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Wild Boar (*Sus scrofa* L.) as the Biomonitor of Cadmium and Lead Pollution in the Republic of North Macedonia

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ABSTRACT

Until the outbreak of African swine fever in almost the entire Europe and consequently in the Republic of North Macedonia (2022), the population of wild boars was in increase. The biological characteristics of this species make it suitable to be used as a bioindicator for environmental pollution. The concentrations of cadmium and lead (Cd and Pb) were measured using atomic absorption spectrophotometry in the liver of free ranging wild boars. Samples were taken from 608 animals, harvested in a period of 7 years (2016-2022), in 11 hunting locations that cover the whole territory of the Republic of North Macedonia. The mean value of Pb in the liver in all hunting areas was 0.225 $mg \cdot kg^{-1}$ wet weight with range of 0.154-0.722 $mg \cdot kg^{-1}$, while mean Cd was 0.366 $mg \cdot kg^{-1}$ and ranged from 0.177 to 0.464 $mg \cdot kg^{-1}$. A statistically significant difference in Cd was found between the hunting locations, while Pb differed between hunting years. The mean Cd concentrations were significantly higher in Pelagonisko, Krivorečansko and Ohridsko-Prespansko hunting areas than in those from Pološko hunting area, while the mean Pb values in 2019 and 2020 were significantly higher than the mean value determined in 2021. For Cd, 18.2% of the total number of samples exceeds the maximum allowed limit provided in the legislation, while for Pb it was 6.7%. The maximum mean value for Pb (0.336 $mg \cdot kg^{-1}$) was measured in 2020 and for Cd (0.449 $mg \cdot kg^{-1}$) in 2016. The mean values for Pb and Cd obtained in this study were in line with data reported for wild boar from other European countries.

Keywords: wild boar; Pb; Cd; liver; hunting areas; pollutants

INTRODUCTION

Regardless of whether it comes from natural processes on the Earth's surface or from human activities, elements and compounds that are released into the environment and cause damage to the biosphere are treated as pollutants (Kłos et al. 2018). About 13,000 tons of cadmium (Cd) are produced annually worldwide, mainly intended for chemical stabilizers, nickel-cadmium batteries, pigments, metal coatings and alloys (Flora et al. 2008). Through the soil and the water, cadmium compounds can be easily adsorbed by plants. With the increased accumulation of toxic metals in the plants and the soil, the risk of their transfer to herbivorous wild mammals, game and livestock may increase (Bilandzić et al. 2010). Lead (Pb) is a highly toxic metal, which with its wide distribution, mainly as a result of human activities (mining, production and combustion of fossil fuels), can cause environmental contamination and health problems in many parts of the world (Jaishankar et al. 2014). The exposure to lead contamination can be manifested in lead-induced encephalopathy, gastroenteritis, and peripheral nerve degeneration (Lénárt et al. 2023). For the domestic and the wild animals and for the humans, acute or chronic exposure to Pb and Cd may have severe consequences because these heavy metals accumulate in almost all tissues, mainly in liver and kidneys as target organs (Satarug et al. 2003, Kramárová et al. 2005, Pandey and Madhuri 2014).

The Republic of North Macedonia, as part of the former Yugoslavia, was an important producer of metals such as copper, steel, ferroalloys, lead, zinc, cadmium, nickel, silver and gold, with long-term mining and mining-related activities contributing greatly to the pollution of the environment (Barandovski et al. 2020). The studies conducted in North

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Macedonia (Rogan et al. 2010, Stafilov et al. 2010, Stafilov 2017, Barandovski et al. 2020, Stafilov et al. 2023), where the concentration of heavy metals was measured in soils and mosses, offer a good contribution to getting to know the situation regarding the representation of heavy metals in this country. On the other hand, very little is known about the exposure of game (herbivores and omnivores) to heavy metals as a direct consumer of food from nature, which is often contaminated around critically endangered areas (industrial zones and mines). The anthropogenic pollution and the environmental areas naturally enriched in toxic heavy metals where game animals reside can increase the risk of contaminants entering the food chain and induce elevated level of them in game (Lehel et al. 2016). The diet is considered to be the most important pathway of metal transfer from soil and plants to the tissues of animals (Stankovic et al. 2014).

A well-known and commonly used bioindicator in modern research is wild boar (*Sus scrofa* L.) (Bilandzić et al. 2009, Bilandzić et al. 2010, Danieli et al. 2012, Gasparik et al. 2012, Lénárt et al. 2023). Wild boar is a potentially good bioindicator for monitoring of heavy metals in the environment because it is an opportunistic omnivore ingesting both flora and fauna (invertebrates, vertebrates, crops (Lee and Lee 2019) and mushrooms (Brzezicha-Cirocka et al. 2016)). Wild boars have a characteristic way of diet; they search for food by browsing or grazing (grasses, herbs, stems, leaves), on the surface of the ground (fruits and fungi) or below the surface of the ground by rooting (roots, invertebrates, rhizomes) (Bueno et al. 2011, Ballari and Barrios-García 2014). They have relatively long lifespan and thus long period of accumulating chemicals (Bilandzić et al. 2010). Also, they are relatively mobile and have the large effective population sizes (Frantz et al. 2012), so they can cover a large area in search for food and thus provide an early warning of adverse toxic effects in the ecosystem as a whole. Wild boars are widely distributed in the world and can be found in Eurasia, the southern part of Asia, and some of the islands od Indonesia (Lénárt et al. 2023).

