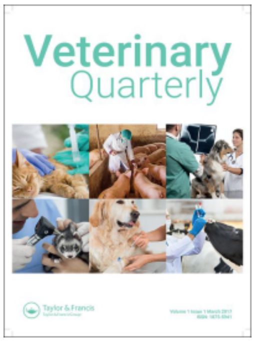

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Influence of age, habitat elevation, and distance to a thermal power plant on pathomorphological findings in the European brown hare (*Lepuseuropaeus* P.)

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ABSTRACT

The lifespan of the European hare (Lepus europaeus P.) is affected by a number of negative factors, including pollutants. In this paper, the individual and joint influence of age and habitat (elevation and distance from the thermal power plant - TPP) on pathomorphological findings of hares shot during three hunting seasons was investigated. Pathomorphological changes were found in 95.12% of hares. In hares up to 1 year of age, the changes were predominant in the lungs, and in older hares, in the kidneys. Degenerative changes in kidneys and liver and inflammatory changes in kidneys and lungs were considered important most in discussing the influence of chemical pollution. The proximity of TPP influenced the type of changes in the liver. A significant joint effect of age and elevation on the type of changes in the lungs of adult hares and on the heart of young hares was found. Elevation and distance from TPP had a joint effect on the occurrence of changes in the lungs, intestines, and heart in hares from the field farther from TPP. The results indicate that the hares were highly exposed to chemical pollutants that may affect their immunity, and lifespan.

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European hare; age; elevation; environmental pollution; thermal power plant; pathomorphology

Introduction

Wildlife is a part of the human ecosystem, an important source of various benefits to local communities, and direct contributor to the well-being of billions of people globally (IPBES 2022). The health of wild animals is closely intertwined with the health of domestic animals, the environment, and humans. Because game meat is used for consumption by humans and domestic animals, it is particularly important to have ongoing insights into wildlife health and the factors that influence it (Niewiadomska et al. 2021; Davies et al. 2019). Wildlife species, including hares, are often used as bioindicators to assess the extent and severity of environmental pollution (Wajdzik et al. 2017; Beuković et al. 2022).

The European hare (*Lepus europaeus* P.), also called the brown hare, is one of the most important wild species inhabiting almost all of Europe, southwestern and central Asia, parts of Australia, New Zealand, North and South America, and some islands in the Atlantic Ocean (Hacklander and Schai-Braun 2019). It is herbivorous and prefers to feed on various grasses and weeds, while in winter its diet includes twigs, shoots, and the bark of shrubs and young trees. In the absence of common foods, the brown hare feeds on agricultural crops: soybeans, clover, corn, winter wheat, carrots, and sugar beets (Reichlin et al. 2006; Santilli et al. 2023).

When they feel safe and have a sufficient variety of feed and other resources to live on, hares stay longer in the same habitat. The size of their habitat and their range of movement vary. The average distance hares travel during the day or night is about 200 m. Over the course of a year, hares gradually shift the center of their habitat, also about 200 m from the starting point (Rühe and Hohmann 2004; Schai-Braun and Hackländer 2014; Mori et al. 2022). Natural population densities range from 2 to 275 individuals/km^2 in particularly suitable habitats. Habitats include terrain at elevations from sea level to 2,400 m (Schai-Braun and Hackländer 2016).

The hare can live 8-12 years (Hackländer and Schai-Braun 2018), although it rarely reaches this age in nature. Population dynamics are primarily influenced by juvenile mortality due to seasonal differences in weather conditions, mechanical activities on agricultural land,

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disease, and predation (Hackländer and Schai-Braun 2018; Viviano et al. 2023). Agricultural intensification and habitat degradation have been identified as major causes of declines in hare populations (Smith et al. 2005), which have been ongoing for decades (Halecki et al. 2017; Hackländer & Schai-Braun 2019; Faehndrich et al. 2023). In Serbia, the total population of hares decreased from 594,023 to 478,111 individuals between 1991 and 2021 (Lazarević 2022), or by 19.51%.

