Oecologia
Montana 2020,
30, 42-49

Influence of car traffic on lead contamination in tissues of juvenile *Parus major* during postnatal development

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Abstract. The main objective of our research was to identify the impact of external factors (spatial and time) on the amount of lead in the tissue great tit nestlings (*Parus major*) during ontogenesis in the submontane region of the High Tatra mountains. The research was carried out in the vicinity of several roads in the municipalities of Štrbské Pleso, Tatranská Lomnica, and Starý Smokovec in 1995-1996. The results confirmed that the amount of Pb found in tissues of great tit nestlings were different between studied sites, likely due to variation in traffic density. The size of each clutch also affects the amount of Pb in tissues. Lead content in tissues of nestlings from nests with a larger number of siblings (a larger clutch), is lower than in the tissues of individuals from a smaller clutch. The amount of lead rises with age; however, it does not depend on individual size predisposition. An insignificant increase in Pb levels were recorded during periods of bone growth (ulna/radius and tarsometatarsus). The amount of lead increased with increasing weight and with growing feathers. This means that Pb is taken up through the process of ontogenesis, mainly via feeding and it quickly accumulates into soft tissues and rapidly growing feathers. The lead level increased with rising weight and growing feathers, which points to accumulated lead derived mostly through feeding. The nestlings at sites with lower intensity traffic exhibited accelerated growth of feathers, as more metabolism was invested in plumage growth. This is considered a positive trend, as plumage growth correlates to an earlier departure from the nest.

Key words: Parus major, lead, ontogenesis, automobile transport

Introduction

There are many studies with a focus on lead poisoning in wild bird populations. However, they primarily focus on lead poisoning in birds caused by lead bullets. It is estimated that annually, lead kills up to one million wild birds in Europe, and results in sublethal poisoning in another three million (Johnson *et al.* 1982). Birds can be poisoned by ingesting lead. Often this occurs when they are struck by a lead bullet, or through fragments of ammunition present in their food. Symptoms of lead poisoning in birds can include lethargy, loss of muscle mass and fat stocks, anaemia, green droppings, a dropped wing, loss of balance and coordination, and other neurological symptoms such as paralysis or twitching in legs (e.g. Wobester 1997; Friend *et al.* 1999; Pattee and Pain 2003). Any or all of these symptoms may occur as a result of lead poisoning.

Lead gradually accumulates in bones throughout the life of the individual. Based on the level of lead present in bones, we can determine whether the individual has been chronically exposed to lead toxication or exposed to a single strong dose of lead toxication. Lead is gradually released from bones into the bloodstream, as a result of bone remodelling; a long-term process over a bird's lifespan. The lead content present in bone is generally considered to be an average lifelong exposure. High levels such as 5 mg.kg^{-1} in bones indicate a lead-polluted environment (Kelly and Kelly 2005).

Bones are one of the best and most reliable indicators of lead content in animals, as the level of lead detected serves as a cumulative dosimeter of lead exposure over many years. Bones are an important sample and indicator of lead exposure in bird bones because they contain 90 % of total lead. Accumulation is not always the same in each bone but may differ between bone types (Scheuhammer 1996).

Bone marrow plays an important role in absorption of lead by bone tissue. Lead deposits in bones through the bloodstream, and bone marrow receives blood from outside the bones. Therefore, bone provides valuable information on lead exposure and can be a very useful tool in investigating mortality from lead poisoning (Llacuna *et al.* 1995).

The great tit (*Parus major* L.) is a small bird. Their reproduction period takes place between March and August. Nesting begins during March and April, and if conditions are favourable, reproduction can occur up to two times per year. Tits build nests in various cavities, so they are a suitable species for settling nest-boxes. The typical clutch size can vary; however, it is most often 6 to 12 eggs. After hatching, both parents feed the chicks until they leave the nest (or longer!), up to 990 times per day (Vilček 1984).

