Cadmium, Lead, and Mercury Concentrations in Tissues of Roe Deer (*Capreolus capreolus* L.) and Wild Boar (*Sus scrofa* L.) from Lowland Croatia

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Abstract

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Cadmium, lead, and mercury concentrations were determined in roe deer and wild boar tissues (muscle, liver, kidney) of three different age groups from lowland Croatia. Cadmium concentrations in the examined tissues increased with age in both species, being the highest in the kidney, and higher in roe deer as compared to wild boar. Lead concentration was higher in younger animals in comparison with both older groups. Contrary to the expectations, roe deer tissues revealed mostly higher lead concentrations than those of wild boar. Mercury concentration in the tissues of the animals examined was relatively low and no correlation with age was found. However, mercury concentration was higher in kidney than in liver with both species. Wild boar had higher mercury concentrations in tissues than roe deer in all age groups. Cadmium and mercury concentrations in both species from lowland Croatia are comparable to those given in similar studies in other European countries, while lead concentration was lower in wild boar and higher in roe deer tissues than those in the same species from European countries. From the hygienic point of view, the muscle samples from roe deer and wild boar were edible as the concentrations of cadmium and lead did not exceed the values prescribed by the official regulations. However, cadmium concentration in liver exceeded the prescribed values in one fifth of all samples while lead concentrations were lower than the allowed concentrations. Most kidney samples from both animal species contained cadmium exceeding the recommended concentrations, while lead concentrations in all samples did not exceed the official value regulation. Mercury concentrations are no longer (since 2008) a matter of legislative.

Keywords: roe deer; wild boar; heavy metal; tissue concentrations; legislation

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The technological progress of the human kind has caused rapid increases in the concentrations of various contaminants (including heavy metals) in the environment (air, soil, water) and, consequently, in different living organisms (plants, animals, humans). Cadmium, lead, and mercury as nonessential metals are the most important and are among the most frequent contaminants due to the poor potential of natural detoxification and their accumulation tendencies. The actual risk presented by these metals to wildlife and humans is mostly demonstrated as a chronic-sub lethal effect (immunopathology, teratogenicity, carcinogenicity, and changes in the reproductive system) and requires pollution monitoring procedures. Wild animals, especially game species like red deer and wild boar, are suitable as bioindicators (Kottferova & Korenekova 1998; Pokorny 2000; Falandysz *et al*. 2005), due to their large geographical distribution, residential way of life, feeding habits, relatively long life-span, and easy sample collection (regular hunting activities).

The aim of this study was to determine the concentrations of selected metals in various roe deer and wild boar tissues in relation to age and different feeding habits. Based on the parameters obtained, evaluation of environmental contamination and potential negative effects on roe deer and wild boar and, subsequently, human health status was discussed. The results obtained were compared with related previous and recent literature data.

MATERIAL AND METHODS

The samples were collected during the regular hunting seasons (2007–2009) from three different open state hunting grounds in lowland Croatia. Two smaller ones, Črnovšćak No. I/3 – covering an area of 2158 ha and Pokupski bazen No. IV/9 – 8183 ha are situated in the western part and the third, and bigger one, Spačva No. XVI/11 – 25018 ha, is situated in the eastern part of Croatia (Figure 1).

All three regions are characterised by intensive agricultural production and thereby the use of various pesticides and fertilisers, but they lack industrial activities inside them. Two smaller hunting grounds are bordered by a major road while it passes through the third one.

All shot animals were classified by age based on the guidelines given by specific literature (Wagenknecht 1984). The samples of muscle, liver, and kidney tissues were packed separately in polyethylene bags, frozen, properly marked, and stored at –18°C until analysis. The tissues damaged with bullets were not sampled. Before analysis, the samples were thawed slightly and rinsed with deionised water. In brief, approximately 1.5 g of the muscle, liver, and kidney cortex were weighed for lead and cadmium determination, dried at 105°C and then dry ashed overnight in quartz crucibles in a muffle furnace at 450°C. The ash residues were then dissolved in concentrated nitric acid, heated and made up to 5 ml with deionised water. For mercury determination, wet digestion in a closed system was used. About 0.8 g of the thawed muscle, liver, and kidney cortex were digested in 1 ml of concentrated nitric acid overnight at room temperature in open long-necked glass tubes. The next day, digestion was performed at 80°C in closed tubes for 5 h in a programmed system (DS-40, Tecator, Höganäs, Sweden). After cooling, the samples were adjusted to 5 ml with deionised water. Blanks were run with each the sample set to control the sample contamination during the procedures. Cadmium and lead were measured by Perkin Elmer Analyst 600 atomic absorption spectrometer (Shelton, Washington, USA) equipped with a transversely heated graphite furnace unit, autosampler, and longitudinal Zeeman effects background correction. Argon was used as the purge gas. A mixture of magnesium nitrate and

