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RESEARCH ARTICLE

Barbary sheep tissues as bioindicators of radionuclide and stabile element contamination in Croatia: exposure assessment for consumers

Maja Lazarus[1] · Pavao Gančević[2] · Tatjana Orct[1] · Delko Barišić[3] · Klemen Jerina[4] · Nikica Šprem[2]

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Abstract

Muscle, liver and kidney of 21 Barbary sheep (*Ammotragus lervia*) from Mosor Mountain, Croatia, were sampled to quantify the activity of caesium and potassium radionuclides and five toxic and ten essential stabile elements in order to establish reference values for this species and to evaluate the potential of Barbary sheep tissues to reflect environmental pollution. We also assessed seasonal diet (botanical composition and dry matter content) of Barbary sheep based on analyses of a rumen content of culled animals. None of the 19 plant species (mostly grasses) identified as part of the Barbary sheep diet is known as a stabile element or radionuclide hyperaccumulator. Measured levels reflected low environmental pollution with arsenic, cadmium, mercury and lead, with levels generally less than those reported for wild herbivorous ungulates. Methodological differences (detection limit of elements in muscle) were shown to hamper interpretation and comparison of the Toxic Contamination Index (TCI) values with those published for other species. There was no homeostasis disturbance of trace elements in Barbary sheep, either due to inadequate intake via food or as an adverse effect due to a high toxic metal(loid) burden. Consumption of the muscle and liver of wild Barbary sheep can be considered safe for the health of adult consumers regarding toxic metal(loid)s and radioactive caesium, though the liver should be avoided as a food item in vulnerable population groups due to the possible adverse effects of cadmium and lead. Otherwise, muscle and liver are a rich source of copper, iron, selenium and zinc for consumers and, as such, can benefit the overall dietary intake of essential elements.

Keywords *Ammotragus lervia* · Exotic species · Toxic Contamination Index · Environmental pollution · Ungulate · Metal accumulation

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✉ Nikica Šprem
nsprem@agr.hr

[1] Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia

[2] Department of Fisheries, Beekeeping, Game Management and Special Zoology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia

[3] Laboratory for Radioecology, Centre for Marine and Environmental Research, Ruđer Bošković Institute, PO Box 160, Bijenička 54, 10002 Zagreb, Croatia

[4] Department of Forestry, Biotechnical Faculty, University of Ljubljana, Večna pot 83, 1000 Ljubljana, Slovenia

Introduction

Barbary sheep (*Ammotragus lervia*, Pallas 1777) is a species of Caprinae (goat-antelope) native to the mountains of Northern Africa (from Mauritania in the west to the Red Sea in the east) (Cassinelo 1998). Barbary sheep was also introduced for hunting or tourism purposes (in most cases illegally) in several parts of Europe (including Croatia) and North and South America (Cassinelo 1998; Cassinello et al. 2008; Mori et al. 2017). It does not adapt to all environments, preferring arid or semi-arid areas (Cassinello 2012). Human consumption and trade of its meat is confirmed on the African continent (Cassinello et al. 2008), though this has not officially confirmed elsewhere. Both the goat and sheep characteristics of this species (Cassinelo 1998) suggest that Barbary sheep meat might be consumed in local hunting communities worldwide. However, no studies are available on the standard nutritional or toxicological properties of this rare species' meat.

Diet largely affects the intake and exposure to environmental pollutants (French et al. 2017; Goretti et al. 2018). Also, diet of this species and thus direct sources of pollutants, are scarcely investigated in Europe (reviewed in Mori et al. 2017) without any data for the small and isolated Croatian population. Recent studies have shown that Barbary sheep is primarily a grazer, selecting forbs and grasses when available (Cassinello 2012; Miranda et al. 2012; Mimoun and Nouira 2015; Cassinello 2018). However, feeding behaviour of this species is highly flexible and can, in specific periods/areas, feed with numerous species of trees, shrubs and climbing plant species (Cassinelo 1998; Mimoun and Nouira 2015).

With regard to inorganic pollutants, both stable and radioactive elements cycle through air, water and soil to enter plant, animal and human organisms. Some organisms bioaccumulate or even biomagnify metals such as ^{137}Cs, Cd, Pb or Hg in their tissues, leading to the risk of exceeding threshold levels for adverse health effects in animals or their consumers (Ramanzin et al. 2010; Berzas Nevado et al. 2012; Patiño Ropero et al. 2016; Durkalec et al. 2018). Numerous studies have been performed using large wild mammals to characterise the element status in the environment inhabited by animals and humans and to estimate the risk for human health due to the similar trophic level (Jarzyńska and Falandysz 2011; Berzas Nevado et al. 2012; Bąkowska et al. 2016; Ertl et al. 2016; Larter et al. 2016; Lehel et al. 2017; Patiño Ropero et al. 2016; Skibniewski et al. 2017; Durkalec et al. 2018; Vukšić et al. 2018; Zacs et al. 2018). Elements in large herbivores have been studied more often than in sympatric carnivores, likely due to the wider distribution, larger populations, hunting status (easier sample collection) and direct link to humans in the food chain via consumption of their meats (Jarzyńska and Falandysz 2011; Dannenberger et al. 2013; Chiari et al. 2015; Ertl et al. 2016; Ferri et al. 2017; Larter et al. 2016; Lehel et al. 2017). Lately, game meat has found its market, not only in traditional communities, but also in urban cultures with growing awareness and demand for meat from non-intensively farmed animals (*organic* food), free of additives and pharmaceuticals (Kudrnáčová et al. 2018). However, free-ranging wild animals tend to have higher levels of toxic metals than farmed domestic animals (Kramárová et al. 2005; Forte and Bocca 2007; Durkalec et al. 2018), and their meats can occasionally exceed the maximum limits of Cd and Pb (Lazarus et al. 2014) set by the European Commission Decision No. 1881/2006 for contaminants in foodstuff (EC 2006). Unlike the marketed meat of wild animals that are subjected to tests for residual contaminants at the national level, non-marketed meat of common and rare species consumed directly by hunters is not controlled (Ramanzin et al. 2010; Lazarus et al. 2014). Therefore, the occurrence data obtained in this study would be the only available source of information for consumers about contaminants in an exotic species such as Barbary sheep. The most common contaminants of terrestrial wild animal meats are Cd and Pb, as their persistent environmental concentrations tend to accumulate with age in soft tissues (primarily kidney and liver as detoxifying tissues) of animals. Lead is often found even in muscle tissue in high amounts due to secondary contamination from lead ammunition (Tsuji et al. 2009). Other elements (e.g. As or Hg) are of minor toxicological relevance for the terrestrial food chain and large wild mammals, although in addition to the direct adverse effects in organ systems, many toxic elements demonstrate indirect damage via impairment of homeostasis of essential macroelements and trace elements (Jarzyńska and Falandysz 2011; Durkalec et al. 2018). Insufficient or excessive levels of Se (Flueck et al. 2012), Cu, Zn, Mo, Co (Frank 2004) and Ca (Bjorå et al. 2001) have been shown to be critical for the health or even life of wild herbivores, which, in turn, can negatively affect population size (Skibniewski et al. 2016).

The aim of this study was to assess (1) the dietary composition of Barbary sheep on Mt. Mosor, Croatia, to scan for possible pollutant hyperaccumulators; (2) the tissue levels of elements and radionuclides to estimate possible adverse effects that those could have on individual animals; (3) the potential of this species to reflect environmental contamination in this area by calculating the Toxic Contamination Index; and (4) the risk and benefit for consumers of Barbary sheep edible tissues.