Both the size of the clutch and the size of the eggs themselves are expressions of the quantity of energy invested by the female great tit into the reproductive process (Pikula 1976; Nilson and Raberg 2001). Individuals that nest in highly urban environments such as city parks, must invest much more energy to provide nourishment due to the poor quality of the habitat; both in terms of food availability and proximity. Therefore, nesting pairs in these environments invest less energy in the clutch (Hinsley *et al.* 2008).

In studies evaluating the influence of several factors on the development of anorganism, it is more appropriate to use the physiological age of the young (expressed by changes in size) than the chronological age. The overall body size is preferable as an estimate of biological age because it is more directly tied to growth than chronological time (Strauss 1987). Because individual birds have a remarkable capacity to vary their mass and volume depending on their nutritional status (Emlen *et al.* 1991), it is biologically most meaningful to define size from skeletal measurements, independent of nutrient reserves (Piersma and Davidson 1991).

The fact that larger and heavier tits also yield larger and more viable eggs, can also be considered crucial (Schifferli 1973; Nager and Zandt 1994; Hegyi 1996). A factor that influences egg size significantly is the dominant status of the female, as well as her age (Desrochers and Magrath 1993; Báldi and Csörgö 1994). Additionally, egg size is also affected by the size of the clutch itself (Batt and Prince 1979). It is known that up to 80 % of eggs are conditioned hereditarily. Nevertheless, the rule that the larger the female, the bigger the eggs, can still be applied (Horak *et al.* 1995). It has been found that chicks hatched in years with a poor food supply exhibit a shorter tarsus than those that hatched in years when there was sufficient supply (Horak 1994).

Our research was conducted between 1995 and 1996 when lead-based fuel in Europe was still prevalent. This kind of fuel had a destructive impact on ecosystems and animals with close proximity to major roads. Therefore, the focus of this research was on birds nesting and reproducting near roads in the Tatras. We focused on the three most wide-spread municipalities in the High Tatras region in terms of vehicle traffic, which are situated directly under the High Tatras. Close attention was paid to the level of lead with respect to the location, the annual period, the number of eggs, the length of the feathers, and the length of the tarsometatarsus. We compared the amount of transport occurring in the selected locations (largely tourist municipalitie). Throughout the year these municipalities experience an influx of many tourists and associated traffic.

Material and Methods

Study area and sampling

In order to investigate the direct impact of car traffic on young birds in the nest, wooden nest boxes were placed in trees near the road in selected localities during early spring. The research was carried out from May to July during the years 1995 and 1996 in three localities: Tatranská Lomnica (850 m asl.; GPS: 49.16472° N, 20.28222° E), Starý Smokovec (1010 m asl.; GPS: 49.14097° N, 20.22102° E), Štrbské Pleso (1350 m asl.; GPS: 49.11889° N, 20.06361° E) in the foothills of the High Tatra Mountains, Slovakia.

In longitudinal transects at an average distance of about 10 m from the road, about 15 nest boxes were placed at each site. The boxes were placed at a height of 2.5-3.0 m above the ground. At Štrbské Pleso the first nesting box was placed at the following GPS coordinates: 49.1067853° N, 20.0755453° E. Every other nesting box was placed 100 meters from the previous one over a distance of 1.5 km. In the village of Starý Smokovec, the first nest-box started at the GPS site: 49.802412° N, 20.1412263° E. In the municipality Tatranská Lomnica the first nest-box was placed at GPS coordinates: 49.917627° N, 20.1901498° E. Nest boxes were placed in each location and arranged in the same way (Fig. 1).

Fig. 1. Placement of wooden nest-boxes near roads in selected locations (Author: Marián Janiga 1995).

From May to July boxes were monitored regularly during the nesting period, and after hatching they were monitored every 2-3 days. Dates corresponding to egg laying and hatching, the number of eggs (clutch size), as well as measurements of egg length and maximum width were recorded consistently during the monitoring period.

The clutch size was determined when no additional eggs accumulated in the box (Gosler 1993). Hatchlings were sampled, and the following parameters were measured: length of tarsometatarsus, wing length (length of ulna / radius), and weight. Juveniles selected for analysis were killed by chloroform inhalation after measurement, then stored in plastic bags and frozen at -18° C.