Figure 1. Sampling area (in black)

palladium nitrate was used as matrix modifier. Total mercury was measured in a mercury analyser (AMA 254, LECO, St. Joseph, USA) using total decomposition, amalgamation, and atomic absorption spectrometry (TDA-AAS) (Boylan *et al.* 2001). At least two replicate determinations were made with each sample. Two certified reference materials were used in duplicates together with each sample series for the method validation in order to check the accuracy: bovine liver 1577b (National Institute of Standards and Technology, Gaithersburg, USA) for Cd and Pb and bovine liver BCR-185R (Institute for Reference Materials and Measurements, Gul, Belgium) for Hg. The results were as follows (mean ± SD; µg/g dry weigh – DW; n= 7): 0.484 ± 0.040 for Cd (certified value: 0.500 ± 0.03 µg/g DW), 0.128 ± 0.010 for Pb (certified value: 0.129 ± 0.004 µg/g DW) and 0.005 ± 0.001 for Hg (reference value: 0.004–0.007 µg/g DW). The detection limits were calculated as three times the standard deviation of at least ten measurements of the reagent blank. The detection limits in the sample, based on the sample fresh weight of 1.5 g (dry ashing) or 0.8 g (wet digestion) and the final sample volume of 5 ml, were: 0.09, 1.84, and 0.417 µg/kg for Cd, Pb, and Hg, respectively. In relation to age, the animals were divided into three age groups: young animals – roe deer 8 to12 months old (n = 10), piglets 4 or 6 months old (n = 10); middle-aged animals – roe deer 3 and 4 years old (n = 11), wild boar around 2 years old (n = 19); old animals – roe deer over 5 years old (n = 13), wild boar 5 or 6 years old (n = 11).

Kolmogorov-Smirnov test was used to verify the normality of distribution of the collected data in each group. The significance of differences between average metal concentrations in tissues of different animal species (roe deer and wild boar) according to the age category was determined. For this purpose, we used Student t-test for the parameters with normal distribution, while those parameters that did not follow the normal distribution were analysed with Mann-Whitney U test. The analysis of variance was used for determining the significance of differences of metal concentrations between three age categories within individual animal species (One way ANOVA, with Unequal n HSD test for post-hoc analysis, or Kruskal Wallis analysis of variance). For each parameter we established the mean, standard deviation, median, as well as upper and lower quartiles.

All the above procedures were performed by Statistics 8.0 software (StatSoft Inc. 2009).

RESULTS

The results are presented in nine figures showing the median values, lower and upper quartiles, and min. and max. values of different roe deer and wild boar age groups from lowland Croatia.

Cadmium median range in muscle tissues of roe deer was 0.0008–0.0060 µg/g while that of wild boar tissues was 0.0007–0.0026 µg/g; in liver tissues of roe deer 0.155–0.527 µg/g while of wild boar 0.053 to 0.458 µg/g; in kidney tissues of roe deer 0.953

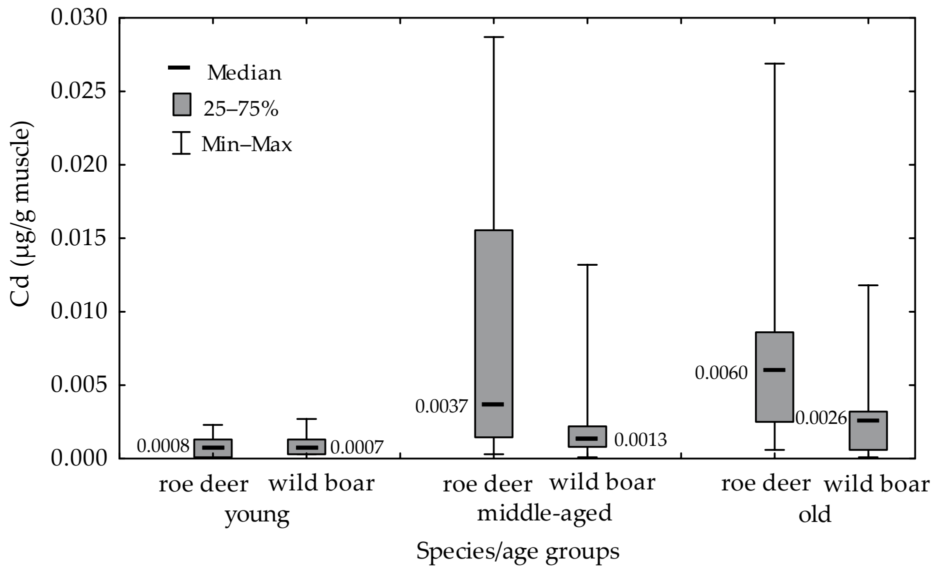


Figure 2. Median cadmium concentrations (µg/g) in muscle tissues of three roe deer and wild boar age groups

