Ecotoxicology and Environmental Safety 163 (2018) 125–135

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Benthos-drift relationships as proxies for the detection of the most suitable bioindicator taxa in flowing waters – a pilot-study within a Mediterranean karst river

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ARTICLE INFO

Keywords:
Urban influence
Contaminant pathway
Protected landscape
Amphipoda
Physico-chemical parameters
Metal contamination

ABSTRACT

Mediterranean karst aquifers are sensitive systems vulnerable to contamination, exhibiting high rates of diversity and endemicity. In the present pilot-study, we aimed to detect the most suitable bioindicators of contaminant accumulation and mobilization within a Mediterranean karst river (Krka River, Croatia), whose lowermost sections belong to a designated protection area (national park). To meet our goal, we sampled water, drift and benthos (macroinvertebrates and periphytic microfauna) at the two Krka River sites, located upstream and downstream from town Knin and its urban influences. We compared: 1) environmental conditions (water physico-chemical parameters, trace- and macro-element concentrations); 2) abundance and diversity of periphyton and macroinvertebrate taxa constituting benthos; and 3) macroinvertebrate benthos-drift relationships between the two sites. Despite higher values of all measured physico-chemical parameters, and most trace- and macro-element concentrations at the urban-influenced site, the concentrations of contamination indicators (i.e., COD, nutrients, metals) at both sites were generally low. This is likely a result of specific "self-purification ability" of the Krka River, mediated by relatively high contaminant retention potential of the underlying tufa (i.e., calcareous) and/or macrophyte substrates. Between-site differences in water quality further affected the spatial variation of macrozoobenthos, drift, and periphytic microfauna. We suggest that increased COD and orthophosphate concentration, and macrophyte presence at the urban-influenced site, supported higher densities and diversity of benthic organisms dominated by eurivalent (i.e., contamination-tolerant) taxa. The most numerous macroinvertebrate taxa in benthos were amphipod *Gammarus balcanicus* and the representatives of the endemic Dinaric karst taxa - gastropods *Emmericia patula* and *Radomaniola curta germari*, and another amphipod *Echinogammarus acarinatus*. Although we expected to observe significantly increased drift at the urban-influenced site due to the degraded environmental conditions, it was not observed. The observed benthos-drift patterns suggest that freshwater amphipods (i.e., gammarids), which were found most numerous in drift, could be considered as the most suitable bioindicators of a contaminant (i.e., metal) accumulation and mobilization within karst aquifers comparable to Krka River.

1. Introduction

Karst aquifers are attributed as extremely sensitive systems vulnerable to contamination – primarily because of their permeability, and highly fragmented and heterogeneous structure (Watson et al., 1997). Mediterranean karst aquifers (e.g., on Iberian, Apennine and Balkan peninsulas) exhibit high rates of diversity and endemicity (Bănărescu, 2004; Klobučar et al., 2013; Tierno de Figueroa et al., 2013; Previšić et al., 2014; Ivković and Plant, 2015), and have thus been identified as "hotspots of biodiversity" and "hyper-hot candidates for conservation

Abbreviations: T, temperature; DO, dissolved oxygen; Cond, conductivity; TDS, total dissolved solids; Alk, alkalinity; TWH, total water hardness; N-NO_2^-, nitrite; N-NO_3^-, nitrate; P-PO_4^{3-}, orthophosphate; COD, chemical oxygen demand; SE, standard error; M-W *U*-test, Man-Whitney *U*-test

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https://doi.org/10.1016/j.ecoenv.2018.07.068
Received 22 January 2018; Received in revised form 6 June 2018; Accepted 18 July 2018
0147-6513/

support" (Myers et al., 2000). However, they suffer great pressures in terms of karst water quantity and quality, mostly due to intense urban development paired with industrial expansion and more "water-intensive lifestyles" (Hartmann et al., 2014). For an effective conservation of the karst aquifers, it is very important to understand the great complexity of these ecosystems, and to detect the way in which all of their abiotic and biotic elements interact (Watson et al., 1997).

The most common types of contamination within aquatic karst ecosystems are eutrophication, excessive inputs of metals and/or a variety of organic and inorganic contaminants sourcing from various waste disposal systems (Watson et al., 1997; Gutiérrez et al., 2014; Milanović and Vasić, 2015). These contamination scenarios might greatly decrease abundance and diversity of benthic freshwater taxa (e.g., periphyton, macroinvertebrates), increase macroinvertebrate drift densities (i.e., the downstream dispersal of the organisms driven by flow), and eventually elicit toxic effects on biota (Matoničkin Kepčija et al., 2011; Sertić Perić et al., 2011; Vukosav et al., 2014). If benthic biota bioaccumulate contaminants from aquatic environments, and are then transported downstream via drift, they could serve as "vectors" of contamination for the downstream aquatic biota and habitats (Sertić Perić et al., 2011). This might be of specific concern if the downstream areas are protected as preserved landscapes and/or habitats of environmental importance.

To achieve the protection of these habitats, the sheer declaration of protected karst areas should be accompanied by environmentally sensitive management, tailored according to the pre-assessment of site-specific environmental conditions and risks (Hamilton-Smith, 2001). For the effective management of the Mediterranean karst aquifers belonging to a high priority protection area (Myers et al., 2000), it is necessary not only to investigate basic environmental and ecological descriptors, but also to detect the most efficient bioindicator organisms within these ecosystems. In the assessment of environmental and ecological indicators within streams and rivers, macroinvertebrate benthos-drift patterns have often been investigated (Principe and del Corigliano, 2006; Tonkin and Death, 2013; Sertić Perić and Robinson, 2015). These investigations usually include comparisons of macroinvertebrate density and taxonomic richness between benthos and drift (i.e., bottom-dwelling and drifting fauna).

