Science of the Total Environment 648 (2019) 1087–1096

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Potentially toxic elements in water, sediments and fish of the Evrotas River under variable water discharges

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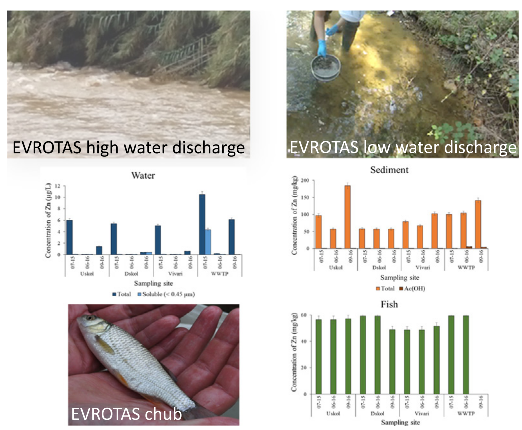
HIGHLIGHTS

- Potentially toxic elements were studied in Evrotas river water, sediments and fish.
- Variable water discharges have influence on contents of analytes in samples analysed.
- Concentrations of potentially toxic elements in water and fish are low to moderate.
- Ecological risk from the presence of potentially toxic elements in sediments is low.
- Contribution of potentially toxic elements to multiple stressor conditions is low.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 July 2018
Received in revised form 9 August 2018
Accepted 9 August 2018
Available online 9 August 2018

Editor: D. Barcelo

Keywords:
Evrotas River
Potentially toxic elements
Water
Sediments
Fish
Variable water discharge

ABSTRACT

Among different stressors like drought, hydro-morphological alterations, and pollution from agricultural activities, nutrients, organic compounds and discharges from wastewater treatment plants (WWTPs), potentially toxic elements (PTE) may also contribute to the overall pollution of the Evrotas River, Greece. Nevertheless, information on pollution of elements in water and sediments in this river is scarcely documented. There is also no information available on the impact of elemental pollution from the aquatic environmental compartments on biota. To fill these gaps, in this study, water, sediment and fish samples were collected from four sampling sites along the Evrotas River under variable flow regimes (July 2015, higher discharge; June 2016, low discharge and September 2016, minimum discharge). Total and dissolved element concentrations in water samples, total and acetic acid extractable contents in sediments, and element concentrations in fish samples were determined by inductively coupled plasma mass spectrometry and significant relationships between samples were established using correlation analysis. The concentrations of PTE (Ni, Cr, Cd, As, Pb, Zn and Cu) in water were generally low, while elevated Ni and Cr contents were found in sediments (up to 150 and 300 mg/kg, respectively), with total Cr concentration in water and sediment being positively correlated. The ecological risk posed by the simultaneous presence of PTE in sediments evaluated by calculating the Probable Effect Concentration Coefficient (PEC-Q), demonstrated that PEC-Qs, which were above the critical value of 0.34, derived mostly from Cr and Ni inputs. Since their mobile sediment fraction was extremely low, Cr and Ni origin is most probably geogenic. The

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https://doi.org/10.1016/j.scitotenv.2018.08.123

analysis of elements in the target fish species, the Evrotas chub, showed low to moderate PTE concentrations, with Pb being positively correlated with total Pb concentration in water. Moderate Zn concentrations found in fish samples from the Evrotas are possibly derived from pesticides and fertilizers.

1. Introduction

Water scarcity is common problem in the Mediterranean, where, as a consequence of global climatic changes, severe droughts are often followed by flash floods (Brilly et al., 2015). Intensive exploitation of water resources for industrial usage, agriculture, tourism and other human activities may create problems in quality and quantity of fresh water available for living beings (Cosgrove and Loucks, 2015). To control and prevent pollution in the European Union (EU) countries, regular water monitoring is applied, considering the requirements of the EU Water Framework Directive (WFD) (Directive 2000/60/EC, 2000) and its Daughter Directives (Directive 2008/105/EC, 2008; Directive 2009/90/EC, 2009; Directive 2013/39/EU, 2013; European Communities (EC) Technical Report 2009–025, 2009; EC Environmental Objectives 272/2009, 2009). As a common implementation strategy for the WFD, the EC issued guidance on chemical monitoring of sediment and biota (EC Technical Report 2010–041, 2010). Monitoring of water, sediment and biota may provide a coherent status of the water bodies within individual river basins. Among elements, WFD and its Daughter Directives prescribe monitoring of potentially toxic elements (PTE) (Cd, Pb, Hg, Ni, As, Cu, Cr and Zn).

Complementary to recommendations of the WFD, numerous sediment quality guidelines, based on biological effects approaches, have been proposed (MacDonald et al., 2000; McCready et al., 2006). For freshwater ecosystems, MacDonald et al. (2000) suggested consensus-based sediment quality guidelines, considering a threshold effect concentration (TEC), below which harmful effects are unlikely to be observed, and a probable effect concentration (PEC) above which harmful effects are likely to be observed. To assess potential hazard posed by the simultaneous presence of Cd, Pb, Hg, Ni, As, Cu, Cr and Zn, Long et al. (2006) proposed the Probable Effect Concentration Quotient (PEC-Q) approach.

The quality of water affects the quality of sediments and has also influence on the aquatic organisms. Contaminants from water, which have been accumulated in river bottom sediments, may be remobilized during heavy rainfalls into overlying waters (Borga et al., 2014; Kwok et al., 2014; Wu et al., 2015), where they are distributed between the suspended particulate matter (SPM) and dissolved fraction, while the partitioning of elements associated with the SPM and dissolved form, strongly depends on hydrological conditions (Vidmar et al., 2017; Milačič et al., 2017).

Since fish occupy a high position in the aquatic trophic web, they can accumulate different contaminants in their tissues, among them also PTE (Subotić et al., 2013; Noël et al., 2013; a; Đikanović et al., 2016; Milošković et al., 2016). Fish are bio-indicators of pollution, and represent also a source of food for other animals and humans (Taconand and Metian, 2009). So, it is important to determine concentrations of PTE in their tissues. To protect public health the EC issued maximum permissible concentrations of contaminants in different foodstuffs, among them Cd, Pb and Hg in fish (Commission Regulation 1881/2006, 2006; Commission Regulation 629/2008, 2008).

The study area of the current work is the Evrotas, a 82 km river located at the Peloponnese Peninsula, in Southern Greece. The natural and anthropogenic characteristics of the Evrotas basin are extensively described in Skoulikidis et al. (2011), Kalogianni et al. (2017) and Karaouzas et al. (2018a). Briefly, its upper section, characterized by sparse rural settlements, is drought impacted, while the lower Evrotas section, with much higher discharge, suffers from diffuse pollution from agriculture and point source pollution from the Sparta wastewater treatment plant (WWTP), receiving also seasonally orange juice factory and olive oil mill wastes. The impact of multiple stressors on the ecological status of the Evrotas River, such as hydromorphological alterations, water scarcity, high nutrient loads and organic contaminants are reported in Kalogianni et al. (2017) and Karaouzas et al. (2018a, 2018b). Data on the presence of contaminants in the Evrotas River is however available mainly for nutrients and organic contaminants (Stamati et al., 2010; Giulivo et al., 2017; Skoulikidis et al., 2017), while evidence on elemental pollution of the Evrotas River is scarce. Basic information exists for water and sediments (Tzoraki et al., 2015), but there is a lack of data on the simultaneous presence of PTE in water, sediments and fish from the Evrotas River.

