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RESEARCH ARTICLE

Alpine accentors as monitors of atmospheric long-range lead and mercury pollution in alpine environments

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Abstract

Mercury and lead are deposited in the West Carpathians as long-range transported air pollution. The Alpine accentor (*Prunella collaris*) was recognized as a cost-effective biomonitor, and used to investigate the bioavailability of contaminants in large alpine areas. The outer tail feathers and blood of the alpine accentors were used for assessment of atmospheric mercury and lead contamination, respectively. Mean mercury levels in feathers of accentors averaged at 1.15 μg/g (SE = 0.105, $n = 40$). There were no temporal variations in mercury concentrations. Mean blood lead levels were at 5.2 μg/dL (SE = 0.5, $n = 27$), showing a slight decreasing trend from July to October. Juveniles were not more susceptible to lead accumulation than adults. Bone lead concentrations that increase with age reflect a bioaccumulation effect. A statistically significant negative correlation was found between the length of erythrocytes and the concentration of lead, which may show the first symptoms of microcytosis. In comparison to aquatic ecosystems, the biogeochemical factors that influence methylmercury availability in alpine habitats are not yet completely known and require further investigation. Our findings show that birds in alpine terrestrial ecosystems may contain surprisingly high levels of methylmercury. The mercury levels in the feathers of accentors probably indicate that alpine autotrophs make sufficient amounts of mercury available to the terrestrial food web. The blood lead levels of accentors likely approach the threshold level for further hematological effects. We found a clear tendency in erythrocytes to change their shape from ellipsoid to smaller and rounder with increasing amounts of lead in their blood. The shape of bird erythrocytes appears to be a very sensitive indicator of critical levels of lead in the alpine environment.

Keywords Lead · Mercury · *Prunella collaris* · West Carpathians · Erythrocytes · Microcytosis · Transport

Introduction

Many studies have confirmed that heavy metals are transported through the atmosphere for thousands of kilometers, interacting with each other and forming secondary pollutants before being deposited. Of these metals, deposits of zinc, cadmium, lead, aluminum, and mercury are of an order of magnitude greater than those of the other metals (Scheuhammer 1987; Steinnes et al. 1988). The particles of these metals are considered to increase in size rapidly in the atmosphere, and smaller particles can be carried by winds and deposited over very large areas (Chamberlain et al. 1979; Ackermann and Hanrahan 1999). The major source-polluted mountains in Europe are in the densely populated and heavily industrialized regions of Central and Eastern Europe. Anthropogenous stress to the alpine ecosystems of the Western Carpathians is caused by local emitters and long-range transport (Šoltés 1992). Since metals may be deposited from the atmosphere onto the feathers of alpine birds as well as incorporated into growing feathers from the blood, use of feathers may be confounded by the combination of these two processes. Lead in plumage likely originates from direct atmospheric deposition on feather surfaces (Janiga et al. 1990; Frantz et al. 2016), whereas ingested lead becomes firmly bound by blood and bone (Johnston and Janiga 1995; Brnušáková and Janiga 2014). Mercury presents a different situation.

