

Trace element levels in the brain tissue of red fox (*Vulpes vulpes* L.) in the Konya region

Niveles de oligoelementos en el tejido cerebral del zorro rojo (*Vulpes vulpes* L.) en la región de Konya

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ABSTRACT

In nature, living beings serve and live as a link in the food chain in the ecosystem. Living beings in the wild are the last link in the food chain. They continue their existence by feeding on the nutritionally valuable foods they find in their natural habitats. People are leaving more residues in nature with developing technology. The effect of these residues is revealed by examining animals living in the wild. In this study, the determination, and histopathological examination of the concentration of trace elements in the brain tissue of red foxes (*Vulpes vulpes*) were carried out. In the study, the measurement of the elemental levels of trace elements manganese, iron, copper, zinc, and cobalt was carried out with the ICP-MS method. Since no other scientific article could be found on red foxes regarding manganese, iron, copper, zinc and cobalt, a comparison could not be made. Histopathologically, hyperemia, hemorrhage, gliosis, neuronal degeneration and necrosis, perivascular space expansion, perivascular inflammatory cell infiltration, demyelination, and perineural edema findings were observed in red fox brain tissue. A statistically significant correlation was detected between manganese and iron. This could be explained by exposure to shared environmental sources or a simultaneous role in brain metabolism. It is thought that such studies should be increased to ensure the continuity of red fox species in wildlife.

Key words: brain; red fox; histopathology; trace element; wildlife

RESUMEN

En la naturaleza, los seres vivos sirven y viven como un eslabón en la cadena alimentaria del ecosistema. Los seres vivos en estado salvaje son el último eslabón de la cadena alimentaria. Continúan su existencia alimentándose de los alimentos de valor nutricional que encuentran en sus hábitats naturales. Con el desarrollo de la tecnología, las personas están dejando cada vez más residuos en la naturaleza. El efecto de estos residuos se revela al examinar animales en estado salvaje. En este estudio, se determinó y analizó histopatológicamente la concentración de elementos traza en el tejido cerebral de zorros rojos. En el estudio, se midieron los niveles elementales de oligoelementos manganeso, hierro, cobre, zinc, cobalto y selenio mediante el método ICP-MS. Dado que no se encontró ningún otro artículo científico sobre zorros en relación con manganeso, hierro, cobre, zinc y cobalto, no fue posible realizar una comparación. Histopatológicamente, se observaron hiperemia, hemorragia, gliosis, degeneración y necrosis neuronal, expansión del espacio perivascular, infiltración de células inflamatorias perivasculares, desmielinización y edema perineural en el tejido cerebral del zorro. Se detectó una correlación estadísticamente significativa entre manganeso y hierro. Esto podría explicarse por la exposición a fuentes ambientales compartidas o un papel simultáneo en el metabolismo cerebral. Se considera que estos estudios deberían incrementarse para asegurar la continuidad de la especie en la fauna silvestre.

Palabras clave: Cerebro; zorro; histopatología; oligoelementos; fauna silvestre

INTRODUCTION

The red fox (*Vulpes vulpes*) is a member of the order carnivora and is a predator with a wide ecological tolerance. This species, which is widely distributed in Europe, Asia, and North Africa, can successfully survive in urban, rural and semi-natural habitats [1]. The feeding habits of red foxes vary with small mammals, birds, reptiles, invertebrates, and occasionally fish as well as carrion and agricultural residues [2, 3, 4].

This wide feeding spectrum increases the possibility of red foxes being exposed to environmental pollutants and makes them stand out among the potential bioindicator species. In this context, the effects of persistent pollutants, especially heavy metals, on biological systems can be evaluated on wild animals such as red foxes. It is known that essential trace elements such as zinc (Zn), copper (Cu), iron (Fe), and manganese can cause harmful effects at high doses [5, 6, 7]. Although trace elements are necessary for the regular functioning of body functions at low levels, they cause toxic effects at high levels [8].

These elements are released into the environment as a result of anthropogenic processes such as industrial activities, mining, agricultural practices, wastewater discharges, and fossil fuel combustion [9]. These pollutants can enter the food chain, especially through water, and soil ecosystems, and accumulate in the tissues of living organisms. Species located at the upper levels of the food chain are more affected by the accumulation of these elements; this situation is explained by the phenomenon of biomagnification [10].

