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Roe deer (*Capreolus capreolus* L.) antlers as an accumulative and reactive bioindicator of lead pollution near the largest Slovene thermal power plant

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ABSTRACT

Due to their morphology, high accumulation rate, intensive annual growth during a well-defined period and wide availability of historical samples, roe deer antlers are particularly suited for assessing temporal and/or spatial variations in environmental pollution with bone-seeking pollutants, such as lead (Pb). Beside determination of pollutant contents they also enable measurements of fluctuating asymmetry (FA), which reflects non-directional and stress-induced differences between the left and the right side of otherwise perfectly symmetrical bilateral traits. Pb levels and FA were determined in a historical set of roe deer antlers, obtained from animals shot in the 1961–2004 period in the vicinity of the largest Slovene thermal power plant of Šoštanj (the Šalek Valley, northern Slovenia). The highest Pb contents were determined in the oldest and the lowest contents in the most recent samples; since the mid-1960s, lead pollution has continuously decreased in the study area. Moreover, environmental (lead) pollution was identified as one of the main stressors causing deviation from bilateral symmetry of roe deer antlers due to the following findings: (i) antler FA was significantly lower after the construction of the desulphurization device in the power plant in comparison with the period before construction; (ii) on the generation scale, a highly significant positive correlation between mean Pb levels in antlers and their mean FA was found. Due to the obvious bioindicative potential of roe deer antlers, we suggest implementation of this extraordinary structure as a bio-sensor of habitat quality/pollution all over Europe.

Key words: roe deer, antlers, pollution, lead, fluctuating asymmetry

Introduction

In recent decades, intensification of anthropogenic pressures on the environment

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(e.g. rapidly ongoing processes of urbanisation, industrialisation and globalisation) has coincided with many efforts orientated toward minimizing of exposure to ecosystems (e.g. initiation of some ecological remediation measures for reducing emissions of toxic substances into the environment). The resulting complex situation demands the development of new monitoring methods and programmes, which enable determination of the recent stage and trends of environmental pollution, its effects on the biocenosis, as well as assessment of the effectiveness of existing ecological remediation measures.

Due to their morphology, high accumulation rate, intensive annual growth during a well-defined period (self-standardisation of the method) and wide availability of historical samples, antlers of different deer species – particularly those of the roe deer (*Capreolus capreolus* L.), which is one of the most suitable species for bioindication of environmental pollution in terrestrial ecosystems (see TATARUCH and KIERDORF, 2003; POKORNY, 2003) – represent a very suitable biomonitoring tool for assessing temporal and/or spatial variations in environmental contamination with bone-seeking pollutants, such as lead (for a review see TATARUCH and KIERDORF, 2003). Beside accumulative bioindication (e.g. determination of Pb contents) antlers also enable reactive bioindication through the measurement of fluctuating asymmetry (FA). FA, which reflects non-directional and stress-induced differences between the left and the right side of otherwise perfectly symmetrical bilateral traits (LEARY and ALLENDORF, 1989; PARSONS, 1990; PALMER, 1994), may be a useful sensitive and early-warning indicator of stressful conditions (CLARKE, 1995). Since antlers constitute secondary sexual traits, which are particularly suitable for FA analysis (THORNHILL and MØLLER, 1998), their asymmetry may perfectly reflect the ecological suitability of the habitat and probably also the quality of the animals carrying them (PELABON and VAN BREUKELEN, 1998). Indeed, the possibility of linking both accumulative and reactive bioindications on the same sample set enables a 'dose-response' approach, which gives antlers a tremendous advantage over many other biomonitoring tools.

Lead is one of the most typical bone-seeking pollutants. In mammals, more than 90% of Pb absorbed is deposited in the skeleton (LANDIS and YU, 1998), which indicates that antlers are a particularly suitable tool for assessing Pb burdens in the environment. Indeed, determination of the temporal variability of lead in roe deer antlers is highlighted, since Pb emissions underwent dramatic changes during the 20th century (intensive coal combustion in the first decades; a rapid increase of traffic in the second half of the century with subsequent introduction of unleaded petrol; introduction of some ecological remediation measures on large emission sources in the last decades). However, many of these changes occurred before adequate environmental monitoring programmes began; therefore, antlers may also help in reconstruction of trends in lead pollution and its effects

on the biocenosis for earlier periods, for which data on emission rates are lacking.

