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ORIGINAL PAPER

Lead concentrations and lead and strontium stable-isotope ratios in teeth of European roe deer *(Capreolus capreolus)*

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Abstract The study reports lead concentrations and lead and strontium stable-isotope ratios in mandibular molars of roe deer from three different areas in western Germany. Lead concentrations in third molars ranged between 0.23 and 36.61 μg/g (dry weight). Comparing lead concentrations in first molars and third molars in a group of ca. 1.5- or ca. 2.5-year-old individuals from the same area revealed an effect of tooth age on tooth lead content. The higher lead concentrations in the first molars were attributed to the longer period of lead accumulation by the dentin of these teeth compared with the later-forming third molars. Differences in lead isotopic signatures of the teeth were observed between the three areas, presumably reflecting variation in exposure to different sources of environmental lead. We also found marked variation in the $^{87}Sr/^{86}Sr$ isotope ratios of the teeth, with only a small overlap in values between two of the areas. Strontium isotope analysis alone or in combination with lead isotope analyses can be a useful means of assessing the provenance of deer teeth of unknown geographical origin.

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Introduction

Several studies in humans have demonstrated that teeth, both deciduous and permanent, are useful indicators of lead exposure of recent and historical populations (Ewers et al. 1982; Cleymaet et al. 1991; Gil et al. 1996; Tvinnereim et al. 1997; Bu-Olayan and Thomas 1999; Budd et al. 2000; 2004; Nowak and Chmielnicka 2000; Bayo et al. 2001; Ericson 2001; Arora et al. 2006; Farmer et al. 2006; Wiechuła et al. 2006; Karahalil et al. 2007). Lead readily accumulates in teeth, where it is incorporated into the crystal lattice during the formation of (impure) hydroxyapatite in the process of biomineralization. Fewer studies have analyzed nonhuman teeth to assess lead exposure of different mammal species employed as bioindicators (Maňkovská 1980, Witkowski et al. 1982; Evans et al. 1995; Appleton et al. 2000; Gdula-Argasińska et al. 2004, Ando et al. 2005; Caurant et al. 2006).

Some investigators used lead isotopic signatures in teeth to distinguish between different sources of lead exposure (Gulson and Wilson 1994; Åberg et al. 1998, 2001; Åberg 2001; Caurant et al. 2006; Farmer et al. 2006). Apportionment of sources of environmental lead is mostly based on $^{206}Pb/^{207}Pb$ ratios (Åberg 2001; Åberg et al. 2001; Bellis et al. 2002; Flament et al. 2002; Bindler et al. 2004). Other workers studied strontium isotope composition of bones and teeth to infer the geographical region inhabited by a human or an animal (Beard and Johnson 2000; Bentley et al. 2003; Knudson et al. 2005; Sykes et al. 2006). The isotopic ratios remain unchanged during transfer through the food chain and thus cause specific signatures in dental and skeletal tissues. In the apatite lattice, both strontium and lead substitute for calcium (Wallach and Chausmer 1990; Bentley et al. 2003).

Several studies have demonstrated that the European roe deer (*Capreolus capreolus*) is well suited as a bioindicator species for assessing lead contamination of the environment (e.g., Tataruch and Kierdorf 2003, Kierdorf and Kierdorf 2005). The species occurs in high numbers in large parts of Europe and occupies a wide range of habitats, including areas strongly influenced by human activities (Andersen et al. 1998). Compared with other wild ruminants, the European roe deer has a rather small home range, enabling a monitoring with a relatively high spatial resolution. Moreover, roe deer are regularly harvested by hunting, and material for study is therefore easily available. Finally, established methods for age estimation exist for the species (e.g., Habermehl 1985). However, thus far, the potential of roe deer teeth as indicators of lead pollution has not received much attention. The same applies to the use of lead isotope ratios in roe deer teeth for source apportionment of environmental lead.

The present paper reports first results on lead concentrations and lead and strontium stable-isotope ratios in molar teeth of roe deer from Germany. Specifically, the following questions were addressed: (1) Are tooth lead concentrations in individual roe deer affected by tooth age, i.e., do earlier-forming permanent teeth exhibit higher lead concentrations than later-forming ones? (2) Do the lead isotopic signatures of the roe deer teeth enable a distinction between possible sources of lead in the animals' habitats? (3) Do strontium and lead isotopic signatures in roe deer teeth allow an assessment of the provenance of an animal?