Laboratory analysis

The samples were thawed at room temperature and were prepared for additional laboratory analysis. In the PTFE autoclave vessel, 0.5–1 g of each sample was weighed and 5 ml of 65 % HNO_3 and 3 ml of double-deionized water were added. The sample was decomposed for 5h in the autoclave (Autoclave ZA-1, Zahnašovice, Czech Republic) at 14° C in a drying box. The cool sample was put in a 25 ml volumetric flask and set up to 25 ml with double-deionized water.

Perkin-Elmer Model 1100B atomic absorption spectrometer (AAS) was used for flame atomic absorption analysis of lead in tissue samples. For graphite furnace atomic absorption spectroscopy (GFAAS) analysis, the same spectrometer was used equipped with a Perkin-Elmer HGA 700 graphite furnace. Samples were introduced into the furnace with a Perkin-Elmer AS 60/70 autosampler and the results were recorded with a Perkin-Elmer EX-80 printer. The instrumental conditions for lead measurement were: lamp current (HCl) 10 mA, wavelength 283.2 nm, slit width 0.7 nm, gas air-acetylene. For GFAAS determination of lead, the pretreatment temperature of 700° C and atomization temperature of 2000° C were used. Ammonium dihydrogenphosphate ($NH_4H_2PO_4$) 0.2 mg was used as a chemical modifier for the measurement of Pb. Deuterium background correction was used throughout the work.

In each nest box, the maximum length and width of each laying egg were measured. The average length and width of an egg per clutch were calculated as the sum of all lengths and all widths and divided by the number of eggs. From this value, the theoretical average volume of eggs was calculated using the Hoyt´ s constant (Hoyt 1979):

$$\mathbf{V = Kv.\ LB^2}$$

Where:

Kv = 0.5228
L = length
B = breadth or maximum diameter

Energy investment of the female in the brood

The size and quantity of eggs in each clutch are not constant and differ among females and between nesting cycles year over year. To better understand the relationship between the energy investment of females and the postnatal development of nestlings, we calculated a female's energy investment in elation to each brood by multiplying the average volume of eggs and the number of all eggs in the clutch.

Car traffic during a typical day

To determine the correlation between the number of cars (emission load) and postnatal development of juveniles, the number of cars in the monitored localities on a typical day of the season (month). Each month (May-July), a random day was selected, in which all cars passing through the location were counted within a time unit (1 hour). The measurement was performed continuously for several hours on the same day and was divided into 2-3 consecutive days.

Statistcal analysis

Data from field observations was further processed using a spreadsheet table in Microsoft Excel. The data included species, place, date of the clutch, month, year, nesting box number and description, number of eggs in the clutch, average length, average width, egg volume, energy investment of the female, sampling date, sample mark, amount of lead, age, and measurements of wings, wing-vane, tarsometatarsus, and weight. Statistica, Ver. 8 was used for data analysis, and one–way ANOVA was used for statistical comparison between selected parameters (95 % confidence level $p < 0.05$).

Results

Lead concentration in great tit tissues collected from the research sites (Štrbské Pleso, Starý Smokovec, Tatranská Lomnica) varies (Fig. 2). The highest levels of lead were recorded in the tissues of jueniles from the Starý Smokovec site (F (2.45) = 3.4, p = 0.04*). The amount of lead (Pb) in bodies of tit chicks in nests depends on each site and its corresponding traffic density (Fig. 3).