A negative anthropogenic influence that affects the longevity and number of hare populations, and also alter its habitat quality, is reflected in the increasing exposure of the brown hare to pesticides and other chemical pollutants (UNDP 2011; Popović and Đorđević 2010; Beuković et al. 2013; Mayer et al. 2020). The type and frequency of pathomorpholocical changes in the organs of the hunted animal indicate whether it has been exposed to pathogens, parasites, and other harmful influences in a given area (Poli et al. 1991; Fuchs and Weissenbock 1992; Posautz et al. 2015; Marinković et al. 2018). The primary objective of this study was to determine the health status of hares harvested regularly during the hunting season. The locations where the hares were harvested were chosen randomly. When we compiled the necropsy reports with the data on the origin of the hares, we found that half of the hunting areas were located near thermal power plants (TPPs) that use solid fuel. It is known that lignite mining and combustion products in TPPs are significant sources of air, soil and water pollution, which seriously affects human health. There is evidence of numerous cases of premature deaths, bronchitis in children and adults, and severe respiratory and cardiovascular symptoms in humans, not only in areas near TPP, but far beyond (Pupovac 2021; Zhang et al. 2022).

There are insufficient data on the effects of TPP near habitats on pathomorphological findings in hares. For this reason, we decided to investigate some factors that might influence the changes in the internal organs of hunted hares. We hypothesized that changes would be more frequent in individuals that are older (and thus exposed to harmful factors for a longer period of time), that live closer to TPP and at lower elevations, as aerosol concentrations typically decrease with increasing terrain elevation (Li et al. 2019). This work presents pathomorphological changes in organs of hares culled during three hunting seasons in Serbia and the results of investigation of individual and joint influence of three factors (age of hare, elevation of habitat and distance of habitat from the nearest thermal power plant) on pathomorphological findings in different organs.

Material and methods

Hunting grounds

The hare bodies examined belonged to the animals culled in three consecutive hunting seasons (2017, 2018 and 2019, in the period October-November) at 12 sites on the territory of Serbia. During this period no carcasses of hares died of natural causes were found on the hunting grounds and also no hares with clinically manifested signs of any disease were noted. Animals were hunted in accordance with the Law on Game and Hunting (Law of the RS 2018), the hunting base and the annual management plan of hunting ground users (Rulebook of the RS 2012). According to the Rulebook of the RS (2010), ethical approval is not required for this study.

Hunting grounds were classified as plain and hilly (≤100 m and >100 m above sea level, respectively) depending on elevation. Six hunting areas were considered as 'near' to the thermal power plant (TPP) because they were within 8 km from the nearest TPP (two hilly and four plain terrains). The remaining six hunting grounds were at least 20 km from these pollution sources (three in hilly and three in flat terrain).

Study area

The elevation of the hunting grounds and power plants was determined using Google Earth Maps (https://earth.google.com): the average elevation of the places where hares were harvested was 92 m in the lowlands and 243 m in the hills. The two thermal power plants near the hunting areas are located at an elevation of about 80 m (TPP in the north of the map) and 90 m (TPP further south), they are located in flat terrain. The chimneys of these TPPs are between 150 and 280 m high. The distance between these two power plants is about 20 km. In the area where the TPPs are located, the air quality at the time of the study belonged to the third category according to an environmental study (Knežević et al. 2020), which means that the air was excessively polluted, i.e. the limits for one or more pollutants were exceeded.

Macroscopic and microscopic examination

Since sexual dimorphism is not pronounced in hares, the sex of hunted hares was determined at necropsy that conveyed within 24 h of death. Age was determined by the weight of dried eye lenses (Beuković et al. 2017). Considering that juvenile hares reach sexual and body maturity at the age of eight months to one year (Beuković and Popović 2014; Hacklander and Schai-Braun 2019), hares in the study were divided into two groups after determining their age: up to one year old ('younger') and over one year old ('older').

The carcasses of the hunted hares were delivered to the Department of Pathology, Faculty of Veterinary Medicine, University of Belgrade for pathomorphological examination. All carcasses were macroscopically examined, and macroscopic changes were noted and recorded. Tissue samples were also collected for microscopic, microbiological and parasitological examinations. The tissue samples of intestine, liver, heart, lung, kidney, spleen and brain for histopathological examination were fixed in 10%

Table 1. Participation (%) share of diseased hares by type of changes in the observed organs*.