Materials and methods

Geographic origin of specimens and collection of teeth

The analyzed teeth originated from 34 roe deer that had been killed in the year 1984 in three different areas (Stolberg, $n=10$; Paderborn, $n=11$; Bergkamen, $n=13$) of the federal state of North Rhine-Westphalia, Germany.

The Stolberg area is located near Aachen in the western part of North Rhine-Westphalia and has a long history of lead and zinc mining and smelting, dating back to Celtic and Roman times (Grabert 1998). The post-Variscan ore deposits are hosted by Carboniferous and Devonian carbonate rocks (Schneider 1982, Large et al. 1983). While ore mining ceased shortly after the First World War (Schneider 1982), smelting activities in the Stolberg area have continued to the present day, including the operation of a primary lead smelter. As a result of the mining and smelting activities, considerable pollution by lead and other heavy metals has occurred in this area (Ewers et al. 1982, Schneider 1982). Increased lead levels (mean, 6.0 µg/g dry weight; range, 1.49–38.5 µg/g) in shed deciduous incisors of children from the Stolberg area compared with children from a rural control region (mean, 3.9 µg/g; range, 1.6 – 9.4 µg/g) were reported by Ewers et al. (1982).

Paderborn and Bergkamen are located, respectively, in the eastern and central part of North Rhine-Westphalia. The bedrock of both areas is formed by sandstones and marls of Cretaceous age. A previous study revealed that lead concentrations in kidneys and livers of roe deer and brown hare (*Lepus europaeus*) collected in 1983–1984 in the three areas were highest in Stolberg and lowest in Paderborn (Lutz 1985).

For study, cleaned and dried mandibles of the roe deer were at our disposal. All animals had a fully erupted permanent dentition. Eruption of the permanent teeth in *C. capreolus* is complete at about 13 months of age. Estimated ages of the animals, using the criteria listed by Habermehl (1985), ranged between ca. 13 months and 9 years. For analyses, the teeth were extracted from the mandibles and thoroughly cleaned from adhering remnants of soft tissues and food remains, first manually using a toothbrush and wooden toothpicks and then by ultrasonication in distilled water. A third molar (M_3) was extracted from each of the 34 specimens and, in addition, a first molar (M_1) in six individuals from Stolberg. These six animals had been killed in January 1984. Assuming that they were born in May or June, the 2 months in which 96% of the births in roe deer from central Europe occur (Rieck 1955), their age at death was estimated on the basis of dental wear at either about 1.5 years (19 to 20 months) or about 2.5 years (31–32 months). After drying in air, the teeth were milled in a mixer mill (type MM 200, Retsch GmbH, Haan, Germany).

Lead and strontium isotope geochemistry

The element lead occurs in nature with four stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. The ratios between the different lead isotopes vary in different geological systems owing to the formation of ^{206}Pb and ^{207}Pb by radioactive decay from ^{238}U and ^{235}U, respectively, while ^{208}Pb is a product of the radioactive decay of ^{232}Th. ^{204}Pb is the only naturally occurring lead isotope that gets no addition from radioactive decay, and thus its abundance in the Earth's crust does not change with time. Strontium also has four stable isotopes, ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr. Three of these isotopes are time-invariant, while ^{87}Sr is formed by the radioactive decay of ^{87}Rb and thus increases in amount with time.

Analytical procedures

For determination of lead concentrations, samples of 0.2 g from each of the powdered teeth were dissolved in concentrated HNO_3 and H_2O_2 under high pressure in a micro-oven at 180°C. The sample solutions were then diluted to a volume of 10 ml with distilled water and analyzed by inductively coupled plasma mass spectrometry (VG Elemental PQ2 + Turbo). The measurement error was 9%. Lead concentrations in the teeth are reported as micrograms per gram on a dry weight basis.