Omnivorous and opportunistic predators such as red foxes can be heavily affected by this process due to their extensive food resources. Therefore, determining trace element levels in the tissues of these species has the potential to provide valuable information in terms of environmental toxicity for different geographies [11, 12, 13].

Although there are studies on heavy metal, and trace element profiles in wild mammal tissues [14, 15], studies on trace element accumulation in brain tissue are quite limited. This gap indicates an important research need in the fields of environmental toxicology, and ecophysiology. As a result of pollution of water resources with heavy metals, and their compounds, they accumulate in the bodies of living beings depending on their age, habitat, and feeding behaviors, and reach the end consumers via the food chain. In this context, red foxes attract attention not only with their roles at the trophic level, but also with their potential as bioindicators of environmental trace element pollution [15].

Especially analyzing the elemental distribution in brain tissues allows for holistic assessments of both animal health and ecosystem health [15]. In this respect, red foxes are among the ideal biological models for environmental monitoring studies.

The trace element Fe has very important roles in the proper myelination of neuronal axons in the central nervous system (CNS) [16, 17], synaptic plasticity [18], brain development, neurotransmitter production, and regulation of neuronal energy needs [19]. Zn, the second most abundant trace element in the brain, has many functions [20]. The trace element Zn is a structural and catalytic component of proteins, a co-factor for more than 300 enzymes, and metalloproteins, provides stability to many

transcription factors, and undertakes neuroprotective functions. It even plays an important role in the defense of the body, and brain against oxidative stress [20, 21, 22]. Zn has important roles in the establishment of neurogenesis, synaptic plasticity, neuronal migration, and differentiation, and regulation of neurotransmission [23, 24]. Thus, it helps maintain healthy cognitive development, and brain functions [25].

Copper is the third most abundant trace element in the brain tissue [26]. Cu in the brain is a cofactor for the enzymes necessary for energy metabolism of nerve cells, myelin formation, neurotransmitter biosynthesis, and mediating the oxidative stress response [27]. Therefore, copper homeostasis is essential for the formation and maintenance of a healthy CNS. For example, Menkes disease, characterized by Cu deficiency in brain tissue in humans, is related to neurodegeneration, and demyelination [28].

Cobalt is usually found in the B12 structure and plays an indirect role in the regulation of neurological functions. Manganese is an element that tends to accumulate in astrocytes and is involved in processes such as mitochondrial function, glycine and the glutamate metabolism.

In addition to the continuity of normal function in the brain tissue, the increase or decrease in the amounts of the mentioned trace elements can cause harmful results in the brain. In this context, their presence in red fox brain tissue may reflect both physiological needs and possible environmental exposures and may shed light on future studies.

Trace elements such as Fe, Zn and Cu have an important physiological role in healthy brain development, and function. Zn in particular is important for neurogenesis, synaptogenesis, synaptic transmission and plasticity, and neurite outgrowth. Considering the essential roles of trace metals in many cellular processes, it is important to maintain adequate levels in the brain. However, the physiological concentration of trace elements, and especially Zn, in the human, and animal brain has not been well determined so far [29, 30].

This study aimed to determine the levels of various trace elements (e.g. Fe, Zn, Mn, Se) in the brain tissues of red foxes living naturally in the Konya region. The findings to be obtained will provide an important contribution to the literature in terms of elemental bioaccumulation studies on wildlife in Türkiye. At the same time, it will allow assessments of regional environmental health, and provide a scientific basis for the usability of red foxes as a regional bioindicator species.

MATERIALS AND METHODS

The materials were collected with permission from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks, dated 16.05.2025 and numbered E-72784983-288.04-19300810. The study material consisted of a total of 6 (3male and 3 female) red foxes (*Vulpes vulpes*) that freshly dead in the Konya region at different times. After the brains of the red foxes were taken, some of the brain tissue was placed in 10% formalin solution and some were kept in the cold chain at -20°C (Vestel, Türkiye) for biochemical analyses. Samples were freeze before heat treatment.

