

Fluoride Levels of Drinking Waters of Farm Animal in Iğdır Province, Turkey ^[1]

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Summary

The aim of this study was to determine fluoride (F) levels of water consumed by animals in Iğdır province located on a volcanic area. As control, water samples were collected from Doğubeyazıt for fluorosis and Cappadocia (Kapadokya) for fluorosis free area. Fluoride level measurements were made by ion selective electrode (ISE) system. In general, F levels of Iğdır, Doğubeyazıt and Kapadokya water samples were 0.292 ± 0.026 mgF/L, 9.460 ± 0.701 mgF/L and 0.303 ± 0.056 mgF/L, respectively. There was a statistically significant difference between Iğdır and Doğubeyazıt ($P < 0.001$) but not Kapadokya ($P > 0.05$). In conclusion, it was observed that the water F levels consumed by animals were not at danger limits according to fluorosis in Iğdır, Turkey. It was also supported by bone fluoride levels and carcass findings.

Keywords: Farm animal, Fluoride level, Water, Volcan, Iğdir, Turkey

Iğdır İli Çiftlik Hayvanları İçme Sularının Flor Düzeyleri

Özet

Bu araştırma, bir yanardağ bölgesi olan Iğdır ilindeki hayvanlar tarafından tüketilen suların flor (F) düzeylerini belirlemek amacıyla yapıldı. Florozis için Doğubeyazıt'tan, florsuz bölge için Kapadokya'dan kontrol numuneleri toplandı. Flor düzeyi ölçümleri iyon ayrımı yapabilen elektrot (ISE) kullanılarak yapıldı. Genel olarak Iğdır, Doğubeyazıt ve Kapadokya su örneklerinin F düzeyleri sırasıyla 0.292 ± 0.026 mgF/L, 9.460 ± 0.701 mgF/L ve 0.303 ± 0.056 mgF/L şeklindedir. Iğdır ve Doğubeyazıt su örnekleri F değerleri arasındaki fark istatistiken önemli bulunurken ($P < 0.001$), Iğdır ve Kapadokya'nın su örnekleri arasında fark istatistiken önemsiz bulundu ($P > 0.05$). Sonuç olarak, Iğdır ili hayvanlarının içme sularının F düzeyleri florozis bakımından tehlike sınırlarında olmadığı gözlenmiştir. Bu bulgular, kemik flor düzeyleri ve karkas bulguları tarafından da desteklenmiştir.

Anahtar sözcükler: Çiftlik hayvanları, Flor düzeyi, Su, Yanardağ, Iğdir, Türkiye

INTRODUCTION

Although fluoride (F) in small amounts is essential for animal and human especially for bone and teeth development, it is toxic when taken in large amounts ^{1,2}. Fluoride is totally found in an ionic form in drinking water, and Hence, it freely passes through the intestinal mucosa, and it may interfere with major metabolic pathways of living system ^{3,4}. It was reported that F could cause skeletal lesions because it tends to accumulate in the bones and affects related functions ⁵⁻⁷.

It is reported that F occurs enormous amounts in volcanic materials ⁸. Endemic fluorosis has been reported

in the south of Mount Ağrı, a volcanic mountain ⁹, but there are no detailed reports associated with the status of fluoride for northern part of Mount Ağrı where Iğdır province is located. Main water source of Iğdır province, especially central county of Iğdır, Karakoyunlu and Aralık countries are supplied from Mount Ağrı.

The aim of this investigation was to reveal fluorine levels in water samples consumed by animals in Iğdır province, Turkey (north of Mount Ağrı), and also to illustrate a map of F levels of water for this area.



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MATERIAL and METHODS

Fluoride concentration was determined in water samples. Control water samples were collected from Kapadokya and Doğubeyazıt, which were reported before according to fluorosis^{9,10}.