For determination of isotopic composition, powdered tooth samples were decomposed in a low-temperature plasma-asher, and the residues were treated with 3 M HNO_3 (sub-boiling quality). The solutions were then evaporated to dryness and the residues dissolved in 3 M HNO_3 before chromatographic isolation of lead and strontium, using a modification of the ion extraction chromatographic method described by Horwitz et al. (1992, 1994). The Pb and Sr fractions were trapped on a column packed with a crown ether resin. After rinsing out the remaining unwanted elements from the columns with 3 M HNO_3, strontium was eluted with water and lead with diluted ammonium carbonate solution. The collected Sr and Pb fractions were subsequently evaporated to dryness. Analyses were performed by thermal ionization mass spectrometry on a Finnigan MAT 261 mass spectrometer, using single-rhenium filaments for Pb and double-rhenium filaments for Sr. The total blanks of Sr and Pb for the separation procedure were <100 pg, and the isotopic ratios of Pb and Sr were corrected for mass fractionation by repeated analyses of the NBS 981 Pb standard and the NBS 987 Sr standard.

Study design

We compared lead concentrations in mandibular first and third molars of the six individuals from Stolberg aged either about 1.5 or about 2.5 years to assess variation in tooth lead content related to tooth age. Formation of the M_1 of roe deer starts prenatally, and the tooth is fully erupted at about 3–4 months postpartum. The M_3 develops completely postweaning, crown formation starting at about 4 months postpartum and eruption being completed at about 11–12 months (Habermehl 1985; Kierdorf and Kierdorf 1989).

Lead isotope ratios ($^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, and $^{208}Pb/^{206}Pb$) were determined in all 34 M_3s and in the six M_1s of the individuals from Stolberg. The $^{86}Sr/^{87}Sr$ ratio was determined in 26 of the third molars (Stolberg, n=10; Paderborn, n=10; Bergkamen, n=6), to test whether the isotopic signature allowed a distinction of the geographical origin of the teeth, and in the six first molars from Stolberg.

Data analysis

Lead concentrations and lead and strontium isotope ratios in mandibular first and third molars of the six individuals from Stolberg were compared with the t test for paired samples (two-tailed). Data on lead and strontium isotope ratios in the M_3s of the roe deer from the three areas were assessed by one-way analysis of variance (ANOVA) followed by post-hoc comparison with Tukey's honestly significant difference (HSD) test for unequal sample sizes to locate significant differences between groups. In all tests, P values <0.05 were considered statistically significant. The statistical analyses were performed using the software package Statistica 6.0 for Windows (Statsoft).

Results

Lead concentrations in the mandibular third molars ranged between 0.23 and 36.61 µg/g (Fig. 1). The latter value was recorded for the tooth of an approximately 7-year-old individual from Stolberg.

Comparison of mandibular first and third molars of the 1.5- or 2.5-year-old individuals from Stolberg revealed higher lead concentrations in the first molars (t=4.1, df=5, P=0.009; Fig. 2). The mean value for the M_1s (2.36 µg/g; SD=1.04 µg/g) more than doubled that for the M_3s (1.09 µg/g; SD=0.35 µg/g). In contrast, no significant differences between first molars and third molars were observed for the lead and strontium isotope ratios (P values between 0.59 and 0.84).

Lead isotope ratios in the third molars differed significantly across the three areas (Table 1). Especially the teeth from Stolberg differed markedly from those of the other two areas. Variation in $^{87}Sr/^{86}Sr$ ratios of the third molars was pronounced with only a small overlap of the data from Paderborn and Bergkamen (Fig. 3). The Stolberg samples in particular were clearly separated from those of the other two areas, with $^{87}Sr/^{86}Sr$ ratios ranging between 0.711336 and

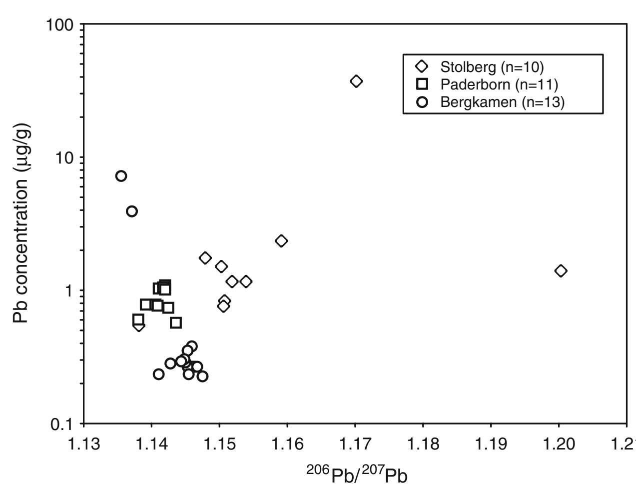

Fig. 1 Plot of lead concentrations vs $^{206}Pb/^{207}Pb$ isotope ratios in mandibular third molars of roe deer from the areas Stolberg, Paderborn, and Bergkamen