Considering the above mentioned facts, the main aims of the study were as follows: (a) the reconstruction of temporal trends in Pb burdens in the environment on the basis on Pb levels in a large set of roe deer antlers, obtained from animals shot in the 1961–2004 period in the vicinity of the largest Slovene thermal power plant of Šoštanj (ŠTPP); (b) the retrospective study of temporal variability in antler asymmetry, which may indicate the influence of anthropogenic emissions on FA (comparison of the antlers' FA before and after the construction of flue-gas cleaning devices on the power plant); and (c) determination of the relationship between Pb levels in antlers and their asymmetry, which may confirm environmental (lead) pollution as one of the main stressors affecting the appearance/viability of roe deer populations (e.g. by reducing the developmental stability of some physiological processes, such as the process of antler formation).

Materials and methods

Antlers of roe deer, shot in the 1961–2004 period, were collected in the Šalek Valley (and its hilly surroundings), which is situated in the northern part of Slovenia, a small country in Central Europe. All animals were shot at altitudes between 300 and 700 m above sea level and within 6 km of the ŠTPP. The study area is exposed to high ambient levels of pollutants due to its close vicinity to the ŠTPP, its position facing the prevailing winds above the power plant and its altitude just below the upper layer of the frequent thermal inversions. Although exact data on heavy metal emissions from the ŠTPP are not available, it should be mentioned that over 90 millions tons of lignite were burned in its boilers after 1980, which resulted in approximately 80,000 tons of emitted dust. Considering the average Pb content in lignite and in residual ash, the average yearly amount of emitted Pb in the 1980–2001 period was assessed at 22 t (POKORNY, 2003). However, it should be emphasised that two flue-gas cleaning devices were constructed at the ŠTPP in 1995 and 2000, resulting in a drastic decrease of annual dust emission as follows: 8121 tons in 1993, 1845 tons in 1996, and 467 tons in 2001, respectively (ROTNIK and RIBARIČ-LASNIK, 2002).

For chemical analysis, antler samples (n = 129) were taken following the method described by TATARUCH (1995) and KIERDORF and KIERDORF (2000). To eliminate the possibility of secondary contamination, antlers were thoroughly cleaned with a nylon brush; moreover, the bone surface (approximately 0.5 mm in depth) was removed by grinding. Afterwards, a hole was drilled into the back of each beam approximately 1.5 cm above the antler-pedicle junction, using a tungsten-carbide cutter fitted to a hand-held electric drill. Between 1 and 3 g of bone powder were collected from both antler beams of a specimen. Prior to analysis, the powder was ground and homogenised using a water

mill. A Milestone Ethos Plus microwave digestion system was used for wet digestion of samples. Bone samples (0.5000 ± 0.0010 g) were weighed into the microwave vessels to which 10 ml of concentrated HNO_3 and 2 ml of H_2O_2 were added. After digestion, samples were diluted to 50 ml with doubly deionised water (Barnstead Nanopure Infinity system). Inductively coupled plasma mass spectrometry (Hewlett Packard 4500 Plus) was used for Pb determination. In the following sections, all results are given as mg/kg of air-dry bone powder.

The age of the bucks carrying the antlers (grouped into three age classes: yearlings, young adults and elderly adults, respectively) was estimated based on tooth wear (when accompanying jaws were at our disposal), general antler shape, height and width of pedicles, ossification of skull sutures and ossification of nose cartilage. However, although it was hypothesised that together with bone minerals previously skeletally deposited Pb is also released by bone resorption during antler growth (TATARUCH and KIERDORF, 2003), we did not find any differences in Pb contents in antlers among the three age classes in preceding statistical analysis, which indicates that the mobilization of previously skeletally deposited Pb is of minor importance in comparison with the deposition of environmental Pb which is taken up by the animal during the antler growth period (POKORNY et al., 2004a). Therefore, for analysis of antler Pb-levels the age of the animals was ignored for the purposes of this study, and only pooled data across all age classes are presented in the following sections.

