Assessment of toxic elements' content in swine kidneys: pathomorphological analysis

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SUMMARY

Background: *In order to ensure the safety of consumers in Serbia, the toxic elements' (Cd, Hg, As, Pb) content in swine kidneys collected from three different sites in Serbia (n=90), were determined by atomic absorption spectrometry. Also, in order to find information on the effects of accumulation of toxic elements in swine kidneys, histopathological examination of kidneys was carried out.*

Methods: *Determination of toxic elements (Cd, Hg, As, Pb) in swine kidneys was performed by atomic absorption spectrometry. For microscopic examination, kidney samples were fixed in 10% neutral buffered formalin and absolute alcohol for 5 to 7 days, processed by routine methods, sectioned at 5-8 μm, and stained with hematoxylin and eosin (HE) for light microscopy.*

Results: *The presence of mercury was found in 33.3% of kidney samples in the range of 0.005-0.055 mg/kg, while presence of cadmium was found in less degree (27.7%) but in higher content (0.05-1,23 mg/kg). The presence of arsenic was found only in one sample, while presence of lead was not found. The metal-to-metal correlation analysis supported the theory that there were different sources of contamination. Histopathological examination of kidneys confirms tubulopathies with edema and cell vacuolization. In addition, hemorrhages and necrosis of proximal kidney tubules' cells were found.*

Conclusion: *This study shows the presence of toxic elements in pigs butchered in Serbia at levels comparable to those reported in other countries., and consequently do not represent any concern from a consumer safety point of view. The lack of strong correlation between histopathological changes and incidence of toxic elements found in our trial may explain the possible synergism among toxic elements and other nephrotoxic compounds, which enhances the toxicity, especially in cases of low contamination.*

KEY WORDS: *Food Contamination; Swine; Kidney; Mercury; Cadmium; Arsenic; Lead; Risk Assessment*

INTRODUCTION

Environmental pollution with toxic elements is a dangerous problem that is recognized worldwide. Toxic elements can be found in water, soil, air, plant, and animal tissues because of industrial and agricultural practices (1,2). Environmental concern pertaining to toxic elements relates to their toxicity, labile nature, bioaccumulation in organisms, and ultimately to their effect on human beings (3). One of the most important aspects of environmental pollution for humans is the intake of toxic elements through nutrition (4). Heavy metals are significantly toxic, even at very low concentrations due to their cumulative nature in the different body organs leading to unwanted side effects (5,6). As metals tend to bioaccumulate in the environment and biomagnify in food chains (7), their levels might reach toxic limits even when found in low concentrations in environmental samples. Since this should be limited to an unavoidable minimum, much attention is paid to the occurrence of these elements in food. Monitoring programs have been carried out in many countries with the purpose of avoiding the distribution of foodstuffs that could pose a risk to human health if consumed. Toxic elements can interfere with the functions of enzymes and are responsible for many diseases, especially cardiovascular, renal, nervous, and even bone disorders (8-12). Toxic elements are also implicated in several major human diseases including carcinogenesis – induced tumor promotion (13,14). Besides, some toxic elements are considered as being carcinogenic, mutagenic, and teratogenic in experimental animals (15,16).

Toxic elements' levels in animal tissues are organ specific. Meat and meat products form an important part of the human nutrition. Although the toxic metal content in muscle is generally low, the entrails, such as liver and kidney, often accumulate higher metal concentrations than most of other food (17-19). In many European countries, internal organs (liver, kidneys, heart, and lungs) are sold and consumed as a valuable food source. Therefore, evaluating toxic metal levels in internal organs is important for safety and health purposes. The risks to health from certain food elements can be assessed by comparing estimates of dietary exposures with the Provisional Tolerate Weekly Intakes (PTWIs) and Provisional Maximum Tolerable Daily Intakes (PMTDIs) recommended by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization of the United Nations and the World Health Organization International Programme on Chemical Safety (20, 21).

In order to ensure the safety of consumers in Serbia, the present study aim was to assess the exposure to toxic elements (Cd, Pb, As, and Hg) through consumption of pork meat in respect to their presence in kidney of healthy slaughtered swine and to assess relationship between pathomorphological changes in kidney with accumulation of toxic elements.

