ORGANSKA ONEČIŠĆIVALA OD RASTUĆEGA ZNAČAJA U KRŠKIM PODZEMNIM VODAMA U HRVATSKOJ

Selak, Ana

Doctoral thesis / Doktorski rad

2024

University of Zagreb

Faculty of Geotechnical Engineering

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EMERGING ORGANIC CONTAMINANTS IN CROATIAN KARST GROUNDWATER

DOCTORAL DISSERTATION

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Supervisors:
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Varaždin, 2024

Sveučilište u Zagrebu

Geotehnički fakultet

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Varaždin, 2024.

BIBLIOGRAPHY DATA

Scientific area: Technical sciences

Scientific field: Interdisciplinary technical sciences

Scientific branch: Environmental engineering

Institution: University of Zagreb, Faculty of Geotechnical Engineering

Mentors:

Assoc. Prof. Ivana Grčić, PhD

Sr. Res. Assoc. Jasmina Lukač Reberski, PhD

Number of pages: 140

Number of references: 190

Date of Defence: 6th of December 2024

Committee members:

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This dissertation has been stored in the library of the Faculty of Geotechnical Engineering in Varaždin, Hallerova aleja 7 and National and University Library in Zagreb, Hrvatske bratske zajednice 4.

The topic of the dissertation was approved at the VII. meeting of the Faculty Council of the Faculty of Geotechnical Engineering on the 22nd of February 2024, and subsequently approved at the X. regular meeting of the Senate of the University of Zagreb on the 9th of July 2024.

BIBLIOGRAFSKI PODACI

Znanstveno područje: Tehničke znanosti

Znanstveno polje: Interdisciplinarne tehničke znanosti

Znanstvena grana: Inženjerstvo okoliša

Institucija: Sveučilište u Zagrebu, Geotehnički fakultet

Mentori:

Izv. prof. dr. sc. Ivana Grčić

Dr. sc. Jasmina Lukač Reberski, viša znanstvena suradnica

Broj stranica: 140

Broj literaturnih navoda: 190

Datum obrane: 6. prosinca 2024.

Stručno povjerenstvo za obranu:

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Tema doktorske disertacije odobrena je na VII. sjedinici Fakultetskog vijeća Geotehničkog fakulteta, održanoj 22. veljače 2024. i na X. redovnoj sjedinici Senata Sveučilišta u Zagrebu, održanoj 9. srpnja 2024. god.

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ACKNOWLEDGMENTS

This doctoral thesis benefited from the support of GeoTwinn project funded by the H2020-EU.4.b. (Grant number 809943) and the Interreg Central Europe boDEREC-CE project (C1412) co-financed by the European Regional Development Fund. The field investigations and analyses were co-financed and supported by the Croatian Geological Survey, Department of Hydrogeology and Engineering Geology.

I am sincerely grateful for the unwavering support and active involvement of my mentor, PhD Jasmina Lukač Reberski, throughout the entirety of my PhD journey. Her commitment is evident in every aspect, from guiding me through field research to providing regular consultations on result interpretation and assisting me in the preparation of scientific papers related to my thesis. PhD Lukač Reberski's expertise and willingness to offer insightful advice have proven instrumental in addressing various research questions. Her encouragement for independent work, the creation of scientific papers, and the pursuit of further professional and academic development is greatly appreciated. Gratitude is also extended to my mentor from The Faculty of Geotechnical Engineering Assoc. Prof. Ivana Grčić as well as members of my doctoral committee - Assis. Prof. Jelena Loborec, Prof. Göran Klobučar, and Sci. Adv. in permanent vocation Renato Buljan - for their constructive feedback.

Special thanks are offered to my colleagues Tomislav Novosel and Joža Kolarić for their invaluable support during the field investigation. Appreciation is also extended to all my colleagues from the Department of Hydrogeology and Engineering Geology for their intellectual camaraderie.

I would like to convey my sincere appreciation to the co-authors of the scientific papers included in this thesis for their contributions and fruitful collaboration.

Acknowledgments are due to the Croatian Meteorological and Hydrological Service and Croatian Waters for providing data.

Lastly, heartfelt thanks are extended to my family and friends for their unwavering encouragement and understanding throughout this demanding academic endeavour.

ABSTRACT

Owing to considerable advancement in analytical techniques and instrumentation, emerging organic contaminants (EOCs) are being detected in both surface water and groundwater at concentrations ranging from ng/L to µg/L, where they have been introduced by ongoing, global daily emissions. Research on the occurrence and behaviour of EOCs in karst aquifers is in its early stages, leading to a dearth of policy measures and legislative controls for these compounds in groundwater. The Dinaric karst aquifers, which provide drinking water to nearly half of Croatia's territory, exhibit heterogeneous, anisotropic structural and hydraulic characteristics, intense karstification, and high fracture-cavernous porosity. These features result in rapid groundwater flow and large karst springs, yet they also render the aquifers highly vulnerable to contamination and their investigation intricate and challenging. The scientific papers published as part of this PhD thesis were the first to identify and quantify EOCs within Croatian karst aquifers at regional (17 karst springs and 1 karst lake) and local (4 locations within Jadro and Žrnovnica catchment) scales. Through two comprehensive campaigns encompassing analysis of 740 EOCs, 65 compounds were detected at the regional level, with pharmaceuticals (n=26) and agrochemicals (n=26) being predominant. Industrials and artificial sweeteners exhibited the highest concentrations up to 90 and 440 ng/L, respectively. At local scale, 22 EOCs were identified in the karst catchment of Jadro and Žrnovnica springs during seven sampling campaigns, with concentrations reaching up to 372 ng/L. The comparatively lower concentrations and detection frequency of EOCs in the Croatian karst, as opposed to other karst regions, suggest significant dilution attributed to substantial discharge of “classical karst” springs and relatively low intensity of agricultural and industrial activities in the area. At both regional and local scales, the majority of compounds were assessed as potentially persistent, mobile and toxic or very persistent and very mobile, unraveling their potential negative impact on (ground)water ecosystem. While current concentrations of EOCs in ng/L in karst drinking water are not expected to pose a significant risk to healthy populations, future research should analyse the potential effects of long-term exposure to EOCs mixtures, particularly for vulnerable or at-risk groups. The importance of establishing regular monitoring protocols for EOCs prioritized with this thesis is underscored, with due consideration given to the implementation of a Voluntary Groundwater Watch List tailored to Dinaric karst characteristics. Future in-depth event analysis at catchment levels is recommended, to elucidate potential background EOCs levels and their behaviour under varying hydrological conditions. Such research will offer crucial insights for risk mitigation, in light of the expected increase in

contamination mass loads in the coming years and the ongoing impact of climate changes on water quantities.

KEYWORDS

Emerging organic contaminants

Karst aquifers

Groundwater

Persistence

Bioaccumulative potential

Mobility

Toxicity

Environmental risk assessment

Drinking water resources

Human exposure

PROŠIRENI SAŽETAK

Napredak analitičkih metoda i instrumenata omogućio je otkrivanje organskih onečišćivala od rastućega značaja (OORZ) u površinskim i podzemnih vodama u koncentracijama reda veličine ng/L do µg/L, a čija je prisutnost u okolišu rezultat kontinuiranoga, svakodnevnoga ispuštanja diljem svijeta. Istraživanje pojavnosti i ponašanja OORZ-a u krškim vodonosnicima je u začecima, što je razlog trenutnog nedostatka političkih mjera i zakonodavne kontrole za ove spojeve u podzemnim vodama. Vodonosnici dinarskog krša opskrbljuju pitkom vodom gotovo polovicu teritorija Hrvatske, a odlikuje ih heterogenost i anizotropnost strukturnih i hidrauličkih značajki, intenzivna okršenost i znatna pukotinsko-kavernozna poroznost. Ove značajke rezultiraju brzim tokovima podzemne vode i izdašnim krškim izvorima, ali istovremeno čine krške vodonosnike izrazito ranjivima na onečišćenje te njihovo istraživanje složenim i izazovnim. Znanstveni radovi objavljeni kao dio ove doktorske disertacije po prvi puta u Hrvatskoj identificiraju i kvantificiraju OORZ-ove u krškim vodonosnicima na regionalnoj (17 krških izvora i jedno jezero) i lokalnoj (izvori Jadro i Žrnovnica, bušotina Gizdavac i rijeka Cetina) razini. U dvije sveobuhvatne kampanje analizirano je 740 OORZ-a, od čega je utvrđena pojavnost 65 spojeva na regionalnoj razini, među kojima su najčešće detektirani farmaceutici (n=26) i poljoprivredni spojevi (n=26). Industrijski spojevi i umjetna zaslađivala imala su najviše koncentracije do 90 ng/L (TPPA) i 440 ng/L (sukraloza). Tijekom sedam kampanja uzorkovanja na lokalnoj razini slijeva izvora Jadra i Žrnovnice, identificirana su 22 OORZ-a u koncentracijama koje su se kretale od 0.3 ng/L (opioidni analgetik tramadol) do 372 ng/L (industrijski spoj 1H-benzotriazol). Repelent DEET bio je najčešće detektiran spoj prosječne koncentracije od 50 ng/L utvrđene u površinskoj vodi rijeke Cetine, izvorima i podzemnoj vodi. Niže koncentracije i manja učestalost detekcije OORZ-a u hrvatskom kršu, za razliku od drugih krških područja, sugeriraju značajno razrjeđenje koje se pripisuje velikim protocima izvora "klasičnog krša" i relativno niskom intenzitetu poljoprivrednih i industrijskih aktivnosti u istraživanom području. Maksimalne koncentracije OORZ-a u izvorima Jadro i Žrnovnica uočene su nakon jesenskih kiša, dok tijekom najviših protoka uslijed razrjeđenja nije zabilježena pojavnost spojeva iznad granica detekcije (LOD). Suprotno izvorima, u podzemnoj vodi iz duboke bušotine Gizdavac, najveća ukupna koncentracija OORZ-a utvrđena je pri uvjetima malih protoka što naglašava značajnu ranjivost krškog vodonosnika. Uz uvid u dinamiku krškog sustava, analiza hidrokemijskih značajki pokazala se korisnom za bolje razumijevanje funkcioniranja i ranjivosti hidrogeoloških sustava na OORZ-ove. Oštri skokovi uočeni na kemografovima izvora Jadra i Žrnovnice dokaz su značajne okršenosti i inherentne

ranjivosti sustava. Zastupljenost ponora, razvijene mreže krških kanala i pukotina mogu pospješiti transport OORZ-a. Utvrđena je snažna, statistički značajna pozitivna korelacija između metformina i iona NO_3^-, kao i koncentracije OORZ-a, broja detektiranih spojeva i iona NO_3^- u rijeci Cetini, što ukazuje na njihovo zajedničko porijeklo, vjerojatno otpadnu vodu. Pojavnost postojanih OORZ-a u izvorima i podzemnoj vodi pri uvjetima niskih voda i pozitivna statistički značajna korelacija DEET-a s Ca^{2+} ionom, ukazuje na potencijalno skladištenje postojanih OORZ-a u pukotinsko-poroznoj epikrškoj zoni vodonosnika. Uočena sezonalnost i sveprisutnost u slijevu indikatora onečišćenja otpadnom vodom, spojeva DEET i 1H-benzotriazola, ukazuje na ograničenu atenuaciju postojanih i mobilnih spojeva unutar krškog vodonosnika. Kako na regionalnoj, tako i na lokalnoj razini, većina otkrivenih spojeva ocijenjena je kao potencijalno postojana, mobilna i toksična ili kao vrlo postojana i vrlo mobilna, ukazujući na njihov potencijalno negativan utjecaj na ekosustav podzemnih voda. Analizom utjecaja na ljudsko zdravlje utvrđeno je kako izmjerene koncentracije OORZ-a u ng/L u krškim izvorima koji se koriste u vodoopskrbi, trenutno ne predstavljaju značajan rizik za zdravu populaciju, no preporuka je da se budućim istraživanja analiziraju potencijalni učinci dugotrajne izloženosti koktelu različitih OORZ-a. Važno je uspostaviti protokole redovitog praćenja OORZ-a koji su prioritizirani ovom disertacijom na temelju analize njihove postojanosti, bioakumulativnosti, mobilnosti i toksičnosti, uz razmatranje mogućnosti provedbe Dobrovoljnog popisa praćenja OORZ-a u podzemnim vodama, prilagođenog karakteristikama dinarskog krša. Radi utvrđivanja potencijalnih pozadinskih koncentracija OORZ-a i njihovog ponašanja pri različitim hidrološkim uvjetima, fokus budućih istraživanja treba usmjeriti na analize događaja na razini pojedinih sljevova. Za slijev izvora Jadra i Žrnovnice predlaže se praćenje spojeva DEET-a, 1H-benzotriazola i metformina. Također, ističe se prednost usporedne analize hidrokemijskih markera i OORZ-a kao novih trasera koji će rasvijetliti zamršeno međudjelovanje čimbenika koji utječu na transport unutar krških vodonosnika i uvjetuju kemiju podzemnih voda podložnih različitim antropogenim pritiscima. Takva istraživanja pružit će uvide ključne za smanjenje rizika, posebice u svjetlu očekivanog porasta opterećenja onečišćenjem u nadolazećim godinama i utjecaja klimatskih promjena na količine vode.

KLJUČNE RIJEČI

Organska onečišćivala od rastućega značaja

Krški vodonosnici

Podzemna voda

Perzistentnost

Potencijal bioakumulativnosti

Mobilnost

Toksičnost

Procjena okolišnog rizika

Resursi vode za piće

Utjecaj na ljudsko zdravlje

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LIST OF ABBREVIATIONS

CID	Commission Implementing Decision
CMR	Carcinogenic, mutagenic, or toxic to reproduction
CZ	Czech Republic
DEET	Diethyltoluamide
ECHA	European Chemical Agency
EOCs	Emerging organic contaminants
ERA	Environmental risk assessment
EU	European Union
GWWL	Groundwater Watch List
IARC	International Agency for Research on Cancer
LOD	Limit of detection
OORZ	Onečišćivala od rastućega značaja
non-PBT	Non-persistent-bioaccumulative-toxic
PBT/vPvB	Persistent, bioaccumulative, toxic / very persistent, very bioaccumulative
PBTr	PBT score per site
PFAS	Perfluoroalkyl substances
PFBS	Perfluorobutanesulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PhD	Doctor of Philosophy

PMT/vPvM	Persistent, mobile, toxic / very persistent, very mobile
POPs	Persistent Organic Pollutants
PPCPs	Pharmaceuticals and personal care products
PFAS	Per- and polyfluoroalkyl substances
REACH	Regulation on the registration, evaluation, authorisation and restriction of chemicals
RQ	Risk quotient
RQ_{site}	Risk quotient per site
SMILES	Simplified molecular-input line-entry system
SVHC	Substances of very high concern
SWWL	Surface Water Watch List
TPPA	Triphenyl phosphate
UK	United Kingdom
UV	Ultraviolet
QSAR	Quantitative structure-activity relationship

LIST OF SCIENTIFIC PAPERS

This PhD thesis comprises five original scientific papers and one data paper, providing comprehensive insights into emerging organic contaminants in the groundwater of the Dinaric karst region in Croatia.

List of published scientific papers:

Published paper	**Database**	**Paper category and quartile**	**Impact factor (IF)**
I. Lukač Reberski, J., Selak, A., Lapworth, D.J., Maurice, L.D., Terzić, J.*, Civil, W., Stroj, A., 2023. Emerging organic contaminants in springs of the highly karstified Dinaric region. J Hydrol 621, 129583. https://doi.org/10.1016/j.jhydrol.2023.129583	WoSCC-SCI-Exp	Engineering, Civil (Q1) Geosciences, Multidisciplinary (Q1) Water resources (Q1)	IF=5.9/2023
II. Selak, A., Lukač Reberski, J.*, Klobučar, G., 2023. Assessing the persistence, mobility and toxicity of emerging organic contaminants in Croatian karst springs used for drinking water supply. Sci Total Environ 903, 166240. https://doi.org/10.1016/j.scitotenv.2023.166240	WoSCC-SCI-Exp	Environmental Sciences (Q1)	IF=8.2/2023
III. Selak, A., Lukač Reberski, J.*, Boljat, I., Terzić, J., 2024. Characterizing occurrence of emerging organic contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs, Croatia. Emerg Contam 10, 100327. https://doi.org/10.1016/j.emcon.2024.100327	WoSCC-ESCI	Environmental Sciences (Q1) Toxicology (N/A)	IF=5.3/2023
IV. Selak, A., Lukač Reberski, J.*, Briški, M., Selak, L., 2024. Hydrochemical characterization of Dinaric karst catchment in relation to emerging organic contaminants. Geol Croat 77(2), 145-158. https://doi.org/10.4154/gc.2024.10	WoSCC-SCI-Exp	Geology (Q3)	IF=1.1/2023

Published paper	**Database**	**Paper category and quartile**	**Impact factor (IF)**
V. Selak, A.*, Lukač Reberski, J., Klobučar, G., Grčić, I., 2022. Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer of Jadro and Žrnovnica springs. Sci Total Environ 825, 153827. https://doi.org/10.1016/j.scitotenv.2022.153827	WoSCC-SCI-Exp	Environmental Sciences (Q1)	IF=9.8/2022
VI. Selak, A.*, Lukač Reberski, J., Klobučar, G., Grčić, I., 2022. Data on occurrence and ecotoxicological risk of emerging contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs. Data Br 42, 108157. https://doi.org/10.1016/j.dib.2022.108157	WOSCC-ESCI	Multidisciplinary Sciences (Q3)	IF=1.2/2022

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1. INTRODUCTION

1.1. Emerging organic contaminants (EOCs)

A substantial quantity of synthetic and natural compounds, including their transformation products and metabolites, is introduced daily into various environmental compartments, due to the intensive development of the world economy and diverse anthropogenic activities. The advancements in analytical techniques have facilitated the global detection of the diverse spectrum of so-called “emerging organic contaminants” (EOCs) (Ferrer & Thurman, 2003; Muter and Bartkevics, 2020), at levels ranging from ng/L to µg/L in both surface water (Loos et al., 2009; Ivešić et al., 2017; Archer et al., 2017; Malnes et al., 2022; Roveri et al., 2022) and groundwater (Loos et al., 2010; Lapworth et al., 2012; Sanchez-Vila et al., 2015; Sui et al., 2015; Koroša et al., 2016; Bunting et al., 2021; Close et al., 2021). The frequently employed term "emerging organic contaminants" encompasses newly synthesized chemicals, as well as substances that have long existed (some for decades) in the environment (Lapworth et al., 2012; Dulio et al., 2018). Most of the emerging substances are not subject to routine inter(national) environmental monitoring, while their behaviour, fate, and (eco)toxicological effects remain poorly understood (Geissen et al., 2015; NORMAN). Divergent definitions exist for EOCs, accompanied by ongoing discussions regarding the types of substances meriting inclusion within this classification. However, EOCs are frequently classified based on their use category rather than their incidence, transport properties, or environmental repercussions (Bunting et al., 2021). The following types of contaminants may be considered to be emerging in the environment (Richardson & Ternes, 2014; Mandarić et al., 2016): pharmaceuticals (human and animal medicines) and hormones; personal care products (fragrances, UV filters, antimicrobials, insect repellents); “life-style compounds” (caffeine, nicotine, artificial sweeteners); drugs of abuse; food additives (for stabilization and preservation); disinfection by-products; perfluorinated substances; industrial chemicals and by-products; flame retardants and surfactants; plasticizers, nanomaterials (organic and inorganic) and microplastics; and plant protection products (e.g. pesticides). This wide spectrum of EOCs is being used daily, worldwide and some of them like medicines, personal care products, hormones or life-style products are a requisite for today’s society. Among the aforementioned categories, the one featuring the largest array of compounds is PPCPs, denoting pharmaceuticals and personal care products (Daughton and Ternes, 1999). Extensive research has been dedicated to PPCPs, surpassing the scrutiny applied to many other anthropogenic substance groups (Lapworth et al., 2019). Pharmaceuticals encompass therapeutic medicines, both prescription and over-the-counter, as well as veterinary medicinal products. Functioning in disease prevention, diagnosis, treatment, and the restoration, correction, or modification of organic functions (Daughton and Ternes, 1999), these substances necessitate bioavailability and biological activity. Fundamentally, they constitute small organic compounds, exhibiting moderate water solubility and lipophilicity (Mandarić et al., 2016). Human and veterinary medications are meticulously formulated to elicit specific physiological responses in organisms (Halling-Sørensen et al., 1998). Hence, the continual release of these EOCs into the environment may result in adverse effects on numerous non-target organisms exposed to diverse mixtures of these substances throughout their entire lifespan (Praskova et al., 2011; Cuthbert et al., 2014; Previšić et al., 2020; Matijević et al., 2023).

The pervasive occurrence of EOCs across environmental matrices (including biological tissues) necessitates a comprehensive multidisciplinary approach to address this both scientific and regulatory challenge, which is imperative for ensuring the sustainable protection of water quality, safeguarding vulnerable aquatic ecosystems, and, subsequently the well-being of human health. The continuous release of EOCs, ineffective elimination during conventional wastewater treatment processes (Senta et al., 2019), coupled with a lack of monitoring activities and established thresholds (Dulio et al., 2018; Lapworth et al., 2019), underscores the necessity for a judicious prioritization methodology (Reemtsma et al., 2016; Rüdel et al., 2020) to mitigate and impede the adverse effects associated with this diverse cohort of chemicals.

1.2. EOCs sources and removal

Although the revelation of their occurrence, sources, and associated detrimental effects has become a recent focus of investigation (Cizmas et al., 2015; Gogoi et al., 2018), the pathway of these substances from their sources to recipients remains a noteworthy matter that should be researched more thoroughly. This holds particular significance for numerous recently introduced compounds, as the pathway from the source to the receptor is often unclear owing to insufficient research data. The emission of EOCs into the environment initiates at the outset of their life cycle through wastewater discharge from manufacturing processes. Subsequently, this emission persists through various stages, encompassing consumption (human and animal excretion, agriculture, aquaculture, etc.) and ultimately culminating in wastewater effluents and waste disposal. Point sources denote contaminants emanating from specific locations, and their introduction into environmental compartments can be concretely delineated. Typically, the spatial extent associated with these sources is more confined (Naidu et al., 2016). Point-source pollution encompasses various origins, including industrial effluents (e.g. manufacturing plants and food processing facilities), hospital effluents, municipal wastewater treatment plants, combined sewage-storm-water overflows, septic tanks, waste disposal sites (including landfills, industrial impoundments, and farm waste lagoons), and manure storages (Lapworth et al., 2012). Contrarily, diffuse pollution emanates from inadequately defined sources, typically manifesting over extensive geographical scales. Non-point diffuse pollution sources involve agricultural runoff, storm-water, and urban runoff. As underscored by Lapworth et al. (2012), these types of sources generally have lower environmental loading compared to point sources, higher potential for natural attenuation, and less direct/obvious links to the "polluter". Consequently, diffuse sources pose a formidable challenge in terms of monitoring and assessing their impact on water resources and regulation. Wastewater treatment plants, which receive wastewater from diverse sources including municipal, hospitals, and industrial sources, are recognized as major global contributors to the presence of EOCs in aquatic environments (Terzić and Ahel, 2006; Bielen et al., 2017; Senta et al., 2019). EOCs were identified in concentrations ranging from ng/L to even mg/L in wastewater effluents, as reported by a Europe-wide survey conducted at 90 European plants (Loos et al., 2013). Wastewater treatment processes, such as widely employed secondary treatment, are not explicitly designed for the removal of EOCs from wastewater (Petrie et al., 2015). Consequently, various studies have observed the incomplete removal of EOCs during conventional wastewater treatment (Gros et al., 2010; Camacho-Muñoz et al., 2012; Köck-Schulmeyer et al., 2013; Senta et al., 2019; Sörengård et al., 2019). This has resulted in the presence of EOCs downstream of wastewater treatment plants (Gago-Ferrero et al., 2017; Česen et al., 2019), with persistent EOCs, such as carbamazepine, being detected at concentrations even higher than those in the influent due to inefficient removal (Gao et al., 2012). Biological removal processes like membrane bioreactor (Ghoshdastidar and Tong, 2013; Monslavo et al., 2014; Lopez-Herguedas et al., 2024), microalgae/fungi (Matamoros et al., 2015; Maryjoseph & Ketheesan, 2020) and activated sludge (Buttiglieri and Knepper, 2008; Elshikh et al., 2022) have demonstrated efficiency in enhancing removal of specific EOCs from wastewater (Ahmed et al., 2017). Furthermore, chemical removal processes, such as ozonation/H_2O_2 (Kim and Tanaka, 2010; Farzaneh et al., 2020), ozonation/UV (Paucar et al., 2019), UV photolysis (Satyro et al., 2017), TiO_2 photocatalysis (Grčić et al., 2023), and photo-Fenton oxidation (Klamerth et al., 2012; Ioannou-Ttofa et al., 2019) have demonstrated effectiveness, at times achieving complete removal of specific types of EOCs such as pesticides, beta-blockers, and pharmaceuticals from wastewater, as indicated by Ahmed et al. (2017).

Wastewater treatment may generate multiple EOCs transformation products, which are subsequently discharged via effluents into receiving water bodies (Li et al., 2016; Rodrigues et al., 2022). Transformation products exhibit notable stability in the environment and can occur at elevated levels compared to the parent compounds, potentially displaying greater toxicity than the original EOC (Funke et al., 2015; Zilberman et al., 2023).

Furthermore, traditional methods employed in drinking water treatment plants have demonstrated inefficacy in eliminating EOCs (Couto et al., 2019). Integration of multiple treatment processes has shown enhanced removal efficiency (Boleda et al., 2011; Huerta-Fontela et al., 2011; Flores et al., 2013; Kim et al., 2020). However, this approach necessitates additional energy and chemical consumption, leading to increased investment and operational costs (Bui et al., 2016). While disinfection is imperative for ensuring microbiologically safe drinking water, it can result in the generation of disinfection by-products (Rubirola et al., 2019), which pose a significant threat to human health due to their toxic properties (Han et al., 2019; Leusch et al., 2019).

1.3. EOCs in groundwater with emphasis on karst

A conspicuous gap exists in the research landscape concerning the occurrence and fate of EOCs in groundwater (Sui et al., 2015), especially in karst aquifers (Lukač Reberski et al., 2022). The predominant focus of research endeavours has been directed toward exploring the occurrence, behaviour, and implications of EOCs in surface water (Kolpin et al., 2002; Loos et al., 2009; Ivešić et al., 2017; Česen et al., 2019; Malnes et al., 2022; Stipaničev et al., 2022) and wastewater (Loos et al., 2013; Bielen et al., 2017; Golovko et al., 2021). In a first global review of EOCs in groundwater, Lapworth et al. (2012) highlighted widespread contamination involving predominantly pharmaceuticals, industrial and life-style compounds, with observed concentrations ranging from 10^2-10^4 ng/L, and degradation products found even more frequently and in larger concentrations than their parent compounds, invoking a serious need for monitoring and effective regulation of EOCs. Loos et al. (2010) presented the outcomes of the first pan-European survey examining EOCs in groundwater across 23 countries. The results indicated that persistent compounds such as diethyltoluamide (DEET), perfluoroalkyl substances (PFAS), atrazine, and 1H-benzotriazole were the most frequently detected substances. In 2021, Bunting et al. conducted a comprehensive review encompassing 39 studies focusing on the occurrence of EOCs in European groundwater. The analysis unveiled carbamazepine and caffeine as the most commonly identified compounds, with pharmaceuticals emerging as the predominant group of contaminants reported across the studies. Large-scale reconnaissance studies and national reviews on EOCs in groundwater have been undertaken in various countries, including Austria (Brueller et al., 2018), Brazil (Marson et al., 2022), China (Dong et al., 2018), France (Lopez et al., 2015), Italy (Meffe and De Bustamante, 2014), Mexico (Vázquez-Tapia et al., 2022), New Zealand (Moreau et al., 2019), Netherlands (van der Aa et al., 2013), Spain (Jurado et al., 2012; Bono-Blay et al., 2012), Slovenia (Koroša and Mali, 2012), Sweden (Banzhaf et al., 2017), the United States (Barnes et al., 2008; Bexfield et al., 2019), and the United Kingdom (Stuart et al., 2012; Manamsa et al., 2016), etc. However, a comprehensive study on the occurrence of EOCs in Croatian groundwater, specifically within karst aquifers is absent.

Karst landscapes encompass approximately 15.2% of the global continental land surface, with an estimated 16.5% of the world's population residing in such areas (Goldscheider et al., 2020). Karst aquifers, known for their capacity to store and transmit substantial volumes of water, serve as a vital source of drinking water for approximately 9.2% of the global population (Stevanović, 2019). The unique and intricate hydrogeological properties of karst aquifers and their subterranean ecosystems render them particularly susceptible to contamination (Ford and Williams, 2007; Goldscheider and Drew, 2007). Investigating these aquifers proves challenging, especially in relation to discerning the presence, transport, and attenuation of trace compounds in such heterogeneous systems. Factors such as highly permeable medium, often barrier-free and direct rapid recharge via sinkholes, and complex flow mechanisms in enlarged fractures and conduits, limit the natural attenuation of contaminants entering karst aquifers (Bakalowicz, 2005; Hartmann et al., 2017). Gaining a more profound understanding of the physio-chemical characteristics, sources, behaviour, and the fate of EOCs in karst aquifers is imperative, particularly in regions where these aquifers constitute the sole source of drinking water. The investigation of EOCs can enhance our understanding of the hydrogeology of the karst systems. Lukač Reberski et al. (2022) conducted a systematic review encompassing 50 distinct studies investigating EOCs in karst formations across 21 countries globally. The findings of this review revealed that pharmaceuticals and pesticides emerged as the most commonly detected types of EOCs. Furthermore, the recorded maximal concentrations surpassed 0.1 mg/L, with half of the detected compounds exceeding 100 ng/L, the established EU drinking water limit for individual pesticides. Although the overall maximum concentrations in karst groundwater were lower compared to other aquifer types, nearly 25% of the detected compounds exhibited concentrations that were comparable to or greater than those identified in other rock formations.

Research focusing on EOCs in karst aquifers offers valuable insights into the contaminant storage, transport mechanisms, and the characteristics of distinct sections of the aquifer (matrix-fractures-channels), which collectively govern the contaminant attenuation capacity of the system. Certain EOCs have proven instrumental as novel environmental tracers or pollution source-specific indicators. Notable examples include pharmaceuticals diclofenac (Einsiedl et al., 2010), carbamazepine (Dvory et al., 2018), ibuprofen and gemfibrozil (Doummar and Aoun, 2018a), artificial sweeteners cyclamate, sucralose and acesulfame (Zirlewagen et al., 2016; Doummar and Aoun, 2018b), life-style compound caffeine (Hillebrand et al., 2012a; Dvory et al., 2018), contrast media iohexol (Doummar and Aoun, 2018a,b), as well as antibacterial and antifungal agent triclosan (Upton et al., 2020). Simultaneous use of conventional tracer uranine with novel tracer caffeine revealed a high attenuation rate, contrary to anticipated behaviour in karst aquifers, as observed by Hillebrand et al. (2012b). Einsiedl et al. (2010) noted the predominant influence of the dilution process on EOCs rather than biodegradation in fractured and vulnerable karst systems. Photodegradation of EOCs in aquifers is not a relevant degradation process (Hillebrand et al., 2012a) due to restricted light access, depth, and turbidity. Dvory et al. (2018) documented an elevated vulnerability of the karst aquifer during fast flow events, where higher concentrations of carbamazepine enter groundwater through the unsaturated zone, leading to a more extensive dispersion of this EOC within the aquifer. Plantak (2024), in her PhD thesis, examined the persistence and dynamics of antibiotic pollution in Croatian coastal aquifer under seawater intrusion, revealing seasonal patterns in antibiotic detection and highlighting aquifer's vulnerability due to agricultural and urban waste. Based on occurrence patterns of specific EOCs like X-ray contrast media, diatrizoic acid, and iopamidol, Zemann et al. (2014) proposed these compounds as potential age markers in groundwater. Similarly, Bexfield et al. (2019) noted EOCs detection frequencies were higher in aquifers where unconfined shallow wells capture "modern-age" groundwater. Moreau et al. (2019) also noted the prevalent occurrence of EOCs in samples of younger groundwater. They underscored the significance of integrating groundwater age stratification with EOCs monitoring, as it provides important insights into the vulnerability of deep groundwater. The persistence of certain EOCs, even after reduced or banned use in recent decades, may also serve as a historical record of past contamination events and indicate the long residence time of the groundwater in the aquifer system (Morasch, 2013; Zemann et al., 2014; Hillebrand et al., 2014; Schiperski et al., 2015).

Karst terrains' catchment delineation poses a significant challenge given the pronounced anisotropy and heterogeneity of these systems. In addition to discerning input sources and pathways, specific EOCs' occurrence, detection frequency, and seasonal variability emerged as valuable indicators for distinguishing and delineating aquifers (Reh et al., 2013). The spatial distribution of EOCs is influenced by their intrinsic physico-chemical characteristics and hydrogeological properties of aquifer and the groundwater residence time. Reh et al. (2015) evaluated the spatial distribution of the industrial compound 1H-benzotriazole to enhance the precision of catchment area delineation. They emphasized the importance of interpreting conventional hydrogeological methods and hydrochemical parameters in conjunction with EOCs to establish a comprehensive conceptual hydrogeological model. Several researchers have noted a positive correlation between the major ion content in karst groundwater, including nitrates and an increasing number of detected EOCs (Zemann et al., 2015), X-ray contrast media (Zemann et al., 2015), and carbamazepine (Doummar et al., 2014), providing insights into the sources of anthropogenic contamination.