Histopathological analyses

Brain tissues that were kept in 10% formalin solution for 24 hours (h) were trimmed to 3 mm thickness and placed in tissue cassettes. They were kept under tap water for 12 h to remove formalin residues. Then, the tissue tracking process was started. They were kept in 60, 70, 80, 90, 96, 100% alcohol containers separately for 45 min. They were kept in 3 different xylene solutions for 50 min each. Finally, they were kept in 3 different liquid paraffins for 30 min each and the tissues were embedded in paraffin. 5 μ m thick sections were taken. Hematoxylin and eosin (H&E) staining and Luxol Fast Blue myelin staining were performed on the sections that were kept in the oven for 45 min. The results were evaluated under a light microscope (Leica DM2500, Germany) [31, 32].

Chemical analyses

The method published by Laur *et al.* [33] was used to determine the element levels in brain tissue. Mn, Fe, Co, Cu, Zn were analyzed in brain tissue samples by ICP–MS method, and Agilent 7700x® brand ICP–MS device was used [34]. Samples were prepared for analysis with microwave (Berghof® PM10, UK) system using microwave dissolution, technique [35]. Precision balance (Radwag®, PS X7, UK), was used in weighing processes during sample preparation, Elga Purelab® ultrapure water systems were used to provide ultrapure water, 65% nitric acid (Merck Suprapur® 65% HNO₃ Darmstadt–Germany), 30% hydrogen peroxide (Merck® 30% H₂O₂ Darmstadt–Germany) was used in working standards, and sample preparation. Brain tissue was studied using the microwave dissolution method of Gutwerk and Krämer [36]. The method is based on the principle of dissolving the organic matrix by microwave effect in high temperature, pressure, and high acid environment in pressure-resistant closed teflon polymer containers, and releasing the elements in the acid matrix. Berghof Microwave® standard software was used for heat–pressure–time calculations in the studied samples.

RESULTS AND DISCUSSION

Histological findings

FIG. 1 shows histopathological sections. Myelin staining with LFB (Luxol Fast Blue, LF), and general tissue structure with H&E are visible. LF: Normally, the blue color indicates myelin. Areas marked with arrows show areas of myelin loss/demyelination. This suggests that myelin integrity in the nervous tissue has been compromised. H&E: Areas marked with an asterisk (*) show gaps/edema or degenerative changes. Areas marked with black arrows indicate neuronal loss, degeneration, and possible gliosis. Blue arrows highlight changes in cellular density (increased glial density or decreased neuron density) (FIG. 1).

These sections demonstrate demyelination, neuronal loss, and degenerative changes in the nervous tissue. These findings suggest a neurodegenerative process, toxic injury, or a lesion induced in an experimental model.

Histopathological examinations of six red foxes revealed hyperemia (3), hemorrhage (3), gliosis (4), degeneration and necrosis (3) in the meninges, expansion of the perivascular space (4), perivascular inflammatory cell infiltration (3), demyelination, (2) and perineural edema (4). Images of H&E, and Luxol Fast Blue staining are given in FIG. 1.

Biochemical findings

The analysis results of the mean, and $\pm$ standard deviation values of the element levels in the brain tissues of 6 red foxes that died in the Konya region are given in TABLE I.

According to Spearman Correlation Analysis (Inter–element), a strong positive and statistically significant correlation was detected between Mn, and Fe: $P=0.88$, $P=0.018$. As Mn increases, Fe levels

