Emerging Organic Contaminants in springs of the highly karstified Dinaric region

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Abstract

Emerging organic contaminants (EOCs) have become of increasing interest due to concerns about their impact on humans and the wider environment. Karst aquifers are globally widespread, providing critical water supplies and sustaining rivers and ecosystems, and are particularly susceptible to pollution. However, EOC distributions in karst remain quite poorly understood. This study looks at the occurrence of EOCs in the Croatian karst, which is an example of the “classical” karst, a highly developed type of karst that occurs throughout the Dinaric region of Europe. Samples were collected from 17 karst springs and one karst lake used for water supply in Croatia during two sampling campaigns. From a screen of 740 compounds, a total of 65 compounds were detected. EOC compounds from the pharmaceutical (n=26) and agrochemical groups (n=26) were the most frequently detected, while industrials and artificial sweeteners had the highest concentrations (range 8 - 440 ng/L). The number of detected compounds and the frequency of detection demonstrate the vulnerability of karst to EOC pollution. Concentrations of 5 compounds (acesulfame, sucralose, perfluorobutane sulfonate, emamectin B1b, and triphenyl phosphate) exceeded EU standards and occurred at concentrations that are likely to be harmful to ecosystems. Overall, most detections were at low concentrations (50 % <1 ng/L). This may be due to high dilution within the exceptionally large springs of the Classical karst, or due to relatively few pollution sources within the catchments. Nevertheless, EOC fluxes are considerable (10 to 10^6 ng/s) due to the high discharge of the springs. Temporal differences were observed, but without a clear pattern, reflecting the highly variable nature of karst springs that occurs over both seasonal and short-term timescales. This research is one of a handful of regional EOC investigations in karst groundwater, and the first regional study in the Dinaric karst. It demonstrates the need for more frequent and extensive sampling of EOCs in karst to protect human health and the environment.

Keywords: Emerging organic contaminants, karst aquifers, Dinaric karst, groundwater, drinking water resources

1 Introduction

Emerging organic contaminants (EOCs) are anthropogenic micropollutants that are typically difficult to identify in aquatic environments due to their low concentrations. In the last decade, technological advances have made detection of a wide range of EOCs in water possible, with increasing numbers of studies (Muter and Bartkevics, 2020; Richardson and Kimura, 2020; Schmidt, 2018). Growing evidence for their presence in surface and groundwater systems has raised lots of new questions about their impact on the environment, related ecosystems, human health, and issues related to monitoring and control of such a large number of diverse compounds (e.g. Bradley et al., 2021; Kolpin et al., 2004; Lapworth et al., 2019; Liu at al., 1997; Masoner et al., 2019; Padilla and Vesper, 2018).

Karst covers around 15.2% of the global continental land surface, and it is estimated that between 16.5% of the world's population lives on karst areas (Goldscheider et al., 2020). About 9.2% of the global population uses freshwater abstracted from karst aquifers (Stevanović, 2019). Karst aquifers are a vital resource for drinking water in many parts of the world (Hartmann et al., 2014). Due to their natural characteristics, they are highly vulnerable to contamination (Goldscheider, 2005). Karst aquifers are often characterized by the contrast of very low matrix porosity and high fracture and conduit porosity which results in rapid groundwater flow and low pollution attenuation (Ford and Williams, 2007; Goldscheider and Drew, 2007). The Croatian karst is part of the Dinaric karst with an extremely high degree of karstification that results in large scale karst landforms, high discharge springs (with average discharges around ten m^3/s), and well developed conduit networks made up of large cave systems. Due to its high degree of karstification, and long history of study, the Dinaric karst is commonly referred to as "classical karst". Moreover, these karst rocks often extend continuously over large areas, uninterrupted by non-karst rocks that would form fixed geological boundaries of underground watersheds. Determining recharge zones, and managing and protecting water resources in such karstified aquifers is difficult and is made even more challenging in the Dinaric region due to the transboundary nature of some catchments.

There are few studies of EOCs in highly karstified aquifers (Lukač Reberski et al., 2022), and EOC contamination of very high discharge karst springs is not well characterised. This study aims to provide some new insights into the impact of EOCs on these types of large springs and on karst aquifers more generally. It is the first regional assessment of the Croatian Dinaric karst, with samples from 17 of the most significant karstic springs in the region and one lake (partially fed by karst springs and lake vruljas). All the sample sites are used for water supply and are geographically distributed across the karst region of Croatia. Each site was sampled during both the spring and autumn seasons. The specific objectives are to: (I) determine which EOCs are present, and at what concentrations; (II) investigate the broad spatial distribution of EOCs in the Croatian karst; (III) determine whether there is a difference in the number and concentrations of EOCs from samples taken in autumn and spring; and, (IV) explore links between EOC detections and land use in karst spring catchments. Results are also compared to EOC data from other karst studies reviewed by Lukač Reberski et al. (2022) and other data from groundwater reviewed by Lapworth et al (2012).

2 Study area

The Dinaric Karst, deposited during the Middle Triassic to the Middle Eocene period, is famous as the type locality for karstic dissolutional landforms (Ford, 2007). In Croatia, this karst covers nearly half of the land area (Chen et al., 2017; Figure 1a), and predominantly comprises limestones and dolomites that are very thick, in some parts more than 8000 m (Vlahović et al., 2005). The high solubility of these carbonate rocks and the intense tectonics in the geological past resulted in extensive karstification, and well-developed karst aquifers with high levels of heterogeneity, unpredictability, and complexity. Groundwater velocities based on results of 199 tracer tests conducted in the Croatian karst range from 0.01 to 32.1 cm/s (or 0.009 to 27.7 km/day), with a median velocity of 2.3 cm/s or 2 km/day (Kuhta and Brkić, 2008).

Figure 1. a) Extent of Croatian karst and sampling locations, b) Jadro Spring, discharging 7.1 m^3/s (photo: Josip Kolarić, 02.03.2020). * Due to the map's scale, the Bistrac spring's catchment and the Zagorska Mrežnica catchment share the same location point. Hydrogeological permeability

background colours are from the Hydrogeological Map of Croatia, scale 1:300,000 (Biondić et al., 1999).

Regarding relief and climate, the Croatian karst can be divided into two major areas: inland hilly and mountainous areas with a moderately warm humid climate; and a coastal belt with a Mediterranean climate. Annual rainfall and average temperature range from 700 mm/17 °C on the Adriatic coast and islands to 3500 mm/5 °C in the highest mountain locations. Despite the high precipitation, due to the rapid vertical infiltration through the epikarst zone, surface rivers networks are generally absent. Because of this, and the rough terrain, the continental karst area is the least populated in Croatia, with few urban areas and very little industrial activity. Most of the population lives along the coast, downstream of the spring catchment zones, which has positive affects on groundwater quality because there are relatively few pollutant sources in the spring catchments. Our working hypothesis is that the karst springs in Croatia might be less impacted by EOCs than other areas with a high level of karstification where there is more intense agricultural land use, and larger urban and industrial areas within groundwater catchments; such as the United Kingdom, France, Germany and USA (Lapworth et al., 2015; Lukač Reberski et al., 2022; Mahler and Musgove 2019).

3 Material and methods

3.1 Sampling and monitoring

Croatian karst groundwater was sampled at 18 locations (16 discharging springs; one intermittent spring that emerges from the cave, but on the days that sampling was undertaken, the groundwater level was below the surface and the spring was not flowing, therefore the sample was taken from the cave; and one karst lake partially fed by springs and lake vruljas of the surrounding karst aquifer, which is the only sampling point for the water supply of the islands of Cres and Lošinj) (Figure 1a). Sampling sites were chosen based on two criteria: 1) ensuring wide coverage of Croatia's karst area, and 2) selecting sites that are used for water supply. Sampling was undertaken in two separate campaigns, spring (19 to 28 March 2019) and autumn (16 to 21 October 2019), to compare EOCs at different times of the year, in generally high and low rainfall periods. Discharge conditions were different on the two sampling occasions, although due to the highly responsive nature of karst springs sampling did not capture discharge extremes.