Material and methods

Study area and species

Barbary sheep (21 individual in total; 15 males and 6 females) samples were collected on Mt. Mosor in the southern Dinarides (surface area 11.3 km^2, Lat 43° 31′ 54.2573″, Lon 16° 38′ 29.1241″) with the highest peak Veliki Kabal (1339 m) (Fig. 1). Mesozoic limestone sediments dominate through the area (Redžić 2011). In the belt higher than 1200 m, the Mediterranean climate, with a mean annual temperature of 10.8 °C and mean annual rainfall of 1665 mm, shifts to a wet boreal climate (Zaninović et al. 2008). Topographically, the study area is highly heterogeneous, interrupted by ditches, bays and rocks. Scrublands and woodlands of sub-Mediterranean and Euro-Mediterranean vegetation represent the typical habitat. To date, 540 vascular plant taxa have been described on Mt. Mosor (Vladović and Ilijanić 1992). Here, Barbary sheep coexists with other large mammal species: European mouflon (*Ovis aries musimon*, also introduced), wild boar (*Sus scrofa*) and grey wolf (*Canis lupus*) (Anonymous 2012). Soil studies obtained in the Dinaric region, including Mt. Mosor, revealed higher levels of toxic Cd, Hg and Pb and lower K than elsewhere in Croatia (Halamić and Miko 2009). The only anthropogenic pollution source of

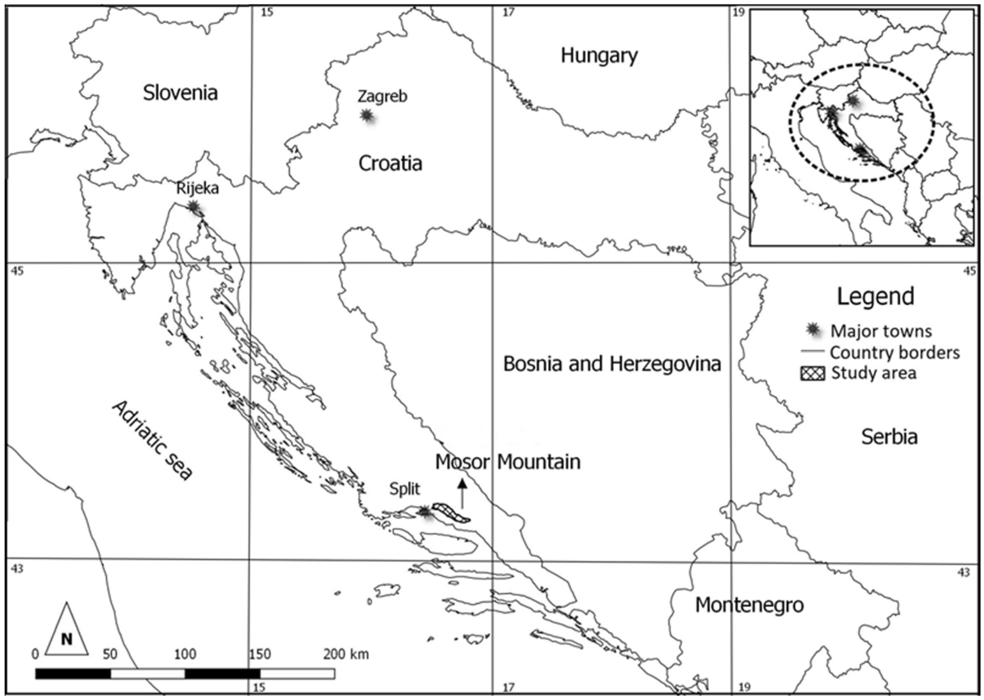

Fig. 1 Study area of Mt. Mosor in the southern Dinarides of Croatia (11.3 km^2)

this otherwise pristine mountain is a cement factory situated several kilometres southwest, in the suburbs of the city of Split (population 180,000; Census 2012). Along with long-range transported airborne particles, the factory is the most significant source of airborne As, Cd, Cr, Cu, Hg, Ni, Pb and Zn (Anonymous 2012), although minimal impacts to the mountain ecosystem are suggested due to prevailing northeaster wind (*Bura*) (Zaninović et al. 2008). Concerning the status of airborne radionuclides in the soil of this mountainous area, the higher ^{137}Cs levels compared to the rest of the country were primarily attributed to a higher degree of precipitation, correlated to higher altitudes (Šoštarić 2017).

Based on the 2008 IUCN Red List, Barbary sheep is categorised as a vulnerable (VU) species in its natural distribution range (Cassinello et al. 2008). Currently in Europe, free-ranging populations of Barbary sheep are present only in Spain, Italy, Czech Republic and Croatia (Cassinelo 1998; Bartoš et al. 2010; Gančević et al. 2016). Barbary sheep in these countries is a non-native species. All these populations were introduced by hunters, confirming the hypothesis of Carpio et al. (2017) that hunting is one of the major pathways for the introduction of exotic species into Europe. In 2002, five Barbary sheep (three females and two males) of unknown origin were illegally released in the southern Dinaric region (Mt. Mosor) of Croatia, and the current population size is estimated at around 140 animals (unpublished data, P. Gančević). This illegal introduction of Barbary sheep has been the source of much controversy between hunters and environmental community. Hunters perceive the introduced animals as an attractive game species and potential source of game meat and income via hunting tourism, while environmentalists stress the possible negative effects of introduced exotic species on native flora and fauna and call for the enforcement of existing laws that prescribe the complete removal of alien animals.

Diet analysis

Four rumen contents of Barbary sheep were examined for their botanical composition according Klansek et al. (1995) during all seasons. Rumen contents were mixed, and at least 500 ml was collected by hunters immediately after culling and stored at − 18 °C until analysis. The rumen contents were thoroughly washed through three sieves with mesh sizes of 6.3 mm, 3.15 mm and 1.0 mm under running water. This enabled separation into differently sized plant particles and also of the unidentifiable microscopic fraction. Subsequently, for the analysis of the qualitative and quantitative composition of the rumen contents, the plant fragments remaining in the sieves with the largest and medium mesh sizes were separated according to their morphological characteristics (e.g. formation of the leaf margin, leaf top and bottom, veining, presence of hair and its morphology) into the following plant groups: (i) herbs and perennials, (ii) grasses, (iii) deciduous trees, (iv) conifers, (v) shrubs, (vi) dwarf shrubs, (vii) feed and (viii) cryptogams. The particles remaining in the sieve with the smallest mesh size or in the washing water were examined microscopically for determination of the type of different plants or concentrates (pollen, spores, starch, etc.). In order to determine the proportions of individual plant groups in the total dry weight of each rumen content sample, the test material was dried in evaporating dishes at a temperature of 65 °C in a desiccator until weight constancy was achieved. The dry matter was determined gravimetrically, and the proportion of the dry matter of the entire rumen content sample was calculated as the percentage of weight.

Radionuclide activity measurement

A total of 10 Barbary sheep muscle tissue samples (minimum of 500 g) were collected during the regular hunting season (hunting season open year-round pursuant to Croatian Hunting Act). Samples were cleaned of fat and connective tissues, placed in counting vessels (125 cm^3) of known geometry and kept frozen at a temperature of − 18 °C until gamma-spectrometric analysis was conducted. Activities of ^{137}Cs and ^{40}K were measured by the gamma-spectrometric method using an HPGe semiconductor detector system coupled to a Canberra 8196 channel analyser. The counting time was 80,000 s. The obtained spectra were analysed by Genie 2000 Canberra software. ^{137}Cs activities were calculated from the photo peak at 661.6 keV while activities of ^{40}K were calculated from the photo peak at 1460.7 keV. The detector system was calibrated using mixed gamma standards supplied by Eckert & Ziegler (Analytics USA). Efficiency of the system was regularly checked during inter-comparison calculations. Precision and accuracy of the system were additionally checked by the simultaneous measurement of IAEA Reference Materials since the laboratory is accredited by the Croatian Accreditation Agency for gamma-spectrometric measurements (HRN EN ISO/IEC 17025:2007). It is noteworthy that efficiency was calculated as a function of energy and geometry at the base of the experimental data. The measured uncertainty, multiplied by the coverage factor $k = 2$, was calculated as the sum of net peak area uncertainty, efficiency uncertainty and background fluctuation uncertainty.

The effective equivalent dose that an individual receives due to radionuclide ingestion from the consumption of contaminated meat was calculated using following relationship:

$$H_{\mathrm{E}} = A_{\mathrm{k}} \times m \times D_{\mathrm{kf(k)}}$$

where H_{E} is the effective equivalent dose (Sv), A_{k} is the average concentration of k radionuclide (Bq kg^{-1}), m is the quantity of consumed meat (muscle tissue) (kg) and $D_{\mathrm{kf(k)}}$ is the dose conversion factor for k radionuclide. A dose conversion factor of 1.3 Sv $Bq^{-1} \times 10^{-8}$ Sv Bq^{-1} was used for ^{137}Cs (IAEA 1996).

Element analyses

The outer layer of the semi-thawed muscle and liver of 15 individuals was removed with a ceramic knife to sample adhering tissue. The kidney cortex was sampled after the removal of the thick renal capsule protecting the tissue from secondary contamination. The percentage of moisture was calculated from sample weights before and after freeze-drying. Freeze-dried samples were digested in an UltraCLAVE IV (Milestone, Italy) microwave digestion system before the elements (As, Ca, Cd, Co, Cu, Fe, Hg, K, Mg, Mn, Mo, Pb, Se, Tl and Zn) were quantified by means of inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500cx, Germany) following the procedure detailed elsewhere (Vihnanek Lazarus et al. 2013). Ultrapure water (18 MΩ cm; GenPure system, TKA, Germany) and purified (duoPUR, Milestone, Italy) nitric acid (p.a. 65%; Merck, Germany) were used for sample preparation and dilution. To monitor the analytical quality of digestion and measurement, duplicate samples of certified reference materials (CRMs) BCR-185R bovine liver, BCR-186 pig kidney (Institute for Reference Materials and Measurements, Geel, Belgium) and DORM-2 dogfish muscle (National Research Council Canada, Ottawa, Ontario) and standard reference material (SRM) 1577a bovine liver (National Institute of Standards and Technology, USA) were included in each digestion series. The obtained and certified values of CRMs and SRM expressed on a dry mass basis are summarised in Table S1 as supplementary material together with the method detection limits for each element. All Barbary sheep tissue data were expressed on a wet mass (w.m.) basis. For the calculation of wet mass concentrations, we used the mean moisture percent for muscle, liver and kidney cortex (75%, 69% and 75%, respectively), corresponding to conversion factors (f) of 4.0, 3.2 and 4.0 (wet mass concentration = dry mass concentration/f), respectively.

