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PAPER

Concentrations of some toxic and trace elements in wild boar (*Sus scrofa*) organs and tissues in different areas of the Province of Viterbo, Central Italy

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Abstract

The aim of the present study was to determine heavy metal reference levels for exposure and risk assessment studies on a local scale. We measured lead (Pb), chromium (Cr), zinc (Zn), copper (Cu) and cadmium (Cd) content in edible tissues and organs of wild boars harvested in different areas of the Province of Viterbo, Central Italy. The average levels of cadmium recorded in 75 wild boars were 0.085, 0.079 and 1.052 mg Cd kg^{-1} wet weight (w.w.) in the liver, muscle and kidney, respectively. The majority of the muscle samples and some of the liver samples contained levels of heavy metal that were over the legal limit [EU Maximum Residue Levels (MRLs)] for pigs. Our data are similar to or lower than the values reported in most of the available literature. For Pb concentration, the average values recorded were 0.318, 0.126 and 0.298 mg kg^{-1} w.w. in the liver, muscle and kidney, respectively. The samples that were non-compliant with regulatory limits (MRLs) for pigs were registered only for muscle. Available data on the presence of Pb content in game meat report lower values than ours, most likely because the area around the bullet path was avoided while sampling. The average values of total Cr were 0.141, 0.139 and 0.097 mg kg^{-1} w.w. in the liver, muscle and kidney, respectively. For Zn, the mean values were 49.76, 53.21 and 32.46 mg kg^{-1} w.w. in the liver, muscle and kidney, respectively. Cu content was 46.12, 12.20 and 5.64 mg Cu kg^{-1} w.w. in the liver, muscle and kidney, respectively. The results obtained have been validated on the basis of the scarce and inconsistent Italian literature available and on international studies.

Introduction

Heavy metals are ubiquitous in soil, water and air. Their transfer to the food chain is an important environmental issue that could represent a risk to human health. Concern over the contamination of game meat with heavy metals is mainly related to toxic elements that accumulate in tissues, such as cadmium (Cd) (Beiglböck *et al.*, 2001), lead (Pb) (Karita *et al.*, 2000), and the metalloid arsenic (As) (Maňkovská and Steinnes, 1995). Some other metals, such as zinc (Zn), copper (Cu) and chromium (Cr), are microelements or trace elements with biochemical and physiological functions (Dosi, 2000). Some of these metals act as functional constituents of one-third of known enzymes. Chromium, which is thought to be an essential element, can lead to intoxication phenomena when organisms are exposed to high concentrations or are exposed to the element for a very long period of time. More generally, the balance between the toxic and useful concentration is variable from element to element and from organism to organism (Tripathi *et al.*, 1997). Several reports indicate that game meat can be an important source of trace metals because of its increasing availability, mainly due to increased hunting activity (Ramanzin *et al.*, 2010). Statistics concerning wild boar harvesting and meat availability in Italy are provided in Table 1, in which the present data are compared with data from the 1998-1999 hunting season. The total culling of ungulates in Italy may be estimated at more than 230,000 heads per year. Wild boar harvesting represents approximately 160,000 heads; however, the data available for this species are incomplete, and figures are most likely underestimated. As reported by Ramanzin *et al.* (2010), wild boar contributes to 80% of the total ungulate meat availability in Italy, with over 5000 tons of carcasses (Table 1). Wild boar is almost the only source of ungulate meat in the Northern and Southern Apennines, and accounts for 75% of the total meat produced in the Western Alps (Ramanzin *et al.*, 2010). Over the last ten years, the total wild boar harvest has increased by approximately 70%, and it is expected that the culling rate will increase in some regions because of crop damage (Amici *et al.*, 2012).

There are often significant regional differences in heavy metal content in the organs and tissues of wild ungulates (Aastrup *et al.*, 2000). Single metals are higher in animals living near mining sites or other sources of pollution (Maňkovská and Steinnes, 1995; Reglero *et al.*, 2009; Pokorny *et al.*, 2009). The accumulation of Cd and Pb increases with age (Falandysz *et al.*, 2005; Hunt *et al.*, 2009). A primary source of Pb contamination originates from residual metal elements in bullets (Falandysz *et al.*, 2005). Very high values have been registered in the muscle area surrounding the bullet pathway. Pb represents an important concern for game meat consumption, and the accurate trimming of the meat around wounds and bullet pathways is recommended (Doborowolska and Melosik, 2008; Hunt *et al.*, 2009). Some muscle, liver and kidney samples contained concentrations of heavy metal that were over European or national legal limits (Swiergosz *et al.*, 1993; Santiago *et al.*, 1998; Zaccaroni *et al.*, 2003; Falandysz *et al.*, 2005; Mandas, 2005; Lazarus *et al.*, 2008); however, the average values recorded in the organs and tissues were under the legal limits. Some authors believe the health risk for game meat consumers to be negligible (Lazarus *et al.*, 2008), even in highly polluted areas (Pokorny *et al.*, 2009). However, there have been no specific exposure assessment studies, especially on a local scale, both for the general population and for particular categories (Danieli *et al.*, 2012). In general terms, many studies on the heavy metal content of edible tissues of wild boar refer to opportunistic sampling (Zaccaroni *et al.*, 2003; Mandas *et al.*, 2005) and are used to provide

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evidence of local sources of pollution (Swiergosz *et al*., 1993; Falandysz, *et al*. 2005; Pokorny *et al*., 2009). These studies often include a small number of samples or small sampling areas (Swiergosz *et al*., 1993) or consider only one edible organ (Guazzetti *et al*., 2001; Orusa *et al*., 2004). There has been no systematic collection of data, and this means that it has been impossible to conduct reliable exposure assessment studies. Collecting useful data on contamination of wild animal tissue by heavy metals is the first step in determining the intake of these elements, both in the overall population and in specific groups, such as hunters and their families.