Organ	Type of histopathological change**							
	1	2	3	4	5	1 & 2	1 & 4	2 & 4
Intestines	–	–	–	**100.00**	–	–	–	–
Liver	**44.26**	–	–	24.59	–	–	31.15	–
Lungs	–	4.48	–	**73.13**	–	1.49	–	20.90
Heart	34.62	15.38	–	**42.31**	–	7.69	–	–
Kidney	**69.49**	–	–	13.56	–	–	16.95	–
Spleen	9.52	–	**76.19**	–	14.29	–	–	–
Brain	**88.24**	5.88	–	5.88	–	–	–	–

*The value of the most frequent change for each organ is bold; **1 – degeneration; 2 – circulatory disturbance; 3 – growth and development disorders; 4 – inflammation; 5 – neoplastic changes.

buffered formalin and, after standard processing in an automatic tissue processor, embedded in paraffin blocks and 5 µm thick sections were stained with hematoxylin and eosin (HE). The stained tissue samples were examined microscopically (Olympus BX51 light microscope, Olympus Optical, Japan). For data analysis, the histopathological changes detected on the organs were classified into one of the following groups: (1) degeneration, (2) circulatory disturbances, (3) growth and development disorders, (4) inflammation, and (5) neoplastic changes. Changes resulting from gunshot wounds were not included.

Statistical analysis

The Chi-square test was used to test the hypotheses, and in cases where the conditions for its use were not met, Fischer's exact probability test was used. When the Chi-square test was used, the results of the test were indicated by the sample χ^2 value and its significance level (p). Fischer's exact probability test is not based on statistics, but only the empirical value of the exact probability of the null hypothesis, p, is calculated, and therefore only this is presented in the paper. The software package GraphPad PRISM (GraphPad Software, Inc., USA) was used for statistical analysis.

Results

In the course of three hunting seasons, a total of 82 hares killed were collected. There were 50.62% younger hares and 49.38% hares older than one year.

The difference in the number of hares from hilly and plain terrain was not significant (χ^2 =1,756; $p=0.185$). Fisher's exact test showed that the sex and age structure of harvested hares is not dependent on the type of terrain ($p=0.502$ and $p=0.405$, respectively) and the proximity of the thermal power plant ($p=0.326$). The number of hares is higher in the terrain distant from the thermal power plants ($\chi^2=$ 15,805; $p<0.001$).

Pathomorphological findings on hare organs

Only 4 hares (4.88%) showed no changes in the internal organs. The other 95.12% showed changes in several organs (most frequently in liver, lungs and kidneys), and in some animals two types of pathological changes were simultaneously present in liver, lungs, heart and kidneys (Table 1).

Intestines. Inflammatory changes were noted in the form of a mononuclear inflammatory infiltrate of lymphocytes, histiocytes, and plasma cells.

Liver. Degenerative changes were predominant, ranging from intracellular edema to vacuolar degeneration and sometimes necrotic changes of hepatocytes. Inflammatory changes were observed in the form of moderate, multifocal, periportal, lymphoplasmacytic hepatitis.

Lung. The most striking finding was chronic interstitial pneumonia characterized by hyperplasia of pneumonocytes of the type II, thickening of the alveolar walls, narrowing of the alveolar spaces, and the presence of a mononuclear infiltrate of lymphocytes, macrophages, fibroblasts, and myofibroblasts in the alveolar interstitium. The second inflammatory change was necrotic desquamative bronchiolitis, often with subsequent alveolar emphysema. Hyperemia, hemorrhage, and pulmonary edema were also noted.

Heart. Myodegeneration and mild multifocal lymphocytic myocarditis were noted as dominant findings.

Kidneys. Degenerative changes of tubulocytes-tubulonephrosis and sometimes necrotic changes of tubulocytes-tubular necrosis were the predominant findings. In addition, inflammatory changes in the form of multifocal mild to moderate chronic interstitial nephritis were also noted.

Spleen. Growth and developmental abnormalities (hyperplastic changes) were the predominant findings.

Brain. Neurodegeneration was most frequently noted, sometimes with subsequent satellitosis and gliosis.

According to the results of the Chi-square test, the number of hares with alterations in the liver, lungs, and kidneys was higher than the number of

Table 2. Involvement of changes in the organs of hares of different ages and significance level of differences between age groups.

Organ	Hares with changes (%)*		Fisher's exact test (p)
	Up to 1 year (younger)	Above 1 year (older)	
Intestines	14.63	15.00	>0.999
Liver	73.17	75.00	>0.999
Lungs	**85.37**	77.50	0.404
Heart	36.59	27.50	0.477
Kidney	63.41	**80.00**	0.139
Spleen	31.71	20.00	0.312
Brain	17.07	25.00	0.424

*The value of the most frequent change for each organ is bold.

Table 3. Participation (%) share of hares of different ages according to the type of organ changes*.