Toxic Contamination Index

The Toxic Contamination Index (TCI) was calculated to estimate the contamination level of the mountainous area where Barbary sheep reside, as the TCI was reported to give more a robust and suitable overview of the biological response to environmental metal contamination than tissue metal levels or liver-to-muscle ratios alone (Goretti et al. 2018). Calculation of the TCI, as proposed by Goretti et al. (2018), is based on metal(loid) level (e.g. Pb) in liver (Pb_L) or kidney (Pb_K) as the tissues where the majority of toxic metals are detoxified and accumulated, metal level in muscle (Pb_M) as inert tissue concerning detoxification and human tolerable weekly intake/benchmark dose values proposed by the European Food Safety Authority (EFSA) for the intake of As (EFSA 2014), Cd (EFSA 2011), Hg (EFSA 2012) and Pb (EFSA 2010) from food. Human tolerable weekly intake (TWI)/benchmark dose lower confidence limit at 1% extra risk ($BMDL_{01}$) was used, as similar safety margins for animals do not exist in the literature. The following equation is used to calculate the TCI (e.g. for Pb):

$$TCI_{Pb} = \log\left((Pb_L)^2/(Pb_M \times BMDL_{01Pb})\right).$$

Exposure assessment

To perform the nutritional and risk analysis of Barbary sheep meat consumption with regard to the intake of essential and non-essential elements, the most recent EFSA data for dietary reference values (DRVs) for essential elements (EFSA 2017) and TWI and benchmark doses (BMDL) for non-essential elements were applied (EFSA 2010, 2011, 2012, 2014). The same estimations were also performed for the consumption of Barbary sheep liver. Calculations were based on the mean and 95th percentile (worst-case scenario) element occurrence in the muscle and liver of Barbary sheep and the assumption that consumers eat only this game species. As in an earlier study (Lazarus et al. 2014), kidney consumption was assumed to be negligible. Consumption frequency of game mammals in the Croatian adult consumer was taken from the EFSA Comprehensive European Food Consumption Database, which published the results of a Croatian food consumption survey conducted in 2011–2012 on 2002 individuals. The estimated daily intake (EDI) of each element was calculated on the basis of both mean (43.4 g) and 95th percentile (80.2 g, for calculation of worst-case scenario) daily portions reported for the Croatian general population, game consumers only (average and P95 consumers).

Statistical procedures

Inter-tissue differences in element levels and TCI values were compared by the Wilcoxon matched-pairs test, as the condition of normality and homogeneity of variance were not met in all data. Statistical procedures were performed using Statistica for Windows 13.0 (StatSoft, Inc., Tulsa, USA).

Results and discussion

This study is one of the first studies to list the plant species consumed by Barbary sheep in Europe (see Miranda et al. 2012 for Spain) and the first one that quantified elements in Barbary sheep tissues transferred from plants and further to humans as game consumers. Element data from mountainous populations of wild animals are very rare due to the somewhat difficult sample collection but have the unique potential to reflect airborne pollutants that enter the soil-plant-animal-human food chain. Namely, certain anthropogenic toxic metals and gamma-emitting radionuclides of high relevance for humans, such as Pb and ^{137}Cs, are found in higher concentrations in mountains due to the higher precipitation and high altitudes that stimulate dry and wet deposition of metal-bound particles (Halamić and Miko 2009; Šoštarić 2017). Contamination of this mountainous habitat and Barbary sheep was assessed using the Toxic Contamination Index.

Animal diet composition

The results of the botanical composition in Barbary sheep rumen contents revealed 19 plant species and 19 genera belonging to 14 families (Table S2). During the winter season, the proportion of dry mass was highest (57.3 g) and the diet was also most variable, consisting of 11 different plant species. The diet analysis showed that this species feeds mainly on grasses, as the quantitative proportion of different grasses amounted to more than 80% in spring, autumn and winter (Fig. 2). During summer, grasses are poorly present in the diet, as the high daily temperatures burn all the grasses in the area; during summer, the main diet of Barbary sheep is deciduous trees, i.e. *Ostrya carpinifolia* (71%) and *Carpinus orientalis* (22%).

In recent studies, Barbary sheep has been classified as grazer, primarily selecting forbs and grasses when available (Miranda et al. 2012; Mimoun and Nouira 2015; Cassinello 2018), so it might be suited to an empty niche in the Mediterranean environment (Cassinello 2012). Although we cannot infer the species feeding selection (we have no data on food availability), our data seems to align with this classification. It is, however, noteworthy that the number and spectrum of species and taxa detected in our food samples were very wide, indicating that the Barbary sheep may be extremely plastic in the utilisation of the available food resources, as previously reported in other studies (e.g. Gray 1980; Krysl et al. 1980; Rodríguez Piñero and Rodríguez Luengo 1992). None of the plant species identified as part of the Barbary sheep diet (Table S1) is known as a stabile element or radionuclide hyperaccumulator (Thijs et al. 2017).

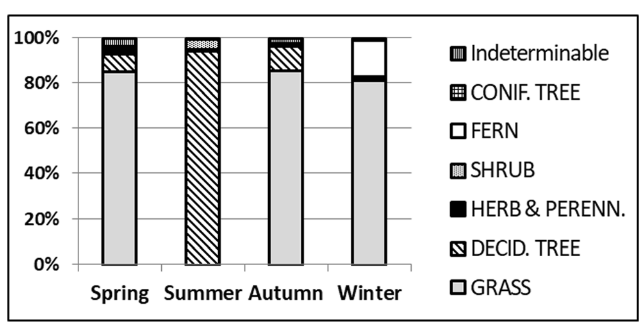

Fig. 2 Percentage of ingested plant groups according to seasons for Barbary sheep from Croatia

Presence of essential elements

The levels of each essential element in muscle, liver and kidney tissues of Barbary sheep are summarised in Table 1. The highest level of macroelements Mg and K was found in muscle tissue, while Ca was the most abundant in the kidney, a critical organ for the regulation of Ca serum levels. Compared to levels from wild herbivorous ungulates from the recent literature, muscle Mg and K in Barbary sheep showed little variation, unlike Ca, which was up to five times lower than that reported for chamois, red deer and roe deer from Austria, Poland and Kazakhstan (Długaszek and Kopczyński 2013; Assenova et al. 2016; Ertl et al. 2016).

Trace elements Cu, Mn, Mo and Zn were most abundant in the liver, Co and Fe in the liver and kidney and Se in the kidney of studied animals (Table 1). Barbary sheep seemed to have the lowest reported Co muscle levels compared to chamois, red deer and roe deer (Jarzyńska and Falandysz 2011; Ertl et al. 2016), although the hepatic and renal ranges fit the values for these species. The renal Cu of Barbary sheep was in accordance with the data reported for Croatian domestic sheep (Bilandžić et al. 2010). In contrast to similar muscle and renal levels, some variation in the hepatic Cu of Barbary sheep was seen in comparison with red deer from Croatia (Lazarus et al. 2008), Poland (Jarzyńska and Falandysz 2011), Italy (Chiari et al. 2015) and Scotland (French et al. 2017) and with roe deer (Wieczorek-Dąbrowska et al. 2013) and European bison (Durkalec et al. 2018). Two to 10 times higher Cu in the liver may be explained by the choice of plant species ingested by the Barbary sheep or due to the vicinity of the cement factory as a source of Cu emissions. Hepatic Cu would reflect the intake and status of the organism, as the liver is the primary organ for Cu metabolism (Davis and Mertz 1986). Fe in all tissues of Barbary sheep was in the same order of magnitude as measured in Croatian fallow deer (Vukšić et al. 2018), red deer (Lazarus et al. 2008), Polish bison (Kośla et al. 2013), chamois, red deer and roe deer (Dannenberger et al. 2013; Długaszek and Kopczyński 2013; Assenova et al. 2016; Ertl et al. 2016). Mn tissue levels were within the range of reported values for chamois (Ertl et al. 2016), red deer (Jarzyńska and Falandysz 2011; Ertl et al. 2016; French et al. 2017), roe deer (Długaszek and Kopczyński 2013; Ertl et al. 2016) and European bison (Durkalec et al. 2018), although the muscle of red deer from Poland (Jarzyńska and Falandysz 2011) and Caspian red deer (Assenova et al. 2016) was reported to contain 10 and 80 times higher levels, respectively, than the Barbary sheep. There is good accordance with liver and kidney levels of Mo in Barbary sheep from Croatia and in red deer from Poland, but as in the case with Mn and Co, muscle levels were the lowest reported. Selenium content in Barbary sheep tissues was in line or even higher than that in Se-supplemented