The aims of the present study were: i) to highlight the content of copper, zinc, lead, cadmium and chromium in the edible tissues and organs of wild boars harvested in 6 hunting areas of the Province of Viterbo; and ii) to describe the distribution pattern and reference levels of heavy metals in the edible tissues of wild boar to be used for *local scale* exposure assessment studies.

Materials and methods

Study area

The study area is represented by 6 wild boar game management districts within the administrative boundaries of the Province of Viterbo, covering an area of 3614 km^2 (Region of Lazio, Central Italy) (Figure 1).

Meteorological records for the area during the 7-year period from January 2004 to December 2010 show a range in monthly mean air temperature between 5.9°C in January and 23.8°C in July. Average annual rainfall was 848 mm, distributed over 203 days with a minimum in the summer and a peak in autumn. The landscape is highly fragmented, especially at altitudes below 600 m, where cultivated fields are interspersed with woodlands and scrublands, with an increase in woodlands and scrublands going inland from the coast to the hill areas. Along the coast, the fields are planted with vegetables. The hinterland plain can be irrigated and summer crops, such as maize and sunflowers, predominate. These are very often sown after the autumn-winter cereals and forages. At the higher altitudes, orchards (vineyards, olive groves, chestnut and hazelnut) and woodlands dominate the landscape. The forests are mainly composed of turkey oak (*Quercus cerris*), downy oak (*Quercus pubescens*), hop hornbeam (*Ostrya carpinifolia*), manna ash (*Fraxinus ornus*), european chestnut (*Castanea sativa*), and several shrub and bush species (*Rosa canina, Arbutus unedo, Erica arborea, Juniperus spp., Cytisus spp., Rosmarinus officinalis*). Most of the forests are coppiced, while timber forests are more frequently to be found in protected areas. The *A. Volta* electrical power plant (in the southwest of the area) and some authorized landfills are located in the province outside the 6 hunting districts. The area is crossed by the Fiora and Paglia rivers that rise from Monte Amiata where cinnabar mines and geothermal phenomena are to be found (Bargagli *et al*., 1991).

Wild boar population status

The particular organization of the agricultural and forest lands in the study area provides abundant feed for the wild boar population throughout the year: cereals such as wheat, oats and barley in the spring, maize in the summer, chestnuts and hazelnuts in autumn, and acorns in winter. Furthermore, drive-hunting teams provide high-energy supplemental feeding, especially during autumn and winter, to ensure the wild boars stay within their own hunting areas. Consequently, the wild boar population in the Province of Viterbo has increased dramatically over the last fifteen years. At the moment, the wild boar population can be considered stable, although some fluctuations have been observed, and the official harvesting range is between 5000 to 7000 heads per year. The main factor that limits an increase in population is hunting. Hunters are active three times a week from the 1st November to the 31st January. Parks, restocking areas, oases and military areas cover approximately 11% of the Province's agro-forestry surface area. Private and free hunting areas cover 13% and 76% of the territory, respectively. Wild boar hunting teams cover approximately 350 km^2 (*i.e.* 13% of the free hunting areas).

Organ and tissue sampling

Wild boars were shot by hunters belonging to 6 different hunting districts within the Province of Viterbo, Central Italy: Acquapendente (ACQ), Monti di Castro (MTI), Montalto di Castro (MTO), Monte Romano (MNO), Graffignano (GRA) and Nepi (NE) (Figure 1). A total of 75 adult animals were culled, aged between 2 and 5 years. The carcasses were randomly selected from the annual hunting bag. Sex was recorded at sampling (38 males, 37 females). Muscle and offal (the liver and kidneys) were collected, individually packed in polyethylene bags, and transferred to the laboratory using refrigerated bags within a few hours. During sampling operations, special care was taken to avoid tissues near the bullet entry or fragmentation (Hunt *et al*., 2009). The muscle, liver and kidney samples were frozen (-20°C) and stored until analysis.

Elemental analysis

Finely ground samples (0.5 and 0.6 g for the meat and offal, respectively) were dispersed in H_2O_2 (30% for trace analysis) and/or HNO_3 (65% for trace analysis) (Sigma-Aldrich S.r.l., Milan - Italy) and then submitted to a specific microwave digestion cycle (Table 2) using a MLS 1200 microwave laboratory system (Milestone Inc., Shelton - USA). At the end of the mineralization procedure, all the samples were quantitatively recovered in polyethylene tubes and diluted to 10 mL using ultrapure

Table 1. Statistics of wild boar harvesting (heads) and meat (tons) availability in different areas of Italy.

Hunting season	1998-1999	2009-2010
Heads harvested, n		
Western Alps°	6700	19,000
Eastern Alps#	430	3000
Northern Apennines§	59,500	155,000
Southern Apennines, Islands^	26,415	28,000
Total	93,045	155,500
Carcass weight,$ tons		
Western Alps°	227.8	646.0
Eastern Alps#	14.6	102.0
Northern Apennines§	2023.0	3587.0
Southern Apennines, Islands^	898.1	952.0
Total	3163.5	5287.0

°Piemonte, Val d'Aosta, Lombardia; #Trentino-Alto Adige, Veneto, Friuli Venezia Giulia; §Liguria, Emilia Romagna, Toscana, Marche, Umbria; ^Abruzzo, Molise, Lazio, Campania, Puglia, Basilicata, Calabria, Sicilia, Sardegna. $Assuming an average carcass weight of 34 kg for wild boar. Adapted from Ramanzin *et al*., 2010

water (specific conductivity 0.055 µS/cm) (Sartorius Stedim, Firenze - Italy). The samples were properly diluted with 1.0% (v/v) HNO_3 before analysis.