Organ	Age	Type of histopathological change** 1	2	3	4	5	1 & 2	1 & 4	2 & 4
Intestines	younger	–	–	–	**100.00**	–	–	–	–
	older	–	–	–	**100.00**	–	–	–	–
Liver	younger	**46.87**	–	–	15.63	–	–	37.50	–
	older	**41.38**	–	–	34.48	–	–	24.14	–
Lungs	younger	–	5.41	–	**75.67**	–	–	–	18.92
	older	–	–	–	**73.33**	–	3.33	–	23.33
Heart	younger	33.33	6.67	–	**53.33**	–	6.67	–	–
	older	27.27	27.27	–	**36.36**	–	9.09	–	–
Kidney	younger	**75.00**	–	–	10.71	–	–	14.29	–
	older	**67.74**	–	–	12.90	–	–	19.35	–
Spleen	younger	7.69	–	**69.23**	–	23.08	–	–	–
	older	12.50	–	**87.50**	–	–	–	–	–
Brain	younger	**87.50**	–	–	12.50	–	–	–	–
	older	**88.89**	11.11	–	–	–	–	–	–

*The value of the most frequent change for each organ is bold; **1 – degeneration; 2 – circulatory disturbance; 3 – growth and development disorders; 4 – inflammation; 5 – neoplastic changes.

individuals without alterations in these organs ($p \leq 0.001$). For the other organs (intestines, heart, spleen, and brain), the number of hares without changes is higher ($p < 0.001$) than those with changes.

Pathomorphological changes in relation to age (younger vs. older hares)

Histopathological changes were present in 97.56% of hares in the group up to one year old and in 92.50% of hares older than one year. Age had no influence on the occurrence of organ changes ($p = 0.092$).

In hares up to one year old, the most frequent changes occurred in the lungs, liver and kidneys (Table 2). In hares more than one year old changes in kidneys, lungs, and liver predominated, respectively. The majority of hares in both age groups had the same type of changes (Table 3).

Pathomorphological changes in relation to the elevation (hilly vs. plain terrain)

The elevation of the terrain did not affect the occurrence of changes in the organs ($p = 0.132$). The difference in the representation of hares with pathological changes from the terrain of different elevations is significant when it comes to lungs, heart and intestine ($p = 0.001$, $p < 0.01$, $p < 0.05$, respectively), Table 4.

Table 4. Participation of changes in the organs of hares from different terrains and level of significance of differences between the terrain.

Organ	Hares with changes (%)* Hilly terrain	Plain terrain	Fisher's exact test (p)**
Intestines	25.71	6.38	**0.024**
Liver	80.00	**70.21**	0.444
Lungs	**97.14**	70.21	**0.001**
Heart	48.57	19.15	**0.008**
Kidney	74.29	**70.21**	0.805
Spleen	25.71	24.53	>0.999
Brain	20.00	21.28	>0.999

*The value of the most frequent change for each organ is bold; **bolded p-values are statistically significant.

Histopathological changes in hares from hilly and plain terrain differed with respect to lungs, heart, spleen, and brain, whereas they were the same for intestine, liver, and kidneys (Table 5).

When studying the simultaneous effect of the age of the individual and the elevation of the terrain from which it originated, the influence of the terrain on the structure of changes in the lungs of hares older than one year ($p = 0.015$) and on the heart of younger hares up to one year ($p = 0.012$) was found.

Table 5. Participation (%) share of hares from different terrains by type of organ changes*.

Organ	Terrain	Type of histopathological change** 1	2	3	4	5	1 & 2	1 & 4	2 & 4
Intestines	Hilly	–	–	–	**100.00**	–	–	–	–
	Plain	–	–	–	**100.00**	–	–	–	–
Liver	Hilly	**42.86**	–	–	21.43	–	–	35.71	–
	Plain	**45.46**	–	–	27.27	–	–	27.27	–
Lungs	Hilly	–	2.94	–	**64.71**	–	2.94	–	29.41
	Plain	–	6.06	–	**81.82**	–	–	–	12.12
Heart	Hilly	35.29	11.77	–	**52.94**	–	–	–	–
	Plain	**33.33**	22.22	–	22.22	–	22.22	–	–
Kidney	Hilly	**76.92**	–	–	11.54	–	–	11.54	–
	Plain	**63.64**	–	–	15.15	–	–	21.21	–
Spleen	Hilly	–	–	**77.78**	–	22.22	–	–	–
	Plain	16.67	–	**75.00**	–	8.33	–	–	–
Brain	Hilly	**71.43**	14.29	–	14.29	–	–	–	–
	Plain	**100.00**	–	–	–	–	–	–	–

*The value of the most frequent change for each organ is bold; **1 – degeneration; 2 – circulatory disturbance; 3 – growth and development disorders; 4 – inflammation; 5 – neoplastic changes.