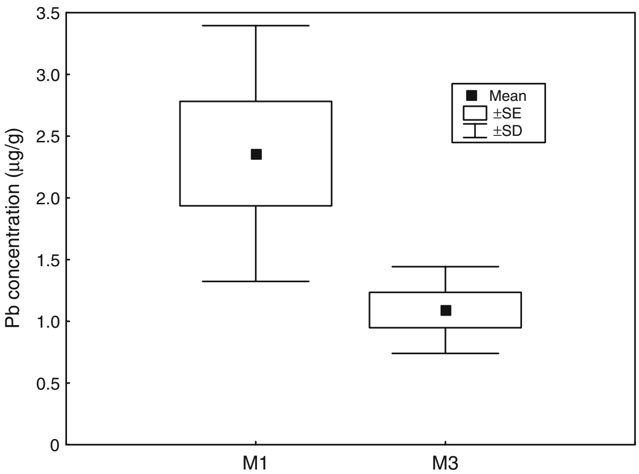

Fig. 2 Lead concentrations in mandibular first and third molars of six roe deer aged about 1.5 or about 2.5 years from the Stolberg area. The difference in lead concentration between the teeth was significant (*t* test for paired samples, $P=0.009$)

0.713733 (mean, 0.712679). Values for the Paderborn samples ranged between 0.708699 and 0.710264 (mean, 0.709714), while those for the teeth from Bergkamen ranged between 0.708309 and 0.708849 (mean, 0.708505). The differences in $^{87}Sr/^{86}Sr$ ratios between the groups were significant (ANOVA, $F_{2,\ 23}=90.012$, $P<0.001$; pairwise comparisons: Stolberg vs Paderborn and Stolberg vs Bergkamen, both $P<0.001$; Paderborn vs Bergkamen, $P=0.005$).

Discussion

To our knowledge, only two studies have previously reported lead concentrations in deer teeth. Maňkovská (1980) determined lead concentrations in teeth (tooth type and animal age not specified) of roe deer from the former Czechoslovakia. Mean lead concentrations in individuals obtained from seven localities in the vicinity of an aluminum plant ranged between 1.4 and 8.8 µg/g (? dry weight), while mean lead concentration in animals from a control region was 5.5 µg/g.

Witkowski et al. (1982) studied mandibular tooth lead levels in white-tailed deer (*Odocoileus virginianus*) killed in 1979 in six different counties (eight animals per county) in Pennsylvania, USA. Mean (±SE) Pb concentrations in the six groups ranged between 34.8±1.5 and 37.7±1.1 µg/g, (ash weight), with no significant difference among localities.

Except for the value of 36.61 µg/g for the M_3 of a ca. 7-year-old individual from Stolberg, lead concentrations in the white-tailed deer teeth were considerably higher than those recorded by Maňkovská (1980) and in the present study for roe deer teeth, even when taking into account the difference between dry-weight-based and ash-weight-based concentrations. The reason for the high tooth lead levels in the white-tailed deer remains unclear, as Witkowski et al. (1982) do not provide information on potential emission sources or geological conditions that could have affected the white-tailed deer populations. Besides differences in lead exposure of the roe and white-tailed deer from natural and anthropogenic sources, also differences in analytical methods might partly explain the differences in reported tooth lead levels between the studies.