Analysis of Cd, Pb, Cr, Zn and Cu was performed using a Varian SpectrAA 30 atomic absorption spectrophotometer equipped with a Zeeman graphite furnace (Varian, Palo Alto – USA). Chemical (or matrix) interference was minimized by using platform atomization techniques with the aid of appropriate matrix modifiers: 1.0% (w/v) $NH_4H_2PO_4$ for Cd and 1.0% (v/v) H_3PO_4 for Pb, while no modifier was added for the Cr, Zn and Cu analysis. Five calibration standards for Cd (1.56-25.00 µg/mL), Pb (3.13-50.00 µg/mL), Cr (0.79-12.50 µg/mL), Zn (15.63-250.00 µg/mL) and Cu (12.50-200.00 µg/mL) were obtained by diluting certified standards (1.000±0.002 g/L) (Merk, Darmstadt, Germany) with 1.0% (v/v) HNO_3. The certified reference material (European Commission, 1998) was treated in the same manner as the wild boar samples, to verify the accuracy of the analytical method. Recoveries for Cd, Pb, Cr, Zn and Cu were then calculated using the certified values as references. The purity of the reagents and water, and the level of cleanness of the laboratory glassware, MW liners and plastics were monitored by including a Reagent Laboratory Blank (RLB) for every MW mineralization run (EPA, 1998). The concentration of all the elements analyzed were expressed in mg/kg w.w. based on the following equation:

$$[El]_{sample} = \frac{(ABS_{\lambda} - Int_{cc}) \times DF \times V_{min}}{Slope_{cc} \times W_{sample}}$$

where

$[El]_{sample}$ is the concentration of element in the tissue sample;

ABS_{λ} the absorbance recorded at 228.8 nm for Cd, 283.3 nm for Pb, 357.9 nm for Cr, 307.6 nm for Zn and 327.4 nm for Cu;

Int_{cc} (in AU) and $Slope_{cc}$ (in mL μg^{-1}) the intercept and slope, respectively, of the calibration curve developed for each element;

DF diluting factor applied;

V_{min} final volume of mineralized samples (mL);

W_{sample} sample weight in grams.

The detection limits (LOD) and quantification limits (LOQ) were also determined for each element following the DIN procedure n. 2345 (DIN, 1994).

Performance values of the method were:

LOD = 0.003 mg kg^{-1}, LOQ = 0.009 mg kg^{-1}, Rec% (mean±s.d.) = 87.1 ± 0.6 for cadmium;

LOD = 0.010 mg kg^{-1}, LOQ = 0.030 mg kg^{-1}, Rec% = 94.1 ± 1.8 for lead;

LOD = 0.012 mg kg^{-1}, LOQ = 0.037 mg kg^{-1}, Rec% = 91.1 ± 2.4 for chromium;

LOD = 0.060 mg kg^{-1}, LOQ = 0.189 mg kg^{-1}, Rec% = 89.8 ± 1.4 for zinc;

LOD = 0.045 mg kg^{-1}, LOQ = 0.140 mg kg^{-1}, Rec% = 93.3 ± 1.3 for copper.

The data presented in this study were not corrected for recoveries.

Statistical analysis

The concentration data obtained using the spectrophotometric readings were stored using MS Excel 2002® (Microsoft Corporation, Redmond – USA) spreadsheets. The statistical analysis and graphic representation of the data were performed using STATISTICA 6.0 (StatSoft Inc., Tulsa - USA) software package. Data distributions were tested using Shapiro-Wilks statistics (Shapiro *et al*., 1968) and were non-compliant with parametric statistics; they were, therefore, log transformed before further analysis. A similar transformation has been used in other papers on the accumulation of heavy metals (Guazzetti *et al*., 2001). The factorial analysis of the distribution and areas for contamination of the tissue was conducted using a GLM-type approach. The statistical model included the harvesting area and the tissue, as well as their interaction as main factors. The significance of the differences was tested using the F statistic; within the relevant factors, the differences were evaluated using the Fisher LSD test (Lowest Significance Differences). A residual probability value of 5% (P=0.05) was adopted as the minimum level of significance.

Results and discussion

Data distribution and statistics for the liver, kidney and muscle samples are presented in Table 3. Overall, left-censorship in the analytical data set was virtually absent. A very low, left-censored rate was only recorded for chromium in the liver (<5%; data not shown). With the exception of the chromium levels in the muscle, all the metal concentration data were adequately fitted by log-normal distributions (Kolmogorov-Smirnov and Pearson chi-square test, P>0.1).

Cadmium

The average levels of Cd (Table 3) recorded in the muscle and kidney were 0.079 and 1.052 mg kg^{-1} w.w., respectively. These mean values are quite high if compared with the European Minimum Risk Levels (MRLs) set by the European Commission Regulation 420/2011

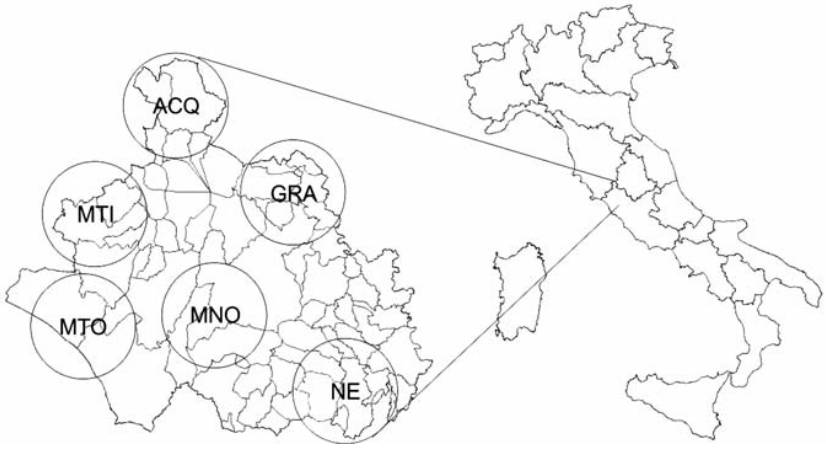

Figure 1. Graphic representation of sampling areas. ACQ, Acquapendente; MNO, Monte Romano; GRA, Graffignano; MTI, Monti di Castro; MTO, Montalto di Castro; NE, Nepi.