Pathomorphological changes depending on the distance to the TPP (near vs. far)

Proximity to thermal power plants in general had no effect on the occurrence of organ changes ($p = 0.092$), Table 6. The structure of the changes was affected by the proximity of the thermal power plant only in the liver ($\chi^2 = 7{,}776$; $p = 0.020$).

Table 6. Participation of changes in the organs of hares from terrains near and far from thermal power plant (TPP) and levels of significance of differences between the locations.

Organ	Hares with changes (%)* Near to TPP	Far from TPP	Fisher's exact test (*p*)
Intestines	7.69	17.86	0.322
Liver	61.54	80.36	0.102
Lungs	**80.77**	**82.14**	>0.999
Heart	19.23	37.50	0.128
Kidney	65.38	75.00	0.432
Spleen	19.23	28.57	0.426
Brain	15.38	23.21	0.562

*The value of the most frequent change for each organ is bold.

The pathological changes differ in hares from areas near or far from the thermal power plant with respect to the lungs, heart, spleen, and brain, whereas the same type of changes was found in the intestines, liver, and kidneys (Table 7).

Considering all factors studied, age of hares and proximity to thermal power plants had no effect on organ changes ($p > 0.05$), while elevation of the terrain had an effect on the type of changes in lungs ($p = 0.005$), intestine ($p = 0.012$) and heart ($p = 0.029$), and only in individuals from the field farther from thermal power plants.

Discussion

In a population with optimal reproductive dynamics, the percentage of young hares should be more than 65% (Beuković et al. 2013); the percentage of young hares in our study (50.62%) represents the lower limit of the 'good' population. This indicates difficulties in maintaining the ideal population structure due to lower reproductive rate or higher mortality. This status could be significantly influenced by climatic factors, as hares are particularly sensitive to adverse weather conditions, as well as to bacterial and parasitic infections caused and easily transmitted under certain meteorological conditions (Beuković et al. 2013). It should also be considered that air pollutants can harm wildlife through disruption of endocrine function, organ damage, increased susceptibility to stress and disease, decreased reproductive success, and possibly death (Newman 1975).

In our study, almost all hares had histopathological changes in the organs. Their occurrence of organ changes was not related to the factors studied when they were observed individually, but only together. All hares without pathological changes were from lowlands; considering their small number (4 individuals), Fisher's exact test showed that the elevation had no influence on the occurrence of organ changes. The lack of individual influence of proximity to the TPP could be due to the fact that a significant number of hares was from areas farther away from the thermal power plant.

The liver, kidneys, and lungs were the most affected organs in hares (Table 2). Inflammation was the predominant type of change in the intestines, lungs, and heart. In considering the possible impact of chemical pollution in the habitat, the most important are the degenerative changes found in the liver and kidneys and inflammatory changes in the kidneys and lungs. No evidence of pathogens was found in the microbiological examinations of the suspicious tissues. Changes due to the presence of parasites, detected by parasitological examination, are not considered.

Regarding the age structure, it is noticeable that changes in the lungs predominate in younger hares and in the kidneys in older hares (Table 2), but without significance between the age groups. The age of the hares and the elevation exerted a joint influence on the type of changes in the lungs of older hares and the heart of young hares. Changes in the lungs are mainly inflammatory in both older and younger hares (Table 1). Inflammation of the mucous membranes of the respiratory tract can be caused by

Table 7. Participation (%) share of hares from areas close and far from TPP by types of organ changes*.