MATERIALS AND METHODS
Sampling

Ninety kidneys from healthy swine slaughtered at three different sites in Serbia were sampled during a six-month period. The pigs were slaughtered at a weight of about 105 kg and age of about 6 months. One kidney per pig, from 5 pigs per farm, was collected at the slaughterhouse. All samples were packed in plastic bags and immediately transported to the laboratory. Visible fat, connective tissue, and major blood vessels were excised and the samples were then homogenized. Sub-samples (10 g approximately) were taken and frozen at -18 °C until analysis.

Digestion

Samples of kidneys for instrumental analysis were prepared using the method of acid microwave digestion. Samples were destroyed in micro-wave digestion unit (*Milestone TC*) with temperature control. Heavy metal contents (Pb and Cd) were determined using GF-AAS technique, atomic absorption spectrophotometer-*Varian Spectra 220*, equipped with *Varian-GTA 110* graphite furnace. Mercury (Hg) concentrations in samples were analyzed by hydride generation atomic absorption spectrophotometry at 253.7 nm (HG–AAS, cold vapor technique), using *Varian Spectra A220*-atomic absorption spectrometer equipped with a *Varian VGA-77* hydride generator. Total arsenic (As) content was measured by HG-AAS, in flame at 197.3 nm, on the same instrument configuration as mentioned above.

Quality assurance

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Samples were generally carefully handled to avoid contamination. Glassware was properly cleaned and the reagents were of analytical grade. Double distilled deionized water was used throughout the study. Reagents blank determinations were used to correct the instrument readings. Recoveries for all examined elements ranged from 95% to 102% and the variation coefficient between 4% and 9%. Quality control included the analysis of standard reference material (BCR No.186).

Microscopic examination

Kidney samples were fixed in 10% neutral buffered formalin and absolute alcohol for 5 to 7 days, processed by standard histological techniques, and stained with hematoxylin and eosin (HE) for light microscopic examination.

Statistical analysis

Differences in the mean levels of OTA contamination across the three groups of positive samples was calculated by analysis of variance and then by a Student's t-test. Additional posttests were applied to evaluate differences between groups with statistically significant variation among the means. The differences with p values smaller than 0.05 were considered statistically significant.

RESULTS

Incidence of heavy metals

The occurrence and content of heavy metals in kidneys of slaughtered pigs from investigated area are summarized in Figures 1 and 2.

Occurrence (regional variations) of toxic elements in kidneys of slaughtered pigs is given in Figure 1. Figure 2 presents the results by the number of samples falling into specified concentration ranges. The occurrence of As was detected in only one sample originating from Vladimirci area (content 0.001 mg/kg), while occurrence of Cd varied from 16.6% (region Bogatić) to 40% (region Vladimirci). Cd concentrations in kidneys from investigated region ranged from 0.05 to 1.23 ng/g. A highest Cd concentration (1.23 ng/g, mean 0.185 ng/g) was found in Vladimirci region in contrast to Bogatić region where the lowest (0.05 ng/g, mean 0.027 ng/g) concentration was found. The occurrence of Hg varied from 23.3% (region Vladimirci) to 43.3% (region Senta). The highest Hg level (0.055 ng/g, mean 0.0033 ng/g) was found in Vladimirci region in comparison to Senta region where the lowest concentration (0.012 ng/g) of Hg was detected, while mean concentration (0.0034 ng/g) was slightly higher. A statistically significant difference (p<0.001) was found between the levels of Cd in examined kidneys. The results in Figure 1 indicate a higher degree of Hg contamination in kidneys (33.3%), when compared with incidence of Cd (27.7%). However, the results showed that Cd concentrations and a mean value in kidneys were several times higher than the concentration of Hg. As shown in Figure 2, all the values measured for As, Pb and Hg were below the limits recommended by the European Regulation (22), while Cd concentration in one kidney from the investigated region was higher and exceeded the maximum limit (1 ng/g) fixed by the European Regulation (22).

