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Abstract

Danube is the river that flows through ten European countries, serving as a resource for human life, agriculture, and industry. Those anthropogenic activities, especially those in the upstream, put environmental pressure on the river, which needs to be monitored and prevented. *Faxonius limosus* sampled in the polluted area of the Danube in Serbia (Slankamen municipality) was studied as the test animal for bioaccumulation of selected toxic and potentially toxic metals. Uptake and potential accumulation of cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg) by abdominal muscles, hepatopancreas, and exoskeleton were measured by MP-AES, and data were compared with metadata from the available references.

The study found that indeed metal contamination in aquatic ecosystems could be an environmental concern with potential implications for aquatic food webs.

Keywords: bioaccumulation; metals; *Faxonius limosus*; Danube basin; Serbia

Abbreviations: BAF - Bioaccumulation Factor; CRM – Certified Reference Material; FTIR - Fourier-Transform Infrared Spectroscopy; IUPAC - International Union of Pure and Applied Chemistry; MAC - Maximum Acceptable Concentration

Introduction

Presence of metals in freshwater basins is a very common indication of anthropogenic pollution by agricultural activities, different industries but also urban sewage (Klavins et al., 2000; Yi et al., 2011). Literature frequently cites this problem as the "heavy metal pollution"; however, the present study will strictly follow the IUPAC's recommendation about the use of this term (Duffus, 2002). IUPAC suggests that the expression "heavy metal" has never been defined, and the present work focuses on understanding bioavailability as a key to assessment of the potential toxicity of metallic elements and their compounds in the tissues of the species examined.

Spiny-cheek crayfish *Faxonius limosus* is a highly invasive North American crayfish species that was first introduced into Europe in 1890 in Germany. Since its initial introduction, spiny-cheek crayfish has progressively expanded its distribution range throughout Europe, including major river basins such as the Danube. Its successful establishment and spread have been facilitated by its high ecological adaptability, broad environmental tolerance, competitive interactions with native crayfish species, and ability to disperse through connected aquatic habitats (Holdich et al,. 2009, Buřič et al., 2013) Due to its invasive potential and ecological impacts, *F. limosus* is currently classified as an invasive alien species of Union concern under European legislation (Regulation EU No 1143/2014; Commission Implementing Regulation (EU) 2016/1141). This crayfish has been identified in Bulgaria and Serbia as an invasive species that tends to migrate even further (Pavlović et al., 2006; Todorov et al., 2020). The first actual population of *F. limosus* in the Danube was reported in 2002 near Apatin municipality (Karaman & Machino, 2004). It has naturalised on the territory of Serbia because it has no natural enemies in this part of the Danube basin and has since had a negative impact on native species of crayfish. Studies show that it alters both the structure of the communities and the functioning of the ecosystem, but also affects the non-biotic component of the ecosystem (Zorić et al., 2020).

Crayfish are frequently suggested as bioindicators for monitoring water status in polluted areas (Alikhan et al., 1990; Kuklina et al., 2013; Pallottini et al., 2015). This is based on earlier research which found that metallic compounds accumulate in crayfish tissues, especially through the food chain (Bruno et al., 2006; Kouba et al., 2010). They have also long been recognised as effective bioindicators for chemical contaminants (Caro, 2010), as they live in contact with polluted sediments. It is known that they resist environmental stress, have a relatively long life span, are easily recognisable and do not migrate (Chambers, 1995; Del Ramo et al., 1987; Roldan & Shivers, 1987; Souty-Grosset, 2006). Most of these studies show that muscles and exoskeleton can be considered specific for the accumulation of mercury and nickel, respectively. On the other hand, Cd, Zn, Cu, Pb and Cr accumulate mainly in the liver and hepatopancreas. According to Sures (2003), pollutants are usually absorbed via the gills or intestines and accumulate until they reach a steady-state concentration. The uptake and accumulation of pollutants is associated with a general physiological response consisting mainly of adverse effects that can be either specific or more generalised and can eventually lead to death (Sures, 2003).

Danube basin through Serbia is exposed to significant anthropogenic pollution (Lenhardt et al., 2020). Agricultural activities, power plant tailings, untreated sewage, industry (Kolarevic et al.,

2011), and pollution from the Sava River and other tributaries (Crnković et al., 2008) have major adverse effects. Other main sources of metal pollution can also be fossil fuel combustion and activities related to shipping and mining (Calmuc et al., 2021). If metals bioaccumulate in the tissues of hydrobionts, these metals can subsequently reach the other components of the trophic chain. The aim of the present research was to measure the bioaccumulation of the selected metals in exoskeleton, hepatopancreas and muscle tissues of the *Faxonius limosus* from the Danube basin in Serbia. Concentrations of Pb, Cd, Hg and As were analysed to compare them with the concentrations in the sediments from the location of the crayfish sampling. In addition, the metal concentrations in two sexes were compared to some level; however, data for females are scarce and are shown only for preliminary informative purposes due to the very small number collected. An analysis of the exoskeleton was additionally conducted using two methods, FTIR and RAMAN. Such an approach is rarely found in the literature; however, it was expected here that it would provide more data on the changes in carapace surface functional group vibrations, and point out potential adsorption sites for metal ions.

Materials and methods

Sampling area

Spiny-cheek crayfish specimens were collected in May 2022 and January 2023. The sampling site was the Slankamen site, Danube River (GPS 45°08'23.64" N 20°15'35.64" E), and 48 crayfish specimens (45 males and 3 females) were collected. The location represents a point 1215.5 Rkm measured upstream from the Danube mouth at the Black Sea (Fig. 1).