Samples were collected in pre-cleaned 1 L glass bottles (1 bottle per sampling location) provided by the National Laboratory Services UK (NLS UK), where EOCs were analysed. Blank and duplicate samples were taken in each campaign to verify the authenticity of the data. Data presented here were first blank corrected to remove compounds detected below concentrations found in the blank samples, and to remove compounds introduced through the sample processing steps. Bottles were immediately stored at a cool place and shipped within one week. Samples were taken from as near to the spring sources as safely possible. Care was taken to minimise the risk of contamination, e.g. bottles were submerged in the spring to minimise local surface contamination, the sampler stood downstream, and bottles were rinsed thoroughly with sample water (which was discarded downstream) before taking the sample.

3.2 Analytical methods

The sample analysis was done at NLS UK with Agilent 6540 Ultra-High-Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) liquid chromatography/mass spectrometry (LC/MS) of Agilent Technologies, Inc. (Santa Clara, CA, USA). A detailed description of the analytical methods can be found in White et al. (2019). The limit of detection (LOD) for each analyte that was detected is available in Supplementary Table S1.

This analytical method (LC-MS/MS, target and non-target screen) returns results for 740 different compounds. The majority of compounds detected are considered to be newly “emerging organic contaminants - EOCs”, i.e. they are not routinely/globally regulated or monitored for, hence we have used the term ‘EOCs’ throughout the manuscript. However, some of the compounds reported, i.e. some pesticides and some industrial compounds, are monitored and regulated in some countries – but this varies considerably from one region to another.

3.3 Land cover and hydrological data

To investigate the impact of land use on groundwater quality, Corine Land Cover (CLC, 2018) spatial data sets were used. The first level of the CLC classification system was applied, which comprise three categories: (1) Urban: This category includes developed/inhabited/industrial areas with many potential sources of EOCs, including both domestic and industrial wastewater, which may provide sources of pharmaceuticals as well as industrial contaminants; (2) Agricultural (crops or livestock): This category is likely to be the main source of pesticides, but also a source of pharmaceuticals; (3) Natural: This comprises forest and upland karst areas which are semi-natural and likely to have few sources of EOCs.

Long-term discharge and water level data were collected from the Croatian Meteorological and Hydrological Service.

4 Results

4.1 EOC compounds in Croatian karst water

Of the 740 compounds analysed, sixty-five different compounds were identified in the Croatian karst groundwater, with a total of 277 detections (see Supplementary information, Table S2 for all results per location). EOCs were detected at all sites and in 34 of the 35 samples. The only sample with no EOCs detected was the sample from the Novljanska Žrnovnica spring in October 2019. Pharmaceutical and agricultural compounds were detected most frequently (Figures 2a and b), and the highest concentrations were from the industrial and personal care product and lifestyle compound (PCP-LS) groups (Figures 2a and c).

Figure 2. EOCs in Croatian karst a) The 20 most frequently detected compounds and their maximum and median concentrations. Bars show frequency (%) of detection (primary y axis), circles and crosses show concentrations (secondary y axis); b) pie chart of the overall % of detections in each of the EOC groups; c) Box-Whisker plots showing the concentrations of the 20 substances with the highest maximum concentrations; numbers inside the boxes are the number of detections.

The maximum concentrations of EOCs in the Croatian karst were compared to those detected in other studies of karst groundwater from around the world (compiled from 32 studies and reported in Lukač Reberski et al., 2022), which include a wide range of karst aquifers with different hydrogeological characteristics (“global karst groundwater” in Figure 3a). A comparison was also made to maximum concentrations of EOCs found in groundwater more generally including non-karst aquifers, using data from 46 studies compiled by Lapworth et al. (2012) (“all types groundwater” in Figure 3a). There have been many studies of EOCs in groundwater since 2012, which are not included here as compiling these data is beyond the scope of the current field study; but the data from Lapworth et al. (2012) provide a good preliminary comparison. Overall maximum EOC concentrations in the Croatian karst are relatively low; almost half of the detected substances had maximum concentrations below one ng/L, which is two or more orders of magnitude lower than most of the maximum concentrations from studies of other karst aquifers. The number of industrial, pharmaceutical and PCP-LSC compounds detected was much higher in other studies of karst groundwaters than in the Croatian karst; although the number of agricultural compounds detected was slightly higher in the Croatian karst (Figure 3b). However, for those compounds that were detected in both Croatian groundwater and in other studies, they were detected more frequently in Croatian karst groundwater (Figure 3c). The detection limits of the analytical methods vary between studies and this could influence the comparisons.

Figure 3. Comparison of EOCs in Croatian karst with other studies: (a) maximum concentrations for different detected compounds ranked from highest to lowest values; (b) the number of detected compounds by different compounds groups; (c) the detection frequency for compounds that were detected in both Croatian karst groundwater and other studies. Data sources used for comparisons are Lukač Reberski et al. (2022) for karst aquifers ("Global karst groundwater" in legend) and Lapworth et al. (2012) for groundwater more generally ("All types groundwater" in legend).

At each site, the total number of detected compounds in both sampling campaigns was compared to the total concentration (the sum of the concentrations of all the pollutants detected at the site from both samples). The objective was to provide insights into how the number of pollutants present compares to the total pollutant load in these waters. Whilst as expected the relationship is positive, it is non-linear (Figure 4). Three clusters of springs can be identified: sites with low numbers of detected compounds and low total concentration; sites with moderate numbers of compounds and relatively high concentrations of detected EOCs; and sites with high numbers of compounds and high concentrations.

Figure 4. The total number of detections versus total concentration for individual sites.

4.2 Spatial and temporal patterns in EOCs in Croatian karst springs

4.2.1 Spatial distribution

There is no clear spatial pattern in EOCs in the Croatian karst: there is no apparent difference in total concentrations or the number of detected EOC compounds at individual sampling locations between the coastal and continental areas (Figures 5a and b).

Personal care products and lifestyle compounds generally had the highest concentrations (14 out of 18 sites) (Figure 5a). Pharmaceuticals are the most commonly detected compounds at most sites (11 out of 18 sites), followed by agricultural compounds (Figure 5b). The catchments range from 24 to 1747 km^2 and cover roughly 30% of the Croatian karst region. This part of Croatia is sparsely populated, and industrial activity is poorly developed, as seen by the low proportion of urban areas (Figure 5c). In most cases, urban areas cover less than 1% of the catchment, except for two coastal springs, Zvir (11) and Golubinka (10). Natural land cover dominates the catchments of all the investigated springs, ranging from 57 to 93% of the catchment areas, as shown in Figure 5c. Agricultural land covers between 7 and 41% of the catchment areas (mean 20%), indicating considerable agricultural activities in the catchments.

Figure 5. Spatial distribution of: (a) total concentrations and (b) the total number of detected compounds of different EOC groups at sampling locations. The size of the pie charts corresponds to: (a) the total concentration (the sum of the concentrations of all EOC compounds detected in both campaigns at the site), and (b) the total number of compounds detected at the site. Each pie chart presents grouped data from both campaigns, and colours correspond to the proportion of different EOC groups that contribute to the total concentration (a) or the total number of compounds (b). Figure c presents proportions of major land cover categories in the spring catchments (Input data source is the European Environment Agency & Copernicus LAND Service Corine Land Cover). Numbers 1-18 correspond to sampling locations. Due to the map's scale, the Bistrac spring's catchment and the

Zagorska Mrežnica catchment share the same location point, therefore the circles are overlaping; the bigger circles correspond to Bistrac spring (18). Hydrogeological background is from the Hydrogeological Map of Croatia, scale 1:300,000 (Biondić et al., 1999) as in Figure 1a.

