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Elements and antioxidants in wild boar from northwestern Russia

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Abstract

To evaluate the nutritional status and the environmental exposure to toxic elements of the wild boar *Sus scrofa* L. ($n = 20$) from northwestern (NW) Russia, we determined the contents of the essential (Co, Cu, Fe, Mg, Mn, Ni, and Zn) and toxic (Cd and Pb) elements in the muscle, kidney, and liver. A second aim was to study the interactions between these elements and several antioxidants, namely, the activity of superoxide dismutase (SOD) and catalase, and the contents of glutathione (GSH), retinol, and α-tocopherol. A third aim was to assess whether the meat and offal of the wild boar are suitable for consumption or unsuitable due to the level of toxic elements. According to reference values of elements reported for domestic pigs, the wild boar from NW Russia was deficient in most of the essential elements (Co, Cu, Mn, Ni, and Zn) but had optimal values of Fe and Mg. The concentrations of Cd and Pb were lower than the values reported for pigs and wild boars living in heavily polluted areas. The correlations between antioxidants and elements could indicate that mineral balance in the body is regulated by antioxidants, among which the SOD activity, GSH, and retinol levels are the most sensitive parameters. Our assessment indicates that consumption of wild boar meat and liver, either rarely (4 times a year) or regularly (monthly), does not pose a health risk to adults and children, although wild boar kidney is not suitable for consumption.

Keywords Essential elements · Toxic elements · Antioxidants · Wild boar · Wildlife toxicology · Suitability for consumption

Introduction

Due to the increase of anthropogenic pressure on the environment, the exposure of wildlife to toxic elements is a major concern today. Some issues at the core of environmental studies include identifying the adverse effects on animals of an excess or deficiency of certain essential elements, including cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn), and others; identifying nonessential elements such as cadmium (Cd), lead (Pb), mercury (Hg), and others that contaminate the food chains;

and estimating the suitability of game meat and offal for human consumption (Kalisińska 2019).

Animals absorb both essential and toxic elements from their diet, and these elements then perform biological functions and/or accumulate in the tissues. Essential elements, such as Co, Cu, Fe, Mg, Mn, Zn, and Ni (which is possible but not proven to be essential), are required for many physiological processes, as they are the components of biological compounds such as enzymes, metalloproteins, and vitamins (Długaszek 2019). Toxic elements, such as Cd and Pb, are environmental contaminants that occur naturally and from anthropogenic activities. A deficiency of essential elements in body fluids and tissues of animals, as well as an excess of toxic metals, disrupts homeostasis, potentially leading to multiple disorders (Długaszek and Kopczyński 2013). To avoid metal-induced toxicity, most organisms possess detoxification pathways controlled through metal-binding proteins or metallothioneins. In addition, the elements interact in vivo. There are known and described relationships between such elements as Pb and Fe, Cu, and Zn; and between Cd and Zn, Fe, and Cu (in

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synergistic and antagonistic interactions) (Długaszek 2019). A well-known example of a synergistic association is the interaction between Cu and Zn, due to their ability to induce synthesis of metallothioneins and to their competition for metallothionein-binding sites (Bremner and Beattie 1995). In an example of an antagonistic interaction, sufficient Fe and Zn stores may reduce Cd absorption and accumulation and prevent or reduce the adverse actions of Cd (Groten et al. 1991; Brzóska and Moniuszko-Jakoniuk 2001).

The characteristic feature of metal toxicity is the disruption of enzyme systems and the induction of oxidative stress by directly or indirectly generating reactive oxygen species (ROS), which causes lipid and DNA damage (Ercal et al. 2001; Isaksson 2010). Redox-active metals (Fe, Cu) undergo redox cycling, whereas redox-inactive metals (Pb, Cd) deplete cells' major antioxidants, particularly thiol-containing antioxidants and enzymes (Ercal et al. 2001).

To maintain the delicate intracellular redox balance and minimize undesirable cellular damage caused by ROS, cells possess a complex of enzymatic and nonenzymatic antioxidants (Isaksson 2010; Espinoza-Diez et al. 2015). Antioxidant enzymes (AOE) include superoxide dismutases (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPx), etc. Such elements as Mn, Cu, and Zn are the cofactors for multiple enzymes including SODs. Cofactor for GPx, glutathione (GSH), is a first line of defense against metal cytotoxicity due to the high affinity of cysteine residues of this tripeptide with cations of such metals as Cd and Pb (Canesi et al. 1999).

Among low molecular-weight antioxidants, there are such lipid-soluble substances as retinol (one of the major forms of vitamin A) and α-tocopherol (the main and most active component of vitamin E). In rats, retinol pretreatment results in tolerance to Cd hepatotoxicity by inducing hepatic metallothionein (Sauer et al. 1997). Treating rats with vitamin E prevented and mitigated the effects of Pb exposure and toxicity, especially on renal structure and function (Alasia et al. 2020). Previously, Rodríguez-Estival et al. (2011) found changes in vitamin A and E status in the wild boar from a Pb-mined area. Animals showed significant reductions in liver retinyl stearate, increased free retinol levels, and a significant negative relationship between liver α-tocopherol and bone Pb. The interactions of antioxidants are presumed to be essential to counteract metal-mediated oxidative damage, but data on interactions between antioxidants and elements in wildlife are scarce (Rodríguez-Estival et al. 2011).

Among many game species, the wild boar is considered to be the most appropriate for use as a bioindicator, due to its large geographic distribution, feeding habits, relatively long life span, and easy sample collection, as they are regularly hunted (Danieli et al. 2012; Crnić et al. 2015). The abundance of wild boar throughout Europe makes it possible to compare the levels of elements in the tissues of this species from different regions. Our study area was chosen because northwestern (NW) Russia is the range border of the wild boar population, its status is currently not endangered, and just one of the NW regions of Russia, Karelia, has an estimated population of more than 1000 wild boars (Panchenko et al. 2019). As in many other European countries, the wild boar is often hunted for food in Russia, and about 300 wild boars are harvested in Karelia annually (State Report 2018, 2019). Furthermore, the wild boar is an omnivorous species: 80–90% of its diet includes food retrieved from the soil and includes cultivated plants, herbs, grasses, roots, the bark of trees and shrubs, berries, fungi, and worms (Danilkin 2002; Danieli et al. 2012; Ballari and Barrios-García 2014). This diet makes the wild boar an appropriate animal for the focus of studies on levels of pollutants in wildlife (Danieli et al. 2012).

Our study area covered the county of Karelia, Lakhdenpoh'ya District, which is located on the northwestern shore of Lake Ladoga. The leading industries of Lahdenpoh'ya district are timber and mining of decorative and building stone, limestone, gypsum, chalk, and shale. In addition, this district receives considerable quantities of contaminants from its neighbors, namely St. Petersburg (Russia) and Finland via long-distance transport (Slukovskii et al. 2020). Contaminants from these surrounding areas enter the study area environment through air and groundwater contamination that affects the soil and plants consumed by the wild boar. Moreover, acidification of the soil in NW Russia may increase the mobility of toxic elements, which migrate from soil to water sources, and to plants and to animals that consume herbs and worms (Medvedev 1999; Jones 2002). Despite the aforementioned sources of pollution, concentrations of such elements as Cd, Co, Mg, Mn, Ni, and Zn were lower than the permissible upper limit in the soil and forest floor of this region (Fedorets et al. 2008). However, the levels of Fe and Pb in the forest floor and the levels of Cu, Mn, Pb, and Zn in the green moss indicator species Bryidae have considerably exceeded the permissible upper limit (Fedorets et al. 2008).

