

Roe and red deer antlers as bioindicators of pollution of deer habitats by lead and fluoride

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ABSTRACT

This paper reviews the authors' studies on the use of the antlers of roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) as pollution indicators for lead and fluoride. Due to their seasonal growth cycle and annual replacement, antlers constitute "naturally standardized" environmental samples whose analysis allows an assessment of the exposure of deer to environmental pollutants with an affinity to mineralized tissues. Historical biomonitoring studies conducted in Germany revealed a marked drop in lead and fluoride concentrations in antlers of roe and red deer in the 1980s and 1990s compared with the 1950s to 1970s, indicating a pronounced reduction of atmospheric deposition of lead and fluoride into the habitats. This is attributed to the phase-out of leaded gasoline and the resulting reduction of lead-emissions from motor traffic and to a decrease of lead and fluoride emissions from stationary sources due to improved emission control measures. In addition, regional differences in lead and fluoride exposure of deer could be demonstrated based on the analysis of roe deer antlers collected during the same period (1990s). There is some evidence that exposure of deer to above-normal levels of fluoride causes an impairment of mineralization in antler bone.

Key words: antlers, environmental pollution, bioindication, biomonitoring, bone mineralization

Introduction

Antlers are cranial appendages that develop from permanent protuberances of the frontal bones (pedicles) of deer. Except for the reindeer (*Rangifer tarandus*), in which both sexes are antlered, pedicles and antlers are normally grown only by male deer. Pedicles are formed at the onset of puberty due to a rise in circulating testosterone, which activates a group of determined periosteal cells (HARTWIG and SCHRUDDE, 1974; GOSS and POWEL, 1985; KIERDORF and KIERDORF, 2001a). The specialized region of the

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frontal periosteum responsible for the formation of pedicles and first antlers was termed “antlerogenic periosteum” (GOSS, 1983; GOSS and POWEL, 1985).

With the casting of its first antlers, a deer enters into a cycle of antler regeneration and casting that in temperate species is controlled by seasonal fluctuations in the levels of circulating sex steroids. Most temperate species, e.g. red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) grow their antlers during spring/early summer; however, in the roe deer (genus *Capreolus*) antler growth takes place in late autumn/winter (early spring). Growing antlers are enveloped in a specialized type of skin, referred to as velvet, and constitute complex organs. Thus a forming antler is composed of skin, mesenchymal tissue, cartilage, bone, blood vessels, and nerves. Antler growth involves both endochondral and intramembranous bone formation (BANKS and NEWBREY, 1983; KIERDORF and KIERDORF, 2004a; PRICE et al., 2005). Rising levels of circulating testosterone cause a cessation of growth and a terminal mineralization of the antler bone. The rise in circulating testosterone also causes shedding of the velvet, thereby exposing the hard (polished) antlers, which are entirely bony structures.

Growing antlers have high requirements for calcium and phosphorus, the main constituents of the antler bone mineral. When the mineral requirements of growing antlers cannot be met by dietary intake, physiological bone resorption occurs in the skeleton of deer to provide the necessary extra amounts of calcium and phosphorus (BANKS et al., 1968a,b; HILLMAN et al., 1973; BAXTER et al., 1999).

During growth, antlers accumulate potential pollutants, like lead and fluoride, which have an affinity to mineralized tissues and that are incorporated into the mineral phase of the antler bone. Because of the seasonally limited life span of antlers, the concentrations of these pollutants in hard antlers reflect accumulation during a species-specific period of some months. Antlers can therefore be viewed as “naturally standardized” environmental samples that contain information on the exposure of deer to pollutants present in their habitats (KIERDORF and KIERDORF, 2000a,c).

Hard antlers are collected by hunters as trophies and regularly kept in private or public collections. Therefore, in many countries larger sets of antlers with known date and place of collection are available for study. These antlers constitute valuable environmental archives, whose analysis can contribute to a reconstruction of past environmental pollution by these contaminants. Comparison of antlers from different regions representing the same time span moreover enables the assessment of spatial variation in the pollution of deer habitats for a given period.

The present paper reports the authors' findings on the use of red and roe deer antlers as biological indicators of lead and fluoride pollution.

Lead and fluoride in the environment

Lead and fluoride are natural constituents of the biogeosphere and ubiquitous in the environment. They enter the biosphere as a result of natural processes such as the weathering of minerals and volcanic activity (WHO, 2002; PATTEE and PAIN, 2003). Natural environmental background levels of lead and fluoride are, however, difficult to establish because of the additional input from anthropogenic sources. Thus global contamination by lead due to human activities has been shown to have a long history (NRIAGU, 1996; HAN et al., 2002).