4.2.2 Spring flows and comparison of sampling campaigns in March and October

The sampled springs generally have very high discharges, with mean discharge ranging from one to a few tens of m^3/s; and some maximum discharges exceeding one hundred m^3/s (Figure 6). Although hydrological extremes were not captured, the discharge did differ between the two campaigns. In the March sampling campaign, discharges ranged from 0 to 12.3 m^3/s, and in the October from 0 to 34.8 m^3/s, although at all but one spring the discharge was higher in March than in October. Most springs in the Dinaric karst have a rapid response to rainfall and therefore discharge can vary substantially in short timescales at all times of the year (Bonacci, 2015). However, typically, discharges are expected to be significantly higher in March than in October (https://hidro.dhz.hr). The hydrological year 2018/2019 was not typical, with very low precipitation during the autumn and winter months, and at most sites, discharges in March were below average (at 16 out of 17 locations).

Figure 6. Long-term spring discharge (Q) statistics and discharge during the two sampling campaigns in March and October 2019. The upper graph shows daily precipitation (P) during the calendar year 2019 for each sampling location; red and green arrows show the timing of the sampling campaigns. Sampling locations not included in the figure: Golubinka spring – long-term data unavailable, water level in March was – 2 cm, and in October – 23 cm; Čikola spring – no discharges during both sampling campaigns when samples were taken from the cave; Vransko lake – water level in March was 10.73 m, and in October 10.32 m; Koreničko vrelo - discharge data are unavailable; Bistrac – sampled only in October, discharge data are unavailable.

Despite the differences in season and spring discharge, the type and number of detected EOC compounds, as well as the concentrations, were similar in both campaigns at most sampling sites (Figure 7a). For example, the four springs with the highest number of detected compounds and highest total concentrations (Golubinka, Prud, Rakonek and Kupica) had the highest concentrations and number of detected compounds in both campaigns, with generally similar types of EOCs present on both occasions (Figures 5b, 6 and 7a). It is also the case that at 11 sample sites, the difference in the total number of compounds between the two campaigns was less than 2 (Figure 7a), and the difference in total concentrations at 14 sites was less than 50 ng/L.

Figure 7. Comparison between the two sampling campaigns (March and October) at all sampling locations: a) total concentrations and number of compounds detected in different EOC groups (Agricultural, Pharmaceutical, PCP-LS, and Industrial); b) total concentrations, discharges and mass fluxes of all detected EOCs at individual sampling sites. Bistrac spring was only sampled in October. Discharge data at Golubinka spring were estimated: March – 1 L/s, October – 5 L/s.

The biggest difference in the total number of detected compounds between the two campaigns was at Zagorska Mrežnica, where more compounds were detected in October. At four sampling locations, differences in the total number of detected compounds between the two sampling campaigns were more than 50 % (Zagorska Mrežnica, Opačac, Tonković and Miljacka; Figure 7a, See Supplementary Material Table S3).

A general comparison of the two sampling campaigns is provided in Table 1. Eleven springs had a higher total number of detected EOCs in March, while total concentrations were higher at half of the springs in March and at the other half in October. Considering the type of EOC, pharmaceutical compounds were detected substantially more in March than in October, although concentrations were higher in October (See Supplementary Material Table S3). Other types of EOC compounds were detected in similar numbers during both campaigns.

Table 1. Summary of EOCs results for the two sampling campaigns, divided by contaminant group.

	Number of locations			
	Agr	Phar	PCP-LS	Ind
No detection during both campaigns	2	2	5	7
Higher number of detections in March	6	11	4	3
Higher number of detections in October	7	4	5	3
Same number of detections	2	0	3	4
Higher concentrations in March	8	5	5	5
Higher concentrations in October	6	10	7	5

Agr-Agriculturals, PCP-LS-Personal Care Products and Lifestyle, Phar-Pharmaceuticals, Ind-Industrials

Despite the broad similarities between the type, number and concentrations observed in the two sampling campaigns, the individual compounds detected were different during the two campaigns. Less than half of the total detected compounds were found in both campaigns, although in these cases, their concentrations in both campaigns were of the same order of magnitude (See Supplementary Material Table S3).

To estimate EOC environmental loads, mass fluxes were calculated for both sampling campaigns for each sampling location. Mass fluxes were obtained by multiplying the total EOC concentrations measured at each sampling site by the spring discharge at the sampling time. Mass fluxes have a very big range from 10 to 10^6 ng/s at the sampled springs, with differences between the two campaigns at most sites (Figure 7b). However, there is no consistent pattern in these differences, with mass fluxes higher in October at five sampling locations and higher in March at eight (Figure 7b).

4.3 Relationship of EOCs with land use and hydrochemical indicators

To analyse the strength and direction of the relationship between individual land cover categories and the type, number or concentration of EOCs, cross plots (Figure 8a-f) were created. These plots do not indicate strong relationships between the land cover categories and the EOCs observed at the springs.

There appears to be a very weak negative correlation ($R^2 \sim 0.2$) between the proportion of natural land cover (where pollution sources would be expected to be low) and the amount of EOC contamination, with those sites with a more natural land cover having lower total concentrations and lower numbers of EOC compounds detected (Figures 8a,b). Figures 8c and d show the relationships between the proportion of catchments with agricultural land cover and the total concentration of agricultural compounds detected at the sampling sites; and the total number of agricultural compounds detected. Due to the seasonal nature of agricultural activities, separate analyses for both campaigns are shown. Although there are positive correlations in these plots, the relationships are weak (R^2 ranges from 0.08 to 0.25) and are not statistically significant at the p= 0.05 level.

Cross-plots of individual EOC groups and the proportion of land use type were also made. Since most pharmaceuticals detected in Croatian karst groundwater are for both human and veterinary use, a relationship between the proportion of agricultural land and pharmaceuticals was analysed (Figure 8e) and the results showed a very weak positive correlation (R^2=0.02) which is statistically significant. Stronger positive correlations (R^2=0.28) were found for the relationship between the number of detected pharmaceuticals and the proportion of urban land (Figure 8f). A comparison of the proportion of urban land and the PCP-LS compounds showed a moderate (R^2=0.34) but statistically significant positive correlation, but there was no correlation between urban land cover and industrial compounds (Figure 8f). Given that only artificial sweeteners, which can be used in animal nutrition, were detected in the group of PCP-LS compounds, the relationship between PCP-LS compounds and the proportion of agricultural land was analysed (8e). They showed weak relationships, and the correlations were not statistically significant.

Figure 8. Correlations between EOC concentrations and land cover: a) correlation between natural land cover (%) and total EOC concentration (Σc EOC total), b) correlation between natural land cover (%) and total number of EOCs, c) correlation between agricultural land cover (%) and concentration of agricultural compounds, d) correlation between agricultural land cover (%) and number of agricultural compounds, e) correlation between agricultural land cover (%) and concentration of PCP-LS compounds and the number of pharmaceuticals, f) correlation between urban land cover (%) and concentration of PCP-LS and industrial compounds and the number of pharmaceuticals. Each graph presents Pearson's r and p-value; $\alpha<0.05$. Total means sum of concentration or number of all detected compounds at each location.

