Original Research

The Effect of Environmental Contamination by Fluorine Compounds on Selected Horse Tissues

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Abstract

Our objective was to evaluate the effects of distance from the source of emission on fluorine content in selected tissues. Studies were conducted in the Central Pomerania region in 2004-06. The studies included stables located in two zones: zone I near the mouth of the Wisła River 50 km west of the source of emission, and zone II near the mouth of the Łeba River 80 to 100 km west of the source of fluorine emission. Soil, feed, and horse tissue (muscles and bones) samples (eight from each type of material) were taken in each zone. The tissues were sampled from 48 horses of different breeds, 24 animals from each zone. Horse tissues were grouped according to age: group 1 – horses to 7 years old, group 2 – horses age over 7 to 15, and group 3 – horses age 15 and over. Fluorine measurements in the soil, feed, and tissues studied were taken by means of the ion-selective electrode working with an ORION ion-meter. Multiple comparison of mean values by Tukey's test was used. Significantly higher fluorine levels were determined in the soils, feeds, and tissues of animals raised in zone I in the vicinity of sources of fluorine emission, compared with zone II. We found influence of horse age on the average muscle and bone levels of fluorine in the animals investigated. A significantly higher level of this element was recorded in the bones of the oldest horses.

Keywords: fluorine, soil, feed, horses, muscles, bones

Introduction

A considerable increase in fluorine concentrations in air, water, food, soil, plants, and animals has been observed in the vicinity of facilities producing phosphorus fertilizers, steel, aluminium, and ceramics, plus glass factories, and heat and power plants. Studies in areas distant from industrial emission locations showed that they were more and more threatened with contamination by fluorine compounds [1-10].

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Fluorine in soil is derived primarily from phosphorus fertilizers containing less that 1 to over 1.5% fluorine [11]. Fluorine behaviour in soil depends to a great extent on the content of clay minerals as well as soil pH [10, 12]. From the standpoint of protection against toxic fluorine, alkalinity of the habitat is the most favourable status for plants [13]. In Polish soils the ranges of natural fluorine contents are as follows: 20-63 $mg\cdot kg^{-1}$ in sandy soils, 168-196 $mg\cdot kg^{-1}$ in silty soils, 250-323 $mg\cdot kg^{-1}$ in loamy soils, and 750-1,660 $mg\cdot kg^{-1}$ in clay soils [8, 14]. Kowalczyk [13], Drzymała et al. [15], and Zbierska [16, 17] conducted studies on the effect of distance from the sources of fluorine emission on the level of soil contamination with this element.

So far we have found no significant impact of fluorine on plants, although the element is common in plant tissues [5, 18]. Physiological fluorine content in plants falls within the range of 1 to 1.6 mg F per 100 g d.m. [19, 20]. Feed levels of fluorine should not exceed 35-40 mg·kg^{-1} d.m. [21]. The permissible fluorine contents in feed concentrated mixtures is 150 mg·kg^{-1}. For cattle, sheep, and goats in lactation, the permissible fluorine content in feed concentrated mixtures is 30 mg·kg^{-1}, the value being 50 mg·kg^{-1} for the non-lactating animals [22].

Fluorine is a microelement that is both an essential and toxic element for living organisms [18]. When in excess in the animal body, fluorine hampers the activity of over 70 enzymes, in particular members of the classes of oxidoreductases, transpherases, and hydrolases. Moreover, it hinders the process of tissue respiration, metabolism of sugars, and lipids, as well as synthesis of thyroid and parathyroid gland hormones. The element permeates the blood-placenta barier to reach fetus tissues [1, 2, 4, 6]. Animals take in fluorine with food (the animal-derived type in particular) and water. Most fluorine, around 95%, is accumulated in hard tissues (bones and teeth), where it is a structural component [23-25]. Fluorine contents in biological liquids as well as soft tissues are low [4, 7]. Fluorine accumulation in animal bones is a sort of protection of their organisms against fluorine toxicity [26]. A safe fluorine dose for livestock should not exceed 1.8 mg·kg^{-1} body weight. The approximate threshold of fluorine toxicological risk is 30-40 mg per kg diet for heifers and dairy cows, 40-50 mg for beef cattle and sheep, and 90 mg dla horses [17]. It is important to improve knowledge of an occurence and biological effects of fluorine activity as it may contribute to improved levels of animal and human health, as well as better understanding of the role this element plays in the environment we live in. Studies on the effect of environmental pollution on fluorine levels in the organisms of different animal species have been conducted by Machoy et al. [20], Żmudzki et al. [27], Zakrzewska and Orowicz [9], Patra et al. [28], Suska [29], Gutowska et al. [30], Salicki and Kalisińska [31, 32], Górecki et al. [24], Piotrowska et al. [33], and Suska and Lubkowska [34].