Location of Samples collected: A total of 74 location including town, villages and other points were randomly selected to provide homogeneity on the map for water samples. The carcass of farm animals obtained from butchers and slaughterhouses was examined for fluorosis while collecting the water samples. As the fluorosis indicator, frangibility, chalky and white appearance, exostoses, thickened growth plates and widened metaphyses, rachitic changes of bones and teeth stains etc. were searched on farm animal carcass^{10,11}. The F content of bones measured to confirm of external bone and carcass examinations for fluorosis.

Abbreviations and numbers in figures and tables: Letters in figures and tables indicate county centers; I: Iğdır (Central county), A: Aralık, T: Tuzluca, K: Karakoyunlu, C: Kapadokya, D: Doğubeyazıt. Each number with letter represents points where samples were collected (villages or other points related to county centers of letters) (Fig 1). Centers collected Samples for Iğdır province illustrated in Figure. For Kapadokya, 13 centers and 15 centers for Doğubeyazıt randomly selected according to previous papers^{9,10}.

Storage of samples: Collected water samples were stored between 2-8°C and analysed in one week. Collected bone samples were kept in deepfreeze (-20 °C) until laboratory procedures.

Bone sample preparation: The bones were initially dried at 125°C for 48 h. Caudal surface of patella was ground by means of stainless steel gang saw and cranial parts of atlas bone were crushed with mortar. Fat was extracted from each ground bone samples by washing with the diethyl ether^{12,13}. Fat-free bone samples were dried at 90°C again. Then, crumbled and double dried bones were weighed as 100±0.3 mg and combusted at 600°C in nickel crucibles. Combustion was increased gradually (200, 400 and 600°C) in one hour for 5 h. Ashes were melted with 1 ml of hydrochloric acid (20%), buffered by means of 1.6 ml of sodium hydroxide (4 mol) and completed to 50 ml with deionized water. After the centrifugation (3000 rpm for 5 min), supernatant was used for F determinations.

Apparatus and chemicals: Orion 4-Star Portable pH/ISE Meter and Fluoride ion-selective electrode (ISE) (96-09) was used for analyses of fluoride. The ISE was combined with a reference filling solution (Orion 900061). As a standard, commercially available solution for fluoride at 0.1 moles per liter diluted with deionized water was used. TISAB II was used as the ion buffer solution for analyses. All chemicals and devices were obtained from Thermo Electron Cooperation Inc. USA. Volumetric flask, baker, mortar and other lab-ware used were made of polypropylene.

Standard solutions containing 0.1, 0.5, 1, 10, 20 and 30 mg/L were prepared from 0,1 mol stock standard solutions described above and hold at 2-8°C. Calibrations of devices were made daily according to sample material (water or bone). The device were calibrated on 5 point; 0.1, 1, 10, 20 and 30 mg/L.

Laboratory analyses: Water samples or bone samples diluted with TISAB II at ratio of 1/1. The measurement with ISE was carried out on the magnetic stirrer. The results were obtained, in mg/L. The bone analyses results were calculated with the consideration of dilution in 25 ml of 100 mg fat-free bone. The solutions were maintained at room temperature (20±0.2°C).

Coefficient of variation (CV) calculation: For the technical precision of the device, 0.1, 1 and 10 mg/L of fluoride solutions for water analysis and 1, 10, 20 and 30 mg/L of fluoride solutions for bone analyses were measured for each 10 samples¹⁴⁻¹⁶.

Statistical Analysis: The data obtained from the water samples and bone F levels were used for statistical analysis. One-Way ANOVA was used to compare the means of groups. Comparisons between the groups were made by Duncan post-hoc¹⁷.

RESULTS

For technical precision of the device coefficient of variation results were as follow; 0.1 mg/L: 11.6%, 1 mg/L: 3.9% and 10 mg/L: 3.05% for water analysis and 1 mg/L: 5.24%, 10 mg/L: 3.89%, 20 mg/L: 2,59% and 30 mg/L: 1.30% for bone analysis.