Table 1 Differences in radionuclide and stabile element levels in the muscle, liver and kidney of Barbary sheep (*Ammotragus lervia*) from Croatia

	N	Muscle			Liver			Kidney cortex		
		Mean (median)	SEM	Range	Mean (median)	SEM	Range	Mean (median)	SEM	Range
H_2O (%)	15	75			69			75		
As (μg kg^{-1})	15	0.725 (0.751)[a]	0.038	0.441–0.960	1.27 (0.982)[b]	0.17	0.571–2.80	1.70 (1.48)[b]	0.23	0.658–3.71
Ca (mg kg^{-1})	15	37.9 (36.3)[a]	2.24	27.8–58.5	53.0 (52.9)[b]	2.89	36.7–73.7	94.4 (82.0)[c]	6.9	59.1–147
Cd (μg kg^{-1})	15	0.671 (0.519)[a]	0.153	0.230–2.61	210 (203)[b]	21	43.9–345	1310 (874)[c]	358	54.7–5560
Co (μg kg^{-1})	15	0.836 (0.683)[a]	0.143	0.398–2.65	35.7 (22.0)[b]	6.1	13.7–75.9	36.4 (31.2)[b]	4.6	14.0–70.0
Cu (mg kg^{-1})	15	1.21 (1.11)[a]	0.16	0.648–3.34	37.1 (35.8)[b]	4.9	8.95–69.7	4.09 (3.64)[c]	0.41	2.04–7.33
Fe (mg kg^{-1})	15	27.8 (25.0)[a]	2.26	17.6–53.5	71.6 (72.7)[b]	2.6	52.7–85.5	82.0 (54.0)[b]	17.6	36.6–304
Hg (μg kg^{-1})	15	0.248 (0.256)[a]	0.027	0.097–0.489	5.60 (6.67)[b]	0.71	1.68–9.94	8.39 (6.92)[b]	1.48	1.52–19.6
K (mg kg^{-1})	15	4051 (4051)[a]	116	3433–5013	2948 (2998)[b]	124	2329–4002	2424 (2341)[c]	164	1615–3704
Mg (mg kg^{-1})	15	251 (246)[a]	5.93	225–310	173 (183)[b]	9.05	68.5–201	192 (165)[b]	22	119–438
Mn (mg kg^{-1})	15	0.087 (0.067)[a]	0.018	0.034–0.327	2.74 (2.90)[b]	0.20	1.47–3.73	1.10 (1.08)[c]	0.08	0.538–1.81
Mo (μg kg^{-1})	15	0.982 (0.757)[a]	0.166	0.508–2.97	805 (722)[b]	120	212–1455	323 (316)[c]	40	71.7–581
Pb (μg kg^{-1})	15	1.93 (0.560)[a]	0.740	0.170–11.3	58.8 (38.5)[b]	10.4	22.2–130.6	70.3 (64.8)[b]	13.3	13.4–223
Se (mg kg^{-1})	15	0.080 (0.080)[a]	0.007	0.041–0.128	0.168 (0.161)[b]	0.01	0.108–0.260	1.72 (1.50)[c]	0.12	1.10–2.55
Tl (μg kg^{-1})	15	1.16 (0.811)[a]	0.160	0.640–2.48	6.25 (4.51)[b]	1.88	0.980–24.3	13.4 (12.8)[c]	2.27	2.46–27.5
Zn (mg kg^{-1})	15	47.5 (50.1)[ab]	4.18	23.5–78.7	42.2 (33.5)[a]	7.1	18.2–105	34.9 (34.5)[b]	3.42	18.9–67.3
^{137}Cs (Bq kg^{-1})	10	3.50 (3.61)	0.065	2.11–4.50	–	–	–	–	–	–
^{40}K (Bq kg^{-1})	10	136 (152)	1.85	82.7–163	–	–	–	–	–	–

Different superscript letters (a, b, c) within each row point to differences between tissues identified by the Wilcoxon matched-pairs test, with significance at $p < 0.05$. All element levels are expressed on a wet mass basis

Croatian fallow deer (Vukšić et al. 2018), but lower than that measured in red deer (Lazarus et al. 2008), both species hunted in eastern Croatia, distant from Mt. Mosor. We found similar muscle levels as in other European countries, though liver Se in Spanish red deer (Berzas Nevado et al. 2012; Patiño Ropero et al. 2016), Polish red deer (Jarzyńska and Falandysz 2011) and bison (Durkalec et al. 2018) was roughly half than that found here. Zinc levels showed a very narrow range of values among species and countries.

To the extent of our knowledge, there are no literature data for Barbary sheep trace elements, so the measured levels were compared with available data for wild herbivorous ungulates. As such, this variation in element levels may originate from inter-species or geographical differences. Mt. Mosor is a relatively pristine area with a cement factory in the foothills, with the prevailing wind carrying the only possible source of metal pollution in the area away from the mountain. Little influence from geographical variation is expected as the essential elements in mammals are regulated to maintain homeostasis in areas and conditions without notable contamination with elements (e.g. Cd or Hg). Namely, biological interactions between non-essential and essential elements are known to affect the distribution and status of essential elements in wild ungulates: Zn/Cu/Se-Cd (Lazarus et al. 2008; Reglero et al. 2009) and Hg-Se (Berzas Nevado et al. 2012).

The normal range of macroelements and essential trace elements has not been defined for wild ungulates, so we compared the measured concentrations in Barbary sheep with tissue values defined as adequate or deficient in domestic animals (goat/sheep). Hepatic and renal levels were in the range of adequate values reported for sheep and goat (Puls 1994). Nevertheless, mean Se in the liver (168 μg kg^{-1} wet mass) appeared to be marginal according to the reference range for sheep (150–250 μg kg^{-1} wet mass; Puls 1994), but even the animal with the lowest Se (108 μg kg^{-1} wet mass) had hepatic levels above those reported for deficient animals (10–100 μg kg^{-1} wet mass; Puls 1994). Farmed red deer affected by white muscle disease had < 35 μg kg^{-1} wet mass of Se in the liver (Wilson and Grace 2001). As such, the occurrence of white muscle disease or other adverse effect due to Se deficiency is unlikely in the examined Barbary sheep.

Presence of non-essential (toxic) elements

The content of toxic metals measured in the tissues of Barbary sheep is presented in Table 1, suggesting the kidney (and liver in case of As, Hg and Pb) is the target organ for the distribution of As, Cd, Hg, Pb and Tl, as noted before in other wild ungulate species (Jarzyńska and Falandysz 2011; Hermoso de Mendoza García et al. 2011; Berzas Nevado et al. 2012; Wieczorek-Dąbrowska et al. 2013; Lazarus et al. 2014; Chiari et al. 2015; Bąkowska et al. 2016; Patiño Ropero et al. 2016; Lehel et al. 2017; Skibniewski et al. 2017; Vukšić et al. 2018).