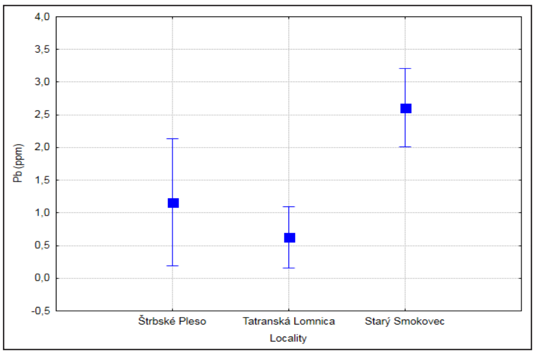

Fig. 2. Mean (± SE) concentrations of lead (ppm; dry weight) in the tarsi of nestlings of great tits from nest boxes located near the roads in the submountain region of the High Tatras. Nestlings were at the age of 6 to 20 days. Chicks from Starý Smokovec contained higher amounts of lead than birds from Tatranská Lomnica or Štrbské Pleso (F (2,45) = 3.4, p = 0.04*). The density of traffic was between two and three times higher in Starý Smokovec than in Tatranská Lomnica or Štrbské Pleso (Fig. 3).

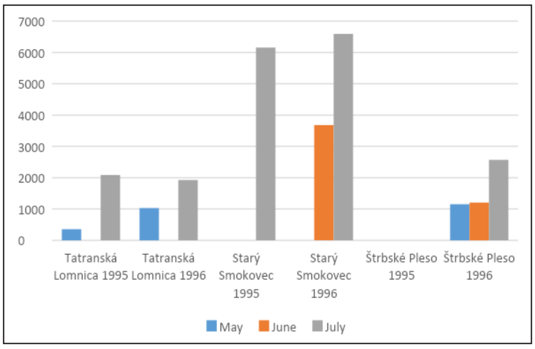

Fig. 3. During the period under review, the lowest car counts were recorded in Tatranská Lomnica and the highest in Starý Smokovec.

Comparison of the first nesting season (spring) and second nesting season (summer) (Fig. 4) indicates that the amount of lead in sampled tissues increases in the summer (F (1.46) = 3.3881, p = 0.7212). However, the differences between the seasons were not statistically significant. This phenomenon is also related to an increased amount of automotive transport in the summer months (Fig. 3).

When examining the dependence of the number of eggs and the level of Pb in the tissue of the nestlings tissues, we recorded a trend that with a larger number of eggs (10-16) the amount of lead in juveniles is lower (F (1.44) = 4.8301, p = 0.3328) (Fig. 5). However, this difference is not statistically significant.

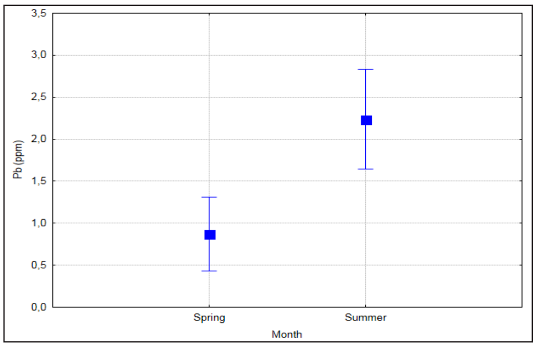

Fig. 4. Mean (± SE) concentrations of lead (ppm, dry weight) in the tarsi of nestlings of great tits from nest boxes located near the roads in the submountain region of the High Tatras. Nestlings were at the age of 6 to 20 days. Nestlings did not differ between summer and spring season (F (1,46) = 3.3881, p = 0.7212).

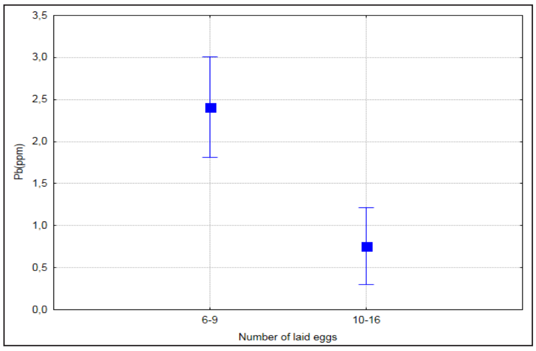

Fig. 5. Mean (± SE) concentrations of lead (ppm, dry weight) in the tarsi of nestlings of great tits from nest boxes located near the roads in the submountain region of the High Tatras. Nestlings were at the age of 6 to 20 days. The clutch size had no significant effect on Hg levels in nestlings (F (1,44) = 4.8301, p = 0.3328).