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Mercury Mercury levels in bird feathers reflect the levels circulating in the blood during the period of feather growth (Westermark et al. 1975; Thompson et al. 1998). Mercury levels in blood may be affected by the level of mercury stored in different tissues of the body. This storage is the main factor that determines the amount of mercury found in the plumage (Furness 1975). At the time of contamination in the area, blood mercury concentration is the most important representation of the metal increase in the growing feathers, while its concentration represents recent dietary uptake and internal tissue redistribution (Furness 1993; Ackerman et al. 2008). The metal can accumulate in bird tissues in its inorganic (total mercury) and organic (methylmercury) form. The methylated form is potentially toxic to lower trophic and considerably toxic to higher trophic organisms (Kim et al. 1996a). Some bird species are capable of demethylating methylmercury in the tissues and store it as an inorganic form in the liver where the percentage of methylmercury varies from 30 to 70% (Kim et al. 1996b). Methylmercury has a strong tendency to bind to sulfur-containing amino acids, which are present in large amounts in keratin, the main protein constituent in feathers. As a result, methylmercury molecules circulating in blood tend to enter into growing feathers (Newman 2014). A very high percentage of the mercury entering feathers is methylmercury, even in birds storing inorganic mercury in the liver (Thompson and Furness 1989). From this point of view, concentrations of mercury in feathers reflect the mercury levels in the blood (Vanparys et al. 2008). Moreover, the mercury level in the growing feathers of nestlings and fledglings is directly related to the dietary intake of methylmercury by young in the breeding period, while mercury concentrations in feathers of adults reflect environmental contamination during molting (Lewis and Furness 1991). Adult feathers usually contain higher concentrations than juvenile feathers (Hughes et al. 1997). Because feathers usually contain higher concentrations of mercury than generally found in other tissues, they allow non-destructive biomonitoring of atmospheric mercury contamination (Hahn 1991). In this paper, the levels of mercury in feathers of alpine accentors (*Prunella collaris*, Scopoli 1786) are presented, and the temporal trends in mercury levels are described. The West Carpathians represent a barrier for the toxic elements in central Europe and bioaccumulation of some heavy metals shows clear seasonal differences (Janiga et al. 2012). Significantly higher depositions are found in birds and mammals immediately following the winter season, which correlates to the wet deposition of contaminants in winter and consequent snow in the spring. However, when compared over historical periods, levels of metal accumulation remain of less important in bioaccumulation patterns (Janiga 2008). We also incorporate analyses of feathers regrown at different time periods to test the hypothesis that mercury concentration varies as the body pool of mercury is depleted through molting during the August/September molt period.

Lead A potential bird species to be used as an indicator organism needs to comply with various requirements in order to be a reliable and continuous biomonitor. The most important factor is that the species can accumulate a sufficient amount of metal without being killed by the excessively large concentrations encountered (Kenntner et al. 2007). Lead concentration levels in bird bones indicate that partially or fully granivorous birds have a higher degree of exposure to lead in the environment than other bird species (Janiga 2001). In mountainous habitats, of particular interest is the relatively high degree of lead exposure that crows, finches, thrushes, and especially alpine accentors have in the environment. Lead concentration in the bones of the most applicable bio-indicator—alpine accentor—evidenced that lead was deposited in the alpine areas in the upper layer of soils as a long-range air pollutant (Janiga 2001, Ciriaková et al. 2011, Krendželák et al. 2018). Lead is relatively immobile in soils, and virtually all of the anthropogenic lead could be found in the topmost 20 cm with a downward migration velocity of lead from 1 to 8 mm/year. Acidic soils have the greatest potential for lead migration (Renberg et al. 2002; Shotyk and Le Roux 2005). The fate of lead in soil is a response to a complex set of parameters including soil texture, mineralogy, pH, conductivity, and abundance of organic matter, in addition to climate and orography (Klamárová and Solár 2017).

Lead usually does not pose a serious hazard to adults of altricial passerines (Grue et al. 1984; Nybo et al. 1996; Arenal and Halbrook 1997) but aging adults (Janiga and Žemberyová 1998) and nestlings may exhibit a high sensitivity to lead. The poor breeding success of passerine birds in polluted areas is often related to the high amount of lead and other heavy metals in their diet (Beľskii et al. 1995; Nyholm 1995; Eeva and Lehikoinen 1995). Lead contamination of female passerine birds affects the required calcium levels during eggshell formation mainly in Ca-poor habitats (Orlowski et al. 2016). Lead intoxication highly influences the hematopoietic system. The enzyme aminolevulinic acid dehydratase (ALAD) is very important in heme-biosynthesis. The marked inhibition of heme-biosynthesis in blood may result in the development of anemia and variation in the size and shape of mature erythrocytes. Small and round erythrocytes with oval nuclei may be found in anemic birds (Morgan 1994; Samour and Naldo 2005). The considerations outlined above led us to examine the concentration of lead present in the blood of alpine accentors and to test if the erythrocyte morphology is a suitable indicator of lead pollution in mountain environments. The results may be

used to consider the potential of alpine accentors to act as a biological indicator for transboundary lead in the alpine environment.