In general there was no statistically significant difference between F levels of water from Iğdır and Kapadokya ($P>0.05$), but statistically significant differences were observed between all samples of Doğubeyazıt and the samples of other localities ($P<0.001$) (Table 1, 2, 3 and 4).

Table 1. The F concentration of water samples of Iğdır province, Cappadocia and Doğubeyazıt (mg/L)**Table 1.** Iğdır ili, Kapadokya ve Doğubeyazıtın su örneklerinin F düzeyleri (mg/L)

Water origin	Number of the Water Samples	Fluoride levels (Mean±SEM)
Iğdır	129	0.292±0.026
Cappadocia	13	0.303±0.056
Doğubeyazıt	15	9.460±0.701*
Total	157	1.169±0.226

* Illustrate that mean values of samples have statistically significant difference from the others, $P<0.001$ **Table 2.** The F concentration of water samples according to counties of Iğdır province and controls (mg/L)**Table 2.** Iğdır ve kontrol bölgeleri ilçelerine göre su örneklerinin F düzeyleri (mg/L)

Countries	Number of the Water Samples	Fluoride levels (Mean±SEM)
Iğdır	24	0.226±0.016
Aralık	39	0.344±0.036
Tuzluca	45	0.332±0.066
Karakoyunlu	21	0.182±0.011
Cappadocia	13	0.303±0.056
Doğubeyazıt	15	9.460±0.701*
Total	157	1.169±0.226

* Illustrate that mean values of samples have statistically significant difference from the others, $P<0.001$ **Table 3.** The F concentration of water samples according to origin (type of watercourse) and controls (mg/L)**Table 3.** Kökenlerine göre (akarsu tipi) kaynak ve kontrol bölgelerinin sularının F düzeyleri (mg/L)

Water origin	Number of the Water Samples	Fluoride levels (Mean±SEM)
Stream	11	0.281±0.068
Big Stream	35	0.251±0.029
River	10	0.243±0.033
Drilled-Well	13	0.261±0.035
Well	16	0.421±0.076
Spring	44	0.300±0.064
Cappadocia	13	0.303±0.056
Doğubeyazıt	15	9.460±0.701*
Total	157	1.169±0.226

Water samples collected from centers coded as; Stream: T, T1, T4, T5, T6, T7, T8, T15, T18x2, A26;

Big Stream: I1, I4, I5, I6, I7, I8, I9, I10, A7, A9, A10, A11, A12, A13, A14, A15, A16x2, A18, A19, T, T2, T3, T11, T13, T14, T16, T18, T21, T22, T24, K, K3, K4, K5;

River: A4, A20, A23, A24, A25, T12x2, T3, T12, K2;

Drilled-Well: Ix2, I3, I4, I11, I12, A, A2, A17, K, K1, K2, K4;

Well: Ix2, I5, I11, I14, A3, A10, A12, A17, A20, K, K1, K2, K4x2, K6;

Spring: I1, I2, I11, I13, I15, A1, A5x2, A6x3, A8x4, A20, A21, A22, T, T3, T5, T9x2, T10, T12, T14, T15x2, T17, T18, T19, T20x2, T21, T22, T23, T25, T26, K6, K7x3, K8x2;

Cappadocia: C1...C13 and Doğubeyazıt : D1...D15.

x: Illustrate that samples collected more than one area in the same centre

* Illustrate that mean values of samples have statistically significant difference from the others, $P<0.001$ **Table 4.** The F concentration of spring water samples according to origin (Lowland, Mount Ağrı, Other Mountains) and controls (mg/L)**Table 4.** Kökenlerine göre (Ova, Ağrı dağı, diğer dağlar) kaynak ve kontrol bölgelerinin sularının F düzeyleri (mg/L)

Water origin	Number of the Water Samples	Fluoride levels (Mean±SEM)
Lowland	8	0.233±0.034
Mount Ağrı	16	0.190±0.021
Other Mountains	20	0.415±0.137
Cappadocia	13	0.303±0.056
Doğubeyazıt	15	9.460±0.701*
Total	72	2.209±0.466