Fluctuating asymmetry (FA) was measured on nine bilateral traits (antler length, length of both front and back tine, total beam length, two circumferences of beam, beam and pedicle diameter, coronet height) of normally developed (without visible morphological abnormalities) and full-grown (after shedding of velvet) antlers of 282 roe deer, shot in the 1961–2002 period. However, necessary statistical pre-treatment of data – evaluation of measurement errors, presence of directional symmetry or antisymmetry, distribution of differences between the left and the right side (PALMER and STROEBECK, 1992; PALMER, 1994; POMORY, 1997) – revealed that only longitudinal antler traits (i.e. all four lengths) were suitable for FA analysis. Among them, total beam length (which was measured with a tape, and represents the sum of the length from the coronet to the top of the antler, length of the front tine and length of the back tine) seems to have the greatest bioindicative potential, because its asymmetry is not affected by the age of the animal analysed (excluding yearlings, whose antler asymmetry is, however, not a suitable bioindicator of environmental quality due to their incomplete ontogenetic development) (see also POKORNY et al., 2004b). Therefore, we employed only FA of the total beam length of adult roe deer antlers for the purposes of this study.

In the study population, the relationship between the trait size (total beam length) and

its asymmetry is rather weak (ibid.); therefore, two indices which are based on absolute rather than on relative differences (FA^1 = arithmetic mean of absolute differences between the left and the right side; FA_4 = variance of differences between both sides) were used for subsequent analysis (for details, see PALMER, 1994). Comparison of antlers FA between two sample sets (before and after the construction of the flue-gas cleaning device on the ŠTPP) was made by t-test (FA_1) and F-test (FA_4); in the later case, the F-test is simply the ratio of the larger over the smaller variance with appropriate degrees of freedom (ibid.). To test the existence of the correlation between Pb levels in antlers and their FA, the Pearson product-moment correlation coefficients (r) were calculated. In all tests, the limit of significance was set at $p < 0.05$. All statistical treatments were performed using the Statistics for Windows 5.5 software package (STATSOFT, 1999).

Results

Pb levels in roe deer antlers obtained from animals shot in the 1961–2004 period in the Šalek Valley, ranged between 0.15 and 7.28 mg/kg, with a median of 0.91 mg/kg and an arithmetic mean of 1.23 ± 0.17 mg/kg. The highest levels were determined in the oldest and the lowest in the most recent samples (Fig. 1). Results for five-year periods are presented in Table 1.

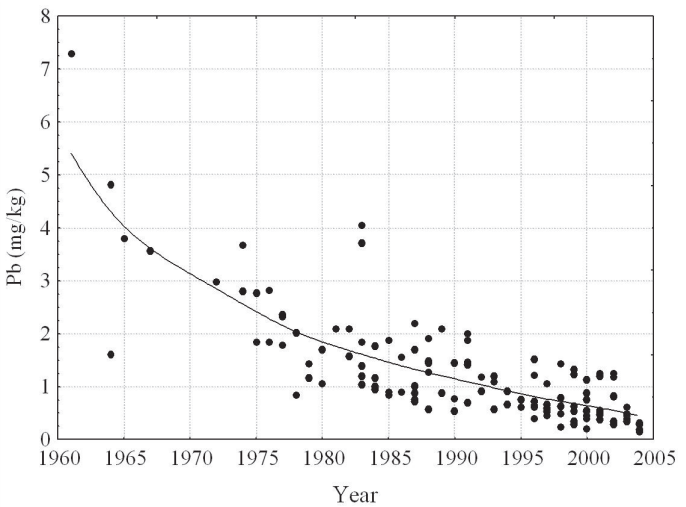

Fig. 1. Temporal decrease of Pb content in antlers of roe deer, shot in the Šalek Valley in the period 1961–2004; hatched line represents best-fitted function obtained by the least squares method (updated from POKORNY et al., 2004a).