Therefore, the aims of the present investigation were (i) to perform an analysis of PTE (Ni, Cr, Cd, As, Pb, Zn and Cu) in water, sediment and fish of the Evrotas River for identifying the areas of possible concern, (ii) to assess the influence of variable river flow conditions on mobilization of PTE from sediments into the overlaying waters of the Evrotas River and estimate their potential impact on fish, (iii) to apply the PEC-Q approach for an overall evaluation of the possible risk posed by the simultaneous presence of PTE in sediments of the Evrotas River.

This investigation was performed as a part of the EU 7th FW founded project GLOBAQUA, in which the Evrotas River is one of the river basins studied (Navarro-Ortega et al., 2015).

2. Materials and methods

2.1. Instrumentation

Total elemental concentrations were determined by ICP-MS (7700x, Agilent Technologies, Tokyo, Japan). ICP-MS operating parameters are presented in Table S1 (Supplementary). A CEM Corporation (Matthews, NC, USA) MARS 5 Microwave System was used for sample digestion. Analytical balance (Mettler AE 163, Zürich, Switzerland) was used for weighting.

Samples were lyophilised at −55 °C and 0.6 mbar in a CHRIST Gamma 1–16 LSCplus (Osterode am Harz, Germany) freeze dryer.

Shaking of samples was performed on an orbital shaker Vibromix 40 (Tehtnica, Slovenia) at 300 rpm and centrifugation on a Hettich (Beverly, MA, ZDA) Universal 320 centrifuge.

2.2. Reagents and materials

Ultrapure 18.2 MΩ cm water (MilliQ) obtained from a Direct-Q 5 system (Millipore, Watertown, MA, USA) was used for preparation of samples and reagents. Merck (Darmstadt, Germany) suprapur acids were used. Samples were filtered using 0.45 μm Minisart cellulose nitrate membrane filters (Sartorius, Goettingen, Germany). The certified reference materials CRM 320 Trace Elements in River Sediment, Community Bureau of Reference (Geel, Belgium), SPS-SW1 Reference material for measurements of elements in surface waters, Spectrapure Standards (Oslo, Norway) and DORM-4 Fish protein certified reference material for trace metals, NRC-CNRC (Ottawa, Ontario, Canada) were used for the accuracy check.

2.3. Sampling

To study the behaviour of PTE under various summer discharges, sampling campaigns were conducted in the Evrotas River, at four water units: Uskol (Us Kolliniatiko, perennial, relatively undisturbed), Dskol (Ds Kolliniatiko, drought impacted), Vivari (perennial, with minor pollution) and WWTP (pollution and drought impacted) during a two year period, i.e. on July 2015 (higher discharge, average discharge during sampling campaign 1.12 m^3/s), followed by June 2016 (low discharge, average discharge during sampling campaign 0.49 m^3/s) and September 2016 (minimum discharge, average discharge during sampling campaign 0.26 m^3/s). Sampling sites are presented in Fig. 1.

Sampling of sediments was performed from the river bank (grab sampling, top 10 cm layer), following WFD guidelines on chemical monitoring of sediment, while sampling of fish was carried out following recommendations for chemical monitoring of biota (EC Technical report 2010–041, 2010). Sampling of water was performed in accordance with the WFD guidance on surface water chemical monitoring (EC Technical Report 2009–025, 2009). Water, sediment and fish samples were obtained concurrently from each individual sampling site.

2.4. Sample preparation and analytical procedures

For water analysis, samples were collected in 2 L plastic bottles. To determine the soluble concentrations of PTE, water samples were filtered (0.45 μm) into 10 mL Teflon tubes and acidified (Official Journal of the EC, 2000; EC Environmental Objectives 272/2009, 2009) with suprapur nitric acid. Filtration blanks did not exceed 10% of the determined element concentration and were in general below the limits of detection (LODs). To determine the total element content, 10 mL of whole water samples were transferred into Teflon vessels and subjected to microwave assisted digestion, using a mixture of nitric, hydrochloric and hydrofluoric acids (Vidmar et al., 2017).

For the determination of the total element concentrations in sediments, approximately 2 kg of samples were collected from a same location in 2 L plastic bottles. Wet sieving was performed through a coarse 2 mm sieve and afterwards through a 63 μm sieve. The sediment fraction <63 μm was analysed for comparison with data from other river basins (European Communities Technical Report 2010–041, 2010). To determine the total element content, approximately 0.25 g of lyophilised sediment was subjected to microwave assisted digestion, using a mixture of nitric, hydrochloric and hydrofluoric acids (Vidmar et al., 2017), and concentrations of PTE (Cr, Ni, Cd, Zn, Pb, As, Cu) were determined by ICP-MS.

In order to determine the easily soluble sediment fraction, 2 g of sediment was shaken with 20 mL of 0.11 mol/L acetic acid on a mechanical shaker (300 rpm) for 16 h. Samples were then centrifuged for 20 min at 8603g, filtered through 0.45 μm membrane filter and PTE contents determined by ICP-MS. The reagent blanks in sediment analysis were below LODs.

Specimens of the Evrotas chub *Squalius keadicus* were collected using an EFKO electrofishing DC unit, following water and sediment collection. The selected species is indigenous and abundant and available in the whole Evrotas basin, whereas other indigenous species are more limited upland. Furthermore, it is the indigenous species of the larger body size, making it easier to test for PTE accumulation in tissue. Fish samples were immediately placed in ice water to euthanize them and then frozen at −20 °C for transport to the laboratory. Subsequently, muscle tissue (the edible part of the fish) was removed, homogenized in a mixer with a titanium knife, lyophilized and stored in plastic bags at −20 °C until analysis. To determine PTE in muscle tissue approximately 0.3 g of lyophilised sample was subjected to microwave assisted digestion, using nitric acid and hydrogen peroxide (Zuliani et al., 2018). The element concentrations were determined by ICP-MS. The reagent blanks in fish analysis were below LODs.

Data for LODs for the determination of PTE in water, sediments, acetic acid extractable fraction of sediments and fish by ICP-MS are provided in Table S2.

All the analyses were performed in triplicate.