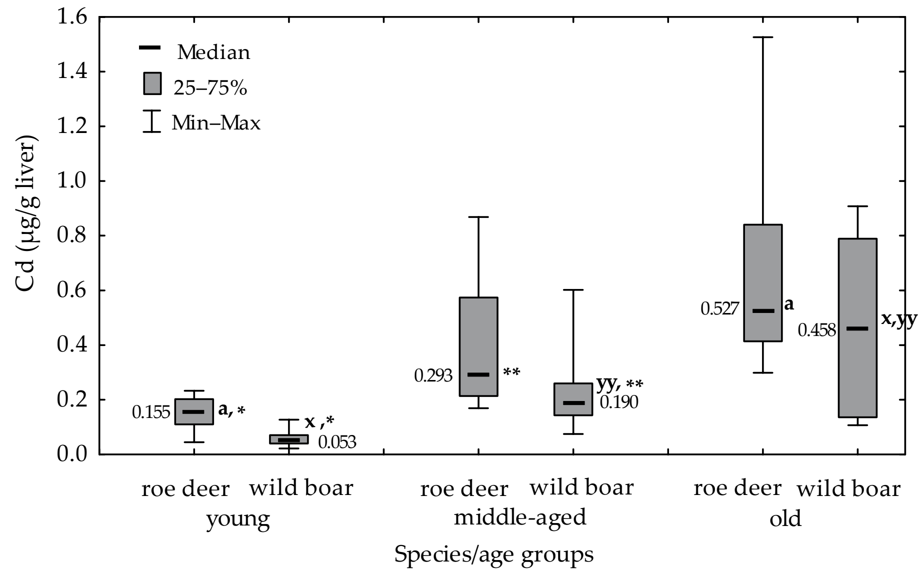


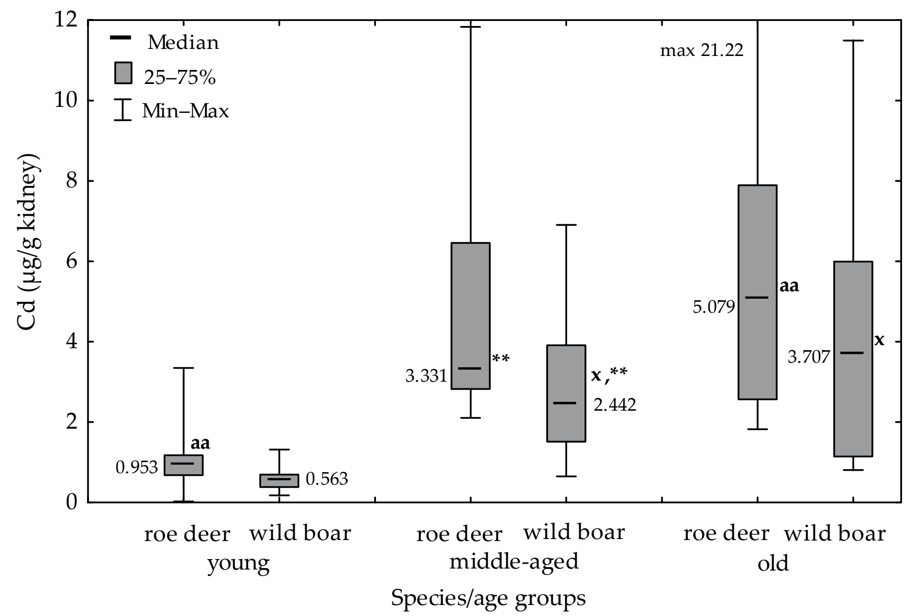
Figure 3. Median cadmium concentrations (μg/g) in liver tissues of three roe deer and wild boar age groups

a,x = $P < 0.01$ between individuals of same species
yy = $P < 0.05$ between individuals of same species
** = $P < 0.05$ between individuals of different species and same age groups