The available literature lacks information on the fluorine levels in horse muscles and bones. Moreover, fluorine content may be a useful inficator of how much animals are threatened with fluorine excess in the environemnt. Thus the aim of our work was to evaluate the effect of the distance from the source of fluorine emission as well as age on fluorine contents in selected tissues of horses in the Central Pomerania region.

Materials and Methods

Studies were conducted in 2004-06 to compare the greatest basin of waters flowing down from the whole area of Poland – the mouth of the Wisła, and a smaller basin – the mouth of the Łeba and Słupia rivers. The area is influenced by the industrial emissions of Gdańska Rafinery, Fosfory of Gdańsk, Gdańsk Shipyard, Keramzyt Gniew Works, industrial waste stockpiles (in particular phosphogypsum), and slag and ash heaps – all emitting many pollutants, including fluorine, into the atmosphere.

The studies included horses from stables located at various distances from the sources of emission. They were situated within two zones: zone I near the mouth of the Wisła and 50 km west of the source of emission, and zone II near the mouth of the Łeba 80 to 100 km west of the emission source.

In each zone, 8 samples of the soil, feed, and horse tissues (muscles, bones) were collected. The tissues were sampled from 48 horses of different breeds, 24 animals from each zone. The tissue samples were divided into three age groups:
group 1 – horses age 7 and below,
group 2 – horses age over 7 to 15, and
group 3 – horses over 15.

The horses in both zones were housed in stables with access to pasture. In summer they consumed mainly pasture that was supplemented with hay and oats. In winter the horses were offered locally-produced meadow hay, which was supplemented with concentrated feeds, in particular oats, grain, and rye. The experimental animals were in good condition, clinically healthy and not parasitized.

Representative soil and feed samples were regularly collected from the area surrounding the stables, and placed in tightly closed plastic bags. Soil pH was determined in a suspension of 1M KCl solution by means of the potentiometric method. The concentration of fluorine in soil was determined according to Ogoński and Samujło [35] in modification of Nowak and Kuran [36].

Feed samples were taken in all the periods when the feeds were offered to the animals for the first time. Plants were cut at a height of 3 cm from the soil surface in three places, which resulted in approximately 1 kg of fresh matter. Feed samples were prepared according to the standard methodology for this kind of analysis. Feed content of fluorine was determined by Szymczak and Grajeta [37].

Muscles and bones were obtained from slaughterhouses. The samples of meat were taken from the m. *longissimus dorsi* at the height of the last thoracic vertebrae and freshly transported to a laboratory, where they were carefully cleansed and stored at -20ºC until analysis. In order to determine fluorine content (following thawing), the muscle samples were homogenized. Bones (central part of the thigh bone) were blanched in distilled water to remove soft tissues. Next, the biological material was dried until the solid matter was obtained. It was then ground down and samples of 0.05 g were weighed. They were mineralized in the oxidizing mixture of HNO_3, H_2SO_4, and $HClO_4$. Spectrally pure acids were used for oxidation. The contents of fluorine in horse tissues were determined by Ogoński and Samujło [35].

In order to maintain pH values within the range of 5-6, 1.5 M citric acid solution and redistilled water were added to the mixture of plant material. Moreover, 1 M sodium citrate was added to dissolved soil samples and examined horse tissues, and was followed by TISAB. Fluorine measurements in the soil, feed and tissues tested were taken by means of the ion-selective electrode working with the ORION ion-meter. Fluorine levels in the soil, feeds, horse muscles, and bones are expressed as mg·kg^{-1} d.m.

The data were characterized by calculating the arithmetical means ($\bar{x}$), extreme values (min., max.), and variation coefficients (V%).