During recent decades, the most important anthropogenic input of lead into the biosphere resulted from the combustion of leaded gasoline (PATTEE and PAIN, 2003). Major stationary sources of lead pollution include lead and other metal smelters, coal-fired power stations, incinerators of solid waste, sewage sludge or waste oil, as well as industrial plants manufacturing lead-containing products (NRIAGU and PACYNA, 1988; PATTEE and PAIN, 2003). Industrial activities known to release larger amounts of fluoride to the environment include coal combustion and the production of aluminum, iron and steel, copper, nickel, glass, ceramics, bricks, glues and adhesives, and phosphate fertilizers (WHO, 2002).

In wild deer, the most important route of elevated lead and fluoride exposure from anthropogenic sources is the uptake of contaminated plant material (FRØSLIE et al., 2001), and in consequence it is basically the degree of contamination of their diet that is reflected by antler lead and fluoride concentrations of deer. It is suggested that most of the lead and fluoride ingested by deer is present on the plant surface as a consequence of dry and wet deposition (EWERS and SCHLIPKÖTER, 1991; WHO 2002).

Historical biomonitoring

Larger sets of hard antlers that were collected in a specific region over several decades or even centuries can be used to retrospectively assess temporal changes in the pollution of deer habitats by “bone-seeking” contaminants. We used roe deer antlers collected by hunters in different regions of western Germany in the period 1948 – 2000 to monitor changes in atmospheric deposition of lead and fluoride into these areas. Lead concentrations in the antlers ranged between 0.3 and 166.3 mg/kg dry wt (Fig. 1, Table 1). Highest individual, mean and median values were recorded for antlers from the 1960s. A marked drop in antler lead concentrations occurred in the last two decades of the 20th century. Antler lead levels < 1 mg/kg dry wt were observed only in antlers collected during the most recent period (1990-2000) The drop in antler lead concentrations coincided with a marked decline in atmospheric lead levels that reflected the phase-out of leaded gasoline and, in addition, a reduction of Pb-emission also from stationary sources (Fig. 2).

Table 1. Lead concentrations in hard antlers (n = 350) of European roe deer from western Germany (federal state of North Rhine-Westphalia) during the second half of the 20th century. Compiled from data in KIERDORF and KIERDORF (2000a, 2001b, 2002a, 2004b) and unpublished data. Lead concentrations varied significantly among the periods (Kruskal-Wallis ANOVA by ranks: $H = 203.02$, $df = 4$, $p < 0.001$). Antlers from periods identified by the same letter did not significantly differ in lead concentrations (a: $p = 0.11$; b: $p = 0.51$); all others were significantly different at $p < 0.001$ (Mann-Whitney U-tests with Bonferroni α -adjustment).

Period	n	Lead concentration (mg/kg dry wt)			
		Mean (SD)	Median	Min.	Max.
1948-1959 _{a,b}	34	8.3 (7.3)	5.9	2.1	42.9
1960-1969 _a	55	13.1 (22.4)	9.2	2.3	166.3
1970-1979 _b	70	7.1 (5.2)	5.6	1.6	37.7
1980-1989	89	3.8 (3.5)	3.0	1.1	26.0
1990-2000	102	2.1 (2.0)	1.5	0.3	14.0

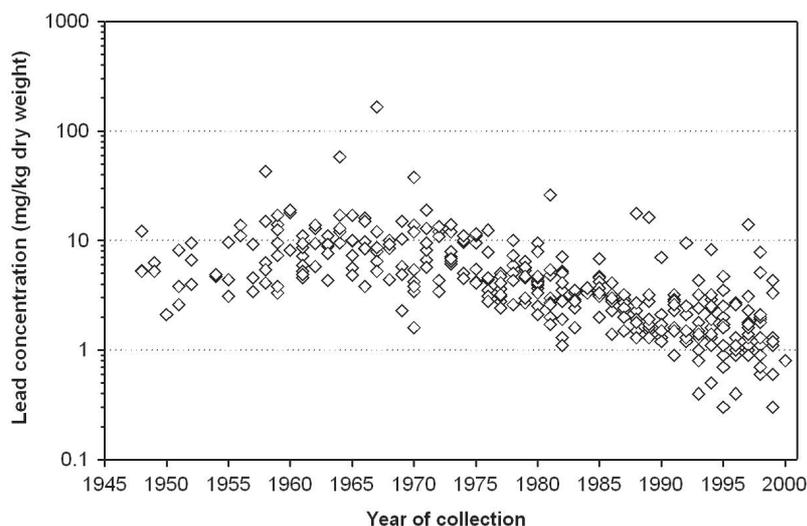


Fig. 1. Lead concentrations in hard antlers (n = 350) of European roe deer (*Capreolus capreolus*) from different regions in western Germany (federal state of North Rhine-Westphalia) during the period 1948 – 2000. See also Table 1.