Material and methods

The focal species, the alpine accentor, is a species of passerine bird that lives in high alpine regions, such as alpine meadows and rocky outcrops. In the West Carpathian Mountains of central Europe, the species breeds between 1600 and 2600 m, often near the highest peaks (Dyrcz and Janiga 1997). Alpine accentors are ground feeders; they forage in alpine meadows and rocky outcrops. Their diet in summer consists of largely nutritionally rich foods (Janiga and Novotná 2006), including gnats, their larvae living in the soils, and caterpillars. The vegetation period is short in alpine habitats, and invertebrate active life and reproduction time is limited, leading to prey abundance. In autumn, after molting, the accentors start to feed on grass seeds in alpine meadows (Janiga and Novotná 2006).

Investigations were carried out in the High and Low Tatra Mountains (Slovakia). The West Carpathians are limited by the following borders: N49°16.556′ E19°31.622′, N48°55.842′ E19°29.645′, N49°14.567′ E20°18.342′, N48°51.725′ E20°10.699′. Our extensive sampling was conducted at 15 peaks and valleys in Northern Slovakia. All birds were trapped between 1990 and 2010. Samples of the feathers and blood of alpine accentors were collected with the permission of the Ministry of the Environment of the Slovak Republic (No. 4638/2016-2.3). Birds were captured using mist nets or walk-in-traps, measured and weighed. One-year-old mature birds (young adults) were aged by the coloring of the middle and greater wing coverts. We collected two outer tail feathers from 40 birds by clipping the calamus close to its insertion point; these were stored in new plastic envelopes prior to mercury analyses. Burger et al. (1992) suggest that mercury concentration in flight feathers grown in the breeding area may simply reflect accumulation of mercury prior to molt and thus indicates contamination of the summer site. Thus, feathers of accentors reflect the conditions present in late summer months, likely between August and September. The molting season begins in alpine accentors in August and continues in some birds until the end of September. Blood was collected from 27 birds caught in 2006 and 2007. Blood samples for lead analyses were collected to heparinized capillary tubes (approximately 50 μL), refrigerated, and stored (−18 °C). Blood samples for smears were collected from the *vena ulnaris cutanea*. In the field, blood smears were air-dried, fixed with methanol, and stained according to Pappenheim (Lucas and Jamroz 1961). In the laboratory, the perimeter, length, and width of 50 randomly chosen, but not deformed, erythrocytes and their nuclei, sampled from each individual, were measured using Micro Image software under × 1000 magnification. A total of 1350 cells were measured. Blood samples from whole blood were sent to the State reference veterinary laboratory, Nitra.

Additionally, we examined the amount of lead in the bones of five nestlings from three broods of accentors which were found dead in nests, during very cold breeding periods. Since the feeding habits of the species determine their risk to lead exposure, we compared the levels in their bones to the levels of nestlings of great tits (*Parus major*, Linnaeus 1758) of the same age. We collected deceased nestlings from the nest boxes located in the Tatra Mountains at lower elevations. Eleven tit nestlings were examined in total. Tits are also insectivorous in the breeding period and granivorous in late autumn and winter.