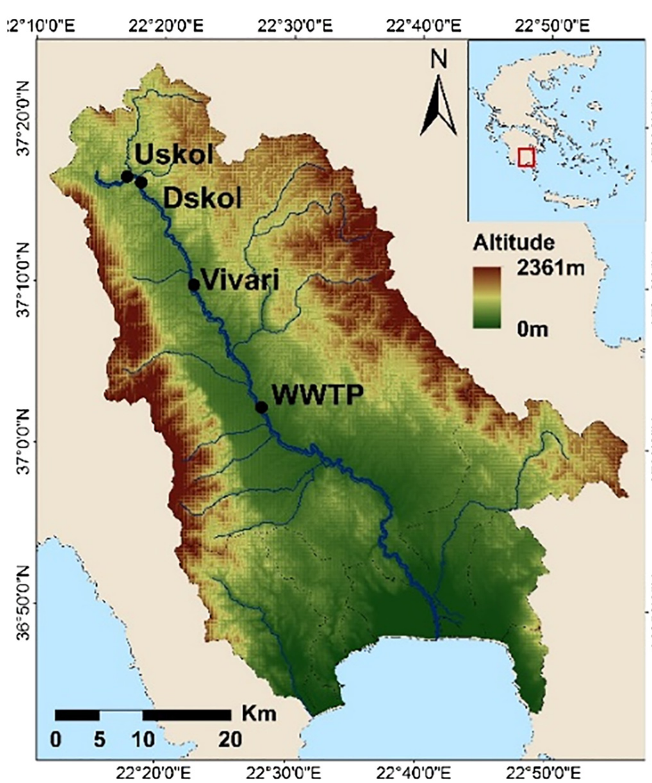

Fig. 1. The Evrotas River sampling sites. Samples were taken under variable water discharges (July 2015 higher discharge, June 2016 low discharge and September 2016 minimum discharge).

2.5. Statistical analyses

To test for differences between samples at variable water discharge levels, i.e. in July 2015 (higher discharge), in June 2016 (low discharge) and in September 2016 (minimum discharge), ANOVA was performed at log-transformed data for each element concentration in water, sediment and fish respectively.

To test for possible correlations between (a) water and sediment samples and (b) water and fish samples, Pearson's correlation analysis was performed using total concentrations of each element. Statistical analysis was carried out in R v.3.3.3 (R Core Team, 2017).

3. Results and discussion

3.1. The accuracy check

The accuracy check was performed by analysing certified reference materials CRM 320 Trace Elements in River Sediment, SPS-SW1 Reference material for measurements of elements in surface waters and DORM-4 Fish protein certified reference material for trace metals. Concentrations of elements were determined by ICP-MS. The results (mean concentration of four parallel samples) are provided in Table S3. Data indicate that the determined concentrations of elements are in good correlation with the reported certified values (the agreement between the results was better than ±5%), which confirmed the accuracy of the analytical procedures applied. The expanded uncertainty of analytical procedures applied was better than ±5% (k = 2).

3.2. The mass fraction of the fine sediment particles <63 μm under variable flow conditions

The content of the sediment fraction <63 μm of the Evrotas River under variable water discharge (samplings July 2015, June 2106, September 2016) is presented in Fig. S1 (Supplementary). Data indicate that the hydrological conditions did not significantly influence the content of the fine sediment fraction. At all sampling locations, mass fraction of fine particles (<63 μm) was in the range between 10 and 20%. Higher fine sediment fraction (around 35%) was found only at sampling site Uskol, taken in 2016 under minimum water discharge.

3.3. PTE concentrations in water, sediments, and fish in relation to water discharge

To evaluate the influence of variable water discharges on PTE concentrations in water, sediment and fish samples, the data from the three sampling campaigns were compared. The results for PTE (Ni, Cr, Cd, As, Pb, Zn and Cu) are presented in Fig. 2.

3.3.1. PTE concentrations in water and sediments

From the data presented in Fig. 2, concentrations of Ni and Cr in water and sediments are highest at Uskol sampling site and decrease downstream. An exception was Ni in July 2015 (higher water discharge) at sampling site Vivari, where its concentration reached a maximum both in the whole water sample (25 μg/L) and in the dissolved fraction (7 μg/L). The latter indicates that perturbation of sediments at higher water discharge enabled remobilization of Ni from sediments. Thus, the dissolved Ni concentration in water slightly exceeded the limit value of 4 μg/L regulated by the WFD and Environmental Objectives for surface waters (Official Journal of the EC, 2013; EC Environmental Objectives, 2009). At other sampling sites, Ni concentrations in whole water samples in July 2015 ranged from 3 to 6 μg/L, while dissolved Ni contents were below 0.3 μg/L. At low and minimum water discharges,