Considering the feeding habits of the wild boar, which is commonly hunted as a food source, an assessment of the elements that are present in its meat and offal provides crucial safety information for consumers. Although wild game meat and offal might contain a high concentration of toxic elements (Rodríguez-Estival et al. 2011; Thomas et al. 2020), to date there is no regulatory limit established in the European Union (EU) for game meat and offal. European Food Safety Authority (EFSA) has estimated the tolerable weekly intake (TWI) value of Cd to be 2.5 µg/kg body weight (BW) (European Food Safety Authority 2009). To assess the risk to human health of the presence of Pb in food, EFSA endorses the "margin of exposure (MOE)" approach, as there is no evidence for a threshold for the critical endpoints, or exact

amount that leads to negative impacts on systolic blood pressure, kidney health (leading to chronic kidney disease), and intelligent quotient scores (European Food Safety Authority 2010). However, we decided also to compare the levels of the elements in game meat and offal with the EU limits set for domestic animals (cattle, pig, etc.) (Commission Regulation (EC) 2008).

This study helps fill a gap in knowledge, as there is only one study reporting the contents of elements in the tissues of wild boar from this region (Medvedev 1999). Because the concentrations of metals in wild animals may vary considerably from location to location, even within the same country (Bilandžić et al. 2009; Amici et al. 2012; Malmsten et al. 2021), we used another study area and identified concentrations of more elements, not just Cd and Pb, expanding beyond the findings of Medvedev (1999).

The aim of this work was threefold: first, to determine the contents of the essential (Co, Cu, Fe, Mg, Mn, Ni, and Zn) and toxic elements (Cd and Pb) in the muscle, kidney, and liver tissues of the wild boar *Sus scrofa* L. from NW Russia. The second aim was to study the interactions between these elements and several indicators of oxidative stress, namely, the activity of SOD and CAT, levels of GSH, retinol, and α-tocopherol. A third aim was to assess whether wild boar meat and offal are suitable for consumption or unsuitable due to the level of toxic elements.

Materials and methods

Animal and tissue sample collection

We took tissue samples from the kidney ($n = 20$), liver ($n = 19$), and hindlimb muscle ($n = 5$) from 20 wild boars (*Sus scrofa* L.) living and feeding in their natural habitats in the Republic of Karelia, NW Russia (Fig. 1). The wild boars
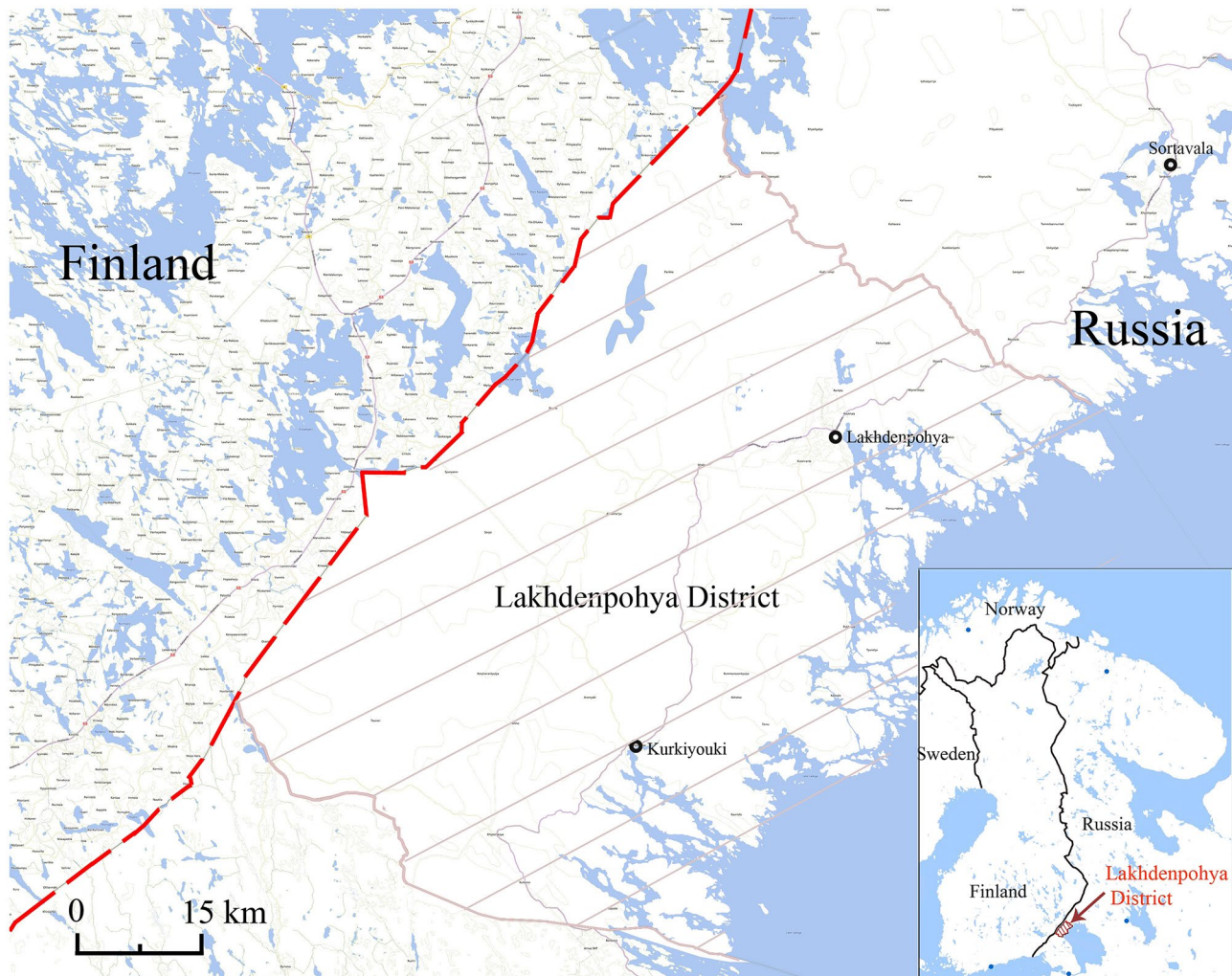

Fig. 1 Geographical position of the study area in northwestern Russia

were shot during the hunting season by hunters between August 2018 and December 2018. Lead exposure to animals may be high if they are shot using lead bullets and the contaminated tissues are not carefully discarded (Thomas et al. 2020). In our study, there was no contamination of kidney, liver, and hindlimb muscle with Pb because we analyzed samples from wild boars, which were shot in the neck, head, or shoulders. None of the collected tissues was damaged by the shot.

All the animals were in good general health before they were shot. We recorded the sex, age, and weight of each animal. There were 10 males and 10 females, with weights from 23 to 90 kg. Age was determined using tooth eruption and tooth replacement (Matshcke 1967). The animals were divided into juveniles (< 1 year), yearlings (1 to 2 years), and adults (> 2 years).

According to the recommendations for storage protocols for the best preservation of tissue (Moore et al. 2015), samples of kidney, liver, and muscle were removed from animals within the first 2 h after death. No samples were analyzed from animals that had been dead for more than 2 h. All samples were immediately placed on dry ice by hunters for transportation to the laboratory, where samples were stored at –80 °C awaiting analyses.

Analysis of elements

Before chemical analyses of Cd, Co, Cu, Fe, Mg, Mn, Ni, Zn, and Pb, the tissue samples were homogenized and 0.5–1 g of the samples was placed in PTFE vessels and mineralized for 30 min at 135 °C in Berghof Speedwave MWS four digestion system (Germany) by adding of a 10-mL mixture of extra-high-purity nitric and hydrochloric acids (4:1 v/v) (Vekton, Russia). After cooling, the final solution was diluted to 15 mL with double-distilled water (Double Distiller GFL 2102, Germany). Elements were measured by atomic absorption spectrometry (AA-7000 and AA-6800 Shimadzu, Japan) equipped with a graphite furnace. Palladium nitrate (Inorganic Ventures) was used as a matrix modifier. We made at least two replicate determinations for each sample. Calibration standards were prepared from certified commercially available multielement standards (Inorganic Ventures). The results are shown in micrograms per gram wet weight (w.w.) of studied tissues.

