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Potentially toxic elements in freshwater (*Alburnus* spp.) and marine (*Sardina pilchardus*) sardines from the Western Balkan Peninsula: An assessment of human health risk and management

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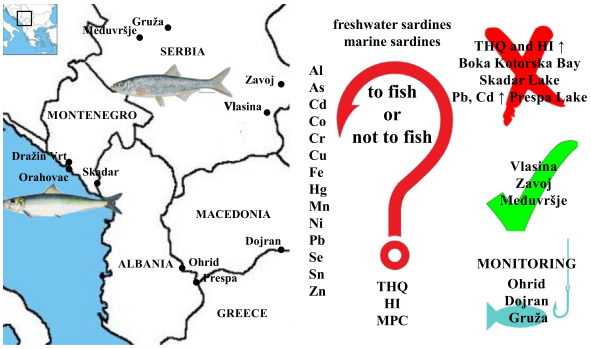
HIGHLIGHTS

- The As concentrations were above the MPC in marine sardines from Boka Kotorska Bay.
- THQ and HI indicated a health risk for Montenegrin consumers.
- Zavoj, Vlasina and Međuvršje reservoirs are potential for commercial fishing and the safe use of freshwater sardines.
- General recommendation for the management is that the use of freshwater sardines should be emphasized.

GRAPHICAL ABSTRACT



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ABSTRACT

The concentrations of 14 potentially toxic elements in freshwater and marine sardines from the Western Balkan Peninsula were determined. In Prespa Lake, a natural lake, the concentrations of toxic Pb and Cd were above the maximum permissible concentrations in freshwater sardines. The As concentrations were elevated in sardines from Boka Kotorska Bay. The Target Hazard Quotient (THQ) and Hazard index (HI) were above their defined limits in Boka Kotorska Bay and Skadar Lake, indicating a health risk for Montenegrin consumers. The results of this study set apart Zavoj, Vlasina and Međuvršje artificial reservoirs as potential ecosystems for developing commercial fishing and for the safe use of freshwater sardines for human consumption. Constant monitoring of Ohrid, Prespa and Dojran natural lakes, as well as Boka Kotorska Bay, may be implemented in the interest of public health.

1. Introduction

From the aspect of fisheries and the sustainable use of fish resources on a global level, there is a strong inequality between the use of freshwater sardines and, based on their body dimensions, similar marine sardines. The main reason for the considerably greater

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presence of marine sardines globally is their natural abundance and the large and developed marine fish market. However, in the Western Balkan Peninsula, this is not the case, since freshwater sardine fishing is not as well-developed as Montenegrin industrial fishing (Pešić et al., 2011).

According to Kottelat and Freyhof (2007), the genus *Alburnus* (bleak), the freshwater sardine, is represented by four species in the western part of the Balkan Peninsula: *Alburnus alburnus* (Linnaeus, 1758), *Aburnus scoranza* (Heckel and Kner, 1858), *Alburnus belvica* (Karaman, 1924) and *Alburnus macedonicus* (Karaman, 1928). The widest distributional pattern is shown by the species *Alburnus alburnus* (Linnaeus, 1758), while the species *Alburnus belvica* (Karaman, 1924) and *Alburnus macedonicus* (Karaman, 1928) are endemic to Prespa and Dojran lakes, respectively. The marine sardine, *Sardina pilchardus* (Walbaum, 1792) is one of the most abundant, and commercially the most important fish species in the fisheries of all countries located along the coast of the Adriatic Sea (Pešić et al., 2010; Sinovčić, 1991, 2000, 2003).

Our previous work (Simić et al., 2016) showed that the degree of sustainability in the populations of the genus *Alburnus* was high to medium in the western part of the Balkan Peninsula. A large fluctuation in the sardine biomass in the entire Adriatic Sea occurred in 1991 and continued until 1997, and since then, sardine stock assessment has indicated a recent slight increase in biomass (Cingolani et al., 2003).

Unlike other pollutants of organic origin, potentially toxic elements are not degraded or eliminated from the ecosystem (Rajkowska and Protasowicki, 2013), and they accumulate in sediments and organisms. Fish normally occupy high positions in aquatic trophic webs, accumulating several kinds of contaminants in their tissues, including potentially toxic elements (Bervoets and Blust, 2003; Noël et al., 2013). By examining published data, we realized that most studies were based on monitoring the potentially toxic element content in different fish species, but a very small number were based on monitoring the potentially toxic element content in freshwater sardines (*Alburnus* spp.) (Al Sayegh Petkovšek et al., 2012; Jovanović et al., 2018; Merciai et al., 2014) and marine sardines (*Sardina pilchardus*) (Walbaum, 1792) (Canli et al., 2001; Ilgar, 2016; Jureša and Blanuša, 2003; Pešić et al., 2010).

Fish and fish products, including resources from freshwater environments (EPA, 2000), are known to be among the food categories that contribute the most to human dietary exposure to contaminants (WHO, 2011). According to our findings, in the countries of the Western Balkans (Serbia, Macedonia, Montenegro and Albania), there have been no studies dealing with the assessment of the human health risk due to consumption of freshwater or marine sardines. The Target Hazard Quotient (THQ) method provides an indication of the risk level associated with pollutant exposure and has been used by many researchers (Chien et al., 2002; Storelli, 2008; Wang et al., 2005), and since it has been shown to be valid and useful, this method was also applied in this study.