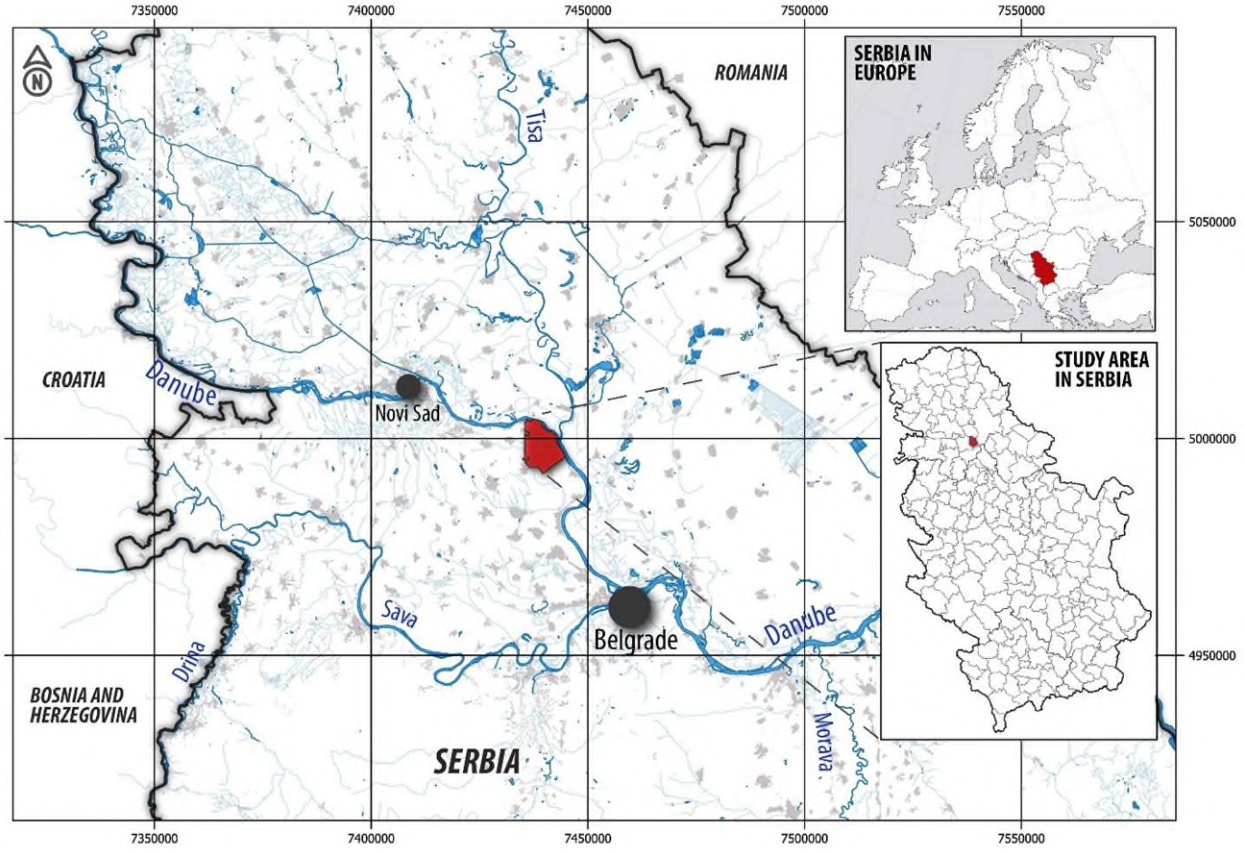


Fig. 1 Map of the sampling location

Biometric Analysis

The collected crayfish were taxonomically identified according to the relevant literature(Gherardi, 2017; Souty-Grosset, 2006). After collection, for each specimen respectively, following data were recorded: (i) sex; (ii) total body length (L_{tot}, from the tip of the rostrum to the tip of the telson); (iii) length of the cephalothorax (L_{cft}, from the tip of the rostrum to the end of the carapace); (iv) total individual weight (W_{tot}) (Garzoli et al., 2020).
Procedure after separation by sex included euthanizing them by freezing at -40°C on site and than stored at -18 °C. Later, frozen specimens of crayfish were thawed in the laboratory at 0°C (Larson & Olden, 2016). Tissue samples were further dissected and then grouped into three subsample groups: abdominal muscle, hepatopancreas, and exoskeleton. Following dissection, for chemical analysis purposes, samples were weighed to four decimals and immediately dried in the drier model VIMS Electric LSW-53, under ventilation, using a temperature program of 12 h at 105º C. Dried-out samples were subsequently burnt at 300º C in a NABERTHERM muffle annealing furnace, for 30 min in the air atmosphere. Sample portions of cca. 2.0 g (dry weight, weighted to four decimals for each sample) were processed in a microwave-digestion system, using a temperature program of 6 h - 148º C. Samples were mineralized by adding 5 mL of 65% HNO_3 and 2 mL of 30% H_2SO_4 (Merck, Darmstadt, Germany). Microwave-assisted digestion was performed in Speedwave CR 4200 WTW, for 1 h according to the standard method. Samples cooled down to room temperature were then diluted with distilled water to a volume of 50 ml (Bridgewater et al., 2017; Jovičić et al., 2015). Metal concentrations were measured by Microwave Plasma-Atomic Emission Spectroscopy (Agilent 4210 MP-AES), allowing the assessment of the concentration of four elements: Pb (λ=405,781), Cd (λ=228,802),
Hg (λ=253,650) and As (λ=193,695). Presence of the analysed metals in chemicals used for digestion was nullified by the blank samples using distilled water.
Along with the tissues, concentrations of analysed metals were measured in the sediment, which was taken at the crayfish collection location. Preparation of the sediment samples also included freeze-drying, fine grinding, homogenization, and sieving (mesh, -150 μm). A sample of sediment, weighted to around 0.05 g, was digested with mixed acids in a beaker, according to the standard (Bridgewater et al., 2017). Following digestion, the dissolved sample was diluted with 2% HNO_3, after which the concentrations of metals were measured at Microwave Plasma-Atomic Emission Spectroscopy (Agilent 4210 MP-AES). The standard used for mercury was Single-Element Mercury Standard, VHG™ (LGC Group). For the measurements of Cd, As and Pb, a Multi-Element US EPA 23 Metals Standard of VHG™ (LGC Group) was used.

FTIR and Raman spectroscopies

An infrared spectrophotometer with Attenuated Total Reflectance method (ATR-S, IRSpirit SHIMADZU) was used to analyse the structure of the raw tissues of the carapace for both sexes

(ventral and dorsal side, respectively), after drying at 105°C. Tissues were positioned and compressed between the ATR crystals, and the spectra were recorded in the zone of 400-4000 cm^{-1} with a resolution of 1 cm^{-1} and using 50 repeated spectral scans.

Raman spectra were collected for carapaces for both sexes (both ventral and dorsal sides, respectively) are measured at Witec R300 system at 785 nm laser with 79mW strength, using 63x magnification, and a grating of 600 g/mm BLZ 500.00 nm. Integration time was set to 60 seconds while accumulations number was 10. Spectral resolution was set to 1.3 cm^{-1}.

FTIR and Raman spectroscopy are complementary vibrational techniques that are widely used to characterize the molecular composition, structural organization, and functional group chemistry of biological matrices. In crustacean cuticle research, FTIR spectroscopy is particularly sensitive to polar bonds and to the vibration modes of the main organic constituents of the exoskeleton (chitin, associated proteins, and the mineral phases (primarily calcium carbonate in its calcite and amorphous forms) enabling the identification of absorption band shifts that reflect conformational re-organization or chemical alteration of these components. Raman spectroscopy is complementary to FTIR because it is highly sensitive to non-polar groups and to skeletal vibrations of both the organic matrix and the biomineral phase, and is therefore well suited to detect variations in the molecular environment of mineral components and pigments such as astaxanthin. Importantly, these two techniques should be regarded as chemically diagnostic tools for the exoskeletal matrix rather than as direct probes of metal binding. They can robustly document changes in functional group composition, in the relative proportion of organic versus mineral phases, and in conformational order of the cuticle, but they do not by themselves provide molecular-scale evidence of the specific functional groups involved in metal coordination or of the geometry of metal–ligand binding. Accordingly, in the present work FTIR and Raman were used as a non-destructive, complementary layer of physicochemical information to be interpreted alongside the quantitative elemental data obtained by ICP-OES/ICP-MS, in order to obtain an integrated view of metal uptake and cuticle alteration in *Faxonius limosuslimosus* (Dib et al., 2026; Morais et al., 2020; Ritschar et al., 2020). Main objectives of these two techniques were: to characterize the baseline molecular composition of the exoskeleton of F. limosus, with particular attention to chitin, associated proteins, and the carbonate mineral phase; to identify and compare structural and chemical alterations of the cuticle between individuals collected at sites with different contamination histories; to assess qualitative changes in the relative distribution of organic and mineral components that may accompany exposure to elevated metal loads; to provide complementary physicochemical context that, in combination with the quantitative metal concentrations determined by ICP-OES/ICP-MS, supports an integrated interpretation of metal uptake in F. limosus. It should be noted that FTIR and Raman spectroscopy, as applied here, are not intended to and cannot directly identify the specific metal sorption sites or resolve the molecular binding mechanisms within the cuticle. Identification of metal coordination environments and binding modes would require additional spectroscopic approaches such as extended X-ray absorption fine structure or X-ray absorption near-edge structure spectroscopy, which are beyond the scope of the present study.