Barbary sheep had the lowest levels of metalloid As in comparison to Croatian fallow deer (Vukšić et al. 2018), chamois, red deer and roe deer hunted in Austria (Ertl et al. 2016). The literature data suggest that Cd is the most widely quantified toxic metal in wild herbivorous ungulates. Its presence in even unpolluted areas, accumulative nature and trophic transfer within the soil-plant-herbivore-human axis (Nordberg et al. 2015) prompted studies aiming at assessing food risk safety (Lazarus et al. 2008; Ramanzin et al. 2010; Jarzyńska and Falandysz 2011; Lazarus et al. 2014; Chiari et al. 2015; Ferri et al. 2017). The muscle tissues tested here contained the lowest levels of Cd, as expected, as Cd distributes mainly to the liver and kidney. Among the reported ungulates from Croatia, the muscle Cd of Barbary sheep was in the lower range and in strong agreement with data from Austria (Ertl et al. 2016) and Spain (Hermoso de Mendoza García et al. 2011) but was lower than that in red deer (Jarzyńska and Falandysz 2011; Chiari et al. 2015) and roe deer (Długaszek and Kopczyński 2013) from Poland and Italy. Bison (Durkalec et al. 2018), moose (Larter et al. 2016; Skibniewski et al. 2017) and mountain caribou (Larter et al. 2016) were species whose offal showed enhanced Cd accumulation compared to Barbary sheep, with 10–50 times higher renal Cd levels. These inter-species differences may be attributed to the affinity of bison and moose to browse on willow (*Salix* sp.) (Larter et al. 2016; Durkalec et al. 2018) and to the high intake of lichen in the caribou diet (Larter et al. 2016). A diet dominated by lichens known to readily absorb atmospheric contaminants, Cd among others, and willow as a known Cd hyperaccumulator plant (Tsuji et al. 2009), differs greatly in comparison with plants used by the Barbary sheep in Croatia (Table S2). Low renal Hg and hepatic and renal Pb levels found in Barbary sheep were closer to the values reported for Croatian domestic sheep (Bilandžić et al. 2010) than those for wild herbivorous ungulates from Croatia and other countries. Muscle Hg and Pb levels were up to 10 times lower than those in other species (Lazarus et al. 2008; Hermoso de Mendoza García et al. 2011; Jarzyńska and Falandysz 2011; Długaszek and Kopczyński 2013; Lazarus et al. 2014; Ertl et al. 2016; Lehel et al. 2017; Vukšić et al. 2018), while hepatic Hg content was comparable (Lazarus et al. 2008; Berzas Nevado et al. 2012; Lazarus et al. 2014; Patiño Ropero et al. 2016; Durkalec et al. 2018; Vukšić et al. 2018). Wild ungulate tissue results should be commented with caution regarding the source of Pb, as Pb incorporated into organ cells after environmental intake can be masked with Pb-based ammunition particles (Tsuji et al. 2009). Although toxic, Tl is, similar to hepatic levels of bison (Durkalec et al. 2018), present in low levels in Barbary sheep tissues, but its level varies compared to the data published for red deer from Poland (Jarzyńska and Falandysz 2011).

Liver and kidney As, Cd, Hg, Pb and Tl of Barbary sheep were in the range of normal values defined for sheep (Puls 1994) and below the terrestrial mammalian effects threshold level for Pb (liver > 30,000 μg kg^{-1} dry mass, kidney > 90,000 μg kg^{-1} dry mass; Ma 1996) and Hg (> 30,000 μg kg^{-1} wet mass; Thompson 1996). However, renal Cd levels in Barbary sheep exceeded the normal range values but were below the levels defined as high (4000–12,000 μg kg^{-1} wet mass) for domestic sheep (Puls 1994) and much lower than the nephropathy threshold of 100,000–200,000 μg kg^{-1} wet mass in mammals (Scheuhammer 1991). In general, Cd is more abundant in free-ranging wildlife offal than in farmed domestic animals, and this difference was confirmed even within the same species (e.g. free-ranging European bison [0.370 mg kg^{-1} wet mass] vs. captive [0.187 mg kg^{-1} wet mass]; Durkalec et al. 2018).

The Toxic Contamination Index applied previously on results for mustelids (Goretti et al. 2018) was calculated for Barbary sheep to test it as an indicator of environmental contamination (Fig. 3). TCI values from 0.7 for As_L to 5.7 for Cd_K in Barbary sheep were higher than values reported for stone and pine marten from Italy and Croatia ($Cd_L < 3$, $Pb_L < 2.5$; Goretti et al. 2018) and for species occupying a higher trophic niche and inhabiting more anthropised environments than Barbary sheep. Such TCI differences are mostly influenced by 30 (for Pb) to 60 (for Cd) times lower muscle levels and similar liver levels measured in Barbary sheep compared to mustelid species (recalculated from dry mass based on the conversion factor 3.18) due to analytical method limitations (detection limit 43 μg Cd kg^{-1} vs. 0.21 μg Cd kg^{-1}, and 42 μg Pb kg^{-1} vs. 1.71 μg Pb kg^{-1} in Goretti et al. (2018) vs. this study, respectively). Thus, TCI (at least for Cd and Pb) is strongly dependent on the analytical properties of the method applied, due to very low levels of toxic metals in meat. Thereby, the TCI comparison between studies is applicable only when similar instrumentation conditions are met. In light of these facts, we suggest that the interpretation of lower than unit and negative TCI values as the one pointing at poorly contaminated site condition and those much higher than unit pointing at polluted sites (Goretti et al. 2018) should be taken with caution. In our case, TCI values for Pb (3.6–3.8) and Cd (4.2–5.7) in Barbary sheep tissues indicate certain pollution in the habitat, but comparison with the reported literature data from ungulates living in uncontaminated areas and the available threshold data for toxicity in domestic animals indicates that Barbary sheep is a species with a very low toxic metal burden and its habitat is a pristine area.

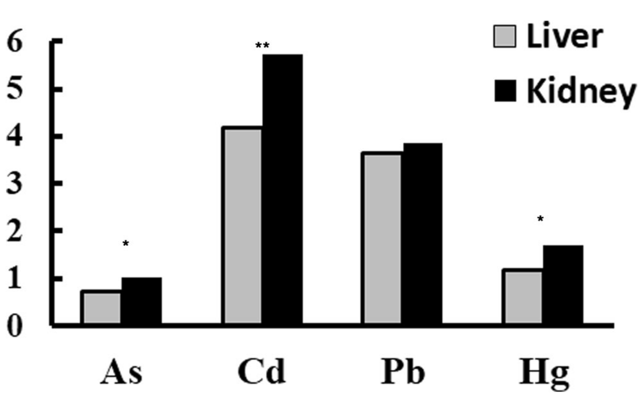

Fig. 3 Mean Toxic Contamination Index (TCI) for As, Cd, Pb and Hg calculated for liver and kidney of Barbary sheep. Differences tested with the Wilcoxon matched-pairs test, *$p < 0.05$, **$p < 0.001$

Risk/benefit assessment for consumers

The presented efforts to quantify toxic metal(loid)s in Barbary sheep meat are in line with the EFSA call for the collection of inorganic contaminant occurrence data in food (with a focus on Cd, Pb, As and Hg) to enable exposure assessment for consumers.

Cadmium in 40% of Barbary sheep kidneys (6/15) exceeded the legislative maximum level (1000 μg kg^{-1} wet mass) set for meat and offal of farmed animals (EC 2006), while Cd and Pb levels in other tissues complied with the legislation. As mentioned by Ferri et al. (2017), muscle and liver, but not kidney, are preferred food items among consumers of wild ungulates. As such, the most frequently consumed tissues are all within the safety margins regarding Cd and Pb.

A risk/benefit assessment was performed for the intake of essential and non-essential elements for consumers of Barbary sheep meat (Tables 2 and 3) and liver (Table S3 in the Supplementary material). In the earlier exposure assessment for Croatian consumers of game (Lazarus et al. 2014), official data of consumption frequency were not available. As such, three proposed consumption scenarios (Lazarus et al. 2014) considered the much lower daily intake of game meat (21.4 g/5 g/1.64 g) than the Croatian food consumption survey revealed (80.2 g/43.4 g). Thus, the amount of game meat eaten per day is the main methodological difference between this and the earlier study. Our estimation (Table 2) showed that the nutritional contribution of essential elements from Barbary sheep muscle consumption is low (0.1% of DRV for Mn and Mo) to significant (39% of DRV for Zn) in average and P95 game consumers in Croatian population. In addition to Zn, muscle is also a good source of Fe, Cu, Se and K, with a daily portion adding 5–27% to the recommended DRV for respective elements (Table 2). When compared to domestic animals, we can conclude that Barbary sheep meat is a similar source of Fe, Mg and Zn as Croatian bovine, sheep and horse meat, but a better source of Zn than pork meat (Bilandžić et al. 2013; Bilandžić et al. 2014). Other authors found red deer meat and offal as a good source of Cu, Zn and Se in human nutrition, and kidney as also a good source of Co, Cr and Mn (Jarzyńska and Falandysz 2011).