Table 2. Microwave digestion condition optimised for wild boar meat, liver and kidney.

Matrix	Sample amount°	Reagents	MW cycles
Muscle	0.600±0.002	HNO_3 (2 mL)	1.0 min - 600 W 5.0 min - 300 W
Liver / kidney	0.500±0.002	HNO_3 (2 mL) H_2O_2 (1 mL)	5.0 min - 300 W 0.5 min - 0 W 5.0 min - 600 W

°Tolerance refers to the precision of the analytical balance used.

(European Commission, 2011) for meat and offal of farmed pigs (0.05 and 1.00 mg Cd kg^{-1} w.w. for muscle and kidney, respectively). From a total of 75 muscle samples tested, 75% contained a Cd value above the limit (1st Q.le = 0.056 mg Cd kg^{-1} w.w.). For a total of 65 kidney samples, 48% contained Cd values over the EU MRL (median 0.975; 3rd Q.le 1.267 mg Cd kg^{-1} w.w.). The median was slightly lower than the mean for all the tissues (Table 3), and a modest asymmetry of the data distribution was recorded. Over 60% variability in cadmium contamination in the three tissue types was observed (Relative Standard Deviation, RSD % = 72.7, 62.1 and 65.0 for the liver, muscle and kidney, respectively). The cadmium content in the kidney tissue was higher ($P<0.01$) than in the liver and muscle tissues, while no differences were observed between the liver and muscle (Figure 2). For all the tissues analyzed, samples belonging to wild boars shot in the ACQ hunting area showed greater cadmium contamination than the samples from MNO and MTO. A comparison with all the other areas showed that the kidney samples harvested from the GRA area were less contaminated ($P<0.01$). If we consider the EU MRLs established for the meat and offal of farmed pigs, the kidney samples collected in ACQ contained the highest number of samples above the EU MRL (1.0 mg kg^{-1} w.w.), with an average value of 1.706 mg Cd kg^{-1} w.w. In addition, both the median and the 1st Q.le values were higher than 1.0 mg Cd kg^{-1} w.w. More than 75% of the samples were non-compliant with the MRL set by European Commission Regulation 420/2011 (European Commission, 2011). In contrast, kidney samples from the MTO and GRA areas contained a mean value of cadmium below the limit (0.792 and 0.594 mg Cd kg^{-1} w.w., respectively), with less than 25% non-conformity of samples (3rd Q.le equal to 0.975 and 0.886 mg Cd kg^{-1} w.w. for the MTO and GRA areas, respectively). All the muscle samples from NE were contaminated with Cd above the EU MRL of 0.05 mg kg^{-1} w.w. established for pork.

The mean values recorded in the liver samples from the ACQ and MTI areas were significantly higher than those for the other 4 areas; however, these results are comparable with those observed in Sardinian wild boars by Mandas (2005) who reported mean values ranging between 0.38 and 0.58 mg Cd kg^{-1} w.w.; these were lower than the mean value of 0.55 mg Cd kg^{-1} w.w. reported by Gasparik *et al*. (2012). For muscle, particularly high values were recorded in the samples from ACQ ($P<0.05$) (Figure 2). A similar value was recorded for the renal tissue for which, even for the MTI harvesting area, the levels of accumulation were significantly higher than those observed in the other areas ($P<0.05$) (Figure 2). There was no difference between data collected on the presence of cadmium in wild boar muscle in the present paper and those values reported in the literature (range 0.001-0.355 mg Cd kg^{-1} w.w.) (Rudy, 2010; Taggart *et al*., 2011). Nevertheless, we observed lower values than those reported by Hernández *et al*. (1985) for cadmium in muscle (geometric means of 0.07 *vs* 0.12 mg Cd kg^{-1} w.w.) or in liver (geometric means of 0.067 *vs* 0.45 mg Cd kg^{-1} w.w.). Our data also show lower values than those reported by Gasparik *et al*. (2012) who reported 0.16 and 3.22 mg Cd kg^{-1} w.w. as the mean values for muscle and kidney, respectively, and those reported by Wolkers *et al*. (1994) who reported a median cadmium concentration of 2.05 mg kg^{-1} d.w. (dry weight) in liver samples of wild boar aged 1.5-5 years. Adopting a conversion factor of 3.4, the cadmium concentration reported by Wolkers *et al*. (1994) corresponds to 0.60 mg kg^{-1} w.w., which is 10 times greater than the median (0.067 mg kg^{-1} w.w.)

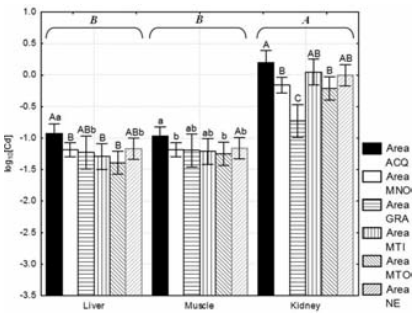

Figure 2. Cadmium content in the liver, muscle and kidneys of wild boars harvested in different hunting areas. Data (log transformed) are expressed as Lsmean ± SEM. ^{A,B}significant differences ($P<0.01$) between tissues; ^{A,B}significant differences ($P<0.01$) between hunting areas within tissues; ^{a,b}significant differences ($P<0.05$) between hunting areas within tissues. ACQ, Acquapendente; MNO, Monte Romano; GRA, Graffignano; MTI, Monti di Castro; MTO, Montalto di Castro; NE, Nepi.

Table 3. Data distribution and statistics of cadmium, lead, chromium, copper and zinc (mg kg^{-1} wet weight, unless otherwise stated) in wild boar liver, muscle and kidney (overall values).