The same trend was also recorded in the energy investment by females in their eggs (transformed into the total volume of eggs). The energy investment of a female into a clutch may vary depending on the size of the eggs and the number of eggs in a clutch. Higher energy investment correlated to lower lead levels in nestling tissues (Fig. 6; F (1.27) = 3.2690, p = 0.8175). Nevertheless, this phenomenon is not statistically significant.

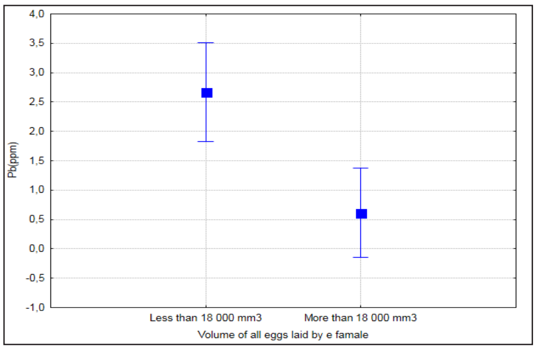

Fig. 6. Mean (± SE) concentrations of lead (ppm, dry weight) in the tarsi of nestlings of great tits from nest boxes located near the roads in the submountain region of the High Tatras. Nestlings were at the age of 6 to 20 days. Pb levels in nestlings did not depend on the total volume of eggs (F (1,27) = 3.2690, p = 0.8175).

We compared lead levels in tissues and physiological age and individual growth factors (plumage growth on the wing and length of tarsometatarsus). Lead levels increased significantly in older nestlings (Fig. 7; r = 0.6273, p = 0.0005). a similar situation was observed when comparing the lead levels and wing plumage growth. As growth of wing plumage increase, lead levels also increased (Fig. 8; r = 0.5331, p = 0.0497). We recorded the same trend in tarsometatarsus growth, although this correlation was not statistically significant (Fig. 9; r = 0.3362, p = 0.1167).

Physiological age of birds and density of traffic

The density of traffic near roads was divided into two categories: less than 2000 cars per day and

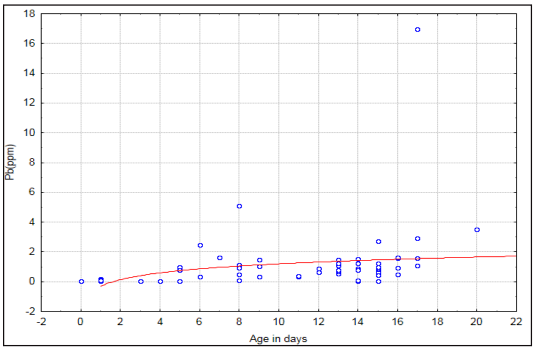

Fig. 7. Concentrations of lead (ppm, dry weight) in the tarsi of nestlings based on age (Pb = 0.3152 + 0.9833 * log10(x), r = 0.6273, p = 0.0005).

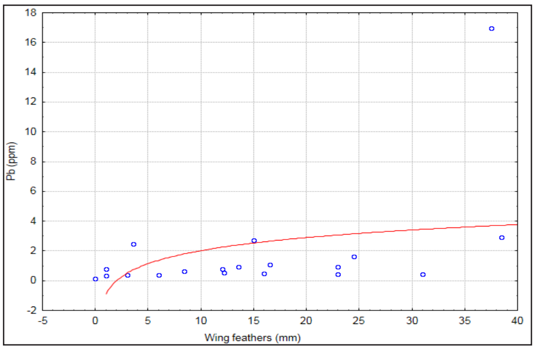

Fig. 8. Concentrations of lead (ppm, dry weight) in the tarsi of great tit nestlings based on wing feather length (Pb = 0.89 + 2.9079 * log10(x), r = 0.5331, p = 0.0497).