The presence of wild boar in the last years in European areas has increased (Jori et al. 2021). In the Republic of North Macedonia, wild boars are represented in the entire territory of the country, while the last decade their number has increased drastically. This is confirmed by data from the State Statistical Office, where the number of the population in 2010 was estimated to be around 2,872 individuals, and in 2022 it was estimated to be around 9,400 individuals (MakStatDatabase 2021a). Since the largest number of hunters in North Macedonia are focused on wild boar hunting, in recent years the number of wild boars harvested during the hunting seasons has increased dramatically (in 2010, about 472 were shot, and in 2022, about 2,800 individuals) (MakStatDatabase 2021b), which makes biomonitoring with this species much easier. The good reflection of environmental pollution in wild boar tissues demonstrated in numerous papers in the last decade and the relevance for the consumers of boar meat (transmission to humans) are just a few of the reasons why we decided to investigate the occurrence of Pb and Cd in the liver of wild boar as the biomonitor of the pollution in the Republic of North Macedonia.

MATERIALS AND METHODS

The Study Area

The Republic of North Macedonia is a country situated in the central part of the Balkan Peninsula, bordering Serbia to the north, Bulgaria to the east, Greece to the south and Albania to the west, and it has a total area of 25,713 km^2. The country is mountainous and has deep basins and valleys with three large lakes and is bisected by the Vardar River. It has a water area of 857 km^2, while its land area is 24,856 km^2.The country has 16 mountains higher than 2,000 m, but most of the surface is between 500 and 1,000 meters above sea level. The highest point (2,764 m) in the country is the peak Golem Korab of Mount Korab and the lowest point (44 m) marks the area at the crossing point of the Vardar River (Barandovski et al. 2020).

In North Macedonia, the state establishes hunting grounds as basic spatial units for game management according to hunting law (2009). However, in the general hunting management plan for the Republic of North Macedonia (period 1997–2016), according to Trpkov and Maletic (1997), the total territory of the country is divided into 11 hunting areas as broader categories for game management. This division was made for the purpose of implementing a unique hunting policy and appropriate measures for protection, breeding and promotion of game management. In fact, the hunting area represents an ecological unit with a large area, conditioned by a complex of environmental factors and the vital characteristics of game populations, where it resides throughout the year and has the greatest impact on the environment. Given the mobility of game, especially large game, and its relatively large radius of action during all seasons to satisfy life's needs, such an organization is fully justified, especially when planning for the joint implementation of hunting policies in the hunting grounds that are part of the same hunting area, especially for the shooting. Also, when monitoring certain types of big game at the hunting ground level, wrong data on the numerical condition of the game are often obtained because the hunting ground is a small spatial unit, so there is a fear of the possibility of counting the same game in two or three hunting grounds which border.

Therefore, this study was carried out on the same 11 hunting areas that cover the territory of the entire country, namely: Pelagonisko hunting area (1) including the municipalities of Prilep, Bitola, Demir Hisar and Kruševo, with a total area of 408,279 ha; Ohridsko-Prespansko hunting area (2) including the municipalities of Resen, Ohrid, Struga and Debar, a total area of 183,743 ha; Kičevsko-Brodsko hunting area (3) including the municipalities of Kičevo and Makedonski Brod, a total area of 173,624 ha; Pološko hunting area (4) including the municipalities of Gostivar and Tetovo, a total area of 168,717 ha; Skopsko-Kumanovsko hunting area (5) including the municipalities of Skopje and Kumanovo, a total area of 276,105 ha; Sredno-Vardarsko hunting area (6) including the municipalities of Veles and Sveti Nikole, a total are of 220,525 ha; Krivorečansko hunting area (7) including the municipalities of Kratovo and Kriva Palanka, a total area of 109,524 ha; Bregalnčko hunting area (8) including the municipalities of Kočani, Vinica, Probištip and Štip, a total area of 214,435 ha; Vlainsko-Maleševsko hunting area (9) including the municipalities of Delčevo, Berovo, Pehčevo and Makedonska Kamenica, a total area of 139,233 ha; Strumičko hunting area (10) including the municipalities of Strumica and Radoviš, a total area of 168,695 ha; Dolno-Vardarsko hunting area (11) including the municipalities of Kavadarci, Negotino, Valandovo and Gevgelija, a total area of 282,772 ha.

Sampling

From 11 hunting areas (number 1 to 11) covering the whole territory of the Republic of North Macedonia, during the regular hunting season, which starts on 1 October and lasts until 31 January every year (between 2016 and 2022), a total number of 608 liver samples of wild boar (aged between 2 and 4 years) harvested by the active hunters were collected. The collected samples do not have a normal numerical arrangement in the hunting areas. From hunting area number 1, 143 samples were collected, from number 2 – 106 samples, from number 3 – 57, from number 4 – 50, from number 5 – 17, from number 6 – 15, from number 7 – 57, from number 8 – 3, from number 9 – 26, from number 10 – 53 and from number 11 – 81. After collection, the samples were individually packed in polyethylene bags and transferred to the laboratory in refrigerated bags. The tissue samples were frozen and stored at -20°C until analysis. During sampling operations, special care was taken to avoid tissues near the bullet pathway; all tissue samples were taken from >40 cm away from the areas of bullet damage (Dobrowolska and Melosik 2008, Danieli et al. 2012).

Method of Analyzing

Approximately 1 g of homogenized samples (measured with accuracy of ±0.01 g) were digested with 5 mL HNO_3 (67% v/v, purity for atomic absorption, Merck, Darmstadt), 1 mL of hydrogen peroxide (30%, analytical grade, Merck Darmstadt) with high-performance microwave oven (model Ethos Up, Milestone Srl, Sorisole, Italy) according to EN 13805:2002 (CEN 2002a). In brief, the temperature was ramped for 20 min to 220°C, after which samples were held at the same temperature for 15 min. During the digestion the potency was automatically adjusted by temperature and pressure control in each digestion vessel. Digested and cooled samples were diluted to the final volume of 25 mL with deionized water with Milli-Q quality.