Determination of the antioxidant enzyme activities and protein content

To determine antioxidant enzyme activity and protein content, we homogenized tissue samples in 0.05 M phosphate buffer, pH 7.0, and centrifuged at $6000 \times g$ for 15 min. All samples were analyzed in triplicate.

The total SOD (EC 1.15.1.1) activity was measured by the adrenochrome method based on the spontaneous autoxidation of epinephrine with the formation of product with an absorbance peak at 480 nm (Misra and Fridovich 1972). This reaction depends on the presence of superoxide anions and is specifically inhibited by SOD. The amount of enzyme that caused 50% inhibition of epinephrine autoxidation is defined as 1 unit (U). SOD activity was expressed as U per mg protein after normalization with estimated total protein in milligrams in the respective tissues.

The CAT (EC 1.11.1.6) activity was evaluated by measuring the decrease in H_2O_2 concentration at 240 nm (Bears and Sizes 1952). One enzyme unit (IU) is defined as the amount of catalase capable of transforming 1.0 μmol of H_2O_2 for 1 min. Catalase activity was expressed as IU per mg protein after normalization with estimated total protein in milligrams in the respective tissues.

Results for antioxidant enzyme activities were standardized to total soluble protein content in tissue homogenates. Total tissue protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Determination of the glutathione level

To measure GSH, tissue samples were homogenized in 0.02 M EDTA and then centrifuged at $5000 \times g$ for 15 min. The GSH content was determined using the Ellman method in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid) (Sedlak and Lindsay 1968). The results were expressed in μmol GSH per gram of protein.

Determination of retinol and α-tocopherol contents

The contents of retinol and α-tocopherol in the tissues were determined by HPLC. Tissue samples were homogenized in 0.25 sucrose solution (pH 7.4) and then centrifuged at $3000 \times g$ for 10 min. Proteins in the samples were precipitated by ethanol. Retinol and α-tocopherol were extracted by n-hexane. Chromatographic separation was carried out by microcolumn chromatography with a UV detector with n-hexane and isopropanol as an eluent (98.5:1.5). The eluate was monitored at 292 nm for α-tocopherol and 324 nm for retinol, and the substances were identified by retention time compared with pure standards (MP Biomedicals, USA). Vitamin contents are given as milligram per gram of tissue.

Assessment of the suitability of meat and offal of wild boar as food

To assess the suitability of the meat and offal of wild boar for human consumption, we compared the data for the Cd and Pb content of wild boar meat and offal with regulatory limits

for Cd and Pb in food (Commission Regulation (EC) 2008). We also evaluated exposure to these toxic elements from wild boar meat and offal, using tolerable weekly intake (TWI) values for Cd and the margin of exposure (MOE) approach for Pb.

Comparison of data for Cd and Pb content of wild boar meat and offal with regulatory limits

European Union Regulations include the Minimum Risk Levels (MRLs) for Cd and Pb in meat (muscle) and offal (liver and kidney) of farmed animals destined for human consumption (Commission Regulation (EC) 2008). The permitted values of Cd are 0.05, 0.5, and 1.00 µg/g w.w. for muscle, liver, and kidney, respectively; the acceptable levels of Pb are 0.1 µg/g w.w. in muscle tissue, and 0.5 µg/g w.w. in the liver and kidney.

Evaluating the exposure to consumers of Cd and Pb from wild boar meat and offal

Due to the lack of reliable data on wild boar meat and offal consumption for NW Russia, the portion size of the meat was assumed to be 200 g for an adult and 100 g for a child (Pilarczyk et al. 2020). The portion of liver or kidney was estimated to be 138.4 g for an adult and 111.2 g for a child (Pilarczyk et al. 2020). The average weight of potential consumers was assumed to be as 60 kg for an adult and 23 kg for a child (European Food Safety Authority 2010; Pilarczyk et al. 2020). Different game meat and offal consumption frequencies were identified according to prior studies (Lazarus et al. 2014; Warenik-Bany et al. 2016; Gasparik et al. 2017; Pilarczyk et al. 2020) as four times a year (rare), once a month (regular), and once a week (often). Frequent consumptions would be most likely associated with hunters and their relatives.

Weekly exposure to Cd and daily exposure to Pb from meat and liver were estimated based on mean Cd or Pb level and the three consumption scenarios (rarely, regularly, and often). Weekly Cd exposure from game meat and offal consumption was expressed as the percentage of TWI (2.5 µg/kg BW) (European Food Safety Authority 2009) and termed "added weekly exposure" because we presumed the existence of other metal intake sources in addition to game consumption (Lazarus et al. 2014).

We estimated risk using Pb exposure levels as estimated daily intake (in µg/kg of BW per day) obtained for each scenario, and we applied the Margin of Exposure (MOE) approach endorsed by European Food Safety Authority (2010). MOE is calculated as the ratio between a defined point on the dose–response curve for the adverse effect (the benchmark dose lower confidence limit, BMDL) and estimated intake with food (MOE=BMDL/estimated intake). In the other words, MOE is a ratio of two factors: the dose at

which a small but measurable effect is first observed and the level of exposure to the substance considered. According to European Food Safety Authority (2010), for cardiovascular effects, the $BMDL_{01}$ value is 1.50 µg Pb/kg BW per day; for nephrotoxic effects, the $BMDL_{10}$ value is 0.63 µg Pb/kg BW per day; and for neurological effects, the $BMDL_{01}$ value is 0.50 µg Pb/kg of BW per day.

Statistical analysis

Statistical analysis was performed using Sigma-Stat 2.03 (SPSS Science Software Ltd., USA). The nonparametric Spearman rank correlation coefficient (r) was used to estimate correlations between the studied parameters that did not have a normal distribution, and the analysis of variance (ANOVA) was used to estimate differences between the samples. Statistical significance was assumed for values of $p < 0.05$.

Results

Results are presented in Tables 1, 2, 3, 4, 5, 6, and 7 and Fig. 2. Similar to the findings of other researchers (Gašparík et al. 2017), we found that sex did not influence the studied parameters (element concentrations, antioxidant and vitamin levels) (ANOVA: $p > 0.05$); hence, data for males and females were pooled in all subsequent analyses. Spearman rank correlation coefficients (r) were calculated to determine the relationship between age, weight, level of elements, antioxidant parameters, and vitamin levels in the tissues of the wild boar.

Concentrations of the elements and their correlations between tissues of the wild boar

The distribution of the elements differed between tissues (Table 1). Levels of Cd and Cu were highest in the kidney; Fe and Mn levels were highest in the liver, and the level of Zn was highest in the muscle, of the tissues we studied (ANOVA: $p < 0.05$). No significant differences in Co, Mg, Ni, or Pb levels were observed among the tissues we studied (ANOVA: $p > 0.05$). Age did not influence the element concentrations (ANOVA: $p > 0.05$) in the studied tissues.

We found a positive correlation between liver and kidney tissues for Co ($r = 0.92$, $p < 0.001$), Mg ($r = 0.50$, $p < 0.05$), and Pb ($r = 0.79$, $p < 0.001$) content. For other studied elements, no significant correlations were found between tissues.