Maximum acceptable concentration

The obtained results were compared with the maximum acceptable concentration (MAC) established by the European Union for fish intended for human consumption (Official Journal of the European Communities, 2001), Food and Agriculture Organization of the United Nations (FAO, 1983), but also with the current regulation of the Republic of Serbia (Official Gazette of FRY, No 28/2011).\

The accumulation rate of metals in the freshwater biota was determined through the bio-indices, where the bioaccumulation factor (BAF) was calculated using GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts, USA. The Bioaccumulation Factor (BAF) was calculated individually for each analyzed tissue (muscle, hepatopancreas and carapace) to evaluate the tissue-specific accumulation patterns of the four metals in both males and females.. The bioaccumulation factor is the value representing the concentration of metals in sediment or water divided by the concentration of metals in the studied biota in mg kg^{-1} as described by Habib et al., 2026; Lovrić et al., 2026). The equation employed is shown below:

$$BAF = \frac{concentration\ of\ metals\ in\ crayfish}{concentration\ of\ metals\ in\ sediment[\frac{mg}{kg}]}$$

Sediment samples' metal concentrations were, on the other hand, evaluated using the MACs values defined in the regulations of the Republic of Serbia (Official Gazette of Republic of Serbia 50/2012, 2012).

Statistical Analyses and Models

We analyzed the data using Statistica 13.0 software (StatSoft, Inc., Tulsa, OK, USA). Data distribution and variance homogeneity were checked using Shapiro-Wilk and Levene's tests. Normally distributed data were analyzed with one-way ANOVA and Tukey's post-hoc test. For non-normal data, we used the Kruskal-Wallis test followed by Mann–Whitney U tests. All results are shown as mean ± SD, with significance defined at $p < 0.05$.

5 Results

Biometric Analyses and Population Size Structure of *F. limosus*

A total of 48 specimens were collected in the present study for the analysis of the main biometric features of *F. limosus*, 93.75% males and 6.25 % females. An overview of biometrics is given in Table 1. These specimens are the same ones used for the study of morphometrics previously (Roljić et al., 2024). All crayfish sampled in this study have reached sexual maturity, which was confirmed by their morphological and biological characteristics. Assessments indicated that all of the specimens were approximately two to three years of age.

Table 1 .Body size measures: distribution range (mean and standard deviation values) for the main biometric features for *F. limosus* studied.M - males, F- females, sd- standard deviation

Sex	Total body length (mm)	Total body length range (mm)	Total weight (g)	Total weight range (g)	Cephalothorax length (mm)
	mean ± sd		mean ± sd		mean ± sd
M	84.19±11.86	55.95 - 108.90	21.83±7.94	6.37-36.30	41.63±6.05
F	76.29±11.89	63.89 - 87.60	12.57±4.90	7.70 - 17.50	34.77±4.15

FTIR and Raman spectrometry

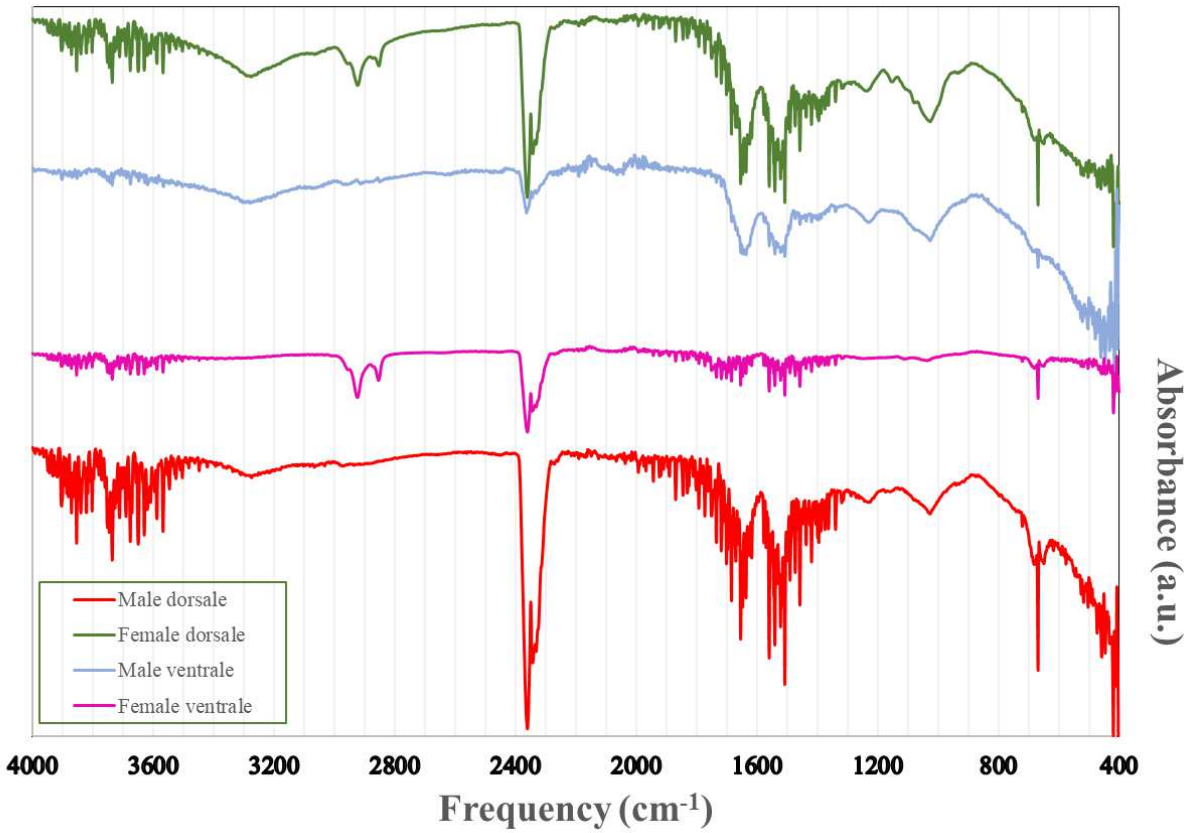


Fig. 2 FTIR-ATR spectra of the carapaces, with corresponding zoom-in spectra for particular areas

Most typical spectral peaks of chitin, which is expected here in the analysed carapace according to the literature, would be: 3400cm^{-1} for NH_2 and OH groups, and these can be observed in all the samples (Fig. 2). 1659 cm^{-1} stands for the NH intrasheet vibrations, 1624 cm^{-1} for C=O vibrations,

and 1556 cm^{-1} for the N-H banding, all of which are observable under some noise present. On the other hand, 1315 cm^{-1} band, which should normally be observed for C-N banding, could not be clearly observed, demanding further discussion.