Analysis of Pb and Cd was conducted by electrothermal atomic absorption spectrometer (ETAAS) with Zeeman background correction, model AAnalyst 600 (Perkin Elmer, Waltham, Massachusetts), and using 0.005 mg Pd (NO_3) as matrix modifier for each atomization of Pb and Cd (CEN 2003). The selected wavelengths for Pb and Cd measurement were 283.3 nm and 228.8 nm, respectively. Furnace programs for ETAAS determination were optimized for liver matrix (Table 1). Calibrations were prepared from commercial solutions of Pb and Cd (1,000 $mg \cdot L^{-1}$) in 2% HNO_3 (Carl Roth GmbH, Karlsruhe, Germany). For recovery and precision validation, reference material offal liver FAPAS test material 07199 was used.

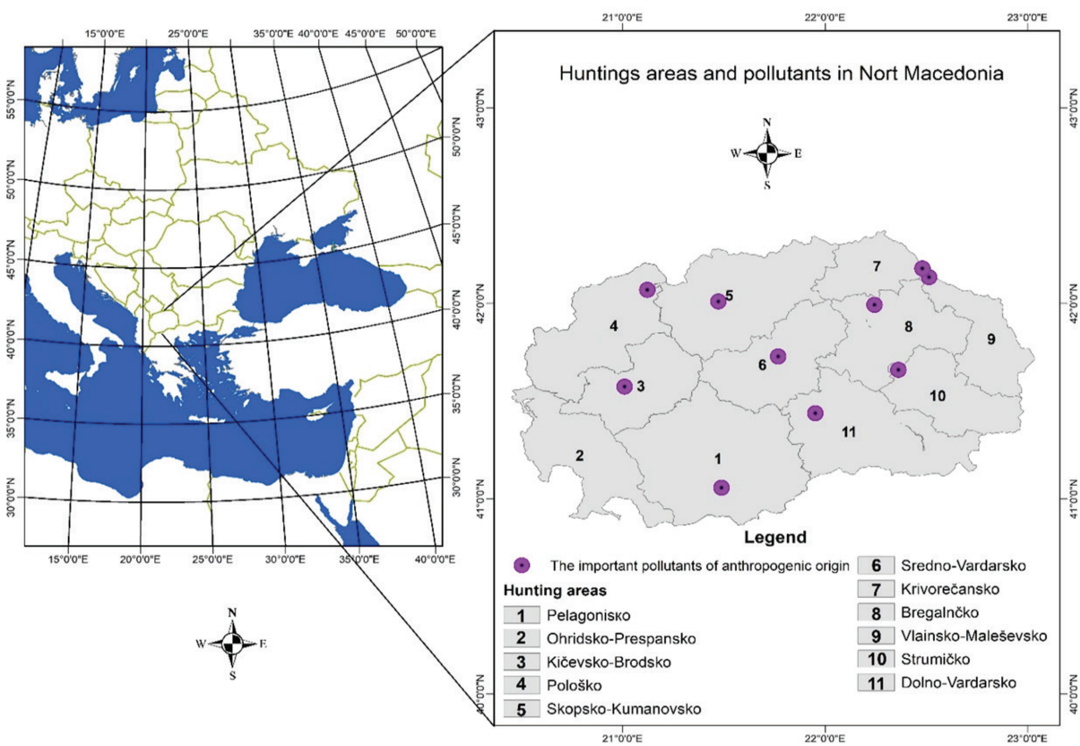

Figure 1. Hunting areas and pollutants of anthropogenic origin in North Macedonia.

Table 1. Furnace programs for lead (Pb) and cadmium (Cd) slit with 0.7 nm.

Step	Element	Temperature (°C)	Ramp time (s)	Hold Time (s)	Internal flow (mL·min^{-1})
1	Pb	110	10	30	250
	Cd	110	15	20	
2	Pb	130	20	30	250
	Cd	130	40	30	
3	Pb	950	35	35	250
	Cd	600	35	20	
4	Pb	1,850	0	5	0
	Cd	1,500	0	5	
5	Pb	2,450	1	3	250
	Cd	2,500	2	2	
6	Pb	20	15	20	250
	Cd	20	15	25	

Method Performance Characteristics

The quality assurance of the ETAAS method was performed by validation in accordance with the requirements proscribed in Commission Regulation (EC 2007). The obtained method of linearity range was 3.716-50 μg·L^{-1}, 0.319-6.0 μg·L^{-1} for Pb and Cd, respectively, with R^2>0.99, with limits of quantification of 12.39 μg·L^{-1} for Pb and 1.06 μg·L^{-1} for Cd. Method precision expressed as relative standard deviation was 6.90% and 8.56% for Pb and Cd, respectively, and the recovery was 104.25% (Pb) and 102.07% (Cd). Internal quality control was performed by running a reagent blank sample and reference material with every batch of samples. External quality control was assured by satisfactory participation in proficiency tests organized by FAPAS and the European Union Reference Laboratory for metals, EURL-MN. Matrix liver was within the ETAAS method scope which was accredited according to EN ISO/ IEC 17025:2017.

Statistical Analysis

Statistical analysis was performed using the Statistica software version 14 (StatSoFT STATISTICA Software) for descriptive analysis and for analysis of variance. To examine differences between sampling areas we used the two-way analysis of variance (ANOVA) test. All statements of significance were based on the 0.05 level of probability (p≤0.05).