To determine whether the exposure of the animals to Cd was chronic or acute, we tested the ratio of liver Cd to kidney Cd (Bilandžić et al. 2009). Chronic exposure to low levels of Cd in food leads to Cd accumulation in the kidneys, whereas

Table 1 Concentrations (µg/g w.w.) of elements in tissues of wild boar

Tissue	Elements	Geometric mean	Median	M ± SEM	Min	Max
Kidney (*n* = 20)	Cd	0.54	0.94	0.84 ± 0.12	0.020	2.225
	Co	0.05	0.05	0.08 ± 0.02	0.006	0.430
	Cu	4.87	5.19	5.11 ± 0.38	3.082	9.903
	Fe	85.85	81.72	96.64 ± 11.18	36.936	196.207
	Mg	235.42	238.95	239.02 ± 10.07	180.125	381.105
	Mn	1.11	1.36	1.27 ± 0.12	0.253	2.064
	Ni	0.07	0.05	0.14 ± 0.07	0.024	1.337
	Pb	0.13	0.12	0.18 ± 0.04	0.039	0.821
	Zn	14.53	14.50	14.95 ± 0.81	9.740	20.899
Liver (*n* = 19)	Cd	0.13	0.17	0.18 ± 0.02	0.006	0.437
	Co	0.06	0.06	0.11 ± 0.04	0.006	0.855
	Cu	2.86	3.41	3.15 ± 0.25	0.672	5.340
	Fe	200.03	253.71	249.09 ± 28.32	8.015	516.208
	Mg	242.10	245.62	246.63 ± 11.11	147.024	372.032
	Mn	1.64	1.87	2.07 ± 0.22	0.105	3.469
	Ni	0.07	0.06	0.12 ± 0.04	0.006	0.679
	Pb	0.14	0.12	0.23 ± 0.07	0.013	1.285
	Zn	23.18	25.14	25.73 ± 3.13	9.456	72.842
Muscle (*n* = 5)	Cd	0.07	0.09	0.08 ± 0.02	0.032	0.138
	Co	0.02	0.01	0.02 ± 0.01	0.009	0.058
	Cu	1.24	1.02	1.50 ± 0.54	0.750	3.644
	Fe	35.80	19.44	122.93 ± 104.68	15.400	541.651
	Mg	276.22	270.68	277.41 ± 13.34	255.966	329.117
	Mn	0.41	0.32	0.88 ± 0.56	0.095	3.170
	Ni	0.05	0.06	0.06 ± 0.01	0.023	0.094
	Pb	0.10	0.08	0.14 ± 0.06	0.047	0.389
	Zn	31.59	41.92	34.00 ± 5.69	15.259	44.532

M ± SEM mean ± standard error of the mean

a single acute exposure results in Cd accumulation in the liver (Jin et al. 2002). The liver Cd/kidney Cd ratio ranged from 0.0097 to 9.64 and was less than 1 in the majority of the samples (84.2%, or 16 of the 19 animals).

Antioxidant and vitamin levels in studied tissues of the wild boar

There were significant differences in the GSH level and CAT activity of tested tissues (ANOVA: $p < 0.01$), with the highest levels as follows (see Table 2): muscle > kidney > liver and kidney > liver > muscle, respectively. Retinol content was highest in the liver and lowest in the muscle among studied tissues (ANOVA: $p < 0.001$). No significant differences in SOD activity and α-tocopherol content were found between tissues of wild boar (ANOVA: $p > 0.05$). Age did not influence the antioxidant parameters and α-tocopherol level (ANOVA: $p > 0.05$) in the studied tissues, but it significantly influenced the retinol level in liver (ANOVA: df = 2, mean square = 477.93, F-ratio = 7.47, $p < 0.05$).

Table 2 Antioxidant parameters in the studied tissues of the wild boar

Antioxidant parameters, M ± SEM	Tissue		
	Kidney (*n* = 20)	Liver (*n* = 19)	Muscle (*n* = 5)
SOD activity, U/ mg protein	3.28 ± 0.31	3.20 ± 0.30	2.26 ± 0.28
CAT activity, U/ mg protein	14.61 ± 1.91	10.77 ± 1.42	1.14 ± 0.25
GSH level, µmol/mg protein	7.89 ± 0.64	5.83 ± 0.57	10.62 ± 0.57
Retinol content, mg/ g tissue	0.80 ± 0.10	21.75 ± 2.55	0.39 ± 0.09
α-tocopherol content, mg/ g tissue	1.95 ± 0.24	2.12 ± 0.24	0.88 ± 0.19

M ± SEM mean ± standard error of the mean

Table 3 Spearman rank correlations (*r*) between studied parameters in the kidney (*n* = 20) of the wild boar

	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn	SOD	Cat	GSH	E	A
Co	ns													
Cu	0.54*	ns												
Fe	0.49*	0.51*	0.47*											
Mg	ns	ns	ns	ns										
Mn	ns	0.60**	0.76**	0.54*	ns									
Ni	ns	0.55*	ns	ns	ns	0.52*								
Pb	ns	0.71**	ns	ns	ns	ns	ns							
Zn	ns	ns	ns	ns	ns	ns	ns	ns						
SOD	ns	ns	ns	ns	ns	ns	ns	ns	-0.58*					
Cat	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns				
GSH	ns	ns	ns	-0.49*	ns	ns	ns	-0.56*	ns	ns	ns			
E	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
A	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.52*	ns	-0.49*	ns	
Age	ns	ns	ns	ns	ns	-0.45*	ns	ns	ns	ns	ns	ns	ns	ns
Weight	ns	ns	ns	ns	ns	ns	ns	ns	-0.54*	ns	ns	ns	ns	ns

E α-tocopherol, *A* retinol, *ns* not significant

$^{*}p$ <0.05; $^{***}p$ <0.01

Correlations between studied parameters

A high positive correlation was found between age and weight in the wild boar (*r* = 0.92, *p* < 0.001). In the muscle, strong negative correlation (*r* = 0.99, *p* < 0.001) was found between Fe level and SOD activity. In the kidney, strong positive correlations (*r* > 0.6) were found between Co and Pb, and between Cu and Mn (Table 3). Renal Mn level was negatively correlated with age, and renal Zn level was negatively correlated with the overall weight of the animal. SOD activity was negatively correlated with both Zn and retinol levels, GSH level was negatively correlated with Fe, Pb, and retinol levels in the kidney.

In the liver, strong positive correlations were found between Cd and Co, between Co and Cu, and between Cu and Mn (Table 4). The hepatic concentration of Mn was moderately negatively correlated with both age and weight of animals. Cu level was moderately negatively correlated with the weight of animals. Liver retinol content was positively correlated with both age and weight of

Table 4 Spearman rank correlations (*r*) between studied parameters in the liver (*n* = 19) of the wild boar

	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb	Zn	SOD	Cat	GSH	E	A
Co	0.80***													
Cu	0.54*	0.63**												
Fe	ns	ns	ns											
Mg	ns	ns	ns	ns										
Mn	ns	0.48*	0.66**	ns	ns									
Ni	ns	ns	ns	ns	0.61**	ns								
Pb	ns	0.50*	ns	ns	ns	ns	0.52*							
Zn	ns	ns	ns	ns	ns	ns	ns	ns						
SOD	ns	ns	ns	ns	ns	ns	ns	ns	ns					
Cat	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns				
GSH	ns	ns	ns	-0.64**	ns	ns	ns	ns	ns	ns	0.50*			
E	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
A	ns	ns	ns	ns	-0.57*	ns	ns	ns	ns	ns	ns	ns	ns	
Age	ns	ns	ns	ns	ns	-0.55*	ns	ns	ns	ns	ns	ns	ns	0.54*
Weight	ns	ns	-0.54*	ns	ns	-0.59*	ns	ns	ns	ns	ns	ns	ns	0.52*

E α-tocopherol, *A* retinol, *ns* not significant

$^{*}p$ <0.05; $^{***}p$ <0.01

Table 5 The percentage (%) of the wild boar tissue samples containing various concentrations of the elements compared with the reference values (bold) of the elements for domestic pigs (Puls 1994; WVDL 2015)