Table 2 Nutritional contribution of Barbary sheep muscle consumption regarding essential elements

Element	Level in muscle (mg kg^{-1} w.m.)	DRV (mg day^{-1})	Consumption frequency			
			Mean		P95	
	Mean (P95)		EDI (mg per 43.4 g)	% DRV	EDI (mg per 80.2 g)	% DRV
Ca	37.9 (58.5)	750[a]–*1000*[b]	1.64 (2.54)	0.2 (0.3)	3.04 (4.70)	0.3 (0.5)
Cu	1.21 (3.34)	1.3–*1.6*[a]	0.053 (0.145)	3 (9)	0.097 (0.268)	6 (17)
Fe	27.8 (53.5)	6[c]–*16*[b]	1.21 (2.32)	8 (15)	2.23 (4.29)	14 (27)
K	4051 (5013)	3500[a]	176 (217)	5 (6)	325 (402)	9 (11)
Mg	251 (310)	300–*350*[a]	10.9 (13.4)	3 (4)	20.1 (24.8)	6 (7)
Mn	0.087 (0.327)	3[a]	0.004 (0.014)	0.1 (0.5)	0.007 (0.026)	0.2 (1)
Mo	9.82×10^{-4} (29.7×10^{-4})	0.065[a]	4.26×10^{-5} (12.9×10^{-5})	0.1 (0.2)	7.88×10^{-5} (23.9×10^{-5})	0.1 (0.4)
Se	0.080 (0.128)	0.07[a]	0.003 (0.006)	5 (8)	0.006 (0.010)	9 (15)
Zn	47.5 (78.7)	*7.5–16.3*[a]	2.06 (3.41)	13 (21)	3.81 (6.31)	23 (39)

P95 95th percentile, *w.m.* wet mass, *DRV* dietary reference values for element (> 18 years), *EDI* estimated daily intake of element (mg per daily portion), calculated by taking into account the mean and 95th percentile (in parenthesis) level of element in tissue and two different consumption frequencies: mean (43.4 g per day) or 95th percentile (80.2 g per day) amount of consumed game mammals per day, based on data from Croatian food consumption survey on adults, general population-consumers only (EFSA Comprehensive European Food Consumption Database)

[a] Adequate intake for Ca, Cu, K, Mg, Mn, Mo, Se and Zn (EFSA 2017); values in italics were taken in the calculation of % DRV

[b] Population reference intake for Ca and Fe (EFSA 2017); values in italics were taken in the calculation of % DRV

[c] Average requirement for Fe (EFSA 2017)

Safe intake levels of non-essential (toxic) As, Cd, Hg and Pb from food considered free of risk of adverse health effects for consumers were not exceeded either with average or with P95 consumption frequency of Barbary sheep muscle (Table 3). With Cd and Pb as the most frequently reported priority contaminants in game meat, consumption of meat from this study would add 0.1 to 3% TWI/$BMDL_{01}$. As such, the contribution of toxic metal(loid)s from Barbary sheep meat to the overall diet of Croatian consumers can be considered low and consumption safe, even in the worst-case scenario conditions predicted here (P95 consumer of meat which contains P95 percentile level of toxic elements). Ferri et al.

Table 3 Risk estimation of Barbary sheep muscle consumption regarding toxic elements

Element	Level in muscle (μg kg^{-1} w.m.)	TWI/$BMDL_{01}$ (μg kg^{-1} b.m.)	Consumption frequency			
			Mean		P95 ($_{wcs}$)	
	Mean (P95)		EDI (ng per 43.4 g kg^{-1} b.m.)	% TWI/$BMDL_{01}$	EDI (ng per 80.2 g kg^{-1} b.m.)	% TWI/$BMDL_{01}$
As	0.725 (0.960)	*0.3*–8[a]	0.449 (0.595)	0.1 (0.2)	0.831 (1.10)	0.3 (0.4)
Cd	0.671 (2.61)	2.5[b]	0.416 (1.62)	0.1 (0.5)	0.769 (2.99)	0.2 (1)
Hg	0.248 (0.489)	4[c]	0.154 (0.303)	0.03 (0.05)	0.284 (0.561)	0.05 (0.1)
Pb	1.93 (11.3)	0.5[d]	1.20 (7.00)	0.2 (1)	2.21 (12.9)	0.4 (3)

P95 95th percentile, *w.m.* wet mass, *TWI* tolerable weekly intake, *$BMDL_{01}$* benchmark dose lower confidence limit at 1% extra risk, *EDI* estimated daily intake of element (ng of element per daily portion per 70 kg body mass person), calculated by taking into account the mean and 95th percentile (in parenthesis) level of element in tissue and two different consumption frequencies: mean (43.4 g per day) or 95th percentile (80.2 g per day) amount of consumed game mammals per day, based on data from Croatian food consumption survey on adults, general population-consumers only (EFSA Comprehensive European Food Consumption Database); *wcs* worst case scenario: 95th percentile of element in tissue and 95th percentile (80.2 g per day) amount of consumed game mammals per day; *b.m.* body mass

[a] $BMDL_{01}$ for As for an increased risk of cancer of the lung, skin and bladder, and skin lesions (EFSA 2014); values in italics were taken in the calculation of % $BMDL_{01}$

[b] TWI for Cd (EFSA 2011)

[c] TWI for Hg (EFSA 2012)

[d] $BMDL_{01}$ for Pb for developmental neurotoxicity (EFSA 2010)

(2017) recorded that liver consumption was often connected with that of game meat, although the consumed amounts of meat largely prevail over liver (Lazarus et al. 2014). In the lack of official data about game offal consumption frequency in Croatia, we used the same scenario as for average muscle consumption, although it should be stressed that the scenario of chronic daily consumption of 43.4 g liver is unlikely. Our findings (Table S3) show that the average consumers of Barbary sheep liver meet their dietary requirements for Cu and Mo (54–189% DRV). Although the adequate intake for Cu (1.3–1.6 mg day^{-1}; EFSA 2017) would be surpassed with liver consumption (1.61–3.02 mg Cu portion^{-1}), those amounts would be similar to the average daily intake assessed by EFSA for adults (1.15–2.07 mg Cu day^{-1}; EFSA 2017) and thus can be considered safe for health. In addition, liver was shown to be a good source of Fe, Se and Zn (16–28% DRV) with also a significant amount of K, Mg and Mn added to their daily nutrition (2–5% DRV). Among the toxic metal(loid)s measured here, Cd intake through liver consumption is of highest concern (Table S3), with the average daily consumption adding 36–60% TWI to the amount ingested daily from other dietary or environmental sources. Exposure to Pb (7–16% $BMDL_{01}$) can be considered low if no other significant occupational or dietary sources enhance the overall intake of Pb in the human body. Hg and As intake from Barbary sheep liver is very low (0.3–1% TWI/$BMDL_{01}$).

Radionuclides

Radiocaesium in Croatia originates from nuclear weapon testing and the Chernobyl accident in the twentieth century. It is persistent, has high gastrointestinal uptake rate in herbivores and humans and, due to its chemical similarity, replaces naturally occurring potassium (^{40}K), thereby elevating the radiological dose. Potassium availability can affect uptake and biomagnification of caesium in the food chain (Šprem et al. 2013) so ^{40}K and ^{137}Cs should be monitored simultaneously. The concentrations of ^{137}Cs and ^{40}K in muscle samples of Barbary sheep from Mt. Mosor are shown in Table 1. Activities of both elements in the analysed samples are found at levels significantly below the statutory values of 600 Bq kg^{-1}. The average mass activity of ^{137}Cs in this study was 3.5 Bq kg^{-1} (maximum 4.50 Bq kg^{-1} ± 0.78 Bq kg^{-1}), which is almost the same activity obtained in other mountain ungulates, such as Alpine chamois, with reported activities of 2.93 Bq kg^{-1} by Šprem et al. (2013) and a few becquerels per kilogram by Pourcelot et al. (2003). European ungulate meat ^{137}Cs contamination has declined since the Chernobyl fallout in 1986 (Ramanzin et al. 2010). ^{40}K mass activity showed equal dispersal with average mass activity of 136.07 Bq kg^{-1} (maximum 163 Bq kg^{-1} ± 22 Bq kg^{-1}), as previously reported for different ungulates (Šprem et al. 2013) and carnivore species (Šprem et al. 2016). The descriptive statistics indicated that the measured ^{40}K mass activity was uniformly distributed among the samples. A similar and parallel trend of the two radionuclides was also confirmed with this study, as Barbary sheep with a lower ^{40}K mass activity of 82.7 Bq kg^{-1} also has the lowest load of ^{137}Cs of 2.11 Bq kg^{-1}. The estimated effective equivalent dose (H_E) for consumption of 1 kg of Barbary sheep meat was 0.046 μSv. This low H_E value is similar to certain other ungulate species, such as Alpine chamois (0.038 μSv), red deer (0.032 μSv) and roe deer (0.030 μSv) (Šprem et al. 2013). Therefore, we can conclude that the meat of herbivore ungulate species can be excluded as being a general risk as a foodstuff regarding caesium concentrations.