RESULTS AND DISCUSSION

The concentrations of lead and cadmium found in livers of wild boars, collected during the period of 7 years on 11 different hunting areas in North Macedonia, are presented in tables 2 and 3. According to Commission Regulation (EC) no.1881/2006 (EC 2006), Food and Veterinary Agency of the Republic of North Macedonia have established maximum levels of Cd and Pb in liver of bovine animals, sheep, pigs, poultry and horses, which amounts to 0.5 mg·kg^{-1} (EC 2006). In this study, no statistically significant difference in hepatic Pb was found between 11 hunting areas locations. The mean concertation for Pb, looking at all hunting areas, ranged from 0.154 to 0.722 mg·kg^{-1}, and mean concentration for all in total was 0.225 mg·kg^{-1}. Compared to other studies, somewhat lower values were found in Croatia (Bilandzić et al. 2009), where the mean values ranged from 0.061 to 0.202 mg·kg^{-1}. Quite similar mean values were obtained in a study carried out in Italy (Danieli et al. 2012), with the mean value of 0.329 mg·kg^{-1}. However, somewhat higher values for Pb were found in Poland (Durkalec et al. 2015), with the mean value of 0.083 to 0.903 mg·kg^{-1}. Significantly higher values than those in our study were obtained in Southern Spain (Santiago et al. 1998), with mean value of 0.44 to 5.05 mg·kg^{-1}. The higher values obtained in Poland and Southern Spain compared to those in our study are probably a result of the choice of locations where the research was conducted. Namely, in Poland, the research was conducted in 3 locations, 2 of which are known as industrial areas, and the third location was taken as a reference area. In Southern Spain, the situation was similar, each of the four zones that were the subject of research had its own sources of possible pollution. In this study, the 11 hunting ground locations on which the research was carried out are of such a setting that they cover the entire territory of the country, and not only the known industrial facilities where higher values can be expected.

Looking at all locations, out of the total number of samples (n=608), in 18% of samples Pb was not detected (<LODs). Seven percent of samples (41 of 608) exceeded the maximum levels for Pb in liver of domestic animals (0.5 mg·kg^{-1}). The maximum measured concentrations of Pb in individual locations ranged from 0.443 to 4.338 mg·kg^{-1}, and the maximum measured value of 4.338 mg·kg^{-1} was obtained from a sample at hunting area no. 7, which is 8.5

Table 2. Lead (Pb) and cadmium (Cd) concentrations in the livers of wild boars from 11 hunting areas of North Macedonia.

Concentration in wet weight ($mg \cdot kg^{-1}$)							
Pb							
Hunting areas	**N**	**N < LODs**	**Mean**	**±SD**	**Min**	**Max**	**N > Max. l.**
1	109	34 (5.6)	0.244	0.384	0.006	2.443	11 (1.8)
2	86	20 (3.3)	0.178	0.184	0.009	0.968	6 (1)
3	44	13 (2.1)	0.190	0.204	0.015	1.067	3 (0.5)
4	50	0	0.247	0.541	0.017	3.512	3 (0.5)
5	14	3 (0.5)	0.154	0.117	0.027	0.443	0
6	15	0	0.205	0.269	0.023	1.060	2 (0.3)
7	44	13 (2.1)	0.352	0.700	0.021	4.338	6 (1)
8	3	0	0.722	1.122	0.021	2.017	1 (0.2)
9	21	5 (0.8)	0.214	0.286	0.030	1.358	1 (0.2)
10	42	11 (1.8)	0.190	0.214	0.015	1.289	2 (0.3)
11	67	14 (2.3)	0.197	0.194	0.004	0.959	6 (0.9)
∑	495	113(18.5)	0.225	0.370	0.004	4.338	41 (6.7)
Cd							
Hunting areas	**N**	**N < LODs**	**Mean**	**±SD**	**Min**	**Max**	**N > Max. l.**
1	134	9 (1.5)	0.423^{a}	0.418	0.012	1.929	40 (6.5)
2	98	8 (1.3)	0.381^{a}	0.315	0.015	1.468	22 (3.6)
3	56	1 (0.2)	0.372^{ab}	0.342	0.006	1.333	12 (2)
4	49	1 (0.2)	0.177^{b}	0.153	0.003	0.732	3 (0.5)
5	14	3 (0.5)	0.305^{ab}	0.138	0.111	0.644	1 (0.2)
6	15	0	0.293^{ab}	0.140	0.026	0.495	0
7	51	6 (1)	0.464^{a}	0.403	0.039	2.531	12 (2)
8	3	0	0.177^{ab}	0.089	0.091	0.268	0
9	22	4 (0.6)	0.387^{ab}	0.242	0.119	1.27	4 (0.6)
10	45	8 (1.3)	0.320^{ab}	0.520	0.008	3.573	3 (0.5)
11	71	10 (1.6)	0.354^{ab}	0.251	0.019	1.093	14 (2.3)
∑	558	50 (8.2)	0.366	0.354	0.003	3.573	111 (18.2)

N – number of samples above the limit of detection; N < LODs – number of samples bellow the limit of detection; N > Max. l. – number of samples exceeding maximum levels of Cd/Pb in liver of bovine animals, sheep, pigs, poultry and horses, set out by law; % of samples from the total number of samples is presented in brackets.

times higher than the maximum allowed concentration for lead. This is most likely due to contamination from Toranica, which is present in this area with a production capacity of 700,000 tons of ore per year. This is confirmed also by the results of the study conducted during the moss biomonitoring of air pollutinon where high contents of Pb and Zn were discovered (average values of 60 and 75 $mg \cdot kg^{-1}$ of moss) in the area very close to the source of pollution (Angelovska et al. 2014).