Although freshwater or marine sardines are small but very tasty and caloric and their fishing importance in some regions is very high, in other regions they are considered to be of little or no importance. European sardines are still an attractive seafood product in Galician and Spanish households (Vázquez-Rowe et al., 2014). The Montenegrin industrial fishing of marine sardines is still undeveloped due to the lack of organization in the fish markets and lack of trained crew (Pešić et al., 2011). Great inequality in the exploitation of the genus *Alburnus* has been shown by relevant surveys of fish catches from the Western Balkan Peninsula in which the percentage of *Alburnus* in the annual fish catch in the Black Sea drainage basin is slightly <1% (Simić et al., 2016). This is not the case in the systems of the Adriatic and the Aegean Sea Basins, where freshwater sardine is one of the most exploited fish species (e.g. 40–70% of the total fish catch from Skadar Lake) (Mrdak et al., 2001). These small but commercially valuable consumable fish, with different commercial potential across the Balkan Peninsula, are an underutilized resource due different region-specific, socio-economic and political conditions, legal regulation, lack of tradition, an underdeveloped market and a low price.

Having in mind the aforementioned, two of the major aims of this study were to determine the concentrations of 14 potentially toxic elements in the freshwater (*Alburnus* spp.) and marine (*Sardina pilchardus*) sardines of the Western Balkan Peninsula, and to estimate the human health risk due to their consumption using the THQ. Finally, the main aim was to constitute a guideline for decision-making in freshwater and marine sardine consumption, which can be a reference point for the future management programs of these species.

2. Material and methods

2.1. Study area and sampling

Eight reservoirs and lakes were chosen in Serbia, Macedonia, Albania, Greece and Montenegro, as well as two sampling sites in Boka Kotorska Bay in Montenegro (Fig. 1). The lentic freshwater systems situated in the territory of Serbia, belonging to the continental Sava (Gruža and Međuvršje) and Danube (Vlasina and Zavoj) and the Black Sea Basin, are artificial reservoirs. On the other hand, within the Adriatic (Skadar, Ohrid, Prespa) and Aegean sea basin (Dojran), all of the systems are natural lakes.

Međuvršje reservoir (ME) is the smallest reservoir investigated, covering an area of 1.5 km^2 with a maximum depth of 12 m (directly below the dam), influenced by numerous contaminants in the catchment area (Đikanović et al., 2016), followed by Zavoj Reservoir (ZA) with an area 5.53 km^2 and a maximum depth of 72 m (Simić et al., 2016). The Gruža Reservoir (GR) is situated in Central Serbia, with a surface area of 9.34 km^2 and a maximum depth of 31 m. The highest and largest is Vlasina Reservoir (VL), with an altitude of 1211 m, and an area of 16 km^2.

On the other hand, the natural lakes are larger and deeper. Skadar Lake (SK) is the largest natural, shallow (mean depth 5 m) lake in the Balkan Peninsula, with a surface area of between 370 km^2 and 530 km^2 (due to seasonal fluctuations), located on the border between Albania (one-third) and Montenegro (two-thirds). In contrast, Ohrid Lake (OH) is the deepest lake in the Balkan Peninsula with a maximum depth of 288 m and a surface area of 358 km^2, of which 248 km^2 belong to the Republic of Macedonia and 110 km^2 belong to Albania (Simić et al., 2016). Prespa Lake (PR) is the highest lake in the Balkan Peninsula (853 m) and it is shared by Albania, Greece, and the Republic of Macedonia with the following ratio: 176.3 km^2 belong to the Republic of Macedonia, 46.3 km^2 to Albania and 36.4 km^2 to Greece. Dojran Lake (DO) has an area of 43.1 km^2, shared between the Republic of Macedonia (27.3 km^2) and Greece (15.8 km^2) (Spirkovski and Bojadzieski, 2013). It is very interesting that all the natural lakes researched in this study are bordered between several countries.

The length of the Montenegrin coastline is about 300 km, of which about two-thirds (200 km) faces the open sea, and one-third forms the Boka Kotorska Bay. The Boka Kotorska Bay is a large bay situated in the southern part of the eastern Adriatic coast and it is one of the most indented parts of the Adriatic coast (Magaš, 2002). Two sampling sites were investigated in Boka Kotorska Bay in order to collect seawater sardines: Dražin vrt (DV) and Orahovac (OR).

The field research was conducted during the summer of 2014. Fishing was carried out using netting tools (gill nets with mesh size 0.5, 10–12 and 14–16 mm), and ten specimens of freshwater and seawater sardines were anesthetized with clove oil, washed with distilled water, and afterwards transported on ice (in individual zipper bags) to the laboratory. From very abundant samples, we chose the biggest individuals of freshwater and marine sardines, guided by the idea that they are the most often consumed. A sample of ten individuals is minimal for statistical analysis, so this number of fish was sampled and the remaining undamaged individuals were returned to the water.