Organ	Location	Type of histopathological change** 1	2	3	4	5	1 & 2	1 & 4	2 & 4
Intestines	Near	–	–	–	**100.00**	–	–	–	–
	Far	–	–	–	**100.00**	–	–	–	–
Liver	Near	25.00	–	–	**50.00**	–	–	25.00	–
	Far	**51.11**	–	–	15.56	–	–	33.33	–
Lungs	Near	–	–	–	**85.71**	–	–	–	14.29
	Far	–	6.52	–	**67.39**	–	2.17	–	23.91
Heart	Near	**40.00**	**40.00**	–	–	–	20.00	–	–
	Far	33.33	9.52	–	**52.38**	–	4.76	–	–
Kidney	Near	**70.59**	–	–	11.76	–	–	17.65	–
	Far	**71.43**	–	–	11.90	–	–	16.67	–
Spleen	Near	40.00	–	**60.00**	–	–	–	–	–
	Far	–	–	**81.25**	–	18.75	–	–	–
Brain	Near	**100.00**	–	–	–	–	–	–	–
	Far	**84.62**	7.69	–	7.69	–	–	–	–

*The value of the most frequent change for each organ is bold; **1 – degeneration; 2 – circulatory disturbance; 3 - growth and development disorders; 4 – inflammation; 5 - neoplastic changes.

various infectious and noninfectious agents, including chemical pollutants in the air (Newman 1975). Espinosa et al. (2020) found toxic-related lesions mainly in young animals during the autumn–winter period.

Sources of pollutant emissions to air can be various facilities for electricity and heat generation (TPPs), combustion in industry, small heating plants and individual stoves, fugitive emissions, traffic, waste, animal husbandry and other areas of agriculture, etc. (Knežević et al. 2020). In Serbia and some countries of the world, thermal power plants using solid fuels (coal) are still the dominant energy producers (Yadav and Prakash 2014; Matković and Kukolj 2020). In the period when our study was conducted, the habitats around the thermal power plants were in the zone with poor quality air (Figure 1); TPPs were the largest source of emissions of sulfur and nitrogen oxides in the air and, to a lesser extent, suspended particulate matters (Knežević et al. 2020). Nitrogen and sulfur oxides, together with carbon dioxide (CO_2), chlorofluorocarbons, and inorganic particles in the air, such as fly ash and soot, are the main components of emissions from coal combustion (Yadav and Prakash 2014). Particles in coal ash contain naturally occurring radioactive materials, polycyclic aromatic hydrocarbons, and heavy metals, including aluminum (Al), arsenic (As), iron (Fe), lead (Pb), cadmium (Cd) and mercury (Hg). Small spherical particles ≤10 µm in diameter (PM10), called 'fly ash', make up about 40-70% of the coal ash product (Hagemeyer et al. 2019). Coal ash can be reused for products such as cement, but the ash that is not recycled is stored in special landfills and ash ponds. The occurrence of respiratory symptoms and mortality is more frequent in people more exposed to fly ash components (Hagemeyer et al. 2019). During poisoning of hares with sulfur dioxide and fly ash in Czechoslovakia 1971, in regions with high sulfur dioxide and fly ash pollution, the ratio of one-year-old hares to adult hares was 30% lower than that observed in pollution-free areas (Newman 1979).

The toxic gasses of TPPs mainly reach the upper layers of the atmosphere. However, the harmful effects of air pollution can be observed at a great distance from its source (Newman 1979). Sulfur dioxide (SO_2) and nitrogen oxides (NO_x) form sulfuric and nitric acids that fall to the ground mixed with rain, snow, fog, or hail. Winds can blow clouds with SO_2 and NO_X over long distances (Grennfelt et al. 2020). Some SO_2 and NO_X are converted to sulfate and nitrate aerosols. In the presence of heat and sunlight, nitrogen oxide can react with methane, other volatile organic compounds, or carbon monoxide to form ozone. Sulfate and nitrate aerosols form gradually over tens (for ozone) to hundreds (for nitrates and sulfates) of kilometers from the source (IEA. 2016).

Ozone levels vary but increase with elevation (Chevalier et al. 2007), as do emissions of some other substances in polluted air. Exposure to ozone triggers bronchial inflammation and respiratory hypersensitivity, and long-term exposure is associated with increased risk of cardiovascular and respiratory mortality in humans (Kim et al. 2020). Studies in animals have shown that acute (1-3 h) low-dose O_3 exposure can cause inflammatory responses in the lungs, damage to epithelial airway tissues, and increased susceptibility to infectious diseases due to modulation of lung defenses (Filippidou and Koukouliata 2011).