Analysis of results for the examined soils and feeds included two-way variance analysis with interaction according to the following formula:

$$y_{ijl} = m + a_i + b_j + ab_{ij} + e_{ijl}$$

...where:

y_{ijl} – value of the examined characteristic from the ith level of factor A (soil, feed) and jth level of factor B (zone);
m – population mean;
a_i – effect of the ith level of factor A (soil, feed);
b_j – effect of the jth level of factor B (zone);
ab_{ij} – effect of interaction factor A x factor B;
e_{ijl} – random effect.

The same variance analysis with interaction was performed for contents of individual elements in the tissues of the examined animals. Stables were assumed as factor A and age of animals as factor B.

Multiple comparison of mean values by Tukey's test was used at the significance level of 0.05. The methodology of statistical calculations is described in the work by Trętowski and Wójcik [38].

Results and Discussion

Soils of the region examined represent light soils belonging to quality classes IV-VI. They are slightly acid soils (pH ranged from 5.6 to 6.4). Zone I soils, in the mouth of the Wisła, had a pH of 5.6 to 6.1. In turn, zone II soils, located in the mouth of the Łeba and Słupia, had higher pH values ranging from 5.9 to 6.4 (Fig. 1).

An assessment of the fluorine level in the soils of Central Pomerania (Fig. 2) showed that there was a significantly higher average level of fluorine in the soils of zone I (70.12 mg·kg^{-1} d.m.) compared with zone II (64.28 mg·kg^{-1} d.m.). The highest concentration of this element was determined in the lessive soil of both zones (137.88 and 121.75 mg·kg^{-1} d.m., respectively) as compared to the remaining soils. Fluorine content in typical river alluvial soil was the lowest in both zones (4.71 in zone I and 3.33 mg·kg^{-1} d.m. in zone II) and, compared with lessive soil, the values were over 30 times lower. The fluorine levels in the lessive and typical river alluvial soils of zone I were significantly higher compared with zone II.

The soils examined were slightly acidic. Soil pH in zone I was slightly lower than in zone II. We found a significantly higher average fluorine level in zone I soils compared with zone II, which could result from greater acidity of zone I soils and the fact that Fosfory of Gdańsk stored phosphogypsum in a nearby heap. The mouth of the Wisła, which is a basin of waters flowing down from a greater part of the area of Poland, might increase the soil level of fluorine in the soils examined, too. According to Kowalczyk [13], phosphogypsum storage in the form of heaps may contaminate soils with fluorine compounds.

The present study indicated that, regardless of the zone, the greatest and lowest fluorine contents were in lessive soil and river alluvial soil, respectively. Kowalczyk [13], as well as Kabata-Pendias and Pendias [5], claim that fluorine contents are the lowest in sandy and organic soils. Heavier soils are characterized by higher fluorine levels compared with light soils [8]. Fluorine concentrations recorded in the present work in podsolic soils were within the limits of natural occurrence of this element [39] and averaged 56.30 mg·kg^{-1} d.m.

Fluorine levels in the soils examined in zone I, located closer to the source of emission, were higher compared with the more distant zone II. The differences were statistically significant for the lessive soil as well as pararendzina.

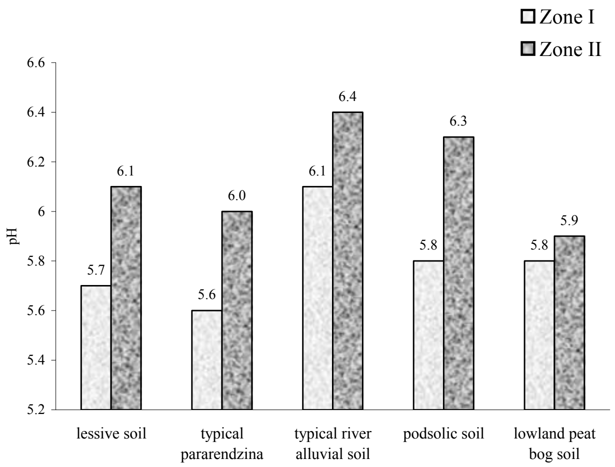

Fig. 1. Soil pH (n=15) in the two zones.