Table 1. Pb (mg/kg) in antlers of roe deer, shot in the Šalek Valley in the period 1961–2004 (sample sizes, arithmetic means with confidence intervals, standard deviations, medians, minimal and maximal values are presented)

Period	n	$\bar{a} \pm t_{0.05}*SE$	SD	Me	Min	Max
1960–1969	5	4.21 ± 2.57	2.07	3.79	1.60	7.28
1970–1974	4	2.82 ± 1.21	0.76	2.89	1.83	3.67
1975–1979	10	1.93 ± 0.47	0.66	1.93	0.83	2.82
1980–1984	15	1.77 ± 0.52	0.94	1.56	0.95	4.04
1985–1989	19	1.26 ± 0.25	0.51	1.01	0.57	2.20
1990–1994	15	1.11 ± 0.25	0.45	1.09	0.53	2.00
1995–1999	25	0.72 ± 0.15	0.36	0.62	0.23	1.51
2000–2004	34	0.58 ± 0.11	0.32	0.49	0.15	1.25

Table 2. Comparison of trait size and asymmetry (arithmetic means with confidence limits) of total antler length of adult roe deer, shot before and after the construction of the flue-gas cleaning device at the ŠTPP in 1995

Period	n	Mean trait size [mm]*	Index FA1 [mm]*	Index FA4*
Before construction	56	261.5 ± 10.7	20.6 ± 4.9	327.7610
After construction	128	261.3 ± 7.1	14.6 ± 2.3	179.7923
Test of differences**		t = 0.0423 P=0.97	t = 2.5425 P<0.01	F = 1.8230 P<0.01

* Formulae: mean trait size = $\Sigma((L+R)/2)/n$; FA1 = $\Sigma|L-R|/n$; FA4 = var(L–R); L: trait size on the left beam; R: trait size on the right beam.
** Differences in trait size and FA1 were tested by t-test and differences in FA4 by F-test (see also Materials and methods).

Indices of fluctuating asymmetry (FA_1 and FA_4; for explanation, see Materials and methods section) of the total length (sum of beam length and both tines lengths) of adult roe deer antlers are presented separately for the period before and after the construction of the first flue-gas cleaning device at the fourth unit of the ŠTPP (Table 2). For assessing the possible effect of Pb exposure on the developmental stability of the process of antler growth, correlation coefficients between Pb levels in antlers and their FA were calculated on an individual level (specimens for which both Pb and FA were determined irrespective of the year of culling; after log-transformation of Pb levels: n=54, r=0.15, P=0.27), on an

annual level (mean yearly Pb and FA values: n=22, r=0.32, P=0.15), and on a 5yr-period level (mean values of Pb and FA in five-year periods: n=5, r=0.99, P<0.001), respectively. Dependence between both parameters on the 5yr-period scale is presented in Fig. 2.