Even though karst aquifers are recognized as groundwater systems that are highly productive and one of most susceptible to anthropogenic contamination (Padilla and Vesper, 2018; Lukač Reberski et al., 2022), a notable scarcity of studies on EOCs still exists, particularly for highly fractured systems characteristic for the Dinaric karst region. Given the intricate nature of karst aquifers' structure and flow regimes, significant uncertainty arising from spatiotemporal heterogeneity, and their indispensable role in water supply, there is a compelling need for comprehensive research on EOCs within these systems. Regular monitoring of EOCs is essential to assess temporal and spatial changes while employing in-depth event analysis can illuminate the potential presence of background levels of specific EOCs. Simultaneous utilization of hydrochemical markers, conventional stable water isotope tracers, and novel tracers like EOCs can provide enhanced clarity in discerning the complex relations between factors shaping flow and contaminant transport pathways, as well as hydrochemical patterns within karst aquifers subject to various anthropogenic pressures.

1.4. EOCs (eco)toxicological implication

Aquatic environments are under increasing threat of various anthropogenic pressures, including globally widespread EOCs (Amoatey & Baawain, 2019; Malev et al., 2022). EOCs occurrence at relatively low concentrations hinders efficient microbial degradation, leading to their persistence in the environment (Griebler et al., 2019). This persistence exposes non-target organisms to a complex "cocktail" of various EOCs in surface and groundwater throughout their entire lifetime, causing detrimental impacts at each stage of development. EOCs in complex mixtures may engage in synergistic, additive, or antagonistic interactions (Białk-Bielinska et al., 2013; Spurgeon et al., 2022; Göbölös et al., 2024). Even at trace levels, the amalgamation of these substances exerts toxicity and may induce irreversible alterations across various biological levels, encompassing cellular to ecosystem dimensions. Bielen et al. (2017) documented the adverse effects observed on freshwater algae (*Pseudokirchneriella subcapitata*), invertebrates (*Daphnia magna*), and the occurrence of various abnormalities in zebrafish (*Danio rerio*) embryos subsequent to exposure to antibiotic-contaminated effluents originating from pharmaceutical industries in Croatia, which are discharged into the Sava River. Additionally, the presence of antibiotic-resistant bacteria was noted, underscoring potential implications for human health. Another study conducted on the Sava River, as detailed by Malev et al. (2022), highlighted the detrimental effects of EOCs on non-target aquatic organisms. The investigation revealed a notable reduction in heartbeat rate and observed failures in pigmentation formation as significant outcomes of EOC exposure to zebrafish embryos. The continual release and ubiquitous environmental presence of EOCs, such as antibiotics, might lead to substantial alterations in microbial cultures that influence their purifying activities within karst environments, potentially resulting in the accumulation of other contaminants (Iker et al., 2010). EOCs present significant threats to public health by transforming water bodies into conduits for the propagation of antibiotic resistance genes (Keenum et al., 2022), and the emergence of antibiotic-resistant bacterial strains (Maravić et al., 2015; Bielen et al., 2017; van Hamelsveld et al., 2023).

Despite oligotrophic conditions, groundwater sustains ecosystems characterized by abundant biodiversity (Gibert et al., 2009), offering significant ecological services associated with water purification. These services involve nutrient cycling, natural attenuation of contaminants via biogeochemical processes facilitated by microorganisms, and the inactivation and elimination of other potentially harmful pathogens and viruses (Griebler and Avramov, 2015). Despite providing valuable ecosystem services, the importance of groundwater biota as a conservation priority is often overlooked (Mammola et al., 2019). Data on the ecotoxicological effects of organic pollutants on stygobiontic fauna are notably scarce, making interpretation challenging (Becher et al., 2022). Moreover, understanding the responses of subterranean groundwater-dependent biota to anthropogenic stressors, especially EOCs, remains challenging due to scarcity and limited research on their difficult-to-access habitats (Castaño-Sánchez et al., 2020, 2021). The significant biodiversity within Croatian karst ecosystems has garnered international recognition in biospeleology, with nearly 70% of identified species in Croatia being endemic (Gottstein et al., 2002). Preserving karst groundwater quality necessitates the prevention and mitigation of adverse effects on the well-being of inherently vulnerable subterranean ecosystems.

Given the scarcity of experimental toxicity data for the vast majority of EOCs, a number of studies employed *in silico* tools such as quantitative structure-activity relationship (QSAR) for ecotoxicological prioritization of chemicals of concern based on the assessment of their environmental risk i.e. their persistence, bioaccumulation potential, mobility, and toxicity (Pizzo et al., 2016; Babić et al., 2018; Huang et al., 2021; Malev et al., 2022; Montes et al., 2022; Roveri & Lopes Guimarães 2023).

1.5. Regulation of EOCs

Owing to the potential adverse effects and/or persistence, EOCs warrant inclusion in water-related legislation. As a member state of the European Union, Croatia is obligated to comply with EU directives and regulations. Under the Directive (2008/105/EC) and with the Commission Implementing Decision (CID 2022/1307), an updated Watch list of substances to be EU-wide monitored in surface water (SWWL) has been developed. The Watch list includes substances that may pose a significant risk to or via the aquatic environment. It provides an indicative analytical method and specifies the maximum acceptable method detection or quantification limit in ng/L, serving as a filter to identify new priority substances (Backhaus, 2023). The latest surface water Watch list includes only 26 substances among which are 3 azole pharmaceuticals, 7 azole pesticides, 3 sunscreen agents, 3 fungicides, 1 herbicide, 1 insecticide, 4 antibiotics, 1 antihyperglycemic and its metabolite, and 1 antidepressant and its metabolite. Moreover, the European Commission revised a List of priority substances in surface water in October 2022, by adding environmental quality standards for 25 substances and total pesticides. This list encompasses substances with a well-documented risk to nature and human health.

The Voluntary Groundwater Watch List (GWWL) was proposed in 2019 by the EU CID Working Group Groundwater, encompassing in total 11 substances (Lapworth et al., 2019). Later in 2022, the European Commission (COM(2022) 540) introduced a proposed amendment to Directive 2006/118/EC on the protection of groundwater against pollution and deterioration, outlining groundwater quality standards. The proposed standards are set at 0.1 µg/L for individual pesticides, 0.5 µg/L for total sum of pesticides, 0.0044 µg/L for sum of PFAS, 0.25 µg/L for total sum of pharmaceuticals, 0.25 µg/L for carbamazepine, 0.01 µg/L for sulfamethoxazole, 0.1-5 µg/L for individual non-relevant metabolites, and 0.5–12.5 µg/L for sum of non-relevant metabolites (depending on the available (eco)toxicological knowledge).

Finally, the Watch list of substances and compounds of concern for water intended for human consumption was first established in 2022 with CID (2022/679) and provides guidance values for only two compounds, namely nonylphenol (300 ng/l) and 17-beta-estradiol (1 ng/l). In Croatia, the Regulation on compliance parameters, methods of analysis, and monitoring of water intended for human consumption (OG 64/2023) defines among other maximal allowed concentrations for total pesticides at 0.5 µg/L and total PFAS at 0.5 µg/L.

Despite recent efforts to incorporate certain EOCs into legislation, the vast majority of these compounds remain unregulated and consequently unmonitored in the water resources.

1.6. Study area

Regional scale

The regional study area encompasses the Croatian Dinaric system, which spans across nearly half of the country's land area and is globally renowned as "classical karst". This geologically complex environment features significant surface and subsurface heterogeneity, deep tectonic faults with a typical NW-SE orientation, and dynamic flow patterns. The region primarily consists of thick Triassic, Jurassic, and Cretaceous limestones and dolomites (Vlahović et al., 2005). The high solubility of carbonate rocks and intense historical tectonic activity have led to a general absence of surface watercourses and extensively karstified, well-developed karst aquifers. Groundwater velocities, based on tracer tests, range from 0.01 to 32.1 cm/s, with a median of 2.3 cm/s (Kuhta and Brkić, 2008). Thin or absent protective soil layers and extensive karstification facilitate rapid infiltration of potential contaminants through numerous swallow holes, fractures, and shafts. Despite the oligotrophic nature of the region, the physical heterogeneity of the karst aquifers supports a rich habitat that is home to several endemic species.

Local scale

The local study area focuses on a typical Dinaric karst catchment, encompassing the large Jadro and Žrnovnica springs in southern Croatia. The estimated catchment size ranges from 250 to 500 km^2 (Bonacci, 1987; Jukić & Denić-Jukić, 2009). The Jadro spring, with a mean discharge of 9.4 m^3/s (2011-2022), is crucial for the regional water supply of Split and its wider area. The Žrnovnica spring, with a mean discharge of 1.7 m^3/s (2011-2022), supplies the nearby settlement and local agricultural areas. Both springs are formed at the contact of permeable carbonate rocks and the impermeable coastal flysch belt at the foot of Mosor Mountain. Previous studies indicate overlapping catchments and groundwater exchange between the springs (Jukić & Denić-Jukić, 2008; Bonacci & Roje-Bonacci, 1997; Bonacci & Andrić, 2015; Kadić et al., 2017). This catchment is primarily composed of highly permeable Mesozoic and Eocene carbonate rocks, resulting in the absence of surface watercourses. Perennial inter-catchment groundwater presumably flows from the neighbouring Cetina River catchment and influences the springs, with hydroelectric power plants altering hydrological regimes since 1961. Tracer tests at the Grabov mlin ponor have demonstrated inter-catchment groundwater flows from the adjacent Cetina River catchment (Geotehnika, 1975; Fritz, 1979), thereby expanding the overall catchment area.

1.7. Objectives and hypotheses

The objectives of this PhD thesis were carefully crafted to steer a thorough exploration of the chosen research domain, with a focus on addressing crucial gaps in knowledge specific to emerging organic contaminants in Dinaric karst aquifers. The following specific objectives guided the research, outlined the primary focus areas, and detailed the intended contributions to the field:

- Identify and quantify EOCs within Croatian karst aquifers at regional (18 locations) and local (4 locations) levels.

- Investigate the interplay between hydrogeological and hydrological conditions and occurrence of EOCs within karst catchment of Jadro and Žrnovnica springs.

- Conduct a spatial analysis to identify potential sources of EOCs within karst aquifers at regional and local levels.

- Assess environmental risks and potential impacts associated with identified EOCs on water ecosystems and human health.

To achieve the research objectives this PhD thesis introduces the following set of hypotheses that were meticulously formulated and substantiated through interdisciplinary research:

- The occurrence and concentrations of EOCs are influenced by their physico-chemical properties and the prevailing hydrogeological and hydrological conditions within karst aquifers.

- The vast array of EOCs, originating from diverse anthropogenic activities, infiltrate Dinaric karst groundwater.

- The occurrence of EOCs within karst water resources poses potential detrimental effects on ecosystem and human health.

2. SCIENTIFIC PAPERS

Paper I

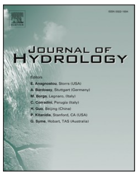
Lukač Reberski, J., Selak, A., Lapworth, D.J., Maurice, L.D., Terzić, J., Civil, W., Stroj, A., 2023. Emerging organic contaminants in springs of the highly karstified Dinaric region. J Hydrol 621, 129583. https://doi.org/10.1016/j.jhydrol.2023.129583

Journal of Hydrology 621 (2023) 129583

Contents lists available at ScienceDirect

Journal of Hydrology

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



Research papers

Emerging organic contaminants in springs of the highly karstified Dinaric region

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ARTICLE INFO

Keywords:
Emerging organic contaminants
Karst aquifers
Dinaric karst
Groundwater
Drinking water resources

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.

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 et 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 (Stevanovic, 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

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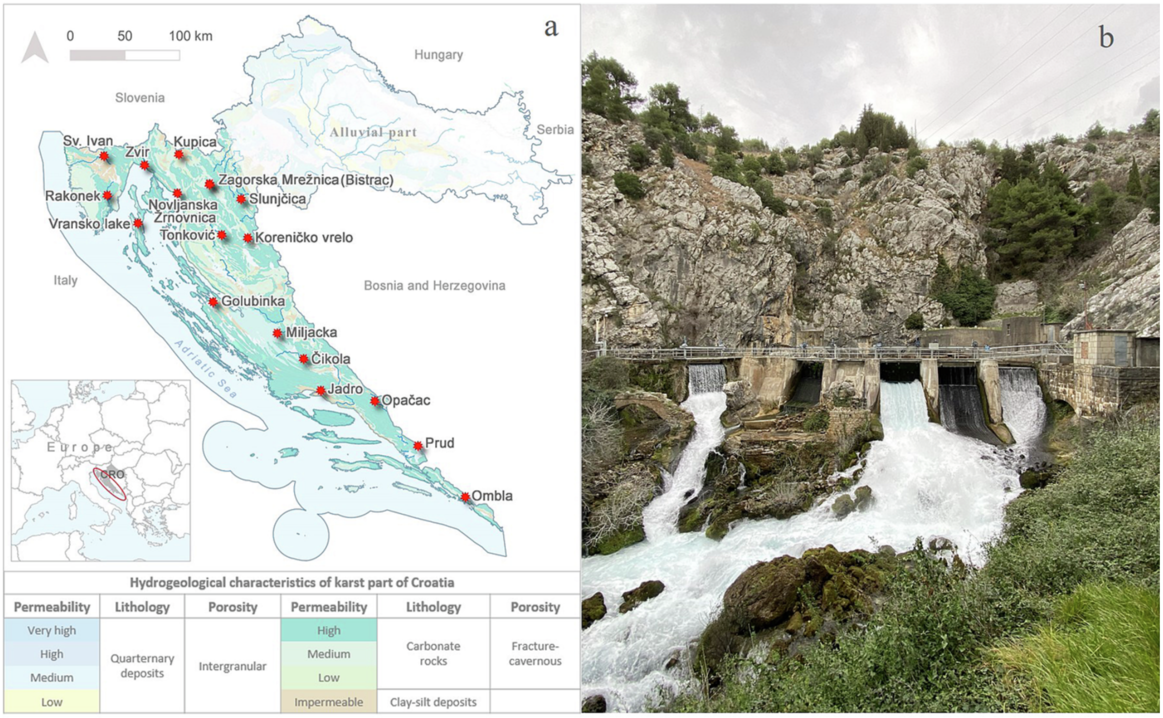
https://doi.org/10.1016/j.jhydrol.2023.129583
Received 28 December 2022; Received in revised form 19 April 2023; Accepted 21 April 2023



Hydrogeological characteristics of karst part of Croatia

Permeability	Lithology	Porosity	Permeability	Lithology	Porosity
Very high	Quaternary deposits	Intergranular	High	Carbonate rocks	Fracture-cavernous
High			Medium		
Medium			Low		
Low			Impermeable	Clay-silt deposits	

Fig. 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).

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; Fig. 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).

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

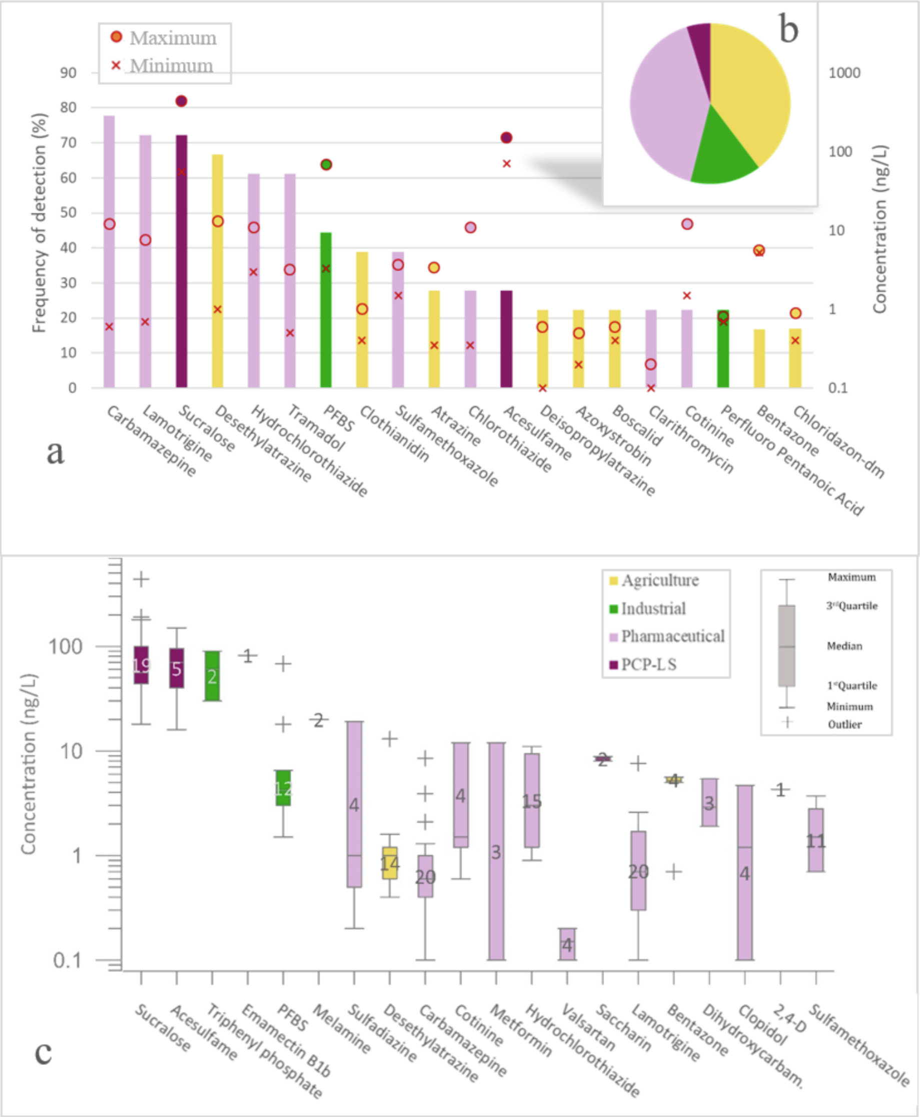


Fig. 2. EOCs in Croatian karst a) The 20 most frequently detected compounds and their maximum and median concentrations. Bars show frequency (%) of detection (primary y axis), circles and crosses show concentrations (secondary y axis); b) pie chart of the overall % of detections in each of the EOC groups; 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.

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 Musgrove, 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) (Fig. 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

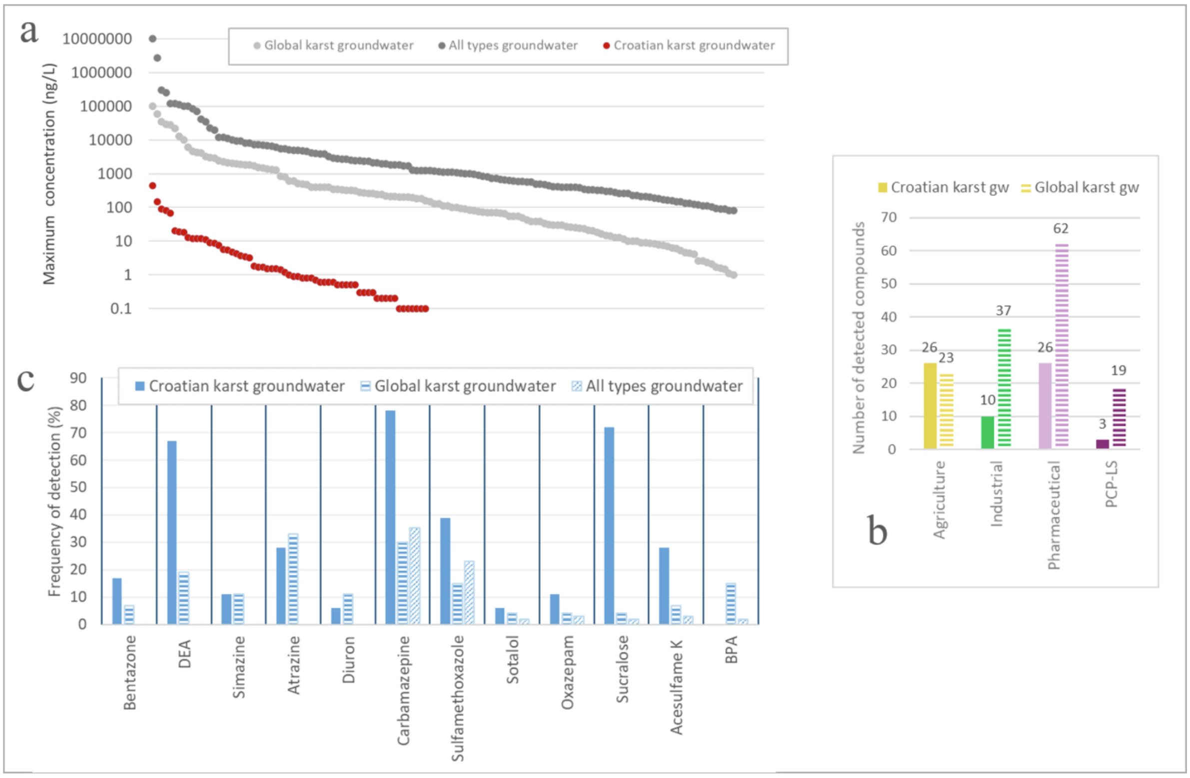


Fig. 3. Comparison of EOCs in Croatian karst with other studies: (a) maximum concentrations for different detected compounds ranked from highest to lowest values; (b) the number of detected compounds by different compounds groups; (c) the detection frequency for compounds that were detected in both Croatian 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).

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 (Fig. 2a and b), and the highest concentrations were from the industrial and personal

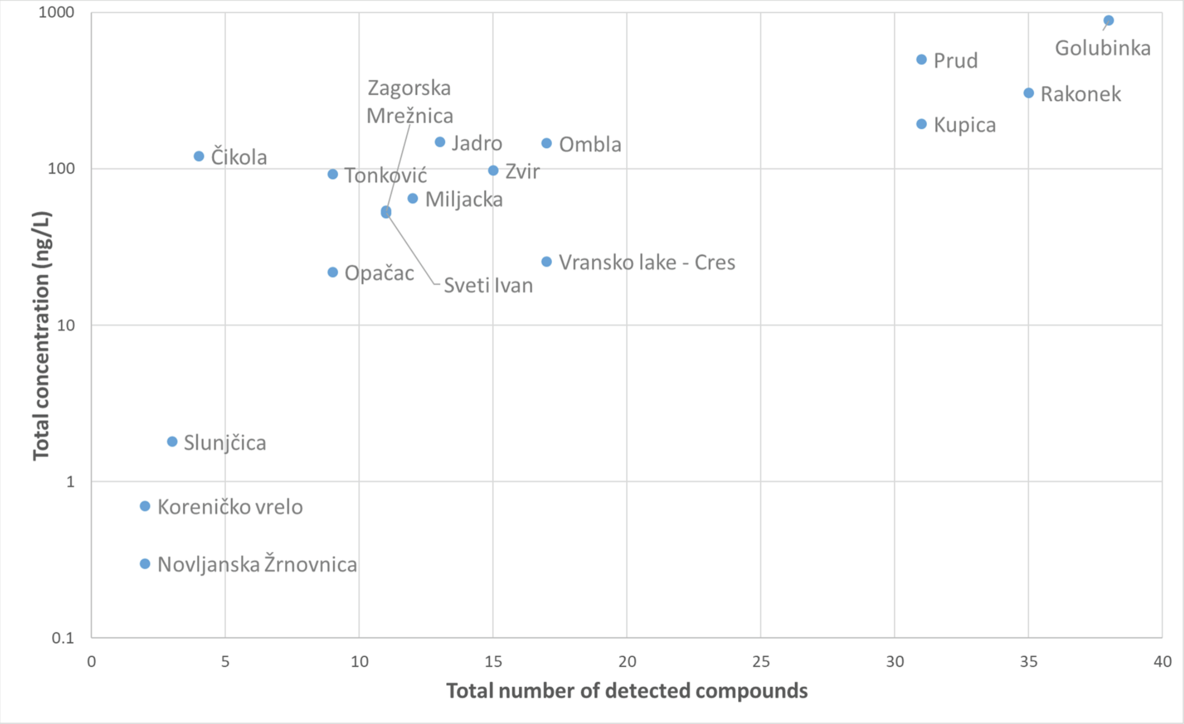


Fig. 4. The total number of detections versus total concentration for individual sites.

care product and lifestyle compound (PCP-LS) groups (Fig. 2a and c).

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 Fig. 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 Fig. 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 (Fig. 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 (Fig. 3c). The detection limits of the analytical methods vary between studies and this could influence the comparisons.

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 (Fig. 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.

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 (Fig. 5a and b).

Personal care products and lifestyle compounds generally had the highest concentrations (14 out of 18 sites) (Fig. 5a). Pharmaceuticals are the most commonly detected compounds at most sites (11 out of 18 sites), followed by agricultural compounds (Fig. 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 (Fig. 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 Fig. 5c. Agricultural land covers between 7 and 41% of the catchment areas (mean 20%), indicating considerable agricultural activities in the catchments.

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 (Fig. 6). Although hydrological extremes were not captured, the discharge did differ between the two campaigns. In the March sampling campaign, discharges ranged from

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

Fig. 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 Fig. 1a.

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).

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 (Fig. 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 (Fig. 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 (Fig. 7a), and the difference in total concentrations at 14 sites was less than 50 ng/L.

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; Fig. 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.

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).

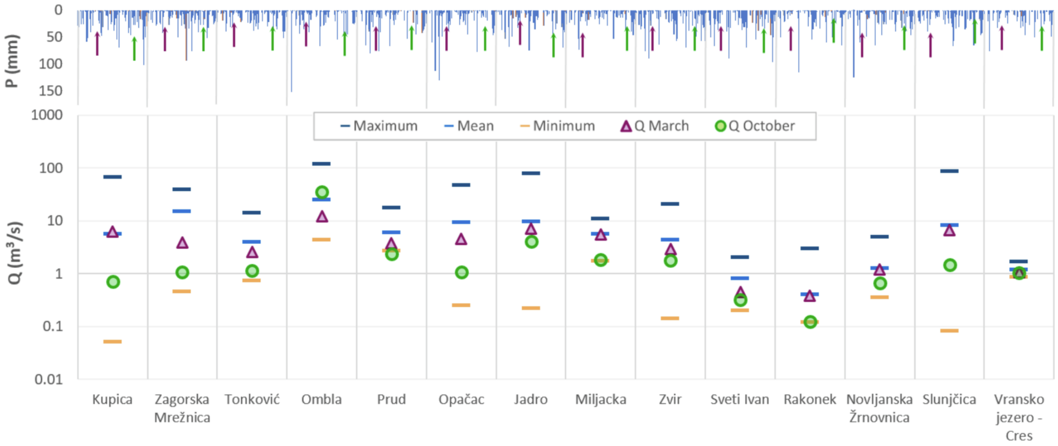


Fig. 6. Long-term spring discharge (Q) statistics and discharge during the two sampling campaigns in March and October 2019. The upper graph shows daily precipitation (P) during the calendar year 2019 for each sampling location; red and green arrows show the timing of the sampling campaigns. Sampling 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

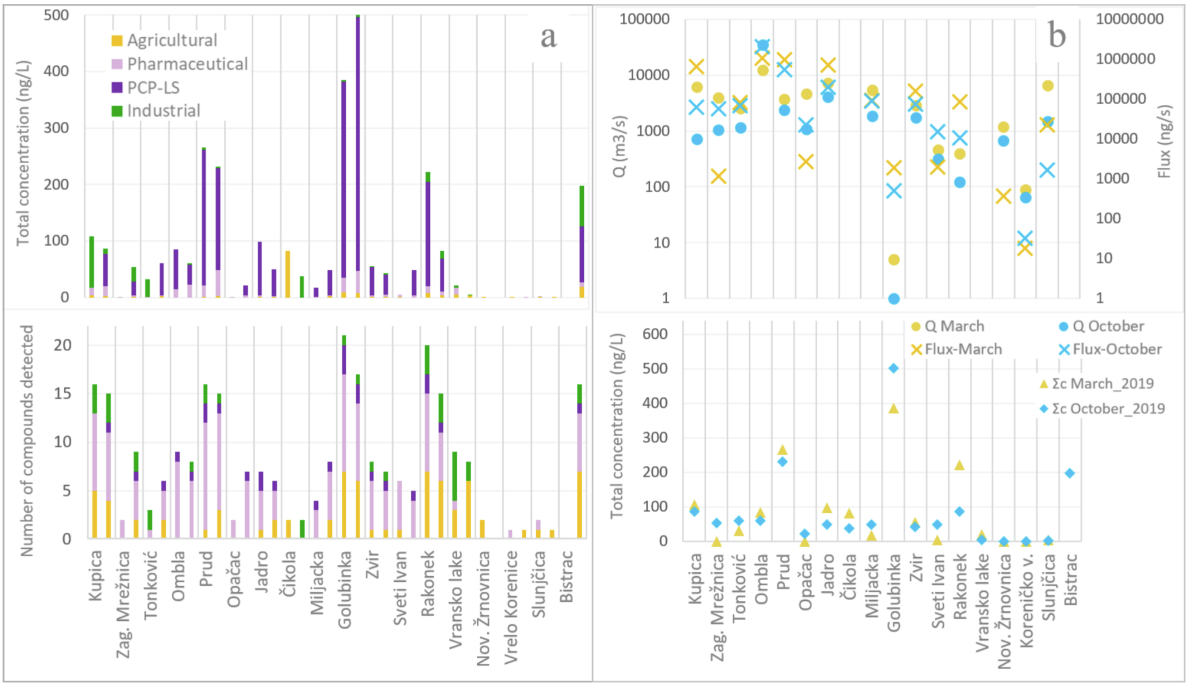


Fig. 7. Comparison between the two sampling campaigns (March and October) at all sampling locations: a) total concentrations and number of compounds detected in different EOC groups (Agricultural, Pharmaceutical, PCP-LS, and Industrial); b) total concentrations, discharges and mass fluxes of all detected 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.

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 (Fig. 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 (Fig. 7b).

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.

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 (Fig. 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 (Fig. 8a, b). Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 8e). They showed weak relationships, and the correlations were not statistically significant.

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 (Fig. 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 et al., 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; Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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

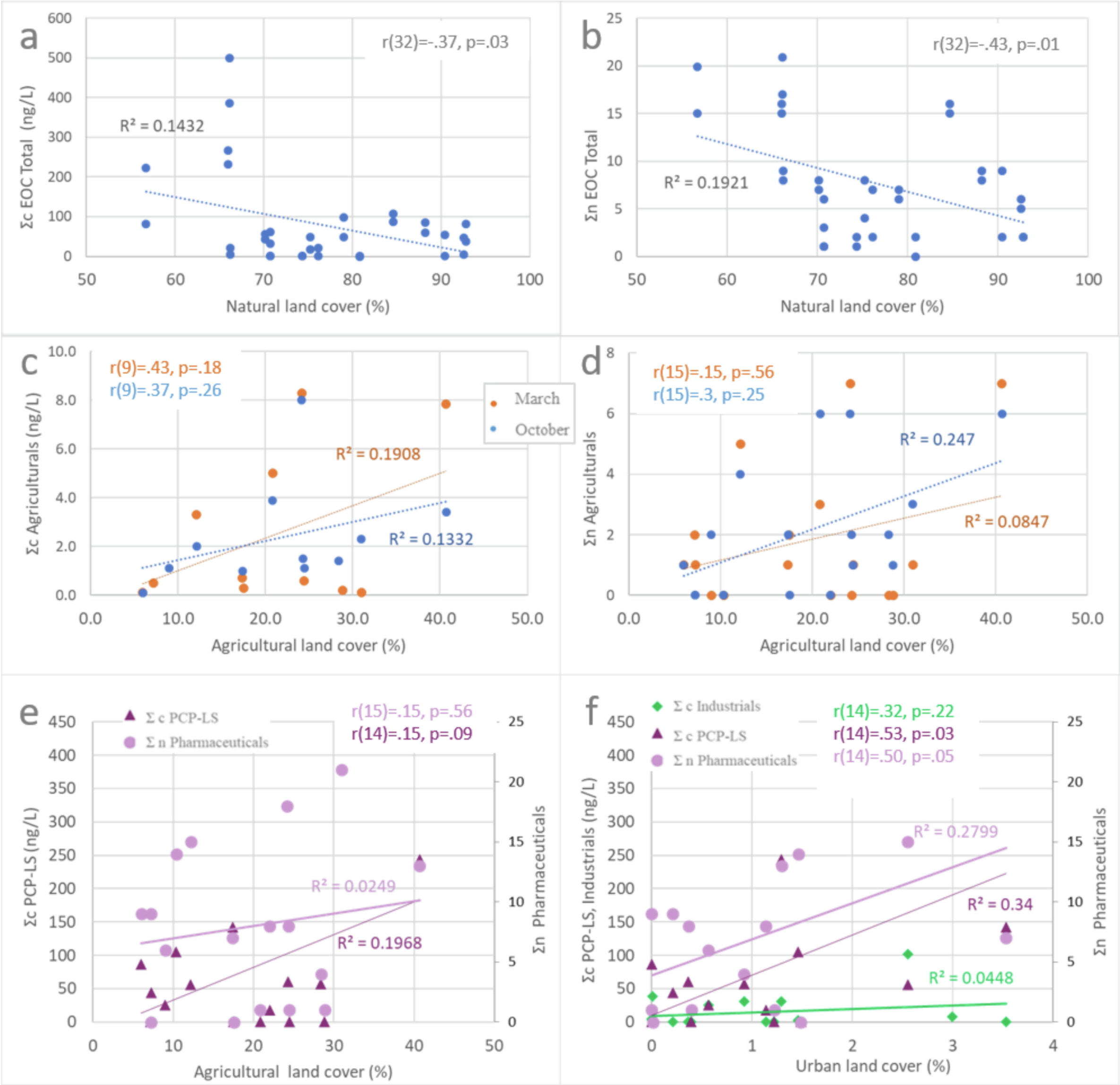


Fig. 8. Correlations between EOC concentrations and land cover: a) correlation between natural land cover (%) and total EOC concentration (Σc EOC total), b) correlation between natural land cover (%) and total number of EOCs, c) correlation between agricultural land cover (%) and concentration of agricultural 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.