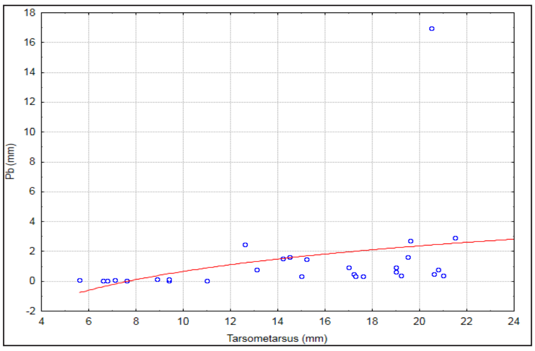

Fig. 9. Concentrations of lead (ppm, dry weight) in the tarsi of great tit nestlings based on tarsus length (Pb = -5.0211 + 5.6849 * log10(x), r = 0.3362, p = 0.1167).

more than 2000 cars per day. Traffic patterns did not appear to impact the physiological growth of young birds' bones (Fig. 10) or soft tissues (bodyweight – Fig. 11).

Taking into account that the amount of Pb in the growing wing feathers of young tits increases (Fig. 8) more quickly than Pb levels in leg bones (Fig. 9), we can state that the physiological growth of young from nests with heavy traffic was delayed. As a result, they stayed in the nest longer (i.e. they were fed longer) and were exposed to lead in the given locality for a longer duration (Fig. 12).

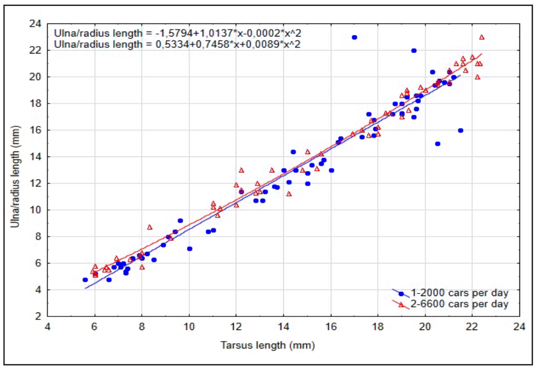

Fig. 10. Growth pattern of great tits (skeleton) in connection to traffic density. Pattern of growth of young did not differ on the number of cars at nearly roads.

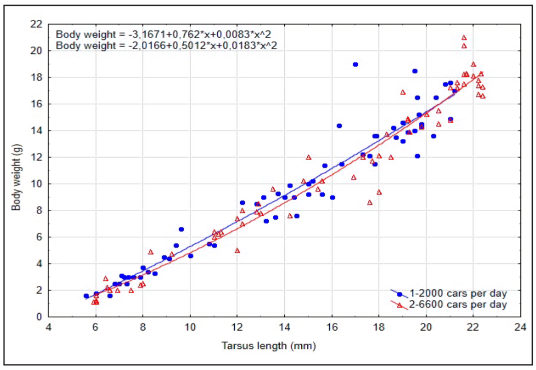

Fig. 11. Growth pattern of great tits (body weigh) in connection to traffic density. Pattern of growth of young did not differ on the number of cars at nearly roads.

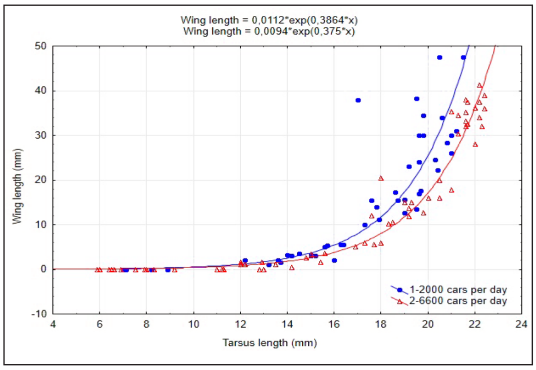

Fig. 12. Growth pattern of great tits (feathers) in connection to traffic density. Wing feathers of young grew relatively faster when related to tarsus bones in localities with less traffic than feathers of nestlings from localities of heavier traffic.