Element	Organ/tissue	n	Mean	Min	Max	SD	RSD, %	Median	1st quartile	3rd quartile
Cadmium	Liver	75	0.085	0.008	0.381	0.061	72.7	0.067	0.047	0.104
	Muscle	75	0.079	0.031	0.381	0.049	62.1	0.071	0.056	0.086
	Kidney	65	1.052	0.016	3.080	0.684	65.0	0.975	0.641	1.267
Lead	Liver	59	0.318	0.179	0.564	0.072	21.8	0.329	0.292	0.362
	Muscle	58	0.126	0.080	0.227	0.028	23.0	0.124	0.109	0.133
	Kidney	61	0.298	0.089	0.440	0.095	32.0	0.311	0.231	0.380
Chromium	Liver	75	0.141	0.003	0.626	0.110	75.9	0.123	0.068	0.165
	Muscle	78	0.139	0.069	0.692	0.082	61.3	0.109	0.100	0.132
	Kidney	65	0.097	0.035	0.590	0.075	77.9	0.080	0.057	0.110
Zinc	Liver	57	49.76	27.78	83.16	11.17	22.4	49.50	42.39	54.09
	Muscle	57	53.21	32.61	80.10	8.11	15.2	53.14	49.10	57.40
	Kidney	62	32.46	12.92	48.30	8.53	26.3	32.64	26.56	37.62
Copper	Liver	75	46.12	7.51	117.54	25.12	54.5	47.19	23.73	61.07
	Muscle	75	12.20	2.95	25.17	4.73	38.8	11.80	8.93	14.97
	Kidney	65	5.64	1.64	13.29	2.23	39.6	5.29	4.05	6.73

RSD, Relative Standard Deviation.

observed in our study. Other authors have indicated that such variations in cadmium levels can be found among wild boars hunted in different areas, even within the same country (Swiergosz *et al*. 1993). However, this figure most likely reflects the high levels of environmental pollution, as also highlighted by Gasparik *et al*. (2012).

Lead

The average lead content in muscle (0.126 mg Pb kg^{-1} w.w.) was over the regulatory limit (0.1 mg Pb kg^{-1} w.w.), with a frequency of non-compliant data over 75% (1st Q.le 0.109 mg Pb kg^{-1} w.w.) (Table 3). In liver, the highest value was slightly higher than the EU MRL for pigs (0.5 mg Pb kg^{-1} w.w.) and non-compliant content was only observed in one sample (Table 3). As a general consideration, there were no significant differences in the lead content in the liver and kidneys but values for both tissue types were higher than in muscle (P<0.01), and the observed variability was significantly smaller than that recorded for cadmium, rarely exceeding 30% RDS.

A consideration of the sampling area could explain the observed differences in the levels of accumulation recorded. However, the lack of scientific studies into the level of anthropogenic pollution and chemical composition of the soil means the causes for this variability cannot be clarified. Higher concentrations were observed for lead in the samples from the MTI area, while the lowest values were recorded in the NE area (P<0.01) (Figure 3). The similar values observed for Pb accumulation in the liver and kidney are in contrast to data reported by Martelli (2005) who investigated Pb accumulation in wild boar from 2 areas in Tuscany with a different degree of anthropogenic disturbance. He reported high accumulation levels in liver (exceeding regulatory limits) but did not observe high values in kidney. Piskorová *et al*. (2003) observed more lead in kidney than in liver (mean concentrations 0.39 mg and 0.24 mg Pb kg^{-1}, respectively) for wild boars hunted in the Slovak Republic. Martelli (2005) observed similar lead concentrations in the liver (0.302-0.674 mg Pb kg^{-1} w.w.) and kidney (0.401 to 0.774 mg Pb kg^{-1} w.w.) of wild boars reared near an industrialized area.

The mean occurrence of Pb in the liver was approximately 0.320 mg kg^{-1} w.w. for all the areas of sampling but differences were without statistical significance (P>0.05). The mean value we have registered in liver was close to that obtained by Mandas (2005) in 3 areas of Sardinia in animals aged 3-5 years (0.11 and 0.28 mg Pb kg^{-1} w.w.). In our study, no differences in the levels of lead concentration of muscle between the areas were recorded. Nevertheless, it should be noted that the area of ACQ showed particularly high right-end values (2 samples with contamination over 0.20 mg Pb kg^{-1} w.w.). In the kidney samples, high values of Pb contamination were recorded for the MTI area, and significantly lower values were recorded for the samples belonging to the NE area (P<0.01) (Figure 3). In wild boars reared in pens near an industrial complex, Martelli (2005) observed a significant presence of lead in muscles and other tissues (0.302-0.674 mg Pb kg^{-1} w.w. in liver, 0.401-0.774 mg Pb kg^{-1} w.w. in kidney, and 0.022-0.082 mg Pb kg^{-1} w.w. in muscle). The high variability of the lead concentration in wild boar muscle and liver has been reported in several studies (Swiergosz *et al*. 1993). Wolkers *et al*. (1994) reported a median concentration of 0.917 mg Pb kg^{-1} d.w. (0.270 mg Pb kg^{-1}w.w.) in the liver of wild boars shot in The Netherlands. These authors also observed that the meat of farmed wild boars had a significantly lower lead concentration (median 0.082 mg kg^{-1}w.w.) than that of free-living animals. Finally, a recent survey performed in Croatia (Bilandzic *et al*., 2009) noted that lead levels in wild boar meat may vary. The mean meat contamination from 7 Croatian hunting areas ranged from 0.028 to 0.150 mg Pb kg^{-1} w.w., with pooled data spanning the 0.001-1.01 mg kg^{-1} w.w. range (Bilandzic *et al*., 2009). In conclusion, our results for lead fall within the range of values observed in the majority of the literature consulted, with the exception of the studies by Morales *et al*. (2011) and Taggart *et al*. (2011). These authors reported that the high lead levels could be related to lead dispersion in the animal body due to bullet fragmentation (Hunt *et al*., 2009). The problem of Pb contamination due to bullets has received great attention in the literature and the possible impact on human health should not be underestimated.