Metal detection and statistics

Mercury The feathers for analysis were washed in double distilled water for 1 week (rewashed 3 ×) and dried at 65 °C for 30 min. The bones were boiled in deionized water. After the remains of fat and muscle were removed, the bones were boiled in 3% H_2O_2, washed in double deionized water at 90–100 °C, and dried. Approximately 0.0020–0.0380 g of dried sample was digested with 2 mL of water, 4 mL of concentrated HNO_3 (Merck, Darmstadt, Germany), and 1 mL of H_2O_2 (Slavus, Bratislava, Slovakia) using the Mars Xpress microwave oven (CEM Corporation, Matthews, USA). Decomposition temperature was 140 °C, ramp time 15 min, and hold time 13 min. The mineralization solution was diluted to 6–10 mL with deionized water. Lead and mercury content in bones and feathers was determined by electrothermal atomic absorption spectroscopy (AAS Perkin Elmer 1100B, Norwalk, Connecticut, USA) equipped with deuterium background correction and an HGA 700 graphite furnace with an automated sampler AS-70 under the following conditions: wavelength 283.3 nm, slit 0.7 nm, and lamp current 10 mA. Temperature: Drying 1:70/10/10; Drying 2:150/2/60; Pyrolysis: 800/15/30; Atomization: 1800/0/3; Cleaning: 2500/0/3 (temperature (°C)/ramp time (s)/hold time (s)). To prepare calibration solutions, aliquots were taken from a stock standard solution 1000 $mg \cdot l^{-1}$ of lead and mercury (Merck, Darmstadt, Germany). The calibration range was 5–20 $\mu g \cdot l^{-1}$. The matrix modifier $NH_4H_2PO_4$ (0.2 mg) was used. The method precision was better than < 5% (RSD). The accuracy of the method was established by analyzing the reference material (bovine liver No. 12-2-01 (Slovak Metrological Institute)). The determined value (0.70 ± 0.05 $mg \cdot kg^{-1}$) agreed well with the certified value (0.71 ± 0.08 $mg \cdot kg^{-1}$), and within the uncertainty limit established for the material.

Lead Whole blood samples were analyzed for lead using atomic absorption spectrophotometry (AAS) using a SOLAAR AA and following standard operational procedures.

Spectrometer parameters for lead: Method: Pb-ETA, instrument mode: furnace, autosampler: FS95/97, wavelength: 283.3 nm, background correction: D2, measurement time: 3 s, signal type: transient, measurement mode: absorbance, bandpass: 0.5 nm, transient type, height, lamp current—90%. Furnace Parameters—lead: cuvette type: ELC, injection temperature: 75 °C, furnace program—68 s, four phases. The matrix modifier $NH_4H_2PO_4$ (5.0 μL) was used. Ash Atomize Parameters—lead: ash (start 600 °C–stop 1000 °C), atomize (start 900°C–stop 1500 °C). The accuracy of analytical methods was determined by the use of a certified bovine blood standard reference material (SRM; 955b; National Institute of Standards and Technology, Gaithersburg, MD, USA). Calibration parameters for Standard 1 and Standard 2 were 9.9 and 15 μg/L, respectively; each sample was measured by the calibration mode: Std. Additions and the rescale limit were 10%. Control measurements agreed well with the certified values within the uncertainty Rsd limits from 0.0 to 2.4%.

Statistics The data was normally distributed; the results were statistically compared using one-way ANOVA and Tukey's test at the 95% confidence level ($P < 0.05$). Simple linear regression analysis was carried out to detect associations between lead levels in the blood and dimensions of erythrocytes. The significance of correlation coefficients was tested by *t* test ($P < 0.05$). All statistical analyses were performed with Statistica ver. 12 software for Windows.

Results and discussion

Mercury and lead in the West Carpathian alpine accentors

Mercury concentrations in the tail feathers of alpine accentors are shown in Table 1. Levels show relatively little difference between adult and young birds as well as between trapped individuals prior to and after the molting period in the second half of August and first half of September. The finding confirms that mountain contamination is not elevated and does not change between different 10-year periods. No significant differences were detected between 1990 and 1999 and 2000–2010 (Table 1).

Blood lead levels in alpine accentors ranged from 0.8 μg/dL to 10 μg/dL, showing a slight decrease from July to October (Table 2). Juveniles did not tend to be more susceptible to lead accumulation in blood than adults.

The negative correlation between the erythrocyte size/shape and lead content in the blood of birds was significant as is the significant negative correlation between red blood cell (RBC) length and mercury content (Table 3). The length of RBCs decreased with increasing levels of lead in the blood but RBC width remained more or less constant. Cell shape changed from ellipsoid or oval to smaller and round. Significant negative correlation between the length of erythrocytes and the concentration of lead probably suggests the first symptoms of microcytosis (round and smaller erythrocytes (Fig. 1)). The shape of RBCs of accentors appears to be a very sensitive indicator for the characterization of critical levels of lead in the alpine environment.