Pathomorphologic examination

The results of pathomorphologic examination are summarized in Figures 3, 4 and 5.
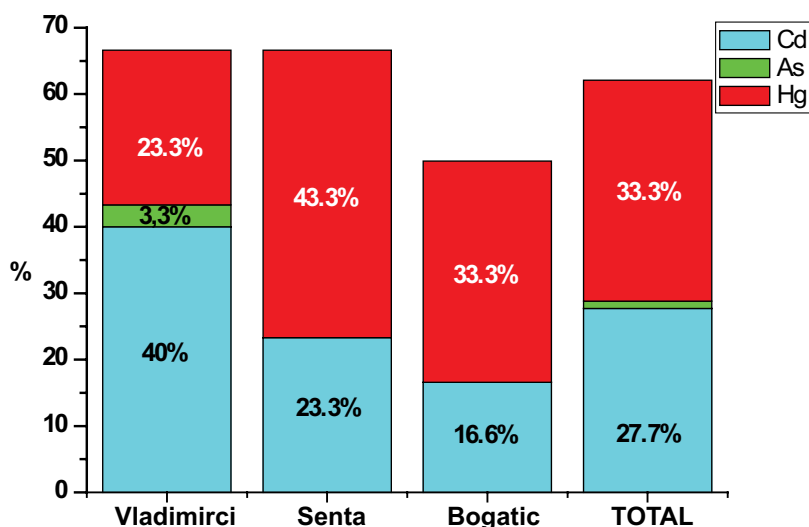

Figure 1. Occurrence of toxic elements in kidneys of slaughtered pigs
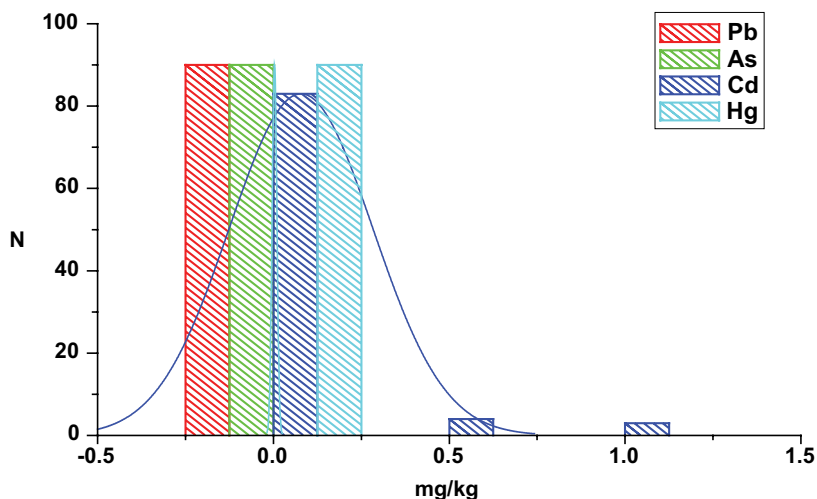(% – Percentage of positives samples)

Figure 2. Content of toxic elements in kidneys of slaughtered pigs (ng/g)
N – Total number of samples

Gross pathology

In all 90 pigs that were slaughtered during the study period, the kidneys which were submitted to the laboratory were pale, swollen and enlarged, and changed in color from the normal mahogany to tan, as follows: 47.7% were mottled or pale kidneys, 30% were enlarged (Figure 3), and 12.2% were smaller kidneys than normal. The only macroscopic lesions in few cases were small grayish-white foci on the kidney surface. No obvious difference was observed between the right and left kidney. No significant changes were seen in other organs. The external surface of kidneys where Hg and Cd were determined is shown in Figure 3.
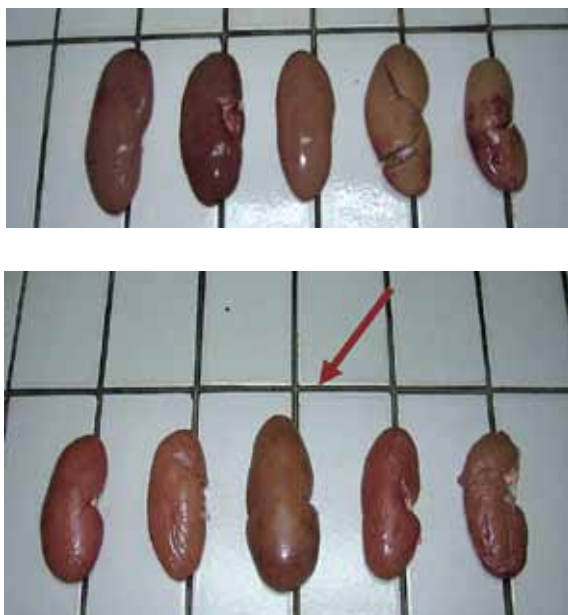

Figure 3. External surface of kidneys where Hg and Cd were determined

Histopathological examination

Histological examination showed two types of changes: degenerative-affecting epithelial cells in some proximal tubules of pigs, and proliferative changes in the interstitium. The major renal histopathological changes were mainly in the epithelium of proximal tubules (Figure 5). Dystrophy (moderate to marked degenerative changes, Figure 5c), swelling, vacuolization and lipidosis, were the main changes in the tubular epithelial cells. The majority of glomeruli exhibited mild or moderate exudates in Bowman's capsular spaces as well as hypercellularity of vascular loops. In addition to vascular changes expressed as a hyperemia of blood vessels, moderate to marked hemorrhages of some renal cortical regions occurred occasionally (Figure 5a). In the interstitium of some renal cortical regions, there was limited proliferation of connective tissue (Figure 5d) and focal infiltration of mononuclear inflammatory cells, which was sometimes accompanied by small granulomas.