Further, it was noted that in all of the samples there was no appearance of the peak at 3400 cm^{-1}. There was only a shifted, similar peak at around 3270 cm^{-1}, in all except the female ventral, where there was none of these. The observed shift or attenuation of the band near 3270 cm^{-1} (and its absence in the female ventral sample) is consistent with an alteration of the N–H stretching vibration of amide groups in the chitin–protein matrix. Such an alteration may, in principle, reflect a modification of the hydrogen-bonding environment of these groups, one possible explanation for which would be an interaction with bound metal ions. However, the present FTIR data do not allow direct identification of the specific functional groups involved in any such interaction, and this interpretation therefore remains a working hypothesis to be examined in combination with the Raman data. One can also observe a series of not-so-intense peaks in the finger-print area around 750 cm^{-1}, and two peaks at proximity to 2360 and 2330 cm^{-1}, which clearly indicate the process of generation of CO_2 gas in the rapid microbiological degradation of defrosted tissue at the time of the sample handling. Raman spectra were taken by measurements directly on carapaces of individuals for both sexes.

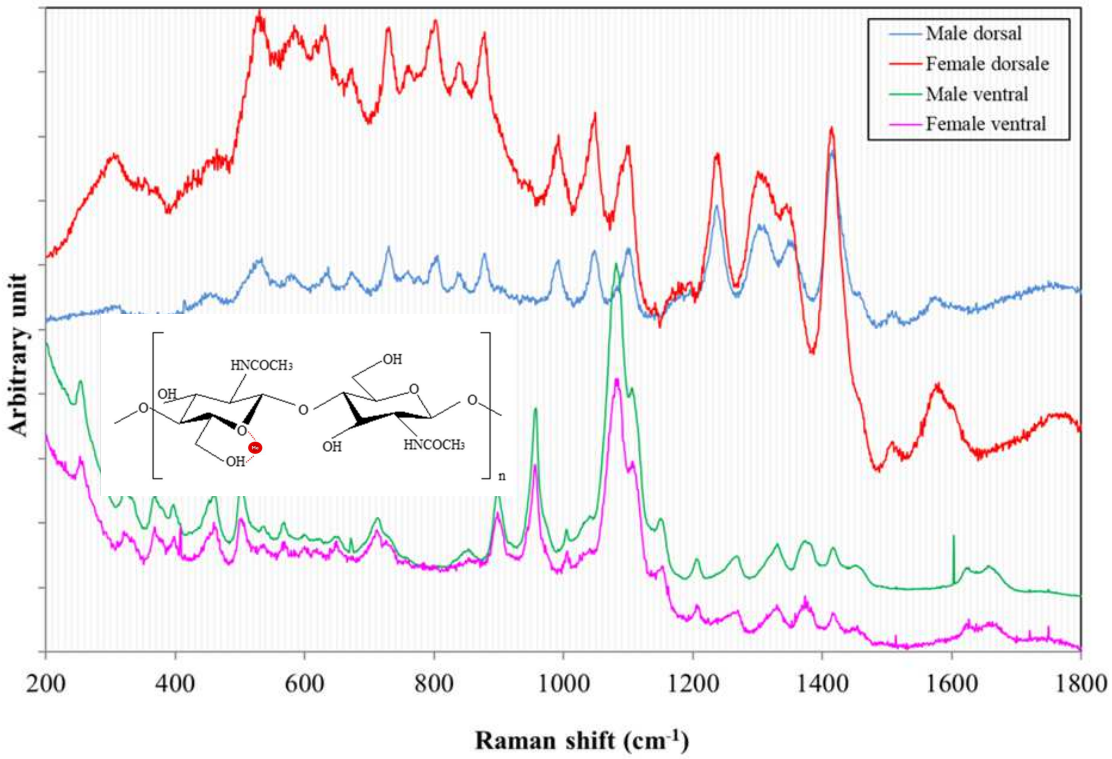


Fig. 3 Comparative Raman spectra (785 nm excitation) of dorsal and ventral carapace samples of male and female Faxonius limosus, showing the dominant α-chitin band at

≈1419 cm^{-1} and saccharide-ring bands at ≈1101, 1048 and 991 cm^{-1} (Kaya et al., 2017); in the ventral samples, a new peak at ≈1083 cm^{-1} overlays the ≈1108 cm^{-1} band. Insert: schematic of the chitin saccharide ring with the ≈1080–1110 cm^{-1} region highlighted as a candidate sorption area.

In the comparative spectra (Fig. 3), one can observe that the spectra of the dorsal for both sexes and ventral for both sexes have high matching of the peak positions. These two groups, on the contrary, show several differences in the analysis of the dominant Raman peaks. For example, for dorsal samples, the most dominant peak is at around 1419 $cm^{-1,}$ which most probably corresponds to the CH_2 ending and CH_3 deformation in the structure of α-chitin. Next, following the literature recommendations, peaks for the glycosidic linkage at 896, 891, or 894cm^{-1} could be expected for α, β, or γ chitin, respectively (Kaya et al., 2017). Although in the present analysis, such peaks could not be observed, there are particular peaks at around 853 for ventral and 880 cm^{-1} for dorsal, which correspond, in order, to the next one to the saccharide peaks.

Corresponding peaks for the saccharide ring in dorsal samples are mostly proportional and with a minor right shift compared to the available literature (in the present study, peaks were at: 1101, 1048 and 991 cm^{-1}). However, the peak at around 1148 cm^{-1} was not observed at all. On the contrary, in the spectra of ventral samples, saccharide peaks are almost a perfect match with the literature ones for α-chitin, but with one observable strong deviation. In particular, the peak at 1108 is mostly overlaid by a new, stronger peak at 1083, which is not even close to any of the suggested peaks from the literature. Taken together, the new peak at ≈1083 cm^{-1} observed in the ventral Raman spectra, the local alteration of the N–H stretching band in the corresponding FTIR spectra, and the deviation of the saccharide-ring peaks from literature values collectively highlight the 1080–1110 cm^{-1} region of the cuticle spectrum as a zone in which the molecular environment of chitin is markedly altered relative to reference α-chitin. Because chitin contains O- and N-containing functional groups that, in principle, are capable of coordinating cations, this region is a reasonable candidate for further investigation as a potential site of metal–matrix interaction in the cuticle. The present data, however, do not provide direct molecular-scale evidence for metal coordination at this site, and the assignment should therefore be regarded as a hypothesis that would require confirmation by techniques capable of resolving metal coordination environments, such as EXAFS or XANES spectroscopy. The pronounced intensity of the ≈1083 cm^{-1} peak in the ventral Raman spectra suggests that the chemical environment of this part of the cuticle differs substantially from that of the dorsal cuticle and from reference α-chitin. This observation is consistent with, but does not by itself demonstrate the hypothesis that metal

ions, when present, may interact with oxygen–containing groups of the saccharide ring, potentially through inner–sphere coordination (commonly referred to as chemisorption). Whether such an interaction actually occurs in the present samples, and whether it involves charge–transfer phenomena, cannot be resolved from vibrational spectroscopy alone and remains a hypothesis to be tested with element– and site–specific techniques. Taken together, the FTIR and Raman observations in the ventral cuticle, namely the attenuation of the N–H stretching band, the appearance of the ≈1083 cm^{-1} peak, and the deviation of the 1100–1148 cm^{-1} saccharide peaks from literature values (Kaya et al., 2017) are mutually consistent and indicate that the molecular organization of the ventral cuticle is altered relative to the dorsal cuticle and to pristine α-chitin. In the context of the present study, where measurable concentrations of Pb, Cd, As and Hg were determined in the exoskeleton by MP-AES, these spectroscopic alterations are interpreted as consistent with, though not proof of the possibility that the ventral cuticle is a region of preferential metal/matrix interaction. The schematic shown in the insert of Fig. 3 is therefore presented as a working model of a candidate sorption region, rather than as a definitive depiction of a metal coordination site, and should be read as a hypothesis-generating illustration. However, the FTIR and Raman data obtained in this study are interpreted at the level of functional group composition, molecular conformation, and organic–mineral partitioning of the exoskeleton, rather than as direct evidence of metal–ligand coordination. Apparent shifts in band positions and relative intensities in contaminated individuals were discussed as indicators of cuticle alteration associated with metal exposure, while the identification of the precise molecular binding sites responsible for metal retention remains an open question that complementary techniques would be required to address.