The higher lead concentrations in mandibular first molars compared with third molars from the same individuals found in the present study demonstrates an effect of tooth age on tooth lead level. This finding highlights the

Table 1 Lead isotope ratios in mandibular third molars of roe deer from the areas Stolberg, Paderborn, and Bergkamen

Area		$^{206}Pb/^{204}Pb$	$^{206}Pb/^{207}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{208}Pb/^{206}Pb$
Stolberg ($n=10$)	Mean (SD)	18.015 (0.288)	1.157 (0.017)	15.564 (0.035)	37.843 (0.218)	2.101 (0.024)
	Median	17.895	1.151	15.563	37.791	2.110
	Min–Max	17.670–18.713	1.138–1.200	15.524–15.629	37.435–38.118	2.037–2.119
Paderborn ($n=11$)	Mean (SD)	17.725 (0.046)	1.142 (0.002)	15.527 (0.050)	37.510 (0.124)	2.116 (0.002)
	Median	17.725	1.142	15.541	37.540	2.116
	Min–Max	17.652–17.805	1.138–1.146	15.409–15.589	37.266–37.730	2.111–2.120
Bergkamen ($n=13$)	Mean (SD)	17.686 (0.085)	1.144 (0.004)	15.464 (0.079)	37.397 (0.194)	2.115 (0.006)
	Median	17.709	1.145	15.495	37.429	2.112
	Min–Max	17.524–17.786	1.135–1.147	15.282–15.549	36.959–37.614	2.109–2.129
ANOVA, $F_{2,\ 31}$		12.551***	8.443**	8.326**	17.666***	3.956*
Post-hoc comparisons[a]	St vs Pa	**	**	$P=0.36$, ns	***	*
	St vs Be	***	**	**	***	$P=0.08$, ns
	Pa vs Be	$P = 0.84$, ns	$P = 0.87$, ns	*	$P = 0.32$, ns	$P = 0.95$, ns

St Stolberg; *Pa* Paderborn; *Be* Bergkamen
*$P<0.05$
**$P<0.01$
***$P<0.001$
[a] Post-hoc comparisons: Tukey's HSD-test for unequal sample sizes

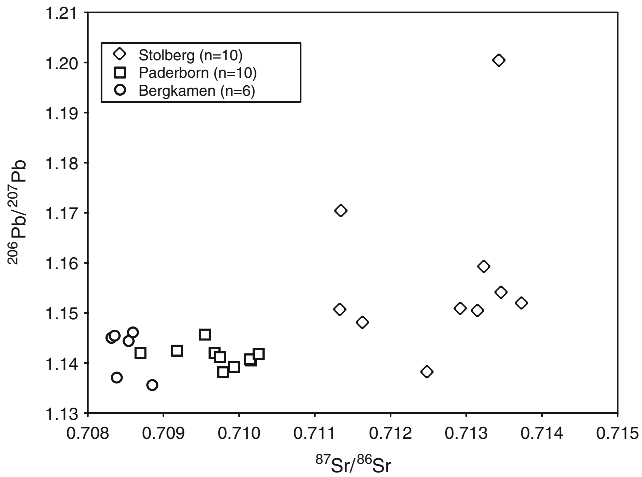

Fig. 3 Plot of $^{206}Pb/^{207}Pb$–$^{87}Sr/^{86}Sr$ ratios for mandibular third molars of roe deer from the areas Stolberg, Paderborn, and Bergkamen

necessity to use corresponding teeth from age-matched individuals when comparing lead exposure of different populations based on lead content of complete teeth.

The powdered tooth samples analyzed in our study consisted of enamel, dentin, and cementum. In contrast to dentin and cementum, which are formed throughout the life of a tooth, enamel is formed only prior to tooth eruption. Lead content of enamel, in particular that of subsurface enamel, which is often used to avoid a contribution of lead taken up posteruptively from the oral environment, is a useful indicator of lead exposure during the pre-eruptive period (Budd et al. 2000, 2004; Farmer et al. 2006). It is, however, the dentin that forms the bulk of a tooth. Because this tissue is formed during the entire life of a tooth, dentinal lead levels reflect lead uptake over a much longer period of time than do enamel lead levels. Especially secondary dentin, which is formed after a tooth has achieved occlusal contact, can accumulate large amounts of lead (Stack 1990; Farmer et al. 2006).

In contrast to the present study, Witkowski et al. (1982) found no increase in tooth lead concentrations with animal age in white-tailed deer. Given the above reasoning and our results in the roe deer, this is a surprising finding, for which Witkowski et al. (1982) do not offer an explanation.