5 Discussion

Out of the 65 detected compounds in the Croatian karst springs, nine were among both the most frequently detected and the compounds with the highest concentration, suggesting that these are potentially the most widespread and significant EOCs in Croatia out of the 740 compounds included in this analysis. These are acesulfame, sucralose, perfluorobutanesulfonic acid (PFBS), carbamazepine, lamotrigine, desethylatrazine, hydrochlorothiazide, cotinine, and bentazone (Figure 2a and c). Carbamazepine is the most frequently detected EOC in both Croatian karst and in other studies of karst groundwater (reviewed by Lukač Reberski et al., 2022). In contrast, paracetamol, which is among the top 20 compounds in terms of both concentration and detection frequency in other karst groundwater studies, does not currently appear to be an important contaminant in the Croatian karst, with just a single detection.

In the Croatian karst, several substances were detected above or near the concentration of 100 ng/L, the current EU drinking water limit for any individual pesticide substance (EU DIRECTIVE 2020/2184). These are acesulfame, sucralose, PFBS, Emamectin B1b and Triphenyl phosphate (TPPA). These substances, except TPPA, are very persistent in the aquatic environment (Belton et al., 2020; Benson et al., 2017; ECHA, 2019; Saeger et al., 1979; Yang et al., 2021). TPPA, a widely used flame retardant and plasticiser (Stapleton, 2009), was detected at two springs situated in the continental part of the

Croatian karst (Tonković and Kupica). It was only detected in the March sample at these sites, but it was present in substantially higher concentrations compared to most other industrial compounds that were detected at other sampling locations. TPPA is indicative of rapid conduit flow from the pollutant source to the karst spring because it is subject to biodegradation in the aquatic environment, with a half-life of 2-4 days (PubChem, 2022), and because groundwater is less likely to contain phosphate esters due to their potential to adsorb to soils and sediments (ATSDR, 2012). Thus, its presence is a helpful indicator of short groundwater residence times.

Since karst environments provide a diversity of habitats for many different species (Gibert et al., 1994, Goldscheider, 2019), we evaluated the impact of detected EOCs on ecosystems. The highest concentrations of chemicals detected in the Croatian karst groundwater were compared with the corresponding PNEC (predicted no-effect concentration) values (Walker et al. 2012). A chemical's PNEC value is the concentration below which there are no observable harmful impacts on an ecosystem from exposure. Increased levels of emamectin B1b and TPPA in the environment may be some of the first to cause negative effects due to their low predicted PNEC values of 0.13 µg/L and 0.74 µg/L, respectively. The PNEC value for PFBS is 4.08 µg/L, while artificial sweeteners have somewhat higher values of 72.40 µg/L for acesulfame and 29.7 µg/L for sucralose. In the Croatian karst springs, acesulfame, sucralose, and PFBS have environmental concentrations 482, 68, and 58 times greater than their PNEC values respectively, whilst TTPA and emamectin B1b have environmental concentrations that are 8 and 1.2 times higher. Thus, the results from this study suggest that these contaminants may pose an imminent threat to ecosystem health in Croatian karst groundwater.

A key observation from the EOC data for the Croatian karst springs is that concentrations of most of the detected compounds are much lower compared to those found in other karst groundwaters (Lukač Reberski et al., 2022; Figure 3a). It is most likely that the lower concentrations reflect the high dilution of contaminants in the Croatian karst springs, as most of the sampled springs have very high discharge rates, and the land use data indicate that much of the catchments comprise natural land use (Figure 5c), and hence may enable recharge with very low contaminant mass to dilute any contaminated groundwater.

However, despite the low concentrations of EOCs in the Croatian karst, the mass contaminant fluxes are often high (Figure 7b). Although higher discharges may lead to higher dilution and consequently lower concentration of compounds, the overall mass flux can be high in springs with large discharges. As a result, it's crucial to consider mass fluxes in addition to concentrations, particularly in the case of high discharge springs, where low concentrations may still reflect an overall high contaminant load, having a more significant impact on dependent ecosystems than would be predicted based solely on concentrations. Very variable and at times high mass fluxes observed in this study are consistent with the investigation of a karst aquifer with similar characteristics reported by Doummar and Aoun (2018).

The number and detection frequency of contaminants in the Croatian karst are considerable (Figure 3b), especially given the area's sparse population and low levels of industrial activity. The detection limits of analytical methods vary between studies and this could influence the comparisons. It also reflects the highly karstic nature of the classical karst aquifer, with fast groundwater flow and lower attenuation capabilities; and is also an indication that pollutant sources are widespread in Croatia despite the relatively low levels of development and urbanisation in the catchments of the investigated karst springs.

The lack of a spatial pattern in total concentration, total number of detected compounds or types of compounds (Figures 5a and b) also reflects the highly heterogeneous nature of karst with local variations in hydrogeological characteristics, as well as variable anthropogenic influences (different land use, pollutant sources and management practices) in the catchment areas. A cross-plot of the total number of detected compounds and concentrations shows three distinct clusters (Figure 4), but a more in-depth analysis failed to establish a link between the sampling sites within individual clusters, which have no geographical pattern. There were also no systematic differences between springs that are located in the coastal areas, and those that are in the inland mountainous areas, suggesting that local variations in karst are more important than geographical patterns.

The highly karstic nature of the aquifer is also reflected in the significant differences in the number of detected compounds and their concentrations between the two sampling campaigns at some locations, with no apparent relationship with discharge or season (Figure 7a and b, Supplementary materials S2 and S3, Table 1). Observed variations are likely to reflect a combination of the change in discharge, different land use practices during different seasons, and the highly variable and localised nature of individual karst spring response to recharge, where even small changes in discharges can lead to significant differences in groundwater flow paths, thus activating different parts of the aquifer system.

Land use is likely to have a significant impact on the type and concentration of EOCs, but the challenges in determining the catchment boundaries in highly karstified areas and the complexities of the karst systems make this relationship unclear (Figures 8). The extremely high discharge of many Croatian karst springs (and hence the large catchment areas), means that identifying specific sources of EOCs is especially difficult. Although the three springs with the highest total concentrations and number of detected EOC compounds (Prud (5), Golubinka (10) and Rakonek (13)) have the highest proportion of urban and/or agricultural land cover in their catchments, relationships between land use and contaminant presence and concentration are generally weak or absent. A slightly better correlation (R^2=0.25) was found between agricultural land use and the number of agricultural compounds detected in the October campaign (Figure 8d), which could be explained by the timing of the ending of the agricultural season when plant protection products are extensively used. The fact that the agricultural land use category also includes pastures (CLC, 2018), and that animal density is typically low throughout the Croatian karst, might be one of the causes of the overall weak correlation between EOCs and the proportion of the catchment with agricultural land use. Another challenge is airborne transport of pesticides (e.g. Clifford et al., 2016; Unsworth et al., 1999), which could result in pesticides in areas with other land uses.

PCP-LS and pharmaceutical compounds can originate from either agricultural or urban sources. The correlation results might suggest that the primary sources in Croatian karst are urban areas, i.e. wastewater (Figures 8e and f). This is in line with previous findings in a highly karstified aquifer (Doummar and Aoun, 2018; Zemann et al., 2015). However, it remains unclear why paracetamol, which is mainly intended for human use (Savides et al., 1984), and proven to be a good wastewater indicator (Godfrey et al., 2007), was only detected once in the Croatian karst. The low detection frequency for paracetamol and the fact that most of the pharmaceuticals detected in this study are intended for both human and veterinary use (e.g. carbamazepine) point to the origin of pharmaceuticals in Croatian karst groundwater being mainly from agricultural sources. The dominance of agricultural land use in study catchments also supports this hypothesis.