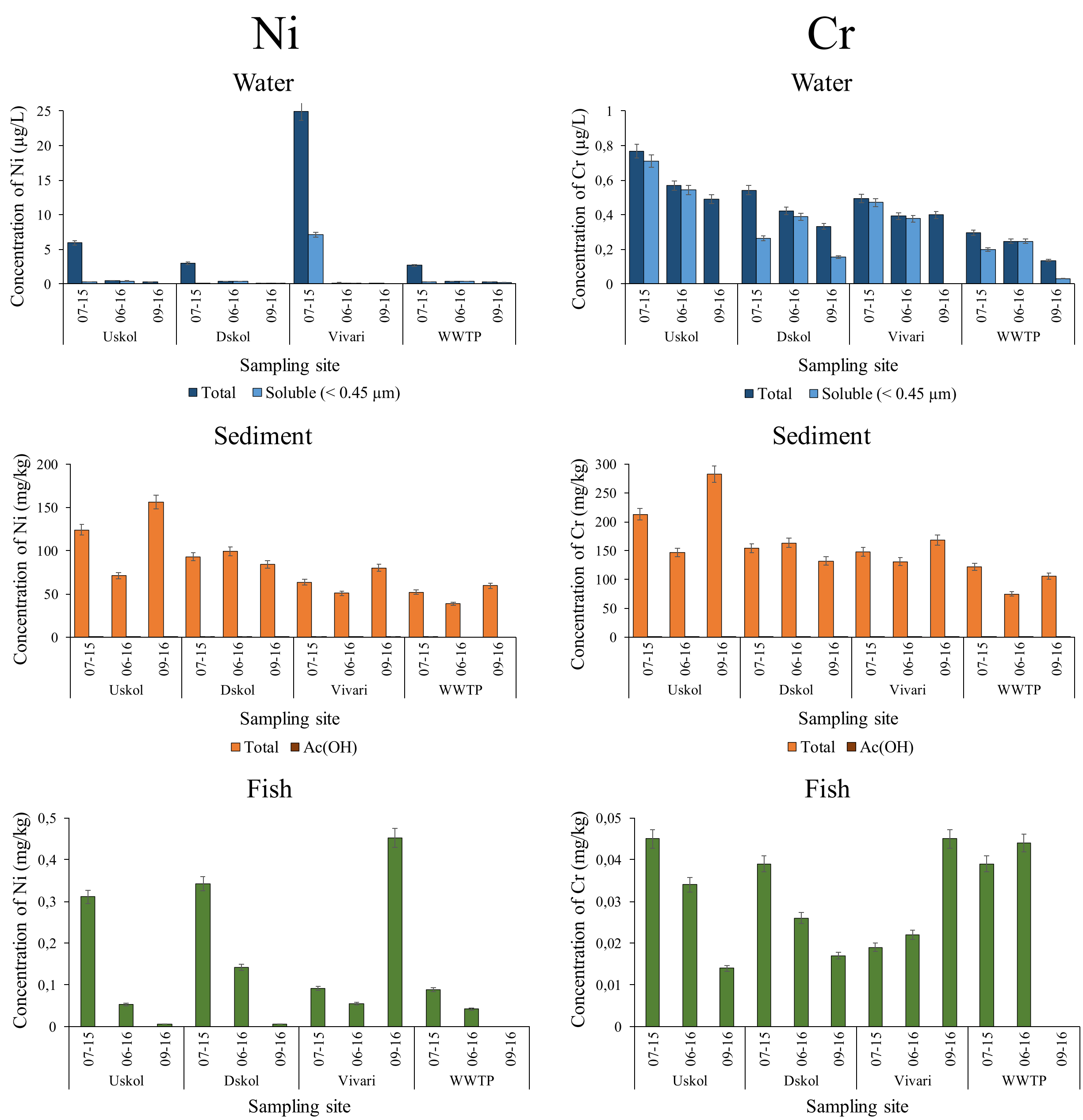

Fig. 2. Elements in water, sediments and fish of the Evrotas River under variable water discharges in July 2015 (higher water discharge), June 2016 (lower water discharge) and September 2016 (minimum water discharge); in September 2016 at the WWTP no chub specimens could be collected. Expanded measurement uncertainty (k = 2) for the applied method is better than 5% for all measured elements. At sampling site WWTP in September 2016, no chub specimens could be collected.

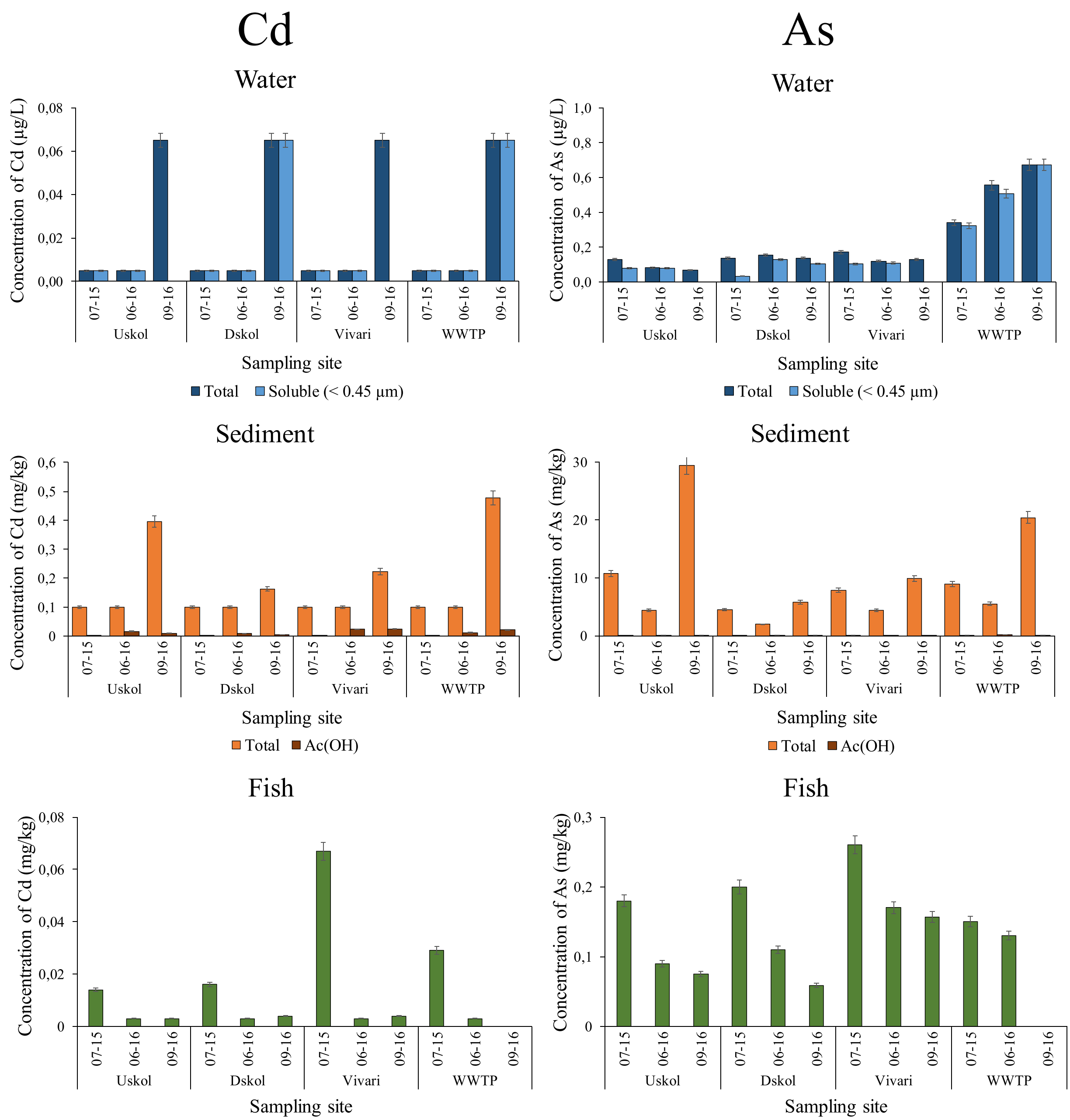

Fig. 2 (*continued*).

Ni concentrations in water samples at all sampling sites were below 0.3 µg/L. Data from Fig. 2 further indicate that Cr in water, contrary to Ni, was present mainly in the dissolved form (concentrations in the whole water and dissolved fraction were almost the same). Cr concentrations ranged from 0.2 to 0.8 µg/L and were all far below the limit value of 4.7 µg/L (Official Journal of the European Communities, 2013; EC Environmental Objectives 272/2009, 2009). Similar concentrations of Ni and Cr in whole water samples were reported by Tzoraki et al. (2015) for the Evrotas River (sampling in 2009 and 2010). Overall, when considering different discharge levels, total Ni concentration was significantly higher at high discharge (ANOVA, $F_{2,9} = 20.13$, *p*-value < 0.001), whereas total Cr concentration did not present a significant difference (ANOVA, $F_{2,9} = 1.16$, p-value = 0.356).

Regarding sediments, total concentrations of Ni and Cr ranged in general from 60 to 150 mg/kg and 120 to 290 mg/kg, respectively, and exceeded the PEC value of 48.6 mg/kg for Ni and of 111 mg/kg for Cr (MacDonald et al., 2000).