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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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.

CRediT authorship contribution statement

Jasmina Lukač Reberski: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Ana Selak:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **Dan J. Lapworth:** Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Louise D. Maurice:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Josip Terzić:** Investigation, Writing – original draft, Writing – review & editing. **Wayne Civil:** Investigation, Writing – review & editing. **Andrej Stroj:** Investigation, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

This paper is partly the result of collaboration, training and education conducted through GeoTwinn project that has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement no. 809943. BGS authors publish with the permission of the BGS-UKRI director. The field investigations and EOCs analysis are funded and supported by the Croatian Geological Survey, Department of Hydrogeology and Engineering Geology. The authors express their gratitude to the Croatian Meteorological and Hydrological Service for the provided data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhydrol.2023.129583.

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Paper II

Selak, A., Lukač Reberski, J., Klobučar, G., 2023. Assessing the persistence, mobility and toxicity of emerging organic contaminants in Croatian karst springs used for drinking water supply. Sci Total Environ 903, 166240. https://doi.org/10.1016/j.scitotenv.2023.166240

Science of the Total Environment 903 (2023) 166240

Contents lists available at ScienceDirect

Science of the Total Environment

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

Assessing the persistence, mobility and toxicity of emerging organic contaminants in Croatian karst springs used for drinking water supply

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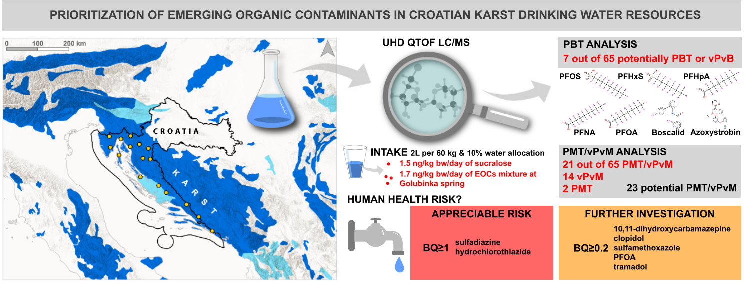
HIGHLIGHTS

- Prioritizing emerging organic contaminants (EOCs) in Croatian karst drinking water.
- 7 of 65 EOCs classified as *PBT/vPvB (persistent, bioaccumulative, toxic; v-very)*.
- 2 of 65 EOCs were evaluated as *not PMT/vPvM (persistent, mobile, toxic; v-very)*.
- 2 out of 65 EOCs pose a potential risk to human health at lifelong exposure.
- PFAS exceed proposed threshold of 4.4 ng/L at 2 springs in March & at 6 in October.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jurgen Mahlknecht

Keywords:
Persistence
Mobility
Toxicity
Emerging organic contaminants
Karst springs
Drinking water

ABSTRACT

Emerging organic contaminants (EOCs) are a vast group of often (very)persistent, (very)mobile and toxic (*PMT/vPvM*) substances that are continuously released worldwide, posing environmental and human health risks. Research on occurrence and behavior of EOCs in karst is in its infancy, thus policy measures and legislative control of these compounds in groundwater are still lacking. The Dinaric karst aquifers are an essential source of drinking water for almost half of Croatia's territory. Intense karstification, complex heterogeneous characteristics, and high fracture-cavernous porosity result in rapid, far-reaching groundwater flow and large karst springs, but also high intrinsic vulnerability due to low contaminant attenuation. To prioritize future monitoring and establish appropriate thresholds for EOCs detected in Croatian karst drinking water resources, in silico tools based on quantitative structure-activity relationships were used in *PBT* (persistence, bioaccumulation, and toxicity) and *PMT/vPvM* analyzes, while toxicological assessment helped identify potential threats to human health. In 33 samples collected during two sampling campaigns in 2019 at 16 karst springs and one lake used for water supply, we detected 65 compounds (EOCs and some legacy chemicals), of which 7 were classified as potentially *PBT* or *vPvB* compounds (PFOS, PFHxS, PFHpA, PFOA, PFNA, boscalid, and azoxystrobin), while only 2 compounds were assessed as *not PMT/vPvM*. This finding underlines that most of detected EOCs potentially endanger karst (ground)water ecosystems and important drinking water sources in Croatia. Comparison of maximum concentrations with existing or derived drinking water guideline values revealed how 2 of 65 detected compounds represent a potential risk to human health at lifelong exposure (sulfadiazine and hydrochlorothiazide), while 5 chemicals warrant additional human health impacts studies and groundwater monitoring.

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https://doi.org/10.1016/j.scitotenv.2023.166240
Received 26 April 2023; Received in revised form 8 August 2023; Accepted 9 August 2023

Although most compounds do not individually pose a significant risk to human health at current environmental levels, their potential synergistic and long-term effects remain unknown.

1. Introduction

A variety of (very)persistent, (very)mobile and toxic (*PMT/vPvM*) organic contaminants ubiquitously occurs in water environments, including drinking water resources, in trace levels (Loos et al., 2009, 2010) consequently posing a serious risk to vulnerable water-dependent ecosystems (Cleuvers, 2003; Fent et al., 2006) and human health (Schriks et al., 2010). Continuous release, inefficient removal during conventional wastewater treatment (Senta et al., 2019), non-existent monitoring activities and thresholds (Dulio et al., 2018; Lapworth et al., 2019) invoke the need for an appropriate prioritization approach (Reemtsma et al., 2016; Rüdel et al., 2020) to impede detrimental effects of this heterogeneous group of chemicals (e.g., pharmaceuticals and personal care products, lifestyle, industrial and agricultural compounds, *per-* and polyfluoroalkyl substances). Neumann and Schliebner (2019) were the first to introduce criteria for identifying *PMT/vPvM* substances under EU REACH regulation, followed by Arp and Hale (2019) who contributed with improvement of proposed assessment approach. Recent prioritization studies conducted by Schulze et al. (2019), Neuwald et al. (2022), and Montes et al. (2022) have demonstrated the widespread occurrence of persistent and mobile substances in water resources across Europe. There is a notable dearth of studies investigating *PMT/vPvM* properties, occurrence, behavior, and potential human health impacts of emerging organic contaminants (EOCs) in groundwater, especially in karst aquifers (Lukač Reberski et al., 2022; Selak et al., 2022). The preservation of chemically safe karst aquifers should be of paramount importance for protecting human health, particularly in regions such as Dinarides, where they serve as vital sources of drinking water (Hartmann et al., 2014; Stevanović, 2019). Substantial heterogeneity in hydrogeological characteristics, absence of protective overlying layers, and direct infiltration via ponors and enlarged fractures make karst aquifers (Ford and Williams, 2007; Goldscheider and Drew, 2007) and their often endemic subterranean species highly susceptible to surface anthropogenic pressures (Di Lorenzo et al., 2019).

Despite providing important ecosystem services such as water storage, purification and active biodegradation of anthropogenic contaminants (Griebler and Avramov, 2015), groundwater biota is still poorly recognized as a priority for conservation (Mammola et al., 2019). Responses of subterranean groundwater-dependent biota to anthropogenic stressors (especially EOCs) remain poorly understood due to scarcity and limited research on their difficult-to-access habitats (Castaño-Sánchez et al., 2020, 2021). The great biodiversity of Croatian karst ecosystems is biospeleologically recognized at global level, with almost 70 % of species detected in Croatia being endemic (Gottstein et al., 2002). To maintain good groundwater quality, it is critical to prevent and mitigate negative impacts on the health of already intrinsically vulnerable subterranean ecosystems.

In 2019, Lukač Reberski et al. (2023) conducted a comprehensive monitoring study consisting of two sampling campaigns (in March and October) in Croatian karst groundwater resources used for drinking water supply. Of 740 compounds included in analytical suite (comprising both EOCs and some legacy compounds, such as widely regulated pesticide atrazine), 65 substances were detected, with carbamazepine (CAS 298-46-4) being the most frequently detected compound, lifestyle compounds having the highest concentrations, and pharmaceuticals and agricultural products being the most often detected contaminant groups. Antihypertensives, antiepileptics, and antibiotics were the most frequently detected pharmaceuticals. Compared to karst in general and other groundwater resources, Croatian karst springs exhibited much lower EOCs concentrations, presumably due to low anthropogenic loading and high dilution from exceptionally high discharges (Lukač Reberski et al., 2023). There are currently no regulatory standards for EOCs present in karst groundwater because their *PMT/vPvM* properties, potential ecotoxicological effects, synergistic and long-term exposure to complex mixtures, and adverse human health effects associated with direct water uptake are still unknown.

Therefore, here we investigate the persistence, mobility, toxicity and bioaccumulation potential (*B*) of EOCs detected in major Croatian karst springs in 2019 and unravel the potential health risk for consumers via drinking water. Selak et al. (2022) conducted *PBT* and *PMT/vPvM* analyses within the Dinaric karst catchment, specifically focusing on Jadro and Žrnovnica springs (Croatia). Here presented research extends beyond the scope of a case study. It offers valuable insights into human exposure to EOCs and constitutes the first comprehensive prioritization of EOCs on a national scale within the Croatian karst region. Being also the first such endeavor in Dinaric karst, our study seeks to fill the underscored knowledge gap and contribute to the wider understanding of the potential environmental and health implications associated with *PMT/vPvM* compounds in karst groundwater. It is aimed at decision-makers in field of water chemistry and environmental policy makers. Our findings primarily serve as an early warning for setting necessary thresholds and including prioritized EOCs in future monitoring activities, as their emission is expected to increase in the future, based on past and current trends observed in other regions (Pronk et al., 2020).

2. Materials and methods

2.1. Sampling and analysis of EOCs at selected karst springs used for drinking water supply

The research area encompasses the Croatian Dinaric system, which spans across nearly half of the country's land. The Dinaric region, renowned worldwide as "classical karst", represents a geologically complex environment characterized by deep tectonic faults, NW-SE strike, considerable heterogeneity of surface/subsurface forms, and flow dynamics. Very thick limestones and dolomites of Triassic, Jurassic, and Cretaceous age predominate (Vlahović et al., 2005). The study area exhibits characteristics of low population density, limited industrial activity, and a prevailing agricultural land use pattern. However, thin or absent protective soil cover, coupled with extensive karstification, facilitates the rapid infiltration of potential contaminants carried by recharging water through numerous swallowholes, enlarged fractures, and shafts.

Two sampling campaigns were conducted at 17 selected sites (16 karst springs and 1 lake partly fed by springs and lake *vruljas* of the surrounding aquifer), one in spring (19–28 March 2019) and the other in autumn (16–21 October 2019) (Lukač Reberski et al., 2023) (Fig. 1). All samples were raw water collected before any treatment. Bistrac spring was sampled only in October 2019. Further detailed information on EOC sampling procedure can be found in Lukač Reberski et al. (2023). Analysis of 740 compounds (both emerging contaminants and some legacy compounds) was performed at National Laboratory Services UK using an Agilent 6540 Ultra-High-Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) liquid chromatograph coupled to a mass spectrometer (LC/MS). Solid phase extraction was conducted, using Waters Oasis HLB SPE cartridges with an automated extraction system. The water sample of 500 mL (flow rate 10 mL/min) was loaded onto the cartridge. More detail information on laboratory analysis can be found in Table S1. An isotopically labelled internal standard Carbutamide-d9 was added to each of the pre-conditioned SPE cartridges to assess instrument performance, while to ensure quality control, field duplicate and blank samples were analyzed. Limits of detection (LOD) for each analyzed compound and the corresponding concentrations observed at each location can be accessed in the Supplementary materials provided by Lukač Reberski et al. (2023).

We examined the chemical diversity of EOCs dataset using Checkmol profiler (Haider, 2010) applied within OECD QSAR Toolbox (v4.5; Dimitrov et al., 2016), which analyzes input molecules and categorizes them per presence of organic functional groups.

2.2. *PBT analysis*

The assessment of EOCs' *PBT/vPvB* (*vPvB* stands for very persistent and very bioaccumulative) properties is based on conceptual scheme for prioritization of chemicals provided by Pizzo et al. (2016a, 2016b) under REACH (EC 1907/2006) and EU(143/2011) regulations. A specific *PBT* score is assigned to each EOC, appertaining to its persistence (*P*), bioaccumulation (*B*), and toxicity (*T*), which were evaluated using the in silico Prometheus tool (Pizzo et al., 2016b). SMILES notations were searched in the PubChem open chemistry database (2023) and used as input data. To compare results with other software, Pizzo et al. (2016b) used a threshold of 0.5 for the overall *PBT* score to distinguish between *non-PBT* (not persistent, bioaccumulative, and toxic; score $<$ 0.5) and potentially *PBT* or *vPvB* compounds (score $\geq$ 0.5). A positive correlation exists between the obtained *PBT* score and corresponding probability, whereby higher scores indicate an increased probability.

We used SARPy software (Ferrari et al., 2013) to identify substructures, i.e. structural alerts related to the *PBT* property. *SMILES* were used as input data, whereas *PBT* and *non-PBT* labels were utilized as activity attributes (active and inactive, respectively). A default option for structural alert was applied (atom number 2 to 18; minimum occurrence 3) and precision was set to *minimize unpredicted rate*.

Lipinski Rule Oasis profiler (Lipinski et al., 2001) was applied in OECD QSAR Toolbox to identify chemicals with poor oral absorption, i.e. bioaccumulation potential, based on computational alerts for molecular weight, hydrogen bonding capacity, and log K_{OW}.

2.3. *PMT/vPvM analysis*

The persistence criterion is evaluated based on high-quality experimental half-lives determined in water (persistent $>$40 days; very persistent $>$60 days) or sediment (persistent $>$120 days; very persistent $>$180 days). In the absence of experimental data, results of inherent/readily biodegradability tests may indicate potentially persistent/very persistent EOCs. The conclusion is based on review of above cut-off criteria as well as predictions from the OECD QSAR Toolbox, Prometheus software, biodegradation BIOWIN models (model 1, 3, 4, and 5, v4.10) in EPI Suite™ (US EPA, 2023a), and half-lives from the CompTox Chemicals Dashboard (Williams et al., 2017; US EPA, 2023b).

Compounds' mobility was assessed using the lowest determined log K_{OC} values, which were either obtained from literature sources or the PubChem database, or predicted with EPI Suite™ KOCWIN v2.00 model in the absence of experimental data. EOCs with log Koc values $<$4.0 or $<$3.0 are categorized as *mobile* (*M*) or *very mobile* (*vM*), respectively.

Input data for the toxicity assessment were searched for hazard categories in the Classification and Labelling Inventory (ECHA, 2023a) and for NOEC values (no observed effect concentration) in the EnviroTox database (HESI, 2023). Cramer class assessment (Cramer et al., 1978) was performed using Toxtree v3.1.0.1851 software (Ideaconsult Ltd., 2015). Substances with Cramer class III (substances with chemical structures permitting no strong initial impression of safety and may suggest significant toxicity) were evaluated as potentially toxic (*potential T*). In addition, we used the software VEGA QSAR (Benfenati et al., 2013) to predict toxicity of EOCs. Information is given on applicability domain index (ADI) of models used in VEGA QSAR, showing reliability of prediction. Substances having mutagenic and carcinogenic properties and/or causing reproductive toxicity, specific organ toxicity, may cause harm to brest-fed children, as reported in Classification and labeling inventory (ECHA), and/or have NOEC$<$0.01 mg/L are evaluated as toxic (*T*) (Arp and Hale, 2019).

Depending on which criteria are met, six categories can be defined within the *PMT/vPvM* analysis (Arp and Hale, 2019):

- *vPvM & PMT* (there is sufficient evidence that EOC meets the *vP*, *vM* and *T* criteria);

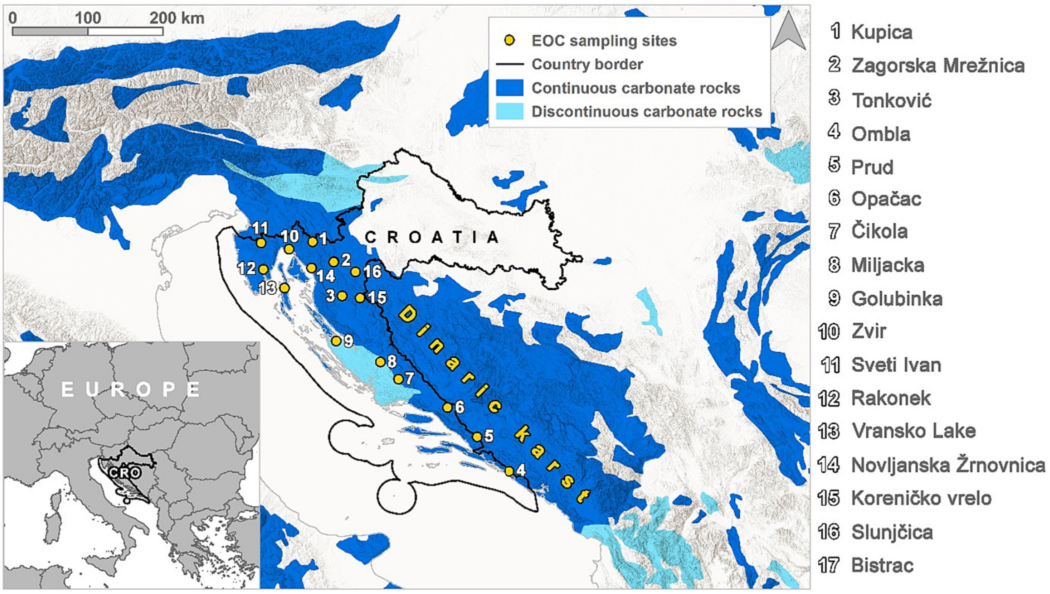

Fig. 1. Selected sampling sites in the Croatian Dinaric karst. Note: Bistrac (17) and Zagorska Mrežnica (2) springs have the same location due to map scale.

- *vPvM* (there is sufficient weight of evidence that EOC meets both the *vP* and *vM* criteria, but not the *T* criterion; this category is also assigned to substances suspected of being potential *P/vP++* if detected in raw or drinking water);
- *PMT* (there is sufficient weight of evidence on EOC meeting *P*, *M*, and *T* criteria);
- *PM* (there is sufficient weight of evidence on EOC meeting both *P* and *M* criteria, but does not meet *T* criterion nor *vPvM* criteria);
- potential *PMT/vPvM* (only screening or low-quality data is available for *P*, *M*, or both, and either a conclusion "*potential P/vP*" and/or "*potential M/vM*" is drawn);
- and *not PMT/vPvM* (criteria "*not P*" or "*not M*" were met with sufficient weight of evidence).

2.4. Evaluation of the potential risk to human health

For the initial assessment of toxicological risk posed by EOCs detected in karstic drinking water resources, we used Cramer's toxic hazard classification. Munro et al. (1996) assigned a Threshold of toxicological concern (TTC) of 1800, 540, and 90 μg/day to Cramer classes I, II, and III respectively (for the 5th percentile NOEL, a 60 kg person and a safety factor of 100). Later, a TTC of 0.15 μg/day (86–97 % probability of cancer risk of $<1 \times 10^{-6}$) was established for compounds with a structural alert or experimental evidence of genotoxicity, whereas a TTC of 1.5 μg/day is used for non-genotoxic compounds (Kroes et al., 2004). Compounds evaluated as genotoxic carcinogens are considered "non-threshold chemicals" that can cause adverse health effects at any dose. These TTC values are considered appropriate for treated drinking water and are consistent with the current limit for pesticides of 0.1 μg/L, as drinking water target values of 0.01 μg/L and 0.1 μg/L can be derived for non-threshold chemicals and other non-genotoxic chemicals, respectively (Mons et al., 2013). The List of Classifications of the International Agency for Research on Cancer (IARC, 2023) was consulted for chemicals that pose a carcinogenic hazard to humans. In addition, carcinogenicity alerts (genotoxic and non-genotoxic) of the ISS profiler in QSAR were used to point out structural alerts for carcinogenic activity of EOCs that were not classified by IARC. Genotoxicity data was searched in literature and also predicted using model In vivo Micronucleus activity (IRFMN) 1.0.1 in VEGA QSAR.

Daily drinking water intake per body weight of 60 kg (DWI_{bw}) was determined using an ingestion volume of 2 L and a drinking water allocation of 10 % (WHO, 2022; Mons et al., 2013). Following the "Concentration addition - CA" concept, indirect exposure to each water sample was determined as the sum of DWI_{bw} of individual detected EOCs.

To further assess the risk of exposure to human health, maximum EOCs concentrations were compared to statutory (*GLV*) or provisional drinking water guideline values (*pGLV*). The *GLVs* were searched in literature, while in their absence *pGLVs* were derived based on toxicological data from previous studies - acceptable daily intake (*ADI*), tolerable daily intake (*TDI*), or a reference dose (*RfD*) (Eq. (1)):

$$pGLV = \frac{(TDI\ or\ ADI\ or\ RfD \times 60\ kg\ bw \times 10\%drinking\ water\ allocation)}{2L} \tag{1}$$

For substances with no reported *ADI*, *TDI*, or *RfD*, the no observed adverse effect level (*NOAEL*) from animal studies was used to calculate provisional *ADI* (*pADI*) as follows (Eq. (2)):

$$pADI = \frac{NOAEL}{(UF_1 \times UF_2 \times UF_3 \times UF_4 \times UF_5)} \tag{2}$$

Appropriate uncertainty/safety (UF) factors were extrapolated and applied to the *NOAEL* (US EPA, 2002; Schwab et al., 2005; Bruce et al., 2010). For pharmaceuticals missing the *ADI* and *NOAEL* values, the lowest daily therapeutic dose (*LTD*) for an adult was looked up in the RxList and previous research studies. Provisional ADI values were derived from therapeutic doses as follows (Prosser and Sibley, 2015; Sengar and Vijayanandan, 2022) (Eq. (3)):

$$pADI = \frac{LTD}{(BWxSF)} \tag{3}$$

where *LTD* is the lowest concentration that produces a desired therapeutic effect in target population, *BW* is body weight, and *SF* is a safety factor of 1000. Comparison between each reported maximum EOC concentration and the existing *GLV* or derived *pGLV* is expressed as the Benchmark Quotient (BQ) value as follows (Schriks et al., 2010) (Eq. (4)):

$$BQ = \frac{max.EOC\ concentration}{pGLV}\ or\ BQ = \frac{max.EOC\ concentration}{GLV} \tag{4}$$

According to Schriks et al. (2010), lifetime consumption of water containing compounds with BQ ≥ 1, i.e. concentrations above statutory or provisional guideline levels, may pose a potential human health concern. Additional monitoring and assessment measures are required for EOCs present in drinking water with BQ $\geq$ 0.1. Recognizing that treatment of drinking water improves its safety, Schriks et al. (2010) proposed a threshold BQ $\leq$ 0.2 for compounds in raw surface and groundwater, as the value denoting absence of appreciable human health concerns.

3. Results and discussion

3.1. Detected dataset description and relevant regulations

Growing concern about the threat to human health and non-target species from the ubiquitous presence of EOCs in the water environment is recognized in some regulatory documents. In 2022, the European Commission (COM/2022/540) proposed an amendment to Directives 2000/60/EC, 2006/118/EC and 2008/105/EC listing the following groundwater quality standards: individual pesticides 100 ng/L, total pesticides 500 ng/L; the sum of 24 PFAS 4.4 ng/L; carbamazepine 250 ng/L; sulfamethoxazole 10 ng/L; total pharmaceuticals 250 ng/L, etc. Only at Golubinka spring, the sum of compounds (≈502 ng/L) was above the thresholds established for both the sum of pesticides and the sum of pharmaceuticals. In March 2019, PFAS sum in two springs (Prud at ≈5 ng/L and Rakonek at ≈18 ng/L) was above the threshold proposed for groundwater. Total PFAS sum recorded at 6 springs in October was higher than the threshold. Furthermore, out of nine pharmaceuticals on proposed Voluntary Groundwater Watch List (GWWL) (Lapworth et al., 2019), four detected in our study were clopidol (CAS 2971-90-6), sulfadiazine (CAS 68-35-9), sotalol (CAS 3930-20-9) and clarithromycin (CAS 81103-11-9). None of the chemicals on first Watch list of substances and compounds of concern for water intended for human consumption (CIS 2022/679) were detected in our study. Under the Directive (2008/105/EC) and with the Commission Implementing Decision (CIS 2022/1307), an updated Watch list of substances to be monitored in surface water (SWWL) has been developed. Fungicide azoxystrobin (CAS 131860-33-8) listed in the SWWL was found in Vransko Lake at 0.2 ng/L during both campaigns. Moreover, PFAS and pesticides detected in Vransko Lake do not exceed the thresholds set with the List of priority substances in surface waters. Similarly, herbicide simazine (CAS 122-34-9) at 0.2 ng/L recorded in Vransko Lake and 0.3 ng/L in Bistrac spring, is well below the environmental quality standard (1000 ng/L) set for river basin-specific pollutants.

Of 65 detected compounds, 22 (33.9 %) are registered under REACH (No 1907/2006), including 9 pharmaceuticals, 7 agricultural products, 4 industrial compounds, and 2 lifestyle products (Table S1). There were 5 detections of REACH-registered compounds with concentration of $\geq$100 ng/L, while only 1 detection of non-REACH registered compound exceeded this value. A total of 7 EOCs, all industrial compounds, are on the Candidate List of substances of very high concern (SVHC) (ECHA, 2023b) (Table S1) because they meet the criteria for classification as carcinogenic, mutagenic, or toxic to reproduction (CMR) or are *PBT/vPvB* compounds per REACH, or substances causing an equivalent concern as CMR or *PBT/vPvB* substances. Perfluorooctane sulfonate (PFOS; CAS 1763-23-1), perfluorohexane sulfonate (PFHxS; CAS 355-46-4), and perfluorooctanoic acid (PFOA; CAS 335-67-1) are on the Stockholm Convention list of Persistent Organic Pollutants (POPs) (SC-4/17; SC-10/13; and SC-9/12). Metformin (CAS 657-24-9), an anti-hyperglycemic medicine detected in Kupica and Ombla springs (12 ng/L) is banned in cosmetic products in the EU (Regulation No. 1223/2009). Furthermore, 10 unauthorized and 10 currently authorized (according to EC Pesticide Database, 2023) active substances used in plant protection products and 5 metabolites were detected in Croatian karst water resources (Table S1).

3.2. PBT analysis results

The reliability of *PBT* properties predicted with Prometheus was generally medium for persistence and bioaccumulation and low to medium for toxicity. The ranking of all detected compounds by *PBT* scores (from 0.17 to 0.73) is shown in Table S2 and Fig. 2A. Most EOCs had *PBT* scores below the threshold of 0.5, with the highest number in range of 0.4–0.5 (Fig. 2B). PFOS, PFHxS, perfluoroheptanoic acid (PFHpA; CAS 375-85-9), PFOA, perfluorononanoic acid (PFNA; CAS 375-95-1), and fungicides boscalid (CAS 188425-85-6) and azoxystrobin stood out with the highest *PBT* scores above the threshold of 0.5, and therefore these compounds are characterized as potentially *PBT* or *vPvB* compounds (listed in Fig. 2A and their molecular structures shown in Fig. 3). Interestingly, none of them is REACH-registered. Industrial compounds stand out as having the largest range, highest score, and highest mean *PBT* value (Fig. 2C). Contrary, lifestyle products had the lowest *PBT* score and mean value.

Rakonek spring and Vransko Lake had the highest number (7) of detected *PBT* substances with the first site containing more industrial and the second more agricultural compounds (Fig. 2D). PFOS (2 detections), PFHxS (2), boscalid (1), and azoxystrobin (2) were detected in Rakonek, while PFHpA (1), PFOA (1), PFNA (1), boscalid (2) and azoxystrobin (2) were detected in Vransko Lake.

Using SARPy software, we extracted C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F as a structural alert that likely causes *PBT* property in our dataset, as it occurs in persistent and bioaccumulative compounds (Fig. 3). Similarly, Strempel et al. (2012) found that PFAS of various chain lengths are one of the most common structural elements in 2930 *PBT*-rated chemicals. As final degradation or metabolism products of other PFAS, both PFOS and PFOA are ubiquitous in the environment, biota, and human organisms (Giesy and Kannan, 2002). In 2023, ECHA published a proposal for PFAS restriction by several EU countries because of increasing exposure of humans, plants, and animals (ECHA, 2023c). In EU groundwater, PFOS was detected at a frequency of 48 %, with maximum concentration of 135 ng/L and an average concentration of 4 ng/L (Loos et al., 2010). The detection frequency of PFOA was 66 %, with maximum concentration of 39 ng/L and an average of 3 ng/L (Loos et al., 2010). In contrast, our karst spring had much lower concentrations of 0.2–0.3 ng/L for PFOS and 0.6 ng/L for PFOA. PFHxS was not detected in the EU-wide survey of persistent organic pollutants in groundwater (Loos et al., 2010), while its concentration in Croatian springs ranged from 2.2 to 11 ng/L. Moreover, PFHpA was found in average concentrations of 1 ng/L in EU surface waters (Loos et al., 2009), similar to Vransko Lake, where it was detected in 0.5 ng/L. PFNA was detected only once in our study in Vransko Lake (0.3 ng/L) at a concentration lower than EU average for surface water of 2 ng/L (Loos et al., 2009). Boscalid, a persistent carboxamide fungicide currently approved for use (Regulation No. 1107/2009) was among 20 EOCs detected in karst water resources worldwide at highest concentrations (>1000 ng/L) (Lukač Reberski et al., 2022). In our study, boscalid was detected at low concentrations of 0.2–0.6 ng/L. It has a low risk of leaching into groundwater (GUS index 2.68) (PPDB, 2023a). Environmentally relevant concentrations of boscalid have effects on neurobehavior of aquatic organisms (*Danio rerio*) by disrupting the visual and nervous systems and inhibiting growth of freshwater algae (*Chlorella vulgaris*) (Qian et al., 2018, 2021). Azoxystrobin, a fungicide that has been used in agriculture for decades, is likely to leach into groundwater given its GUS of 3.10 (PPDB, 2023b). Review of its occurrence in the aquatic environment reports mean concentrations ranging from 0.8 ng/L to 3030 ng/L (Rodrigues et al., 2013). In our study azoxystrobin was found in karst springs and lake at concentrations between 0.1 and 0.5 ng/L. Azoxystrobin is very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment (EFSA, 2010).

Of 65 compounds, 9 were predicted as non-persistent/persistent (*nP/P*), 8 as very persistent (*vP*), and 28 as persistent/very persistent (*P/vP*) (Table S2). Only for metformin, the Prometheus software was not able to predict persistence. Strempel et al. (2012) caution that the primary source of uncertainty in identifying potential *PBT* chemicals may lie in limited availability and reliability of persistence data. However, it is worth noting how Prometheus had far better performance in correctly classifying PBT compounds than other in silico tools or method proposed by Strempel et al. (2012) (Pizzo et al., 2016a).

Most compounds (59) in our dataset are characterized as bioavailable according to Lipinski Rule Oasis profiler, whereas only 6 substances are suspected to have a low bioaccumulation potential. Prometheus provided divergent results, as most chemicals were classified as *non B*. Bioaccumulation factor values were predicted to range from 0.5 for 2,4-dichlorophenoxyacetic acid (2,4-D; CAS 94-75-7) to 3.73 for PFOS (Table S2). Besides PFOS, only PFHxS and PFHpA had predicted bioaccumulation above the threshold of 3.3 (log 2000 for bioaccumulative compounds, defined by Pizzo et al., 2016b). PFOS is classified as very bioaccumulative (*vB*) because the predicted value exceeds the threshold of 3.7 (log 5000 for *vB* compounds). According to Lipinski Oasis profiler, PFOS was one of 6 compounds classified as less bioavailable, which is inconsistent with *PBT* assessment. Nendza and Müller (2010) explain this misclassification by different exposure regimes between oral pharmaceuticals' absorption and contaminants uptake by aquatic organisms. According to their proposed thresholds for deprioritization of *non B* substances (molecular weight $>$ 650 g/mol and log $K_{OW} < 3$ or >10), PFOS with a molecular weight of 500.13 g/mol and predicted log K_{OW} of 4.49 (EPI Suite™) should not be deprioritized, i.e. considered as *non B* substance. To achieve more reliable discrimination of *non B* substances, Nendza and Müller (2010) highlight how a combination of parameters (lipophilicity, dissociation, Henry's law constant, water solubility, hydrolysis, and ready biodegradability) should be considered. Regarding the initial EOCs prioritization per their bioaccumulation potential, we opt for Prometheus results rather than the Lipinski Oasis profiler, because Prometheus had the highest reliability compared to other in silico tools (Pizzo et al., 2016a).