to 5.079 μg/g while of wild boar 0.563–3.707 μg/g. Lead median range in muscle tissues of roe deer was 0.001–0.034 μg/g while of wild boar it was 0.002–0.015 μg/g; in liver tissues of roe deer 0.018 to 0.055 μg/g while of wild boar 0.025–0.041 μg/g; in kidney tissues of roe deer 0.024–0.057 μg/g while of wild boar 0.020–0.032 μg/g. Mercury median range in muscle tissues of roe deer was 0.0012–0.0014 μg/g while of wild boar it was 0.0071–0.0116 μg/g; in liver tissues of roe deer 0.0027–0.0112 μg/g while of wild boar 0.0225–0.0395 μg/g; in kidney tissues of roe deer 0.024–0.096 μg/g while of wild boar 0.063–0.129 μg/g.

Positive correlation between muscle cadmium concentration and age was established for both species, but it was not statistically significant (Figure 2). Young animals of both species showed similar cadmium concentrations, while middle-aged and older roe deer revealed higher cadmium concentrations in comparison with wild boar, but again with no statistical significance.

Liver cadmium concentrations increased with age in both species. Statistical significance was established between young and old roe deer, young and old wild boars, and between middle-aged and old wild boars. Cadmium concentration was higher in roe deer compared to wild boar in all three age groups, while statistical significance was found between young animals and middle-aged animals (Figure 3).



x = $P < 0.01$ between individuals of same species
aa = $P < 0.05$ between individuals of same species
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Figure 4. Median cadmium concentrations (μg/g) in kidney tissues of three roe deer and wild boar age groups

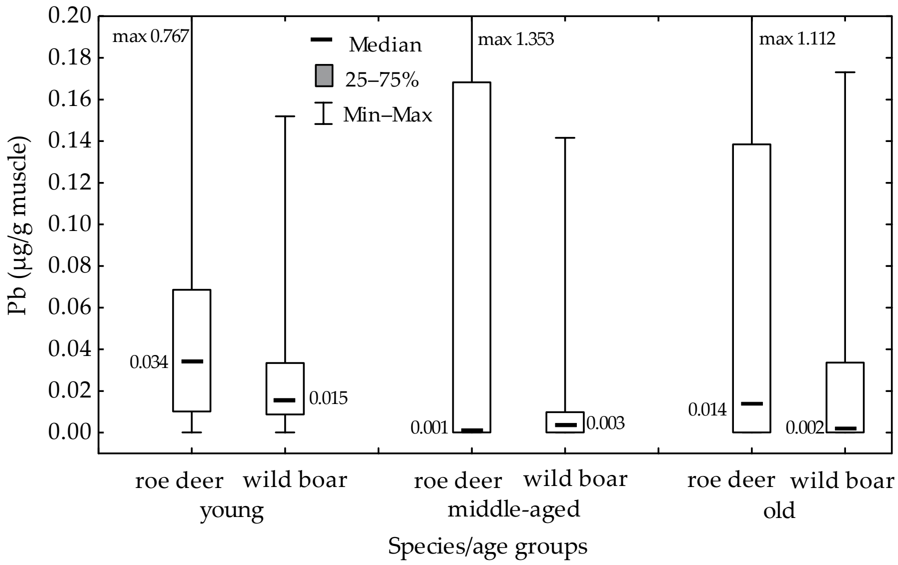


Figure 5. Median lead concentrations (μg/g) in muscle tissues of three roe deer and wild boar age groups

Kidney cadmium concentration increased with age. Statistically significant difference was established between young and old roe deer, and middle-aged and old wild boars. Roe deer accumulated higher concentration of cadmium compared to wild boar in all three age groups. Statistically significant difference was established only between the middle-aged individuals (Figure 4).

The highest lead concentration was found in muscle tissues of young animals of both species. Middle-aged roe deer had slightly lower lead concentration compared to old individuals, while middle-aged wild boar showed a similar concentration compared to old animals. Roe deer revealed higher muscle lead concentration in relation to wild boar with the exception of middle-aged animals (Figure 5). No difference was statistically significant.

The liver of the middle-aged roe deer contained the highest lead concentration, a slightly lower concentration was present in the young individuals, while the old individuals demonstrated the lowest concentration. In wild boar, the highest concentration was found in the old animals, followed by the middle-aged and young animals. Roe deer showed a higher liver lead concentration in relation to wild boar with the exception of the old animals (Figure 6). No difference was statistically significant.