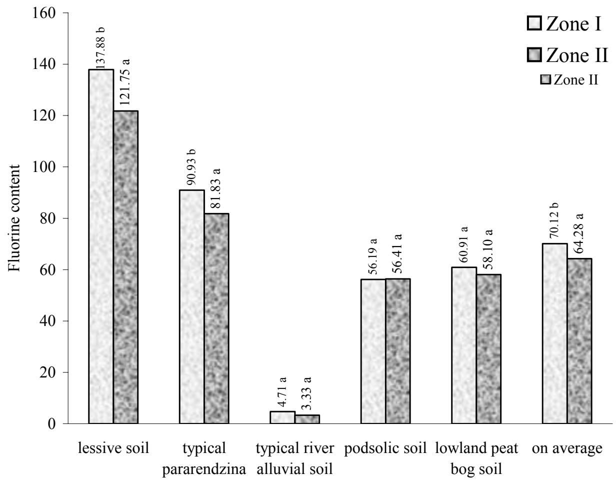

Fig. 2. Soil content of fluorine (mg·kg^{-1} d.m.) according to zone. a, b, .. – means marked with different letters differ significantly (P≤0.05).

Zbierska [17] confirmed that there was an influence of contamination with fluorine on soil and vegetation in the area surrounding "Luboń" Chemical Works. The fluorine levels in her study were higher than the levels found in the present study. At a distance of 200 m from the works the average fluorine amount in the soil was 29.4 mg per 100 g soil, whereas at a distance of 3 km it ranged from 4.2 to 11.4 mg per 100 g soil. The author suggested that the increased fluorine level might result from an impact of other factors, like flooding by the Warta River waters. The highest fluorine level was determined in the soils located east of the chemical works, that is along the main direction of emission influence [16]. In studies conducted by Drzymała et al. [15] and Kłódka et al. [40], fluorine content in soils was much higher compared with the present study and ranged between 40 to 399 mg·kg^{-1} d.m. However, it did not exceed the permissible standard for cultivated soils, that is 500 mg·kg^{-1}.

Analysis of fluorine contents in feeds (Fig. 3) revealed that the level of this element was more than five-fold higher in zone I feeds (16.86 mg·kg^{-1} d.m.) compared with zone II (3.53 mg·kg^{-1} d.m.). We found a significantly higher fluorine concentration in oat grains and rye straw (29.90 and 25.95 mg·kg^{-1} d.m., respectively) produced in zone I compared to the same kinds of feeds from zone II (2.85 and 3.28 mg·kg^{-1} d.m., respectively). Fluorine content in greed fodder harvested from pastures ranged between 3.30 mg·kg^{-1} d.m. in zone II, which was situated quite far away from the source of fluorine emission, to 5.19 mg·kg^{-1} d.m. in zone I, which was located in the vicinity of the emission sources. The fluorine level in meadow hay ranged from 4.68 in zone II to 6.41 mg·kg^{-1} d.m. in zone I.

Evaluation of fluorine content in feed offered to horses in the area of Central Pomerania showed that feed concentration of fluorine in zone I was significantly higher compared with zone II. It was found that fluorine contents in oat grains and rye straw sampled from zone I were 8 and 10 times higher, respectively, compared with zone II. Studies by Wędzisz [41] on fluorine content in cereals grown in the vicinity of a power plant demonstrated that the fluorine level was 10 times higher (48.97 mgF·kg^{-1}) compared with samples collected from uncontaminated areas (4.1-4.6 mgF·kg^{-1}).

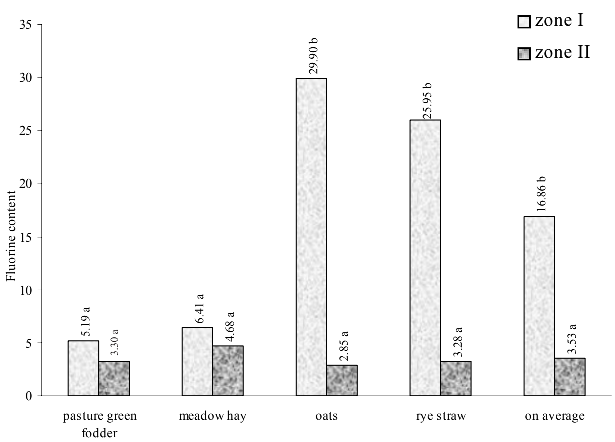

Fig. 3. Horse feed content of fluorine (mg·kg^{-1} d.m.) according to zone.
Explanations: see Fig. 2.