Chromium

The median values for the total chromium in the wild boar liver were slightly higher (0.123 mg kg^{-1}w.w.) than in the muscle (0.109 mg kg^{-1}w.w.), with a proportionally slight difference in the mean values (Table 3). The average and median chromium contents of the kidney were slightly lower than those of the muscle and liver, although differences did not reach significance. Great variability was seen in all tissues with an RDS range of between 61.3% and 77.9% (Table 3). Among tissues or organs, the preferential accumulation of chromium appears to be significantly lower in the kidney compared with the liver and muscle tissue (Figure 4), although no differences were observed. There was very little difference in the presence of chromium in the kidney of wild boar (Figure 4) between different study areas (P>0.05) although, on average, those coming from the MTI area were more contaminated. A

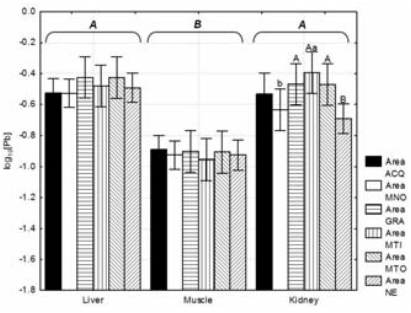

Figure 3. Lead content in the liver, muscle and kidney of wild boars harvested in different hunting areas. Data (log transformed) are expressed as Lsmean ± SEM. ^{A,B}significant differences (P<0.01) between tissue types; ^{A,B}significant differences (P<0.01) between hunting areas within tissue types; ^{a,b}significant differences (P<0.05) between hunting areas within tissue types. ACQ, Acquapendente; MNO, Monte Romano; GRA, Graffignano; MTI, Monti di Castro; MTO, Montalto di Castro; NE, Nepi.

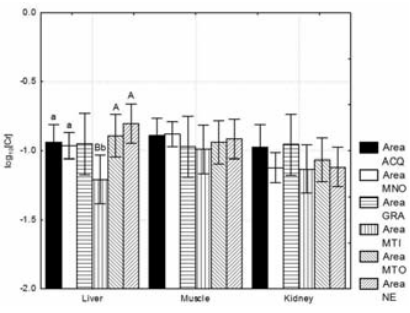

Figure 4. Chromium content in the liver, muscle and kidney of wild boars harvested in different hunting areas. Data (log transformed) are expressed as Lsmean ± SEM. ^{A,B}significant differences (P<0.01) between hunting areas within tissue types; ^{a,b}significant differences (P<0.05) between hunting areas within tissue types. ACQ, Acquapendente; MNO, Monte Romano; GRA, Graffignano; MTI, Monti di Castro; MTO, Montalto di Castro; NE, Nepi.

certain uniformity was also observed for muscle (Figure 4), although the highest values were recorded in tissue samples from animals harvested in ACQ and MNO. A similar trend towards uniformity was also observed in kidney samples (Figure 4).

Chromium could play different roles in the living organism and is a contaminant that can be ingested via the diet (Gunderson, 1995); however, it is also a trace element essential for many biochemical processes. Currently, there is no EU limit for chromium in animal food products. For this reason, only a few studies have reported the chromium content in either small or large game animals. Swiergosz *et al*. (1993) observed a mean chromium level in wild boar liver of 0.705 mg kg^{-1}w.w. This result is most likely caused by environmental pollution, and it is 5 times greater than the level we observed in the same organ (0.134 mg Cr kg^{-1}w.w.). Wlostowski *et al*. (2006) observed that wild animals, although living in unpolluted habitats, contain higher amounts of trace elements in their tissues and organs compared with farmed animals. However, we cannot ultimately affirm or exclude that our data might reflect low-to-moderate environmental pollution because no reference values in wild boars hunted in unpolluted areas are available.

Seňczuk (1990), indicated that physiological levels of chromium in wild boar liver and kidney should be within the range of 0.015-0.220 mg Cr kg^{-1} d.w. (approximately 0.004-0.064 mg Cr kg^{-1} w.w.). For muscle tissue, Bratakos *et al*. (2002) reported that the chromium content of pork ranged from 0.05 to 0.14 mg Cr kg^{-1} w.w. Because chromium supplementation is not allowed in animal feed in the EU (Mantovani *et al*. 2009), these values may be considered a reference level for chromium in pork, even though higher levels should be expected in feral pigs or wild boars living in the same areas (Wlostowski *et al*., 2006). In our study, the chromium level in wild boar muscle, liver and kidney ranged from undetectable to 0.692 mg kg^{-1} w.w. This range is 4 times wider than that recorded by Bratakos *et al*. (2002) in pigs. The values we observed also fall in the range reported by Piskorová *et al*. (2003) for the muscle (0.02-0.49 mg kg^{-1} w.w.) of wild boars harvested in a polluted region of the Slovak Republic. In Italy, Martelli (2005) reported similar values for a small number of animals. A general consideration of the available literature leads us to conclude that the high values reported in our data are most likely due to the accumulation of chromium related to the aging of the animals and/or a moderate load of chromium from the environment. However, it must be underlined that the relationship between chromium accumulation and aging is unclear (Gamberg *et al*. 2005), and further investigation is needed to ascertain whether our data reflect a moderate environmental load of this element.