Lead concentration and isotopic composition in the roe deer teeth reflect exposure to environmental lead originating from different sources. Because the analyzed teeth originated from animals killed in 1984, car-exhaust emissions containing lead from organolead additives to gasoline can be considered to have been an important source of atmospheric lead in the roe deer habitats. Thus, in 1985 lead originating from motor traffic accounted for 72.2% of the total annual lead emissions in Germany, which were estimated at 5,014 metric tons (Umweltbundesamt 1997). Unleaded gasoline (lead content not exceeding 0.013 g/l) was first introduced in Germany in October 1984, and its availability at all German gasoline stations became mandatory in 1985 (Von Storch et al. 2003). The $^{206}Pb/^{207}Pb$ ratios of gasoline samples obtained in 1988 or 1990 in different European countries ranged between 1.062 (Netherlands) and 1.174 (Poland; Hopper et al. 1991). The $^{206}Pb/^{207}Pb$ signature for lead from industrial emissions is typically higher, ranging between about 1.14 and 1.22, depending on the emission source (Flament et al. 2002). According to Hopper et al. (1991), $^{206}Pb/^{207}Pb$ ratios in Western European aerosol and gasoline samples are generally lower than in Eastern European samples.

Our study revealed differences in lead isotope ratios among the three areas. In particular, the isotopic composition in the teeth from Stolberg differed markedly from those in the teeth from the other two areas. The higher $^{206}Pb/^{207}Pb$ ratio in the teeth from Stolberg compared with the other two areas might indicate a higher contribution of industrial lead sources from the smelting activities in Stolberg, to the exposure of the resident roe deer population. However, also an influence of the local geological situation on the Pb isotope signatures in the teeth of the resident roe deer is suggested by the isotopic data. Large et al. (1983) reported the following isotope ratios for a galena (PbS) sample from the Stolberg deposits: $^{206}Pb/^{204}Pb$, 18.495; $^{207}Pb/^{204}Pb$, 15.738; $^{208}PB/^{204}Pb$, 38.704. The respective Pb isotope ratios for the teeth from Stolberg are, on average, closer to these values than the ratios recorded for the teeth from the other two areas. This suggests that the roe deer from Stolberg had been exposed to lead from geogenic sources in the area. Isotopic ratios ($^{206}Pb/^{204}Pb$, 18.247; $^{207}Pb/^{204}Pb$, 15.593; $^{208}PB/^{204}Pb$, 38.098) in the tooth from Stolberg with the highest lead content (36.61 μg/g) were quite close to those in the galena sample from the area. It may therefore be assumed that the home range of the respective roe deer included a Pb-contaminated “hot spot”. This could have been an ore outcrop or one of the mining-waste heaps in the area, for which high soil-lead concentrations (sometimes exceeding 10,000 μg/g, dry weight) have been reported (Schneider 1982, Trüby 1994).

In the Paderborn and Bergkamen areas, for which no data of isotopic signatures in the bedrock could be obtained, a contribution of sources other than motor traffic to the lead exposure of the roe deer can also be deduced based on the $^{206}Pb/^{207}Pb$ ratios. One possible additional source is the combustion of coal, which mostly exhibits $^{206}Pb/^{207}Pb$ ratios of about 1.17 to 1.18 (Bellis et al. 2002; Flament et al. 2002).

The $^{87}Sr/^{86}Sr$ isotopic signatures differed markedly between the teeth from the three areas, the values forming three distinct clusters. There was only a slight overlap between the values for Paderborn and Bergkamen, whereas all signatures for the teeth from Stolberg were higher than those in the other two tooth samples. These differences are regarded to reflect differences in the geological situation between the three areas.

The observed clear differences in dental Sr isotopic signatures between the three areas underscore the value of the $^{87}Sr/^{86}Sr$ ratio in teeth as a provenance indicator. Sykes et al. (2006) recently arrived at a similar conclusion in their study on the provenance of fallow deer (*Dama dama*) from an archaeological site in England.

In conclusion, the present study has demonstrated that teeth are useful indicators for assessing lead exposure of roe deer populations. By studying lead isotope ratios, it is possible to assess the role of different lead sources for the overall Pb burden on the deer. It was also shown that strontium isotope ratios in deer teeth can be used as a provenance indicator. Combining the analysis of lead and strontium isotope ratios may sometimes allow a better discrimination between samples of unknown geographical origin than strontium isotope analysis alone.

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