According to Kabata-Pendias and Pendias [5], fluorine content in cereals was 1.58-6.69 mg·kg^{-1} d.m., on average. In the present study the concentration of this element in zone I oat grains was much higher and amounted to 29.90 mg·kg^{-1} d.m. Pasternak and Truchliński [18] reported that fluorine content in oat straws collected 0.2-2.5 km from an industrial facility ranged between 6.05-18.43 mg·kg^{-1} d.m. In their work, Rosik-Dulewska et al. [42] found that fluorine content in barley ranged from 11 to 32 ppm, higher fluorine levels being in tissues of barley grown on soils of more contaminated areas.

Fluorine content in pasture green fodder of zone I, located close to an emission source, was much higher compared with zone II situated a greater distance away. Fluorine amounts in pasture green fodder sampled from points in the vicinity of the emitters did not exceed natural contents in plants that range between 1 mg·kg^{-1} d.m. in green fodder and 30 mg·kg^{-1} d.m. in potato leaves [9]. In a study by Zakrzewska and Orowicz [9], fluoride content in green fodder was comparable with the results obtained in the present study and amounted to 4.50-6.38 mg·kg^{-1} d.m. A markedly higher fluorine level was found by Zbierska [17]. It amounted to 67 mg·kg^{-1} d.m. within a 0.2-kilometre distance east of the source of emission and, beyond this point, it remained within the natural limits of 0.9 to 6.1 mg·kg^{-1} d.m. Żmudzki et al. [27] observed very high fluorine concentration of 59.3 mg·kg^{-1} d.m., on average, in green feeds and silage in a contaminated environment. According to the aforementioned authors, high fluorine concentrations in foliage indicate that the 1.5-kilometre area around the fluorine-emitting facility should not be used for pasture purposes.

Fluorine levels in meadow hay were higher in zone I, in the vicinity of emission source, but it did not exceed values of natural fluorine content of 10.6 mg·kg^{-1} d.m. [43]. Borowiec and Zabłocki [43] recorded 2.1-2.8 times higher fluorine levels in hay within a distance of 1-2 km from the "Police" Chemical Works, which decreased as the distance from the facility increased.

Fluorine content in horse meat (Table 1) was significantly higher in the muscles of animals raised in zone I at the mouth of the Wisła River (3.49 mg·kg^{-1} d.m.) compared with horses raised in zone II at the mouth of the Łeba and Słupia rivers (2.96 mg·kg^{-1} d.m.). The average fluorine concentration in horse muscles increased significantly as the animals grew older, and it ranged from 2.70 mg·kg^{-1} d.m. in animals under age 7, to 3.79 mg·kg^{-1} d.m. in horses age 15 and over. A similar tendency was observed in the muscles of horses raised in individual zones, but no significant differences were found as far as individual groups were concerned. The variation coefficient for the horse muscles content of fluorine slightly varied and ranged from 12.1% in group 3 horses in zone I, to 15.9% in young group 1 horses in zone II.

In the present study we confirmed the impact of distance from the emission source on horse muscle levels of fluorine. The average fluorine level in the muscles of horses raised in the Central Pomerania region significantly increased as the animals grew older. The available literature

Table 1. Variation and average fluorine contents in horse muscles (mg·kg^{-1} d.m.).

Zones	Basic characteristics	Age			Means for zones
		1	2	3	
I	$\bar{x}$	2.99 a	3.37 a	4.12 a	3.49 b
	min.-max.	2.25-3.50	2.79-4.00	3.39-4.78	
	V%	13.6	15.4	12.1	
II	$\bar{x}$	2.42 a	2.98 a	3.47 a	2.96 a
	min.-max.	2.05-3.01	2.28-3.55	2.72-3.91	
	V%	15.9	13.2	13.2	
Means for age		2.70 a	3.17 b	3.79 c	3.22

$LSD_{0.05}$ for zones 0.26
$LSD_{0.05}$ for age 0.38
$LSD_{0.05}$ for interaction zones x age n.s.
$\bar{x}$ – arithmetic mean
V% – variation coefficient
a, b, .. – means marked with different letters differ significantly ($P \leq 0.05$)
Age: 1 – horses at the age of up to 7; 2 – horses aged over 7 to 15; 3 – horses aged 15 and over

lacks information on fluorine concentration in horses. The fluorine levels recorded in horse muscles was higher than values prevailing in mammalian meat, that is 0.2 to 2 mg·kg^{-1}, but it did not exceed 8 mg·kg^{-1} [5, 18, 41]. Studies on different animal species showed higher fluorine levels in the tissues of animals found in an area influenced by industrial emissions, compared with the control [9, 24, 27, 29]. Fluorine concentrations in goat meat were found to range from 0.5 to 5 mg·kg^{-1} [5].