Lipidosis of proximal tubules` cells, renal hemorrhages and swelling of proximal tubules` cells were seen in 45.5%, 37.7%, and 34.4% kidneys of slaughtered pigs, respectively. Thirty percent of examined pigs' kidneys had a dystrophy of proximal tubules cells, while exudate in Bowman's space as well as focal interstitial fibrosis was seen in 22.2% kidneys of slaughtered pigs. Vascular changes expressed as a hyperemia of blood vessels was seen in 13.3% kidneys, while hypercellularity of vascular loops, necrosis of proximal tubules' cells and renal adenoma occurred in a less degree.
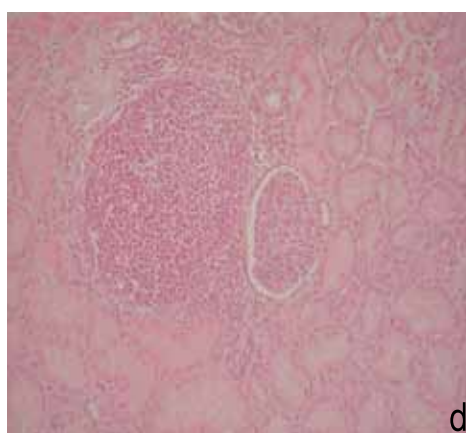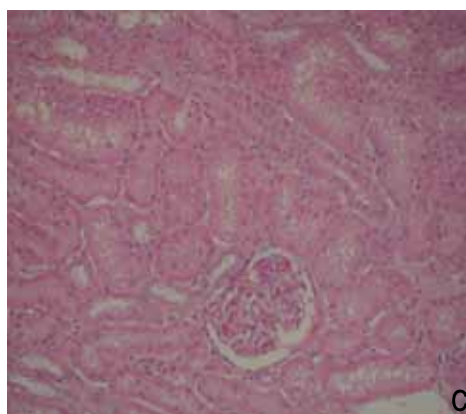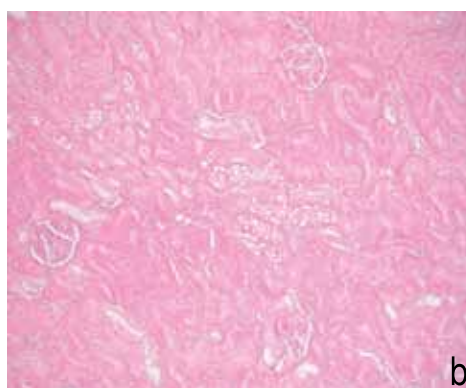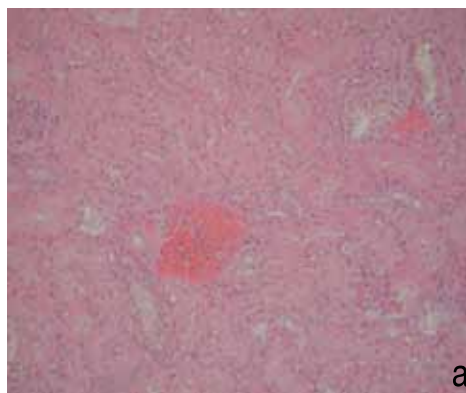

Figure 4. Hemorrhages in cortex (a), Fatty change (b), Necrosis of proximal tubules' cells (c), Dystrophy and vacuolar degeneration in the epithelium of proximal tubules' cells and Focal interstitial fibrosis (d)