As previously indicated, chromium is also an essential nutrient for animals, and an adequate ingestion level (35 μg day^{-1} for a 60 kg adult) has been recommended by the Institute of Medicine of the U.S. National Academy of Science (IOM, 2000). Nevertheless, a high intake can be toxic (Anderson, 1997; Bielicka *et al*. 2005). A higher range of intake levels (from 50 to 200 μg day^{-1} $person^{-1}$) was assessed by Anderson (1997). The scarce number of toxicological reports has prevented international health organizations (US-FNB, 2001; UK-EVM, 2003; SCF, 2003) from establishing limits for ingestion of chromium and its compounds. Currently, upper limits of 250 μg Cr day^{-1} $person^{-1}$ (WHO, 1996) and the oral MRLs of 5 μg Cr^{VI} day^{-1} kg^{-1} b.w. (body weight) for a brief exposure period (15-365 days) and 1 μg Cr^{VI} day^{-1} kg^{-1} b.w. for exposure for periods of over one year (ATSDR, 2008) should be adopted. Uncertainty also arises from the scarce data on chromium speciation in feed (Boon *et al*. 2012). In fact, $chromium^{III}$ is most likely the predominant form occurring in feed because it is the most stable (EPA, 1998, IOM, 2000; Levina and Lay, 2008). In addition, $chromium^{III}$ is gastrointestinally absorbed at 0.4-2.5% (IOM, 2000). Currently, no chromium deficiency has been reported in human populations (Stallings and Vincent, 2006), and the measure of its essentiality is still controversial (Stearns 2000; Levina *et al*. 2003; Stallings and Vincent 2006). In contrast, there is a large volume of literature that discusses the genotoxic effect in animals and humans (Snow, 1991; Bridgewater *et al*., 1994) due to the accumulation and excessive intake of chromium.

Zinc

Similar mean values were registered in the liver and muscle for zinc, and those for the kidney are significantly lower ($P<0.01$) (Table 3; Figure 5). A similar trend was also registered for the median, maximum and minimum values. Variability for the different tissues (SDR) ranged between 15.2% and 26.3%. The content of zinc in the liver was significantly higher in GRA compared with MNO ($P<0.01$) and all the other areas ($P<0.05$) (Figure 5). In muscle, the highest value was registered in MNO compared with ACQ ($P<0.01$) and GRA ($P<0.05$).

There was a high variability between the different study areas in the accumulation of zinc in the kidney (Figure 5) and significant differences were registered when a comparison was made between the NE hunting area and the other areas ($P<0.01$), excluding MNO ($P<0.05$).

The suggestion that zinc accumulation in the liver and muscle is similar, and higher than that in the kidney, already suggested above, was therefore confirmed. Sporadic cases of contamination by zinc over 70 mg kg^{-1}w.w. were recorded in samples of liver harvested from ACQ, MNO and GRA. Considering the kidney samples analyzed, the average contamination was variable in samples from ACQ, MTI and MTO, approximately 40 mg Zn kg^{-1} w.w. in GRA, decreased to 30 mg Zn kg^{-1} w.w. in MNO, and decreased further to approximately 20 mg Zn kg^{-1} w.w. for the samples from NE ($P<0.01$).

Falandysz (1994) reported average wild boar muscle values of 28-37 mg Zn kg^{-1}w.w. with a range oscillating between 4.3 and 130 mg Zn kg^{-1}w.w. Gasparik *et al*. (2012) reported a mean value of 28.20 mg Zn kg^{-1} w.w.. These average data are lower than those reported in our study; however, they have a wider range (Table 3). Falandysz (1994) reported mean values of 37 to 48 mg kg^{-1} w.w. and 30 to 31 mg kg^{-1} w.w. in liver and kidney, respectively, that were similar to those of our study and with a similar range of variation. Similar mean values were also reported by Swiergosz *et al*. (1993): 114-143 mg Zn kg^{-1} d.w. and 82-104 mg Zn kg^{-1} d.w. in muscle and kidney, respectively. Gasparik *et al*. (2012) reported 28.20 and 20.98 mg Zn kg^{-1} w.w. in the same organs, respectively. Literature data on zinc from Italy are limited; the only data are reported by Zaccaroni *et al*. (2003) with an average of 94.76 mg Zn kg^{-1} d.w. in kidney. Average and maximum values recorded for zinc levels muscle do not appear to cause particular concern if compared with the acceptable exposure doses in humans (Gupta and Gupta, 1998). However, the high values of zinc in the liver mean it is not recommended for human consumption. Careful consideration should be given to the fact that the role of zinc in humans exposed to a cadmium load is still unclear, as highlighted by Brzóska and Moniuszko-Jakoniuk (2001). Although the accumulation of cadmium in some human tissues and organs has been proven if zinc-deficient diets are administered, further research on this topic is needed (Brzoska and Moniuszko-Jakoniuk, 2001). Our data confirm this hypothesis given that the zinc content in the liver was high in MNO and low in ACQ and *vice versa* for cadmium (Table 3).

Copper

There were significant differences in copper content of different tissues (P<0.01) showing a clear accumulation order (Figure 6): liver > muscle > kidney. In liver, highly significant differences were observed between samples from the NE and GRA hunting areas (P<0.01); however, significant differences were also observed between other sampling areas (P<0.05).

Particularly high values of Cu accumulation in wild boar liver were recorded for the ACQ area where 2 samples exceeded 100 mg kg^{-1} w.w. Different results were obtained for kidney tissue, as the highest value was observed in GRA and the lowest in NE (P<0.01). Significant differences were also observed between other sampling areas (P<0.05). No differences were observed between muscle samples from different areas. It is interesting to highlight the wide range of copper content, mainly in liver (54.5% SDR).