Metals bioaccumulation analysis

Zoobenthos, including a crayfish, live in direct contact with the bottom, where typically a polluted sediment is located. They are in direct contact with the sediment and are because of that, potentially increasingly exposed to the pollutants present there. In the present study, average metal concentrations analysed in the sediment decreased in the order Pb > As > Cd > Hg.

In each crayfish specimen, respectively, accumulated concentrations of four studied metals were measured in the abdominal muscle, hepatopancreas and exoskeleton. Mean values of these concentrations are given in Table 2. Differences in concentrations and distribution could be seen between these data for the analysed crayfish organs.

Clearly, the highest concentrations are those of Cd, found in the abdominal muscle of male individuals, followed by the concentration of Pb in the exoskeleton of females. On the other hand, the lowest concentrations were those of Hg in all the analysed crayfish tissues. Concentrations of As were increased in all three types of tissues for males compared with females.

Table 2 Average values of metals in abdominal muscles, hepatopancreas and carapax (exoskeleton) and sediment

Metals (ppm)	Males			Females			Sediment
	Abdominal muscle	Hepatopancreas	Exoskeleton	Abdominal muscle	Hepatopancreas	Exoskeleton	
Pb	0.060	0.050	0.050	0.040	0.050	0.140	0.380
Cd	0.140	0.010	0.010	0.010	0.010	0.010	0.020
Hg	0.001	0.001	0.001	0.001	0.001	0.001	0.001
As	0.050	0.040	0.030	0.020	0.020	0.020	0.060

Survey and experimental analysis significance was controlled by an ANOVA test (results shown in Table 3).

A significant difference between the Pb concentrations in tissues and in sediment was found (a low p-value, significance level of less than 0.05) . Same trend is found for Concentrations of the As between males, females and sediment, have shown the same trend.

Table 3 . Results of Analysis of variance between groups (tissues and sediment), within groups (males and females), and total in metal concentration

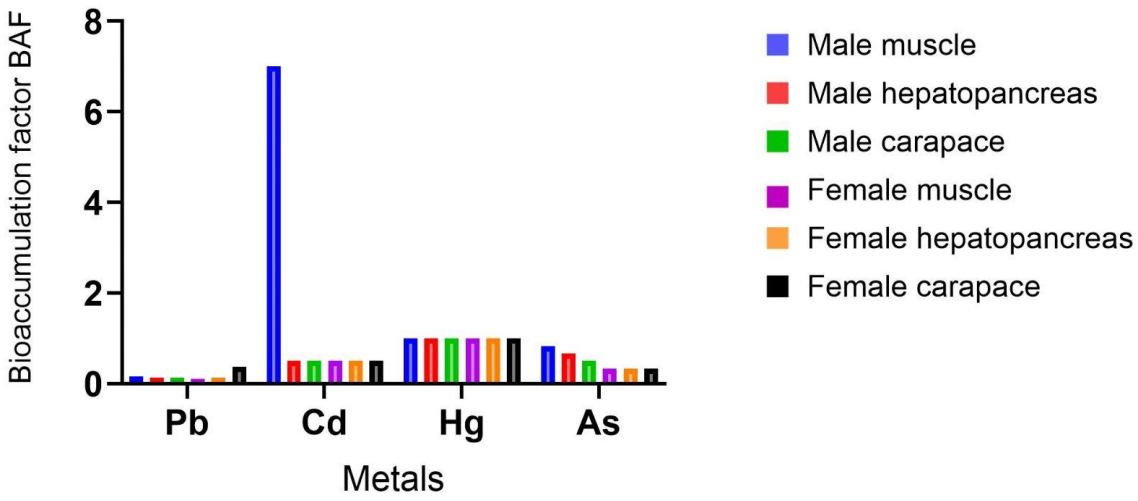
Metals		Sum of Squares	df	Mean Square	F	p-value (significance level at 0.05)
Pb	Between Groups	0.203	2	0.102	98.462	0.000
	Within Groups	0.006	6	0.001	/	/
	Total	0.210	8	/	/	/
Cd	Between Groups	0.003	2	0.001	0.782	0.499
	Within Groups	0.011	6	0.002	/	/
	Total	0.014	8	/	/	/
Hg	Between Groups	0.000	2	0.000	1.000	0.422
	Within Groups	0.000	6	0.000	/	/
	Total	0.000	8	/	/	/

As	Between Groups	0.002	2	0.001	35.548	0.000
	Within Groups	0.000	6	0.000	/	/
	Total	0.003	8	/	/	/

Table 4 shows results of the Kruskal Wallis test, pointing out precise differences between females and sediment. Asymptotic significances are displayed. The significance level is 0.050.

Table 4 Result of Kruskal Wallis test

	Null Hypothesis	**Test**	**Significance**	**Decision**
1.	The distribution of Pb is the same across categories of the group	Independent-Samples Kruskal-Wallis Test	0.059	Retain the null hypothesis
2.	The distribution of Cd is the same across categories of the group	Independent-Samples Kruskal-Wallis Test	0.144	Retain the null hypothesis
3.	The distribution of Hg is the same across categories of the group	Independent-Samples Kruskal-Wallis Test	0.368	Retain the null hypothesis
4.	The distribution of As is the same across categories of the group	Independent-Samples Kruskal-Wallis Test	0.024	Reject the null hypothesis



Tissue-specific bioaccumulation factors (BAF) of Pb, Cd, Hg and As in different organs (muscle, hepatopancreas, and carapace) of male and female *Faxonius limosus*.Criteria for the analysis were found in the literature by other authors who studied bioaccumulation (Brooks, 1998; Demina et al., 2009; Anani et al., 2019; Nour and Garoub, 2026), in particular, to define the bioaccumulation factor. BAF, as already defined, is the ratio of metal concentration in tissue

compared to soil, and if BAF was greater than 1, this was an indication of an increased level beyond the defined threshold. On the other hand, if BAF was lower than 1, values indicated safe limits.

Biological accumulation confirmed for metals subject of present research, indicated to the ability of crayfish to accumulate these specific metals..The tissue-specific bioaccumulation factors revealed distinct accumulation patterns across different organs, metals, and sexes (Figure 4). The highest bioaccumulation potential was recorded for Cd in male muscle tissue (BAF 7.0), indicating a strong biomagnification capacity. In contrast, Cd accumulation in all other tissues and in females remained low (BAF 0.5). For Pb and As BAF values were generally below 1, indicating low bioaccumulation relative to the sediment. The highest BAF for Pb was observed in the female carapace (BAF 0.37), while the highest BAF for As was found in male muscle tissue (BAF 0.83). Hg exhibited a uniform BAF value of 1.0 across all tissues and sexes, reflecting its very low concentrations near the detection limit in both the sediment and crayfish organs..