Increased use of fossil fuels threatens to accelerate climate change as well as metal pollution. Current investigations have demonstrated that there is a high level of metal pollution in many alpine regions, i.e., in the ecosystems which substantially form the national parks and protected areas throughout the world (Shotyk et al. 2002; Shotyk and Le Roux 2005). In alpine habitats, the relative importance of more poisonous organolead increases. Consideration must be also given to altitudes above the planetary boundary layer (ca. 1500 m), which, by the study of Shotyk et al. (2002), are more influenced by long-range transport processes. In general, very incomplete information is available on field studies, especially regarding chronic exposure of mercury and lead to alpine biota (Janiga 2008; Janiga et al. 2012). Results from some European regions suggest that deposition of metals occurs within 200 km of the source (De Caritat et al. 1997; Reimann et al. 1997). From this point of view, the West Carpathians combine two important centers for potential heavy metal emissions: North

Table 1 LS means (SE) of mercury concentrations (μg/g dry weight) in the feathers of alpine accentors, divided according to age and pre/post-molting period

Variable	*n*	Mercury (μg/g) mean	SE	One-way ANOVA
Mature birds	32	1.092	0.117	$F(1, 38) = 1.47$ $P = 0.23$, NS
Juveniles	8	1.409	0.234	
Pre-molting	13	1.147	0.187	$F(1, 38) = 0.003$ $P = 0.95$, NS
Post-molting	27	1.160	0.129	
1990–1999	15	1.348	0.169	$F(1, 38) = 2.06$ $P = 0.18$ NS
2000–2010	25	1.041	0.131	
Total *P. collaris*	40	1.156	0.105	
		Minimum 0.228–maximum 3.300		

Table 2 LS means (SE) of lead concentrations (μg/dL) in the blood of alpine accentors, divided according to age and months of blood collection

Variable	*n*	Lead mean (μg/dL)	SE	One-way ANOVA
Matures	20	4.9	0.6	$F(1, 25) = 1.48$ $P = 0.23$, NS
Juveniles	7	6.4	1.0	
July	12	6.0	0.7	$F(2, 24) = 1.23$ $P = 0.31$, NS
August	9	5.2	0.9	
October	6	3.9	1.1	
Total *P. collaris*	27	5.2	0.5	
		Minimum 0.8–maximum 10.4		

Moravia in the Czech Republic and the Małopolska district in Southern Poland. Due to their high metabolic rate and intensity of exposure, alpine accentors could be expected to be a top bio-indicator of metal contamination in the mountains (Janiga 2001).

The alpine accentor is an insectivorous bird in spring and summer, when it collects insects from upper soil layers. The occurrence of significant levels of lead and mercury contamination in the Carpathian accentors is consistent with results from a survey of lead in snow voles—alpine rodents living in the alpine habitats of the West Carpathians. Dietary ingestion was recognized as the primary source of increased metal intake in voles in the winter/spring period (Janiga et al. 2012, 2016). In the Tatra Mountains, metals accumulate in the upper soil layers (Ciriaková et al. 2011; Krendželák et al. 2018) and increase with altitude (Janiga 2008). The rhizosphere of wild vascular plants significantly influences the uptake of lead (Sivertsen et al. 1995). Metals are bioaccumulated from the soil by herbivorous larvae of insects along the food chain (Metcheva et al. 2008; Belcheva et al. 1998). The imagos and insect larvae are a major source of food for accentors.