Only 3 compounds (azoxystrobin, lamotrigine, and griseofulvin) were classified as toxic because their *T* values (0.0001, 0.008, and 0.01 mg/L respectively) were below the 0.01 mg/L limit (Table S2). Dong et al. (2013) classified the anticonvulsant medicine lamotrigine (CAS 84057-84-1) as high risk based on its potential toxicity across various endpoints. The defined daily dose per 1000 inhabitants per day for lamotrigine was 1.29 in 2019, making it one of the most commonly used antiepileptic drugs in Croatia (Draganić and Oštarčević, 2021). Lamotrigine was detected in 12 of 16 monitored springs with concentrations ranging from 0.1 to 7.6 ng/L (mean 1.3 ng/L). Contrary, Jurado et al. (2022) reported much higher average concentration of 397 ng/L in Spanish urban groundwater, while Bollmann et al. (2016) detected lamotrigine in German groundwater with a maximum concentration of 70 ng/L. Griseofulvin (CAS 126-07-8), used as an antifungal agent for humans and as veterinary drug, can cause genotoxic, carcinogenic, and teratogenic effects in laboratory animals (Knasmüller et al., 1997). In our study, it was detected only once at concentration of 0.1 ng/L in Prud spring. To our knowledge, this is the only reported detection of

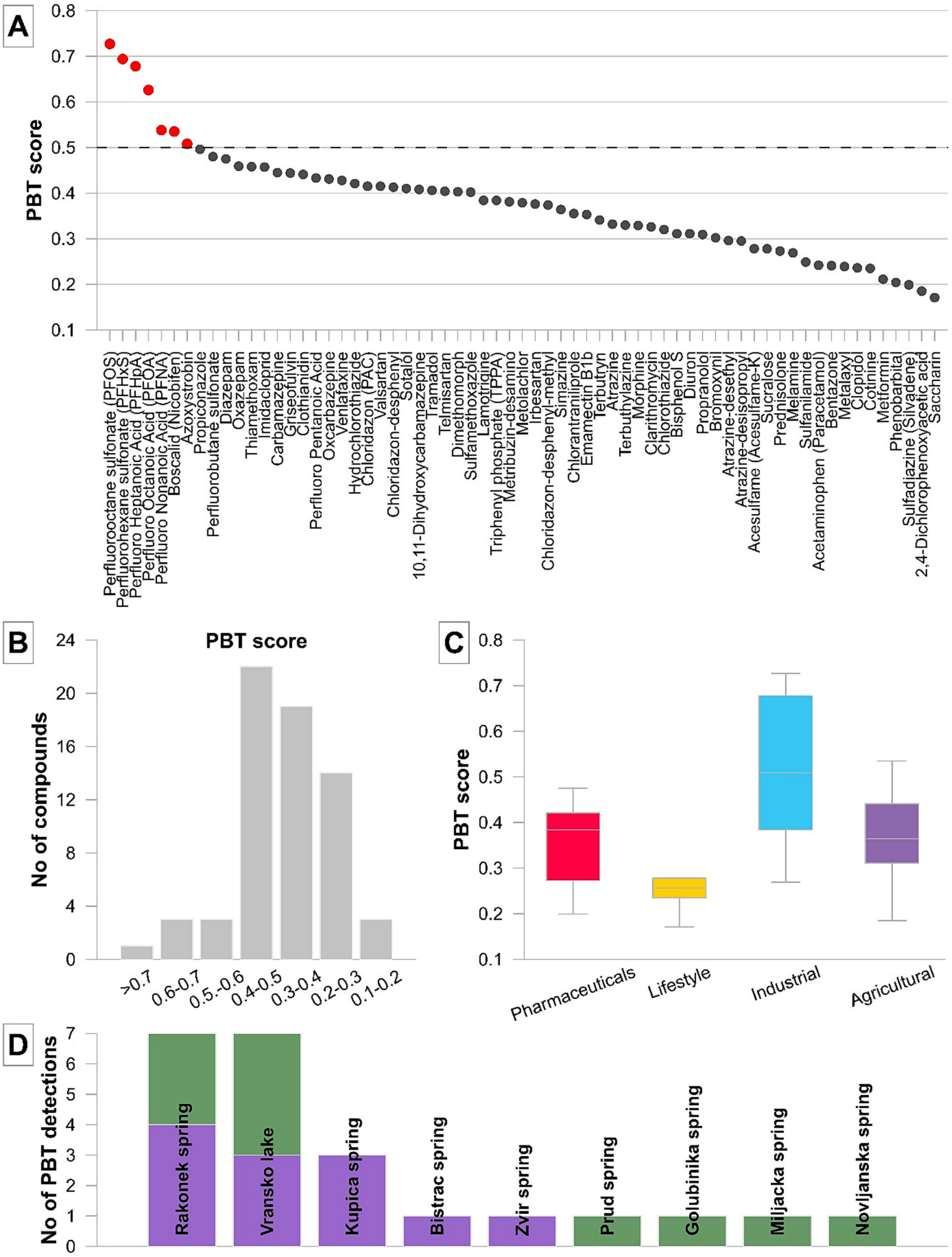

Fig. 2. In silico *PBT* prediction in Prometheus software; the threshold of 0.5 distinguishes *PBT* substances from *non-PBT* substances (A); number of compounds in different *PBT* score ranges (B); statistics of *PBT* scores in different use categories (C); *PBT* detections at each location and in each category (sites without *PBT* detections are omitted from graph) (D).

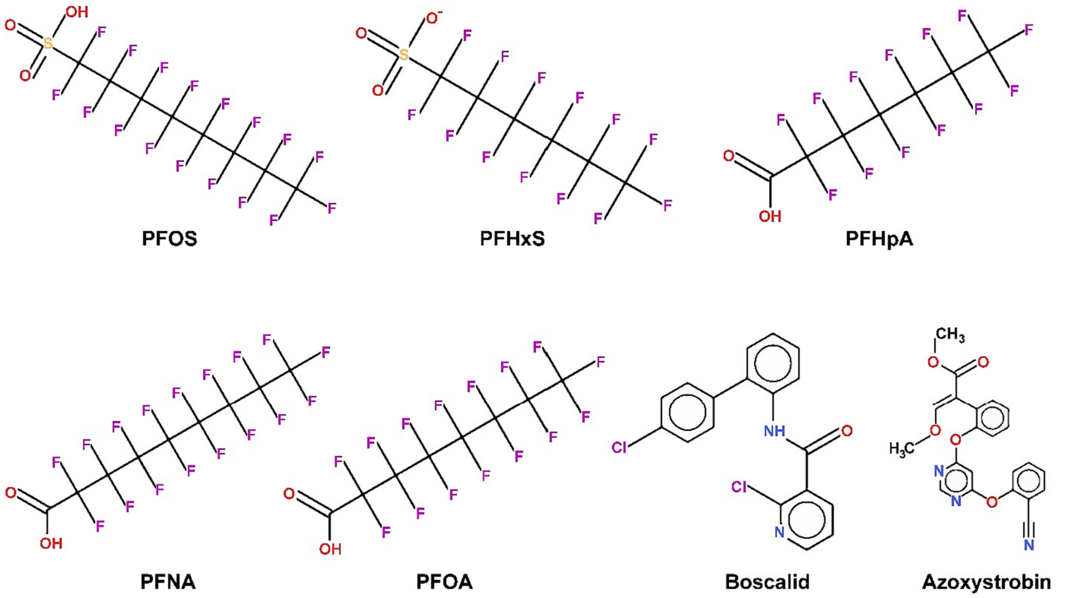

Fig. 3. Molecular structure of substances meeting PBT or vPvB criteria.

griseofulvin in karst springs worldwide.

A high degree of uncertainty in toxicity assessment due to lack of data for many EOCs may account for the generally low reliability of Prometheus toxicity results and for about 30 % of EOCs (19 of 65 compounds) that were not assigned a toxic category. In particular, Prometheus could not predict the toxicity for PFOS, PFHxS, PFHpA, PFOA, and PFNA, which had the highest *PBT* values. Toxicological data for aquatic species is limited considering how extensive the group of PFAS is and the need for toxicity testing on multiple test species for each of them (Zodrow et al., 2020). The toxicological effects of PFOS and PFOA are the best studied among other PFAS (Stahl et al., 2011). Zodrow et al. (2020) defined chronic risk-based screening values for aquatic species ranging from 220 ng/L to 3.4 mg/L PFAS. Acute toxicity tests on freshwater species and plants showed that PFOS was moderately toxic, while PFOA was slightly toxic (Li, 2009). Reported 48-h NOEC values for aquatic invertebrates ranged from 10 to 100 mg/L for PFOS and from 125 to 500 mg/L for PFOA (Li, 2009).

3.3. PMT/vPvM analysis results

Most of the chemicals detected in our study met the PMT criteria. Of 65 compounds, 23 (35.4 %) were evaluated as *potential PMT&vPvM*, 21 (32.3 %) as *PMT&vPvM*, 14 (21.5 %) as *vPvM*, 3 (4.6 %) as *PM,* and 2 (3.1 %) as *PMT* (Fig. 4A, Table S3). The level of concern for *PMT/vPvM* substances is the same as for *PBT/vPvB* substances (Neumann and Schliebner, 2019). The rapid and concentrated infiltration of water through swallowholes or shafts in karst systems enables very mobile compounds to bypass the soil and epikarst layers, which typically play a crucial role in attenuating contaminants. Consequently, these mobile compounds can be swiftly transported through interconnected conduits, reaching the aquifer without undergoing the same level of natural filtration and degradation experienced in other geological formations. Persistent compounds, may be stored in aquifer matrix and slowly leach with arrival of recharge waters (Morasch, 2013; Hillebrand et al., 2014). Storm pulses may activate karst conduits and flush previously stored persistent compounds towards karst springs (Doummar et al., 2014). Similar to our study, Huang et al. (2021) assessed nearly half of compounds detected in Chinese groundwater and drinking water as potential *PMT/vPvM*. In over half of the analyzed groundwater samples from German drinking water resources, Neuwald et al. (2022) identified already prioritized *PMT/vPvM* substances. In contrast, out of 32 analyzed persistent and mobile compounds Kolkman et al. (2021) only recorded cotinine in Dutch and Flemish groundwater used for water supply. Cotinine prioritized in this study as *potential PMT/vPvM* substances was identified in two of our karst springs and a karst lake. Only a small proportion (around 3 %) of our dataset was identified as *not PMT&vPvM* (an angiotensin II receptor blocker telmisartan (CAS 144701-48-4)), which did not meet *M* criteria; and a plasticizer triphenyl phosphate (TPPA, CAS 115-86-6), which was assessed as *not P*) (Fig. 4A). Artificial sweeteners sucralose (CAS 56038-13-2; REACH-registered) and acesulfame (CAS 33665-90-6), detected at the highest concentrations of 440 ng/L and 150 ng/L respectively, were both evaluated as *potential PMT&vPvM* substances (Fig. 4B). Doummar and Aoun (2018) demonstrated persistence of sucralose and acesulfame in the matrix of karst aquifer or epikarst, even in the presence of substantial dilution. Similarly, Zirlewagen et al. (2016) reported constant background concentrations suggesting long-term contamination.

Of the 22 REACH-registered substances, 9 (40.9 %) were evaluated as *PMT&vPvM*, 7 (31.8 %) as *potential PMT&vPvM*, 3 (13.6 %) as *vPvM*, and only 1 (4.6 %) as *PMT*, *PM*, and *not PMT/vPvM*. Of 7 SVHC compounds, 3 were assessed as *PMT&vPvM*, 3 as *vPvM*, and 1 as *potential PMT&vPvM*. Of the non-approved pesticides detected in Croatian karst, 3 were evaluated as *PMT&vPvM*, 3 as *vPvM*, 2 as *potential PMT&vPvM,* 1 as *PMT* and *PM* (Table S3). These findings reinforce the need for stricter regulatory control and effective prevention strategies to address the presence of harmful compounds. In similar vein, Moreau et al. (2019) advocate for a review of pesticide list for national surveys, having also found restricted pesticides in groundwater (e.g. atrazine and its metabolites, clothianidin, simazine, imidacloprid, thiamethoxam). As pointed out by Morasch (2013), implementation of extensive, sustainable farming practices in recharge areas of karst systems, coupled with the prohibition of treatments identified as sources of EOCs further supports the protection of karst groundwater quality.

We attribute the distribution of EOC groups across *PMT/vPvM* categories to widespread contamination emission from diffuse sources through either municipal wastewater or agricultural products use.

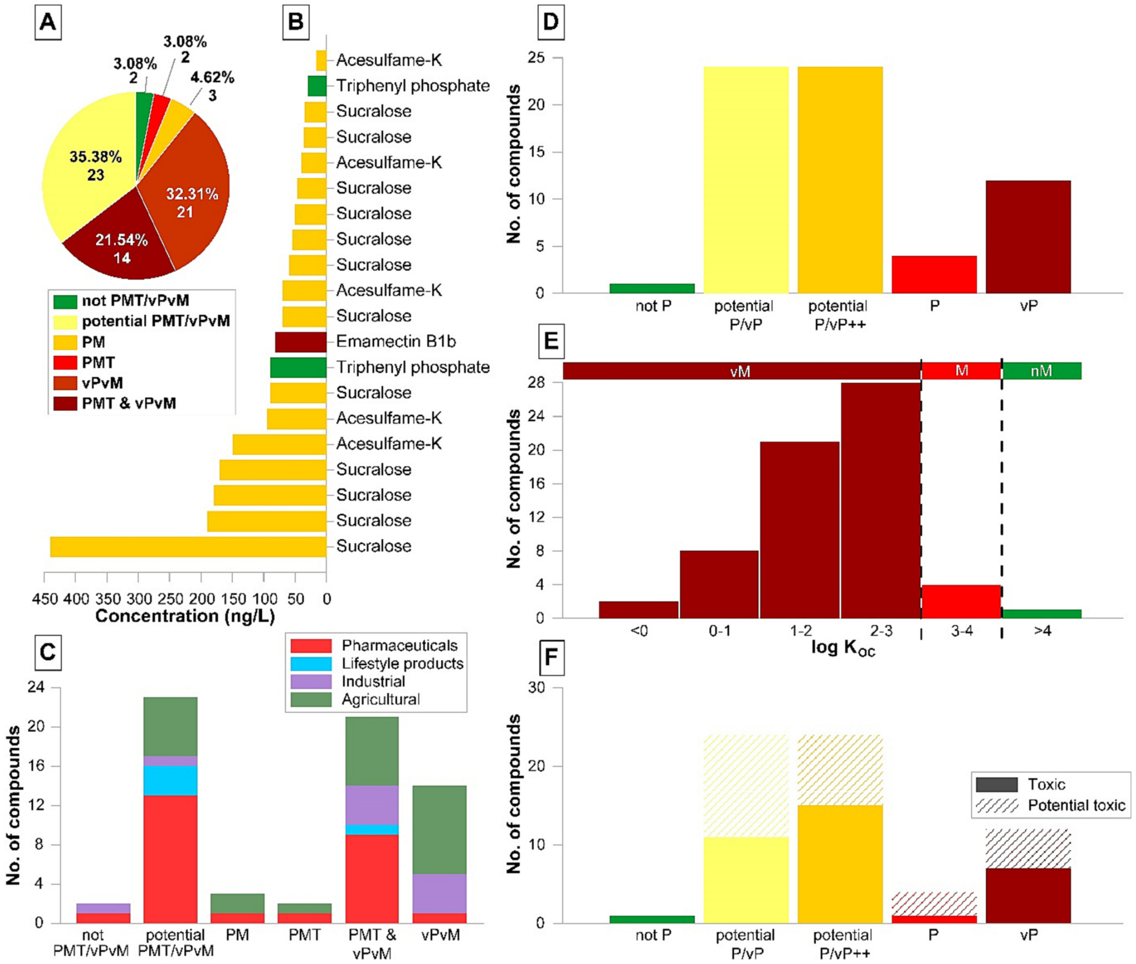

Fig. 4. Number and percentage of detected compounds per PMT/vPvM category (A); PMT/vPvM categories of 20 detected compounds with highest concentration (B); number of compounds per PMT/vPvM assessment category and use group (C); number of compounds per persistence category (D); number of compounds per mobility category and corresponding log K$_{OC}$ range (E); count of toxic and potentially toxic compounds per persistence categories (F).

Slightly smaller number can be attributed to industrial point sources (Fig. 4C). This confirms the findings of Lukač Reberski et al. (2023) that agriculture appears to be an important EOC source in the Croatian karst. Neuwald et al. (2022) also observed a diverse range of emission patterns for *PMT/vPvM* substances, with a prevalence of diffuse sources originating from urban wastewater. Within the most diverse and abundant category of *potential PMT&vPvM* in terms of intended use, pharmaceuticals and agricultural products predominate in our study, followed by lifestyle products (Fig. 4C). The *PMT&vPvM* evaluated herbicides include atrazine, diuron, and simazine, all REACH-registered substances that are not currently approved in the EU. Moreover, PBT fungicide boscalid was also classified as *PMT&vPvM*. Among *PMT&vPvM* pharmaceuticals, phenobarbital (CAS 50-06-6), sulfadiazine, valsartan (CAS 137862-53-4) and venlafaxine (CAS 93413-69-5) are REACH-registered substances. The category *PMT&vPvM* was also assigned to industrial compounds PFNA, PFOA, and PFOS, which were also prioritized with PBT analysis.

The persistence data of *PMT/vPvM* assessment exhibits that most compounds detected in this study are not readily biodegradable (12 were assessed as *vP*, 4 as *P*, 24 as *potentially P/vP++*, and 24 as *potentially P/vP*). PFAS, flame retardant melamine, anxiolytic oxazepam (CAS 604-75-1), herbicide metolachlor (CAS 51218-45-2), pesticide metabolite metribuzin-desamino (CAS 35045-02-4), fungicide propiconazole (CAS 60207-90-1), and pharmaceutical metabolite 10,11-dihydroxycarbamazepine (CAS 35079-97-1) were all identified as very persistent compounds. All these compounds were also assessed as *P/vP* in PBT analysis, except for metribuzin-desamino, which was classified as *nP/P*. Kupica and Rakonek springs stood out with the highest number (5) of detected *vP* compounds, similar to the PBT classification.

The chemicals detected in groundwater were mainly classified as *very mobile* (*vM*) (60 of 65 compounds), based on their log K$_{OC}$ values < 3 (Fig. 4E, Table S3). This is of particular concern given that large fractures and conduits in karstified aquifers with rapid and concentrated groundwater flow allow contaminants to travel large distances in a relatively short time. Lifestyle products stood out with the lowest mean log K$_{OC}$ value of 0.7, in contrast to industrial compounds that had the highest mean log K$_{OC}$ value of 2.1. Only the human medicine telmisartan, detected in Kupica and Golubinka springs, was evaluated as *not mobile* (*not M*) given its log K$_{OC}$ value of 5.6. In contrast, sucralose detected in the highest concentrations and several locations, had the lowest log K$_{OC}$ value of 0.7, making it the most mobile compound detected.

According to ECHA Classification and labelling inventory, NOEC values, Toxtree toxic hazard estimation, and VEGA QSAR toxicity prediction evaluation, 30 compounds were characterized as *potentially toxic* (*potential T*), while 35 of them were classified as *toxic* (*T*) (Table S3). Among the 30 potentially toxic EOCs, VEGA QSAR models demonstrated limited reliability in predicting toxicity of 19 compounds. Similarly, Roveri and Lopes Guimarães (2023) deemed VEGA QSAR toxicity results for 115 investigated pharmaceuticals, which were classified as potentially toxic, as unreliable. Of the compounds meeting toxicity criteria, 15 were characterized as *potential P/vP++*, 11 as *potential P/vP*, 7 as *vP*, and 1 as *P* and *not P* (Fig. 4F). The majority of toxic compounds were pharmaceuticals (17) and agricultural products (11). For toxicity assessment, minimum NOEC values for surface water species (particularly algae, fish, and invertebrates) were sought in literature because of the lack of ecotoxicological data and standardized protocols for stygobiotic taxa, especially concerning EOCs. However, we acknowledge how the sensitivity of subterranean groundwater-dependent biota to anthropogenic stressors differs significantly from the responses of surface water relatives (Di Lorenzo et al., 2019). Caution should be exercised when further interpreting toxicity assessment results of *PMT/vPvM* substances (Neuwald et al., 2022), as limited availability of toxicokinetic data may introduce uncertainties in assessment (Kim et al., 2023). Findings are aimed at preliminary prioritization of EOCs, as their potential ecotoxicological impacts in groundwater are poorly understood and may be more severe for subterranean biota due to their intrinsic vulnerability. Thus, to adequately protect the significant endemism of subterranean aquatic species in the Croatian karst, it is critical to develop specific guidelines for ecotoxicological testing of groundwater dwellers and to establish a regulatory framework.

Given the complex and heterogeneous nature of karst aquifers, sampling sites have locally different hydrogeological characteristics that affect contaminant transport pathways, while different geomorphological forms determine ground cover and, consequently, contaminants leaching from the surface. Lukač Reberski et al. (2023) analyzed the relationships between land use, land cover, spring discharges and occurrence of EOCs' use groups. Although lower than average, discharges at almost all springs were higher in March, when pharmaceutical, agricultural, and industrial compounds were detected in lower numbers than in October. Authors report how temporal disparities among observed campaigns do not show a clear pattern but rather reflect the highly variable nature of karst springs' responses to recharge, with no apparent geographic pattern. Likewise, slight differences in temporal patterns of occurrence of *PMT/vPvM* categories can be observed at most sites between the March (Fig. 5A) and October (Fig. 5B) campaigns (Table S3), while spatial variation between locations is more pronounced. In March campaign there were more detections within the *potential PMT&vPvM* (39) and *PM* (10) categories, while in October there were more *PMT&vPvM* (41) and *vPvM* (37) detections. Temporal difference between spring and autumn campaigns is most evident in the case of Opačac spring (Fig. 5, marked 6) with zero detections of *potential PMT&vPvM* substances in March, but 4 detections in October, and Rakonek spring (Fig. 5, marked 12) with 7 detections of *PMT&vPvM* substances in March and only 3 in October.

The highest total concentrations per site were observed in both campaigns for the *potential PMT&vPvM* category (≈357 ng/L in March and ≈456 ng/L in October at Golubinka spring, respectively). In March, Golubinka spring (Fig. 5A, marked 9) stood out with the highest detection number (21) among which 8 substances were *potential PMT&vPvM*, 6 *PMT&vPvM*, 5 *vPvM*, 1 *PM,* and 1 *PMT*. Same spring also had the highest detection number in October, but fewer *potential PMT&vPvM* and *PMT&vPvM* compounds. Compounds considered *not PMT & vPVM* occurred during both campaigns, with TPPA (*not P*) having high concentrations in March (30 and 90 ng/L) along with higher discharges and telmisartan (*not M*) having low concentrations in October (0.1 and 0.2 ng/L) when discharges were lower. No detection >LOD for TPPA was recorded in October, while telmisartan was not detected in March.

3.4. Human exposure to EOCs via drinking water

All sampled locations are captured for water supply, except for Bistrac spring which, as part of Zagorska Mrežnica catchment, may indirectly affect its water quality. Thus, we present the potential risk that EOCs and detected legacy compounds may pose to human health through consumption of drinking water from all locations.

Conventional processes used in water treatment plants have been

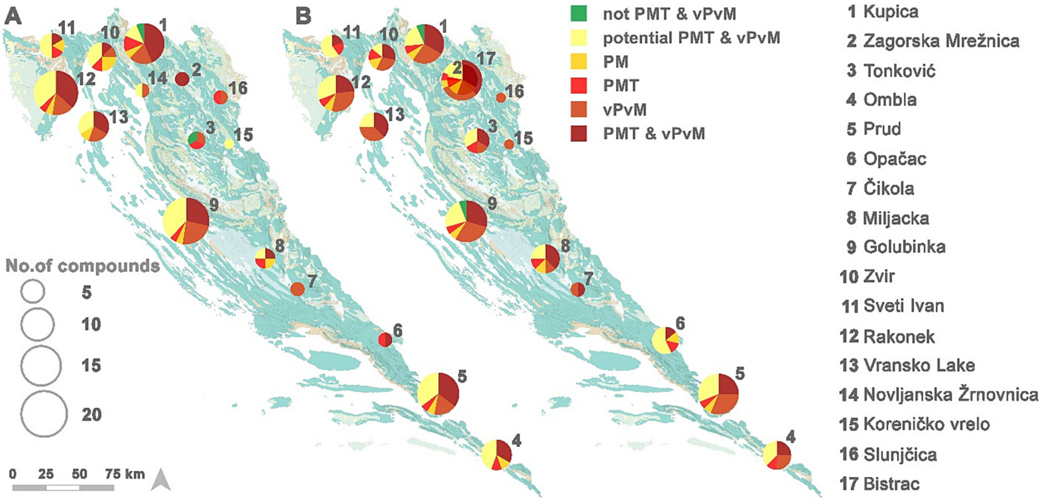

Fig. 5. The spatial and temporal analysis of PMT/vPvM patterns for March (A) and October 2019 (B) campaigns; the size of pie charts corresponds to number of detections per site, for representation purposes size in qGIS was scaled by area and not by diameter; Hydrogeological map of Croatia is used as background showing the prevalence of carbonate rocks with high permeability (dark green) in the study area. Note: no EOCs were detected at Novljanska Žrnovnica (marked 14) in October above LOD; Bistrac spring (marked 17) was not sampled in March; due to map scale Bistrac and Zagorska Mrežnica circles overlap, with the larger circle marking Bistrac spring.

ineffective in removing EOCs (Gibs et al., 2007). However, even some advanced treatment processes like UV irradiation or ozonation can only completely eradicate some EOCs from drinking water (Barbosa et al., 2016). Combining multiple treatment processes shows higher removal efficiency (Kim et al., 2007; Huerta-Fontela et al., 2008; Boleda et al., 2009; Flores et al., 2013), but requires additional energy and chemical consumption, resulting in higher investment and operating costs (Bui et al., 2016). Schriks et al. (2010) excluded compounds with n-octanol–water partition coefficients log Kow > 3 from further human health risk assessment, as these compounds are less likely to pass drinking water treatment plants (Westerhoff et al., 2005). In our case, 6 industrial compounds (PFAS), 5 agricultural products, and 6 pharmaceuticals have either predicted (KOWWIN v. 1.68) or experimental log Kow > 3 (Table S3), with angiotensin II receptor blocker telmisartan having the highest lipophilicity/hydrophobicity (estimated log Kow of 8.42). Water captured in Croatian springs is generally disinfected with NaClO only. Although disinfection is essential for microbiologically safe drinking water, it is inefficient in removing EOCs and can lead to an increase in disinfection by-products that pose a substantial threat to human health due to their carcinogenic properties (Van Leeuwen, 2000). For example, transformation products of antiepileptic drug carbamazepine, the most frequently detected pharmaceutical in our study, showed increased genotoxicity in chlorinated samples (Han et al., 2018).

In a preliminary classification of toxic hazard in Toxtree, 62 of 65 compounds were assigned the most severe hazard level or Cramer Class III. Only 3 substances, namely analgesic acetaminophen (CAS 103-90-2), fungicide metalaxyl (CAS 57837-19-1), and insecticide thiamethoxam (CAS 153719-23-4) were assigned Cramer Class I. However, all compounds had a maximum daily drinking water intake DWI_{bw} that was several orders of magnitude lower than the TTC values assigned to Cramer Class I and III. DWI_{bw} ranged from 3.33E-07 μg/kg bw/day for 21 different compounds occurring at several karst springs to 0.0015 μg/kg bw/day calculated for sucralose at Golubinka spring in October (Table S4). The DWI_{bw} of detected pharmaceuticals ranges up to 6.33E-05 μg/kg bw/day for sulfadiazine recorded in Prud spring sample in October (Table S4). The calculated DWI_{bw} of this REACH-registered sulfonamide antibiotic is well below the relevant ADI of 20 μg/kg bw (APVMA, 2022) and the lowest therapeutic dose of 2.0E+06 μg/day for an adult (RxList, 2023). Carbamazepine, as most frequently detected pharmaceutical in Croatian karst and karst aquifers worldwide (Lukač Reberski et al., 2022), had a maximum DWI_{bw} of 4.0E-05 μg/kg bw/day measured for Golubinka spring in October. This compound is considered an effective marker of effluent contamination and a breakthrough indicator of the particular vulnerability of karst springs (Doummar et al., 2014; Dvory et al., 2018). We estimate that 1 μg of carbamazepine (0.0003 % of its clinical dose) can be ingested via drinking water from Golubinka spring during pregnancy (exposure during 36 weeks), which is two orders of magnitude lower than the 0.03 % determined by Collier (2007), who observed a potential concern for pregnant women ingesting pharmaceuticals via drinking water. It should be noted that Collier used a much higher concentration of carbamazepine in treated drinking water (258 ng/L) for his estimate. Pregnant women may ingest 0.6 μg of sulfadiazine (0.00005 % of its therapeutic dose) via drinking water from Prud spring. Despite low concentrations compared to clinical doses, Collier (2007) highlights how ingested pharmaceuticals may not have a linear dose-response relation when causing congenital abnormalities, and there is also a question about synergistic effects of EOCs mixtures. Among agricultural products, macrocyclic lactone insecticide emamectin B1b had the highest DWI_{bw} of 2.73E-04 μg/kg bw/day recorded in Čikola spring in March, followed by DEA with a DWI_{bw} of 4.33E-05 μg/kg bw/day calculated for October sample of Bistrac spring (Table S4). Approximately 12 % of emamectin ADI (EFSA, 2012) and 0.11 % of DEA *GLV* can be ingested via drinking water during pregnancy.

Indirect exposure calculated for each water sample had values ranging from 6.67E-07 μg/kg bw/day or 4.0E-5 μg/day calculated for March Vrelo Koreničko spring sample (1 pharmaceutical and 1 agricultural compound) to 0.0017 μg/kg bw/day or 0.1 μg/day derived for October Golubinka spring sample (17 EOCs, including 8 pharmaceuticals, 6 agricultural, 2 lifestyle, and 1 industrial compound) (Table S4). In mixtures, substances with similar modes of action as pharmaceuticals may have additive interactions. It should be noted that the concept of concentration addition does not take into account properties and modes of action of the individual EOCs in mixture, nor their potential antagonistic or synergistic interactions, but rather outlines potential exposure. We emphasize that a better understanding of the short or long-term dose-additive or synergistic effects of EOCs mixtures on human health is needed to establish safe future guideline levels for EOCs mixtures in drinking water resources.

To evaluate possible health risks of individual compounds, statutory and provisional guideline values were compared with maximum detected concentrations as benchmark quotients. Currently, World Health Organisation drinking water guideline values (*GLV*) (WHO, 2022) are set only for 7 compounds present in Croatian karst drinking water resources, namely atrazine and its chloro-s-atrazine metabolites (100 μg/L), 2,4-D (30 μg/l), metolachlor (10 μg/L), simazine (2 μg/L), and terbuthylazine (7 μg/L). These levels represent a contaminant concentration that is not expected to cause a significant health risk for a lifetime consumption (2 L/day and 10 % source contribution). Health-related indication values (HRIV) for drinking water were recommended by German Federal Environment Agency (UBA) at 0.3 μg/L for carbamazepine, lamotrigine, PFHpA, PFHxS, phenobarbital, and valsartan, at 3 μg/L for PFBS and PFPeA, and at 1 μg/L for metformin (Dieter, 2014).

According to IARC List of Classifications, 2,4-D, phenobarbital, hydrochlorothiazide (CAS 58-93-5), griseofulvin, oxazepam, and PFOA are evaluated as possible human carcinogens. Of 6 compounds not classified as carcinogenic to humans (IARC), 2 had WHO-derived *GLVs*, while 4 exhibited genotoxic properties. For these possibly carcinogenic and genotoxic compounds, a drinking water target value of 0.01 μg/L for "non-threshold chemicals" was applied to calculate BQ values, except for PFOA for which a TWI of 4.4 ng/kg bw/day (EFSA et al., 2020) was used to calculate *pGLV*. Even though other 53 substances have not been evaluated by IARC, they cannot be presumed as non-carcinogenic. The genotoxicity and carcinogenicity of artificial sweeteners are still debated, as both positive and negative test results have been reported (Table S4), but based on weight of evidence, recent comprehensive review indicates that acesulfame, sucralose, and saccharin are not associated with genotoxic or carcinogenic effects (Pavanello et al., 2023). For 12 of 53 compounds, *GLVs* were previously proposed by WHO or UBA. QSAR ISS profiler for carcinogenicity detected structural alerts for non-genotoxic carcinogenicity in 5, and for genotoxic carcinogenicity in 5 other non-IARC-assessed compounds without *GLVs*. Structural alerts for both non-genotoxic carcinogenicity and genotoxic carcinogenicity were found in 2 compounds. Hence, these 12 substances are seen as non-threshold compounds and a conservative drinking water target value of 0.01 μg/L was applied, instead of deriving *pGLVs* from toxicological data. The same applies to 12 other compounds for which genotoxicity is assigned based on literature findings and/or predictions of In vivo Micronucleus activity (IRFMN) 1.0.1 model in VEGA QSAR. Toxicological data was used to derive *pGLVs* for 19 compounds. ADI/TDI values were available in literature for 15 substances. The NOAEL was used once to calculate provisional ADI values, while LTD and RfD were used in 2 cases each. The *pGLVs*, which denote concentration of compounds below which no adverse health risks to consumers are suspected, ranged from 0.002 μg/l for PFOS and PFOA to 210 mg/L for TTPA (Table S4). We evaluated PFOS as a *PBT/vPvB* and *PMT & vPvM* substance, while TTPA was classified as *non-PBT* and *not PMT & vPvM*. Calculated *pGLVs* are generally up to several orders of magnitude higher than target value for non-genotoxic compounds proposed by Mons et al. (2013).