Discussion

For the present discussion, it is convenient to start with a reminder that river sediment is comprised of complex systems containing accumulated or dispersed eroded particles, organic matter, nutrients, and anthropogenic particles which are transported long distances along a basin by water (Mackenzie, 2005). Sediments are an essential, dynamic component of all aquatic ecosystems as they have a pronounced tendency to accumulate and retain toxic and non-degradable anthropogenic compounds (Barrento et al., 2009). This is why studies like the one presented here are necessary. Undoubtedly, accumulation of toxic substances in sediments has numerous negative impacts on the total aquatic ecosystem, some of these impacts highly visible, ranging to some individual/isolated and invisible impacts.

Reasons behind the application of aquatic organisms as bioindicators of ecotoxicity are multiple. Macroinvertebrates and fish, which live their entire life cycle within a particular aquatic ecosystem, are thus suitable for water quality monitoring. As indicators of current pollution levels, organisms with shorter life cycles and high reproductive potential are useful. Logically, species which live longer and could bioaccumulate more, are better for monitoring long-term pollutants (Pavlović et al., 2006; Reddy et al., 1997).

It is generally accepted that freshwater decapods are capable of accumulating metals in their muscles to fulfil their basic metabolic needs. This explains why this makes them suitable bioindicators of metals in the ecosystem, as was also studied here.

The present study confirms that spiny-cheek crayfish found at the marked location of the Danube accumulate cadmium, lead, mercury, and arsenic in the abdominal muscles and also in the exoskeleton to certain levels (Table 2). Despite the fact that the statistical analysis of the variances between the groups did not provide too many significant p-values, it is necessary to focus on the absolute measured concentrations of particular metals given in Table 2. Although the observed accumulation trends of Cd, Pb, and As in the abdominal muscles of males were identified within a limited number of analyzed samples, particularly regarding female specimens, they certainly provide a valuable basis and motivation for further research. These

preliminary data deviate to some extent from the patterns described in earlier literature. It has been clearly demonstrated that decapods absorb and accumulate Cd and Pb from both water and food, but the hepatopancreas remains the primary organ where the majority of this accumulation occurs (Barrento et al., 2009). This typical distribution of toxic metals between abdominal muscles and the hepatopancreas in various decapod species worldwide is further confirmed by a comprehensive literature review covering the period from the early 1970s to 2007 (Kouba et al., 2010).

In contrast, our study revealed elevated Cd and Pb levels in the abdominal muscles of males, while elevated Pb concentrations were recorded in the exoskeleton of females, where mercury accumulation is otherwise primarily expected. Given the limited sample size, especially for female specimens, we do not interpret these results as definitive biological regularities. Instead, we view them solely as preliminary indications and working hypotheses that require further verification through studies involving a substantially larger number of samples.

Unexpectedly, the overall concentration of Hg in studied sediments was 0.001 ppt in all analyzed tissues, indicating negligible Hg accumulation.

Like other toxic or potentially toxic metals, As tends to accumulate in the tissues of freshwater crayfish. Studies have shown that As is absorbed from both water and food, with the liver and pancreas being the major organs for accumulation, similar to Cd and Pb (Rodríguez Martín et al., 2006; Reynolds & Souty-Grosset, 2011). Arsenic concentrations in crayfish were highest in muscles, slightly lower in the hepatopancreas, and the lowest in the exoskeleton. The present discussion would like to suggest that such bioaccumulation of As in crayfish could serve as an important indicator of sediment pollution, especially in areas affected by the studied metals. Arsenic in sediments can also originate from natural sources such as volcanic eruptions and rock erosion; however, it could also evolve from human activities such as industrial pollution, the use of pesticides, and the burning of fossil fuels, as suspected here (Roldan & Shivers, 1987).

In discussing the BAF differences found between the two sexes, multiple factors could potentially play a role. Most probably, seasonal activity of individual crayfish, dependent on the reproductive period, is the most decisive factor. In this study, sampling was performed in May and January and it was confirmed that all the females had eggs attached, which confirms that they were in the reproductive period. In the reproductive period, females typically do not move a lot and stay calm near to their habitat (Simon et al., 2000). *F. limosus* is characterized by a high level of reproductive plasticity, and this is why it is hard to define their exact reproductive periods. They depend more on the water body temperature than on the season (Smith et al., 1996). There is a possibility that they are susceptible to depending on the local climate change as well. On the other hand, males are known to move more intensively, up to 20 km/year or about 7-8 km/year, in searching for new habitats and for females (Souty-Grosset, 2006). These increased movements, combined with pronounced search for food, can explain why they might accumulate more metals, as their food is available mostly in potentially contaminated sediment. It is necessary to study these considerations further. The tissue-specific BAF analysis provides insights into metal partitioning in crayfish. The

exceptionally high BAF of Cd in male muscle tissue indicates high Cd bioavailability in Danube sediment and a specific physiological affinity of crayfish muscle for this toxic metal, which is highly relevant for environmental risk assessment. In contrast, the greater accumulation of Pb in the carapace, particularly in females, agrees with literature showing that decapod crustaceans often deposit divalent metals like Pb into their calcified exoskeleton as a detoxification mechanism. However, because of the limited number of analyzed females, the observed sex-based differences in BAF, such as elevated Pb in the female carapace, should be considered preliminary and require further investigation with larger, more balanced sample sizes.

At this place it is important to once more compare the present findings with those from the used literature. The sediment metal ordering reported here (Pb > As > Cd > Hg) and the absolute tissue concentrations measured in Faxonius limosus are now compared with (i) Calmuc et al. for the Lower Danube in Romania, which reports a similar Pb > Cd ranking at moderately impacted sites; (ii) Jovičić et al. on wels catfish (Silurus glanis) from the same stretch of the Serbian Danube, whose muscle Pb and Cd burdens are of the same order of magnitude as those we measured in F. limosus; and (iii) the sediment studies of Crnković et al. and Kolarević et al. at the Belgrade area of the Danube–Sava confluence, which identify the same anthropogenic pressure sources (urban sewage, industry, shipping). This positions the Slankamen site consistently within the regional pattern of Pb and Cd dominance along the middle/lower Danube.

If referred to the decapod review of Kouba et al., which places the hepatopancreas as the main Cd and Pb reservoir, and uses this comparator to flag the unusually high male-muscle Cd accumulation (BAF 7.0) in the present study as a divergent pattern that warrants further investigation.

Conclusion

Obtaining more data sources, both about different species, tissues, and sampling sites, will produce more relevant and reliable biomonitoring surveys. Hopefully, this study will fulfil the aim of drawing attention to emerging risks in the human consumption chain related to the studied crayfish, but also to its potential use as the convenient bioindicator of the pollution status of river sediments. The FTIR and Raman spectra of the carapace indicate alterations of vibrational bands attributable to chitin, amide groups and the carbonate mineral phase in individuals collected at the studied Danube site, where measurable metal concentrations in the exoskeleton were determined by MP-AES. These spectroscopic alterations are compatible with, but do not, in themselves, prove the hypothesis that a fraction of the metals associated with the exoskeleton may be bound to the cuticle via inner-sphere coordination. The mechanistic nature of metal retention in the cuticle, and the relative contribution of physisorption versus chemisorption-like interactions, therefore remain open questions that will require element-specific, coordination-sensitive techniques to be resolved.

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Data availability statement: Raw data are available on request.