In general, lower Ni and Cr concentrations were determined in the Evrotas sediments by Tzoraki et al. (2015). Concentrations of Ni and Cr from the present study are however similar to the reported values found in sediments at industrially exposed sites of the Sava River (Vidmar et al., 2017; Milačič et al., 2017) and the Danube River (Sakan et al., 2010; Vignati et al., 2013; ICPDR, 2008). Since the Uskol area is almost pristine, without known sources of pollution, concentrations of Ni and Cr in sediments are most probably of geogenic origin; they may originate from phyllites and quartzites, containing pyroxenes, which

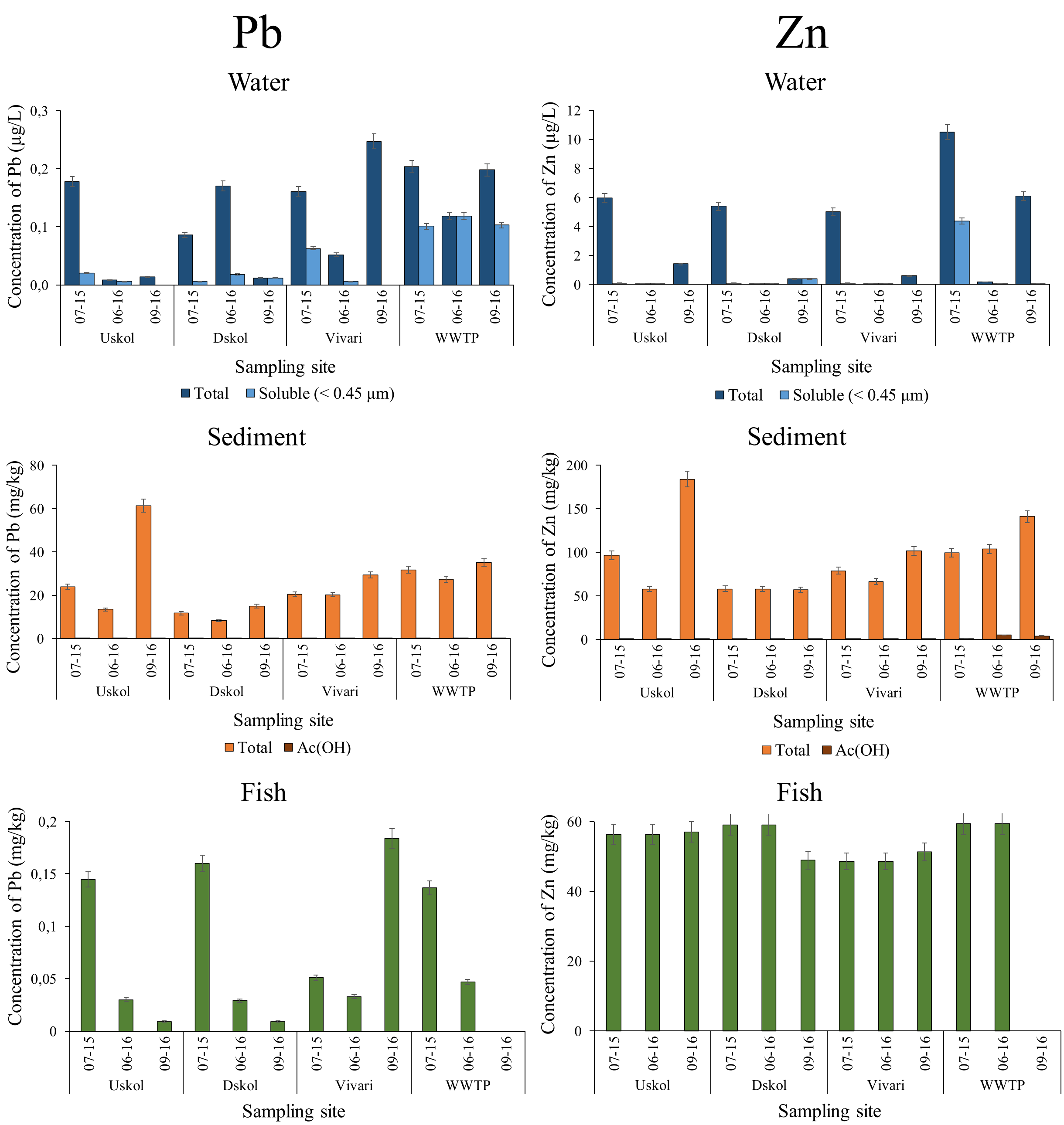

Fig. 2 (*continued*).

are mainly distributed at the upper part of the basin (e.g. Katagas, 1980). The acetic acid extractable concentrations, which involve the water-soluble, exchangeable and carbonate-bound Ni and Cr compounds, did not exceed 0.3% of the total Ni and 0.05% of the total Cr sediment content, indicating their low mobility and bioavailability in the Evrotas sediments. Similar low extractable Cr concentrations were reported for the Sava River (Milačič et al., 2017), while higher extractable Ni contents were found in the Sava sediments (up to 8% of total Ni concentration, Milačič et al., 2017) and were also reported for rivers in Serbia (up to 13% of total Ni and 1% of total Cr content, Sakan et al., 2016).

The data presented in Fig. 2 further indicate that, at all sampling sites, concentrations of Cd and As in water were low; for Cd below 0.065 µg/L and As below 0.6 µg/L. Cd and As in water were present in the dissolved form (concentrations in the whole water and dissolved form were similar). The dissolved Cd concentrations in water samples did not exceed the limit values set for surface waters (0.08 µg/L), while As concentrations were far below regulated concentrations (25 µg/L, Official Journal of the EC, 2013; EC Environmental Objectives 272/2009, 2009). At high and low discharge conditions, Cd concentrations in water were 0.005 µg/L and at minimum water discharge 0.065 µg/L. Higher concentration of the dissolved Cd at minimum discharge is probably related to the lower water volume, and thus, lower factor of dilution. Similarly, the dissolved concentration of As in water was the highest at minimum discharge. A slight increase of dissolved As concentration was observed at the WWTP sampling site (from about 0.1 at upstream locations to 0.7 µg/L at WWTP), indicating a weak influence from anthropogenic activities. Similar Cd and As concentrations in water were previously reported for the Evrotas River by Tzoraki et al. (2015) and for the Sava River (Vidmar et al., 2017; Milačič et al., 2017). As in water samples, total concentrations of Cd and As in