Water samples collected from centers coded as; Lowland: I1, I2, I11, I13, A1, A5x2, A20;

Mount Ağrı: I15, A6x3, A8x4, A21, A22, K6, K7x3, K8x2;

Other Mountains: T, T3, T5, T9x2, T10, T12, T14, T15x2, T17, T18, T19, T20x2, T21, T22, T23, T25, T26; Cappadocia: C1...C13 and Doğubeyazıt : D1...D15.

x: Illustrate that samples collected more than one area in the same centre

* Illustrate that mean values of samples have statistically significant difference from the others, $P<0.001$

No signs of fluorosis on bones and teethes were found in animal carcass from which bones were collected, in neither Iğdır nor Kapadokya. Fragile, chalky and excessive white bones, exostoses, thickened growth plates or widened metaphyses, rachitic changes of bones and teeth stains were not encountered on carcass. However, fragility and chalky appearance were observed in the bone samples of Doğubeyazıt which was more than the other localities.

The F levels of cattle bones obtained for Iğdır, Kapadokya and Doğubeyazıt were 0.21±0.02% mg (n: 36), 0.21±0.01% mg (n: 9) and 0.28±0.04% mg (n: 10), respectively. The fluoride levels of sheep bones from Iğdır, Kapadokya and Doğubeyazıt were 0.20±0.02% mg (n: 32), 0.21±0.02% mg (n: 9) and 0.29±0.06% mg (n:7), respectively. There was no statistically significant difference between Iğdır and Kapadokya, but statistically significant difference ($P<0.001$) was found between Iğdır and Doğubeyazıt for each cattle and sheep bone samples.

DISCUSSION

Endemic fluorosis was mostly reported at volcanic areas^{9,10,18}. The majority of Iğdır province is located in a volcanic area of the Mount Ağrı, but no investigation was conducted before. Detection of fluoride status of this province was investigated in this study.

Most recorded occurrences of fluorosis are in cattle. Sheep are less susceptible than cattle. A continuous

intake of 1 mg/kg BW is maximum safe limit for ruminants, an intake of 2 mg/kg/BW produces clinical signs ¹¹. WHO reported that maximum fluoride (F) level of drinking water should be 1.5 mg/L but also water consumption must be considered especially in hot air conditions ^{19,20}. In this study mean value of F in water samples of Iğdır province was found as 0.29±0.03 mg/L (*Table 1*). There was no statistically significant difference between the results of Kapadokya samples ¹⁰. In another study, Tokaloğlu et al.⁸ reported that mean value of F levels for drinking waters of Kayseri province was 0.17±0.10 mg/L. Kayseri province is neighboring city to Kapadokya and located on the volcanic Mount Erciyes. However, similar results were not observed for Doğubeyazıt, located in south of Mount Ağrı (*Table 2*). For this area, water fluorid levels were reported as 10.26-12.54 mg/L in 1973 ⁹. There was a great difference between these two close neighboring areas. These differences might result from different water origins and contaminations by different F sources under the ground. In addition, no statistically significant difference was found in waters of Iğdır, classified according to water origin as stream, big stream, river, drilled-well, well, spring (P>0.05) (*Table 3*).

Fluoride is highly reactive halogen and has a strong affinity for calcium ²¹. Accumulation of this element (96%) in bones and teeth is reported ^{4,5,7,22}. For this reason, bone F levels were measured to countenance of external bone and carcass examinations for fluorosis. The results of F measurement showed that there was no statistically significant difference between Iğdır and Kapadokya (P>0.05), but not Doğubeyazıt (P<0.001).