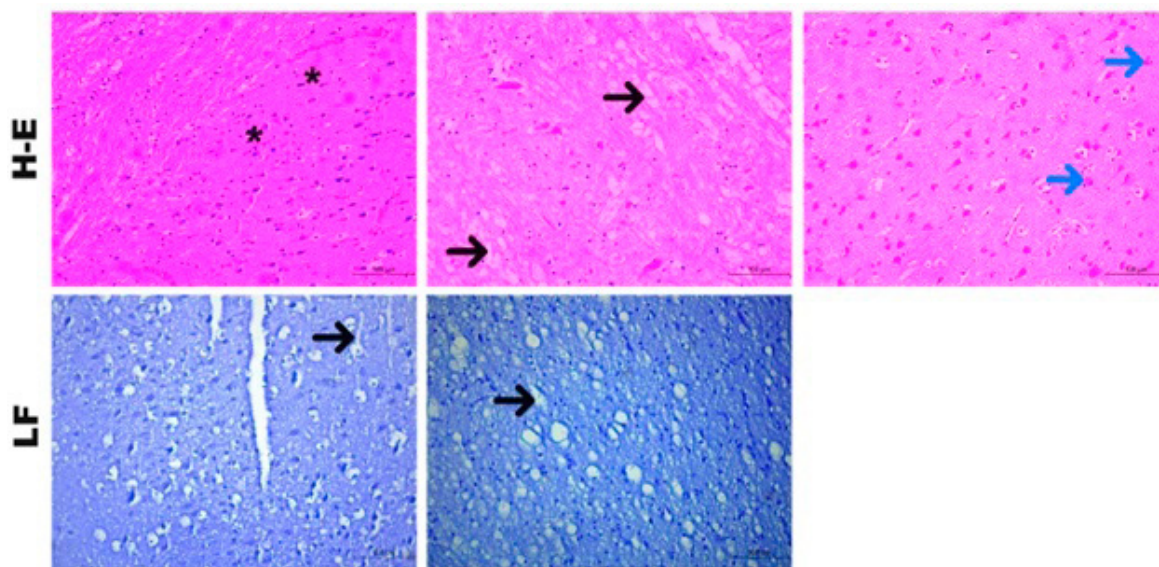


FIGURE 1. H-E: Hematoxylin–Eosin, LF: Luxol Fast Blue. Blue arrows: Gliosis, Black arrows: Demyelination, degeneration and gliosis, asterisk (*): Edema, degenerative changes

TABLE I
Evaluation results of element levels in red fox brain tissue

Elements	Mn	Fe	Co	Cu	Zn
Concentration (mg·kg ⁻¹)	9.44 ± 4.1	500.00 ± 221.11	562.50 ± 298.47	36.66 ± 11.18	261.50 ± 105.64
n=6, mean and ± standard deviation values					

also increase. This suggests accumulation from shared metabolic pathways or similar environmental sources.

A significant correlation was detected between Mn, and Fe. This could be explained by exposure to shared environmental sources or a simultaneous role in brain metabolism.

The correlation matrix showing the relationship between elements is presented in FIG. 2.

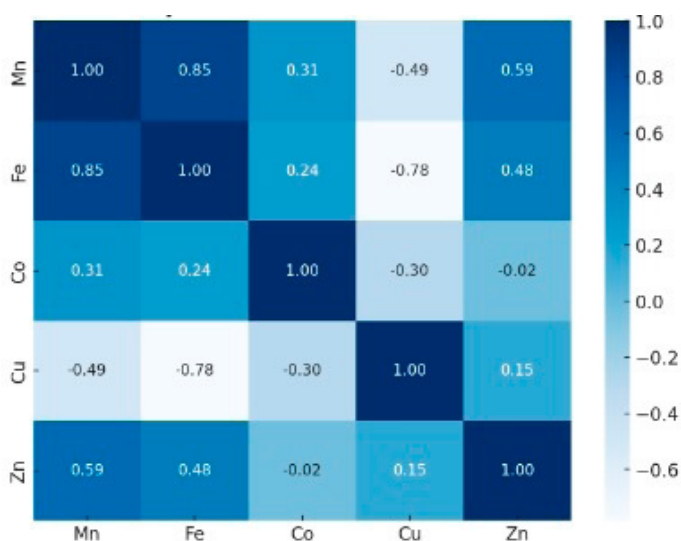


FIGURE 2. The correlation matrix (Trace Elements) in red fox brain tissue

The boxplot comparing Fe levels in foxes with and without gliosis is presented in FIG. 3.

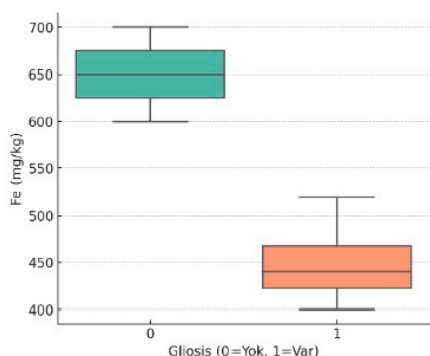


FIGURE 3. Fe levels vs. Gliosis in red fox brain tissue

Trace elements are of vital importance for the metabolic activity, structural integrity, and continuity of function of brain tissue. Both deficiencies, and excessive accumulations of trace elements can cause toxic effects in the CNS [37]. In the present study, copper, Co, Mn, Zn, Fe levels were examined, and Cu, Co, Mn, Zn and Fe elements were detected in the brain of all red foxes.