Coincidence of histopathological findings of renal tissues and toxic elements in kidneys from slaughtered pigs are summarized in Figure 5.
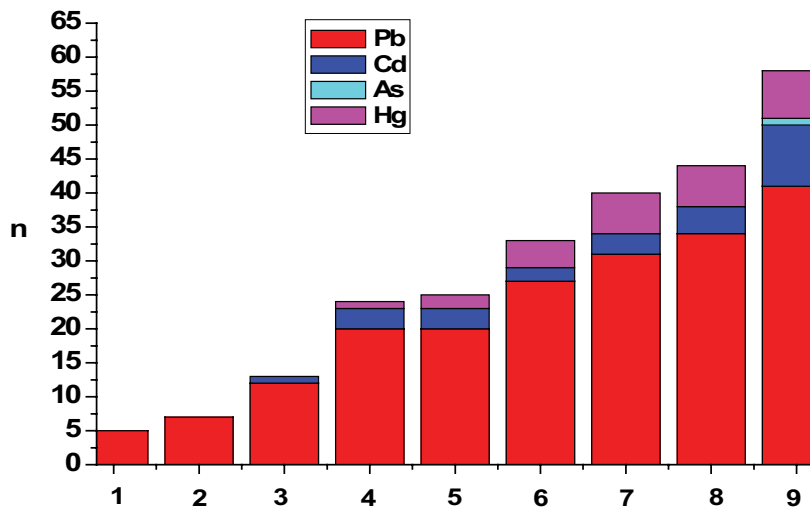

Figure 5. Summary of histological findings of renal tissues and incidence of toxic elements in kidneys from slaughtered pigs (n=90). Necrosis of proximal tubules' cells (1), hypercellularity of vascular loop (2), vascular changes (3), exudate in Bowman's space (4), focal interstitial nephritis (5), dystrophy of proximal tubules' cells (6), swelling of proximal tubules' cells (7), renal hemorrhages (8), fatty changes of proximal tubules' cells (9).

DISCUSSION
Incidence of toxic elements

The concentration of the four toxic elements analyzed was very variable in relation to the region where samples were collected. Also, the present study has shown that toxic elements' levels in kidneys are comparable with those of other countries, especially within Europe (23-26). The results of this study confirm that Hg, whose toxicity is well known particularly in the organic form, is one of the main contaminants of the environment (Figure 1). In contrast, our results showed that the Cd level was higher than the levels of Hg (Figure 2). In only one sample an excessive concentration of total Cd was found (>1.0 ng/g).

Reduction of Cd contamination can be explained by good agricultural practice (GAP), which uses fertilizer low in Cd and restrict disposal of sewage sludge on cropland. The presence of Pb was not found because of global reduction in usage. Strict environmental legislation has led to reduction of this metal discharge in the last 15 years (27). The present work indicates that regional variations on the occurrence of toxic elements in kidneys of slaughtered pigs were observed. The regional differences might thus partially explain the concentration differences in the corresponding formulas, as could differences in the contamination of foodstuffs by toxic elements. The main source of exposure to mercury is from food. Mercury is present in feed naturally (e.g. in fish which take up mercury from marine sediments), or because of pollution (e.g. emissions from industrial processes, fossil fuel combustion). For non-plant feed materials, fishmeal is to be identified as the most common source of mercury for farmed animals under normal farming conditions (28,29). Mercury is a naturally occurring element that is released from a variety of sources including human activities. Once released into the environment, mercury undergoes a series of complex chemical and physical transformations as it cycles between atmosphere, soil, and water. Humans, plants, and animals are routinely exposed to mercury and accumulate it during this cycle, potentially resulting in a variety of health impacts. In addition, fishmeal normally contains lower cadmium contents, but mineral feed is usually structurally contaminated with Cd.

However, these findings suggest that industrial activities in the studied areas introduce more Cd than Hg into the food chain. Higher concentrations are found in hot spots related to human activities (13,19) and on agricultural land where high concentrations of phosphate fertilizers and manure are applied (30,31). Since cadmium is taken up by plants, an increased soil concentration can result in increased levels in food and feeds (32). The concentrations of total toxic elements found in the analyzed kidneys were usually low and did not exceed the maximum permissible levels established by the Serbian law. Since vegetable foods constitute the major source of Cd for humans (33), the values of total toxic elements found in the tested kidneys do not represent an imminent toxicological risk. However, food that was consumed in larger quantities made the greatest contribution to the dietary exposure. It is very important to keep steady control on the level of this element in staple food in the country in view of the potential health hazard that toxic elements may pose.