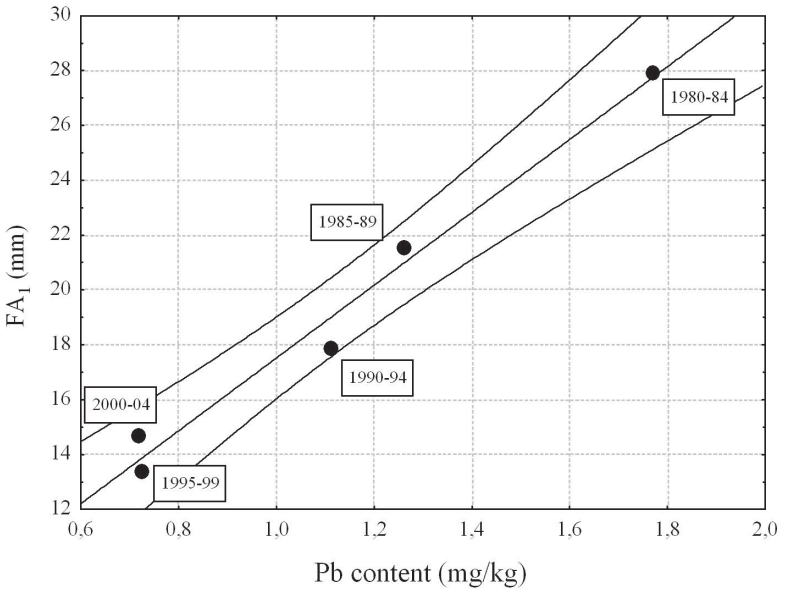

Fig. 2. Correlation between mean Pb level and asymmetry (FA_1) of the total length of antlers of roe deer, shot in the Šalek Valley in five-year periods (1980–84, 1985–89, 1990–94, 1995–99 and 2000–04, respectively).

Discussion

A continuous temporal decline of Pb levels in roe deer antlers (Figure 1) indicates that the ambient lead pollution has continuously decreased in the Šalek Valley over recent decades. This trend is strongly in accordance with previous European studies (e.g. MEDVEDEV, 1995; TATARUCH, 1995; CHYLA et al., 1996; KIERDORF and KIERDORF, 2000, 2001, 2002). However, it should be emphasised that in comparable periods, Pb concentrations in antlers from the Šalek Valley were lower in comparison with other European data – the highest Pb contents in antlers of different deer species were determined in the sixties/seventies (up to 166.3 mg/kg for a roe deer shot in Germany in 1967; KIERDORF and KIERDORF, 2004), while the lowest values (generally <2 mg/kg) were measured towards the end of the twentieth century (for a review, see POKORNY et al., 2004a). This indicates

that the Šalek Valley has never been heavily polluted with lead, which has already been demonstrated by some other bioindicators, such as roe deer kidney (POKORNY, 2000) or higher fungi (AL SAYEGH-PETKOVŠEK et al., 2002). Nevertheless, three important milestones of decreasing Pb pollution are obvious in the Šalek Valley (Table 1): (i) in the middle seventies, as the consequence of a rapid decrease in coal combustion due to the construction of the remote heating system (today, over 90% of households in the Šalek Valley as well as industry as a whole are supplied with heat from the ŠTPP); (ii) in the late eighties, as a result of the introduction of unleaded petrol in Slovenia and other European countries; (iii) after 1995 and 2000, when the first and the second large flue-gas cleaning devices were constructed at the ŠTPP.

The almost identical trends obtained across a broad scale of environmental conditions (e.g. different climatic, geographical and orographic regions, availability of different food sources, large differences in the levels of ambient pollution) undoubtedly confirm deer antlers as a reliable and effective accumulative biomonitoring tool. This is further supported by the existence of a strong correlation between the annual emissions from the ŠTPP and the mean yearly Pb contents in antlers (POKORNY et al., 2004a), which confirms that the ecological remediation of the ŠTPP is already reflected in the lower exposure to the biocenosis in the Šalek Valley.

Although the contents of bone-seeking pollutants (such as lead) in antlers perfectly reflect exposure of wildlife in space and time, they do not provide adequate information on the response of organisms. Therefore, by simultaneous measuring of fluctuating asymmetry of roe deer antlers we tried to link chemical agents with their possible effects on a selected physiological process in the target monitoring species (i.e. dose-response approach).

When using fluctuating asymmetry for assessing exposure to different environmental stressors it has to be considered that disruption of developmental stability cannot be detected within individuals; rather, it may become evident on a population (generation) scale (see GRAHAM et al., 1993; PALMER, 1994). Considering historical biomonitoring, mean values of FA across different time periods may be of particular importance. In this respect it is important that FA of the total antler length significantly decreased after the construction of the desulphurisation device on the fourth unit of the ŠTPP in 1995 (Table 2). Because mean trait size does not differ between the two periods (which means that the decrease of FA was not influenced by changes in trait size per se), this indicates that exposure to environmental pollution might be one of the main stressors causing deviation from perfect bilateral symmetry of roe deer antlers. This hypothesis is supported by the existence of a highly significant positive correlation between mean Pb levels in antlers and their FA in five-year periods (Figure 2).