Fluorine content (Table 2) was significantly higher in the bones of horses from zone I (359.5 mg·kg^{-1} d.m.) compared with horses from group 2 (261.0 mg·kg^{-1} d.m.). Significant differences between the investigated zones were found when older animals (group 2 and 3) were examined. It was found that the average fluorine content in horse bones significantly increased as the animals grew older, and ranged from 184.6 mg·kg^{-1} d.m. in young animals making up age group 1, to 456.4 mg·kg^{-1} d.m. in the bones of group 3 horses, regardless of whether the animals were raised in zone I or II. The coefficient of fluorine content variation in horse bones ranged from 6.1% in group 1 to 24.1% in group 2, both groups raised in zone II.

In the present work substantial fluorine accumulation was found in horse bones. It was over 100 times higher compared with fluorine levels in the muscles of the animals examined. The literature on this topic indicates that fluorides accumulate in hard tissues of both animals and humans [30, 33]. The maximum fluorine level in bones should not exceed 1000 mg·kg^{-1} [9]. In cattle, it ranges from 172 do 856 ppm [20]. Assuming that these values are typi-

Table 2. Variation and average fluorine contents in horse bones (mg·kg^{-1} d.m.).

Zones	Basic characteristics	Age			Means for zones
		1	2	3	
I	$\bar{x}$	190.6 a	336.2 b	551.6 c	359.5 b
	min.-max.	174-210	275-480	449-621	
	V%	6.6	20.0	11.0	
II	$\bar{x}$	178.6 a	243.0 b	361.2 c	261.0 a
	min.-max.	164-192	200-370	290-419	
	V%	6.1	24.1	13.0	
Means for age		184.6 a	289.6 b	456.4 c	310.2

$LSD_{0.05}$ for zones 28.3
$LSD_{0.05}$ for age 41.7
$LSD_{0.05}$ for interaction zones x age 59.0 (3) 49.0 (2)
Explanations: see Table 1.

cal of mammals, it can be inferred that the fluorine levels obtained for horse bones in the present study were close to these normal values. Fluorine content in deer mandibles was significantly higher in animals from more contaminated areas [30]. The authors believe that excess fluorine content in bones may disturb its mineralization, which in turn leads to substantial loss of mechanical resistance, despite the fact that bone mass increases. In their study, Piotrowska et al. [33] found differences in fluorine content in antlers and skull bones of roe deer, which depended on both animal age and degree of contamination of the natural environment. Lubkowska et al. [25] showed that, when exposed to fluorine activity lasting for 4 months, fluorine accumulation was observed in bones, whereas when the exposure time was shorter, fluorine was accumulated in the animals' serum. Górecki et al. [24] found that the greatest fluorine concentration was in chicken bones. The average range of fluorine content in long bones of lying hens was 149.3-463.8 mg mgF·kg^{-1}. In the present study it was found that the average fluorine content in horse bones significantly increased as the animals grew older. Such a relation was observed in both zones examined. There is no information on the topic in the available literature. Studies by Salicki and Kalisińska [31, 32] on bones of the wood pigeon showed a rapid accumulation of fluorine as the birds grew older, as well as substantial variation in fluorine content in individual years. The authors concluded that bones of herbivorous animals are much more reliable in an evaluation of environmental contamination with fluorine than the bones of carnivores, scavengers, and animals consuming mixed diets.

Conclusions

Both the soils and feeds sampled from zone I, located at the mouth of the Wisła at up to 50 km west of the source of emission, were characterized by significantly higher average fluorine levels compared with zone II in the mouth of the Łeba 80 to 100 km west of the source of emission. Fluorine content in the examined soils did not exceed the permissible standards. In contrast, the permissible concentrations of the element were exceeded in zone I feeds.

The distance from the source of emission influenced fluorine levels in muscles and bones of horses. We found a significantly higher fluorine concentration in the tissues tested of animals raised in zone I, compared with zone II. Moreover, age of horses significantly increased the average fluorine level in the muscles and bones of the animals examined. Also, muscle and bone accumulation of fluorine significantly increased as the animals grew older.

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