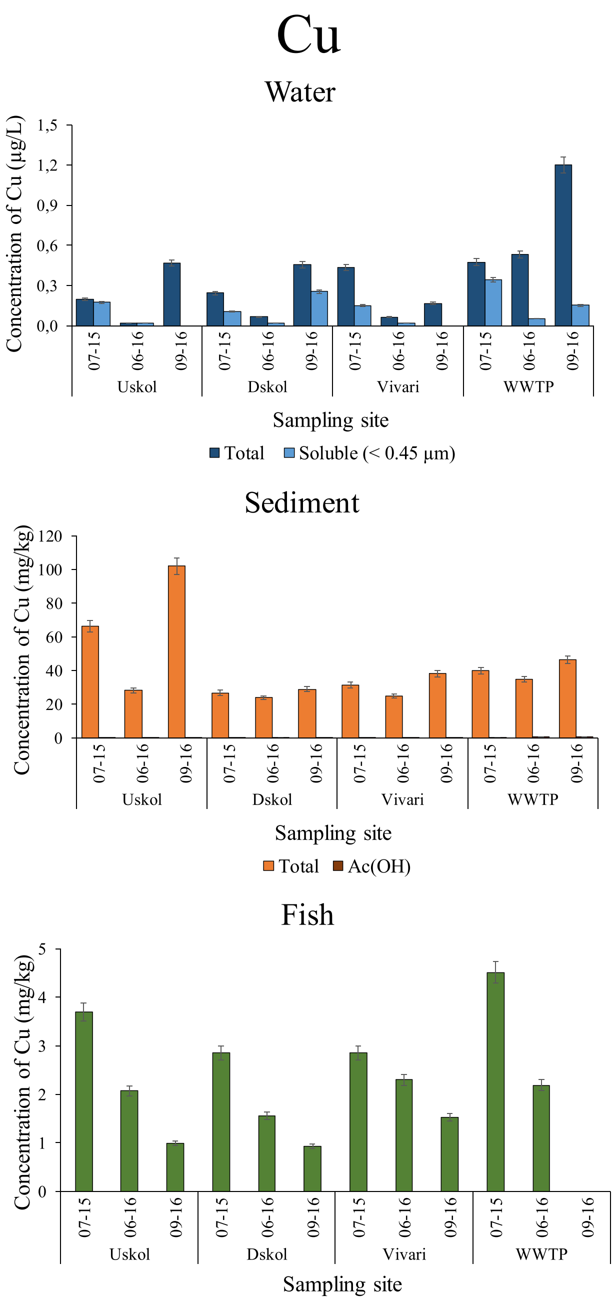

Fig. 2 (*continued*).

sediments, were higher at minimum water discharge and lower at low water discharge, with the described changes in total As concentration being significantly different (ANOVA, $F_{2,9} = 5.53$, p-value < 0.05). At Uskol and WWTP sampling sites, Cd concentrations were 0.45 and 0.5 mg/kg, and As concentrations 30 and 15 mg/kg, respectively. At Dskol and Vivari sites, Cd and As concentrations were lower, ranging for Cd from 0.1 to 0.25 mg/kg, and for As from 5 to 10 mg/kg, respectively. At Uskol reference site, Cd and As concentrations in sediments reflect most probably their natural background, or, in the case of As, airborne sources of lignite combustion (Skoulikidis et al., 2008) in the Megalopolis area, where the Megalopolis power plant is located, while at the WWTP site, they predominately derive from anthropogenic sources. Total Cd and As concentrations were however below PEC values (4.98 mg/kg and 33 mg/kg, respectively) (MacDonald et al., 2000) and are comparable to those determined previously in the Evrotas River (Tzoraki et al., 2015) and the Sava River (Vidmar et al., 2017; Milačič et al., 2017), but much lower than those reported for the Bílina (around 2 mg/kg, Kohušová et al., 2011) and Danube rivers (around 3 mg/kg) (ICPDR, 2008). At all sampling sites, total As concentrations in sediments were in general around 10 mg/kg, with the exception of Uskol (30 mg/kg) and WWTP (20 mg/kg) at minimum water discharge. At the WWTP site, As concentrations found in the present study were lower than previously reported for low water level conditions at the Evrotas River (135 mg As/kg) by Tzoraki et al. (2015), and are in the concentration range found in Sava and Serbian rivers' sediments (Vidmar et al., 2017; Milačič et al., 2017; Sakan et al., 2010). Considerably higher As concentrations (around 70 mg/kg) were determined in the Danube River sediments (Comero et al., 2014). The acetic acid extractable fraction of Cd comprised in general between 2 and 10% of total sediment content in the Evrotas, while for As it was much lower, approx. 0.3%. These data show that Cd and As in sediments of the Evrotas River are present mostly in sparingly soluble compounds. The acetic acid extractable fractions of Cd and As are much lower than reported by Milačič et al. (2017) for the Sava River (up to 55% of total Cd content and 3% of total As content) and for Serbian rivers (up to 67% of total Cd content, Sakan et al., 2016).

Our data (Fig. 2) also indicate that concentrations of Pb, Zn and Cu in water were low; for Pb below 0.3 µg/L, for Zn below 12 µg/L, and for Cu below 1 µg/L. Pb, Zn and Cu were associated mainly with the particulate matter (concentrations in the whole water were higher than in the dissolved form). When considering different discharge levels, total Zn concentration was significantly higher during high discharge and presented the lowest values during low discharge (ANOVA, F2,9 = 30.119, p-value < 0.001). The dissolved Pb, Zn and Cu in water samples were all below the limit values regulated for surface waters (1.2 µg/L, 8 µg/L and 5 µg/L, respectively, Official Journal of the EC, 2013; EC Environmental Objectives 272/2009, 2009). The concentrations of Pb, Zn and Cu in whole water samples are in general similar to those previously reported for the Evrotas River (Tzoraki et al., 2015). Vidmar et al. (2017) and Milačič et al. (2017) determined similar Zn concentrations, but higher Pb (0.2 to 2 µg/L) and Cu (2 to 8 µg/L) concentrations in the Sava River water. Considering the total Pb, Zn and Cu concentrations in sediments, the highest were found in the Uskol reference site (up to 60 mg Pb/kg, up to 200 mg Zn/kg and up to 100 mg Cu/kg), while at the other sampling sites they were in general 50% lower. These Pb, Zn and Cu concentrations did not however exceed PEC values (128 mg/kg for Pb, 459 mg/kg for Zn and 149 mg/kg for Cu) (MacDonald et al., 2000). The highest total concentrations of Pb, Zn and Cu in sediments of the Uskol site, are probably related to the geological background and, possibly, to lignite combustion. Tzoraki et al. (2015) reported considerably lower Pb, Zn and Cu concentrations for the Evrotas River sediments. Similar concentrations of Pb and Zn, as in this study, were found in the Sava River sediments (Milačič et al., 2017). Cu concentrations in Sava sediments were in general around 20 mg/kg (Milačič et al., 2017), and were comparable to those reported in this study for Dskol, Vivari and WWTP sites. Concentrations of Pb, Zn and Cu from the present work are in general lower than those found in sediments of the Danube River (Sakan et al., 2010; Vignati et al., 2013; Comero et al., 2014) and the Bílina River (Kohušová et al., 2011). The acetic acid extractable fraction of Pb, Zn and Cu representing <0.05% of their total element content, indicate the very low mobility of Pb, Zn and Cu in the Evrotas sediments. In contrast to the Evrotas, significantly higher extractable Pb, Zn and Cu concentrations were determined in the Sava (Milačič et al., 2017) and Serbian rivers' sediments (Sakan et al., 2016) from industrially exposed sites. For Pb, these comprised up to 2% and 10% of its total contents in sediments from Sava and Serbian rivers, for Zn up to 20% and 45%, while for Cu up to 10 and 57%, respectively.