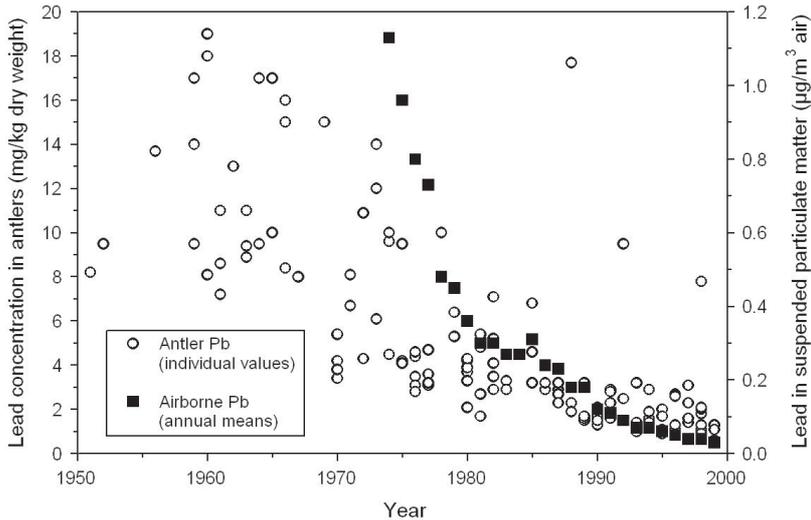


Fig. 2. Lead concentrations in hard antlers ($n = 132$) of European roe deer (*Capreolus capreolus*) killed between 1951 and 1999 in the northern Ruhr area (western Germany), and mean lead concentrations in suspended particulate matter of the Rhine-Ruhr area for the period 1974 – 1999. For the period 1974 – 1999, annual means of lead concentrations in antlers and in suspended particulate matter were positively correlated (Spearman rank correlation, $r_s = 0.74$, $p < 0.001$). Modified after KIERDORF and KIERDORF (2002a).

Fluoride concentrations in antlers from western Germany showed a very similar temporal variation as antler lead levels (Fig. 3, Table 2). Thus, fluoride concentrations in antlers collected during the 1980s and 1990s were markedly lower than those in antlers collected earlier during the studied period. Individual antler fluoride concentrations < 400 mg/kg ash wt were recorded only for antlers collected during the two most recent periods (1980-1989 and 1990-2000). This finding is indicative of a pronounced reduction also of atmospheric deposition of fluoride into the deer habitats.

In a study on fluoride levels in antlers of red deer from four regions in western Germany (KIERDORF and KIERDORF, 2000c), we found very low concentrations (28 to 79 mg F-/kg ash wt) in antlers collected prior to the year 1860, i.e., before the onset of large-scale industrialization (Fig. 4). These low values were suggested to be close to natural baseline levels for red deer antlers from the study areas. As for the roe deer, also

Table 2. Fluoride concentrations in hard antlers (n = 384) of European roe deer from western Germany (federal state of North Rhine-Westphalia) during the second half of the 20th century. Compiled from data in KIERDORF and KIERDORF (2000a, 2001b,c, 2003) and unpublished data. Fluoride concentrations varied significantly among the periods (Kruskal-Wallis ANOVA by ranks: $H = 187.66$, $df = 4$, $p < 0.001$). Antlers from periods identified by the same letter did not significantly differ in fluoride concentrations (a: $p = 0.18$; b: $p = 0.23$); all others were significantly different at $p < 0.01$ [1960-1969 vs 1970-1979] or $p < 0.001$ [other comparisons] (Mann-Whitney U-tests with Bonferroni α -adjustment).