6 Conclusion and future outlook

1) A total of 65 different contaminants were present, with 277 detections from 35 samples. Five compounds were found at concentrations close to or exceeding EU standards, and concentrations of some EOCs exceeded PNEC values indicating that they are likely to be impacting aquatic ecosystems. Agricultural, Industrial, Pharmaceutical and PCP-LS compounds were all detected at most sites. Pharmaceutical and agricultural compounds were detected most frequently, whilst the highest concentrations were in industrial and personal care product and lifestyle compound groups. Of 35 samples, only one had no detected EOCs.

2) EOC compounds were detected frequently and often with high mass fluxes, further indicating the vulnerability of the Croatian karst. TPPA, with a half-life of 3-4 days, was present at two sites, and could be useful in vulnerability assessments as an indicator of rapid groundwater flow.

3) The lower concentrations found in this study compared with other karst groundwater studies may reflect pollutant dillution due to the exceptionally high discharge of the “classical” karst springs. It could also reflect the relatively large proportion of “natural land cover” present in the studied catchments. However, due to the high spring discharges, the mass fluxes of EOC pollutants were considerable (10 to 10^6 ng/s).

4) Agriculture appears to be a major source of EOC contamination in the Croatian karst, with high proportions of agricultural land use; and many of the EOCs detected are likely to have an agricultural source. However, the percentage of agricultural land use in the catchment generally had no or only a very weak correlation with the number of detected compounds/concentrations. This is likely to reflect the large size of the studied catchments, the complexity of karst pollution transport, and the potential for long range atmospheric transport of pesticides. Further work at the individual catchment scale is needed to understand the relationship between land use and EOCs in the Croatian karst, which would also provide valuable insights into the transport and attenuation of EOCs in karst more generally.

5) Data from two sampling campaigns show the high variability of EOC contamination in karst springs. Such changes would be expected in karst where spring discharges and karst conduit flow paths vary substantially on both seasonal and sometimes hourly/daily timescales. These results highlight the need for future studies focussed on temporal variations in EOCs in karst.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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https://hidro.dhz.hr Gauge stations of Croatian Meteorological and Hydrological Service

Abstract

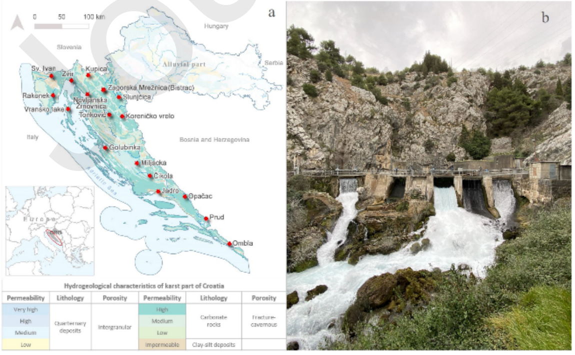
Emerging organic contaminants (EOCs) have become of increasing interest due to concerns about their impact on humans and the wider environment. Karst aquifers are globally widespread, providing critical water supplies and sustaining rivers and ecosystems, and are particularly susceptible to pollution. However, EOC distributions in karst remain quite poorly understood. This study looks at the occurrence of EOCs in the Croatian karst, which is an example of the “classical” karst, a highly developed type of karst that occurs throughout the Dinaric region of Europe. Samples were collected from 17 karst springs and one karst lake used for water supply in Croatia during two sampling campaigns. From a screen of 740 compounds, a total of 65 compounds were detected. EOC compounds from the pharmaceutical (n=26) and agrochemical groups (n=26) were the most frequently detected, while industrials and artificial sweeteners had the highest concentrations (range 8 – 440 ng/L). The number of detected compounds and the frequency of detection demonstrate the vulnerability of karst to EOC pollution. Concentrations of 5 compounds (acesulfame, sucralose, perfluorobutane sulfonate, emamectin B1b, and triphenyl phosphate) exceeded EU standards and occurred at concentrations that are likely to be harmful to ecosystems. Overall, most detections were at low concentrations (50 % <1 ng/L). This may be due to high dilution within the exceptionally large springs of the Classical karst, or due to relatively few pollution sources within the catchments. Nevertheless, EOC fluxes are considerable (10 to 10^6 ng/s) due to the high discharge of the springs. Temporal differences were observed, but without a clear pattern, reflecting the highly variable nature of karst springs that occurs over both seasonal and short-term timescales. This research is one of a handful of regional EOC investigations in karst groundwater, and the first regional study in the Dinaric karst. It demonstrates the need for more frequent and extensive sampling of EOCs in karst to protect human health and the environment.

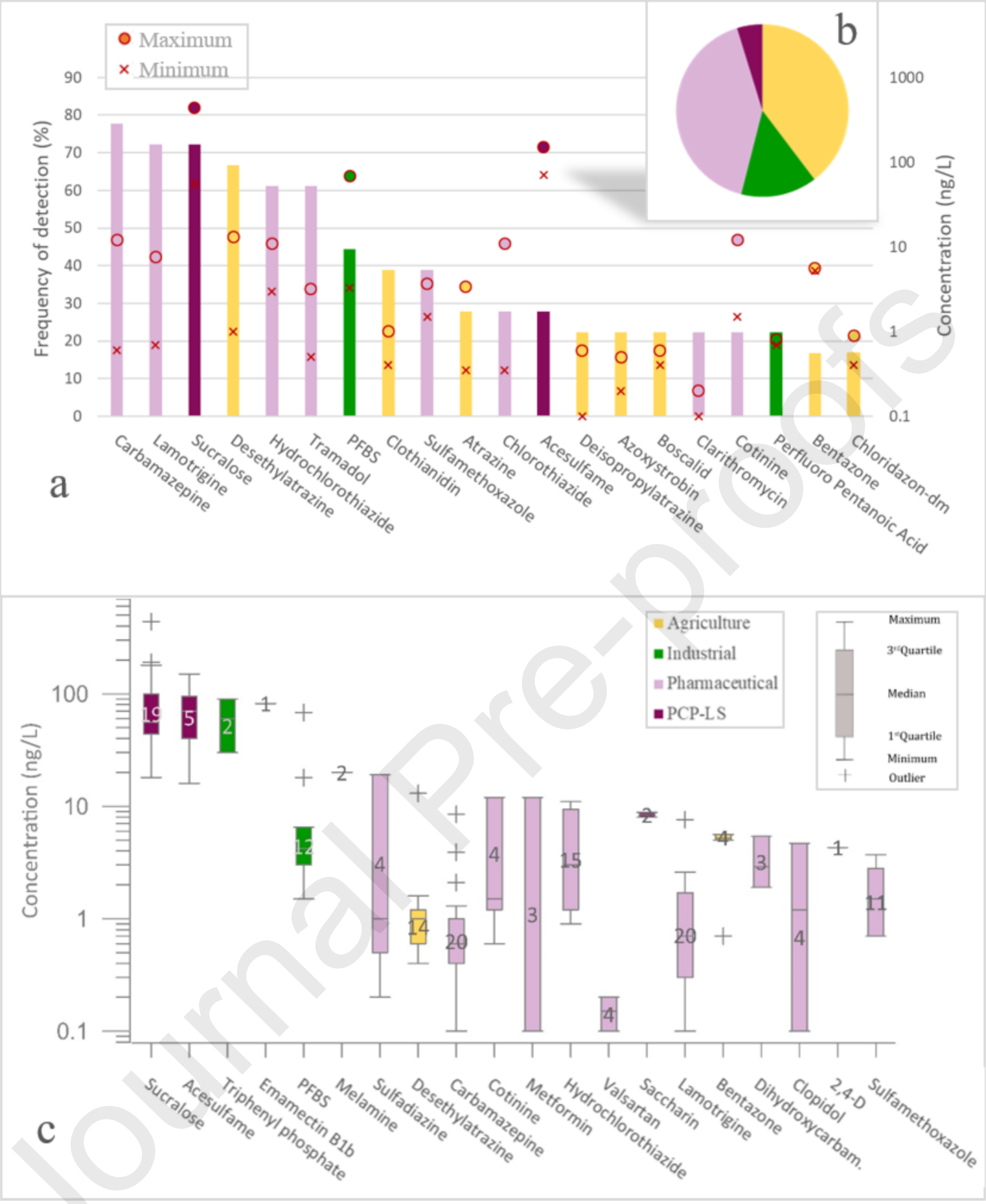
Highlights

- First regional investigation of EOCs in Dinaric karst aquifers
- 65 different EOCs detected, conc. < 1 ng/L for almost half
- EOC concentrations are two orders of magnitude lower than in other groundwater types
- EOCs are detected more frequently than in other types of groundwater
- EOCs are detected in greater or comparable numbers than in other groundwater types