Declarations

Ethics approval According to national regulations **(Annex 4 of the "Official Gazette of RS", No. 39/10)**, ethical approval is not required for research involving invertebrate animals. Regarding the permitting, collection of non-native species does not require any permit in Serbia

Competing interests: The authors declare no competing interests

References

Alikhan, M. A., Bagatto, G., & Zia, S. (1990). The crayfish as a "biological indicator" of aquatic contamination by heavy metals. *Water Research*, *24*(9), 1069–1076. https://doi.org/10.1016/0043-1354(90)90168-6

Anani, O. A., Olomukoro, J. O., Anani, O. A., & Olomukoro, J. O. (2019). Assessment of Metal Accumulation and Bioaccumulation Factor of Some Trace and Heavy Metals in Freshwater Prawn and Crab. In *Crustacea*. IntechOpen. https://doi.org/10.5772/intechopen.88103

Barrento, S., Marques, A., Teixeira, B., Carvalho, M. L., Vaz-Pires, P., & Nunes, M. L. (2009). Accumulation of elements (S, As, Br, Sr, Cd, Hg, Pb) in two populations of *Cancer pagurus*: Ecological implications to human consumption. *Food and Chemical Toxicology*, *47*(1), 150–156. https://doi.org/10.1016/j.fct.2008.10.021

Bridgewater, L. L., Baird, R. B., Eaton, A. D., Rice, E. W., American Public Health Association, American Water Works Association, & Water Environment Federation (Eds). (2017). *Standard methods for the examination of water and wastewater* (23rd edition). American Public Health Association.

Brooks, R. R. (1998). *Plants that Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining*. CAB International.

Bruno, G., Volpe, M. G., Luise, G. D., & Paolucci, M. (2006). DETECTION OF HEAVY METALS IN FARMED CHERAX DESTRUCTOR. *Bulletin Français de La Pêche et de La Pisciculture*, (380–381), Article 380–381. https://doi.org/10.1051/kmae:2006039

Buřič, M., Kouba, A., & Kozák, P. (2013). Reproductive plasticity in freshwater invader: from long-term sperm storage to parthenogenesis. *PLoS ONE, 8*(10), e77097. https://doi.org/10.1371/journal.pone.0077097

Dib, O., Assaf, A., Pean, A., Yannick, F., Zalouk-Vergnoux, A., Thouand, G., & Durand, M.-J. (2026). Evaluation of cadmium's impact on marine mussels: Insights from Raman spectroscopy and chemometric analysis. Analytical and Bioanalytical Chemistry, 418(4), 1347–1356. https://doi.org/10.1007/s00216-025-06293-1

Calmuc, V. A., Calmuc, M., Arseni, M., Topa, C. M., Timofti, M., Burada, A., Iticescu, C., & Georgescu, L. P. (2021). Assessment of Heavy Metal Pollution Levels in Sediments and of Ecological Risk by Quality Indices, Applying a Case Study: The Lower Danube River, Romania. *Water*, *13*(13), Article 13. https://doi.org/10.3390/w13131801

Caro, T. (2010). *Conservation by Proxy: Indicator, Umbrella, Keystone, Flagship, and Other Surrogate Species*. Island Press.

Chambers, M. G. (1995). *The effect of acute cadmium toxicity on marron, Cherax tenuimanus (Smith, 1912) (Family Parastacidae)*. (10), 209–220. https://www.astacology.org/FCmobileAbstracts.asp?uid=Guest&mid=450&v=10&i=1

Crnković, D. M., Crnković, N. S., Filipović, A. J., Rajaković, L. V., Perić-Grujić Jr., A. A., & Ristić, M. Đ. (2008). Danube and Sava river sediment monitoring in Belgrade and its surroundings. *Journal of Environmental Science and Health, Part A*, *43*(12), 1353–1360. https://doi.org/10.1080/10934520802231974

Del Ramo, J., Díaz-Mayans, J., Torreblanca, A., & NÚñez, A. (1987). Effects of temperature on the acute toxicity of heavy metals (Cr, Cd, and Hg) to the freshwater crayfish, *Procambarus clarkii* (Girard). *Bulletin of Environmental Contamination and Toxicology*, *38*(5), 736–741. https://doi.org/10.1007/BF01616694

528 Demina, L. L., Galkin, S. V., & Shumilin, E. N. (2009). Bioaccumulation of some trace elements in the
529 biota of hydrothermal fields of the Guaymas Basin (Gulf of California). *Boletín de la Sociedad*
530 *Geológica Mexicana*, *61*(1), 31–45. https://doi.org/10.18268/BSGM2009v61n1a4
531 Duffus, J. H. (2002). 'Heavy metals' a meaningless term? (IUPAC Technical Report). *Pure and Applied*
532 *Chemistry*, *74*(5), 793–807. https://doi.org/10.1351/pac200274050793
533 Garzoli, L., Mammola, S., Ciampittiello, M., & Boggero, A. (2020). Alien Crayfish Species in the Deep
534 Subalpine Lake Maggiore (NW-Italy), with a Focus on the Biometry and Habitat Preferences of
535 the Spiny-Cheek Crayfish. *Water*, *12*(5), Article 5. https://doi.org/10.3390/w12051391
536 Gherardi, F. (Ed.). (2017). *Crayfish in Europe as Alien Species*. Routledge.
537 https://doi.org/10.1201/9781315140469
538 Habib, S. S., Latif, F., Rind, K. H., Masud, S., Fazio, F., Kesbiç, O. S., ... & Al-Rejaie, S. S. (2026).
539 Seasonal bioaccumulation and sources of heavy metals in water, sediment, and commercial fish
540 species from Taunsa Reservoir, Indus River: implications for human health risk. *Biological Trace*
541 *Element Research, 204*(6), 3759-3775.
542 Holdich, D. M., Reynolds, J. D., Souty-Grosset, C., & Sibley, P. J. (2009). A review of the ever-
543 increasing threat to European crayfish from non-indigenous species. *Knowledge and Management*
544 *of Aquatic Ecosystems*, *11*(394-395) https://doi.org/10.1051/kmae/2009025
545 European Commission. (2016). Commission Implementing Regulation (EU) 2016/1141 of 13 July 2016
546 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No
547 1143/2014 of the European Parliament and of the Council. Official Journal of the European
548 Union, L 189, 4–15.
549 Jovičić, K., Nikolić, D. M., Višnjić-Jeftić, Ž., Đikanović, V., Skorić, S., Stefanović, S. M., Lenhardt, M.,
550 Hegediš, A., Krpo-Ćetković, J., & Jarić, I. (2015). Mapping differential elemental accumulation
551 in fish tissues: Assessment of metal and trace element concentrations in wels catfish (Silurus
552 glanis) from the Danube River by ICP-MS. *Environmental Science and Pollution Research*,
553 *22*(5), 3820–3827. https://doi.org/10.1007/s11356-014-3636-7

Karaman, I., & Machino, Y. (2004). *Occurrence of the spiny-cheek crayfish (Orconectes limosus) and the Chinese mitten crab (Eriocheir sinensis) in Serbia*. 19–20. https://www.astacology.org/docs/cn/CrayfishNews_26(2)_lr.pdf

Kaya, M., Mujtaba, M., Ehrlich, H., Salaberria, A. M., Baran, T., Amemiya, C. T., Galli, R., Akyuz, L., Sargin, I., & Labidi, J. (2017). On chemistry of γ-chitin. *Carbohydrate Polymers*, *176*, 177–186. https://doi.org/10.1016/j.carbpol.2017.08.076

Kolarevic, S., Knezevic-Vukcevic, J., Paunovic, M., Tomovic, J., Gacic, Z., & Vukovic-Gacic, B. (2011). The anthropogenic impact on water quality of the river Danube in Serbia: Microbiological analysis and genotoxicity monitoring. *Archives of Biological Sciences*, *63*(4), 1209–1217. https://doi.org/10.2298/ABS1104209K

Kouba, A., Buřič, M., & Kozák, P. (2010). Bioaccumulation and Effects of Heavy Metals in Crayfish: A Review. *Water, Air, & Soil Pollution*, *211*(1), 5–16. https://doi.org/10.1007/s11270-009-0273-8

Kuklina, I., Kouba, A., & Kozák, P. (2013). Real-time monitoring of water quality using fish and crayfish as bio-indicators: A review. *Environmental Monitoring and Assessment*, *185*(6), 5043–5053. https://doi.org/10.1007/s10661-012-2924-2

Larson, E. R., & Olden, J. D. (2016). Field Sampling Techniques for Crayfish. In *Biology and Ecology of Crayfish*. CRC Press.