Lead concentration in the kidney tissue of roe deer decreased with age without any statistical significance. In wild boar, similar lead concentrations were found in the young and middle-aged

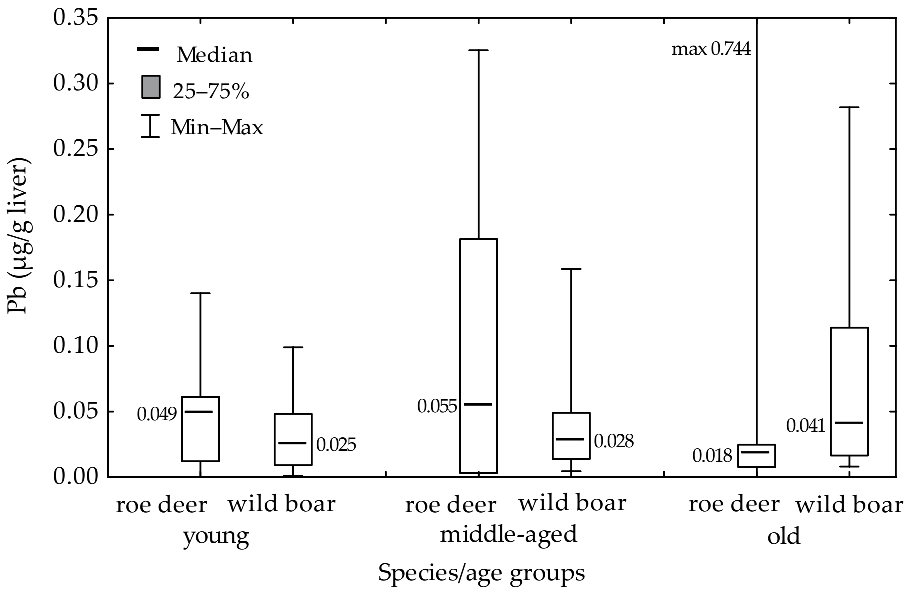


Figure 6. Median lead concentrations (μg/g) in liver tissues of three roe deer and wild boar age groups

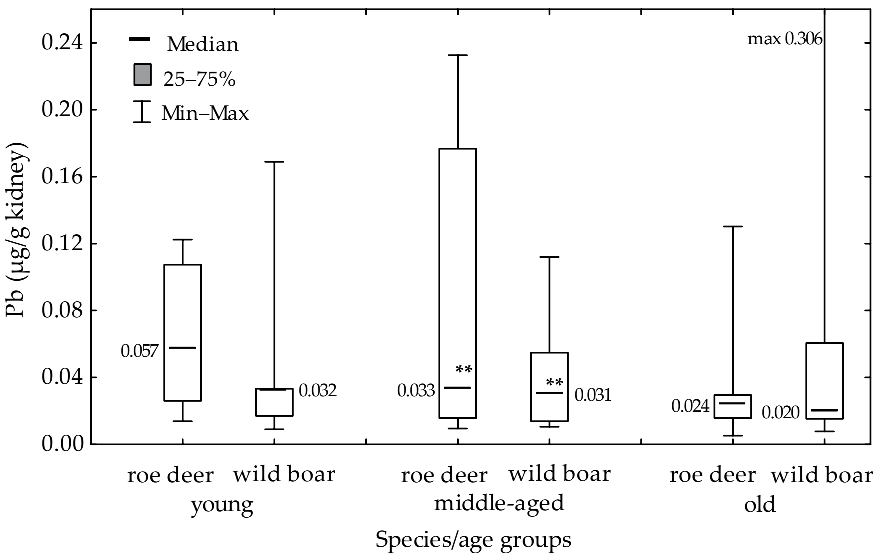


Figure 7. Median lead concentrations (μg/g) in kidney tissues of three roe deer and wild boar age groups

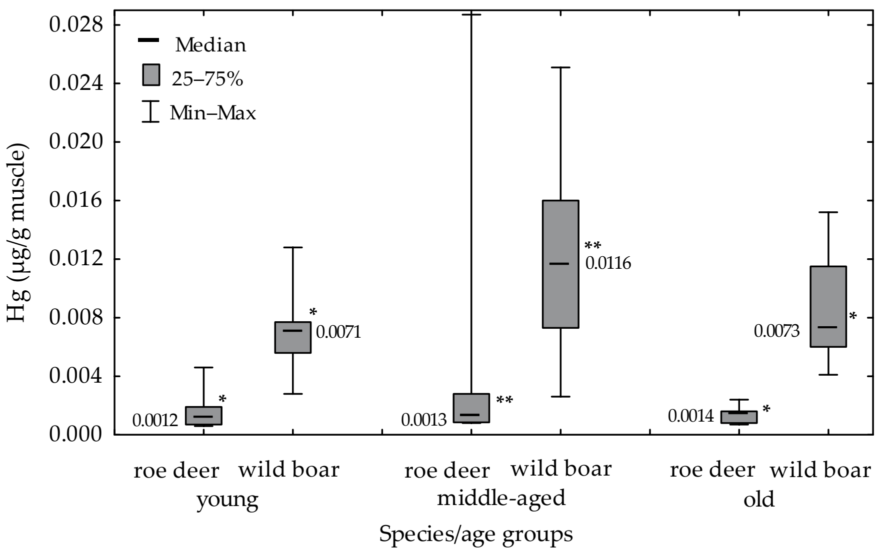
** = $P < 0.05$ between individuals of different species and same age groups