The Benchmark Quotient (*BQ*) values ranged from 4.29E-07 for TTPA to 1.9 for sulfadiazine (Table S4). For most compounds, no significant risk to human health is presumed. Only two compounds (sulfadiazine > possibly carcinogenic hydrochlorothiazide) had $BQ \geq 1$ (Fig. 6), i.e. maximum detected concentration above used guideline value (in this case, TTC of 0.01 μg/L for non-threshold chemicals). For these compounds, a potential health risk could be presumed at lifelong exposure. Although they were detected in raw spring water, previous studies reported their inefficient removal by conventional drinking water treatment commonly used in Croatia (Huerta-Fontela et al., 2011; Gaffney et al., 2016). Sulfadiazine was evaluated as *PMT & vPvM*, while hydrochlorothiazide as *potential PMT/vPvM*. Both substances have very low $log\ K_{ow} < 0$ and low $\log K_{OC} < 3$ values, indicating that they are hydrophilic, very mobile, and with no tendency to bioaccumulate.

Further examination and environmental monitoring are needed for 5 additional contaminants present in raw spring water that have $BQ > 0.2$ (Fig. 6) and either structural alerts for carcinogenicity or evidence of genotoxicity or carcinogenicity. These include the industrial SVHC PFOA, pharmaceuticals 10,11-dihydroxycarbazepine, clopidol, sulfamethoxazole, and REACH-registered tramadol. Poor removal of sulfamethoxazole and PFOA during chlorination was observed in previous studies (Gibs et al., 2007; Rahman et al., 2014). Cheng et al. (2015) reported occurrence of tramadol transformation products during chlorination treatment, which exhibited increased genotoxicity compared to parent compound. PFOA, which we assessed as PBT/vPvB and a PMT & vPvM compound, has an elimination half-life of 3.5 years in human serum (Olsen et al., 2007).

As highlighted by Kolkman et al. (2021), lack of toxicity data for many persistent and mobile compounds limits the reliability of drinking water guideline values. Here presented results serve as an early warning of the chemical status of karst drinking water resources and point out the need for more comprehensive monitoring activities and thorough analysis of compound-specific sources, exposure pathways and organism responses.

4. Conclusions

This is the first systematic study to prioritize a diverse chemical array of 65 organic contaminants (emerging and some legacy) detected in Croatian karst groundwater resources (16 karst springs and 1 lake) used for drinking water supply and to assess the potential health risk to consumers. In our detected dataset, 22 compounds are registered under REACH (No. 1907/2006), 7 are included on the Candidate List of substances of very high concern (SVHC), 10 are unauthorized pesticides (EC Pesticide Database, 2023), and 9 pharmaceuticals can be found on Voluntary Groundwater Watch List. The detected PFAS exceeded proposed groundwater quality standards threshold (COM(2022)540) at two karst springs in March and six springs in October. We recorded 5 detections of REACH-registered compounds with concentrations exceeding current pesticide threshold $\geq$100 ng/L. These findings underline the importance of proactively addressing presence of compounds of concern in the environment. It is imperative to raise awareness within the agriculture and industry sectors regarding the necessity of substituting unauthorized compounds with safer and sustainable alternatives. Specifically in karst recharge areas, water managers should promote responsible use and disposal of chemicals and educate the local population on adoption of environmentally friendly practices to prevent EOCs pollution of groundwater. Furthermore, concerted efforts should be made to phase out the use of harmful PFAS worldwide.

While both PBT and PMT/vPvM methods are employed in our study to assess the priority of EOCs, their outcomes are not directly comparable due to the variations in input data and distinct workflows. However, the individual results from each one provide complementary perspectives, contributing to a more comprehensive understanding of EOCs characteristics and potential impacts in the karst groundwater environment. Both prioritization methods has demonstrated their efficacy in identifying chemicals of concern within Dinaric karst groundwater. Moreover, these methods exhibit broad applicability to other karst or intergranular water resources worldwide. Nevertheless, it is essential to exercise caution when interpreting the results, primarily owing to the limited availability of input data for numerous EOCs. Muir et al. (2019) warn that the use of in silico models may lead to prioritization of only those chemicals that are similar to well-characterized compounds based on whose physical-chemical properties are models trained. Therefore, the obtained findings should be viewed as guidance, serving as a valuable reference for future research endeavors and decision-making processes pertaining to the management and protection of water resources. In our study, a total of 7 compounds (PFAS, fungicides boscalid and azoxystrobin) were classified as potentially *PBT* or *vPvB* compounds. Most of detected compounds met *PMT/vPvM* criteria, with 12 assessed as *vP*, 4 as *P*, 24 as *potentially P/vP++*, 24 as potentially *P/vP*, 60 out of 65 assessed as *vM*, 35 characterized as *T*, and 30 as *potential T*. Between two sampling campaigns (March and October 2019), slight temporal and more pronounced spatial variations in the occurrence of different *PMT/vPvM* categories were observed, but without a clear pattern. We stress the necessity of incorporating those prioritized chemicals into groundwater watch lists for Dinaric karst and monitoring protocols. This is a vital step in comprehensive assessment of

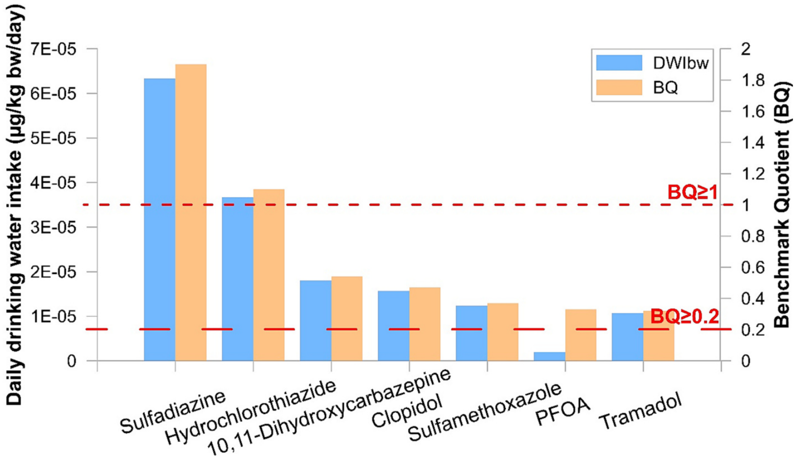

Fig. 6. Calculated daily drinking water intakes (DWI_{bw}) and Benchmark Quotients (*BQ*) for EOCs posing appreciable risk to human health ($BQ \geq 1$) and EOCs warranting further research and monitoring ($BQ > 0.2$).

their emission patterns and understanding of their behavior, in order to facilitate timely prevention or mitigation strategies. The outcomes of *PMT/vPvM* assessment conducted on the drinking water resources of Dinaric karst, represent a significant scholar contribution in the context of the Chemicals Strategy for Sustainability Towards a Toxic-Free Environment (COM(2020)667). Specifically, our findings provide direct support for the proposed amendment of REACH Article 57 to add *PMT* and *vPvM* substances in the SVHC list. By advocating for the inclusion of prioritized EOCs in legislative framework and water monitoring activities, our research aligns with the global ongoing efforts aimed at preserving environmental integrity and human health. Prioritized chemicals can be further studied using contaminant transport models, can help to elucidate vulnerability and attenuation capacity of karst aquifers (e.g., carbamazepine studied by Doummar et al., 2014; acesulfame-K and sucralose investigated by Doummar and Aoun (2018); carbamazepine attenuation explored by Dvory et al. (2018)), provide insights into karst aquifers' contaminant storage potential or evidence of long water residence time (e.g., banned atrazine and its metabolites observed in research by Reh et al. (2013) and Hillebrand et al. (2014)). Considering the prioritization of the majority of detected compounds as *PMT/vPvM*, it is important to acknowledge that the absence of detection, with only 65 compounds detected out of 740 analyzed, does not imply the absence of potential environmental pollution risks. The sensitivity of the analytical techniques employed, the utilized sampling strategies, quality assurance as well as contaminants' properties play a critical role in determining detection rates. To gain a more comprehensive understanding of the presence of potentially persistent, mobile, and toxic compounds in karst aquifers, further research efforts at catchment scale and more frequent sampling are warranted.

For only 16 of 65 detected compounds, (statutory) drinking water guideline values are reported. By comparing maximum detected concentrations with the calculated provisional or existing statutory drinking water guideline values, we conclude that most substances occurring in Croatian karst groundwater resources do not pose significant risk to human health. However, pharmaceuticals sulfadiazine and hydrochlorothiazide are suspected to pose a potential health risk at lifelong exposure. We endorse future investigation of their sources and trends at aquifers level, to gain comprehensive understanding of their dynamics and fate. Additionally, we recommend an in-depth research focusing on efficient water treatment methods. Another 5 compounds, in particular industrial PFOA and pharmaceuticals 10,11-dihydroxycarbazepine, clopidol, sulfamethoxazole, and tramadol also warrant additional research and monitoring. We emphasize that short or long-term, dose-additive or synergistic effects of EOCs mixtures on human health remain unexplored and that insights are critical for establishing safe future guideline values for EOCs mixtures.

Findings reported in this study hold particular importance for water managers and policymakers, as they provide valuable insights for informed decision-making. The efficient management of chemically heterogeneous group of predominantly persistent and very mobile contaminants, which exhibit high fluxes in Dinaric karst springs characterized by seasonally varying discharges and intricate groundwater flow through networks of conduits and enlarged fractures, is significant technical challenge. Thus, we encourage stronger collaboration between scientific community and water practitioners to facilitate adequate prevention, early detection of *PMT/vPvM* substances in karst water resources and timely responses to potential pollution events. Our study serves as an early warning, highlighting areas of concern and identifying gaps in current water-related legislation and management. It provides a robust basis for enhancing existing policies and points out that prevention measures at national (Croatia) and regional (Dinaric karst) levels with objective of achieving the zero pollution of water resources are urgently needed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166240.

Funding sources

This paper is partly the result of collaboration, training and education conducted through GeoTwinn project that has received funding from the H2020-EU.4.b., [grant number 809943]. The field investigations and EOCs analysis are funded and supported by the Croatian Geological Survey, Department of Hydrogeology and Engineering Geology.

CRediT authorship contribution statement

Ana Selak: Investigation, Conceptualization, Data curation, Methodology, Writing – original draft, Visualization. **Jasmina Lukač Reberski:** Investigation, Supervision, Writing – review & editing, Data curation. **Göran Klobučar:** Methodology, Data curation, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Paper III

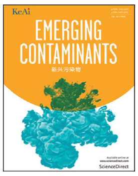
Selak, A., Lukač Reberski, J., Boljat, I., Terzić, J., 2024. Characterizing occurrence of emerging organic contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs, Croatia. Emerg Contam 10, 100327. https://doi.org/10.1016/j.emcon.2024.100327

Emerging Contaminants 10 (2024) 100327

Contents lists available at ScienceDirect

Emerging Contaminants

journal homepage: www.keaipublishing.com/en/journals/emerging-contaminants



Characterizing occurrence of emerging organic contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs, Croatia

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ARTICLE INFO

Article history:
Received 29 December 2023
Received in revised form
12 February 2024
Accepted 29 February 2024
Available online 1 March 2024

Keywords:
Karst groundwater
Emerging organic contaminants
Pharmaceuticals
Human exposure
DEET
1H-benzotriazole

ABSTRACT

The occurrence of emerging organic contaminants (EOCs) was investigated in vulnerable Dinaric karst catchment of Jadro and Žrnovnica springs in Croatia, under varying flow conditions and across three different water resource types (groundwater, springs, and surface water). The maximal EOCs concentration in both springs were observed following autumn recharge events, while during peak discharge no detection above the limits of detection (LOD) was recorded due to dilution process. Contrarily to springs, groundwater from deep borehole exhibited highest total EOCs concentration under low flow conditions, underscoring the considerable karst aquifer vulnerability and its oligotrophic nature. The peak EOCs concentration in karstic Cetina River coincided with the river's lowest discharge. The highest mass flux of 1013 g/day was determined for very mobile pharmaceutical metformin detected in Cetina. The presence of potentially persistent to very persistent compounds, like DEET and 1H-benzotriazole, which exhibited highest detection frequencies across all sampling sites, was observed in association with varying hydrological conditions. Hypotheses regarding the occurrence of identified EOCs include surface contamination infiltrating directly through ponors and highly karstified areas, potential persistence in the epikarst and aquifer matrix, and site-specific contamination sources for compounds such as 1H-benzotriazole, gabapentin, and ketoprofen found in groundwater. To evaluate the risk of inadvertent human exposure to EOCs across various age groups, we utilized measured spring concentrations and calculated drinking water equivalent levels (DWEL), which ranged from 1.4 μg/L for cotinine to 503 mg/L for sucralose, both detected in Jadro spring. Although EOCs concentrations in ng/L are unlikely to pose a significant risk to healthy population, long-term exposure to EOCs mixture remains unknown. Given scarcity of research on EOCs in karst environments on both global and national levels, our study enhances comprehension of their occurrence and behaviour across different karst water resources that hold crucial importance for drinking water supply in regions like Dinarides.

1. Introduction

Emerging organic contaminants (EOCs), a specific group of existing or newly synthesized anthropogenic chemicals, have been ubiquitously ascertained in all water environment compartments in ng/L to μg/L concentrations [1]. Despite growing concerns regarding their continual emission and potential detrimental effects on aquatic ecosystems and human health, still an immense array of these natural or synthetic organic compounds remain unmonitored and unregulated in groundwater [2].

The substantial permeability of karst medium, direct infiltration via *ponors*, shafts and caves, preferential and rapid groundwater flow through systems of enlarged fractures and connected conduits, result in water resources of great production potential but also cause high intrinsic vulnerability to contamination [3,4]. Because of distinctive anisotropy and heterogeneity of surface and underground structures, karst aquifers present perplexing transport systems of highly variable flow dynamics, posing challenges for identifying and quantifying contaminants moving through them [5].

Contrarily to numerous EOCs studies recently conducted for surface waters [6,7], the paucity of EOCs studies in groundwater [8–10] and especially karst aquifers (reviews: [11,12]; nation-wide

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Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.emcon.2024.100327

studies: [13–16]) is conspicuous. Pharmaceuticals and pesticides are the predominant EOCs types in karst groundwater worldwide and Dinaric karst, typically exhibiting lower detection frequencies and maximum concentrations compared to other aquifer types [12,16]. However, these compounds can induce harmful effects in non-target aquatic organisms even at low concentrations [17–20], with human and veterinary antibiotics as well as some non-antibiotic pharmaceuticals facilitating spread of antibiotic resistance genes [21,22].

Investigating EOCs has the potential to enhance our comprehension of karst hydrogeology, by offering insights into the contaminant storage [23], transport mechanisms [24,25], and the characteristics of distinct sections of the aquifer (matrix-fractures-conduits), which collectively govern the contaminant attenuation capacity of the system [26]. Moreover, certain EOCs have proven instrumental in aquifer vulnerability assessment [27], as novel tracers used simultaneously with conventional ones [26] or as pollution source-specific indicators [24–26,28–30].

The primary objective of this research paper was to gain comprehensive insights into the EOCs dynamics in Dinaric karst aquifer, particularly focusing on two large springs used for local and regional water supply. One of the hypotheses put forward in this study was that EOCs occurrence and dynamics would mirror the karst aquifer behaviour under varying hydrological conditions. We assume EOCs concentrations to be low or absent in both surface and groundwater during the peak discharge due to significant dilution effect within the expansive karst system. Additionally, we postulate that mobile and persistent EOCs will be recorded in water resources during baseflow period.

In a complementary paper by Selak et al. [31], the focus shifted to assessing the environmental risks of identified EOCs in terms of persistence, mobility and toxicity, particularly concerning ecological health. Consequently, a pivotal research question emerged: Do the detected emerging organic contaminants present a potential risk to human health through the consumption of drinking water? By addressing this research question and investigating various aspects of EOCs dynamics and associated risks, this study aimed to enhance the understanding and management of water resources in the Dinaric karst.

2. Study area

A typical Dinaric karst catchment of large Jadro and Žrnovnica springs, in southern Croatia, was chosen as a study area (Fig. 1). From Jadro spring situated at 35 m a.s.l. to the elevated peak of Mosor Mountain at 1339 m a.s.l., the immediate catchment exhibits a dynamic and intricate morphology characterized by rolling hills and mountains adorned with sparse Mediterranean vegetation. Interspersed between these geographical features are several karst *poljes*, each hosting smaller settlements and areas of arable land. Morphological structures have a predominant and typical Dinaric orientation of NW-SE direction. The estimated catchment size ranges from 250 to 500 km^2 [32,33], as only southern and northern catchment boundaries (mainly topographic) are determined with a higher level of certainty. The western and eastern boundaries are hypothetically delineated based on several tracer tests [34–38]. The geological and hydrogeological features of study area have been thoroughly documented in previous studies [39–44].

The catchment is dominantly made of highly permeable carbonate rocks of Mesozoic and partly Eocene age (Fig. 1), resulting in absence of surface watercourses. Impermeable Eocene flysch deposits in coastal area and Triassic clastites to the north of Muć *polje*, act as hydrogeological barriers, limiting and directing groundwater flow [42]. Superficial deposits of low or medium permeability found in karst *poljes* (Muć, Dugopolje, Bisko) cause a temporary accumulation of water and its gradual release into karst underground. During long-lasting intensive rainfall, *ponors* at the edge of karst *poljes* are activated, contributing to quick-flow component. The substantial aquifer's storage capacity was confirmed by groundwater balance calculations, showing water accumulation during autumn season [33]. Jadro and Žrnovnica springs are formed at the contact of permeable carbonate rocks of hinterland and impermeable coastal flysch belt at the foot of Mosor Mountain. Both springs' discharge is generated by the same hydrological processes, and groundwater exchange between them exists [46]. The mean, maximal, and minimal measured discharge of Jadro spring is 9.51 m^3/s, 56.62 m^3/s, and 3.73 m^3/s respectively (10-year period 2011–2021). The mean, maximal and minimal measured discharge of Žrnovnica springing zone is 1.76 m^3/s, 17.0 m^3/s, and 0.27 m^3/s respectively (2011–2021). Jadro holds regional water supply importance for City of Split and surrounding municipalities, while Žrnovnica supplies adjacent settlement and is used for local irrigation.

Several studies reported inter-catchment perennial groundwater flows from Cetina River catchment in the east (Fig. 1) [33,34,47], dependent on groundwater levels [48]. Hydroelectric power plants constructed along Cetina altered hydrological regimes of Jadro (increased spring discharge) and Žrnovnica (prevented drying up during hydrological minimums since 1961) [49,50].

The ecotoxicological implications of EOCs occurrence within the present Dinaric karst aquifer, specifically in the Jadro and Žrnovnica springs, Gizdavac borehole, as well as Cetina River, have been comprehensively reported in the study conducted by Selak et al. [31,51].

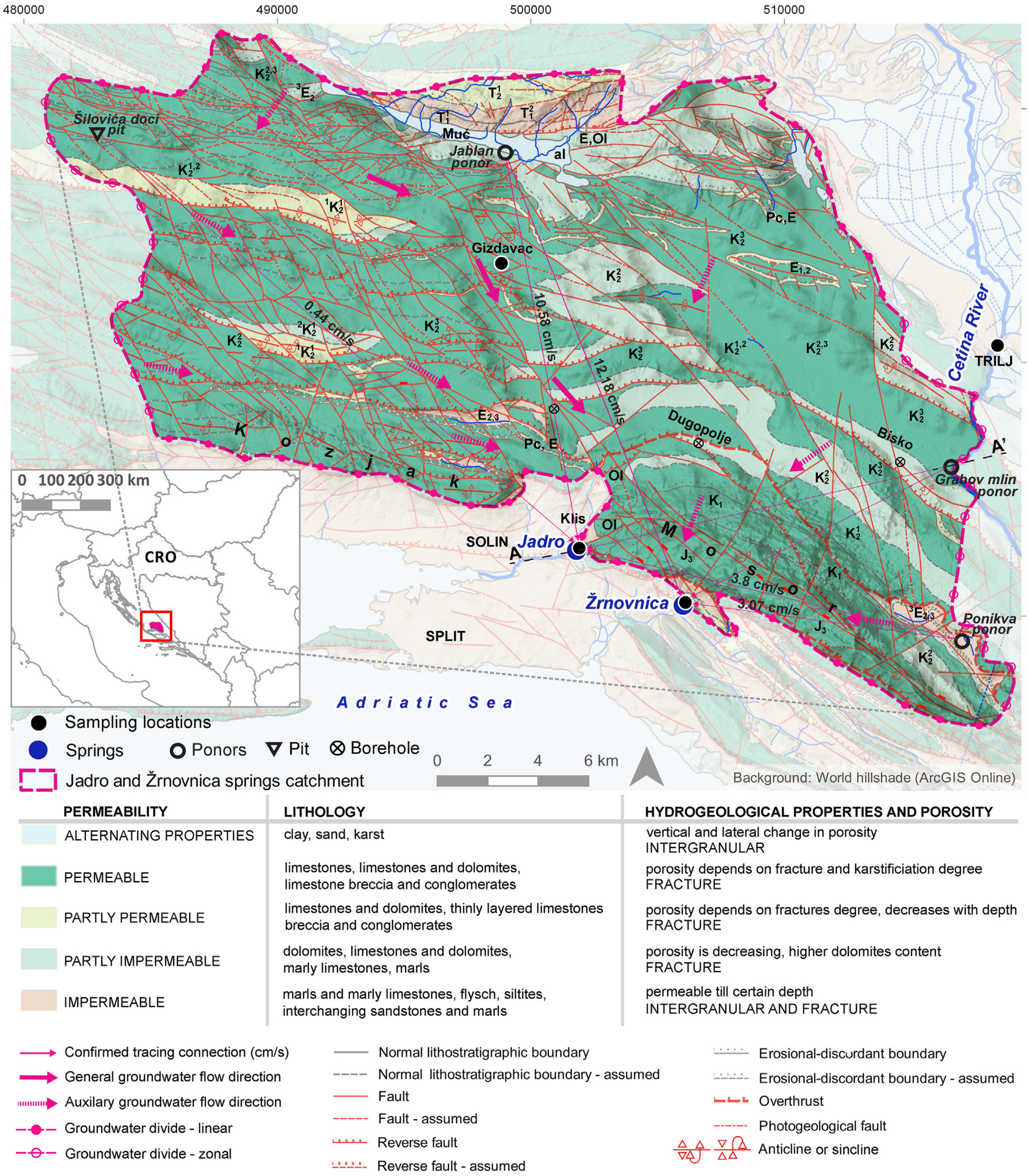
3. Materials and methods

3.1. Sampling and analysis of emerging organic contaminants

To assess EOCs occurrence in investigated catchment, we collected samples at 3 types of observation points: two large karst springs (8 samples at Jadro and 7 at Žrnovnica), one deep borehole (6 samples from Gizdavac), and a karstic river (7 samples from Cetina) (Fig. 1). Sampling campaigns, procedures, and transport preservation methods were described by Selak et al. [31,51]. Vltava River Basin Authority laboratory in Czech Republic employed 1290 Ultra-High-Performance Liquid Chromatograph (UHPLC in electrospray ionization ESI+ and ESI− modes) coupled with an Agilent 6495B Triple Quad Mass Spectrometer (MS/MS) for the analysis of 102 compounds in samples from campaigns of October 2019 to December 2021, at all locations. 740 compounds in Jadro samples from two campaigns (March and October 2019) were analyzed using Agilent 6540 Ultra-High-Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) Liquid Chromatography coupled with Mass Spectrometry (LC/MS) in the UK National Laboratory Services. Sample preparation, analyte list with limits of detection (LOD), analytical methods utilized at both laboratories, and EOCs types were previously reported by Selak et al. [31,51].

3.2. Spatial analysis of potential contamination sources and conceptual transport model

The handling and graphical data processing on a spatial scale were carried out using QGIS Version 2.18.21. Insights into spatial distribution of potential EOCs sources were obtained by following European (COST 620) guidelines for identifying and mapping



PERMEABILITY	LITHOLOGY	HYDROGEOLOGICAL PROPERTIES AND POROSITY
ALTERNATING PROPERTIES	clay, sand, karst	vertical and lateral change in porosity INTERGRANULAR
PERMEABLE	limestones, limestones and dolomites, limestone breccia and conglomerates	porosity depends on fracture and karstifiication degree FRACTURE
PARTLY PERMEABLE	limestones and dolomites, thinly layered limestones breccia and conglomerates	porosity depends on fractures degree, decreases with depth FRACTURE
PARTLY IMPERMEABLE	dolomites, limestones and dolomites, marly limestones, marls	porosity is decreasing, higher dolomites content FRACTURE
IMPERMEABLE	marls and marly limestones, flysch, siltites, interchanging sandstones and marls	permeable till certain depth INTERGRANULAR AND FRACTURE

Fig. 1. Study area - Dinaric karst catchment of Jadro and Žrnovnica springs (cross-section is marked with A-A′) (modified from Ref. [45]; tracer test data: Jablan ponor and Grabov mlin ponor [34], Ponikva ponor [36], and Šilovića doci pit [37]).

hazardous activities within karst catchment [52] (see Supplementary materials for input data sources details). The hazards inventory included: point (industrial and municipal effluents, landfills and illegal dump sites), linear (traffic corridors), and polygonal diffuse sources (settlements' sewerage systems and cesspits, agricultural land, industrial sites).

For conceptual EOCs transport model, Plugin Profile tool 4.1.8 in QGIS and a digital relief model of Croatia were used to obtain a cross-section A-A' of Jadro and Žrnovnica catchment.

3.3. Hydrogeological data for characterization of karst catchment

Hourly electrical conductivity data was collected using Onset's HOBO U24 data loggers at Jadro and Žrnovnica springs (installed on March 10, 2021). Additional specifications and technical details of the data loggers are available in the Supplementary materials. Regular monthly field surveys for years 2019 and 2020 was impeded due to the unprecedented Covid-19 pandemic. Discharge and precipitation data from 2019 to 2021 were acquired from the Croatian Meteorological and Hydrological Service. The Thiessen polygons method was employed to delineate the influence area of each rain gauge station within catchment. Croatian Waters provided Cetina discharge (2019–2021), groundwater levels and electrical conductivity data for the logger installed in Gizdavac borehole, covering period from 2010 to 2021.

3.4. Human exposure to EOCs in drinking water

We evaluated potential risk of EOCs in drinking water sources for different age groups by comparing the recorded maximum concentrations with drinking water equivalent levels (*DWEL*). *DWELs* were quantitatively estimated using an approach similar to that employed in recent research papers [53–56] (Eq. (1)):

$$DWEL = \frac{ADI \times BW \times HQ}{DWI \times AB \times FOE} \quad (1)$$

where *ADI* represents acceptable daily intake, *BW* denotes the 50th percentile value of body weight per specific age group, *HQ* indicates Hazard Quotient set at 1, *DWI* stands for drinking water intake, *AB* represents gastrointestinal absorption rate set at 1, and *FOE* is the frequency of exposure calculated as 0.96 (equivalent to 350 days out of 365). This frequency accounts for a two-week vacation away from home each year, as specified by US EPA [57]. The *BW* and *DWI* values utilized in the estimation were sourced from the study conducted by de Jesus Gaffney et al. [53].

To derive Risk Quotients (*RQs*) for detected EOCs, we utilized the ratio between maximal recorded concentrations and estimated *DWELs*. Specifically, EOCs with $RQ \geq 1$ are considered to have the potential to induce adverse effects on human health over a lifetime of water consumption. EOCs with $RQ \geq 0.1$ are deemed to warrant close monitoring in drinking water [58].

4. Results and discussion

4.1. Identification of EOCs sources in a karst catchment

The EOCs are mainly synthetic products not occurring in nature. Detection of pharmaceuticals in springs of Jadro, Žrnovnica, groundwater and Cetina River is a direct indication of an anthropogenic influence through wastewater or manure, as these substances are solely used in human and veterinary treatment. According to Loborec et al. [59], Jadro spring experiences increasing anthropogenic pressures on water quality. The study area is characterized by sparsely populated and spatially dispersed settlements with active agriculture and present industrial activities, as depicted in Fig. 2. Smaller communities predominantly rely on septic tanks, of questionable impermeability, as sewerage systems are absent. Larger settlements with sewer networks are potential EOCs source due to possible leaks. Fig. 2 illustrates a map depicting potential EOCs sources related to industrial, agricultural, and urban activities within the study area (Table S1). Similarly, Loborec et al. [59] developed hazards map for Jadro and Žrnovnica catchment based on the COST 620 recommendations. Apart from most recent information from relevant databases, we included data from the newly established Register of illegally disposed waste locations. Groundwater flow directions determined with tracer test studies are given as well. The identified general groundwater flow direction from NW to SE (towards Jadro spring) corresponds to regional Muć-Gizdavac-Klis fault (Fig. 1). Using COP + K method, Loborec et al. [60] assigned a high to very high intrinsic groundwater vulnerability to this area. Other regions of high intrinsic groundwater vulnerability include Mosor Mountain and Dugopolje. Inoperable state of a deep borehole in Dugopolje (Fig. 1) hindered investigating whether wastewater effluents from industrial, commercial sites and a pharmaceutical laboratory in Dugopolje could be EOCs sources.

The conceptual transport model illustrates cross-section (A-A') positioned along the proven groundwater flow direction between Grabov mlin *ponor* near Cetina River and Jadro spring (Fig. 3). Tracer test verified the existence of inter-catchment groundwater flow facilitated by a well-developed conduit network connecting this specific region and Jadro spring [34]. The model highlights EOCs pathways from potential contamination sources to the water resources of Jadro and Žrnovnica catchment. The contamination origin-pathway-target schematics was consulted in previous research [9,61,62] and adapted to our study area. Guided with EOCs sources map (Fig. 2), we marked wastewater effluents, agriculture and waste dumps as potential major contamination pathways on conceptual model. Fig. 3 illustrates physical and biochemical processes governing solute transport and groundwater chemistry, as described by Perrin et al. [63]. Contaminant retardation and biochemical processes listed in Fig. 3 primarily occur within the soil layer. However, the area under investigation is distinguished by the prevalent absence of protective soil layers, which are limited to karst poljes (Bisko, Muć polje and Dugopolje, Figs. 1 and 2), where they are typically found in up to several meters thickness.

4.2. Characterising the occurrence of EOCs

EOCs main categories and concentrations per location can be found in Selak et al. [31,51]. The October (only detections > LOD were pharmaceuticals diclofenac in 28.7 ng/L and metformin in 23.6 ng/L in Cetina River) and December 2021 (no detection > LOD) sampling campaigns were not included in Selak et al. [31,51]. To the best of our knowledge, this was the first detection of diclofenac in Croatian karst water. Despite differences in detection limits and analysis conducted in various laboratories, the EOCs concentrations in Jadro and Žrnovnica springs are comparable to those observed by Lukač Reberski et al. [16] in other karst springs across Croatia. Both springs exhibit concentrations ranking among the highest ones observed in Croatian karst. The total mass fluxes of detected EOCs per spring sample reached 7.9 g/day at Žrnovnica and 66.7 g/day at Jadro, aligning with findings of Lukač Reberski et al. [16], who reported total mass fluxes in Croatian karst springs of up to 90 g/day. The highest mass flux of 1013 g/day in Cetina River was determined for very mobile pharmaceutical metformin. Readers are referred to Table S2 showing mass fluxes of three most frequently detected EOCs in spring and river water. EOCs detected within Jadro and Žrnovnica catchment were classified as persistent or potentially persistent to very persistent and mobile to very mobile [31].