Conclusions

As the first study reporting Barbary sheep tissue contents of radionuclide and stabile elements, it can be concluded that the measured levels reflected low environmental (likely atmospheric) pollution with As, Cd, Hg and Pb, with levels generally less than those reported for wild herbivorous ungulates. Methodological differences (lower detectable levels of elements in muscle of our animals) hampered the interpretation and comparison of TCI values with the previously published data (for mustelids). Our results suggest that there was no homeostasis disturbance of trace elements in Barbary sheep, either due to inadequate intake via food or as an adverse effect due to high toxic metal(loid) burden. Consumption of the muscle and liver of wild Barbary sheep can be considered safe for the health of adult consumers regarding toxic metal(loid)s and radioactive caesium, though the liver should be avoided as a food item in vulnerable population groups (children and child-bearing and lactating women) for the possible adverse effects of Cd and Pb. Otherwise, muscle and liver are shown to be a rich source of Cu, Fe, Se and Zn for consumers and, as such, can benefit the overall dietary intake of essential elements.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Anonymous (2012) Izvješće o stanju okoliša u Splitsko–dalmatinskoj županiji za razdoblje od 2008. do 2011. godine. Splitsko – dalmatinska županija, Upravni odjel za graditeljstvo, komunalne poslove, infrastrukturu i zaštitu okoliša, Split, p 398 [in Croatian]

Assenova B, Okuskhanova E, Rebezov M, Korzhikenova N, Yessimbekov Z, Dragoev S (2016) Trace and toxic elements in meat of maral (red deer) grazing in Kazakhstan. Res J Pharm, Biol Chem Sci 7(1):1425–1433

Bąkowska M, Pilarczyk B, Tomza-Marciniak A, Udała J, Pilarczyk R (2016) The bioaccumulation of lead in the organs of roe deer (*Capreolus capreolus* L.), red deer (*Cervus elaphus* L.), and wild boar (*Sus scrofa* L.) from Poland. Environ Sci Pollut Res Int 23(14): 14373–14382

Bartoš L, Kotrba R, Pintíř J (2010) Ungulate and their management in the Czech Republic. In: Apollonio M, Andersen R, Putman R (eds) European ungulates and their management in the 21st century. Cambridge University Press, Cambridge, pp 243–261

Berzas Nevado JJ, Rodríguez Martín-Doimeadios RC, Mateo R, Rodríguez Fariñas N, Rodríguez-Estival J, Patiño Ropero MJ (2012) Mercury exposure and mechanism of response in large game using the Almadén mercury mining area (Spain) as a case study. Environ Res 112:58–66

Bilandžić N, Đokić M, Sedak M (2010) Survey of arsenic, cadmium, copper, mercury and lead in kidney of cattle, horse, sheep and pigs from rural areas in Croatia. Food Addit Contam Part B Surveill 3(3): 172–177

Bilandžić N, Sedak M, Đokić M, Varenina I, Solomun-Kolanović B, Božić Đ, Brstilo M, Sokolić-Mihalak D, Jurković Z (2013) Comparative study of iron, magnesium and zinc and daily intakes in certain meats and meat products. Slov Vet Res 50(3):103–110

Bilandžić N, Sedak M, Đokić M, Solomun-Kolanović B, Božić Đ, Brstilo M, Šimić B (2014) Determination of zinc concentrations in foods of animal origin, fish and shellfish from Croatia and assessment of their contribution to dietary intake. J Food Compos Anal 35(2):61–66

Bjorå R, Falch JA, Staaland H, Nordsletten L, Gjengedal E (2001) Osteoporosis in the Norwegian moose. Bone 29(1):70–73

Carpio A, Guerrero-Casado J, Barasona J, Tortosa F, Vicente J, Hillström L, Delibes-Mateos M (2017) Hunting as a source of alien species: a European review. Biol Invasions 19:1197–1211

Cassinelo J (1998) *Ammotragus lervia*: a review on systematics, biology, ecology and distribution. Ann Zool Fenn 35:149–162

Cassinello J (2012) The Mediterranean's mosaic landscape and its survival: from pastoralism to the role of exotic species. Lychnos 9:48–55

Cassinello J (2018) Misconception and mismanagement of invasive species: the paradoxical case of an alien ungulate in Spain. Conserv Lett 11:e12440

Cassinello J, Cuzin F, Jdeidi T, Masseti M, Nader I, De Smet K (2008) *Ammotragus lervia*. The IUCN Red List of threatened species 2008: e.T1151A3288917. https://doi.org/10.2305/IUCN.UK.2008.RLTS.T1151A3288917.en, Accessed 5 April 2018

Census (2012) Census of population, households and dwellings 2011. Croatian Bureau of Statistics, Zagreb http://www.dzs.hr/, Accessed 20 March 2018

Chiari M, Cortinovis C, Bertoletti M, Alborali L, Zanoni M, Ferretti E, Caloni F (2015) Lead, cadmium and organochlorine pesticide residues in hunted red deer and wild boar from northern Italy. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32(11):1867–1874

Dannenberger D, Nuernberg G, Nuernberg K, Hagemann E (2013) The effects of gender, age and region on macro- and micronutrient contents and fatty acid profiles in the muscles of roe deer and wild boar in Mecklenburg-Western Pomerania (Germany). Meat Sci 94(1):39–46

Davis GK, Mertz W (1986) Copper. In: Mertz W (ed) Trace elements in human and animal nutrition, vol 1, 5th edn. Academic, Orlando, pp 301–364

Długaszek M, Kopczyński K (2013) Elemental composition of muscle tissue of wild animals from central region of Poland. Int J Environ Res 7(4):973–978

Durkalec M, Nawrocka A, Krzysiak M, Larska M, Kmiecik M, Posyniak A (2018) Trace elements in the liver of captive and free-ranging European bison (*Bison bonasus* L.). Chemosphere 193:454–463

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

EFSA (2010) Scientific opinion on lead in food. European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM). EFSA J 8(4):1570

EFSA (2011) Statement on tolerable weekly intake for cadmium. European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM). EFSA J 9(2):1975

EFSA (2012) Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM). EFSA J 10(12):2985

EFSA (2014) Dietary exposure to inorganic arsenic in the European population. European Food Safety Authority (EFSA). EFSA J 12(3): 3597

EFSA (2017) Dietary reference values for nutrients. Summary report. European Food Safety Authority (EFSA) supporting publication 2017:e15121, pp. 92

Ertl K, Kitzer R, Goessler W (2016) Elemental composition of game meat from Austria. Food Addit Contam Part B Surveill 9(2):120–126

Ferri M, Baldi L, Cavallo S, Pellicanò R, Brambilla G (2017) Wild game consumption habits among Italian shooters: relevance for intakes of cadmium, perfluorooctanesulphonic acid, and ^{137}cesium as priority contaminants. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 34(5):832–841

Flueck WT, Smith-Flueck JM, Mionczynski J, Mincher BJ (2012) The implications of selenium deficiency for wild herbivore conservation, a review. Eur J Wildl Res 58(5):761–780

Forte G, Bocca B (2007) Quantification of cadmium and lead in offal by SF-ICP-MS: method development and uncertainty estimate. Food Chem 105(4):1591–1598

Frank A (2004) A review of the "mysterious" wasting disease in Swedish moose (*Alces alces* L.) related to molybdenosis and disturbances in copper metabolism. Biol Trace Elem Res 102(1–3):143–159

French AS, Shaw D, Gibb SW, Taggart MA (2017) Geochemical landscapes as drivers of trace and toxic element profiles in wild red deer (*Cervus elaphus*). Sci Total Environ 601–602:1606–1618

Gančević P, Šprem N, Jerina K (2016) Space use and activity patterns of introduced Barbary sheep (Ammotragus lervia) in southern Dinarides, Croatia. In: Hadjisterkotis, E. (Ed.), Abstract book of 6th world congress on mountain ungulates and 5th international symposium on mouflon, Nicosia, Cyprus, pp. 41

Goretti E, Pallottini M, Cenci Goga BT, Selvaggi R, Petroselli C, Vercillo F, Cappelletti D (2018) Mustelids as bioindicators of the environmental contamination by heavy metals. Ecol Indic 94(1):320–327

Gray GG (1980) Aspects of Barbary sheep (*Ammotragus lervia*): biology in Palo Duro Canyon, Texas [Ph.D. thesis]. Texas Tech University, Lubbock, pp. 175

Halamić J, Miko S (2009) Geochemical atlas of the Republic of Croatia. Croatian Geological Survey, Zagreb, p 87 [in Croatian]

Hermoso de Mendoza García M, Hernández Moreno D, Soler Rodríguez F, López Beceiro A, Fidalgo Alvarez LE, Pérez López M (2011) Sex- and age-dependent accumulation of heavy metals (Cd, Pb and Zn) in liver, kidney and muscle of roe deer (*Capreolus capreolus*) from NW Spain. J Environ Sci Health A Tox Hazard Subst Environ Eng 46(2):109–116

IAEA (1996) International Atomic Energy Agency safety standards series. Safety series no. 115. IAEA, Vienna

Jarzyńska G, Falandysz J (2011) Selenium and 17 other largely essential and toxic metals in muscle and organ meats of red deer (*Cervus elaphus*)—consequences to human health. Environ Int 37(5):882–888