individuals, while in the old animals it was lower. No statistical significance was observed. Young roe deer contained higher lead concentrations than young wild boar, old roe deer and wild boar showed similar concentrations without any statistical significance. In middle-aged roe deer was found statistically significant higher lead concentration compared to wild boar of the same age (Figure 7).

Mercury muscle concentration in roe deer slightly increased with age. In middle-aged wild boar, mercury concentration was higher compared to young and old individuals, but without any statistical significance. Wild boar accumulated significantly higher concentration of mercury in comparison with roe deer in all three age groups (Figure 8).

Mercury concentration in liver of middle-aged roe deer was the lowest, old roe deer followed while the highest concentration was found in the young individuals. These differences were not significant. In wild boar, mercury concentration was higher in the middle-aged compared to the young individuals, but in the old animals it was intermediate. These differences were statistically significant. Roe deer contained a significantly lower concentration of mercury in relation to wild boar in all three age groups (Figure 9).

In roe deer kidney, mercury concentration insignificantly decreased with age. On the contrary, in wild boar mercury concentration significantly increased with age in relation of young to old and middle-aged to old animals. The middle-aged and



* = $P < 0.01$ between individuals of different species and same age groups

** = $P < 0.05$ between individuals of different species and same age groups

Figure 8. Median mercury concentrations (μg/g) in muscle tissues of three roe deer and wild boar age groups

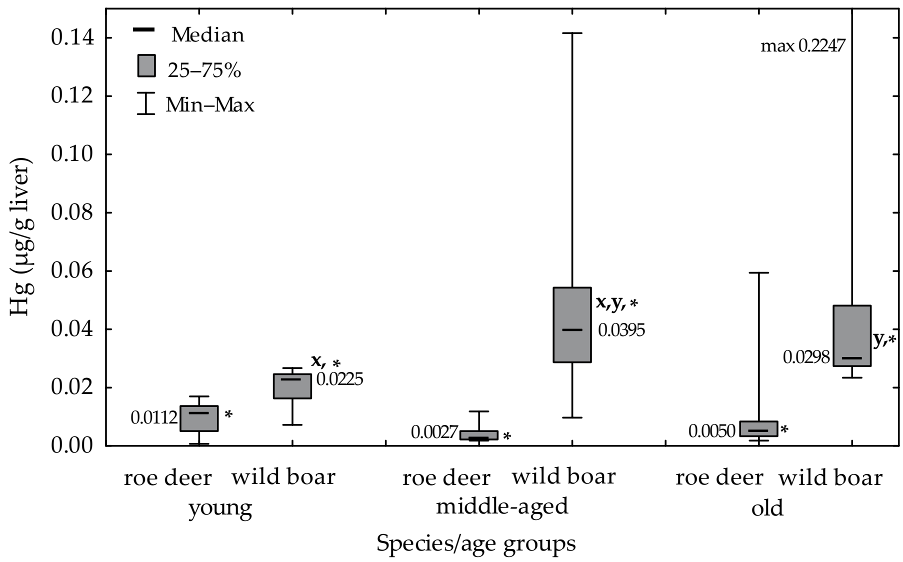


Figure 9. Median mercury concentrations (µg/g) in liver tissues of three roe deer and wild boar age groups