Period	n	Fluoride concentration (mg/kg ash wt)			
		Mean (SD)	Median	Min.	Max.
1948-1959a,b	36	2775 (1757)	2501	425	8383
1960-1969a	59	3165 (1661)	2741	458	8178
1970-1979b	79	2304 (1311)	2090	409	7140
1980-1989	96	901 (814)	1286	110	4800
1990-2000	114	471 (370)	574	116	2713

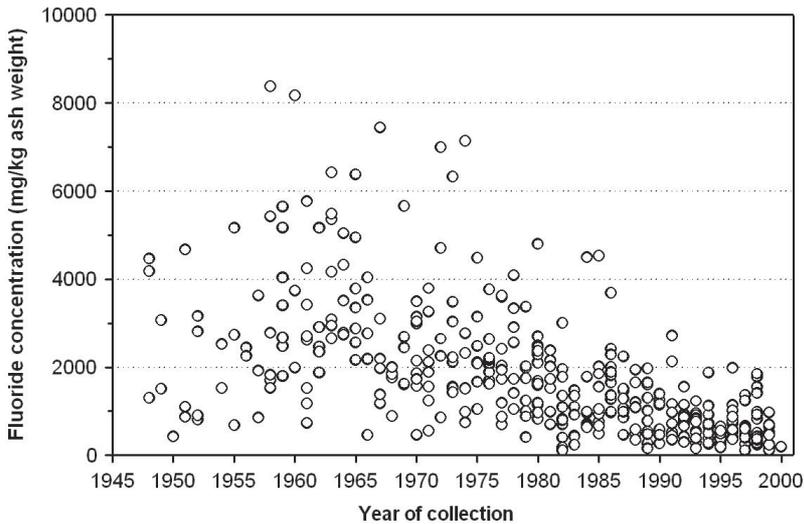


Fig. 3. Fluoride concentrations in hard antlers (n = 384) of European roe deer (*Capreolus capreolus*) from different regions in western Germany (federal state of North Rhine-Westphalia) during the period 1948 – 2000. See also Table 2.

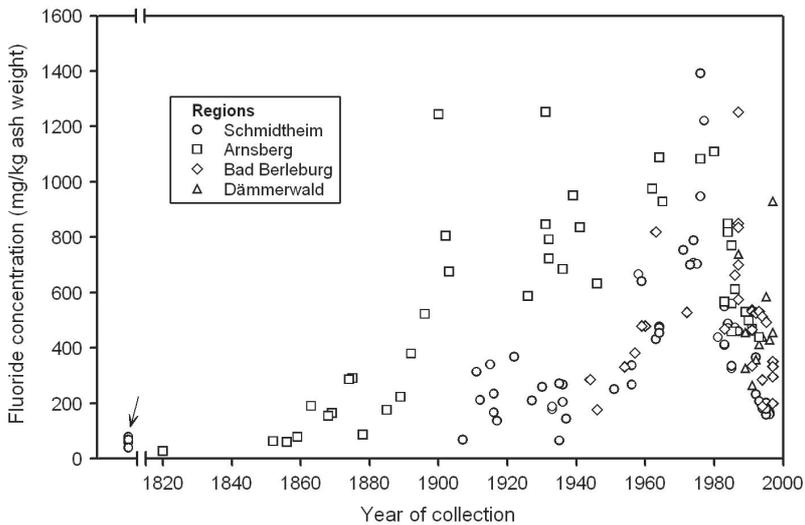


Fig. 4. Fluoride concentrations in hard antlers (n = 141) of red deer (*Cervus elaphus*) from four regions in Western Germany. Except for 4 specimens from the 17th/early 18th century (marked by arrow; exact years of collection not known), the antlers were collected between 1820 and 1997. Redrawn from KIERDORF and KIERDORF (2000d).

for the red deer antlers a decline of fluoride concentrations compared with the immediately preceding decades was observed during the 1980s and 1990s (Fig. 4). Antler fluoride levels recorded for the latter periods were, however, higher than the concentrations recorded for the antlers collected before 1860, indicating that the red deer were still exposed to an additional fluoride load from anthropogenic sources in the late 20th century.

Regional comparisons

Antlers collected in different regions over the same period of time can be used to assess regional differences in the contamination of deer habitats. Using roe deer antlers collected by hunters in western Germany in the period 1990-1999, such regional differences could be demonstrated for lead and fluoride (KIERDORF and KIERDORF, 2000b, 2002b) (Fig. 5).