Swiergosz *et al*. (1993) reported mean values from 6.4 to 7.4 mg Cu kg^{-1} d.w. and from 17.2 to 24.5 mg Cu kg^{-1} d.w., respectively, in the muscle and kidney of wild boars shot in Poland. In Italy, Zaccaroni *et al*. (2003) reported 30 and 21.2 mg Cu kg^{-1} d.w. in kidney and liver tissue, respectively. Wolkers *et al*. (1994) reported 15-20 mg Cu kg^{-1} d.w. and 17-18 mg Cu kg^{-1} d.w. in liver and kidney tissue, respectively, with a very high variability (over 60% SDR). Gasparik *et al.* (2012) reported 1.61, 3.41 and 3.80 mg Cu kg^{-1} w.w. These are similar to our data for kidney tissue; however, the muscle and liver contents in our study were higher than those recorded in the aforementioned studies. A possible explanation for these differences in the mean level of copper accumulation may be due to the particular geo-environmental context in which our wild boars lived. The soil of the Province of Viterbo is prevalently of volcanic origin. Some metals that occur in the soil and vegetables, such as copper and zinc, are strictly linked to volcanic activity (Bargagli *et al*., 1991), and, most likely, the high value of copper observed, especially in the wild boar liver, may have an alimentary exposure due to the naturally high level of this metal in the wild boar diet constituents. Absorbed copper in the portal blood binds to albumin and transcuprein (Weiss and Linder, 1985) for transport to the liver, which is the major storage organ for metallothionein-bound Cu (Van Pamel *et al*., 2010)

Average and maximum values of copper concentration were recorded for muscle, although the high values do not appear to cause particular concern if compared with the exposure doses in humans (Gupta and Gupta, 1998). However, as also indicated for zinc, the high values in the liver mean it is not recommended for human consumption.

Conclusions

Game meat can be an important source of trace metals because of the increasing availability of this food, especially in hunting communities. This study demonstrates that for the trace elements considered, our data fall in the range observed by other authors, except for copper content in muscle and liver.

The average level of cadmium recorded in the wild boar muscle, liver and kidney were often higher than the EU MRLs for pigs (75% and 50% of non-compliant samples for muscle and kidney, respectively). The mean values recorded in the liver are comparable with those reported in Sardinia and lower than those recorded in the Slovak Republic. There was no difference between the data collected on cadmium occurrence in wild boar muscle and that available in the literature, but these values are generally lower than those recorded in other European countries.

The average lead concentration in muscle was greater than the regulatory limit for pork, with over 75% non-compliance. For the liver, the mean value was less than the regulatory limit, and only one sample was non-compliant with this limit. Similar values of lead content were observed in the liver and kidney but this was higher than in muscle, and the observed variability was low, rarely exceeding 30%. The mean values observed in liver were close to the values recorded in Sardinia. It should be noted that the maximum lead values in different tissues is 2-4 fold higher than the mean, as the areas near the bullet pathway were avoided while sampling. In those studies, high lead levels could be related to lead dispersion into the animal body due to bullet fragmentation. Although very important, the problem of lead dispersion along the bullet pathway is not the focus of the present paper.

Currently, there is no EU limit for chromium in animal food products. For this reason, only a few studies have reported on the chromium content in either small or large game animals. Some authors observed higher chromium levels than those found in the present study; however, result is most likely caused by the environmental pollution of the area, even though to date no reference values in wild boars hunted in unpolluted areas are available. Considering the available literature, it is most likely that our levels of contamination are high because of chromium accumulation due to aging and/or to a moderate environmental chromium load from the environment. Although chromium is an essential element, its genotoxic role has been proven, and attention should be given to

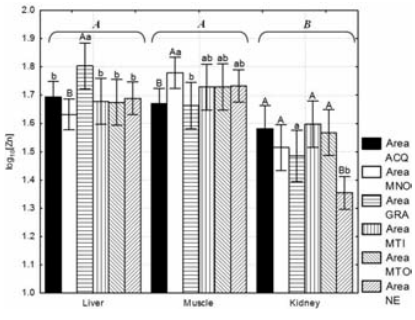

Figure 5. Zinc content in the liver, muscle and kidney of wild boars harvested in different hunting areas. Data (log transformed) are expressed as Lsmean ± SEM. [A,B]significant differences (P<0.01) between tissue types; [A,B]significant differences (P<0.01) between hunting areas within tissue types; [a,b]significant differences (P<0.05) between hunting areas within tissue types. ACQ, Acquapendente; MNO, Monte Romano; GRA, Graffignano; MTI, Monti di Castro; MTO, Montalto di Castro; NE, Nepi.

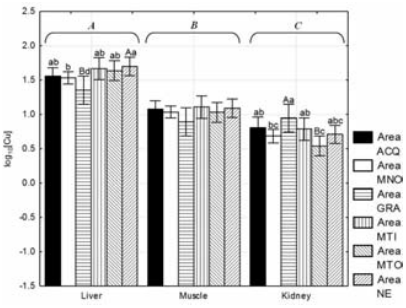

Figure 6. Copper content in the liver, muscle and kidney of wild boars harvested in different hunting areas. Data (log transformed) are expressed as Lsmean ± SEM. [A,B]significant differences (P<0.01) between tissue types; [A,B]significant differences (P<0.01) between hunting areas within tissue types; [a,b]significant differences (P<0.05) between hunting areas within tissue types. ACQ, Acquapendente; MNO, Monte Romano; GRA, Graffignano; MTI, Monti di Castro; MTO, Montalto di Castro; NE, Nepi.

its intake. Zinc content in the wild boar liver, muscle and kidney differed among the areas investigated. These values were lower than those registered in other countries but similar to the only report from Italy. The role of zinc in humans should be carefully considered, as the accumulation of cadmium in some human tissues and organs was proved when zinc-deficient diets were administered.

The copper content in the wild boar liver was higher than in the other tissues, and a significant difference was also observed between the content in the muscle and liver. The order of accumulation in these tissues differed from that reported in other studies and content was generally higher. These contrasting results highlight the need for further studies on this element. Nevertheless, the average and maximum values recorded in muscle do not appear to raise particular concern if compared with the exposure doses in humans. However, as also indicated for zinc, the high values in the liver mean it is not recommended for human consumption.

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