Mercury in the feathers

It is now generally accepted that more than 90% of atmospheric mercury is in the vapor phase and in inorganic form. Methylmercury species are present in ambient air in minor quantities (Petersen et al. 1998). Trace elements are removed from the atmosphere by dry deposition (sedimentation, interception, and impaction) and by wet deposition (rainout, washout). The first process is strongly dependent on particle size, wind velocity, and surface characteristics, while precipitation scavenging is largely a measure of the liquid water content in the precipitation clouds (UNECE 1995). Small wetlands and frequently saturated soils in alpine ecosystems may offer an opportunity for methylation that transforms ionic mercury into highly bioavailable methylmercury (Benoit et al. 2003; Evers et al. 2007). The methylation process is also supported by the addition of sulfur pollution, acidification (Steffan et al. 1988), and snow cover (Poissant et al. 2008). As sulfur is increased from summer to autumn in the Tatra alpine habitats (Krendželák et al. 2018), the activity of sulfur-reducing bacteria probably increases in the conversion of inorganic mercury to methylmercury (cf. Jeremiason et al. 2006). Recent findings show that animals in some terrestrial ecosystems may contain surprisingly high levels of methylmercury (Bianchi et al. 2008; Tsipoura et al. 2008; Leonzio et al. 2009). For example, elevated methylmercury has been measured in birds of subalpine ecosystems, such as the blackpoll warbler (*Setophaga striata*) and the endemic Bicknell's thrush (*Catharus bicknelli*). The level of methylmercury in the thrushes ranged from 0.3 to 1.2 μg/g (Rimmer et al. 2005), and were significantly lower than in alpine accentors from the Tatra Mountains. Magpies (*Pica pica*) from the vicinity of zinc smelters in Poland contained from 0.07 to 0.7 μg/g methylmercury in their feathers (Dmowski 1997). Currently, it appears that autotrophs make sufficient amounts of methylmercury available to the terrestrial food web. Schwesig and Matzner (2000) measured sufficient methylmercury concentrations in the layer of forest soils and Miller et al. (2005) suggest that leaves of plants assimilate mercury from the atmosphere.

In the present study, we did not find significant differences in mercury concentrations between immature juvenile and adult birds or between feather types from the pre- and post-molt period. For accentors, body molt in males begins during mid-August and in females, it may continue sometimes until the end of September. Therefore, a sample of two outer tail feathers chosen from an individual bird was representative of

Table 3 Product—moment correlations of erythrocyte characters (weighted means of characters of 50 cells per bird) to the levels of lead in the blood of alpine accentors

Variable	Correlation (*r*) to amount of lead	*t* test	*P*
RBC perimeter	− 0.35	− 1.90	0.061 NS
RBC length	− 0.39	− 2.11	0.045*
RBC width	0.06	0.31	0.756 NS
Nucleus perimeter	− 0.28	− 1.47	0.150 NS
Nucleus length	− 0.28	− 1.49	0.150 NS
Nucleus width	0.29	1.54	0.136 NS

Only erythrocyte length significantly decreased with the increasing amount of lead in the blood. Blood from 27 alpine accentors was collected and dimensions of 1350 erythrocytes were measured

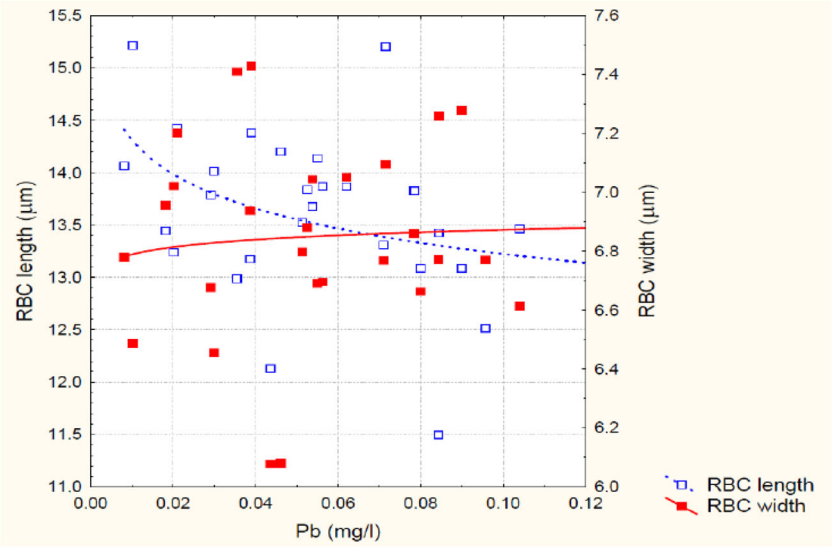

Fig. 1 Scatterplot of red blood cells (RBCs) length and width against amount of lead in blood of alpine accentors (RBC length = 12.1316–1.0918*log10(Pb); RBC width = 6.9553 + 0.0833*log10(Pb)). Points denote weighted means of 50 RBC lengths and widths of 27 alpine accentors