Discussion

To determine the heavy metal load of an environment, different species of birds are often used dependent on their position in the food chain. A potential animal species needs to comply with various requirements in order to be a reliable and contiguous biomonitor (Van Eeden and Schoonbee 1996). Great tit (*Parus major*) is often used as suitable model in behavioural and ecological research and are very useful as biomonitors for local contamination (e.g. Eens *et al.* 1999; Masahiko and Noriko 2001; Brnušáková and Janiga 2014). Due to the fact that tits readily use man-made nesting boxes, breeding populations can be easily established to monitor and study them in an area of interest, where it is possible to identify and evaluate local heavy metal pollution (Janssens *et al.* 2003). In our research, nesting boxes installed at selected sites were inhabited by tits during the first year of study in 30 % of cases. Species included *Parus major, Periparus ater* and *Lophophanes cristatus*. The dominant species was *P. major*.

Pb levels in young tit tissues varied between the three localities. Great tit nestlings from the site closest to the pollution source have significantly higher concentrations of metals (Dauwe *et al.* 2004). In our research, the highest concentration of Pb in tissues of tit juveniles was found in Starý Smokovec. This road is the most frequented, as it runs directly between Poprad, the largest foothill town and the town administrative center of the High Tatras, St. Smokovec. This location was observed to have the highest number of vehicles per day (Fig. 3).

The natural environment has been gradually contaminated by various forms of pollution, mainly as a consequence of urbanization and the increasing use of fuels by households, vehicles and industry (Swaileh and Sansur 2006). Lead remains a frequent contamination of vertebrates, especially in alpine habitats (Kompišová Ballová *et al.* 2020). The mobility of European Union residents has been growing as well, generating a substantial increase in the number of vehicles on the roads. According to Schafer and Victor (2000), the total mobility of the world's citizens at the turn of the millennium was 23 billion km, and will reach 105 billion km in 2039. In recent years, traffic congestion is becoming a significant problem for the majority of large cities all over the world, resulting in a reduction in transportation efficiency as well as increasing traffic exhaust emissions (Pan *et al.* 2018, 2019). Increased tourism in mountain areas results in increasingly toxic levels of lead in the environment as well as the growing prevalence of noise pollution; one of the most significant adverse factors disturbing a given habitat. The continuous movement of vehicles on roadways results in disturbing effects on nearby fauna and flora (Reijnen *et al.* 2002).

One of the consequences of increased traffic is an increase in emissions. As part of our research, we found that lead levels in the bodies of tit chicks are influenced considerably by the density of automotive transoport at a given site. Our research took place in 1995-96, when emissions from cars contained lead in addition to CO_2, due to additives in combustion mixtures. Current standards include construction and operation of low-emission vehicles and improvement of traffic flow (building ring roads, viaducts and gradeseparated junctions) (Szwalec *et al.* 2020), but road transport remains detrimental to soil ecosystems along roadways. Heavy metals (Cd, Pb, Zn and Cu) with cumulative coefficients of 10–600 are among the substances emitted by vehicular traffic (Xu *et al.* 2014). Leaded gasoline is not the only source of rising lead concentrations in the environment. Other human activities, such as fuel combustion, industrial processes and solid waste combustion are also contributors.

In summer, measured Pb levels were higher than in spring. This phenomenon can also be attributed to an increased number of cars during tourist season. An interesting hypothesis is presented by Markowski *et al.* (2014), who observed higher concentrations of heavy metals in nestlings indirectly related to precipitation. According to Leech and Crick (2007), heavy rain decreases the number of available invertebrates by washing them out of vegetation. Such conditions may cause food limitation for canopy feeders and force them to forage for their young on the ground where food is more contaminated.