In areas characterized by elevated lead and/or fluoride concentrations in antlers, the influence of particular point sources of pollution could often be ascertained. For example, fluoride concentrations between 2067 and 11995 mg/kg ash wt were recorded for hard

antlers of roe bucks living in the direct vicinity of an aluminum smelter in Germany (KIERDORF and KIERDORF 2002b). For comparison, fluoride concentrations in two antler samples obtained from roe deer living in areas of western Germany lacking major emission sources ranged between 156 and 496 (n = 20) and 113 and 605 mg/kg ash wt (n = 10), respectively. This indicates a drastically increased exposure to fluoride of the roe deer living in the vicinity of the smelter, attributable to fluoride emissions from this source.

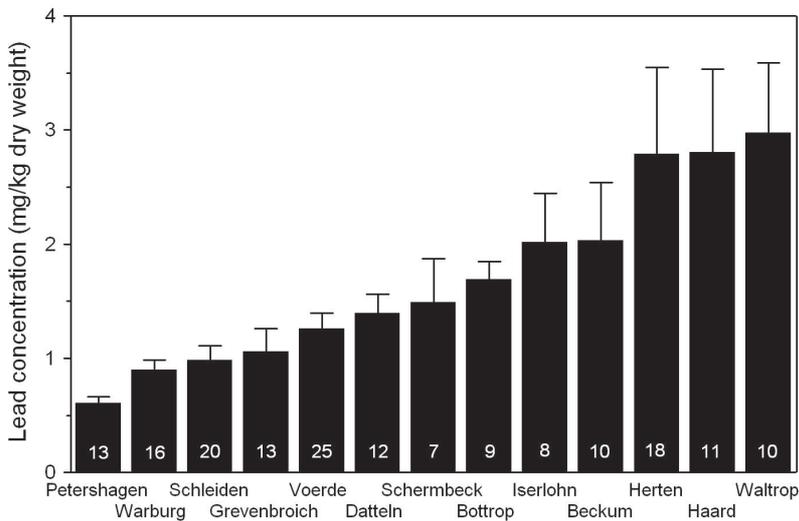


Fig. 5. Lead concentrations (Means and SEMs) in hard antlers of European roe deer (*Capreolus capreolus*) collected by hunters between 1990 and 1999 in 13 areas of North Rhine-Westphalia. The number of analyzed antlers per area is indicated in the columns. From KIERDORF and KIERDORF (2000b).

Pollutant exposure and antler quality

Only few investigators addressed the potential effect of elevated pollutant exposure of growing antlers on antler quality. JOP (1979) found that the average weight of roe deer antlers from a forest region in southern Poland decreased drastically 2 to 3 years after a large iron and steel works started to operate in the immediate vicinity of the forest in 1957. He suggested that the observed decline in antler quality was likely caused by the contamination of the animals' food by emissions from this source.

SAWICKA-KAPUSTA (1979) measured high sulfur concentrations in roe deer antlers from this forest area and from forests in Silesia. In the latter region, mean sulfur concentration in antlers (dry matter) increased by about 29 percent from the period 1938 – 1950 to the period 1951 – 1973. SAWICKA-KAPUSTA (1979) interpreted this increase as “reflecting the higher acidification of the forest environment by sulphur dioxide”. Later, also MEDVEDEV (1995) attempted to use the sulfur concentration in antlers as an indicator of sulfur contamination of deer habitats.

In our view, it may, however, be doubted that sulfur concentration in antlers can be used as an indicator of environmental pollution by sulfur-compounds. In bone, sulfur is present mainly in the organic matrix, i.e., in collagen and different glycosaminoglycans. A decrease in the mineral content of antler bone will go along with a proportional increase of organic matrix, and thus of sulfur concentration, in antler dry matter. Such an inverse relationship (correlation coefficient of $r = -0.98$, $n = 20$, $p < 0.001$) between calcium concentration (measure of mineralization) and sulfur concentration in antler dry matter was demonstrated in red deer (Fig. 6). An elevated sulfur concentration in the dry matter of hard antlers thus primarily indicates a reduced degree of antler mineralization rather than an increased exposure to sulfur-compounds of the respective individual.