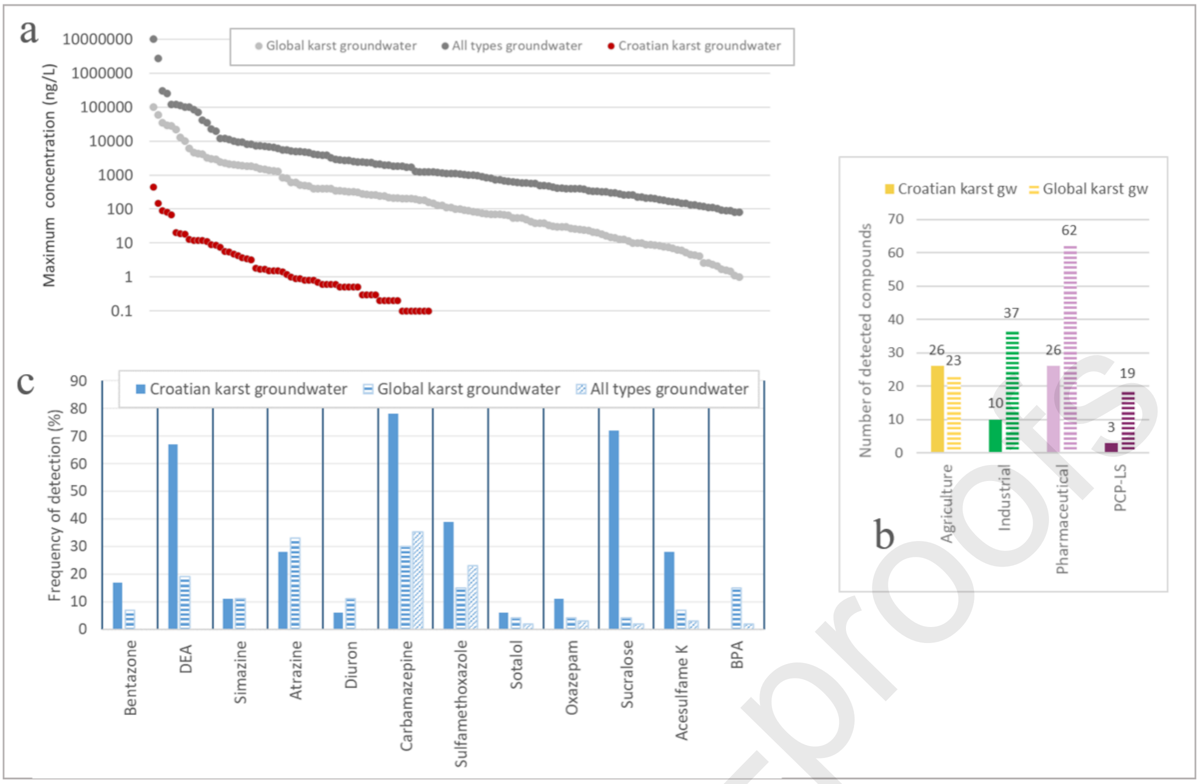
	Number of location			
	Agr	Phar	PCP-LS	Ind
No detection during both campaigns	2	2	5	7
Higher number of detections in March	6	11	4	3
Higher number of detections in October	7	4	5	3
Same number of detections	2	0	3	4
Higher concentrations in March	8	5	5	5
Higher concentrations in October	6	10	7	5

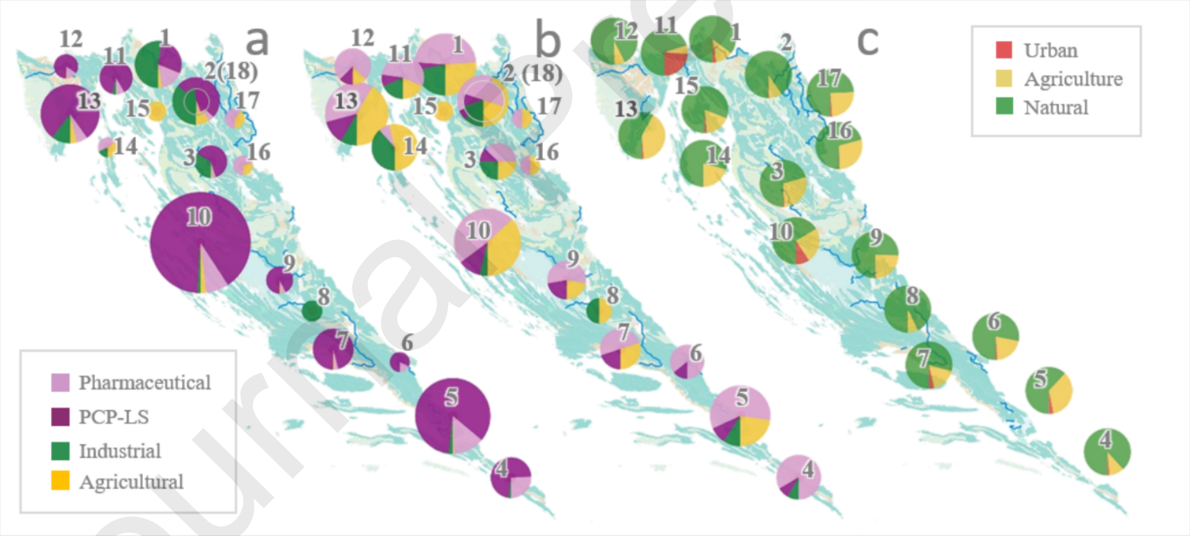
Agr-Agriculturals, PCP-LS-Personal Care Products and Lifestyle, Phar-Pharmaceuticals, Ind-Industrials




Maximum
Minimum
Frequency of detection (%)
0
10
20
30
40
50
60
70
80
90
Concentration (ng/L)
0.1
1
10
100
1000
b
a
Carbamazepine
Lamotrigine
Sucralose
Desethylatrazine
Hydrochlorothiazide
Tramadol
PFBS
Clothianidin
Sulfamethoxazole
Atrazine
Chlorothiazide
Acesulfame
Deisopropylatrazine
Azoxystrobin
Boscalid
Clarithromycin
Cotinine
Perfluoro Pentanoic Acid
Bentazone
Chloridazon-dm
Concentration (ng/L)
0.1
1
10
100
19
5
2
1
2
12
4
14
20
4
3
15
4
2
20
4
3
4
1
11
Agriculture
Industrial
Pharmaceutical
PCP-LS
Maximum
3rdQuartile
Median
1stQuartile
Minimum
Outlier
c
Sucralose
Acesulfame
Triphenyl phosphate
Emamectin B1b
PFBS
Melamine
Sulfadiazine
Desethylatrazine
Carbamazepine
Cotinine
Metformin
Hydrochlorothiazide
Valsartan
Saccharin
Lamotrigine
Bentazone
Dihydroxycarbam.
Clopidol
2,4-D
Sulfamethoxazole