This study provides important information about the physiological roles of the trace elements detected in the normal functions of the CNS, and their possible neurotoxic effects. The levels of trace elements in the brain tissue play a decisive role in both the maintenance of neurological functions, and the oxidative balance [38].

Mammals need trace elements such as Fe, Mn, Zn, Co and Cu [39]. Fe is the trace element found in the highest concentration in the brain. After Fe, Zn and Cu trace elements are found in high amounts in the brain, respectively [20]. When the values of the trace elements analyzed in this study are considered, we observe that the levels of Co, Fe, Zn, Cu, and Mn are ranked from the most to the least.

In the literature searches, no elemental analysis results were found for red foxes, except for heavy metal elements, from brain tissue.

The comparison of the results obtained as a result of the studies sometimes reveals that there are large differences in the amounts of trace elements detected in the brain. These differences may be due to the use of different methods for tissue preparation, a nonstandard protocol for measurements, and differences in sex, age, or diet. For example, one study found a Zn concentration of $32.93 \pm 0.12 \text{ mg} \cdot \text{kg}^{-1}$ in the cerebellum of Wistar albino rats [40], while a different study reported the amount in the same brain region as $15.00 \pm 5.50 \text{ mg} \cdot \text{kg}^{-1}$ [41].

In the present study, various pathological changes were detected in histopathological examinations performed on the 6 red fox brain tissues. The most common changes were gliosis (66.7%), perineuronal edema (66.7%), neuronal degeneration and necrosis (50%), hyperemia (50%), hemorrhage (50%), demyelination (33%), perivascular inflammatory cell infiltration (50%), and neuronophagia (50%).

The results obtained from the brain tissue examinations of the relevant red foxes indicate acute, subacute or chronic damage in the CNS. In addition, the findings obtained are compatible with neuropathological findings that may develop due to infectious, toxic or traumatic factors [42, 43, 44].

Gliosis, which develops in response to damage to nerve tissue in the central nervous system, indicates reactive proliferation of astrocytes [45]. In the presented study, its detection in the 4 red foxes indicates the response to damage in the brain tissue. In addition, the findings of degeneration, necrosis, and neuronophagia

indicate irreversible damage of nerve cells. This may indicate the presence of possible viral encephalitis, toxic exposure or hypoxic conditions.

The blood–brain barrier in the CNS has a highly dynamic structure [46]. In this study, the presence of perineuronal edema in the 4 red foxes suggests increased capillary permeability, and/or impaired blood–brain barrier integrity. In addition, hemorrhage, and hyperemia findings may indicate any disorder developing on the vascular structure. In particular, hemorrhage findings may indicate focal vascular destruction, while hyperemia findings may indicate an active inflammatory response in the brain tissue or a congestive circulatory disorder. The current histopathological results may be related to diseases or environmental toxins in the environment that cause neurological symptoms in red foxes. Although an etiological diagnosis was not established in our study, our results provide remarkable data in terms of both animal health, and zoonotic risks by documenting the presence of neuropathological changes in wildlife species.

CONCLUSIONS

In conclusion, the histopathological findings observed in this study indicate serious structural, and functional disorders in the brain tissue. It is important to conduct further etiological studies to elucidate the pathogenesis of the current results and to exclude some zoonotic agents. In addition, trace element findings may constitute an important preliminary source for the evaluation of environmental toxic element exposure, and potential neurodegenerative processes in wildlife.

It is thought that more samples should be analyzed in order to contribute to the healthy survival of red foxes in wildlife. It does not seem possible to examine red foxes by taking blood from them in the wild. For this reason, it is important to examine the tissue element levels of red fox cadavers that have not deteriorated. According to the results obtained, comments can be made about the environmental residues originating from elements in the region where red foxes live. The vital trace elements that are seen to be deficient can be left in the habitats where red foxes can consume, thus contributing to their healthier survival in wildlife.

Conflict of interest

There is no conflict of interest between the authors.

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