Consequents of this study were as follows;

1. The waters of Iğdır province were clear for F toxicity and there was no sign of fluorosis on farm animals.

2. The water F content of Iğdır illustrated with a detailed map.

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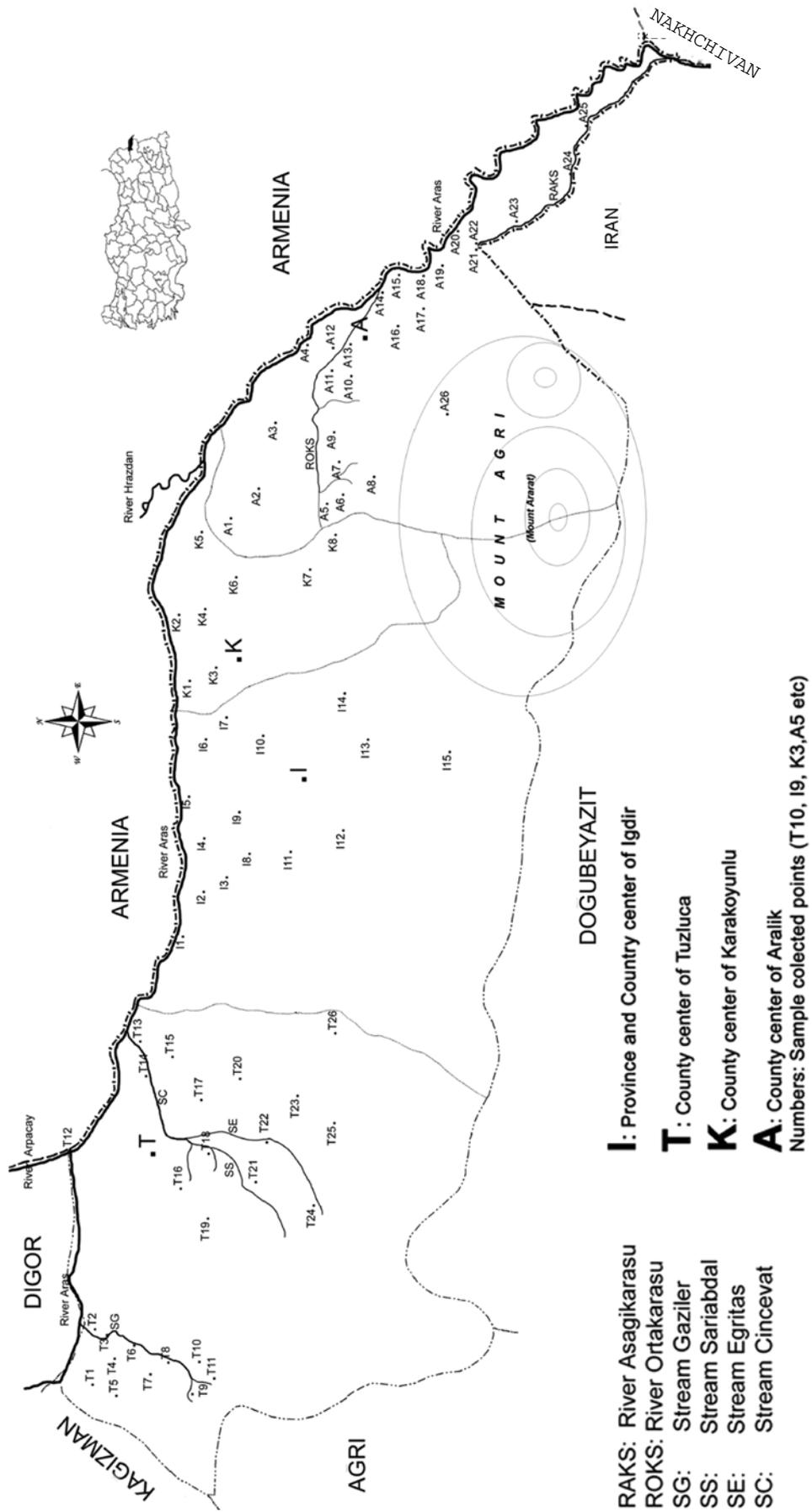


Fig 1. Map of Iğdır according to sample collection points
Şekil 1. Örnek toplama merkezlerine göre Iğdır haritası