DEET and 1H-benzotriazole, two most frequently detected compounds in our study area, were also listed among the top 20 compounds in global karst resources, based on their maximum concentrations. 1H-benzotriazole was identified as one of the top 20 most frequently detected EOCs in karst [12]. Unlike other Croatian karst springs investigated by Lukač Reberski et al. [16] in 2019 and analyzed in NLS laboratory along Jadro samples of March

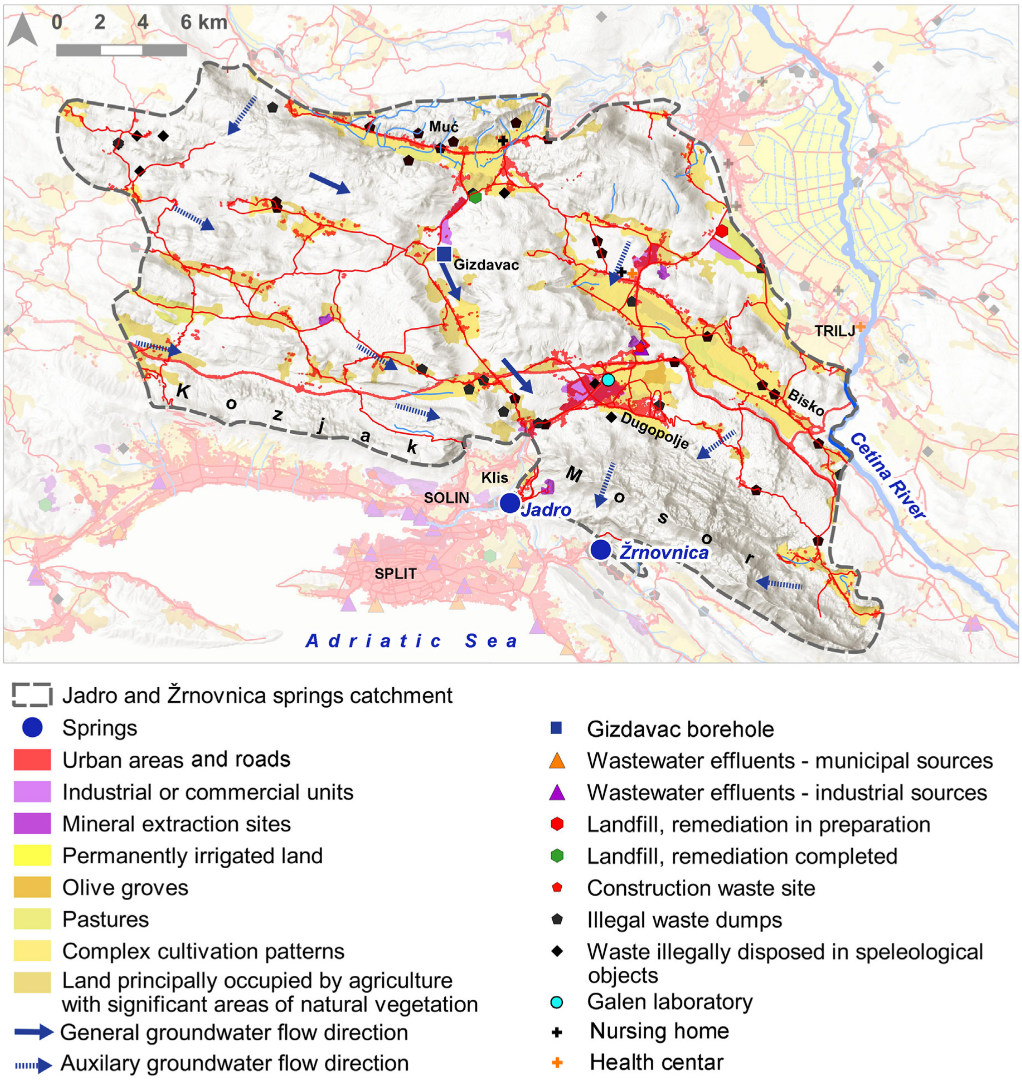


Fig. 2. Potential EOCs sources in Jadro and Žrnovnica springs catchment.

and October 2019, the presence of DEET has solely been observed within Jadro and Žrnovnica catchment. Despite relatively comparable LODs of both laboratories, with NLS laboratory using an LOD of 10 ng/L and Czech laboratory having an LOD of 3 ng/L, DEET detection was only confirmed in aforementioned catchment. This peculiarity merits further investigation to comprehend the underlying factors contributing to disparate occurrences of DEET in these specific locations. The NLS laboratory exhibited a significantly higher LOD for 1H-benzotriazole, set at 5000 ng/L than the Czech laboratory, which employed a considerably lower LOD set at 7 ng/L. Consequently, only Jadro and Žrnovnica catchment showed detectable levels of 1H-benzotriazole. This highlights the importance of considering analytical capabilities of laboratories when conducting environmental monitoring and assessment of EOCs in karst aquifers. The widespread detection of DEET and 1H-benzotriazole across water resources of our research area suggests their potential as markers for anthropogenic contamination [31]. DEET mass fluxes ranged from 0.4 g/day in Žrnovnica to 31.52 g/day in Jadro, while 1H-benzotriazole had values of 5.3 g/day in Žrnovnica up to 47.7 g/day in Jadro spring (Table S2). Cetina River displayed much higher mass fluxes of DEET (up to 466.5 g/day) and 1H-benzotriazole (983.5 g/day). Another highly mobile and frequently detected EOC, namely metformin, exhibited mass fluxes ranging from 129.1 g/day to 1012.6 g/day in Cetina River (Table S2). Metformin ranked among the top 20 most frequently used medications in Croatia between 2019 and 2021. Intriguingly, the presence of pharmaceuticals like paracetamol, ibuprofen, and ibuprofen-carboxy was exclusively recorded in conjunction with the peak mass flux of metformin, particularly in October 2019. This co-occurrence strongly suggests the potential influence of wastewater discharge, possibly originating from leaking sewer systems or septic tanks in the neighbouring settlements. Contrarily to our

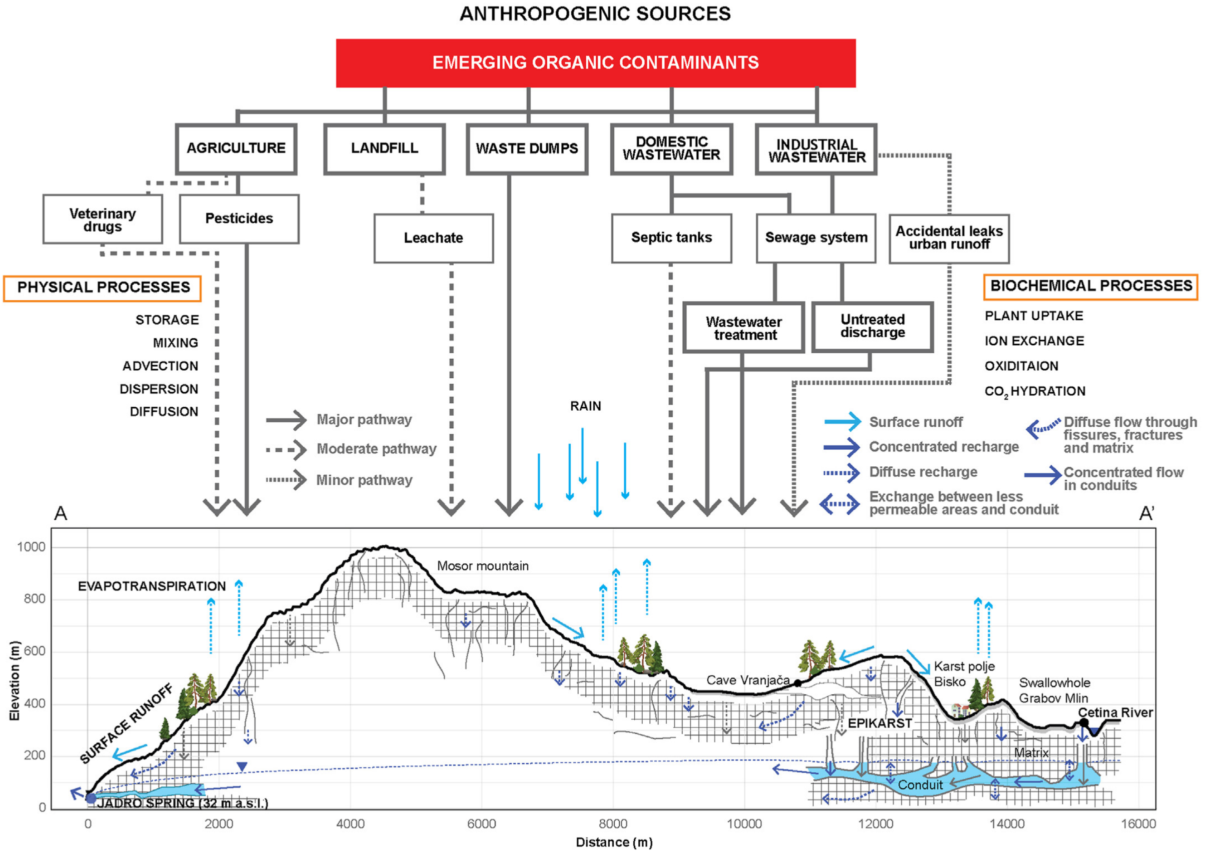


Fig. 3. The conceptual schematic model of potential EOCs sources and transport in karst aquifer of Jadro and Žrnovnica (Žrnovnica spring and Gizdavac borehole are not visible in cross-section A-A′). The cross-section position is visible in Fig. 1.

findings for Cetina River, Doummar & Aoun [64] report lower mass fluxes of ibuprofen, diclofenac and caffeine in a rural karst river, which they also attributed to domestic wastewater effluents. Among EOCs detected in our study, ibuprofen stands as the sole compound featured in the Voluntary Groundwater Watch List proposed in 2019 [2]. In the 2022 proposed Directive [65] amending Directive 2000/60/EC, Directive 2006/118/EC, and Directive 2008/105/EC, a groundwater quality standard for pharmaceuticals was introduced, set at a cumulative total of 250 ng/L. Our findings reveal cumulative EOCs concentrations in springs and groundwater well below this proposed limit.

4.2.1. *Linking EOCs occurrence with hydrogeological characteristics*

Depending on catchment structural characteristics, temporal variability in spring discharge, pollution sources, and contaminant properties (importantly solubility and sorbability), EOCs occurrence and magnitude of concentrations can differ considerably [12,66]. The aquifer's hydrogeological properties determine how quickly contaminants enter, are attenuated, transported or stored in the system. Surface water inputs through ponors or large fractures allow contaminants to enter aquifer with little to no filtration, impeding physio-chemical attenuation. Verifying the link between spring discharges and EOCs proves challenging due to insufficient information on the contamination sources, lack of site-specific monitoring, and considerable temporal variation in spring discharge trends and heterogeneity of groundwater flow in karst. The hydrographs of Jadro and Žrnovnica springs exhibit typical characteristics observed in coastal Dinaric karst catchments, with rising limb of discharge curve starting in early autumn and ending in late spring, followed by prolonged recession periods with minimal effective rainfall during summer months (Fig. 4). Recession periods and groundwater recharge episodes observed in Gizdavac borehole align with springs' hydrographs (Fig. 4). Both springs respond to rainfall events in less than 24 h, exhibiting important quick-flow component in wet and dry years similarly [67]. Sharp drop in electrical conductivity in both springs are direct evidence of a highly karstified system, underscoring its substantial vulnerability to potential surface-originated contamination. Electrical conductivity exhibits rising values in summer to autumn periods, while minimums occurred during elevated discharges in winter and spring (Fig. 4).

To gain insights into EOCs dynamics in monitored water resources under varying hydrological conditions, concentrations of target EOCs can be observed. Fig. 5 illustrates noticeable variations in total EOCs concentrations across sampling campaigns. In December 2021, during elevated discharge at Jadro and Žrnovnica springs (29.9 m^3/s and 4.8 m^3/s, respectively) and increased groundwater levels at Gizdavac borehole, no EOCs were detected above LOD (Figs. 4 and 5). The sharp drop in electrical conductivity followed the discharge peak, observed in this month, pointing out

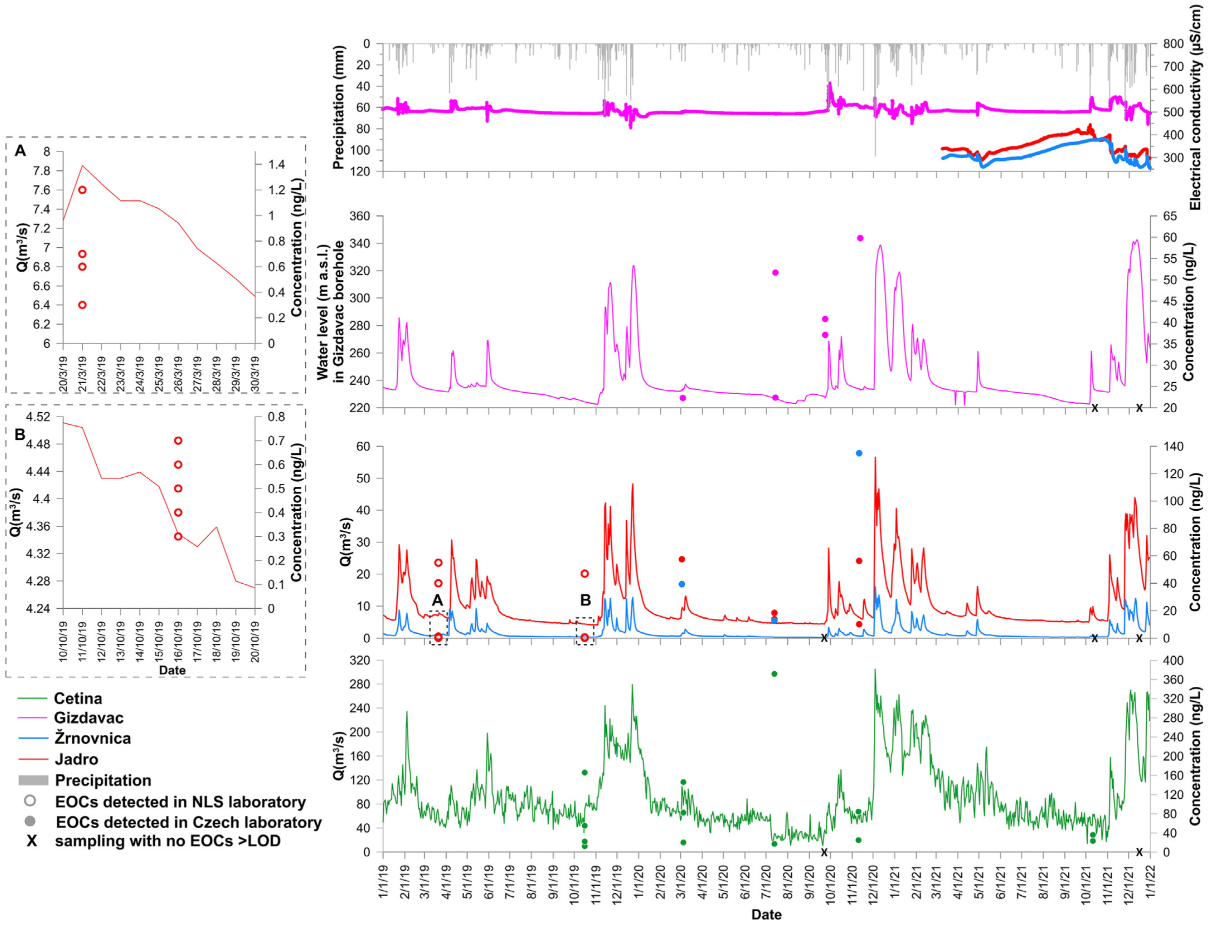


Fig. 4. Temporal variations of discharge and electrical conductivity at Jadro and Žrnovnica springs, groundwater level and electrical conductivity at Gizdavac borehole, Cetina River discharge, catchment area total precipitation, and detected EOCs concentrations.

the rapid water infiltration. As expected, no EOCs were detected in the Cetina River, which had discharge reaching 181 m^3/s. This lack of detections can be attributed to the significant dilution processes that occur under high-flow conditions. Due to the limited sample number and low detection frequency, assessing the statistical significance of seasonal variability for individual EOCs was not feasible. There was no significant correlation (p-value <0.05) between total EOCs concentration per sampling site and same-day discharge values of Jadro, Žrnovnica, and Cetina.

In October 2021, when baseflow was interrupted by infiltrated rainfall that was falling from the start of the month, there were no EOCs detections above LOD. No detection above LOD was also recorded in September 2020 when at the end of longest recession period both springs exhibited their lowest discharges, and electrical conductivity was reaching its highest peak as observed by Jukić et al. [68] at Jadro. Contrarily to springs and river, groundwater sample collected in September 2020 exhibited a peak total EOCs concentration of 77.9 ng/L, namely a nonsteroidal anti-inflammatory drug ketoprofen (40.8 ng/L) used in the treatment of acute pain and chronic arthritis and an antiepileptic adjunctive and neuropathic pain reliever gabapentin (37.1 ng/L). The average concentration of ketoprofen reported in an EU-wide groundwater survey was 26 ng/L [8]. Ketoprofen is potentially persistent to very persistent compound [31,51], while gabapentin is rapidly biodegradable [69] and does not persist in the environment. Both compounds are very mobile with log *Koc* values of 0.2 [70] and 0.36 [71], respectively. Regardless of fast biodegradation, gabapentin was observed in deep aquifer parts probably due to its high mobility, the oligotrophic nature of karst system, and potential continuous release from a contamination source. This potential source of both medicines could be traced to a nursing home situated upstream of the borehole in Muć *polje*. The existence of an underground connection between Jablan *ponor* at the south edge of Muć *polje* (Fig. 1) and Jadro and Žrnovnica springs is confirmed with the tracer test with high apparent groundwater flow velocities of 10.58 cm/s and 12.18 cm/s, respectively [34]. Despite the concentration higher than the EU average, mobility, persistency, and detection in aquifer's part where rapid groundwater flow towards Jadro and Žrnovnica exists, ketoprofen was not detected at springs. Bearing in mind the significantly karstified medium with expected limited to no attenuation, we assume how this result indicates proximity of contamination source to Gizdavac borehole. According to Jukić et al. [68], rainfall from Muć exerts minimal influence on Jadro spring, with observable effects confined to the quick-flow component.

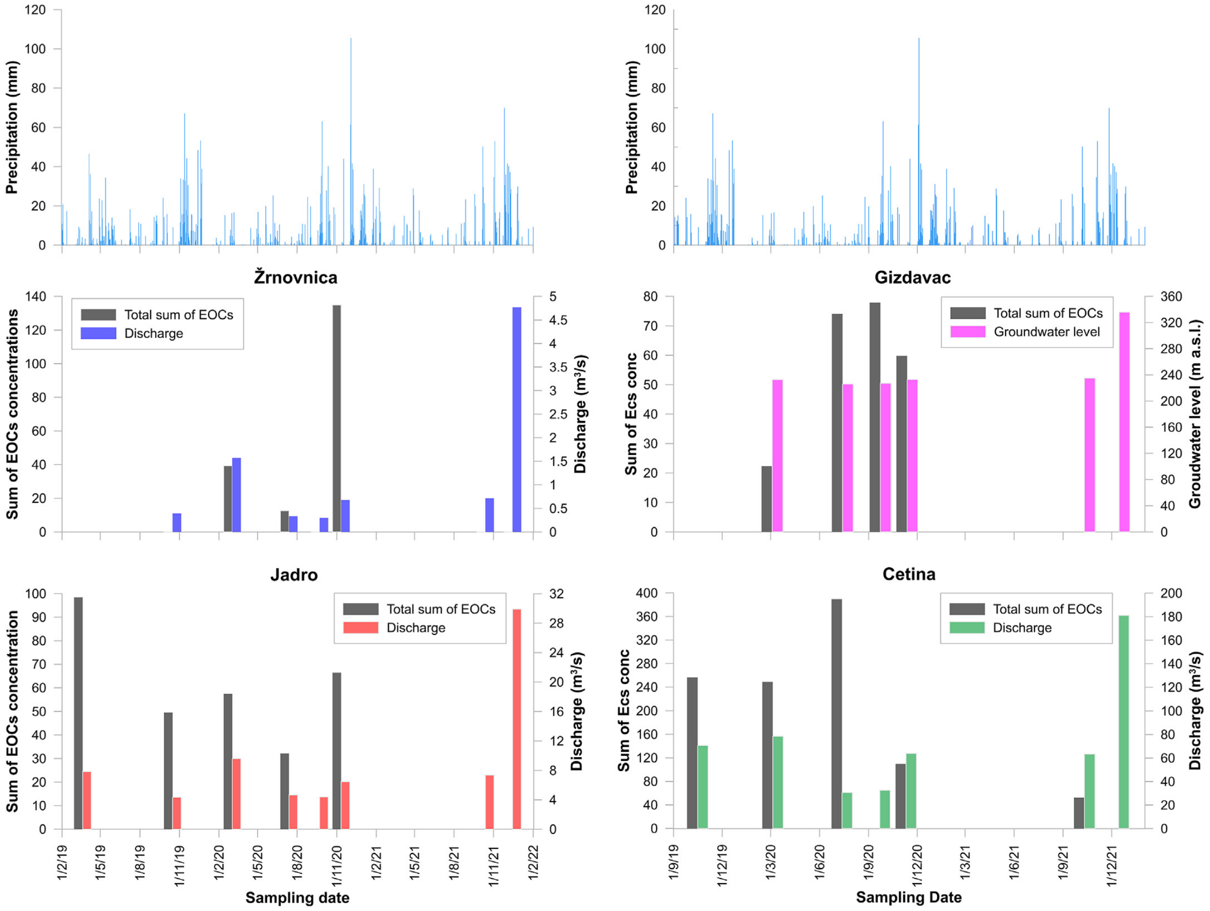


Fig. 5. Total EOCs concentration per sampling campaign versus daily discharge measured at chosen locations and total calculated precipitation for the catchment area. Discharge values are shown only for conducted campaigns.

Ketoprofen was detected during baseflow conditions. For any further conclusion, more detailed research is needed.

The highest total EOCs concentration at Jadro was observed in March 2019 in between winter and spring aquifer recharge periods, reaching a value of 98.4 ng/L. These peak concentrations occurred with slightly elevating discharge attributed to the seasonal spring rains, which also caused a decrease in electrical conductivity [68]. Reported EOCs results of March and October 2019 for Jadro incorporate data from both laboratories. When exclusively considering Czech laboratory results starting from October 2019, the highest total EOCs concentrations at Jadro and Žrnovnica coincided in November 2020, measuring 66 and 135 ng/L, respectively. This occurred after several autumn aquifer recharge events, likely flushing contaminants accumulated in various parts of the system during summer (Figs. 4 and 5). Contrarily, Cetina River exhibited highest total concentration in July 2020 (398.3 ng/L) which coincided with the lowest river discharge of 30.6 m^3/s at Trilj žičara station.

During six concurrent campaigns from March 2020 to December 2021 at Gizdavac and springs, groundwater from borehole exhibited both higher total EOCs concentration and a greater number of detections above LOD (6 detections) than Jadro (5 detections) and Žrnovnica (3 detections). Bexfield et al. [66] noted a significant decrease in detection number with increasing well depth. Despite Gizdavac being a deep borehole of considerable depth (entrance at ~350 m a.s.l., screen interval 185–277 m depth), its location in highly karstified area allows rapid infiltration with presumably no attenuation. The borehole is situated in a sparsely populated area, comprised of smaller villages and an industrial zone containing various establishments, including a metalwork facility, boatyard, building stone exploitation zone, and two food-processing companies. Industrial applications as anticorrosive in metalworking or use as a dishwashing agent could be potential contamination sources of 1H-benzotriazole in sampled groundwater. Several studies [72–74] have substantiated the potential of benzotriazoles as effective tracers of wastewater impacts on groundwater and as a valuable tool for delineating catchment areas [27]. The observed concentrations of 1H-benzotriazole in our study area suggest its occurrence at springs and in groundwater during spring runoff episode but also amid baseflow conditions in groundwater and river. In March 2020, 1H-benzotriazole had concentrations abundance order of 57.5 ng/L at Jadro, 39.3 ng/L at Žrnovnica, and 22.3 ng/L in groundwater from Gizdavac. October 2020 campaign recorded 372 ng/L of this EOC in Cetina River and 51.7 ng/L in groundwater. Such elevated concentrations in river and groundwater highlight the likelihood of location-specific contamination sources. Additionally, 1H-benzotriazole persistence in the aquifer is evident, emphasizing the potential risk associated with long-lasting presence. The groundwater linkage between Grabov mlin *ponor* and both Jadro and Žrnovnica, as well as the connection between Jablan *ponor* and springs, as substantiated by the tracer tests and illustrated in Fig. 1, provides support for the potential utilization of 1H-benzotriazole in delineating karst aquifer. Nonetheless, the limited sample number and highly karstified nature of the area warrant additional sampling campaigns to validate this finding.

Seasonal variations in DEET concentrations, though studied sparingly, tend to show higher levels in summer and lower in winter periods [75,76]. In summer, DEET outflowed in lower concentrations with groundwater's long-term component at our springs opposite to late autumn periods when discharge episode started and DEET was detected in around 5-fold higher total concentration at catchment scale. Sorensen et al. [77] reported ubiquitous DEET in karst groundwater, with median concentrations 5-fold higher during wet season, possibly due to diffuse source and contaminant pulse accompanying recharge. In July 2020, DEET concentrations had an abundance order as follows: groundwater 22.4 ng/L > Cetina 17.3 ng/L > Jadro 13.8 ng/L > Žrnovnica 12.7 ng/L. The significant increase in electrical conductivity values during this sampling period (as seen in Ref. [68]), indicates draining of water stored in aquifer matrix. In November 2020, the concentrations were higher at Žrnovnica 135 ng/L > Cetina 84.5 ng/L > Gizdavac 59.8 ng/L > Jadro 56.3 ng/L. Given its common use as an insect repellent, DEET is readily available in consumer products and is frequently applied during summer and early autumn seasons against mosquitos. Elevated DEET concentrations observed during dry period in groundwater, contrarily to springs and river, suggest a constrained biodegradation process within the aquifer. This observation suggests limited attenuation capacity of potentially persistent and highly mobile compounds like DEET in karst aquifer, rendering groundwater resources highly susceptible to surface contamination.

4.3. Human exposure to EOCs via Jadro and Žrnovnica springs

Despite growing public concern about potential health implications caused by EOCs in water environment, there are currently no guidelines specifying the assessment of indirect human exposure. The first Watch list of compounds of concern to be monitored in drinking water, adopted in January 2022, includes guidance values for endocrine-disrupting substances 17-beta-estradiol (1 ng/L) and nonylphenol (300 ng/L) [78]. Nonylphenol was not analyzed in our study, while 17-beta-estradiol was analyzed in 4 out of 9 sampling campaigns with no detections > LOD.

Assessing EOCs risk to consumers can be approached through measured or predicted environmental concentrations [79]. Predicting contaminant levels and exposure in karst is complex task with substantial uncertainty, due to impossibility to fully capture the system's intrinsic properties (heterogeneity, anisotropy, and non-linearity) and account for numerous parameters affecting intricate hydrodynamics and contaminant transport.

To address this challenge and the absence of specific guideline values for EOCs, our study adopted a methodological approach consistent with established practices in the field [54–56]. By employing a validated methodology, our aim was to provide a preliminary assessment of the potential human health risk posed by EOCs in the drinking water sources of Jadro and Žrnovnica catchment. We evaluated *RQs* using measured EOCs concentrations in drinking water resources and calculated *DWELs*, while considering the absence of contaminants removal during treatment, as water at observed springs is solely disinfected with NaClO. Table S3 shows the calculated *DWEL* and *RQ* values assigned to different age groups for EOCs detected in Jadro and Žrnovnica springs. Except for sulfamethoxazole, none of the compounds detected in spring water samples, do not appear on the List of classification by the International Agency for Research on Cancer (IARC). Sulfamethoxazole falls under the category of "not classifiable as to its carcinogenicity to humans" according to IARC. All identified EOCs are considered threshold chemicals per WHO Guidelines for drinking water quality [80].

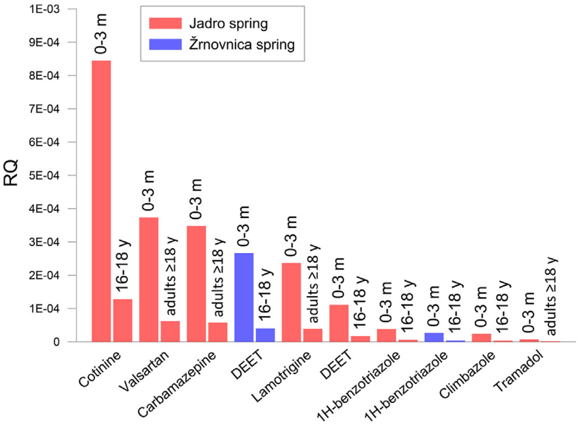


Fig. 6. Human health risk quotients (*RQ*) per age group for 10 detections of EOCs associated with the highest risks within study area. Graph also includes the lowest *RQ* values identified for those 10 EOCs detections.

DWEL values ranged between 1.4 μg/L for nicotine metabolite cotinine (age group 0–3 months, *BW* of 5.6 kg and *DWI* of 1.15 L) and 503 mg/L for artificial sweetener sucralose (age group 16–18 years, *BW* of 57 kg and *DWI* of 1.77 L) recorded at Jadro. The *RQs* for all detected EOCs were considerably below the threshold of 1, demonstrating that the current environmental levels of EOCs in Jadro and Žrnovnica springs do not pose a potential adverse risk to human health. *RQs* reached 0.0008 determined for cotinine in Jadro sample, while the highest although negligible human health risk at Žrnovnica spring was posed by DEET (Fig. 6, Table S3). The literature has documented a negligible human health risk posed individually by the majority of EOCs found in drinking water resources [53,58,81,82].

In our study *RQ* values were the highest for infants of age 0–3 months (Fig. 6). Sharma et al. [54], Kibuye et al. [55], and Sengar and Vijayanandan [56] also reported higher *RQ* values in children compared to adults, which supports the notion of increased susceptibility to EOCs in younger population. While our findings are indicative, it is crucial to acknowledge that these risk calculations have limitations. Specifically, the scope of our study, in terms of sampling size and frequency, may not fully capture the extent and variability of EOC presence and their associated human health risks. Additionally, the employed methodology does not account for the combined impacts of EOCs or potential chronic effects, a concern echoed in recent studies. Water managers and policymakers must consider this when setting drinking water quality standards, as synergistic and cumulative toxicological effects of EOCs mixtures are still not fully understood, and undesirable pharmacological effects may emerge with future elevated EOCs concentrations.

5. Conclusion

This study underscores the intricate nature of Dinaric karst aquifers and the necessity for comprehensive research on EOCs within these systems of vital water supply importance for countries like Croatia. Electrical conductivity fluctuations observed at springs indicated highly karstified system that due to rapid infiltration pathways is particularly vulnerable to potential surface-originated contamination. Analysis of springs, groundwater and river samples yielded evidence of EOCs presence within karst aquifer under varying hydrological conditions. As postulated, EOCs were not detected during peak high-flow owing to substantial dilution processes. The highest total concentrations were measured at springs following several significant autumn recharge events. This suggests that any surface contamination either infiltrates directly through *ponors* and highly karstified areas or it persisted in the epikarst and aquifer matrix, later being pushed to the springs by freshly infiltrated water. Contrarily, groundwater from deep borehole exhibited highest total EOCs concentration during baseflow period, indicating site-specific contamination source. The occurrence of gabapentin in groundwater, a biodegradable yet highly mobile EOC, indicated the oligotrophic nature of investigated system. Observed seasonality in markers of wastewater contamination, namely DEET and 1H-benzotriazole, along with their ubiquitous presence across sampling sites, suggest limited attenuation of persistent and mobile compounds within karst aquifer.

Quantitative estimation of human health exposure suggested that consumption of water containing recorded EOCs concentrations is unlikely to pose health risks. However, applied method does not consider potential long-term and synergistic effects of EOCs mixtures. Moreover, the limited sample number may not fully depict the overall contamination scenario. Therefore, we strongly advocate for additional research and the establishment of routine monitoring protocols targeting EOCs in vulnerable karst aquifers. This is pertinent in light of anticipated rise in contamination mass loads projected in forthcoming years.

To reinforce the protection of karst groundwater resources, we further suggest initiating implementation of a voluntary Groundwater Watch List tailored to unique Dinaric karst characteristics. This list should be revised and expanded to include frequently detected EOCs, like DEET and 1H-benzotriazole. Aligning the current drinking water monitoring suite with the latest scientific findings on EOCs occurrence is essential for safeguarding water quality and public health. Despite limitations in presented research, like limited sampling due to Covid-19 restrictions and analysis cost constraints, there remains a crucial need for high-frequency sampling to better comprehend the EOCs behaviour under varying hydrological conditions. In-depth event analysis can shed light on potential existence of background levels of certain EOCs, indicative of their persistence in aquifers, and furnish additional evidence essential for future risk assessments.

Funding sources

This work was financially supported by Interreg Central Europe boDEREC-CE project co-funded by the European Regional Development Fund, GeoTwinn project funded under H2020-EU.4.b., [grant number 809943] and NONa project funded by National Recovery and Resilience Plan 2021–2026 of the European Union – NextGenerationEU. The field investigations and EOCs analysis are funded and supported by the Croatian Geological Survey, Department of Hydrogeology and Engineering Geology.

Conflict of interest

All authors declare that there are no competing interests.

CRediT authorship contribution statement

Ana Selak: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Jasmina Lukač Reberski:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Ivana Boljat:** Writing – review & editing. **Josip Terzić:** Writing – review & editing.

Acknowledgements

The authors express their gratitude to the Croatian Meteorological and Hydrological Service and Croatian Waters for the provided data. Presented research was conducted in the scope of the internal research project „NONa“ at the Croatian Geological Survey, funded by the National Recovery and Resilience Plan 2021–2026 of the European Union – NextGenerationEU, and monitored by the Ministry of Science and Education of the Republic of Croatia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2024.100327.