Moreover, this hunting area is bordered by two more lead and zinc mines, , Zletovo and , Sasa, which are characterized by high deposition of Pb and Zn, especially near the source of pollution (Balabanova et al. 2014, Balabanova et al. 2017) . This has also been confirmed by one of the latest studies conducted in the Republic of North Macedonia (Stafilov et al. 2023) in which the distribution of various chemical elements was determined in soils and vegetables from gardens in the region of Probištip, North Macedonia (with the main point being the Zletovo mine). The researchers obtained the results in which the lead content in 8 of 19 soil samples studied was higher than the target value (85 $mg \cdot kg^{-1}$) according to the Dutch standards, and the highest lead content measured in soil was 478 mg·kg^{-1}, which is 5.6 times higher than the target value (the target values indicate the level at which there is a sustainable soil quality). In the Republic of North Macedonia, the regulations for the maximum allowed content of pollutants in the soil and vegetables have not yet been adopted, so in the previous studies regarding the soil and vegetables, the values are compared with the Dutch regulations.

The results of the occurrence of lead in vegetables and cereals showed that a larger number of samples (18 samples out of 38) had an elevated value that exceeds the maximum allowed levels of 0.10 mg·kg^{-1} for vegetables, 0.30 mg·kg^{-1} for leafy vegetables and 0.20 mg·kg^{-1} for cereals. In the mentioned study, the highest content of Pb was found in the barley samples with the concentration 6.65 mg·kg^{-1}, exceeding the maximum allowed content by 33 times. Since the wild boar does not know natural boundaries and obstacles, and since omnivores when searching for food can cover a large area, the occasional high values that exceed the maximum permitted limit may be the result of contamination right near such industries.

The mean concertation for Cd, looking at all hunting areas together, ranged from 0.177 to 0.464 mg·kg^{-1}, and the total mean concentration was 0.366 mg·kg^{-1}. Similar values were obtained in two studies conducted in Croatia, where the mean values ranged from 0.3 to 0.49 and 0.162 to 0.308 mg·kg^{-1} (Bilandzić et al. 2009, Florijancic et al. 2015), and to one study in Spain (0.17 to 0.34 mg·kg^{-1}) (Santiago et al. 1998), while the lower mean value was obtained in Italy amounting to 0.084 mg·kg^{-1} (Danieli et al. 2012). From a total of 608 samples, cadmium was not detected in 50 samples or 8.2% of the total number of samples (Cd<LODs). This study indicates location differences, so the mean Cd concentrations were significantly higher in Pelagonisko, Krivorečko and Ohridsko-Prespansko hunting areas than in the samples collected from Pološko hunting area (p=0.004 both.). The most acceptable explanation for these differences is the availability and distance from pollutants and mining activities, although it is a fact that there is no large-scale pollutant in the Ohrid-Prespa hunting ground. However, Cd and Pb are transported over long distances by air, so the dry and wet deposition of the particles may be due to pollutants from greater distances. In the Krivoreško hunting area or near its borders, as mentioned previously, there are 3 potential sources of pollution on a huge scale. In the research conducted near the Zletovo mine (Stafilov et al. 2023), high concentrations of cadmium were found in soil samples (16 out of 19 exceeded the maximum allowed limits of 0.8 mg·kg^{-1}) and vegetables (14 out of 19 samples exceeded the maximum allowed limits of 0.05 mg·kg^{-1} for vegetables, 0.20 mg·kg^{-1} for leafy vegetables, and 0.10 mg·kg^{-1} for cereals). In the vicinity of the Sasa and Toranica mines, high concentrations of cadmium in moss were detected, especially near the source of contamination (Angelovska et al. 2014, Balabanova et al. 2017). Compared to the area where much lower concentrations of cadmium were determined, near the eastern border of the Pološko hunting area, a polluter of this scale is the previously active high-melting ferrochromium plant, Jugohrom - Tetovo, which stopped working in 2016. In the research carried out in North Macedonia, and referring to the Tetovo region, in general this area is characterized as highly contaminated with Sc, Cr, Fe, Co, and Ni, as a consequence of the former operation of the ferrochromium plant, and not with cadmium (Barandovski et al. 2008). Also, some of the hunting grounds in this area border the Mavrovo National Park, and most of them today are already part of the newly formed Shar Planina National Park. Characteristic of the Pelagonian hunting area, which applies to all other hunting areas and can be seen from the obtained results, is the fact that the largest number of samples that exceed the maximum permissible limit for cadmium (N>0.5 mg·kg^{-1}), i.e. 40 out of 111 samples or 6.5 out of 18.2%, were determined exactly in this area. The major polluter of the environment in the region is the thermo-electric power plant REK Bitola. The average value of cadmium of 0.423 mg·kg^{-1} for this locality does not exceed the maximum permissible limit, but it is still significantly higher than the average value of the Polog hunting area. Our results correspond with the recent study (Stafilov et al. 2018)indicating that the concentrations of cadmium in the soil in the Bitola region do not exceed the maximum allowed according to Dutch Standards. Furthermore, compared to the average values of European soils, they are lower in relation to them, but some samples in the area of REK Bitola have slightly increased content over the target value ranging from 1.0 to 1.3 mg·kg^{-1}. Pollution of soil with some elements present in fly ash deposited in this region influence the appearance of high content of arsenic, cadmium, lead and zinc.

Table 3 shows Pb and Cd concentration in livers of wild boars during the period of 7 years (2016-2022) in North Macedonia. The mean values for lead over the years ranged from 0.095 to 0.336 mg·kg^{-1}, or the mean for all years was 0.225 mg·kg^{-1}. The highest mean value for lead (0.336 mg·kg^{-1}) was measured in 2020. In the same year, the largest number of liver samples exceeding the maximum allowed limits (N>0.5 mg·kg^{-1}) were determined, 20 out of 41 samples or 3.4 out of 6.7%. The maximum measured lead concentration in wild boar liver was 4.333 mg·kg^{-1} and it was measured in 2019.