current intake in late summer and early autumn, because the tail feathers regrow in the second half of the molt period. Levels of mercury in first-molt feathers may indicate a combination of current dietary intake plus stored organic mercury accumulated between molts (Furness 1993), while flight feathers grown in the breeding area simply reflect accumulation of mercury prior to molt (Burger et al. 1992). Moreover, our adult birds had comparable metal levels in their feathers as did immature juveniles. Because the diet of accentors during the breeding period has remained constant over decades (flying tipulid imagos, tipulid larvae, and Colembolla living in the upper soil levels), birds should reflect any mercury pollution in the alpine soil environment. When compared to aquatic ecosystems, the biogeochemical factors that influence methylmercury availability in alpine soils are not yet completely known and require further investigation. This issue is of particular concern because alpine fauna is restricted to high elevation habitats for breeding.

Lead in the blood

The symptoms of lead intoxication in birds include increased kidney weight, the presence of renal intranuclear inclusion bodies, altered mitochondrial structure, partial inhibition of hematopoietic systems, and anemia (Haig et al. 2014). Anderson and Havera (1985) considered 0.5 μg/g lead as a threshold in blood for lead-poisoned waterfowl, while Tsipoura et al. (2008) reported 0.4 μg/g lead as a threshold in blood for small passerine birds. Reding et al. (1983) based on enzyme assays concluded that blood concentration as low as 0.2 μg/g indicates sublethal lead exposure in eagles. Passerines from locations not exposed to lead contamination generally display low concentrations of lead in the blood during the breeding period: from 2 to 10 μg/dL. However, in highly contaminated industrial areas, the birds exhibit concentrations between 20 and 40 μg/dL (Nyholm 1994, 1995, 1998; Chapa-Vargas et al. 2010), and may be classified as subclinically poisoned (Kelly and Johnson 2011). Johnston and Janiga (1995) published a review on lead concentration in feral pigeons from American and European cities, wherein the concentrations usually increased progressively with proximity to the centers of large cities. Bone lead levels in central London ranged from 108 to 670 μg/g (blood—from 16 to 101 μg/dL), and in central Paris from 174 to 500 μg/g (blood—from 42 to 65 μg/dL). Pigeons absorbed the lead through the respiratory tract. Bones from alpine accentors contain lead at 90 μg/g (Janiga 2001), while the blood levels are relatively low—from 0.8 to 10 μg/dL, confirming the gastrointestinal route of lead into the body. In our study area, feces of accentors contain relatively high concentrations of lead. The highest concentrations were found in early spring (31 μg/g), while in summer, the amount decreased to 11 μg/g (Janiga 2002). In spring, lead from many layers of melting snow penetrate into the alpine soils and birds collect insects from these wet soils or snowfields (Apostoli et al. 1988). Alpine accentors are relatively small, with short lifespans and a high metabolic rate, and are significant accumulators of environmental lead. Concentrations of lead in the blood of accentors suggest that birds are representatives of acute exposure to lead in the spring. Increased lead in bone represents chronic exposure; the lead in bone accumulates over the lifetime of the bird. Their nestlings have approximately five times lower lead levels in the bones (Fig. 2) than adults but blood levels of young and adults do not differ. Our study has confirmed the assumption that accentors accumulate more lead than atmospheric measurements of lead concentration in the West Carpathians (Tužinský and Chudíková 1991). The blood of some specimens of accentors shows the first symptoms of non-regenerative anemia. This type of anemia develops quickly in birds because of their short erythrocyte half-life. Anemia can be caused by lead toxicosis (Martinho 2012) and it is usually associated with microcytosis. In accentors, we found a clear tendency in