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Paper IV

Selak, A., Lukač Reberski, J., Briški, M., Selak, L., 2024. Hydrochemical characterization of Dinaric karst catchment in relation to emerging organic contaminants. Geol Croat 77(2), 145-158. https://doi.org/10.4154/gc.2024.10

Geologia Croatica
Journal of the Croatian Geological Survey
and the Croatian Geological Society

2024 | 77/2 | 145–158 | 6 Figs. | 2 Tabs. | 2 Supplements |

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Hydrochemical characterization of a Dinaric karst catchment in relation to emerging organic contaminants

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doi: 10.4154/gc.2024.10

Article history:
Manuscript recieved: April 18, 2024
Revised manuscript accepted: May 27, 2024
Available online: June 21, 2024

Abstract

The main findings of a hydrochemical investigation conducted within a typical Dinaric karst catchment located in Southern Croatia are outlined. The studied aquifer is drained by the Jadro and Žrnovnica springs, which are important for the regional and local water supplies, respectively. Presumably, there is intercatchment groundwater flow coming from the neighbouring Cetina River catchment. Various factors governing aquifer hydrochemistry and their interplay with emerging organic contaminants (EOCs) that were detected at different water resources in ng/L concentrations was assessed. A total of 26 sampling campaigns (October 2019 – October 2022) were conducted at two springs, in a river and at a deep borehole, all representative of this complex hydrogeological system. Assessment of major ion constituents and saturation indices calculated with PHREEQC revealed the sampled water resources are of a Ca-HCO_3 type due to the predominant weathering of the carbonate mineral calcite. Sharp spikes observed in chemographs indicated a highly karstified system with an effective conduit network allowing rapid spring responses to precipitation events. Water resources are of good chemical status, as affirmed by anthropogenic contamination indicators, with nitrates, chlorides and sulphates all below maximum threshold values. Strong positive correlations were found between EOCs concentration, number of detected compounds, and nitrates in the Cetina River, indicating a common origin, most likely wastewater. Identification of persistent EOCs including widely used repellent N,N-diethyl-meta-toluamide (DEET) during base flow conditions and its strong positive correlation with the Ca^{2+} content in both the Cetina and Jadro samples, suggests potential storage in the epikarst and aquifer matrix. This coupling of conventional hydrochemical indicators and novel markers of anthropogenic impacts, including EOCs, in vulnerable karst water resources is a crucial advancement in the assessment and management of emerging environmental and potential human health risks. Such an approach is pivotal for the sustainable protection of hydrogeologically intricate sites.

Keywords: karst hydrogeology, major ions, isotopes, time series analysis, emerging organic contaminants, Jadro and Žrnovnica springs

1. INTRODUCTION

Karst aquifers are intricate, highly dynamic and heterogeneous systems with enlarged fractures, an often well-developed conduit network, direct surface-subsurface connection via ponors, and high hydraulic conductivity, leading to preferential flow pathways and significant drinking water resources susceptible to anthropogenic contamination (PADILLA & VESPER, 2018; ŽIVANOVIĆ et al., 2022). Interpreting hydrochemical spring responses and identifying the origins of chemical compounds in karst water, some of which as natural traces allow the understanding of system functioning and structure (PERRIN, 2003; MUDARRA et al., 2011; SHEIKHY NARANY et al., 2019), can help to pinpoint contamination sources and pathways (HILLEBRAND et al., 2014). The hydrochemical research of the karst aquifer in question is fundamental in characterising the behaviour and origin of emerging organic contaminants (EOCs). In 26 sampling campaigns within the Jadro and Žrnovnica Dinaric karst catchment (Croatia), water samples were collected for ion and stable isotope analysis. Notably, in seven of these campaigns, samples were specifically taken for EOC analysis. EOCs are typically encountered in karst water resources at concentrations ranging from ng/L to µg/L, encompassing a myriad of anthropogenic organic compounds (LUKAČ REBERSKI et al., 2023). Some EOCs exert adverse effects on aquatic ecosystems and human health (CIZMAS et al., 2015; SCHRIKS et al., 2010).

Numerous uncertainties persist regarding the presence and behaviour of EOCs within large hydrogeological domains (LLAMAS et al., 2022). Due to diverse physico-chemical properties and sources, coupled with the intricacy of Dinaric karst structures and (ground)water pathways, datasets and analyses necessary to comprehend EOCs behaviour can be extensive. Multivariate statistical analyses have a broad application in simplifying large datasets, facilitating characterization of interrelationships among numerous hydrogeological variables, uncovering main hydrogeological processes, and providing insights into temporal and spatial variations (MATIATOS et al., 2014; JEBREEN et al., 2018). These invaluable analytical tools were employed in this study for the reliable interpretation of intricate karst dynamics and hydrochemical fluctuations (CAETANO BICALHO, 2012; VASIĆ et al., 2020; ĆUK ĐUROVIĆ et al., 2022).

The hydrological and hydrogeological features of the Jadro and Žrnovnica aquifer have been extensively documented in prior studies by JUKIĆ & DENIĆ-JUKIĆ (2008), KAPELJ et al. (2012), BONACCI & ANDRIĆ (2015), KADIĆ et al., (2019), and SELAK et al. (2024). Additionally, the (eco)toxicological aspects related to EOCs occurrence in this karst aquifer were previously explored by SELAK et al. (2022, 2024). This study seeks to enhance our understanding of karst aquifer behaviour under varying hydrological conditions by utilizing major ion and stable water isotopes data. The analysis of stable isotopes of oxygen δ^{18}O and hydrogen δ^{2}H has been effective in discerning groundwater provenance and karst aquifer recharge processes (CLARK & FRITZ, 1997). We posited that certain identified EOCs could correlate with physico-chemical parameters of water resources within the studied system. These relationships may facilitate the identification of potential contamination sources, behaviour, and transport. We pursued the objective of elucidating factors that govern the hydrochemistry of the Jadro and Žrnovnica springs catchment. This involved monthly sampling at two aforementioned karst springs, a deep borehole, and the adjacent Cetina River from which presumably groundwater inflows into the studied catchment (FRITZ, 1979; JUKIĆ & DENIĆ-JUKIĆ, 2015).

2. MATERIALS AND METHODS

2.1. Study area

The immediate catchment of the Jadro and Žrnovnica springs in Southern Dalmatia (Croatia) extends to an area of around 500 km^2 of heterogeneous relief defined by interchanging mountains and karst poljes. Both Jadro (35 m a.s.l.) and Žrnovnica (spring zone 78 to 90 m a.s.l.) emerge at the foot of Mosor Mountain where an impermeable coastal flysch belt meets with the permeable carbonate rocks of the hinterland (for detailed hydrogeological map readers are referred to SELAK et al., 2024). Typical karst, well-expressed geomorphology has a predominant NW-SE Dinaric orientation. The catchment mainly consists of karstified carbonate rocks of Mesozoic and partly Eocene age, characterized by their high permeability, lack of soil cover and consequently absence of surface watercourses (KAPELJ et al., 2012). Jadro with a mean discharge of 9.4 m^3/s (data period 2011-2022) is used for the regional water supply of Split and its wider area. Žrnovnica with mean discharge of 1.7 m^3/s (data period 2011-2022) supplies the nearby settlement and local agricultural areas. Previous studies denote overlap of the springs' catchments and groundwater exchange between them (JUKIĆ & DENIĆ-JUKIĆ, 2008; BONACCI & ROJE-BONACCI, 1997; BONACCI & ANDRIĆ, 2015; KADIĆ et al., 2017). Moreover, a tracer test undertaken at the Grabov mlin *ponor* proved the presence of intercatchment groundwater flows coming from the adjacent Cetina River catchment (Fig. 1) (GEOTEHNIKA, 1975; FRITZ, 1979), thereby expanding the overall catchment area.

The diverse relief affects the local climate, with a prevalence of temperate humid Köppen climate type *Cfa*, while the mountainous areas of Kozjak and Mosor exhibit a temperate humid climate type *Cfb* (ŠEGOTA & FILIPČIĆ, 2003). The mean daily air temperature, recorded at Split Marjan meteorological station at 122 m a.s.l. (data period 2009-2022), is 17.4°C. Annual precipitation averages 1304 mm (data period 2009-2022) with non-uniform spatial distribution within the catchment.

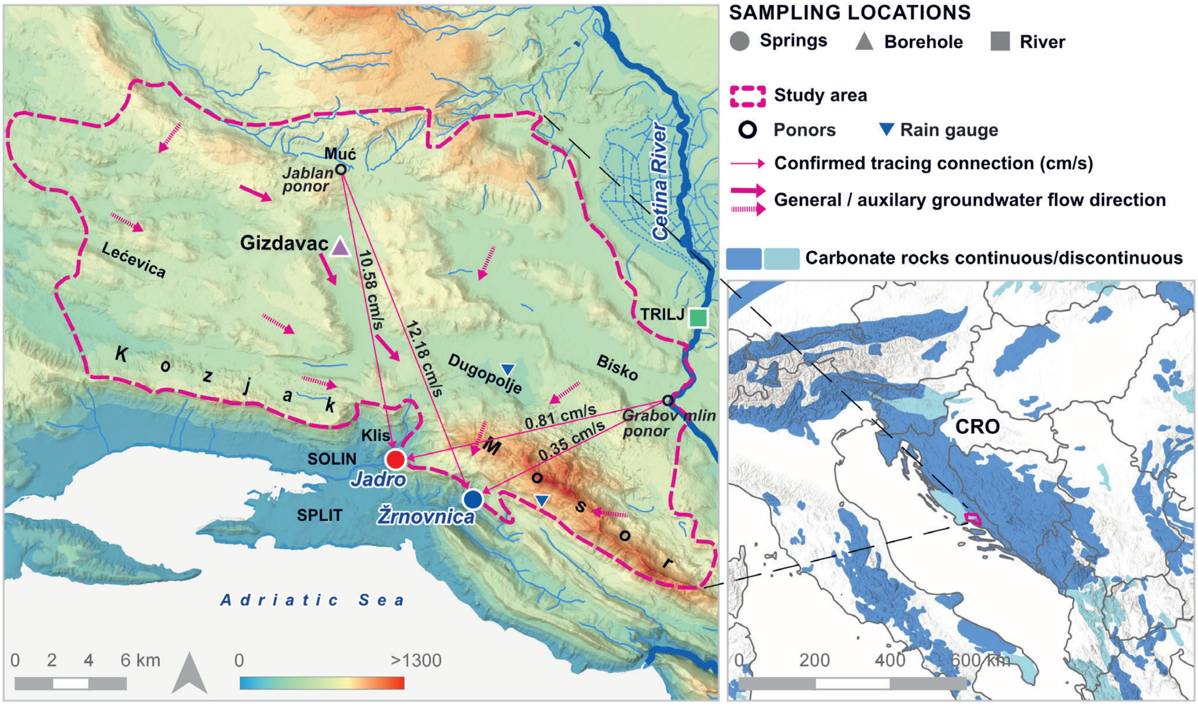


Figure 1. Location of the study area (World Karst Aquifer Map by BGR et al. (2017)).

Both springs respond rapidly (within 24 hours) to rainfall, indicating the presence of a substantial rapid flow component (KADIĆ et al., 2019) primarily generated by rainfall from the Dugopolje area (JUKIĆ et al., 2022). Consequently, rapid alterations in spring hydrochemistry, accompanied by prompt arrival of potential anthropogenic inputs can be anticipated.

2.2. Sampling and analysis of physico-chemical and hydrochemical parameters

In 26 sampling campaigns between October 2019 and October 2022, physico-chemical parameters were measured *in situ* using a WTW multi-parameter probe at the Jadro and Žrnovnica springs, Cetina River and Gizdavac borehole. Only Jadro was monitored in March 2019. The Covid-19 pandemic disrupted regular monthly surveys in 2019 and 2020. Measurements and sampling were undertaken directly at the springs, while a bucket of water was collected from the midstream of the Cetina River. Before conducting sampling and measurements in the bucket, three volumes of groundwater were pumped from the borehole Gizdavac to ensure a representative sample of the aquifer. During our research, among the three deep boreholes used for groundwater monitoring, only the Gizdavac borehole featured a functional pump (installed at the depth of 266 metres).

Alkalinity was determined by volumetric titration using 1.6N H_2SO_4 until reaching a pH 4.5, employing HACH digital titrator Model 16900 with bromocresol green-methyl red as an indicator. The water temperature and electrical conductivity data in the Jadro and Žrnovnica springs, as well as the Cetina River (upstream of Trilj City at Vedrine hydrological station), were recorded using HOBO data loggers U24 from Onset Computer Corporation (Bourne, MA, USA). The data was captured with hourly readings, commencing on 10th March 2021 for the Jadro and Žrnovnica springs, and on 11th March 2021 for the Cetina River.

A total of 84 water samples were collected, including 26 from each of the Cetina River, Jadro and Žrnovnica springs, and an additional 6 samples from the Gizdavac borehole. Samples were collected in 200 ml and 100 ml polyethylene bottles for analysis of major anions, cations, and stable water isotopes. For conservation purposes, samples were transported to the laboratory at 4°C and were analysed within 48h at the Hydrochemical laboratory of the Croatian Geological Survey. Principal ion composition was determined using ion chromatography on a DIONEX ICS-6000 DP. Water quality analyses were assessed for cation-anion balance by calculating the relative deviation from charge balance ($\Delta meq = 100 \times (\Sigma meq+ - \Sigma meq-)/(\Sigma meq+ + \Sigma meq-) < \pm 5\%$) (DOMENICO & SCHWARTZ, 1990). Stable water isotopes $\delta^{18}O$ and $\delta^{2}H$ were measured using isotope-ratio mass spectrometry with a Picarro L2130i (Santa Clara, USA) employing Cavity Ring-Down Spectroscopy (CRDS) technology. Isotope measurements were crosschecked against Picarro's standards, which are periodically verified against International Atomic Energy Agency (IAEA) standards: Vienna Standard Mean Ocean Water 2 (VSMOW2) and Standard Light Antarctic Precipitation 2 (SLAP2). Measurement precision was ± 0.3 ‰ for δ18O and ± 1 ‰ for δ2H. Deuterium excess (d-excess), defined as $d = \delta^{2}H - 8 \times \delta^{18}O$ (DANSGAARD, 1964), was used as an indicator of precipitation air mass origin and non-equilibrium conditions during evaporation. In order to take into account the infiltration of precipitation into the system, mean isotopic precipitation values were weighted with the amount of precipitation using the following equation (YURTSEVER & GAT, 1981):

$$\delta_w = \sum_{i=1}^{n} \frac{P_i \times \delta_i}{\sum P_i} \tag{1}$$

where P_i is the monthly precipitation amount (mm), δ_i is the isotopic composition (‰), and n is the number of months.

The Croatian Meteorological and Hydrological Service provided discharge data for the Jadro and Žrnovnica springs for the period 2011-2022 and precipitation data for 2009-2022. Groundwater temperature and electrical conductivity data from the Gizdavac borehole logger (period 2010-2022) were provided by Croatian Waters. They also provided discharge data for the Cetina River, measured at the Trilj žičara station.

A Piper plot was used for classifying the predominant water types for the springs, groundwater, and surface water. This plot characterizes water based on major cation and anion content in meq/L, enabling analysis of the chemical composition for each water source (PIPER, 1944; APPELO & POSTMA, 2005).

Saturation indices (SI) were calculated to determine the chemical equilibrium between minerals and groundwater using PHREEQC v. 3.7.3.15968 (PARKHURST & APPELO, 1999; USGS, 2021). When SI values are negative, minerals will be dissolved, since the water is undersaturated with respect to them. Conversely, positive SI values indicate oversaturation and mineral precipitation.

2.3. Multivariate statistical analysis

Statistical analyses were performed in R (v. 1.4.1717; R CORE TEAM, 2021) using packages stats (R CORE TEAM, 2023), dunn.test (DINNO, 2017), corrplot (WEI & SIMKO, 2021), vegan (OKSANEN et al., 2022), and Hmisc (HARELL, 2023). The Shapiro-Wilk test was performed to investigate whether the hydrogeochemical data is normally distributed ($p\text{-value} > 0.05$). A nonparametric Kruskal-Wallis test with a post hoc Dunn test with Bonferroni correction (HOLLANDER & WOLFE, 1973; OGLE et al., 2023) was used to test for a statistically significant difference between the hydrochemical parameters from different sampling locations.

To gain insights into the relationships between the hydrochemical parameters in each sample, a correlation matrix was performed using Spearman's rank correlation coefficient (SPEARMAN, 1904) on a normalized dataset. Furthermore, the total sum of EOCs and their detection rate (number of detected compounds per sampling location) were correlated with major cations and anions.

4. RESULTS AND DISCUSSION

4.1. Hydrochemical composition of the springs, river and groundwater

Temporal variability in water quality inherent to the karstic system of Jadro and Žrnovnica springs was comprehensively monitored for three years. This facilitated the inclusion of the

Table 1. Main statistical descriptors of the physico-chemical parameters and discharge (m^3/s) observed within the study area. Water temperature (°C) and electrical conductivity (EC, µS/cm) data encompass the period from October 2019 to October 2022 for the Jadro, and Žrnovnica springs, and the Cetina river, and from 9.11.2010.-31.10.2022 for the Gizdavac borehole. Commencing in March 2021, logger data for both springs and the river were incorporated into the statistical analysis. pH data is given for the period from October 2019 to October 2022, while discharge data is from January 2011- December 2022 for the Jadro and Žrnovnica springs and from January 2019 – December 2022 for the Cetina river (Trilj žičara hydrological station). Major ion data (mg/L) is shown for the period October 2019 – October 2022.

Site	Statistics	T	EC	pH	Q	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	SO_4^{2-}	HCO_3^-
Jadro spring	Min	12.47	259.62	7.25	3.73	2.38	0.15	2.21	70.71	3.69	1.50	3.17	204.50
	Max	13.99	433.24	8.02	56.62	21.44	0.95	8.03	79.50	40.89	4.32	22.70	274.50
	Mean	13.12	355.36	7.47	9.42	9.43	0.52	5.56	75.55	14.84	2.31	12.47	236.07
	Median	13.09	351.45	7.46	6.62	6.39	0.50	5.75	75.62	9.47	2.27	11.61	236.07
	Variance	0.18	1573.60	0.03	49.9	36.17	0.03	2.60	4.01	120.00	0.36	31.48	171.41
Žrnovnica spring	Min	12.32	263.50	7.35	0.27	2.96	0.22	1.49	66.52	4.10	0.88	2.87	192.76
	Max	13.38	399.84	8.52	17.00	20.47	1.97	6.77	73.10	39.31	3.89	15.99	256.20
	Mean	12.84	320.01	7.79	1.73	8.99	0.72	4.44	70.50	14.29	1.66	9.00	222.36
	Median	12.88	312.20	7.76	0.87	6.88	0.57	4.59	71.08	10.49	1.50	8.20	224.48
	Variance	0.08	1311.65	0.05	4.96	30.57	0.18	2.34	4.51	104.19	0.45	12.23	176.59
Cetina River	Min	6.89	263.10	7.80	11.10	1.71	0.32	3.32	55.55	2.53	0.52	9.36	176.90
	Max	16.90	562.00	8.57	305.00	52.76	2.25	7.37	73.07	85.92	3.26	28.49	267.18
	Mean	11.98	362.64	8.25	86.76	20.02	0.83	5.26	65.13	30.11	1.24	15.99	202.40
	Median	11.85	334.10	8.29	70.90	12.06	0.80	5.15	64.93	18.80	1.05	14.43	198.86
	Variance	8.26	5359.92	0.03	2771.38	304.56	0.19	0.91	10.57	713.30	0.43	23.10	316.55
Gizdavac borehole	Min	12.00	329.60	7.26	/	2.36	0.19	2.30	91.92	3.83	2.47	2.59	296.46
	Max	12.80	501.60	7.58	/	3.38	0.78	6.43	108.89	9.93	4.22	5.65	318.42
	Mean	12.44	399.82	7.38	/	2.89	0.61	5.43	97.96	5.59	3.02	3.73	306.73
	Median	12.40	396.17	7.36	/	2.94	0.68	6.24	96.39	4.98	2.72	3.54	307.44
	Variance	0.005	0.0003	0.01	/	0.20	0.05	2.64	40.79	5.07	0.42	1.06	65.85

entire spectrum of hydrological conditions and detailed hydrochemical characterization of the Dinaric karst aquifer during both dry and wet seasons. Table 1 summarizes the descriptive statistics of eleven physico-chemical parameters (T, pH, EC, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, SO_4^{2-}, and HCO_3^-) ascertained across 26 distinct sampling campaigns and different hydrological conditions.

Consistent with findings for sizable karst aquifers (PULIDO-BOSCH, 2021), water temperature in both the springs and groundwater exhibited limited variability over the observed period. As expected, the Cetina River displayed a broader temperature range with pronounced daily oscillations due to direct contact with the atmosphere (Table 1). All water constituents in the investigated catchment exhibited a slightly alkaline pH typical of carbonate aquifers (MATIĆ et al., 2012; PATEKAR et al., 2022; MALDINI et al., 2023). Given the relatively low anthropogenic interference in the studied catchment (LOBOREC et al., 2015), we postulate that the electrical conductivity oscillations are predominantly governed by the dissolution of karst aquifer rocks. Comparatively, groundwater exhibited somewhat higher mean EC values, reflecting prolonged residence periods.

The Piper plot (Fig. 2) revealed a Ca-HCO$_3$ hydrogeochemical facies present in all the examined samples. This facies is indicative of prevailing carbonate weathering processes, aligning with the characteristic hydrochemical profile of Dinaric karst water resources, as substantiated in prior studies (MATIĆ et al., 2012; TERZIĆ et al., 2014; FILIPOVIĆ et al., 2023; MALDINI et al., 2023). In contrast to Ca^{2+}, Mg^{2+} exhibits relatively low abundance (Table 1, Fig. 1), suggesting the predominance of limestone deposits over dolomite. This observation aligns with the lithological analysis of the

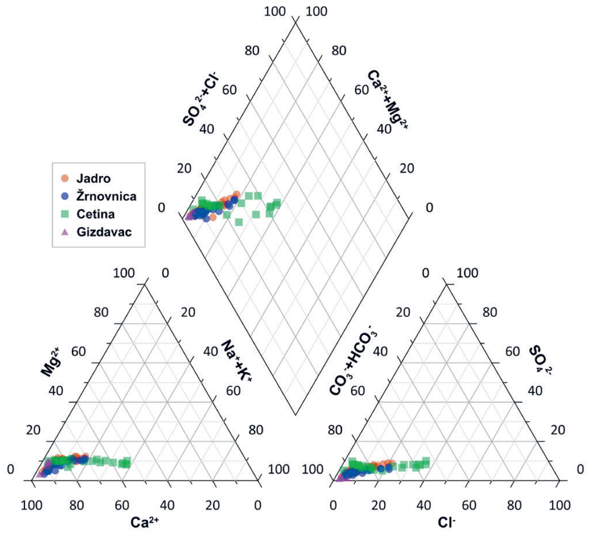


Figure 2. Piper plot presentation of the hydrochemical composition of the springs, river, and groundwater samples within the study area.

Gizdavac borehole, which identified limestone as the sole aquifer rock constituent in that area (KAPELJ et al., 2006).

SI values calculated using PHREEQC (Table S3) indicate that most water samples are oversaturated with respect to calcite (Fig. 4). $SI_{calcite}$ values ranged between -0.02 and 1.2 in the springs, ranged from 0.2 to 0.5 in groundwater, and varied between 0.4 and 1 in the Cetina River. According to WHITE (1997), a $SI_{calcite}$ of -0.3 marks the kinetic threshold for slowdown in dissolution processes, while a $SI_{calcite}$ value of +0.5 indicates the onset of precipitation. In contrast to the Jadro spring and groundwater samples, the Cetina River exhibited oversaturation with respect to dolomite, while over half of the Žrnovnica spring samples showed dolomite undersaturation. These carbonate minerals are the prominent constituents of the host rocks within the studied aquifer. Accordingly, river and spring samples exhibited Ca^{2+} prevalence for cations, with an abundance order generally observed as $Ca^{2+} > Na^{+} > Mg^{2+} > K^{+}$. Groundwater samples demonstrated a distinctive abundance order of cations, with Ca^{2+} being the most prevalent, followed by $Mg^{2+} > Na^{+} > K^{+}$. In groundwater and springs, a robust positive correlation (0.69 and 0.67, respectively) between Ca^{2+} and HCO_3^- suggests their common origin from calcite dissolution. Most water samples were enriched with Ca^{2+} and Mg^{2+} relative to HCO_3^- (Fig. 3a) which confirms how the dissolution of calcite and dolomite by carbonic acid is not the only source of alkaline earths. Excess Ca^{2+} and Mg^{2+} charges must be balanced by other major anions through dissolution or ion exchange processes. Figure 3b shows samples aligning with the 1:1 line, suggesting gypsum and anhydrite dissolution alongside calcite and dolomite dissolution. This observation is further supported by gypsum saturation indices averaging around -2.5 for the springs, -2.9 for groundwater, and -2.4 for the Cetina River, along with anhydrite saturation indices averaging around -3 for the springs, -3.3 for groundwater, and -2.8 for the Cetina River (Table S3). According to FRITZ'S hydrogeological study (1979), gypsum and anhydrite are expected near the surface in the northern part of the catchment along a fault delineating contact with Triassic deposits and subsequent strata, north of Muć *polje*.

The average $[Mg^{2+}]/[Ca^{2+}]$ molar ratios observed in all water samples approximated 0.1, as expected in aquifers draining predominantly limestone rocks. Such ratios imply dolomite dissolution and calcite precipitation processes (APPELO & POSTMA, 2005). We noted a conspicuous decline in $[Mg^{2+}]/[Ca^{2+}]$ during the hydrograph peaks and higher values in the recession periods. The highest values occurred at the later stages of recessions, consistent with the prevailing interpretation of increased mean residence time of karst groundwater during recession periods. Elevated $[Mg^{2+}]/[Ca^{2+}]$ ratios can be indicative of matrix water or diffuse recharge through the epikarst, whereas lower $[Mg^{2+}]/[Ca^{2+}]$ values observed in spring water are associated with conduit recharge (TORAN & REISCH, 2013). The observed lower $[Mg^{2+}]/[Ca^{2+}]$ values, distinct positive and negative fluctuations in chemographs (Fig. 4), and rapid hydrodynamic responses corroborate a highly karstified system with effective conduit network that transmits infiltration to springs under high flow conditions and the absence or presence of only thin overlying layers that fail to dampen the springs' response to rainfall events. Mobile compounds including Cl^- and SO_4^{2-}, which tend to accumulate in soil solution due to elevated evapotranspiration processes during the summer months (GOLDSCHEIDER & DREW, 2007), display a concentration peak with the first significant autumn rainfall events (Fig. 4). The measured levels of Cl^-, SO_4^{2-} and NO_3^- at all sampling sites remained appreciably below the maximum allowed concentrations (250 mg/L, 250 mg/L, and 50 mg/L, respectively) specified in the Drinking Water Directive (2020/2184).

In contrast to the springs and groundwater, the Cetina River displayed a greater degree of variability in chemical composition, particularly with respect to concentrations of Cl^-, Na^+, HCO_3^-, and K^+ ions (Table 1, Fig. 4). The strongest positive correlation of R=0.99 was observed between Na^+ and Cl^-

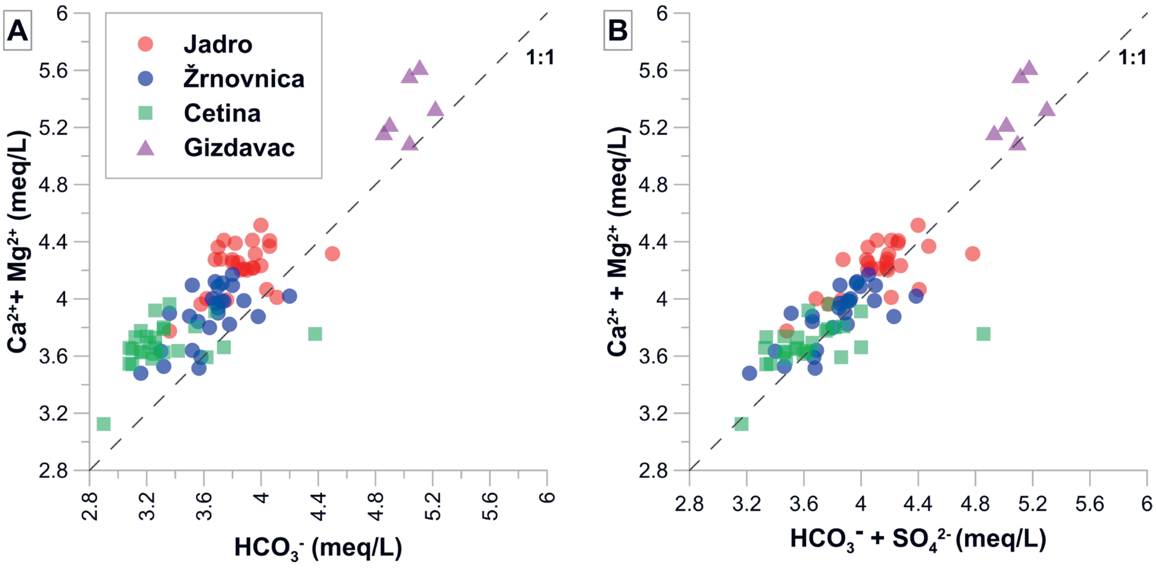


Figure 3. A) Biplots of $(Ca^{2+}+Mg^{2+})$ versus (HCO_3^-). **B)** $(Ca^{2+}+Mg^{2+})$ versus $(HCO_3^-+SO_4^{2-})$.

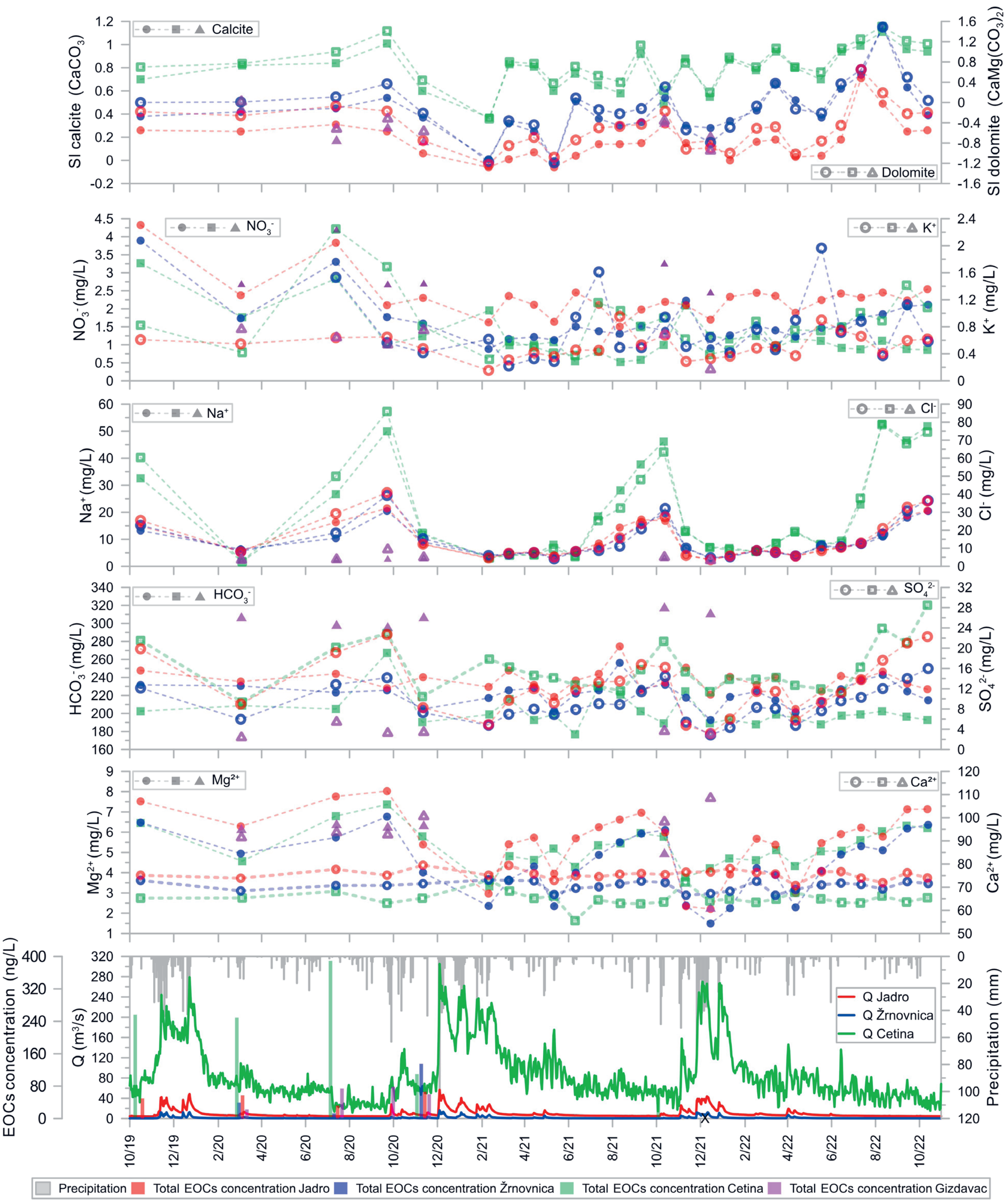


Figure 4. Multivariate chemographs showing the temporal variation in major ion and saturation indices for calcite and dolomite observed at the Jadro and Žrnovnica springs, Cetina River and in groundwater from the Gizdavac borehole. Total EOCs concentration per location (bar graph) were reported in SELAK et al. (2022a). X on the x-axis marks the sampling campaign with no EOCs detected above limits of detection.

ions for the Jadro, Žrnovnica, springs and the Cetina River (Fig. S1). Kruskal-Wallis post hoc Dunn test revealed no statistically significant distinction in terms of Cl$^-$ ion content among the observed locations (except for Cetina vs. Gizdavac) (Table S2). This lends support to the presence of intercatchment groundwater flow, whereby groundwater from the Cetina catchment traverses towards the Jadro and Žrnovnica springs. Elevated Na$^+$ and Cl$^-$ concentrations are usually anticipated within coastal aquifers affected by seawater intrusion or input of airborne salts (LUKAČ REBERSKI et al., 2020; PLAN-

TAK et al., 2021; PATEKAR et al., 2022; FILIPOVIĆ et al., 2023). In our case, the impermeable Eocene flysch coastal belt hinders Adriatic Sea intrusion into the land, as observed by FRITZ (1979). However, strong positive correlations between SO_4^{2-}, Mg^{2+}, Na^+, and Cl^- in all water samples, and between those ions and K^+ in the Jadro spring and Cetina River may indicate sea spray influence (Fig. S1). Furthermore, the presence of chloride may stem from evaporite dissolution (SCHMIDT et al., 2013) or anthropogenic sources including wastewater and fertilizers (DOGDEN et al., 2017), warranting additional research for comprehensive elucidation.