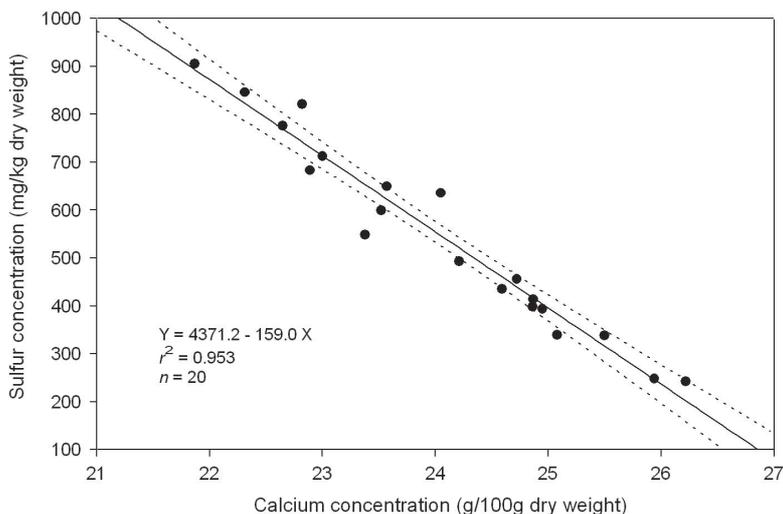


Fig. 6. Relationship between calcium and sulfur concentrations (determined by quantitative x-ray fluorescence analysis) in hard primary antlers of red deer from Eastern and Central Europe. Dashed lines indicate 95% confidence limits for the regression line. D. STOFFELS and U. KIERDORF, unpublished data.

Only two studies have analyzed the relationship between fluoride concentration in hard antlers and the degree of bone mineralization (KIERDORF et al., 1997, 2000d). It was shown that primary antlers of red deer from a fluoride-polluted region (North Bohemia, Czech Republic) contained significantly more fluoride and were significantly less mineralized than antlers from two largely unpolluted "control" regions in Western Germany. For the combined antlers from the two regions, significant negative correlations existed between fluoride concentration in bone ash and different mineralization parameters. Based on these findings it was suggested that higher levels of fluoride impair antler mineralization and that the rapidity of their growth and mineralization makes antlers particularly susceptible to fluoride action. It was further hypothesized (KIERDORF et al., 1997) that deer populations exposed to elevated levels of fluoride might be expected to show a higher frequency of antler breakage than those from "unpolluted" areas. Since then, BORRERO-YU and SCANLON (1998) reported on brittle antlers and unusual antler fractures in mule deer (*Odocoileus hemionus*) from a military base in Colorado Springs (USA) that had significantly higher fluoride concentrations in their bones and teeth than deer from reference sites, and that exhibited tooth lesions characteristic of dental fluorosis. An analysis of antler mineralization or strength in relation to fluoride concentration was, however, not performed by these authors. Further studies addressing the potential detrimental effect of high fluoride intake by deer on antler mineralization and biomechanical competence are therefore needed.

In conclusion, it has been demonstrated that antlers are useful bioindicators of pollution of deer habitats by lead and fluoride. Further studies using antlers as pollution bioindicators are therefore encouraged.

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SAŽETAK

Pregledno je prikazana upotreba rogovlja srnjaka (*Capreolus capreolus*) i jelena običnog (*Cervus elaphus*) kao pokazatelja onečišćenja olovom i fluorom. Zahvaljujući sezonskom ciklusu rasta i redovitim godišnjim zamjenama, rogovlje punorožaca predstavlja "prirodno standardizirane" uzorke čija nam analiza

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omogućuje procjenu izloženosti jelenske divljači onečišćivačima s izraženom sklonošću za mineralizirana tkiva. Istraživanja provedena u Njemačkoj pokazala su očit pad koncentracije olova i fluora u rogovlju jelena i srnjaka prikupljenima tijekom 80-ih i 90-ih godina prošloga stoljeća u odnosu na uzorke prikupljene 50-ih i 70-ih godina. Takve razlike upućuju na naglašeno smanjenje odlaganja olova i fluora u staništima ove divljači. Smanjenje onečišćenja olovom jednim je dijelom uvjetovano smanjenjem uporabe olovnog benzina i posljedičnim smanjenjem emisije olova putem vozila, a drugim dijelom boljom kontrolom izlučivanja olova i fluora iz industrijskih postrojenja. Osim toga, područne razlike u izloženosti jelena olovu i fluoru mogu se uočiti promatranjem rogovlja prikupljenoga u istom razdoblju (1990.) na različitim lokalitetima. Prekomjerna izloženost jelenske divljači fluoru uzrokuje nedostatnu mineralizaciju rogovlja.

Ključne riječi: rogovlje, onečišćenje okoliša, pokazatelji, biomonitoring, okoštavanje