Lead is one of the most widely distributed toxins in our environment. It cannot be broken down, only converted to other forms. Studies on lead contamination in birds conducted in industrial areas showed a definite influence of environmental pollution on the levels of metals accumulated by birds (Dmowski 1993; Adout *et al.* 2007; Berglund *et al.* 2010). Lead can be accumulated in the bodies of soil organisms. Soil functions are disturbed by lead intervention, especially near highways and farmlands, where extreme concentrations may be present. Soil organisms suffer from lead poisoning and subsequently, these organisms can influence entire food chains (www.lenntech.com 2021). Tits as a nidicolous bird spend their first days of life inside nest boxes. Their main source of heavy metals exposure is through food supplied by their parents (Furness 1993). The great tit is versatile and often forages in bushes and even on the ground (Lack 1971). It is known to prefer caterpillars as the main food source for nestlings (Cholewa and Wesołowski 2011).

Diet may be an important variable in the manifestation of metal concentrations. The juvenile great tits are good indicators of lead toxicity from automobile transport, because these individuals easily absorb and retain thie pollutant due to an extremely rapid metabolism. Food provided by parents from the environment surrounding the nest had a high level of lead, resulting in an increase in lead levels in the chicks during the growth stage. Chicks that exhibited greater so-called ‘selfish activity’ did not receive food in larger quantities. Interestingly, tit can precisely determine the quantity of food required by a particular individual depending on its size. According to a study by Neuenschwander *et al.* (2003), tit of higher biological age also exhibit a greater intensity of begging behaviour and higher competition for food. However, development quality and the ability to leave the nest often decreases in these individuals.

This organism is characterized by a specific ability to discharge toxic elements of lead to their fast growing feathers, bones, or faeces (Dauwe *et al.* 2002, Deng *et al.* 2007). The liver discharges lead into feathers and bones in a way that prevents distribution to other body parts (Nybø *et al.* 1996). Lead content present in the body increases with rising age in nestlings, based on contamination levels present in the food supply. This phenomenon is also confirmed by the gradual increase in Pb levels with age, and both feather and tarsometatarus growth (Figs. 8-10). The same trend was observed in study by Brnušáková and Janiga (2014).

We concluded that nestlings from nests with more eggs tended to have lower Pb levels in their tissues. Taking into account the female’s energy investment in laying, we found that in nestlings where the female had a higher energy expenditure (larger total egg volume), Pb levels were also lower. It can be assumed that a tit living during the nesting period in a biotope with higher lead concentrations will be much more contaminated, and therefore, can pass a higher percentage of lead to the clutch than a tit that lives in a less contaminated environment. Reproduction is usually described as one of the most energetically costly phases in the life cycle of an organism (Stearns 1992; Charnov 1993). In birds, reproduction is divided into nest construction, egg laying, incubation and chick rearing (Hansell 2000; Heenan 2013). Females occupied by taking care of a larger number of young tits are less able to effectively collect food than a tit caring for a smaller number of chicks. A larger number of young individuals results in a higher energy investment by the female into care but cannot compete with a tit that uses a lower energy investment in more frequent and intense feeding intervals. Therefore, young birds from large clutches leave the nest at a lighter weight and have a substantially reduced post-fledging survivorship. Evidence exists for this in a population of great tits, that varied their average clutch size from eight to twelve over a 17-year period, likely in response to crowding and the resulting changes in the density of the caterpillar population; their primary food source (Pianka 2008). In their study, Fritsch *et al.* (2019) found that *Turdus merula* showed a correlation between Pb levels and nesting success, where the breeding success of females decreased with increasing exposure to Pb. This research indirectly confirms our finding that higher levels of Pb are found in less successful clutches, with lower numbers of chicks and smaller eggs (Figs. 6-7).

Lead levels did not increase with body or bone growth. Weight and growing feathers proved to be the most important factors. In these two cases, the increasing curve of the lead level in the body of tit chicks was significant. Wing feathers of young grew relatively faster when related to tarsus bones in localities with less traffic than feathers of nestlings from localities with heavier traffic. It is possible that this is a detoxification method where Pb is deposited more readily in growing feathers. Condition parameters (body mass and hemoglobin concentration) were not related to heavy metal concentrations in the nestlings’ excreta of *Sylvia communis*, which may correlate to the ability of young to detoxify their body, according to Turzańska-Pietras *et al.* (2018).

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Received 21 April 2021; accepted 29 June 2021.