x,y = $P < 0.01$ between individuals of same species

* = $P < 0.01$ between individuals of different species and same age groups

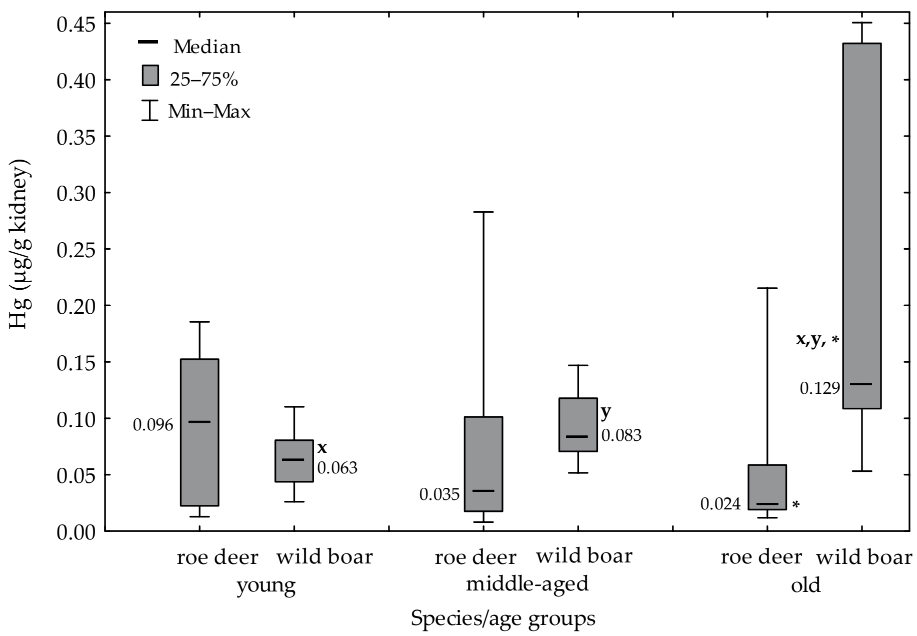
old wild boars contained higher mercury concentrations compared to roe deer, with statistical significance only in the old animals. On the other hand, young roe deer showed insignificantly higher mercury concentration in comparison with wild boar (Figure 10).

DISCUSSION

As the literature data on the contamination of roe deer tissues, especially with lead and mercury, are rather scarce, we used the literature on the same topic in relation to red deer.

Cadmium

Probably in all animal species, cadmium exhibits two regularities, the first one is the positive correlation between the cadmium concentration and age, pointing out the fact that the animals are exposed to Cd during the whole life. The second regularity is the relatively low concentration of Cd in muscles, while this is higher in livers, and the highest in kidneys. Both of these regularities were recorded in this study (Figures 2–4). The results obtained are in accordance with the previous reports by Kuiters (1996) and Falandysz *et al*. (2005) on red deer, Pokorny (2000), Pompe-



x,y = $P < 0.01$ between individuals of same species

* = $P < 0.01$ between individuals of different species and same age groups

Figure 10. Median mercury concentrations (µg/g) in kidney tissues of three roe deer and wild boar age groups

Gotal and Prevendar Crnić (2002) on roe deer, and Wolkers *et al*. (1994), Medvedev (1999) and Piskorova *et al.* (2003) on wild boar. The highest levels of Cd in red deer kidneys is a combination of a high affinity of Cd for kidneys, where it is deposited indirectly from the liver and directly from the blood stream, and a low excretion rate (up to 30 yars in mammals) (Cook & Johnson 1996). Our results point out that the tissues of roe deer contain higher amounts of Cd than those of wild boar of the similar age group. This is in accordance with the results obtained by Wilke *et al*. (2000). The fact that Cd enters the green parts of the plants and that roe deer is an exclusively herbivorous species (browser), while wild boar is omnivorous, can explain such findings. More over, the main plant foods of wild boar are various grains, not leaves or stems.

Cadmium concentrations in the tissues of roe deer at found in our investigation are similar to those reported in the investigations made on the deer population in other European countries (Santiago *et al*. 1998; Čelechovska *et al*. 2008; Reglaro *et al*. 2009). Wild boars from our study were somewhat less contaminated with cadmium than those from other European countries (Wolkers *et al.* 1994; Kuiters 1996; Medvedev 1999). Precise comparison of the results is not always possible as many authors do not divide animals (frequently large number of samples) according to the age, which is of particular importance for the interpretation of Cd levels.

Lead

Although such regularity related to age and cadmium is not seen with lead, in some reports as well as in our study lead concentration was the highest in young individuals with a decreasing trend in older groups (Figures 5–7). Similarly, Wolkers *et al.* (1994) and Kuiters (1996) determined a decreasing trend in lead concentration with age in liver and kidney tissues of red deer and wild boar. This trend can be explained by the increased need for minerals in young animals, especially for calcium whose kinetics is closely related to that of lead.

