important issue for the health of people and animals living in contaminated areas.[13] The consequences of air pollution depend, in part, on the chemical nature of the emitted pollutants and the type of area in which they operate. The actual effects of pollution are usually distant in time and space, and the causal relationship between the emission and the environmental damage is not always easily discernible.[14] The results of our study show a positive relationship between the proximity to the source of emission, the chemical plant Grupa Azoty Zakłady "Police" SA, and the fluoride levels in the eggshells of the blackbird. The fluoride levels in the eggshells in contaminated Bartoszewo, 8 km from the chemical plant, were almost three-times greater than those in the non-polluted area in Wełtyń, 36 km from the plant.

Originally, the European blackbird (*Turdus merula*) was a forest species.[15] In the mid-1880s, it invaded western European cities, and at the turn of the 19th century it reached the north-western part of Poland. The urban population described in this study (Szczecin) is mostly resident in the area,[16] while the rural populations (Bartoszewo and Wełtyń) are migratory.[17] Females lay 2–6 eggs at one day intervals. The urban population has 1 to 5 broods during the breeding season[18] while the rural population usually has no more than 3 broods.[15] The

breeding season lasts from March to July and depends on the climatic conditions[19] and the breeding experience.[20]

Blackbird females have one to five broods in a single breeding season, the first is usually in April, and the subsequent ones appear from April to July.[18] In the case of the studied population of blackbirds from the Żeromski park, we decided to analyze the order of broods but not their dates as fluoride accumulates in the female's body and its level in the egg shell should depend on the level in the mother's body. Therefore, the fluoride levels should be the highest in the egg shells of the first brood. However, our study does not show this correlation. The finding of no differences related to the order of the broods suggests that the fluoride deposited in the shell is probably dependent on its content in the food eaten by the female during the egg-laying. It has been found that doses of 1,000 and 1,300 mg/kg bw fluoride decreased the feed intake, weight gain, and the efficiency of egg production. The long-term administration of high doses of sodium fluoride does not, however, lead to a permanent loss in egg production ability because the changes related to the lack of appetite subside within six months of the cessation of the fluoride exposure. An increase in fluoride in the diet is accompanied by a reduction in the volume of eggs with a tendency for a better quality eggshell (resistance to deformation and fracture) and also a decrease in the retention of phosphorus, magnesium, and calcium, resulting in lower levels of these elements and less calcium being available for bone formation. There is also a slowdown in yolk synthesis.[21] The results of our previous studies show that the optimal age for breeding for males and females is the period between the fourth and sixth year of life. The youngest birds, in their second calendar year, and the oldest birds, older than six years, are less successful at finding a partner,[16,22] breed later,[20] and have a lower likelihood of breeding success.

Experimental studies show that differences in the content of fluoride in the bones of poultry are gender-related due to physiological factors associated with the production of eggs. Bone fluoride levels are higher in hens than cocks, due to an increased metabolism of minerals in the bones of females during egg production. Calcium is removed from bones to create eggshells. The increased absorption of calcium is also accompanied by increased fluoride absorption. Probably, fluoride moves with calcium to the bone, but when the calcium is removed from bones to form the eggshell, the fluoride remains in the bones and the concentration there increases proportionally with each cycle of egg production. In total, there are three ways to eliminate fluoride from circulation: excretion by the kidneys, and deposition in the bones and eggshells. Interestingly, in chickens, even at a concentration of F^- in the diet reaching 1,300 mg/kg, the amount of fluoride in the egg white did not exceed 1 mg/kg.[21]

Research by other authors also indicates that eggshell F^- levels correlate with environmental exposure. In an experiment on Rhode Island laying hens receiving 45 mg/L NaF in drinking water for 4 weeks, eggshell F^- levels also increased, although only twofold.[23] It seems likely that the mechanism of fluoride deposition in bones and also in eggshells may be related to the limited ability of the kidneys

to excrete F^-. When this threshold is exceeded, fluoride is also deposited in soft tissues, mainly in the liver, kidneys, and muscles, and as permanent deposits in the bones.[24] However, it is worth noting that the research referred to breeding birds that produce relatively large numbers of eggs, compared to free living birds with only one or two hatches per year. The results of studies on the impaired reproductive efficiency of owls (*Otus asio*) living in areas contaminated with fluoride showed that, despite there being no significant differences in the biochemical blood parameters between the control and treatment groups, the addition of 200 mg/kg of sodium fluoride to the diet of the birds produced a significant reduction in the volume and weight of the eggs.[25]

The blackbird eggshell fluoride concentrations observed in our study were relatively high, which indicates the constant presence of fluorine in the local environment. Part of the West Pomeranian Voivodeship is permanently exposed to fluoride pollution from the Grupa Azoty Zakłady "Police" SA chemical plant which is located 8 km from Bartoszewo and 16 km from the city of Szczecin. Research conducted in 1977–1992 showed that the plant emitted up to 97 million tonnes of fluorine to the atmosphere per year.[26] The modernization of the plant in recent years, including the introduction of chimney filters, has substantially improved its environmental record. However, this has resulted in the abandoning of systematic monitoring of fluorine pollution in the area. The aim of our research was to fill this gap and provide valuable information on the state of the local natural environment.

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