In our study, results show that the mean lead values in 2019 and 2020 are significantly higher than the mean value determined in 2021 (p=0.0002). It is very difficult to explain and determine the specific reason why the mean value for lead was significantly lower in 2021 compared to the previous two years. Additional research is needed, where many more factors that could influence the differences that occur over the years will be monitored to determine the specific causes.

In general, the mean values for cadmium over the years have showed no statistically significant difference. Mean values ranged from 0.324 to 0.449 mg·kg^{-1} and mean value for all years was 0.366 mg·kg^{-1}. The maximum mean value for Cd (0.449 mg·kg^{-1}) was measured in 2016. It can be indicated that cadmium is present in the environment, but its average amount over the years has remained stable and no changes in an upward or downward direction have been observed. Also, in each of the researched years, certain values exceeding the maximum allowed limits for North Macedonia were observed (N>0.5 mg·kg^{-1}), namely 7 in 2016, 18 in 2017, 18 in 2018, 19 in 2019, 19 in 2020,

Table 3. Lead (Pb) and cadmium (Cd) concentrations in the livers of wild boars for the period 2016-2022 in North Macedonia.

Concentration in wet weight (mg·kg^{-1})							
Pb							
Years	**N**	**N < LODs**	**Mean**	**±SD**	**Min**	**Max**	**N > Max. l.**
2016	25	4 (0.6)	0.318^{ab}	0.710	0.004	3.512	2 (0.3)
2017	78	18 (3)	0.207^{ab}	0.116	0.026	0.549	1 (0.2)
2018	82	21 (3.4)	0.197^{ab}	0.392	0.012	2.443	4 (0.7)
2019	59	13 (2.1)	0.322^{a}	0.625	0.006	4.338	7 (1.1)
2020	92	20 (3.3)	0.336^{a}	0.379	0.016	2.017	21 (3.4)
2021	84	14 (2.3)	0.095^{b}	0.103	0.021	0.684	1 (0.2)
2022	75	23 (3.8)	0.174^{ab}	0.157	0.030	0.968	5 (0.8)
∑	495	113 (18.5)	0.225	0.370	0.004	4.338	41 (6.7)
Cd							
Years	**N**	**N < LODs**	**Mean**	**±SD**	**Min**	**Max**	**N > Max. l.**
2016	29	0	0.449	0.460	0.017	1.551	7 (1.1)
2017	92	4 (0.6)	0.406	0.288	0.006	1.333	18 (3)
2018	99	4 (0.6)	0.372	0.363	0.008	1.929	18 (3)
2019	65	7 (1.2)	0.406	0.394	0.003	1.522	19 (3.1)
2020	100	12 (2)	0.324	0.423	0.005	3.573	19 (3.1)
2021	93	5 (0.8)	0.346	0.315	0.015	2.531	17 (2.8)
2022	80	18 (3)	0.328	0.266	0.012	1.316	13 (2.1)
∑	558	50 (8.2)	0.366	0.354	0.003	3.573	111 (18.2)

N – number of samples above the limit of detection; N < LODs – number of samples bellow the limit of detection; N > Max. l. – number of samples exceeding maximum levels of Cd/Pb in liver of bovine animals, sheep, pigs, poultry and horses, set by law; % of samples from the total number of samples is presented in brackets.

17 in 2021, and 13 in 2022, or 111 of total 608 samples during 7 years. This indicates that cadmium can accumulate in larger amounts in game organs, which has also been confirmed by the numerous studies conducted in the surrounding countries of the region, where different species of game such as brown hare, roe deer and red deer were used as bioindicators for the presence of heavy metals in the environment (Bilandzić et al. 2009, Durkalec et al. 2015, Beuković et al. 2022, Beuković et al. 2023).

CONCLUSION

From this study it can be concluded that wild boars, as free-migrating animals on the hunting areas of North Macedonia, are show low contamination with Cd and Pb. The mean values for Pb and Cd obtained in this study were in line with data reported for wild boars from some other European countries. The research was conducted on the entire territory of the country, and not only on the industrially polluted areas, as it is the case in most studies where game is used as a bioindicator. Even though the average values of these heavy metals did not exceed the maximum permissible limits, in each year and on each location a certain number of samples exceeded the maximum permissible limits. This indicates the need for additional research, in order to assess the risk while consuming liver, which is especially important for the population of hunters who more often incorporate wildlife in their nutrition.

Author Contributions

All authors listed under the title contributed to the research and development of the paper. KC, VM, DB and MPH conceived and designed the study, KC, VM and DB carried out the field activities in the collection of wild boar samples, EDS and VE performed the laboratory analysis, MPH and MV performed the statistical analysis, KC, DB and MPH wrote the manuscript except the part of method of laboratory analyzing which was written by EDS and VE.

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REFERENCES

Angelovska S, Stafilov T, Balabanova B, Šajn R, Bačeva K, 2014. Applicability of Atomic Emission and Atomic Absorption Spectrometry for Variability Assessment of Trace and Macro-Elements Content in Moss Species from Pb-Zn Mine Environment. *Modern Chem Appl* 2(1). http://dx.doi.org/10.4172/2329-6798.1000123.

Balabanova B, Stafilov T, Šajn R, Andonovska KB, 2017. Quantitative assessment of metal elements using moss species as biomonitors in downwind area of lead-zinc mine. *J Environ Sci Heal A* 52(3): 290-301. https://doi.org/10.1080/10934529.2016.1253403.

Balabanova B, Stafilov T, Šajn R, Bačeva K, 2014. Variability assessment of metals distributions due to anthropogenic and geogenic impact in the lead-zinc mine and flotation „Zletovo" environs (moss biomonitoring). *Geol Maced* 28(2): 101–114.