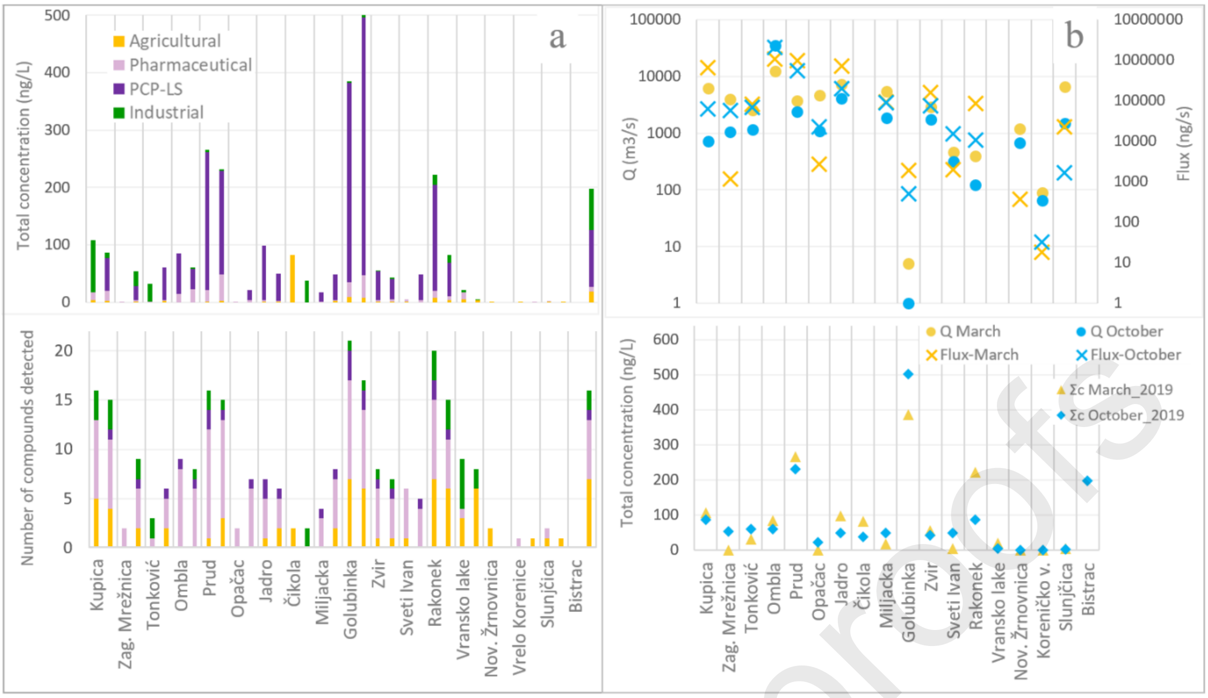
628

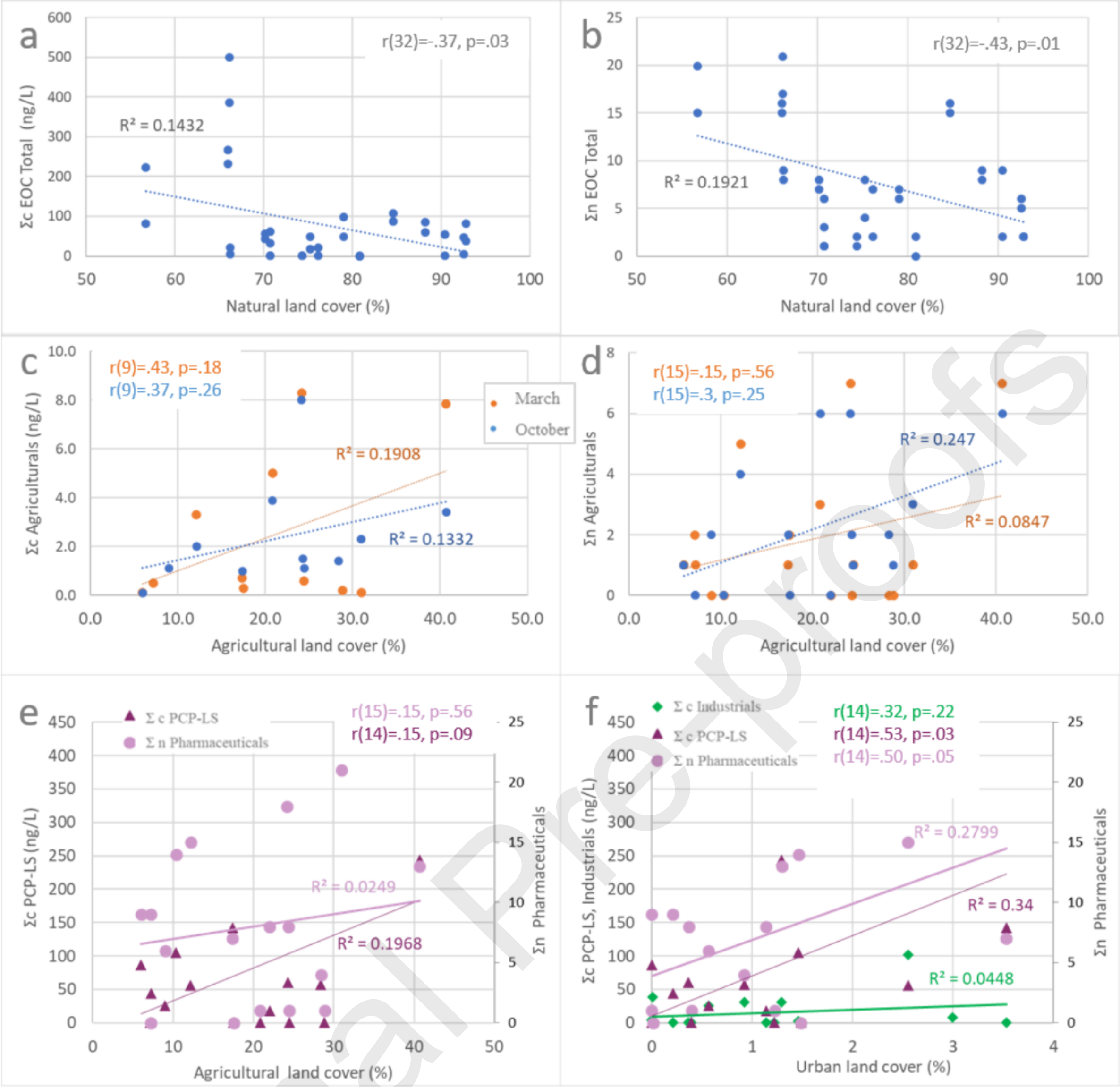


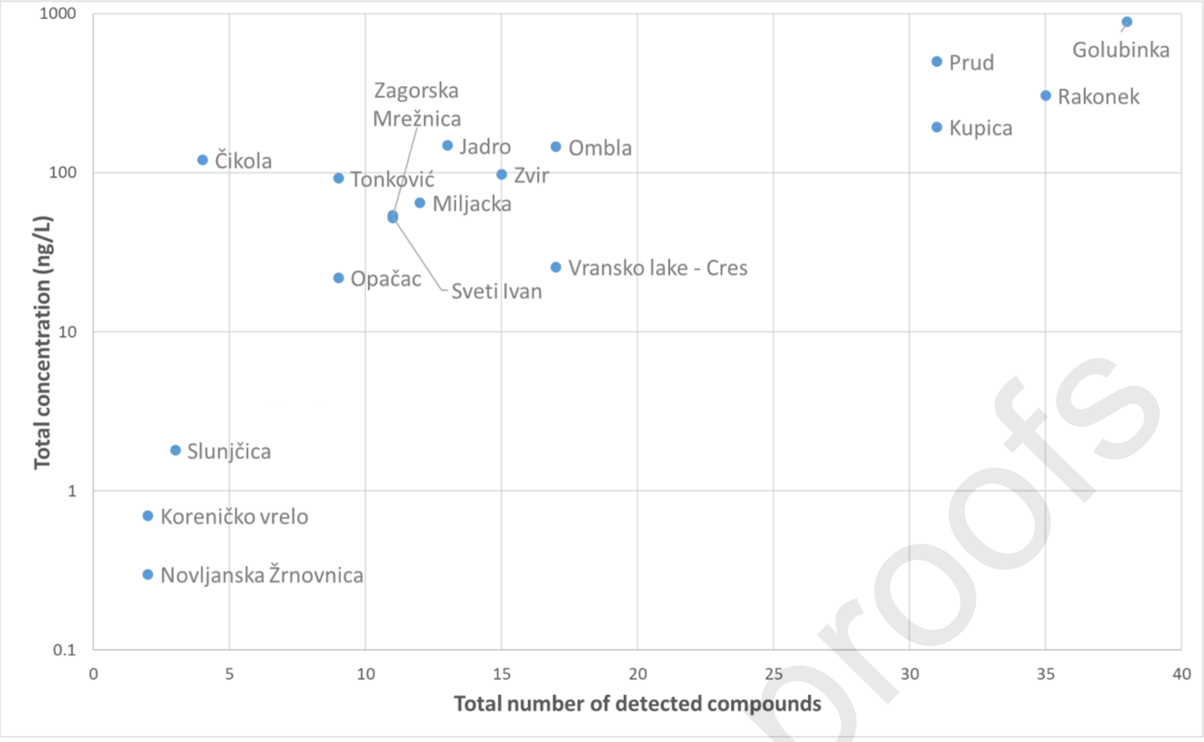


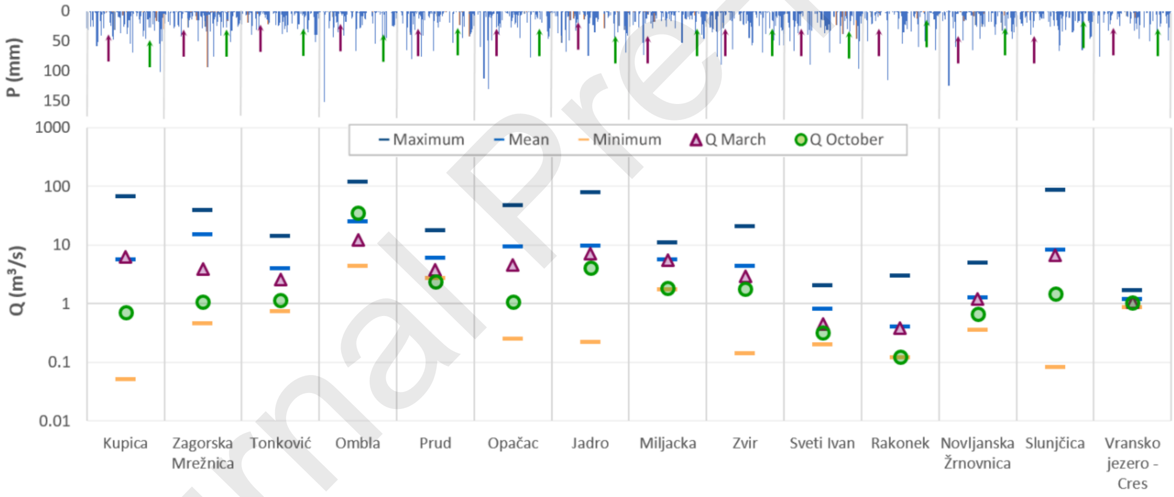
Sample locations					
1	Kupica	7	Jadro	13	Rakonek
2	Zagorska Mrežnica	8	Čikola	14	Vransko lake - Cres
3	Tonković	9	Miljacka	15	Novljanska Žrnovnica
4	Ombla	10	Golubinka	16	Koreničko vrelo
5	Prud	11	Zvir	17	Slunjčica
6	Opačac	12	Sveti Ivan	18	Bistrac

Pie chart size		
Concentration (ng/L)		Number
1000	○	25
100	○	15
10	○	5


a
Agricultural
Pharmaceutical
PCP-LS
Industrial
Total concentration (ng/L)
500
400
300
200
100
0
Number of compounds detected
20
15
10
5
0
Kupica
Zag. Mrežnica
Tonković
Ombla
Prud
Opačac
Jadro
Čikola
Miljacka
Golubinka
Zvir
Sveti Ivan
Rakonek
Vransko lake
Nov. Žrnovnica
Vrelo Korenice
Slunjčica
Bistrac
b
Q (m3/s)
100000
10000
1000
100
10
1
Flux (ng/s)
10000000
1000000
100000
10000
1000
100
10
1
Q March
Q October
Flux-March
Flux-October
Σc March_2019
Σc October_2019
Total concentration (ng/L)
600
500
400
300
200
100
0
Kupica
Zag. Mrežnica
Tonković
Ombla
Prud
Opačac
Jadro
Čikola
Miljacka
Golubinka
Zvir
Sveti Ivan
Rakonek
Vransko lake
Nov. Žrnovnica
Koreničko v.
Slunjčica
Bistrac


a
Σc EOC Total (ng/L)