Contrary to our expectation and to literature data (Wolkers *et al.* 1994; Santiago *et al.* 1998; Reglaro *et al*. 2009), lead concentrations in roe deer tissues were generally higher than those in wild boar from lowland Croatia (Figures 5–7). We expected that wild boar as an omnivorous species whose feeding habits enable the contact with and ingestion of soil would accumulate higher concentrations of lead than the herbivorous species like roe deer. Moreover, lead is generally present in much higher amounts in soil than cadmium and, contrary to this, only trace amounts of lead can pass into the top parts of plants (Hoffman *et al*. 2001).

Few samples of roe deer and wild boar muscle tissues contained high lead concentrations which was not in agreement with the concentrations in liver and kidney (max values Figure 5). In spite of the precision taken during the sampling procedure, some muscle samples were obviously contaminated by lead particles from the bullet.

Lead concentrations in tissues of wild boar coming from our investigation were lower in comparison to some European countries (Wolkers *et al.* 1994; Kuiters 1996; Santiago *et al*. 1998; Medvedev 1999; Piskorova *et al.* 2003; Reglaro *et al.* 2009). The literature data on the lead contamination in roe deer are scarce. Pokorny (2000) recorded 0.11–0.71 μg/g of lead in roe deer livers from four areas in Slovenia which is much higher compared to our investigation. Similarly, lead concentrations in the tissues of red deer from some European countries (Wolkers *et al.* 1994; Kuiters 1996; Santiago *et al.* 1998) were also higher than in roe deer coming from lowland Croatia.

Mercury

Tissue mercury concentrations in the animals analysed were low, representing the usual finding in terrestrial animals (exceptions are areas with heavy anthropogenic pollution), especially in the deer population (Froslie *et al.* 2001). In water environment, the situation is opposite because of the well known biomethylation and biomagnification of organic mercury, but this is not relevant for this study.

The relation between age and metal concentration as seen with cadmium and lead was not present in the case of mercury, but it can be seen in Figures 8–10 that kidneys accumulate mercury in higher amounts than livers in both species. These findings suggest an inorganic form of mercury which is excreted mainly by kidney. Similar findings can be seen in Froslie *et al*. (2001) review of mercury concentration in wild boar and deer species.

Comparing the mercury concentrations in wild boar to those in roe deer, the majority of wild boar tissues contained significantly higher concentrations of mercury with respect to the other two metals analysed in all age groups (Figures 8–10), despite the fact that the young animal group i.e. piglets of up to 6 months of age, were compared with roe deer old up to 1 year. The reason for that can be strongly bound mercury in soil and the consequent prevention of its passage into plants. Additionally, roots are an efficient barrier for mercury passing into the upper parts of the plant. From all that mentioned, it can be concluded that wild boar that digs up with snout and eats food of plant as well as animal origin, will accumulate more mercury than roe deer.

Mercury concentrations in livers and kidneys of roe deer and wild boar from our study are comparable with similar results obtained from other European countries (Froslie *et al.* 2001) but the reference mentioned does not present data on muscle mercury concentrations.

CONCLUSIONS

In spite of the fact that lead is present in much higher amounts in soil (25.3–27.0 μg/g DW) than cadmium (0.2 μg/g DW) (Halamić & Miko 2009), the tissues of the investigated animals contained much higher concentrations of cadmium. The reason for this is the previously mentioned low excretion rate of cadmium whose retention time is measured in decades, while with lead the biological half-time is measured in weeks (Ma 1996). Mercury concentrations in the tissues of all animals analysed were low because of its low amount in soil, 35–50 μg/kg DW (Halamić & Miko 2009) and a low bioavailability.

From the hygienic point of view, the muscle samples from roe deer and wild boar were edible as the concentrations of cadmium and lead did not exceed the values (Cd – 0.05 μg/g; Pb – 0.10 μg/g) prescribed by the official regulations. However, cadmium concentration in liver exceeded the prescribed value (0.50 μg/g) in one fifth of all samples while lead concentrations were lower than the allowed concentration (0.50 μg/g). Most kidney samples from both animal species contained cadmium above the recommended concentration (1.0 μg/g), while lead concentration in any sample did not exceed the official value regulation (0.5 μg/g). Mercury concentration is no longer (since 2008) a matter of legislative.

The results obtained from lowland area will be compared in future with similar results from two other characteristic game habitats situated in Croatia, namely Karst and Mediterranean habitats. The main reason for such a kind of investigation resides in significant differences in natural concentrations of the investigated metals in soil (Halamić & Miko 2009), but also in lower industrial, agricultural, and traffic contaminations in the two latter habitats.

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