Ballari SA, Barrios-García MN, 2014. A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. *Mammal Rev* 44(2): 124-134. https://doi.org/10.1111/mam.12015.

Barandovski L, Cekova M, Frontasyeva M, Pavlov S, Stafilov T, Steinnes E, Urumov V, 2008. Atmospheric deposition of trace element pollutants in Macedonia studied by the moss biomonitoring technique. *Environ Monit Assess* 138: 107-118. https://doi.org/10.1007/s10661-007-9747-6.

Barandovski L, Stafilov T, Šajn R, Frontasyeva M, Bačeva Andonovska K, 2020. Atmospheric heavy metal deposition in north macedonia from 2002 to 2010 studied by moss biomonitoring technique. *Atmosphere* 11(9): 929. https://doi.org/10.3390/atmos11090929

Beuković D, Vukadinović M, Krstović S, Polovinski-Horvatović M, Jajić I, Popović Z, Lavadinović V, Beuković M, 2022. The European Hare (*Lepus europaeus*) as a Biomonitor of Lead (Pb) and Cadmium (Cd) Occurrence in the Agro Biotope of Vojvodina, Serbia. *Animals* 12(10): 1249. https://doi.org/10.3390/ani12101249

Beuković D, Vukadinović M, Polovinski-Horvatović M, Popović Z, Lavadinović V, Đorđević N, Beuković M, 2023. The brown hare (*Lepus europaeus*) as a biomonitor for the presence of lead and cadmium in Vojvodina, Serbia. *Contemporary agriculture* 72(1-2): 1-5. https://doi.org/10.2478/contagri-2023-0001.

Bilandzić N, Sedak M, Dokić M, Simić B, 2010. Wild boar tissue levels of cadmium, lead and mercury in seven regions of continental Croatia. *B Environ Contam Tox* 84(6): 738-43. https://doi.org/10.1007/s00128-010-9999-7.

Bilandzić N, Sedak M, Vratarić D, Perić T, Simić B, 2009. Lead and cadmium in red deer and wild boar from different hunting grounds in Croatia. *Sci Total Environ* 407(14): 4243-7. https://doi.org/10.1016/j.scitotenv.2009.04.009.

Brzezicha-Cirocka J, Mędyk M, Falandysz J, Szefer P, 2016. Bio-and toxic elements in edible wild mushrooms from two regions of potentially different environmental conditions in eastern Poland. *Environ Sci Pollut R* 23(21): 21517-21522. https://doi.org/10.1007/s11356-016-7371-0.

Bueno C, Reiné R, Alados C, Gómez-García D, 2011. Effects of large wild boar disturbances on alpine soil seed banks. *Basic Appl Ecol* 12(2): 125-133. https://doi.org/10.1016/j.baae.2010.12.006.

CEN 2002a E, Foodstuffs – Determination of trace elements – Pressure digestion, European Committee for Standardization, 2002.

CEN 2003 E, Foodstuffs – Determination of trace elements – Determination of lead, cadmium, zinc, copper and iron by atomic absorption spectrometry (AAS) after microwave digestion, European Committee for Standardization, 2003.

Danieli P, Serrani F, Primi R, Ponzetta M, Ronchi B, Amici A, 2012. Cadmium, lead, and chromium in large game: a local-scale exposure assessment for hunters consuming meat and liver of wild boar. *Arch Environ Con Tox* 63: 612-627. https://doi.org/10.1007/s00244-012-9791-2.

Dobrowolska A, Melosik M, 2008. Bullet-derived lead in tissues of the wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). *Eur J Wildlife Res* 54: 231-235. https://doi.org/10.1007/s10344-007-0134-y.

Durkalec M, Szkoda J, Kolacz R, Opalinski S, Nawrocka A, Zmudzki J, 2015. Bioaccumulation of lead, cadmium and mercury in roe deer and wild boars from areas with different levels of toxic metal pollution. *Int J Environ Res* 9(1): 205-212. https://doi.org/10.22059/ijer.2015.890.

European Commision, 2007. Commission Regulation (EC) 333/2007 of 28 March laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCDP and benzo(a)pyrene in foodstuffs. *Off J Eur Union*, L88.

European Commission E, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off J Eur Union* 364: 5-24.

Flora S, Mittal M, Mehta A, 2008. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J Med Res* 128(4): 501-523.

Florijancic T, Ozimec S, Jelkic D, Vuksic N, Bilandzic N, Boskovic G, Boskovic I, 2015. Assessment of heavy metal content in wild boar (*Sus scrofa* L.) hunted in eastern Croatia. *J Environ Prot Ecol* 16(2): 630-636.

Frantz A, Bertouille S, Eloy M-C, Licoppe A, Chaumont F, Flamand M-C, 2012. Comparative landscape genetic analyses show a Belgian motorway to be a gene flow barrier for red deer (*Cervus elaphus*), but not wild boars (*Sus scrofa*). *Mol Ecol* 21(14): 3445-3457. https://doi.org/10.1111/j.1365-294X.2012.05623.x.

Gasparik J, Dobias M, Capcarova M, Smehyl P, Slamecka J, Bujko J, Gasparik Jr J, 2012. Concentration of cadmium, mercury, zinc, copper and cobalt in the tissues of wild boar (*Sus scrofa*) hunted in the western Slovakia. *J Environ Sci Heal A* 47(9): 1212-1216. https://doi.org/10.1080/10934529.2012.672065.

Hunting law, 2009. In Official Gazette of the Republic of Macedonia, Skopje North Macedonia. num. 26/2009; 82/2009; 136/2011; 1/2012; 69/2013; 164/2013; 187/2013; 33/2015; 147/2015 and 193/2015.