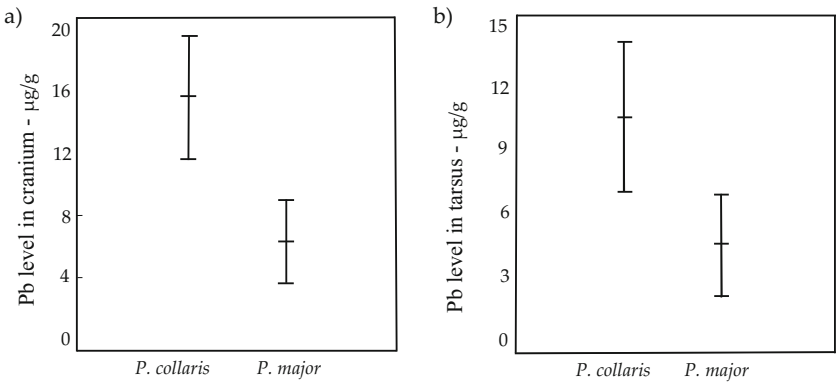

Fig. 2 Average levels of lead (with 95% confidence intervals for factor means) in the bones of 10-day old nestlings of alpine accentors ($n = 5$) and great tits ($n = 11$) from the Tatra mountains

erythrocytes to change their shape from ellipsoid to smaller and round with an increasing amount of lead in their blood (cf. Kostelecka-Myrcha et al. 1997). Lead inhibits aminolevulinic acid dehydratase (ALAD) enzyme activity, which is important in the biosynthesis of heme. Some authors mention that Pb concentration around 15 μg/dL in blood can cause sublethal effects such as inhibition of δ-ALAD activity in birds (Martínez-López et al. 2004; Gómez-Ramírez et al. 2011; Espín et al. 2015), but lower concentration of Pb in blood also causes enzymatic inhibition (Gómez-Ramírez et al. 2011). Another enzyme that is seriously inhibited by lead is 6-Phosphogluconate dehydrogenase (6PGD). This enzyme is essential to cytoplasmic production and is necessary for erythrocyte viability. Microcytosis in lead poisoning occurs due to lack of heme, which leads to a lack of hemoglobin and thus a deficiency, causing smaller erythrocytes (Apostoli et al. 1988; Tejedor and Gonzáles 1992; Urrechaga et al. 2015). Our results indicate that blood lead levels of accentors may have been approaching the threshold level for further hematological effects. From this point of view, *Prunella collaris* looks to be a suitable physiological bio-indicator of lead pollution in the high mountains.

Conclusions

Data on lead concentrations in the blood of alpine accentors suggests that alpine biota in the West Carpathians is probably under permanent long-term stress from exposure to metals existing in the atmosphere in different physical and chemical forms. Additionally, we found five dead nestlings of alpine accentors and compared the amount of lead in their bones to the amount of lead in the bones of great tit (*Parus major*) nestlings living in the same study area. Mean bone lead concentrations in ten-day-old accentor nestlings ranged from 11 μg/g (tarsus) to 16 μg/g (cranial bones), and were significantly higher than in nestlings of great tits from the lower parts of the valleys in the Tatra Mountains (Fig. 2). Mean lead levels between the cranium and tarsus significantly differed at $P = 0.001$ and $P = 0.009$ (one-way ANOVA), respectively between the two species of passerines. Because lead is referred to as one of the few elements which can be transported through the air, our results encourage additional research to ascertain the effect of climate change on the environmental cycle of lead in the high mountains. The effect of increased temperature on lead deposition is expected to influence the distribution of lead within the bodies of alpine birds and mammals, and will probably change their habitats in these sensitive alpine regions (Matz et al. 2011)

Over the course of our study, alpine accentors from the Slovakian West Carpathians maintained mercury concentrations in feathers at the significantly comparable level between 1990 and 2010. Determination of long-term trends in mercury pollution indicates that the contamination of the diet of alpine birds has remained constant over decades. Because all of the mercury entering feathers is methylmercury, even in birds storing inorganic mercury in the liver (Thompson and Furness 1989), plants and bacteria of alpine soils must play an important role in the methylation of mercury, such as in the rhizosphere where bacteria transform inorganic mercury to methylmercury in alpine soils. At sites that are highly contaminated, this may be a concern, and further studies are required to assess whether mercury levels in internal tissues have become hazardous at this time.

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Author contributions MJ initialized study, collected samples in the field, interpreted the data, and wrote the manuscript. MH collected samples in the field, made laboratory examination of blood smears, and helped with the preparation of the manuscript. Authors have reviewed and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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