Klansek E, Vavra I, Onderscheka K (1995) Die Äsungszusammensetzung des Alpensteinwildes (*Capra i. ibex* L.) in Abhängigkeit von Jahreszeit, Alter und Äsungsangebot in Graubünden. Z Jagdwiss 41:171–181

Kośla T, Skibniewski M, Skibniewska E, Urbańska-Słomka G (2013) The iron content in organs of free ranging European bison from the Białowieża herd. Ann Anim Sci 13(2):357–364

Kramárová M, Massányi P, Slamecka J, Tataruch F, Jancová A, Gasparik J, Fabis M, Kovacik J, Toman R, Galová J, Jurcik R (2005) Concentration of cadmium in liver and kidneys of some wild and farm animals. Bull Vet Inst Pulawy 49(4):465–469

Krysl LJ, Simpson CD, Gray GG (1980) Dietary overlap of sympatric Barbary sheep and mule deer in Palo Duro Canyon, Texas. In: Simpson CD (ed) Proceedings of the symposium on ecology and management of Barbary sheep. Texas Tech University Press, Lubbock

Kudrnáčová E, Bartoň L, Bureš D, Hoffman LC (2018) Carcass and meat characteristics from farm-raised and wild fallow deer (*Dama dama*) and red deer (*Cervus elaphus*): a review. Meat Sci 141:9–27

Larter NC, Macdonald CR, Elkin BT, Wang X, Harms NJ, Gamberg M, Muir DC (2016) Cadmium and other elements in tissues from four ungulate species from the Mackenzie Mountain region of the Northwest Territories, Canada. Ecotoxicol Environ Saf 132:9–17

Lazarus M, Orct T, Blanuša M, Vicković I, Šoštarić B (2008) Toxic and essential metal concentrations in four tissues of red deer (*Cervus elaphus*) from Baranja, Croatia. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25(3):270–283

Lazarus M, Prevendar Crnić A, Bilandžić N, Kusak J, Reljić S (2014) Cadmium, lead, and mercury exposure assessment among Croatian consumers of free-living game. Arh Hig Rada Toksikol 65:281–292

Lehel J, Zwillinger D, Bartha A, Lányi K, Laczay P (2017) Food safety aspects of primary environmental contaminants in the edible tissues of roe deer (*Capreolus capreolus*). Environ Sci Pollut Res Int 24(32):25372–25382

Ma WC (1996) Lead in mammals. In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife: interpreting tissue residues. CRC Press Inc, Boca Raton, pp 281–296

Mimoun JB, Nouira S (2015) Food habits of the aoudad *Ammotragus lervia* in the Bou Hedma mountains, Tunisia. S Afr J Sci 111:11–12

Miranda M, Sicilia M, Bartolomé J, Molina-Alcaide E, Gálvez-Bravo L, Cassinello J (2012) Contrasting feeding patterns of native red deer and two exotic ungulates in a Mediterranean ecosystem. Wildl Res 39:171–182

Mori E, Mazza G, Saggiomo L, Sommese A, Esattore B (2017) Strangers coming from the Sahara: an update of the worldwide distribution, potential impacts and conservation opportunities of alien aoudad. Ann Zool Fenn 54(5–6):373–386

Nordberg GF, Nogawa K, Nordberg M (2015) 32. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M (eds) Handbook on the toxicology of metals, vol 2, 4th edn. Academic, Amsterdam, pp 667–716

Patiño Ropero MJ, Rodríguez Fariñas N, Mateo R, Berzas Nevado JJ, Rodríguez Martín-Doimeadios RC (2016) Mercury species accumulation and trophic transfer in biological systems using the Almadén mining district (Ciudad Real, Spain) as a case of study. Environ Sci Pollut Res Int 23(7):6074–6081

Pourcelot L, Renaud P, Louvat D, Gurriaran R, Richon P (2003) Influence of hot spots on cesium-137 contamination of an alpine food chain and doses associated with it. Environ Risque Santé 2: 112–120

Puls R (1994) Mineral levels in animal health. Diagnostic data. Sherpa International, Clearbrook

Ramanzin M, Amici A, Casoli C, Esposito L, Lupi P, Marsico G, Mattiello S, Olivieri O, Ponzetta MP, Russo C, Trabalza M (2010) Meat from wild ungulates: ensuring quality and hygiene of an increasing resource. Ital J Anim Sci 9(3):318–331

Redžić S (2011) Phytogeographic and syntaxonomic diversity of high mountain vegetation in Dinaric Alps (Western Balkan, SE Europe). J Mt Sci 8:767–786

Reglero MM, Taggart MA, Monsalve-González L, Mateo R (2009) Heavy metal exposure in large game from a lead mining area: effects on oxidative stress and fatty acid composition in liver. Environ Pollut 157(4):1388–1395

Rodríguez Piñero JC, Rodríguez Luengo JL (1992) Autumn food habits of the Barbary sheep (*Ammotragus lervia* Pallas, 1772) on La Palma Island (Canary Islands). Mammalia 56:385–392

Scheuhammer AM (1991) Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environ Pollut 71(2–4):329–375

Skibniewski M, Skibniewska EM, Kośla T, Olbrych K (2016) The content of copper and molybdenum in the liver, kidneys, and skeletal muscles of elk (*Alces alces*) from North-Eastern Poland. Biol Trace Elem Res 169:204–210

Skibniewski M, Skibniewska EM, Kośla T, Olbrych K (2017) Relationship between Cd and Zn concentration in the kidneys, liver, and muscles of moose (*Alces alces*) from North-Eastern Poland. Environ Sci Pollut Res Int 24(1):598–604

Šoštarić M (2017) Radiological properties of soil in the Republic of Croatia, [Ph.D. thesis]. Faculty of Science, University of Zagreb, Zagreb. [in Croatian]

Šprem N, Babić I, Barišić D, Barišić D (2013) Concentration of ^{137}Cs and ^{40}K in meat of omnivore and herbivore game species in mountain forest ecosystems of Gorski Kotar, Croatia. J Radioanal Nucl Chem 298:513–517

Šprem N, Piria M, Barišić D, Kusak J, Barišić D (2016) Dietary items as possible sources of ^{137}Cs in large carnivores in the Gorski Kotar forest ecosystem, Western Croatia. Sci Total Environ 542A:826–832

Thijs S, Langill T, Vangronsveld J (2017) The bacterial and fungal microbiota of hyperaccumulator plants: small organisms, large influence. Adv Bot Res 83:43–86

Thompson DR (1996) Mercury in birds and terrestrial mammals. In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife: interpreting tissue residues. CRC Press Inc, Boca Raton, pp 341–356

Tsuji LJ, Wainman BC, Jayasinghe RK, VanSpronsen EP, Liberda EN (2009) Determining tissue-lead levels in large game mammals harvested with lead bullets: human health concerns. Bull Environ Contam Toxicol 82(4):435–439

Vihnanek Lazarus M, Sekovanić A, Kljaković-Gašpić Z, Orct T, Jurasović J, Kusak J, Reljić S, Huber Đ (2013) Cadmium and lead in grey wolf liver samples: optimization of a microwave-assisted digestion method. Arh Hig Rada Toksikol 64:395–403

Vladović D, Ilijanić LJ (1992) A contribution to the flora of Mosor Mountain (Croatia). Acta Bot Croat 51:143–150 [in Croatian with English abstract]

Vukšić N, Šperanda M, Lončarić Z, Đidara M, Ludek E, Budor I (2018) The effect of dietary selenium addition on the concentrations of heavy metals in the tissues of fallow deer (*Dama dama* L.) in Croatia. Environ Sci Pollut Res Int 25(11):11023–11033

Wieczorek-Dąbrowska M, Tomza-Marciniak A, Pilarczyk B, Balicka-Ramisz A (2013) Roe and red deer as bioindicators of heavy metals contamination in North-Western Poland. Chem Ecol 29(2):100–110

Wilson PR, Grace ND (2001) A review of tissue reference values used to assess the trace element status of farmed red deer (*Cervus elaphus*). N Z Vet J 49(4):126–132

Zacs D, Rjabova J, Ikkere LE, Bavrins K, Bartkevics V (2018) Brominated flame retardants and toxic elements in the meat and liver of red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), and moose (*Alces alces*) from Latvian wildlife. Sci Total Environ 621:308–316

Zaninović K, Gajić-Čapka M, Perčec Tadić M, Vučetić M, Milković J, Bajić A, Cindrić K, Cvitan L, Katušin Z, Kaučić D, Likso T, Lončar E, Lončar Ž, Mihajlović D, Pandžić K, Patarčić M, Smec L, Vučetić V (2008) Klimatski atlas Hrvatske / Climate atlas of Croatia 1961–1990, 1971–2000. Državni hidrometeorološki zavod, Zagreb [in Croatian]