Krka River (Croatia) is one of the most prominent Mediterranean karst aquifers. Owing to its biological and geological characteristics, the lower part of the Krka River is protected as a national park. However, upstream of its entrance into the national park, Krka River is affected by the wastewaters of the town of Knin, located a few kilometers downstream the Krka River source and upstream from the national park border (Cukrov et al., 2008, 2012). Krka River is composed of soft and porous calcite deposits (i.e., tufa, travertine), built up from a mixture of inorganic (i.e., calcite) and organic material (i.e., cyanobacteria, algae, moss, leaf litter, branches, fine detritus). Such calcite deposits are also met within many other Mediterranean karst aquifers (e.g., Ruidera National Park, Spain; Pamukkale, Turkey) (Pedley, 1990; Woodward, 2009). Because of its porosity, permeability and biofilm accumulation, tufa has been identified as a well-favored habitat for many aquatic organisms (e.g., periphyton and macroinvertebrates) (Matoničkin Kepčija et al., 2011; Sertić Perić et al., 2011, 2014), but susceptible to heavy metal bioaccumulation (e.g., Prohić and Juračić, 1989; Gümgüm et al., 1994; Liu et al., 2009; Zhao et al., 2012). However, due to its great capability of self-purification, such as remediation of the heavy metal contamination by the intensive calcite sedimentation processes, tufa has been revealed to decrease the elevated trace metal concentrations from the water column (Cukrov, 2008; Liu et al., 2009).

Few studies prior to the present pilot-study have investigated the benthos-drift relationships in order to choose an appropriate model organism for investigating ecotoxicological effects and distribution of contamination within aquatic environment (e.g., Piggott et al., 2015; Magbanua et al., 2016). This exactly was the overall aim of the present pilot-study conducted within a Mediterranean karst Krka River (Croatia). The specific aims of the present pilot-study were to investigate: 1) environmental conditions (i.e., water physico-chemical parameters, and dissolved trace- and macro-elements concentrations); 2) abundance and diversity of periphyton and macroinvertebrate taxa constituting benthos; and 3) macroinvertebrate benthos-drift relationships, at two sites within Krka River located upstream and downstream from an urbanized area (i.e., town Knin and the belonging industrial facility for production of screw elements by method of cold forging). We hypothesized that at the downstream site, periphyton and macrozoobenthos would demonstrate predictable species turnover, such as lower taxa density and diversity - especially of the endemic and contamination-intolerant taxa, and significantly increased drift due to the degraded environmental conditions. We anticipated that the increased macroinvertebrate drift could indicate a stress response of organisms induced by contamination (Lauridsen and Friberg, 2005), and a dispersal potential of benthic macroinvertebrates with potentially bioaccumulated contamination to downstream reaches (cf. Sertić Perić et al., 2011). We presumed that an insight into the benthos-drift composition might help us identify the best available bioindicator taxa within Krka River and their distributional potential. Our pilot findings could reveal the most efficient bioindicators for the potential future studies of a contaminant bioaccumulation by benthic macroinvertebrates within Mediterranean karst aquifers, especially those with designated protection areas.

2. Materials and methods

2.1. Study area

Krka River is a karst river located in the central part of the eastern Adriatic coast, draining carbonate-dominated terrain of the Croatian Dinaric karst area (Fig. 1). The hydrogeological drainage area of the Krka is around 2427 km^2. The length of Krka's freshwater section is 49 km, whereas its karstic estuary to the Adriatic Sea is extended around the town of Šibenik for additional 23.5 km (Fig. 1). With an average annual discharge of 47.4 m^3/s (1990–2009), Krka represents the medium-size river in Croatia (cf. Čanjevac and Orešić, 2015).

The first sampling site (K1), selected as the reference site without any anthropogenic influence, was located 800 m downstream of the main source of the Krka River. The second sampling site (K2) was located 5.4 km downstream from the Krka River source, 2 km upstream from the border of the Krka National Park, and approximately 2.7 km downstream from the town of Knin (Fig. 1). This site comprises inflow of the Krka River's tributary Orašnica River, which flows by industrial wastewater pools of the screw factory, but is located before the confluence of another tributary, Butižnica River (Fig. 1). Therefore, K2 was influenced by the inflows of municipal and industrial wastewaters emerging from urbanized Knin area, including the impact of factory producing screw elements by method of cold forging (Švač et al., 2010) sourcing from Orašnica River (Fig. 1). In the previous studies, only Ca, Mg, Zn, Cd, Pb and Cu concentrations in water downstream of Knin were assessed, indicating anthropogenic contamination of the Krka River (Cukrov et al., 2008, 2012).

2.2. Measurements of water physico-chemical parameters

The present pilot-study was conducted in autumn and spring as moderate seasons in terms of hydroclimatic extremes within karst regions (cf. Hartmann et al., 2014; Bonacci et al., 2006). At both sampling sites (K1, K2), on each sampling occasion (November 14, 2015 – autumn, and May 2, 2016 – spring), in situ measurements of the following physico-chemical parameters were made using the respective portable field meters, whose probes were calibrated before each sampling occasion: temperature (T) and concentration and saturation of dissolved oxygen (DO) (oximeter OXI 96, WTW GmbH, Weilheim, Germany), pH (pH-meter 330i, WTW GmbH, Weilheim, Germany), conductivity