All hares from our study with pathomorphological changes in tissues, especially the young ones, had changes in respiratory organs (Table 2). It could be assumed that hares in hilly terrain were more exposed to toxic substances from the air, because the inflammatory changes in the lungs were found in a statistically higher number of hares whose habitat was at a higher elevation (Table 4). The average elevation of the hilly terrain (234 m) is approximately equal to the height of the chimneys of the two TPPs. Another possible source of toxic aerosols which can harm the lungs is pesticides. After application to crops, pesticides may volatilize, or droplets of sprayed pesticides may adhere to dust particles and be carried by the wind to other areas, posing a potential hazard to wildlife. Repeated application during a growing season results in regular exposure and accumulation of pesticides in the body (Gryz and Krauze-Gryz 2022). Brown hares use agricultural fields shortly after pesticide application. Pesticide uptake can be very high, and uptake from overspray/oral grooming could be seven times higher than foraging (Mayer et al. 2020).

Pesticides are one of the possible causes of degenerative changes in the liver and kidneys, in addition to mycotoxins and various infectious agents (Poli et al. 1991). In our study, the structure of changes in the liver was under the influence of distance from the TPP, and changes in these organs were more frequent in hares from more distant hunting areas, probably because more animals were harvested in hunting areas far from the TPP. Liver and kidneys are the organs responsible for detoxification and elimination of various toxic substances from the body, such as Pb (Wajdzik et al. 2017). Degenerative changes found in our study (in the liver in the form of hepatosis of varying degrees and in the kidneys in the form of nephrosis of varying degrees, which has progressed somewhere to necrosis), are caused by toxic cell damage. Cd and Pb from contaminated feed caused degenerative changes in the kidneys of bank voles (Salińska et al. 2012). Massányi et al. (2003) found significantly higher concentrations of Cd in the liver as well as kidney in adult hares in comparison with juveniles, and Petrović et al. (2014) found increasing accumulation of Cd and Hg concentrations in the kidneys and liver of hares with age. This is consistent with our finding that the changes in kidneys and liver were found more frequently in older hares than in younger ones. Petrović et al. (2014) also considered the findings of Pb in the kidney and liver as the consequences of direct lead exposure in hare habitats due to proximity to roads and aerial deposition.