Sediments represent a sink for PTE. The potential toxicity of elements studied in the Evrotas sediments was thus evaluated by the Probable Effect Concentration Quotient (PEC-Q) methodology proposed by

Long et al. (2006). PEC-Q enables an overall estimation of the possible risk posed by the simultaneous presence of several trace elements and is expressed as:

$$\text{PEC}-\text{Q} = \frac{\sum_{i=1}^{n} \frac{[\text{Me}]_i}{\text{PEC}_i}}{n} \tag{1}$$

where $[\text{Me}]_i$ is the concentration of the i^{th} element determined in the sediment sample (Table S4), PEC_i is the corresponding PEC value (Table S4), and n is the total number of measured elements. Two critical values were provided for PEC-Qs: 0.25 and 0.34 (Long et al., 2006). PEC-Q 0.25 classifies conditions, where in laboratory toxicity tests with marine (i.e., *Ampelisca abdita* and *Rhepoxynius abronius*) and freshwater (i.e., *Hyalella azteca*) organisms, the incidence of toxicity exceeds 20%, whereas a PEC-Q value of 0.34 indicates sites where a decrease in the population of amphipods, gastropods, and capitellid polychaetes and in the total abundance of benthic organisms is expected. On the basis of data from Table S4, PEC-Q values for Evrotas sediment samples were calculated using formula (1). Data presented in Fig. 3A demonstrate that in all sediments analysed, PEC-Qs exceeded the critical value of 0.34, and were the highest at minimum water discharge. At Dskol, Vivari and WWTP sites, PEC-Qs were between 0.34 and 0.6, while at Uskol reference site, PEC-Q, at minimum water discharge conditions, reached a value of 1.2. These PEC-Q data indicate a potential ecological risk posed by the simultaneous presence of PTE in sediments of the Evrotas River. The high PEC-Q values derived mostly from high Ni and Cr contents in sediments. This can be seen from Fig. 3B, which shows significantly lower PEC-Q values, when these are calculated excluding Ni and Cr concentrations in sediments (PEC-Qs in general below 0.34). For the Uskol site, with minimal anthropogenic impacts, high Ni and Cr concentrations in sediments are most likely associated with their geogenic origin, while in sediments collected at the WWTP site, Ni and Cr concentrations may indicate potential point source contamination. Although PEC-Qs in the Evrotas River sediments were above 0.34, the low mobility and bioavailability of Ni, Cr, Cd, As, Pb, Zn and Cu (acetic acid extractable concentrations of Cd <10%, and of all other elements <0.3% of their total sediment content) indicate that the environmental risk posed by PTE in the Evrotas sediments is very low. Vignati et al. (2013) reported that in sediments of the Danube Delta, 35% of PEC-Q values ranged between 0.34 and 0.6. When, however, Cr and Ni were excluded from calculations, PEC-Q values were below the critical value of 0.34. Since, in that case too, there were no known anthropogenic sources of Cr and Ni, Vignati et al. (2013) concluded that their origin is also geogenic. Milačič et al. (2017) reported PEC-Q values in Sava sediments ranging from 0.4 to 1.4, while with exclusion of Cr and Ni concentrations, PEC-Qs were also below 0.34. The elevated Cr and Ni concentrations in Sava sediments, which contributed to high PEC-Qs, contrary to the Danube Delta and Evrotas, are related to industrial pollution.

In conclusion, the data of the present study indicate that PTE in water samples of the Evrotas River are present at very low concentrations. In sediments, the relatively high PTE concentrations determined at the Uskol reference site, probably derive from the geological background or are affected by airborne ash from lignite combustion at the Megalopolis power plant. Since the acetic acid extractable fractions of PTE in the Evrotas sediments are very low, PTE do not pose an environmental burden. At the other sampling locations, the contamination of Evrotas sediments with PTE is low. Regarding the variable water flow conditions, results indicate that PTE in water of the Evrotas River are the highest for Ni, Cr and Zn at high water discharge, while for Cd, As, Pb and Cu under minimum water discharge. In sediments, concentrations of PTE were also found to be the highest at minimum water discharge.

Furthermore, total Cr concentration was found to be significantly and positively correlated in water and in sediment samples (Pearson's $r = 0.696$, p-value < 0.05).

3.3.2. Concentrations of PTE in fish

In order to assess PTE concentrations in fish from the Evrotas River, individuals of the Evrotas chub (*S. keadicus)* that can reach a maximum size of 25 cm in total length over 5 years, were collected and analysed. Concentrations of PTE were determined by ICP-MS in muscle tissue (the edible parts of the fish) as described in paragraph *2.4*. There are only few articles in the literature, describing concentrations of PTE in chub fish species. Among them, Milošković et al. (2016) reported PTE concentrations in chub from the Morava River, and Zuliani et al. (2018) from the Sava River. The results from this study presented in Fig. 2 (concentrations of elements expressed in mg/kg dry weight) indicate a general trend of a decrease of PTE concentrations with decreasing water discharge, i.e. the highest concentrations of PTE were generally found at high water discharge (July 2015), and the lowest, at minimum water discharge (September 2016). This observation may be related to the presence of higher amounts of suspended particulates in water, bearing PTE, at high water discharge. Similarly, considerably higher PTE concentrations were reported for chub species (of similar isometric parameters) from the Sava River, at the Litija sampling site, at high water discharge, than at low discharge conditions (Zuliani et al., 2018). Concentrations of elements determined in the Evrotas chub decreased in the following order: Zn > > Cu > Ni > Cr > As > Pb > Cd. A similar order of PTE concentrations was found for chub species (of similar isometric parameters) collected form Sava River at high water discharge (Zuliani et al., 2018). Concentrations of Zn in the Evrotas chub ranged between 50 and 60 mg/kg and are in general higher from those determined in chub species from the Sava River (30 to 40 mg/kg, dry weight, Zuliani et al., 2018) and chub species, with similar isometric parameters, from the Kolubara River (approx. 20 mg/kg, expressed on dry weight basis, Milošković et al., 2016). When comparing between different water discharge levels, Cu concentration presented a significant decrease following decreasing discharge, with highest values during high discharge and lowest during minimum discharge (ANOVA, $F_{2,9} = 22.322$, p-value<0.001). The slightly higher Zn concentrations found in the Evrotas chub are possibly derived from pesticides and fertilizers. No negative effects, due to slightly higher Zn concentrations, can, however, be expected in Evrotas chub. Cu concentrations in the Evrotas chub ranged from 1 to 4.5 mg/kg and were in concentration similar range for those reported for chub species from the Sava River and Kolubara River (Zuliani et al., 2018; Milošković et al., 2016). Ni concentrations in the Evrotas chub ranging from <0.006 to 0.45 mg/kg are comparable to concentrations determined in chubs from the Sava and Kolubara River (Zuliani et al., 2018; Milošković et al., 2016). Cr concentrations in the Evrotas chub, which ranged from 0.015 to 0.045 mg/kg are in general five to ten times lower from those determined in chubs of the Sava River (Zuliani et al., 2018) and two times lower from chubs of the Kolubara River (Milošković et al., 2016). Concentrations of As in the Evrotas chub, ranging from 0.06 to 0.16 mg/kg, are generally lower than those reported for chubs caught at most of the sampling sites along the Sava River (0.2 to 0.8 mg/kg, Zuliani et al., 2018) and Kolubara River (approx. 0.4 mg/kg, Milošković et al., 2016). As concentration significantly decreased following decreasing discharge, presenting the lowest values during minimum discharge (ANOVA, F2,9 = 4.8688, p-value < 0.05). Very low Pb concentrations determined in the Evrotas chub, ranging from 0.06 to 0.16 mg/kg (dry weight), or when expressed on wet weight, from 0.02 to 0.05 mg/kg, were all below the limit value of 0.3 mg/kg (wet weight), regulated for Pb in fish muscle tissue (Commission Regulation 1881/2006, 2006). Despite the low concentrations, total Pb concentration was significantly and positively correlated in water and fish tissue samples (Pearson's $r = 0.764$, p-value<0.01).