Lenhardt, M., Smederevac-Lalić, M., Hegediš, A., Skorić, S., Cvijanović, G., Višnjić-Jeftić, Ž., Djikanović, V., Jovičić, K., Jaćimović, M., & Jarić, I. (2020). Human Impacts on Fish Fauna in the Danube River in Serbia: Current Status and Ecological Implications. In D. Bănăduc, A. Curtean-Bănăduc, F. Pedrotti, K. Cianfaglione, & J. R. Akeroyd (Eds), *Human Impact on Danube Watershed Biodiversity in the XXI Century* (pp. 257–279). Springer International Publishing. https://doi.org/10.1007/978-3-030-37242-2_13

Lovrić, A., Ilić, K., Čermak, A. M. M., Pavičić, I., Volarić, N., & Petrinec, B. (2026). Bioaccumulation of metal pollutants and radionuclides in dragonflies (Odonata). *Environmental Research*, 124579.

579 Mackenzie, F. T. (2005). *Sediments, Diagenesis, and Sedimentary Rocks: Treatise on Geochemistry,*
580 *Second Edition, Volume 7* (Vol. 7). Elsevier.
581 Morais, C. L. M., Lima, K. M. G., Singh, M., & Martin, F. L. (2020). Tutorial: Multivariate classification
582 for vibrational spectroscopy in biological samples. Nature Protocols, 15(7), 2143–2162.
583 https://doi.org/10.1038/s41596-020-0322-8
584 Nour, H. E., & Garoub, M. M. (2026). Heavy metal bioaccumulation and risk assessment in intertidal
585 molluscan shells and sediments of Suez Bay. *Marine Pollution Bulletin, 224*, 119141
586 Official Gazette of Republic of Serbia 50/2012, Pub. L. Nos 110-3320/2012-1, 10 (2012).
587 https://www.pravno-informacioni-
588 sistem.rs/SlGlasnikPortal/eli/rep/sgrs/vlada/uredba/2012/50/1/reg
589 Pallottini, M., Goretti, E., Gaino, E., Selvaggi, R., Cappelletti, D., & Céréghino, R. (2015). Invertebrate
590 diversity in relation to chemical pollution in an Umbrian stream system (Italy). *Comptes Rendus.*
591 *Biologies*, *338*(7), 511–520. https://doi.org/10.1016/j.crvi.2015.04.006
592 Pavlović, S., Milošević, S., Borković, S., Simić, V., Paunović, M., Žikić, R., & Saičić, Z. (2006). A
593 Report of *Orconectes (Faxonius) Limosus* (Rafinesque, 1817) [Crustacea: Decapoda: Astacidea:
594 Cambaridae: Orconectes: Subgenus Faxonius] in the Serbian Part of the River Danube.
595 *Biotechnology & Biotechnological Equipment*, *20*(1), 53–56.
596 https://doi.org/10.1080/13102818.2006.10817304
597 Reddy, P. S., Tuberty, S. R., & Fingerman, M. (1997). Effects of Cadmium and Mercury on Ovarian
598 Maturation in the Red Swamp Crayfish,Procambarus clarkii. *Ecotoxicology and Environmental*
599 *Safety*, *37*(1), 62–65. https://doi.org/10.1006/eesa.1997.1523
600 Reynolds, J., & Souty-Grosset, C. (2011). *Management of Freshwater Biodiversity: Crayfish as*
601 *Bioindicators*. Cambridge University Press.
602 Ritschar, S., Bangalore Narayana, V. K., Rabus, M., & Laforsch, C. (2020). Uncovering the chemistry
603 behind inducible morphological defences in the crustacean Daphnia magna via micro-Raman
604 spectroscopy. Scientific Reports, 10(1), 22408. https://doi.org/10.1038/s41598-020-79755-4

Rodríguez Martín, J. A., Arias, M. L., & Grau Corbí, J. M. (2006). Heavy metals contents in agricultural topsoils in the Ebro basin (Spain). Application of the multivariate geoestatistical methods to study spatial variations. *Environmental Pollution*, *144*(3), 1001–1012. https://doi.org/10.1016/j.envpol.2006.01.045

Roldan, B. M., & Shivers, R. R. (1987). The uptake and storage of iron and lead in cells of the crayfish Orconectes propinquus) hepatopancreas and antennal gland. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *86*(1), 201–214. https://doi.org/10.1016/0742-8413(87)90165-4

Roljić, R. L., Nikolić, V. P., Đikanović, V. Đ., Zorić, K. S., Urošević, A. M., & Marković, V. M. (2024). Morphometric characteristics of spiny-cheek crayfish Faxonius limosus (Rafinesque, 1817) from the Danube River on the territory of Serbia. *Archives of Biological Sciences*, *76*(76), 5–5. https://doiserbia.nb.rs/Article.aspx?ID=0354-46642400005R

Simon, O., Ribeyre, F., & Boudou, A. (2000). Comparative Experimental Study of Cadmium and Methylmercury Trophic Transfers Between the Asiatic Clam Corbicula fluminea and the Crayfish Astacus astacus. *Archives of Environmental Contamination and Toxicology*, *38*(3), 317–326. https://doi.org/10.1007/s002449910042

Smith, S. L., MacDonald, D. D., Keenleyside, K. A., Ingersoll, C. G., & Jay Field, L. (1996). A Preliminary Evaluation of Sediment Quality Assessment Values for Freshwater Ecosystems. *Journal of Great Lakes Research*, *22*(3), 624–638. https://doi.org/10.1016/S0380-1330(96)70985-1

Souty-Grosset, C. (2006). *Atlas of Crayfish in Europe*. Muséum national d'histoire naturelle.

Sures, B. (2003). Accumulation of heavy metals by intestinal helminths in fish: An overview and perspective. *Parasitology*, *126*(7), S53–S60. https://doi.org/10.1017/S003118200300372X

Todorov, M., Trichkova, T., Hubenov, Z., & Jurajda, P. (2020). Faxonius limosus (Rafinesque, 1817) (Decapoda: Cambaridae), a New Invasive Alien Species of European Union Concern in Bulgaria. *72*, *1*, 113–121. https://www.acta-zoologica-bulgarica.eu/002367

631 Zorić, K. S., Atanacković, A. D., Ilić, M. D., Csányi, B., & Paunović, M. M. (2020). The Spiny-cheek
632 Crayfish Faxonius limosus (Rafinesque, 1817) (Decapoda: Cambaridae) Invades New Areas in
633 Serbian Inland Waters. *Acta Zoologica Bulgarica*, (72 (4)), 623–627.
634