Pathomorphological examination

Kidney is clearly the major target organ of chronic Cd and Hg toxicity (19,34-36). Accumulated evidence also indicates that kidney is a target organ of As toxicity. Since kidney is the major organ for As elimination and most of the As is rapidly eliminated through the kidney (37), renal cells are, thus, exposed to a major portion of the absorbed As dose. After absorption, most of the toxic elements are accumulated in the liver where they induce the production of metallothioneins (MT), a family of low-molecular-weight metal-binding proteins that aid in the intracellular processing of metal ions (38). Cadmium may also bind to other SH-rich low-molecular weight peptides or amino acids such as glutathione and cysteine respectively (39). Metallothioneins exist in most tissues, have a high cysteine content, and hence may be similar to metal chelators in providing tolerance against metal-induced hematotoxicity and immunotoxicity (40). The toxic effects of mercury on the kidney are well characterized and include acute tubular necrosis and reduced glomerular filtration rate. In small doses, the S3 segment in the cortico-medullary area is the primary target site. As the dose of mercury is increased, the injury spreads to involve the S1 and S2 segments of the proximal tubules (41). The ability of Hg to interact with phospholipids and specific enzyme systems may help explain the cell degeneration, apoptosis, and necrosis, and overall toxicity observed in immune system cells. The effects of I-Hg on transduction at cellular membrane channels have been investigated through studies of Hg2+. In a study on the effects of inorganic Hg on cell membranes, Liang et al. (42) found that Hg2+ induced channelopathies in guinea pig sensory cells by impairing K+ channels and changing the permeability of the cell membrane. Leong et al. (43), for example, found that Hg2+ ions suppressed neuronal somata sprouting, thus inhibiting neurite growth in snails. These and other experimental animal studies demonstrate a wide range of biological effects from exposure to different species of Hg (44-47).

Enlarged kidneys are indicative of renal inflammation and proliferative lesions as a result of chronic exposure to Cd (48,49) or As (50). The

increased kidney injury from Cd and As co-exposure might be due to increased oxidative stress. It has been proposed that both Cd and As (51) may produce oxidative stress as a cellular mechanism of toxicity. Two mechanisms play an important role in cadmium genotoxicity: 1) induction of reactive oxygen species (ROS) and 2) inhibition of DNA repair. A more recent study has shown that Cd nephrotoxicity is also associated with alterations in the localization of the tight junction protein claudin-2 in the proximal tubule (52). Additional studies have shown that N-cadherin and its associated proteins may be involved in the nephrotoxic responses to other metals such as mercury (Hg) (34,53) and bismuth (Bi) (54). Other recent studies have shown that alterations in the activity of focal adhesion kinase may play a key role in the malignant transformation of cells by arsenic (55,56) In this regard, Cd and As co-exposure produces significantly more lipid per oxidation in liver and kidney than either inorganic given alone. Cadmium may also potentiate diabetes-induced effects on kidneys (57,58). Kidney damage may further progress to end stage renal disease (ESRD) and death if exposure is high and prolonged. Recent evidence suggests that cadmium may also play a role in the development of other cancers, such as testicular cancer, bladder cancer, pancreatic cancer and cancer of the gall bladder (59). Concerning aetiology of porcine nephropathy, the production of multiple toxins, as is sometimes the environmental situation, presents a problem that has not been sufficiently investigated.

CONCLUSION

It could be concluded that presence of toxic elements, and consequently, daily intake for the studied toxic elements, is below that reported by the FAO/WHO who has set a limit for toxic elements intake based on body weight for an average adult (60 kg body weight). Thus, the consumption of average amounts of pork meat and meat products, which include internal organs (liver, kidneys, heart, and lungs), does not pose a health risk for the consumer. The present study provides additional data on toxic elements pollution in Serbia and can help in risk assessment of consumer exposure to the expected toxic elements. It is therefore suggested that regular survey of toxic elements should be done on all food commodities in order to evaluate whether any health risks from toxic elements exposure do exist, to assure food safety and to protect the end users from food that might injure their health. Regular survey and monitoring programs of toxic elements and heavy metal contents in foodstuffs have been carried out for decades in most developed countries.

In summary, there is compelling evidence that both higher and lower vertebrates are at risk to toxic elements through multiple exposure scenarios. It is apparent that exposures to chemical toxicants, such as toxic elements, may be substantial especially during early life or with highly exposed populations such as subsistence anglers. Therefore, efforts to evaluate health risks associated with environmental toxicants should be given high priority in testing, as these chemicals are directly or indirectly consumed by individuals at particular risk.

Conflict of interest

We declare no conflicts of interest.

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