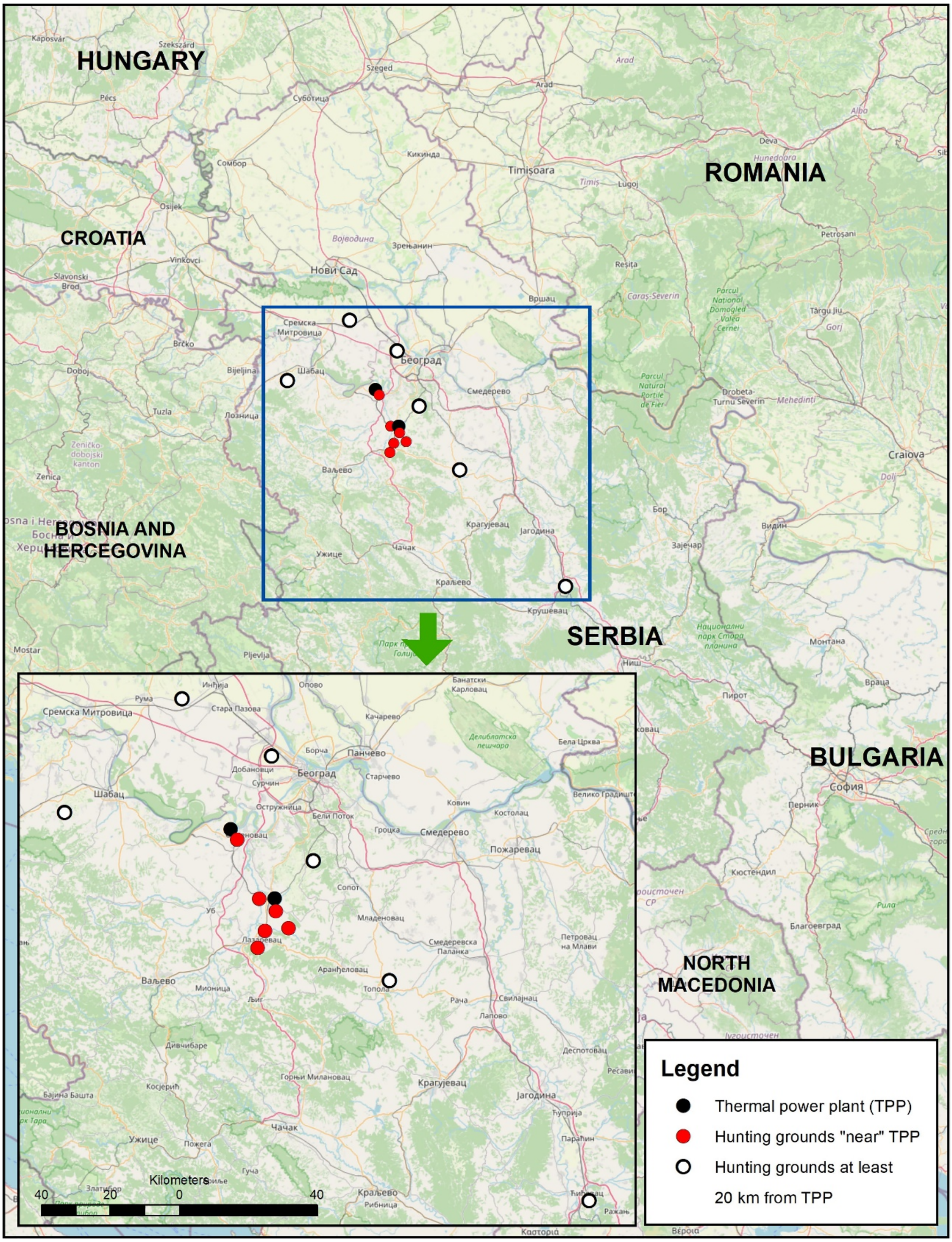

Figure 1. Location of hunting grounds in relation to thermal power plants: a large map shows the location of thermal power plants and hunting grounds in Serbia (framed part) in relation to neighboring countries; the small map represents an enlarged, framed part of the large map, with a scale that provides information about the distance between thermal power plants (TPP - black dots), hunting grounds near the TPP - red dots and hunting grounds located at a greater distance from the TPP - white dots (source of the map: https://www.openstreetmap.org/about).

Long-term Pb exposure in hares negatively affect their oxidative status and caused the strongest toxicity in brain and muscle, threatening their survival and/or population number (Linšak et al. 2022). In our study, the most common changes found in the brain of both age groups were degenerative changes (Table 1). They occurred, in statistically insignificant numbers, more frequently in older hares (Table 2). In a postmortem study, similar neuropathologic lesions were found in the prefrontal cortex of both children and dogs exposed to air pollution. Examination of animals exposed to air pollution shows the same pattern of neurotoxic effects (increased markers of oxidative stress and neuroinflammation, age-related susceptibility) as in humans, suggesting that animal studies may be useful predictors of human outcomes (Costa et al. 2014). Other components of fly ash, such as As and Hg, may also have neurotoxic effects (Zhang et al. 2022). Soil tests at one of the locations near the thermal power plant detected an elevated level of arsenic and nickel in the ash dump, which also can be found in vegetation used for recultivation (Simić et al. 2022).

The intestines of the hares in our study showed only inflammatory changes, and the feces inside the intestines had a pasty consistency. As a possible cause of these changes, apart from parasites, the microbiological examination of the feces excluded pathogenic microorganisms, so the finding was due to the action of one of the physical or chemical agents. Other studies provided the evidence of disturbance of the intestinal flora of hares with inflammation and the development of severe enteritis and changes in the intestinal mucosa, leading to diarrhoea, weight loss and death. It was considered that exposure to environmental contaminants might, beside pathogens, also contributing to the observed changes. In accordance with that, Hornek-Gausterer et al. (2021) found microplastics in the feces and intestine samples of hares.

The histopathological changes we found in the spleen could be the result of a viral infection (Abrantes et al. 2012).

Conclusions

Our study points out some of the mechanisms by which pollutants can reach the organism of hares and cause changes in their cells, i.e. organs. Toxic chemicals, by irritating the skin and mucous membranes of the respiratory, digestive, and genitourinary tracts, make the body's defenses less effective in contact with microorganisms and parasites in both animals and humans. Thus, when it comes to the changes in the organs as a result of exposure to infectious agents, it is possible that chemical pollutants were also involved in the development of the changes. Changes in the organs of animals can be analogous to those of humans living in the same area.

The results of this study show that the hares were exposed to pollutants to a high degree. Pollution from thermal power plants likely had a strong influence on the histopathological findings in the hares, perhaps even stronger than the results suggest. To determine the origin of pollution, a larger number of hares and additional analyzes are needed. That would provide better insight into the extent of health risk not only to hares but also to people living in rural areas and 'sharing' resources with wildlife.

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Authors declare that Ethical Commission approval was not needed due to the nature of the study.

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