Pb concentrations in the Evrotas chub were lower than the concentrations reported for chubs from the Kolubara River (approx. 0.3 mg/kg,

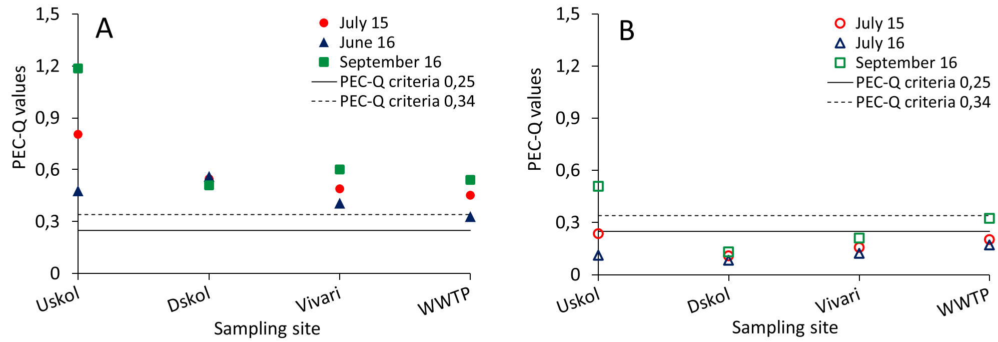

Fig. 3. PEC-Q values for sediment samples with (A) and without inclusion of Cr and Ni (B), collected along the Evrotas River under variable water discharges (samplings July 2015 higher discharge, June 2016 low discharge, September 2016 minimum discharge). Horizontal lines correspond to PEC-Q criteria of 0.25 and 0.34.

Milošković et al., 2016) and significantly lower than most of Pb concentrations found in chubs from different locations along the Sava River (0.1 to 0.55 mg/kg, Zuliani et al., 2018). Similarly to Pb, Cd concentrations in Evrotas chub specimens collected from all sites along the Evrotas River were very low, ranging from <0.002 to 0.07 mg/kg (dry weight), or when expressed on wet weight, from 0.0007 to 0.02 mg/kg. These Cd concentrations are all below the limit value of 0.050 mg/kg (wet weight) set for Cd in fish muscle tissue (Commission Regulation 1881/2006, 2006). Similar Cd concentrations (0.02 mg/kg) were reported for chubs caught in the Kolubara River (Milošković et al., 2016), while higher Cd concentrations were found for chubs from industrially exposed locations of the Sava River (approx. 0.05 mg/kg, Zuliani et al., 2018).

The data of the present study revealed that PTE in the Evrotas chub, caught at various locations along the Evrotas River basin, were low. Regarding PTE analysed, Evrotas chub fish is thus considered safe for human consumption. With the exception of Zn, PTE in the Evrotas chub were similar or lower than those determined in chubs from the Sava and the Kolubara River. Zn is the only element that was found present in slightly higher concentrations in the Evrotas chub in comparison to chubs caught at the Sava and Kolubara River.

4. Conclusions

To the best of our knowledge, this is the first report on the presence of PTE (Ni, Cr, Cd, As, Pb, Zn and Cu) in sediments and water of the Evrotas River (Southern Greece), under variable water discharges, and evaluation of their impact on fish. The data revealed that river flow conditions significantly influence the concentrations of PTE in water and sediments. In water samples, PTE contents were in general very low. The highest Ni, Cr and Zn concentrations in water were found at high water discharge, whereas Cd, As, Pb and Cu concentrations peaked at minimum water discharge, however this was statistically significant only in the case of Ni and Zn. In their soluble fractions (<0.45 μm), PTE concentrations were below the limit values regulated for surface waters by the WFD and EC Environmental Objectives. The only exception was Ni in one water sample collected at higher water discharge at the Vivari sampling site, which slightly exceeded the limit value of 4 μg/L. Concentrations of PTE in sediments were found to be higher at minimum water discharge. The highest were determined at the Uskol reference site, which corresponds to an almost pristine area without known sources of pollution. At the other sampling sites, PTE concentrations were low to moderate. Potential ecological risk posed by the simultaneous presence of PTE in sediments, expressed in PEC-Q values, exceeded the critical value of 0.34 and derived mostly from Cr and Ni inputs. Since, however, the acetic acid extractable content of these elements in sediments was extremely low (below 0.3% of their total concentration in sediments), anthropogenic inputs of Cr and Ni in the study area appeared to be low, and considering the geological background, it can be assumed that their origin is geogenic. In addition, the low mobility of PTE in sediments indicates their low environmental impact. In conclusion, regarding PTE studied in water and sediments, the Evrotas can be considered as a river with low levels of pollution. This was reflected also in PTE concentrations in fish samples, which were very low to moderate. However, even though low, Pb concentration in water was significantly correlated with Pb concentration in fish samples. The slightly higher Zn concentrations found in fish specimens are possibly derived from pesticides and fertilizers. The data from this study provide information on the behaviour of PTE in the Evrotas River under variable discharge regimes, and indicate that the contribution of PTE to multiple stressor conditions and overall pollution of the area investigated, is low.

The holistic approach, which was applied in the Evrotas River, and includes determination of the total PTE concentrations in water and their soluble fraction, total PTE concentrations in sediments and their mobile fraction, calculations of PEC-Q values for evaluating the impact of sediment pollution on biota, and determination of the total PTE concentrations in fish, using also statistical analysis, enabled the comprehensive investigation of the behaviour of PTE in the Evrotas River under variable river water discharges. Such advanced methodology could also be applied to other river basins, where information on the behaviour of pollutants under variable flow discharges is still scarcely documented.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.08.123.

Conflict of interest

Authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

Acknowledgments

This work was supported by the European Communities 7th Framework Programme Funding under Grant agreement no. 603629-ENV-2013-6.2.1-Globaqua, the MASSTWIN project, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 692241 and by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia (Programme group P1-0143).

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