r(32)=-.37, p=.03
R² = 0.1432
Natural land cover (%)
b
Σn EOC Total
r(32)=-.43, p=.01
R² = 0.1921
Natural land cover (%)
c
Σc Agriculturals (ng/L)
r(9)=.43, p=.18
r(9)=.37, p=.26
March
October
R² = 0.1908
R² = 0.1332
Agricultural land cover (%)
d
Σn Agriculturals
r(15)=.15, p=.56
r(15)=.3, p=.25
R² = 0.247
R² = 0.0847
Agricultural land cover (%)
e
Σ c PCP-LS
Σ n Pharmaceuticals
r(15)=.15, p=.56
r(14)=.15, p=.09
R² = 0.0249
R² = 0.1968
Σc PCP-LS (ng/L)
Σn Pharmaceuticals
Agricultural land cover (%)
f
Σ c Industrials
Σ c PCP-LS
Σ n Pharmaceuticals
r(14)=.32, p=.22
r(14)=.53, p=.03
r(14)=.50, p=.05
R² = 0.2799
R² = 0.34
R² = 0.0448
Σc PCP-LS, Industrials (ng/L)
Σn Pharmaceuticals
Urban land cover (%)


1000
100
10
1
0.1
Total concentration (ng/L)
0
5
10
15
20
25
30
35
40
Total number of detected compounds
Golubinka
Prud
Rakonek
Kupica
Zagorska Mrežnica
Jadro
Ombla
Čikola
Tonković
Zvir
Miljacka
Vransko lake - Cres
Opačac
Sveti Ivan
Slunjčica
Koreničko vrelo
Novljanska Žrnovnica


0
50
100
150
P (mm)
1000
100
10
1
0.1
0.01
Q (m³/s)
Maximum
Mean
Minimum
Q March
Q October
Kupica
Zagorska Mrežnica
Tonković
Ombla
Prud
Opačac
Jadro
Miljacka
Zvir
Sveti Ivan
Rakonek
Novljanska Žrnovnica
Slunjčica
Vransko jezero - Cres