Element	Tissue	Reference values for pigs, µg/g w.w				
		Deficient	Marginal	Optimal	High	Toxic
Cd	Kidney			**0.15–1.0**	**2.0–50**	**>270**
				95% (n=19)	5% (n=1)	
	Liver			**0.04–0.5**	**3–30**	**>13**
				100% (n=19)		
Co	Kidney	**<0.4**		**0.4**	**10–17**	**25–40**
		95% (n=19)		5% (n=1)		
	Liver	**<1**		**1–2**	**6–8**	**10–20**
		100% (n=19)				
Cu	Kidney	**2–4**	**4–7**	**7–10**	**12–25**	**30–1200**
		35% (n=7)	50% (n=10)	15% (n=3)		
		0.3–1.02	**4–7**	**5–25**	**15–200**	**150–15,000**
		10.5% (n=2)	84% (n=16)	5% (n=1)		
Fe	Kidney	**<50**		**50–100**	**>100**	
		15% (n=3)		55% (n=11)	30% (n=6)	
	Liver	**30–50**		**100–200**		**>400**
		5.2% (n=1)		79% (n=15)		15.8% (n=3)
Mg	Kidney			**140–180**	**>180**	
				5% (n=1)	95% (n=19)	
	Liver			**150–200**	**>200**	
				16% (n=3)	84% (n=16)	
Mn	Kidney	**0.35–0.45**	**0.75–1.13**	**1.3–2.0**	**2–3**	
		20% (n=4)	20% (n=4)	60% (n=12)		
	Liver	**0.53–0.97**	**2.8–3.1**	**3.3–4.0**	**4–5**	
		10% (n=2)	74% (n=14)	16% (n=3)		
Ni	Kidney	**<0.15**	**0.15–0.2**	**0.25–1.0**	**>1.0**	
		90% (n=18)		5% (n=1)	5% (n=1)	
	Liver	**<0.08**	**0.08–0.1**	**0.1–0.2**	**>0.25**	
		63% (n=12)		26% (n=5)	11% (n=2)	
Pb	Kidney, liver			**<5**	**5–25**	
				100% (kidney n=20, liver n=19)		
Zn	Kidney	**<15**		**15–30**		**190–370**
		45% (n=9)		55% (n=11)		
	Liver	**9.6–25**	**25–35**	**40–90**	**>200**	**500–3100**
		47% (n=9)	42% (n=8)	11% (n=2)		
	Muscle	**<21**		**21–24**	**>24**	
		20% (n=1)			80% (n=4)	

animals, and it was negatively correlated with liver Mg level. GSH content was positively correlated with CAT activity and negatively correlated with Fe level in the liver.

Comparison of data for element content of wild boar kidney and liver with reference values for domestic pigs

According to the reference values of the elements reported by Puls (1994) and the Wisconsin Veterinary Diagnostic Laboratory (WVDL 2015) for pigs (Table 5), the levels of Cd, Fe, and Pb in wild boar kidney and liver tissues fell into normal ranges for the majority of our samples. Concentrations of Co, Cu, and Ni in most of the wild boar tissue samples were lower than optimal values for domestic pigs. The level of Mg in wild boar tissues was high compared to reference values for domestic pigs. Tissue levels of Mn were defined as deficient, marginal, or adequate in kidney samples (20%, 20%, and 60%, respectively) and liver samples (10%, 74%, and 16%, respectively). The level of Zn fell into normal ranges in 55% of the kidney samples

Table 6 Cadmium exposure estimations for consumers of meat, kidney, and liver of the wild boar from NW Russia

Tissue, n	Added weekly exposure to Cd as % TWI by:					
	Rare consumption (4 times a year)		Regular consumption (once a month)		Often consumption (once a week)	
	Adult	Child	Adult	Child	Adult	Child
Kidney (n = 20)	5.97	12.51	17.90	37.52	77.87	163.22
Liver (n = 19)	1.24	2.61	3.73	7.81	16.22	33.99
Muscle (meat, n = 5)	0.85	1.11	2.54	3.32	11.07	14.43

Calculations were made for mean Cd levels in wild boar tissues, taken from Table 1

TWI tolerable weekly intake

but was deficient or marginal in most of the liver samples. Overall, the majority of the elements we measured in wild boar tissues showed values similar to the values for the domestic pigs, with the notable exceptions of high levels of Mg, and low levels of Mn, Co, Cu, Ni, and Zn.

Assessment of suitability of meat and offal of wild boar as food

Comparison of data for Cd and Pb content of wild boar meat and offal with regulatory limits for Cd and Pb in food

European Union Regulations include the Minimum Risk Levels (MRLs) for Cd and Pb in meat (muscle) and offal (liver and kidney) of farmed animals destined for human consumption (Commission Regulation (EC) 2008). Figure 2 shows the percentage of the wild boar tissue samples exceeding MRL for Cd and Pb. In the case of Cd, levels exceeding permissible values were observed in 80% of the muscle samples and 35% of the kidney samples. Liver tissue contained a Cd concentration lower than the maximal allowed value. Concentrations of Pb exceeding permissible values for kidney, liver, and muscle were detected in 5%, 10%, and 40% of samples, respectively.

Evaluating the exposure to Cd and Pb from wild boar meat and offal

Calculations of the added weekly exposure to Cd as % TWI for the three consumption scenarios (rarely, regularly, and often) are presented in Table 6. The highest values were observed for the worst-case scenario (often consumption) for adults and children in the kidney. Moreover, in the case of children consuming wild boar kidney often, the estimated weekly exposure to Cd exceeded the TWI. Muscle and liver were the tissues most relevant for consumption on the rare and regular bases, given the 0.85–7.81% TWI of Cd. However, weekly consumption of wild boar meat and liver could significantly contribute to exposure to Cd (11.07–33.99% TWI). Children had greater exposure to Cd than adults in all consumption scenarios.

The estimated daily intake of Pb is presented in Table S3, and the calculation of MOE for the three consumption scenarios (rarely, regularly, and often) is presented in Table 7. According to EFSA's data, a MOE value of 10 or greater ensures that there is no noticeable risk of relevant toxic effects. If MOE is equal to or slightly higher than 1, it is understood that there may be a low risk; with MOE lower than 1, there is a higher possibility of negative effects on consumers (European Food Safety Authority 2010). Mean MOE values equal to or greater than 10 were observed for adults and children in all the studied tissues when rare and regular consumed. In general, mean MOE values were lower than 10 when adults and children consumed wild boar meat and offal often. The lowest MOE values (< 1) were observed when children consumed wild boar often. In this case, there is a risk of nephrotoxic and neurological effects from consuming the kidney and liver tissues.

Discussion

Determining the levels of essential and nonessential elements in the tissues of the wild boar allows us to evaluate its nutritional status, i.e., to assess the deficiency or excess of these elements and thereby determine the environmental exposure of the wild boar to beneficial and toxic elements. Understanding the interactions between the studied elements and antioxidants allows us to evaluate the importance of each parameter and understand how the elements are metabolized by the wild boar. The measurements in this study and the explanation of the correlations of the elements will help determine the possible threat to humans of consuming the meat and offal of the wild boar inhabiting NW Russia.

Nutritional status and environmental exposure of wild boar from NW Russia

There are no published reference values for the elements found in wild boar tissues, so we compared our data with the reference values reported for domestic pigs (Puls 1994; WVDL 2015). The values for pigs might not be identical to

Table 7 Estimated MOEs of Pb for different endpoints by the intensity of consumption of wild boar meat and offal