In recent decades, environmental pollution has often been confirmed as an important factor causing increased FA of many different traits in a wide range of wild-living species (for a review, see POKORNY et al., 2004c). Indeed, some studies have revealed the significant influence of heavy metals on FA of terrestrial vertebrates – e.g. a significant decrease of FA of wing feathers and tarsus length of the great tit (*Parus major* L.) and pied flycatcher (*Ficedula hypoleuca* L.) with distance from a copper smelter (EEVA et al., 2000), as well as significantly higher FA of different traits in mandibles of common shrews (*Sorex araneus* L.) living near a lead smelter in comparison with a control population (PANKAKOSKI et al., 1992) were found in Finland. Moreover, in the study of shrews, the average FA of mandibles of different populations were significantly correlated with the mean Pb levels in the inner organs of the same populations (PANKAKOSKI et al., 1994). A strong accordance with the results of our study indicates that Pb should be viewed as an important stress-inducing factor, which may influence developmental stability of wildlife in terrestrial ecosystems.

Although the principle of the influence of lead pollution on the process of antler formation is less clear, it may represent developmental noise in terms of changed mass and energy drifts in the biocenosis (i.e. influence on the quality and species composition of ingested plants) as well as in an individual (i.e. substitution of Ca_2+ with Pb_2+ in the process of antler formation, influence on the nervous system or on enzymatic activity). However, it should be noted that FA, which is a universal indicator of stress rather than a specific bioindicator of environmental pollution (e.g. PARSONS, 1990), may also be influenced by some other stress-inducing factors. For example, parasites (for reindeer: FOLSTAD et al., 1996) or high population density (for roe deer: PELABON and VAN BREUKELEN, 1998) may influence the FA of deer antlers.

In spite of the possible confounding effect of some other environmental factors, we believe that FA measurements should be continued in the future as a supplement to chemical analyses of roe deer antlers. Indeed, due to its great potential with respect to both accumulative and reactive bioindication we suggest implementation of this extraordinary structure as a bio-sensor of habitat quality/pollution all over Europe.

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POKORNY, B.: Rogovlje srnjaka (*Capreolus capreolus* L.) kao kumulativni i reaktivni pokazatelj onečišćenja olovom u blizini najveće slovenske termoelektrane: Vet. arhiv 76, S131-S142, 2006

SAŽETAK

Građa, visoki kumulativni potencijal, intenzivan rast u tijeku točno određenoga vremenskoga razmaka te dostupnost starijih uzoraka čine rogovlje srnjaka naročito prikladnim za određivanje vremenskoga i/ili prostornoga onečišćenja okoliša elementima s afinitetom za kosti, poput olova (Pb). Osim određivanja sadržaja onečišćivača, rogovlje također omogućava mjerenje fluktuacijske asimetrije (FA), što odražava posredne i stresom uzrokovane razlike između lijeve i desne strane inače potpuno simetričnih uzoraka. Prisutnost Pb i razina FA određeni su vremenom u kojem su dotični rogovi stečeni, odstrjelom unutar perioda od 1961. do 2004. godine, u blizini termoelektrane Šoštanj (dolina Šalek, sjeverna Slovenija). Najviši sadržaj Pb u najstarijim uzorcima rogovlja ukazuje na postupno smanjenje onečišćenja promatranog okoliša olovom od 1961. godine do danas. Onečišćenje okoliša olovom okarakterizirano je kao jedan od glavnih uzroka otklona od bilateralne simetrije rogovlja zbog: (i) FA rogova je značajno niža nakon ugradnje uređaja za odsumporavanje, (ii) generacijski gledano utvrđena je značajna pozitivna sprega između srednjih vrijednosti sadržaja Pb u rogovima i FA. Na temelju razvidnog bioindikatorskog potencijala rogovlja predlaže se njihova uporaba u smislu pokazatelja kvalitete okoliša/onečišćenja diljem Europe.

Ključne riječi: srnjak, rogovlje, onečišćenje, olovo, fluktuacijska asimetrija