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN, 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology* 7(2): 60-72. https://doi.org/10.2478/intox-2014-0009.

Jori F, Massei G, Licoppe A, Ruiz-Fons F, Linden A, Václavek P, Chenais E, Rosell C, 2021. Management of wild boar populations in the European Union before and during the ASF crisis. In: Understanding and Combatting African Swine Fever: A European Perspective. Leiden, The Netherlands: Wageningen Academic, pp. 263-271. https://doi.org/10.3920/978-90-8686-910-7_8.

Kłos A, Ziembik Z, Rajfur M, Dołhańczuk-Śródka A, Bochenek Z, Bjerke JW, Tømmervik H, Zagajewski B, Ziółkowski D, Jerz D, Zielińska M, Krems P, Godyń P, Marciniak M, Świsłowski P, 2018. Using moss and lichens in biomonitoring of heavy-metal contamination of forest areas in southern and north-eastern Poland. *Sci Total Environ* 627: 438-449. https://doi.org/10.1016/j.scitotenv.2018.01.211.

Kramárová M, Massányi P, Slamecka J, Tataruch F, Jancová A, Gasparik J, Fabis M, Kovacik J, Toman R, Galová J, 2005. Distribution of cadmium and lead in liver and kidney of some wild animals in Slovakia. *J Environ Sci Heal A* 40(3): 593-600. https://doi.org/10.1081/ESE-200046605.

Lee S-M, Lee E-J, 2019. Diet of the wild boar (*Sus scrofa*): implications for management in forest-agricultural and urban environments in South Korea. *PeerJ* 7: e7835. https://doi.org/10.7717/peerj.7835/supp-1.

Lehel J, Laczay P, Gyurcsó A, Jánoska F, Majoros S, Lányi K, Marosán M, 2016. Toxic heavy metals in the muscle of roe deer (*Capreolus capreolus*)—food toxicological significance. *Environ Sci Pollut Res* 23: 4465-4472. https://doi.org/10.1007/s11356-015-5658-1.

Lénárt Z, Bartha A, Abonyi-Tóth Z, Lehel J, 2023. Monitoring of metal content in the tissues of wild boar (*Sus scrofa*) and its food safety aspect. *Environ Sci Pollut Res* 23: 4465-4472. https://doi.org/10.1007/s11356-022-23329-6.

MakStatDatabase, 2021a. *Game population, by region, by year*. State Statistical Office, North Macedonia. Available from: https://makstat.stat.gov.mk/PXWeb/pxweb/mk/MakStat/MakStat__Zemjodelstvo__LovRibolov/150_Lov_Reg_Brojna_sostojba_divech_god_ml.px/table/tableViewLayout2/?rxid=46ee0f64-2992-4b45-a2d9 cb4e5f7ec5ef.ž

MakStatDatabase, 2021b. *Game shot, by region, by year*. State Statistical Office, North Macedonia. Available from: https://makstat.stat.gov.mk/PXWeb/pxweb/en/MakStat/MakStat__Zemjodelstvo__LovRibolov/175_Lov_Reg_Otstrel_broj_god_ml.px/table/tableViewLayout2/?rxid=46ee0f64-2992-4b45-a2d9-cb4e5f7ec5ef

Pandey G, Madhuri S, 2014. Heavy metals causing toxicity in animals and fishes. *Res J Anim Vet Fishery Sci* 2(2): 17-23.

Rogan N, Dolenec T, Serafimovski T, Tasev G, Dolenec M, 2010. Distribution and mobility of heavy metals in paddy soils of the Kočani Field in Macedonia. *Environ Earth Sci* 61: 899-907. https://doi.org/10.1007/s12665-009-0405-x.

Santiago D, Motas-Guzmán M, Reja A, María-Mojica P, Rodero B, García-Fernández AJ, 1998. Lead and cadmium in red deer and wild boar from Sierra Morena Mountains (Andalusia, Spain). *B Environ Contam Tox* 61(6): 730-7. https://doi.org/10.1007/s001289900822.

Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PE, Williams DJ, Moore MR, 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol Lett* 137(1-2): 65-83. https://doi.org/10.1016/S0378-4274(02)00381-8.

Stafilov T, 2017. Environmental pollution with heavy metals in the Republic of Macedonia. *Contrib Sect Nat Math Biotech Sci* 35(2).

Stafilov T, Šajn R, Arapčeska M, Kungulovski I, Alijagić J, 2018. Geochemical properties of topsoil around the coal mine and thermoelectric power plant. *J Environ Sci Heal A* 53(9): 793-808. https://doi.org/10.1080/10934529.2018.1445076.

Stafilov T, Šajn R, Pančevski Z, Boev B, Frontasyeva MV, Strelkova LP, 2010. Heavy metal contamination of topsoils around a lead and zinc smelter in the Republic of Macedonia. *J Hazard Mater* 175(1-3): 896-914. https://doi.org/10.1016/j.jhazmat.2009.10.094.

Stafilov T, Stojanova K, Tašev K, Bačeva Andonovska K, 2023. Accumulation and distribution of heavy metals in soil and food crops in the Pb–Zn mine environ. Case study: Region of Probištip, North Macedonia. *J Environ Sci Heal A* 58(3): 171-182. https://doi.org/10.1080/10934529.2023.2174331.

Stankovic S, Kalaba P, Stankovic AR, 2014. Biota as toxic metal indicators. *Environ Chem Lett* 12: 63-84. https://doi.org/10.1007/s10311-013-0430-6.

Trpkov B, Maletic V, 1997. General hunting management plan for the period 1997-2016. Edited by Ministry of Agriculture Forestry and Water Management. Skopje: Government of the Republic of North Macedonia.