Tissue, n	Endpoints	Mean MOEs of Pb (min–max)					
		Rare consumption (4 times a year)		Regular consumption (once a month)		Often consumption (once a week)	
		Adult	Child	Adult	Child	Adult	Child
Kidney (n=20)	Cardiovascular effects	333.33 (72.46 – 1500.00)	159.57 (34.48 – 750.00)	111.94 (24.12 – 517.24)	53.38 (11.49 – 245.90)	25.73 (5.55 – 118.11)	12.27 (2.65 – 56.18)
	Nephrotoxic effects	140.00 (23.33 – 630.00)	67.02 (14.48 – 315.00)	47.01 (10.13 – 217.24)	22.42 (4.83 – 103.28)	10.81 (2.33 – 49.61)	5.16 (1.11 – 23.60)
	Neurological effects	111.11 (24.15 – 500.00)	53.19 (11.49 – 250.00)	37.31 (8.04 – 172.41)	17.79 (3.83 – 81.97)	8.58 (1.85 – 39.37)	4.09 (0.88 – 18.73)
Liver (n=19)	Cardiovascular effects	258.62 (46.15 – 5000.00)	122.95 (22.03 – 214.29)	86.21 (15.40 – 1500.00)	41.10 (7.35 – 714.29)	19.84 (3.54 – 348.84)	9.46 (1.69 – 166.67)
	Nephrotoxic effects	108.62 (19.38 – 2100.00)	51.64 (9.25 – 90.00)	36.21 (6.47 – 630.00)	17.26 (3.09 – 300.00)	8.33 (1.49 – 146.51)	3.97 (0.71 – 70.00)
	Neurological effects	86.21 (15.38 – 1666.67)	40.98 (7.34 – 71.43)	28.74 (5.13 – 500.00)	13.70 (2.45 – 238.10)	6.61 (1.18 – 116.28)	3.15 (0.56 – 55.56)
Muscle (meat, n=5)	Cardiovascular effects	294.12 (105.63 – 882.35)	223.88 (81.08 – 681.81)	97.40 (35.21 – 294.12)	75.00 (27.03 – 223.88)	22.46 (8.12 – 67.26)	17.22 (6.22 – 51.55)
	Nephrotoxic effects	123.53 (44.37 – 370.59)	94.03 (34.05 – 286.36)	40.91 (14.79 – 123.53)	31.50 (11.35 – 94.03)	9.43 (3.41 – 28.25)	7.23 (2.61 – 21.65)
	Neurological effects	98.04 (35.21 – 294.12)	74.63 (27.03 – 227.27)	32.47 (11.74 – 98.04)	25.00 (9.01 – 74.63)	7.49 (2.70 – 22.42)	5.74 (2.07 – 17.18)

Calculations were made for estimated Pb intake values by the intensity of consumption of wild boar meat and offal, taken from Table S3

For cardiovascular effects, the MOE was calculated by dividing the $BMDL_{01}$ intake value of 1.50 μg/kg BW per day by the respective daily intake taken from Table S3

For nephrotoxicity, the MOE was calculated by dividing the $BMDL_{10}$ intake value of 0.63 μg/kg BW per day by the respective daily intake taken from Table S3

For neurological effects, the MOE was calculated by dividing the $BMDL_{01}$ intake value of 0.50 μg/kg BW per day by the respective daily intake taken from Table S3
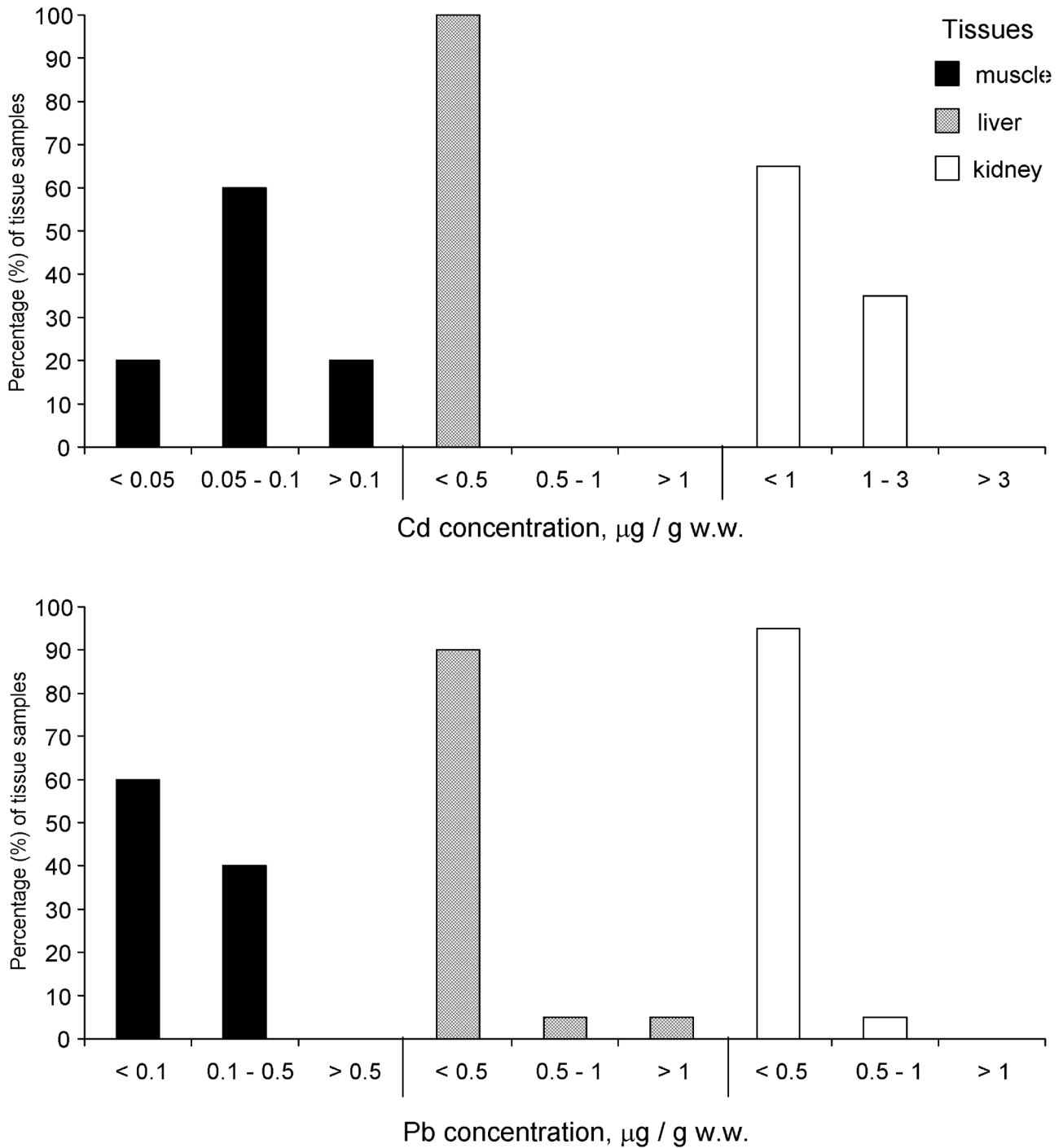
MOE margin of exposure

Fig. 2 The percentage (%) of the wild boar tissue samples containing various Cd and Pb concentrations

elements present in wild boar tissues, but a comparison can give some indication if the levels of the elements are high or low. According to Puls (1994) and WVDL (2015), the wild boar from NW Russia was deficient in most of the tested essential elements (Co, Cu, Mn, Ni, and Zn), although the values of some essential elements (Fe and Mg) and toxic elements (Cd and Pb) fell into normal ranges reported for pigs.

Essential elements in wild boar tissues

The concentration of **Cu** in the liver is considered to be a good indicator of its reserves in the animal (Reglero et al. 2008) because the hepatocyte is crucial in the control of Cu homeostasis (De Romaña et al. 2011). However, in our study, Cu levels were deficient or marginal in both liver and

kidney tissues for most of the animals. Compared to other studies on wild boar from different countries and geographic regions, we detected intermediate to low levels of Cu in the animals from NW Russia (Amici et al. 2012; Długaszek and Kopczyński 2013; Gašparík et al. 2017; Dannenberg et al. 2013; Pilarczyk et al. 2020; Malmsten et al. 2021). In mammals, Cu deficiency causes anemia, loss of appetite, bone deformities, cardiovascular hypertrophy, and other negative effects (Shields et al. 1962). The Cu deficiency in wild boar from NW Russia could be due to the inadequate Cu level in its food (plants, herbs, grasses, roots, etc.), which is dependent on the level of bioavailable Cu in the soil, in keeping with the findings of Fedorets et al. (2008), who reported a low level of Cu in soils of our study area (NW Russia). In our study, Cu was positively correlated with Cd, Fe, and Mn, as it was shown previously on cattle (Alonso et al. 2004).

The **Zn** level was highest in the muscle tissue of wild boar compared to levels in other studied tissues. This is probably due to the important role of Zn in the development of muscles (Baltaci et al. 2018). In our study, 80% of the muscles samples contained high Zn levels as compared to the reference values for pigs. A total of 55% of the kidney samples and only 11% of the liver samples contained optimal levels of Zn. The remaining liver samples showed deficient or marginal levels of Zn. Compared to reports from other countries (Amici et al. 2012; Długaszek and Kopczyński 2013; Dannenberg et al. 2013; Gašparík et al. 2017; Pilarczyk et al. 2020; Malmsten et al. 2021), the levels of Zn in wild boar from NW Russia were intermediate to low.

In animals, Zn deficiency results in many pathological changes, including growth retardation, increased susceptibility to infection and damage induced by oxidative stress, etc. (Dardenne 2002; Kalisińska 2019). In our study, Zn level was negatively correlated with one of the antioxidant parameters, SOD activity, in the kidney. Factors affecting the Zn absorption from the gut include Zn and protein levels in the diet, which have a positive effect on this process, and the presence of Cd, Co, Cu, Fe, or Ni, which impairs Zn absorption (Baltaci et al. 2018). However, we found no correlation between Zn and other studied elements in the tissues of wild boar.

Mn is mainly found in low levels in all types of animal diets (Kalisińska 2019). After absorption from the gut, Mn is efficiently accumulated in the liver, where it is metabolized. In our study, the Mn level was adequate in 16% of the liver samples and 60% of the kidney samples, and it was either deficient or marginal in the other tissue samples. Kalisińska (2019) suggests that the natural demand for Mn is lower in the domestic pig's ancestor than in modern intensively fattened farm animals. Dietary Mn deficiency can result in growth disorders and neurological disorders, skeletal deformities, and glucose intolerance (Erikson et al. 2005). In our study, liver Mn level was negatively correlated with

the weight of the animals. Our observations also suggest that the level of Mn in the kidney and liver may decrease with increasing age. Previously, Parmalee and Aschner (2016) suggested that exposure to Mn may be related to some of the mechanisms of aging: neurogenesis, oxidative stress, and microglial activation and inflammation. This idea requires further study.

We found **Co** deficiency and possible related changes in Ni and Fe status in wild boar from NW Russia. Cobalt is an active center of cobalamin (vitamin B_{12}), which is synthesized in microorganisms and enters the food chain of humans and other mammals through incorporation into the food of animal origin. After absorption, Co is distributed to all tissues, but mainly to the liver and kidney (Jorhem et al. 1989; Pilarczyk et al. 2020). In our study, we found significant associations between hepatic and renal concentrations of Co.

In the wild boar from NW Russia, the mean kidney and liver Co levels were higher than those for Swedish domestic pigs (Jorhem et al. 1989), lower than the levels noted in prior studies on wild boar (Gašparík et al. 2017; Pilarczyk et al. 2020), and similar to optimal values reported for domestic pigs (Puls 1994). In our study, Co level was deficient or marginal in 95% of kidney samples and 100% of liver samples. In mammals and humans, Co deficiency leads to cobalamin deficiency, and, therefore, to megaloblastic anemia and neurological dysfunction.

Vitamin B_{12} deficiency is associated with diminished serum and liver concentrations of vitamin B_{12} and folate, as well as an accumulation of Fe in the liver, and hyperhomocysteinemia (Stangl et al. 2000). A few studies have demonstrated that experimentally induced vitamin B_{12} deficiency can change the metabolism of such trace elements as Fe, Cu, and Ni in rats and cattle (Brown and Strain 1990; Stangl et al. 2000). Additionally, Co had significant positive relationships with essential elements (Fe, Mn, and Ni in the kidney; Cu, and Mn in the liver) as well as nonessential elements (Pb in the kidney; Cd in the liver). Similar associations between elements were reported by Alonso et al. (2004) in the cattle from NW Spain and by Gasparik et al. (2012) in the wild boar from western Slovakia.

Fe has an essential role in transporting oxygen in the body, and it is involved in many enzymatic reactions. A deficient or excessive amount of iron in the body can cause a broad spectrum of diseases with diverse clinical manifestations, ranging from anemia to iron overload, and possibly to neurodegenerative diseases (Kalisińska 2019). Despite the prevalence of plants in the diet of the wild boar (80–90% of the diet) (Danilkin 2002; Danieli et al. 2012; Ballari and Barrios-García 2014), the animals can accumulate high amounts of Fe: 16% of the liver samples contained toxic levels for pigs (> 400 µg/g w.w.). However, the majority of the wild boars had an adequate Fe level in their tissues. Our data on Fe content in wild boar from NW Russia had similar to or

higher values than reports of levels in wild boar from other countries (Skobrak et al. 2010; Długaszek and Kopczyński 2013; Dannenberg et al. 2013; Babicz and Kasprzyk 2019; Pilarczyk et al. 2020; Malmsten et al. 2021).

Ni is considered to be a possibly essential element according to the National Research Council (NRC 2005). Although no specific biochemical role for Ni has been identified in mammals (Genchi et al. 2020), signs of Ni deficiency have been reported in miniature pigs, lambs, and goats, including reduced growth rate and increased mortality (Spears 1984). Nielsen et al. (1989) suggested that Ni has a biological function in a metabolic pathway in which vitamin B_{12} is important. According to the reference values of the elements reported for domestic pigs (Puls 1994; WVDL 2015), Ni levels were deficient in the wild boar from NW Russia in 90% of the kidney samples and 63% of the liver samples. Compared to a report from Ukraine (Pilarczyk et al. 2020), our study showed a wider range of Ni levels in tissues.

Another essential element analyzed in this study was **Mg**, which is required for protein metabolism, and which performs specific actions in the neuromuscular and cardiovascular system. Compared to the reference values for pigs (Puls 1994; WVDL 2015), tissues of the wild boar from NW Russia had high levels of Mg. The lower Mg content in the muscles of wild boar compared to values in domestic pigs may result from the low content of this element in the diet. Compared to other studies on wild boar from different countries (Długaszek and Kopczyński 2013; Malmsten et al. 2021), the Mg levels in the animals from NW Russia were intermediate.

Nonessential toxic elements in wild boar tissues

To study the environmental exposure of the wild boar to toxic elements, we determined the content of such nonessential elements as **Cd** and **Pb** in animal tissues. The kidney is essential for the excretion of toxins from the organism, and Cd and Pb are primarily accumulated in this organ. In our study, as in others (Sedki et al. 2003; Pilarczyk et al. 2020), the mean concentration of Cd was found to be the highest in the kidney compared to values in other tissues. Unlike concentrations of Cd, the concentrations of Pb were not significantly different among the tissues we tested, although we found a high positive correlation between hepatic and renal contents of Pb. We did not observe any correlation between the concentration of Cd and age or between Pb and age of the animals.

Renal Cd concentrations had a greater range (0.02–2.23 µg/g w.w.) than optimal values (0.15–1.0 µg/g w.w.) reported for pigs (Puls 1994; WVDL 2015), but the levels did not reach toxic status (>270 µg/g w.w.). The levels of Pb did not exceed optimal values reported for pigs (Puls 1994; WVDL 2015) in any of the kidney and liver samples in this study. The estimated ratio of liver Cd to kidney Cd

indicates that most of the tested animals were chronically exposed to low levels of Cd (Bilandžić et al. 2009). Our data on the level of toxic elements in the tissues of the wild boar from NW Russia were in agreement with most prior reports from other regions and countries (Medvedev 1999; Amici et al. 2012; Danieli et al. 2012; Pb values in Gašparík et al. 2017; Cd values in Pilarczyk et al. 2020; Pb values in Malmsten et al. 2021), but our study showed lower values compared to some reports from uncontaminated areas (Cd values in Długaszek and Kopczyński 2013; Cd values in Gašparík et al. 2017; Pb values in Pilarczyk et al. 2020; Cd values in Malmsten et al. 2021) as well as reports from highly polluted areas (Bilandžić et al. 2009; Chiari et al. 2015; Durkalec et al. 2015).

Most previous studies reported that Cd causes a disruption in Cu and Zn homeostasis (Doyle and Pfander 1975; Sedki et al. 2003; Alonso et al. 2004). In our study, Cd was positively correlated with Cu in both kidney and liver, as it was reported in cattle (Alonso et al. 2004). In addition, Pb was positively correlated with Co in both kidney and liver, and with Ni in the liver. Długaszek and Kopczyński (2011) also reported a positive correlation between Pb and Ni in the liver of wild boar from Poland.

Antioxidant status of wild boar from NW Russia

The activation of an organism's antioxidant defense mechanisms depends on the environment, and is a result of many factors including environmental pollution. Although previous studies revealed changes in antioxidant activity in response to Cd- or Pb-induced oxidative stress (Rodríguez-Estival et al. 2011; Wieloch et al. 2012), in our study, the Cd and Pb concentrations in wild boar tissues did not reach values considered toxic for pigs. We found a few moderately strong correlations between studied elements and antioxidants in wild boar tissues. SOD activity, retinol, and low-molecular thiol, GSH, were correlated with studied parameters more often than others. For example, SOD activity was negatively correlated with Zn in the kidney, and with Fe in the muscle. Retinol level was negatively correlated with Mg in the liver. GSH level was negatively correlated with Fe in the kidney and liver, and with Pb in the kidney.

The reduced form of glutathione (GSH) and its associated enzymes are considered to be the most reliable biomarkers of oxidative stress, which underlies the toxicity of anthropogenic pollution (Isaksson 2010). It is well known that toxic elements inhibit antioxidant enzyme activity and cause lipid peroxidation (Casalino et al. 2002), but we did not find any correlations between Cd and antioxidants. However, GSH level was negatively correlated with Pb in the kidney. Rodríguez-Estival et al. (2011) found an increase in GSH content in the liver of wild boar from a highly polluted area, which may suggest that GSH synthesis was being induced by Pb-driven oxidative stress.

Wieloch et al. (2012) consider that the increase of SOD activity in blood is one of the defense mechanisms of an organism against oxidative stress caused by environmental factors, whereas non-enzymatic mechanisms are the main antioxidant defense system concerning Pb concentrations in humans from an unpolluted area. Our study showed significant inverse relationships between Fe and SOD activity in muscle tissue, and between Fe and GSH levels in the kidney and liver. It is well known that Fe is an element with redox properties and that it is an initiator of lipid peroxidation through generating hydroxyl radicals via the Fenton reaction, and through creating lipid peroxyl radicals via decomposing lipid hydroperoxides (Wieloch et al. 2012; Shi et al. 2021). The thiol group of GSH is a binding site for many metals, including Fe, which blocks the formation of a hydroxyl radical (Koivula and Eeva 2010). So, our results are in an agreement with the important role of the GSH redox system in the homeostasis of Fe as identified in prior studies (Shi et al. 2021).

Such antioxidant enzymes as SODs and CAT are among the so-called triad of enzymes. SODs catalyze the dismutation of the extremely toxic superoxide radical into potentially less toxic hydrogen peroxide; CAT suppresses the formation of hydroxyl radicals by converting hydrogen peroxide to oxygen and water; glutathione peroxidase (GPx) metabolizes hydrogen peroxide and lipid hydroperoxides. In our study, CAT activity was positively correlated with GSH content in the liver, which confirms these relationships between antioxidants. Another group of antioxidants that we studied included α-tocopherol and retinol. No significant correlation between α-tocopherol and other studied parameters was found. However, other studies suggest that α-tocopherol may act as an up- or downregulator of certain antioxidant enzymes (Vertuani et al. 2004) and that it plays a protective role in terms of vitamin A stores (Rodríguez-Estival et al. 2011).

Age and weight of the wild boar were significant factors influencing retinol content in its liver tissue. Similar results were obtained previously in several species of birds and mammals (Majchrzak et al. 2006). The retinol level was negatively correlated with Mg in the liver in the wild boar muscle tissue. Previous studies suggest that retinoids are involved in metabolic pathways of Mg (Rayssiguier et al. 2008). Although this aspect requires more future study, we found a moderate activation of antioxidant defense mechanisms in wild boars from NW Russia.

Assessment of suitability of meat and offal of wild boar from NW Russia as food

Game meat and offal are often consumed by hunters and their relatives, and the assessment of the concentration of toxic elements in these products is crucial to the health and safety of the consumers. Cd is efficiently retained in the kidney, muscle, and liver tissue of the human body, and excess amounts of Cd may cause renal dysfunction and bone demineralization. Lead toxicity in humans has been attributed to inhibition of enzymes (e.g., those involved in heme synthesis) and to interference with Ca, Mg, and Zn homeostasis. In humans, Pb is absorbed more by children than by adults, and it accumulates in soft tissues and bones (European Food Safety Authority 2010). In humans, the central nervous system is the main target organ for Pb toxicity. In addition, the International Agency for Research on Cancer has classified Cd as a human carcinogen and Pb as probably carcinogenic to humans (European Food Safety Authority 2009, 2010). The biological half-life of Cd is reported to be 10–30 years in the kidneys and 4.7–9.7 years in the liver (European Food Safety Authority 2009); the half-lives of Pb in blood and bone are approximately 30 days and 10–30 years, respectively.

Although wild game meat and offal could be sources of human exposure to such toxic elements as Cd and Pb (European Food Safety Authority 2009, 2010), there are no legislative maximum limits of nonessential elements in the meat and offal of wild game species. To assess the suitability of meat and offal of wild boar from NW Russia as the food, we compared the data for Cd and Pb content of meat and offal of the wild boar with regulatory limits for Cd and Pb in food. We also evaluated the exposure to Cd and Pb from wild boar meat and offal in three consumption scenarios (consumed rarely, regularly, or often).

Wild boar kidney is not suitable for consumption because it could be a significant source of Cd even if consumed rarely (4 times a year). Consuming wild boar kidney could be dangerous, particularly for children. Levels of Cd and Pb were higher than EU MRL for pig meat and offal in 35% and 5% of the wild boar kidney samples, respectively. Consumption of wild boar meat and liver on a rare or regular basis does not pose a health risk to adult and child consumers. However, 80% of the muscle samples and none of the liver samples from wild boar were found to contain Cd levels higher than EU MRL for pig meat and offal. In contrast to Cd, the Pb level was higher than EU MRL for pig meat and offal in 40% of the muscle samples, and 10% of the liver samples. These results can guide risk management and food monitoring programs. Future research is required to obtain reliable data on game meat and offal consumption for NW Russia and to study the food safety of game meat and offal, especially for hunters and their relatives, who are the primary consumers of this type of food product.

Conclusions

Our findings indicate that, according to reference values of elements reported for domestic pigs, the wild boar from NW Russia was deficient in most of the essential elements we measured (Co, Cu, Mn, Ni, and Zn) but had optimal values of Fe and Mg. The concentrations of Cd and Pb were lower than the values reported in studies of pigs and wild boars living in heavily polluted areas. The correlations between antioxidants and elements (Co, Cu, Fe, Mg, Mn, Ni, and Pb) probably indicate that mineral balance in the body is regulated by antioxidants, among which the SOD activity, GSH, and retinol levels are the most sensitive parameters. Assessment of the suitability of meat and offal of the wild boar from NW Russia as food showed that consumption of meat and liver on a rare (4 times a year) or regular basis (monthly) does not pose a health risk to adult and child consumers, whereas the kidney of wild boar is not suitable for consumption.

Author contribution All the authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Svetlana Kalinina, Danila Panchenko, Viktor Ilyukha, Irina Baishnikova, Ekaterina Antonova, and Kseniya Nikerova. The first draft of the manuscript was written by Svetlana Kalinina and all the authors commented on previous versions of the manuscript. Conceptualization, review, and editing were performed by Andrea Canfield. All the authors read and approved the final manuscript.

Availability of data and material All data can be found in Supplemental Information.

Declarations

Ethics approval All samples were collected during regular hunting activities and no animals were sacrificed for the sake of this study; therefore, no ethical approval was needed for compliance with our institutional or governmental organizations.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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