Elevated nitrate concentrations are typically associated with agricultural practices, encompassing the application of synthetic fertilizers (e.g. N/P/K and ammonium sulphate), the utilization of manure in cultivated fields, and septic systems discharges (DOGDEN et al., 2017). The mean NO_3^- concentrations observed within the research area remained below the recorded average in Croatian Dinaric karst groundwater, which stands at approx. 5 mg/L (ONDRASEK et al., 2021). Over the course of six sampling campaigns at the deep borehole site, groundwater exhibited NO_3^- concentrations somewhat higher than those observed at other sampling locations, with mean values of 3.02 mg/L compared to 2.31 mg/L for the Jadro, and 1.66 mg/L for the Žrnovnica springs, and 1.24 mg/L for the Cetina River (Table 1, Fig. 4). LOBOREC et al. (2015) observed the trajectory of increasing nitrate concentrations in the Jadro spring over nearly four decades (1975-2014), substantiating the growing anthropogenic pressures exerted upon the catchment area. The presence of nitrates in groundwater may suggest rapid infiltration processes (CELLE-JEANTON et al., 2003), which is also indicated by the occurrence of biodegradable EOCs gabapentin identified in groundwater from the Gizdavac borehole (Selak et al., 2024). NO_3^- displayed a strong positive correlation with SO_4^{2-} in the Žrnovnica spring (R=0.77) and groundwater from the Gizdavac well (R=0.81) (Fig. S1), as well as with K^+ in groundwater (R=0.75). These ions could have similar anthropogenic sources in fertilizers and wastewater (HAN et al., 2016; MASS et al., 2019; TORRES-MARTÍNEZ et al., 2020). As previously mentioned SO_4^{2-} may originate from the dissolution of gypsum deposits north of Muć, highlighting the intricate interplay between inherent hydrogeological processes and potential site-specific pollution sources. Given the absence of concurrent stable nitrogen isotope analysis alongside nitrate measurement, a definite attribution of nitrate concentrations in groundwater to wastewater or agriculture pollution sources cannot be conclusively established. The strategically situated borehole along the regional Muć-Gizdavac-Klis fault, delineates a prevailing groundwater flow direction from the NW to the SE towards the Jadro and Žrnovnica springs (identified through a tracer test from the Jablan ponor with high apparent groundwater flow velocities of 10.58 cm/s and 12.18 cm/s, respectively) (FRITZ, 1979). According to this, there is the potential conveyance of agricultural pollutants including fertilizers and pesticides from Muć *polje* towards the sampled groundwater and spring locations within a relatively short time. It should be noted how recent research questions the reliability of the tracer tests conducted, as the electrical conductivity of the Jadro spring does not display the rainfall effect from Muć despite the highest apparent groundwater velocity (JUKIĆ et al., 2022). Further investigation is necessary to deepen our understanding of the sources and pathways of anthropogenic contaminants within the studied catchment.

4.2. Stable water isotopes

Table 2 provides a descriptive statistical overview of the stable water isotopes $\delta^{18}O$ and δ^2H, including deuterium excess, for springs, river, groundwater, and precipitation. Cetina River $\delta^{18}O$ data deviated from a normal distribution (Table S1). The results for the Jadro and Žrnovnica springs corroborates earlier research by KAPELJ (2001; published in BRKIĆ et al., 2020) and POLJAK (2014), aligning with the average stable water isotopic values observed in Dalmatian karst springs, which typically vary between -6.7‰ and -8.7‰ for $\delta^{18}O$ and -39.7‰ and -58‰ for δ^2H (BRKIĆ et al., 2020). The isotopic content of precipitation is slightly depleted compared to the data presented by HUNJAK et al. (2013) for the Split station, (located outside of catchment), that had an average of -5.5‰ for $\delta^{18}O$, -34.7‰ for δ^2H and d-excess of 9.2 (for period 2007-2010). This is to be expected as our rain gauges were positioned in the hinterland and at higher altitudes than the Split station (located near the coast at 40 m a.s.l.; Fig. 1). The average d-excess values of karst groundwater, (springs and borehole) and surface water, correspond to the d-excess values of West Mediterranean precipitation (GAT & CARMI, 1970; CELLE-JEANTON et al., 2001). Precipitation at the Dugopolje rain

Table 2. Elementary statistics of isotopic signatures for all sampling points (data period October 2019 – October 2022 for springs, river, and groundwater, and data period March 2021 – October 2022 for precipitation).

Sampling location	Altitude (m.a.s.l.)	$\delta^{18}O$ (‰)				δ^2H (‰)				d-excess (‰)
		min	max	mean	sd	min	max	mean	sd	mean
Jadro	35	−7.82	−7.05	−7.47	0.17	−47.31	−41.44	−44.76	1.43	15
Žrnovnica	90	−8.05	−7.09	−7.56	0.22	−47.58	−40.79	−44.97	1.94	15.53
Cetina	~294	−8.46	−7.20	−8.13	0.3	−52.93	−44.06	−50.1	2.02	14.96
Gizdavac	~356	−7.09	−6.66	−6.94	0.17	−41.85	−39.31	−40.89	0.96	14.62
Rain gauge Dugopolje*	497	−9.09	−1.59	−6.42***	1.94	−58.45	−6.33	−39.72***	14.16	11.66***
Rain gauge Mosor**	869	−10.25	−3.43	−7.45***	1.85	−62.53	−13.69	−43.33***	13.47	16.28***

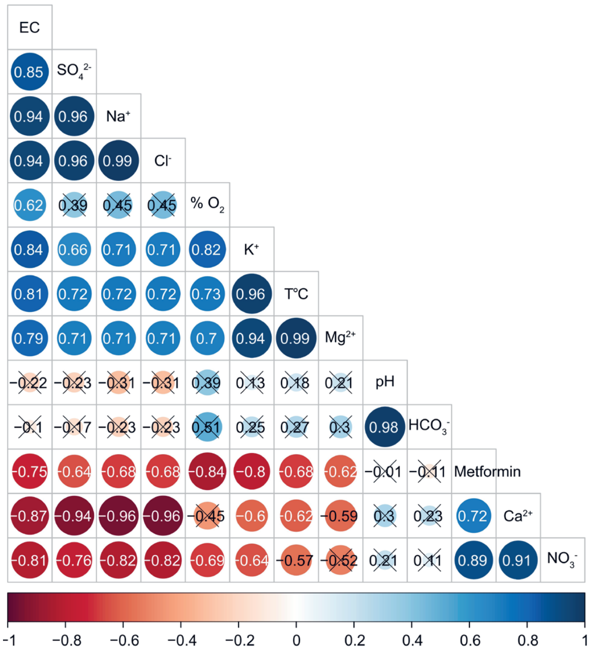
Due to insufficient rain amounts collected in the rain gauge, the following data is missing: *no data for July 2021 and 2022; **no data from July to November 2021, and for July 2022. *** amount-weighted mean values.

gauge displayed a weighted-mean d-excess of 11.66‰, which is close to the global mean d-excess of 10‰ associated with Atlantic air masses, but indicates a slight Mediterranean influence. The Mosor rain gauge recorded predominantly higher weighted-mean d-excess values (16.28‰), signifying a stronger Mediterranean influence, but also the altitude effect (the rain gauge is located at 869 m a.s.l. in a mountainous area).

Water isotopic content is affected by meteoric processes and orographic effects (BRKIĆ et al., 2020). KAPELJ et al. (2012) determined an altitude gradient variation in $\delta^{18}O$, ranging from -0.2‰ to -0.4‰/100 m. Slightly more negative $\delta^{18}O$ and $\delta^{2}H$ values for Žrnovnica than Jadro can be attributed to the altitude effect, i.e. recharge with precipitation from higher altitudes. The Cetina River had an even lower average $\delta^{18}O$ and $\delta^{2}H$ values, which is attributed to a recharge area of higher altitudes and a stronger continental influence. In June 2021, there were notable similarities in the stable isotopic composition, particularly for $\delta^{18}O$ and Cl^- content (Fig. 4), between both springs and the Cetina River. This observation hints at inter-catchment groundwater flow coming from the Cetina River to the Jadro and Žrnovnica springs. Such inflows from the adjacent Cetina River catchment were identified by JUKIĆ & DENIĆ-JUKIĆ (2015) in the partial auto-correlation function of the discharge signal. This phenomenon is likely more pronounced during dry periods, as indicated by the increase in the minimum flow rates at both springs (KADIĆ et al., 2017).

4.3. Correlation between hydrochemical parameters and emerging organic contaminants

Exploring relationships between emerging organic contaminants and other chemical indicators helps to understand the sources, occurrence, and transport of emerging contaminants in karst aquifers (KATZ & GRIFFIN, 2008; HILLEBRAND et al., 2014; ZEMANN et al., 2015). Owing to a limited quantity of samples, the correlation between the hydrochemical parameters and concentrations of a particular EOC was solely feasible for the Cetina River. Readers are referred to the work by Selak et al. (2022b) for a comprehensive inventory of analysed and identified EOCs along with their limits of detection and physico-chemical properties. A very mobile pharmaceutical compound metformin ($C_4H_{11}N_5$) was the only EOC detected more than twice at a single location (recorded in 4 out of 7 campaigns). Metformin exhibited a statistically significant and strong positive correlation with Ca^{2+} and NO_3^- ions in the Cetina River, with correlation coefficients of R=0.72 and R=0.89, respectively (Fig. 5).



EC												
0.85	SO_4^{2-}											
0.94	0.96	Na^+										
0.94	0.96	0.99	Cl^-									
0.62	0.39	0.45	0.45	% O_2								
0.84	0.66	0.71	0.71	0.82	K^+							
0.81	0.72	0.72	0.72	0.73	0.96	T°C						
0.79	0.71	0.71	0.71	0.7	0.94	0.99	Mg^{2+}					
−0.22	−0.23	−0.31	−0.31	0.39	0.13	0.18	0.21	pH				
−0.1	−0.17	−0.23	−0.23	0.51	0.25	0.27	0.3	0.98	HCO_3^-			
−0.75	−0.64	−0.68	−0.68	−0.84	−0.8	−0.68	−0.62	−0.01	−0.11	Metformin		
−0.87	−0.94	−0.96	−0.96	−0.45	−0.6	−0.62	−0.59	0.3	0.23	0.72	Ca^{2+}	
−0.81	−0.76	−0.82	−0.82	−0.69	−0.64	−0.57	−0.52	0.21	0.11	0.89	0.91	NO_3^-

Figure 5. Correlation matrix of hydrochemical parameters for Cetina (correlation coefficients order with AOE algorithm - the angular order of the eigenvectors) and EOC metformin. Values marked with X are not significant.

Additionally, a strong positive correlation (R=0.87) was observed between the total concentration of EOCs and NO_3^- ion in samples collected from the Cetina River (Fig. 6). Nitrates, often indicative of anthropogenic contamination from wastewater or agriculture, were previously associated with particular EOCs, including herbicides (HILLEBRAND et al., 2014), X-ray contrast media (ZEMANN et al., 2015), and carbamazepine (DOUMMAR et al., 2014). Nitrates showed a positive correlation with increasing EOCs detection number (ZEMANN et al., 2015; RICHARDS et al., 2023) and total EOC sum (SCHAIDER et al., 2014). Similar to findings of ZEMANN et al. (2015) and RICHARDS et al. (2023), this research identified a statistically significant correlation (R=0.90) between the EOCs number and NO_3^- concentrations in the Cetina River (Fig. 6). These positive correlations validate the EOCs sources map and conceptual model presented by SELAK et al. (2024).

In groundwater samples, EOCs detection number and total concentration were strongly negatively correlated with HCO_3^- and Ca^{2+} ions, while a strong positive correlation was observed between the EOCs detection number, the total concentration and Mg^{2+} ion (Fig. 6). The prevalence of calcium and bicarbonate ions in groundwater, attributed to the predominance of limestone in the aquifer rocks, suggests that in this tectonically disturbed and highly permeable part of the aquifer with low matrix porosity typical of Dinaric karst aquifers, the detected EOCs undergo swift infiltration. This explains the presence of rapidly biodegradable gabapentin in the sample extracted from the Gizdavac borehole. In the Cetina River, we observed strong negative correlations (in descending order of correlation coefficient strength) between the number of EOCs detections, the total concentration sum of EOCs, and SO_4^{2-}, Cl^-, and Na^+, as well as between EOCs detection rate and Mg^{2+} and K^+ ions, as depicted in Fig. 6. Similar patterns were noted in Jadro samples, where the total EOCs concentration and detection number displayed strong negative correlations with K^+, Na^+, Cl^-, SO_4^{2-}, and Mg^{2+} (Fig. 6). Moreover, Žrnovnica spring samples exhibited strong negative correlations between EOCs detection rate and Mg^{2+}, Na^+, Cl^-, SO_4^{2-}, and K^+ ions. Since Cl^-, Na^+, K^+, SO_4^{2-}, and Mg^{2+} ions can partly originate from sea spray, the negative correlation discerned with the EOCs number and total concentration may suggest the absence of atmospheric input of EOCs in the study

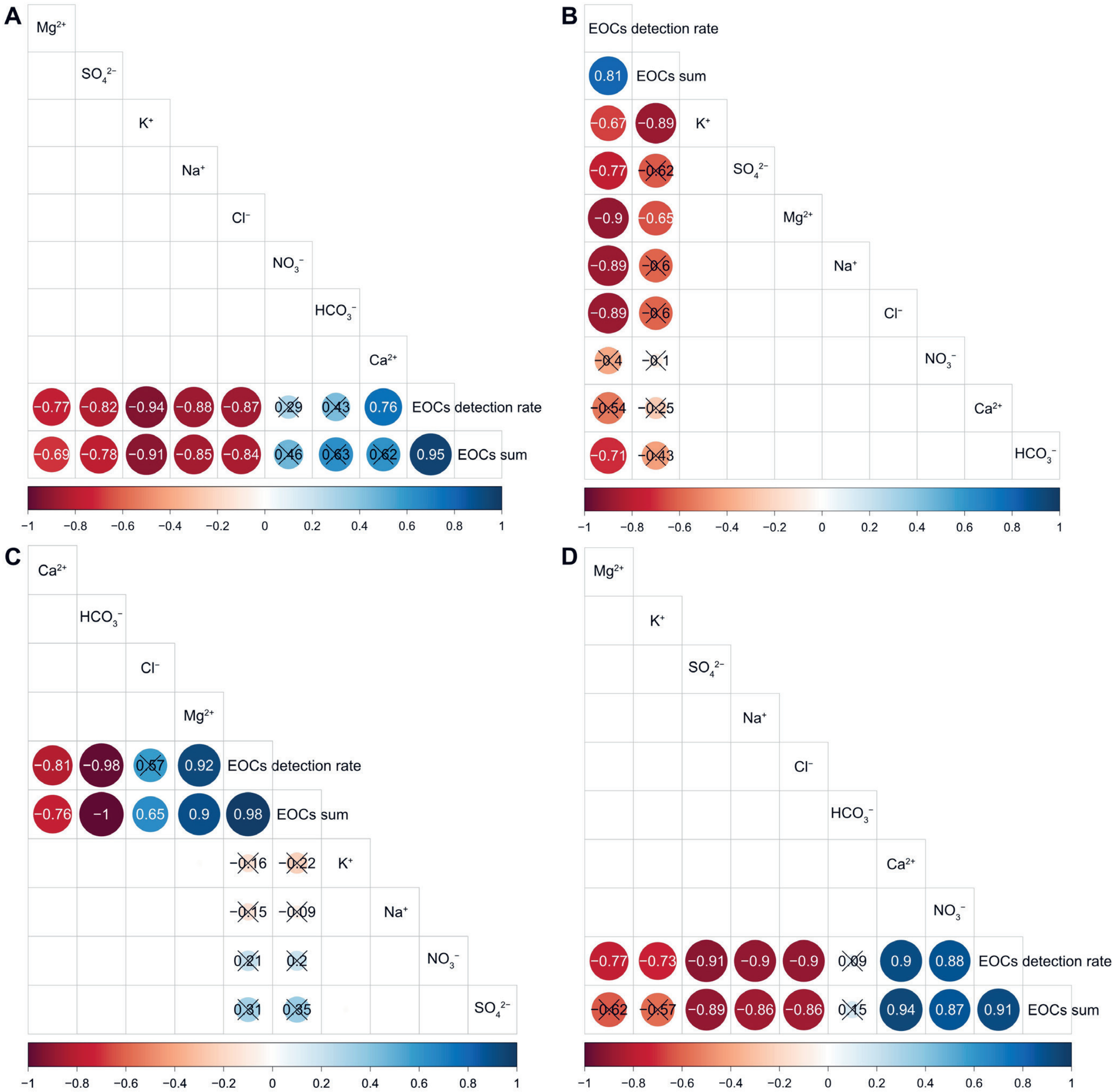


Figure 6. Correlation matrix of hydrochemical parameters for the Jadro (A), Žrnovnica (B), Gizdavac (C), and Cetina (D) (correlation coefficients order with AOE algorithm - the angular order of the eigenvectors), EOCs detection number and total concentration sum. Values marked with X are not significant.

area. Conversely, a strong positive correlation was observed between the EOCs detection number and total concentration sum with respect to Ca^{2+} content in the Cetina River and EOCs detection number and Ca^{2+} in the Jadro spring samples. HILLEBRAND et al. (2014) established a robust correlation between calcium ions and the herbicide atrazine, which they attributed to the gradual release of atrazine from the karst rock matrix into the groundwater. Persistent and mobile compounds including DEET detected in the Cetina River and Jadro spring, showed limited attenuation within the studied karst aquifer (SELAK et al., 2024). This compound showed elevated concentrations during autumn runoff and persisted even during base flow conditions, suggesting potential storage within the epikarst and matrix.

5. CONCLUSIONS

This hydrochemical characterization study has provided a comprehensive examination of the Jadro and Žrnovnica karst aquifer in both spatial and temporal dimensions, while analyzing factors influencing the quality of its water resources. The hydrochemical fingerprint of this aquifer is typical for coastal Dinaric aquifers characterised by highly karstified structures, a prevalence of limestone, as indicated by the Ca-HCO_3 hydrogeochemical facies, and clear evidence of seaspray influence. Stable isotope signatures confirmed a common catchment area shared by the Jadro and Žrnovnica springs while also revealing intercatchment groundwater flow originating from the Cetina River.

Alongside the hydrodynamics, hydrochemical characterization has a pivotal role in comprehending the complexity of karst systems and assessing their susceptibility to EOCs. Sharp spikes in chemographs, alongside rapid hydrodynamic responses are evidence of a highly karstified and inherently vulnerable system. Its well-developed conduit network can swiftly transport EOCs via infiltrated water to springs under high flow conditions, as the absence of or thin overlying "protective" layers fail in their attenuation. While conventional indicators of anthropogenic pollution including NO_3^-, Cl^-, and SO_4^{2-} remain below their respective maximum allowable concentrations in drinking water, detections of EOCs reflect the anthropogenic impact on water resources within the studied catchment. Significant strong positive correlation between metformin and NO_3^- concentration in the Cetina River, as well as significant strong positive correlation between total EOCs concentration, number and NO_3^- ion, indicates potential contamination stemming from wastewater or agriculture. Robust significant positive correlation between EOCs detection rate and Ca^{2+} content observed in the Jadro spring samples suggests the persistence of certain EOCs including DEET within the epikarst and aquifer matrix. To enhance the reliability of multivariate analyses of hydrochemical parameters and EOCs, we recommend that future research should involve a larger sample number and focus on monitoring prioritized EOCs. In the context of this Dinaric karst catchment, we propose directing research efforts towards persistent and mobile EOCs, for example; DEET, 1-H benzotriazole, and metformin. The future long-term monitoring and concurrent utilization of hydrochemical markers and novel tracers including EOCs will offer enhanced clarity in discerning the intricate interplay of dominant factors shaping hydrochemical patterns within karst aquifers subject to various anthropogenic pressures.

ACKNOWLEDGEMENT

The authors express their gratitude to the Croatian Meteorological and Hydrological Service and Croatian Waters for the provided data. This work was financially supported by Interreg Central Europe boDEREC-CE project co-funded by the European Regional Development Fund, GeoTwinn project funded under H2020-EU.4.b., [grant number 809943] and NONa project funded by National Recovery and Resilience Plan 2021–2026 of the European Union – NextGenerationEU, and monitored by the Ministry of Science and Education of the Republic of Croatia. The field investigations and EOCs analysis were funded and supported by the Croatian Geological Survey, Department of Hydrogeology and Engineering Geology.

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Paper V

Selak, A., Lukač Reberski, J., Klobučar, G., Grčić, I., 2022. Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer of Jadro and Žrnovnica springs. Sci Total Environ 825, 153827. https://doi.org/10.1016/j.scitotenv.2022.153827

Science of the Total Environment 825 (2022) 153827

Contents lists available at ScienceDirect

Science of the Total Environment

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

Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer of Jadro and Žrnovnica springs

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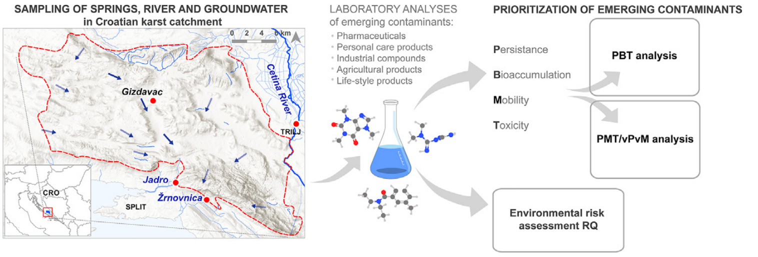
HIGHLIGHTS

- Karst catchments harbor specific ecosystems vulnerable to artificial contamination.
- Identification of 21 emerging contaminants at ng/L levels in karst catchment
- Compounds were assessed as non-persistent-bioaccumulative-toxic (non-PBT).
- Only 4 out of 21 compounds were not assessed as PMT/vPvM.
- Caffeine posed the highest risk and Cetina River had a moderate site risk quotient.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 October 2021
Received in revised form 19 January 2022
Accepted 8 February 2022
Available online 11 February 2022

Editor: Jurgen Mahlknecht

Keywords:
PBT ranking
PMT assessment
Environmental risk assessment (ERA)
Risk quotient (RQ)
Karst
Water resources
Jadro

ABSTRACT

Karst aquifers are globally important source of drinking water and harbor specific ecosystems that are vulnerable to anthropogenic contamination. This paper provides insights into the occurrence and ecotoxicological characterization of 21 emerging contaminants (ECs) detected in the karst catchment of Jadro and Žrnovnica springs (Dinarides, Croatia). Karst springs used for water supply, surface water, and groundwater were sampled during seven campaigns. The ECs concentration levels ranged from 0.3 ng/L (tramadol in Jadro spring) to 372 ng/L (1H-benzotriazole in Cetina River). DEET was the most frequently detected ECs with an average concentration of around 50 ng/L in both surface water and groundwater. To prioritise detected ECs, their persistence (*P*), bioaccumulation (*B*), mobility (*M*) and toxicity (*T*) were assessed based on in silico strategy for *PBT* assessment and recently developed REACH *PMT* guidelines. *PBT* scores ranging below the threshold of 0.5, indicated *non-PBT* compounds of expected low concern. However, only 4 out of 21 detected ECs were not assessed as *PMT/vPvM*. Concerningly, 20 ECs were categorised as very mobile. Karst springs exhibited larger proportions of ECs meeting *PMT/vPvM* criteria than surface water. To characterise the contamination extent and estimate the incidence of adverse effects of detected ECs, a preliminary environmental risk assessment (*ERA*) was conducted. Most ECs posed no environmental risk with *RQ* values predominantly below 0.01. The total risk quotient RQ_{site} accentuated Cetina River as having the highest risk compared to other sampling sites. This is the first study on ECs in Croatian karst, contributing to a growing need to understand the impacts of emerging contaminants in karst aquifers, which are still largely unexplored.

1. Introduction

The ubiquitous presence of emerging contaminants (ECs) across the environmental matrices requires a multidisciplinary approach for overcoming this both scientific and regulatory conundrum, in order to safeguard water quality, protect aquatic ecosystems, and consequently human health. Owing to significant advances in analytical techniques and instruments, this vast spectrum of natural and synthetic organic compounds (including their metabolites and transformation products) is detected worldwide at ng/L to μg/L levels in both surface water (Loos et al., 2009) and groundwater bodies (Sui et al., 2015). Global and daily use of ECs compounds such as

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http://dx.doi.org/10.1016/j.scitotenv.2022.153827

pharmaceuticals and personal care products, hormones, lifestyle products, industrial, and agricultural chemicals entails their continual emission into the environment, which is still not adequately monitored nor regulated. Given how many of ECs are developed to induce a specific physiological effect, their ability to retain the chemical structure and often-lipophilic nature allow them to persist in the environment and impose detrimental effects on many non-target organisms. Accordingly, the persistence, bioaccumulation potential, and toxicity of various ECs to aquatic species became a subject of many recent studies (Cleuvers, 2003; Fent et al., 2006; Brausch et al., 2012; Cizmas et al., 2015; Babić et al., 2018). However, the synergistic and cumulative toxicological effects of ECs mixtures (Cleuvers, 2004; Pomati et al., 2008; Białk-Bielińska et al., 2013; Flaherty and Dodson, 2005; Spurgeon et al., 2010; Vasquez et al., 2014), as well as their environmental behaviour and transport are still insufficiently explored (Lapworth et al., 2012).

Karst aquifers provide drinking water for 9.2% of the world's population (Stevanović, 2019), whereas in some Europe countries they significantly contribute to water supply (e.g. 50% in Austria and Slovenia, 36% in Croatia, etc.) (COST 65, 1995; Hartmann et al., 2014). The specific and complex hydrogeological properties make karst aquifers and their subterranean ecosystems particularly vulnerable to contamination (Ford and Williams, 2007; Goldscheider and Drew, 2007) and their investigation quite intricate, namely regarding the presence, transport, and attenuation of trace compounds. The often-thin protective soil layers, highly permeable medium, rapid infiltration via sinkholes, and flow mechanisms in enlarged fractures and conduits are factors that limit the natural attenuation of contaminants entering karst aquifers (Bakalowicz, 2005; Hartmann et al., 2017). Gaining a better understanding of ECs physio-chemical characteristics, sources, behaviour, and fate in karst aquifers is crucial and challenging, specifically in areas where they are the only source of drinking water. To date, only a few studies have examined ECs in karst aquifers (Lapworth et al., 2012; Lukač Reberski et al., 2022) and their effects on karst ecosystems (Goldscheider, 2019). It should be stressed how most studies on ECs in Croatia have focused on a narrow range of compounds that are mainly found in wastewater or effluent recipients in the alluvium (Terzic and Ahel, 2011; Bielen et al., 2017; Ivešić et al., 2017; Senta et al., 2013, 2017, 2019; Česen et al., 2019).

Given the scarcity of ECs research in groundwater and karst systems our paper is the first study of its kind conducted on karst water resources (springs, river, and groundwater) in Croatia, contributing significantly to filling the knowledge gaps on the occurrence and fate of ECs in karstic environments at national and global scales.

The aim of this study was to identify ECs occurring in Jadro and Žrnovnica karst catchment of a regional and historical water supply importance, and to characterise the potential impacts and environmental risks they pose to ecological health. Through use of QSAR-obtained data and database research, ECs were prioritised based on their persistence (*P*), bioaccumulation (*B*), mobility (*M*) and toxicity (*T*) assessed according to existing *PBT* method (REACH Commission regulation (EU), 2011; Ortiz de García et al., 2013; Pizzo et al., 2016a,b) and *PMT*/vPvM guidelines (Arp and Hale, 2019). In addition, conducted environmental risk assessment (*ERA*) (Hernando et al., 2006; Köck-Schulmeyer et al., 2021), allowed us to pinpoint the locations with the highest potential environmental risk. The findings of our study stress out the importance of ECs monitoring and provide an opportunity to support decision-makers in improving existing legislation and water safety plans to protect not only aquatic ecosystems but also human health.

2. Materials and methods

2.1. Study area

The present research focuses on a typical Dinaric karst catchment of Jadro and Žrnovnica springs, situated in southern Croatia (Fig. 1). Both Jadro and Žrnovnica rivers flow into the Adriatic Sea. Jadro springs at an altitude of 35 m a.s.l. east of the town of Solin, at the foot of the Mosor Mountain (1339 m a.s.l.). The mean measured discharge of Jadro spring is 9.24 m^3/s (2011–2019). The public water supply of the city of Split and its wider surroundings (about 270,000 inhabitants) relies on the water intake at Jadro spring from the 3rd century CE. Depending on the groundwater level, the multiple springs of Žrnovnica River occur northeast of the city of Split and near Žrnovnica settlement, at altitudes ranging from 77 to 90 m a.s.l. All tributaries of the Žrnovnica River are mostly dry during the year. The mean measured discharge of the Žrnovnica springing zone is 1.94 m^3/s (2009–2019). Due to lower discharges in the summer months, Žrnovnica spring is captured only for the water supply of the nearby settlements and for irrigation of the surrounding agricultural land (Kapelj et al., 2012). Before distribution to end users, the water on both springs is disinfected with NaClO.

The estimated catchment area of Jadro and Žrnovnica springs is between 250 and 500 km^2 (Kapelj et al., 2012). Cetina River flows along the presumed eastern catchment boundary, at an aerial distance of about 15 km from the springs and at an elevation of about 300 m a.s.l.

The catchment is mostly made of highly permeable carbonate rocks (Fig. 1), which is why there are no surface flows. An array of karst features and phenomena occur throughout the catchment. The entire catchment is characterised by a dynamic and complex morphology of intertwined mountains and hills extending to the coast, while in between there are several karst *poljes* (Muć, Dugopolje, and Bisko) with smaller settlements and arable land. A predominantly typical Dinaric orientation (NW-SE) of morphological structures corresponds to the groundwater flow direction (Fig. 1). The perennial inter-catchment groundwater flows coming from the Cetina River catchment depend on groundwater levels (Denić-Jukić, 2002).

Regardless of the oligotrophic nature, the physical heterogeneity of karst aquifers enables habitat and species richness (Gibert et al., 1994). Reportedly, Jadro spring is a habitat for an endemic species *Proteus anguinus* and is the *locus typicus* for at least three subterranean freshwater gastropod species *Costellina turrita, Kerkia jadertina,* and *Iglica elongata* (Kuščer, 1933), which adds to the intrinsic natural value of this catchment.

The catchment is sparsely populated, with spatially scattered smaller settlements. Both springs are located upstream of the most populated urban areas of Split and Solin and out of their direct pollution reach. The most likely sources of ECs are potentially leaking sewage networks of settlements without wastewater treatment plants, and smaller communities, which rely only on septic tanks. Few unsanitary landfills are currently in the process of remediation, and waste is reportedly illegally disposed of in some karst sinkholes and caves (documented in 2020, by various speleological associations). Loborec et al. (2015) highlighted the increasing trend of nitrogen and nitrate content in Jadro and Žrnovnica springs, and the occasional presence of coliform bacteria in Jadro spring, as clear evidence of anthropogenic contamination. The Cetina River is the main recipient of the wastewater treatment plant effluents, which are collected only in certain parts of the town and discharged downstream (Spatial plan of Trilj City, 2020). The potential contamination sources are also permanently irrigated areas and arable land along the river and upstream of the town of Trilj. Considering the general groundwater flow direction (Fig. 1) and the results of tracer tests, we assume how contamination from agricultural activities in the karst *poljes* (pesticides and fertilizers), and from industrial and urban areas (such as Dugopolje and Trilj) could eventually reach the downstream Jadro and Žrnovnica springs.

2.2. Sampling and monitoring

Seven sampling campaigns were conducted to detect the presence of ECs categorised into following groups: pharmaceuticals and personal care products, industrial, agricultural and life-style products. Four sampling sites (Fig. 1) included Jadro and Žrnovnica springs, Cetina River, and Gizdavac borehole (pumping depth of 266 m) (Table 1, Data in Brief). Sampling campaigns in March and October of 2019 were carried out only at Jadro within pilot activities of the Horizon 2020 GeoTwinn project. Further five sampling campaigns (October 2019, March, July, September, and

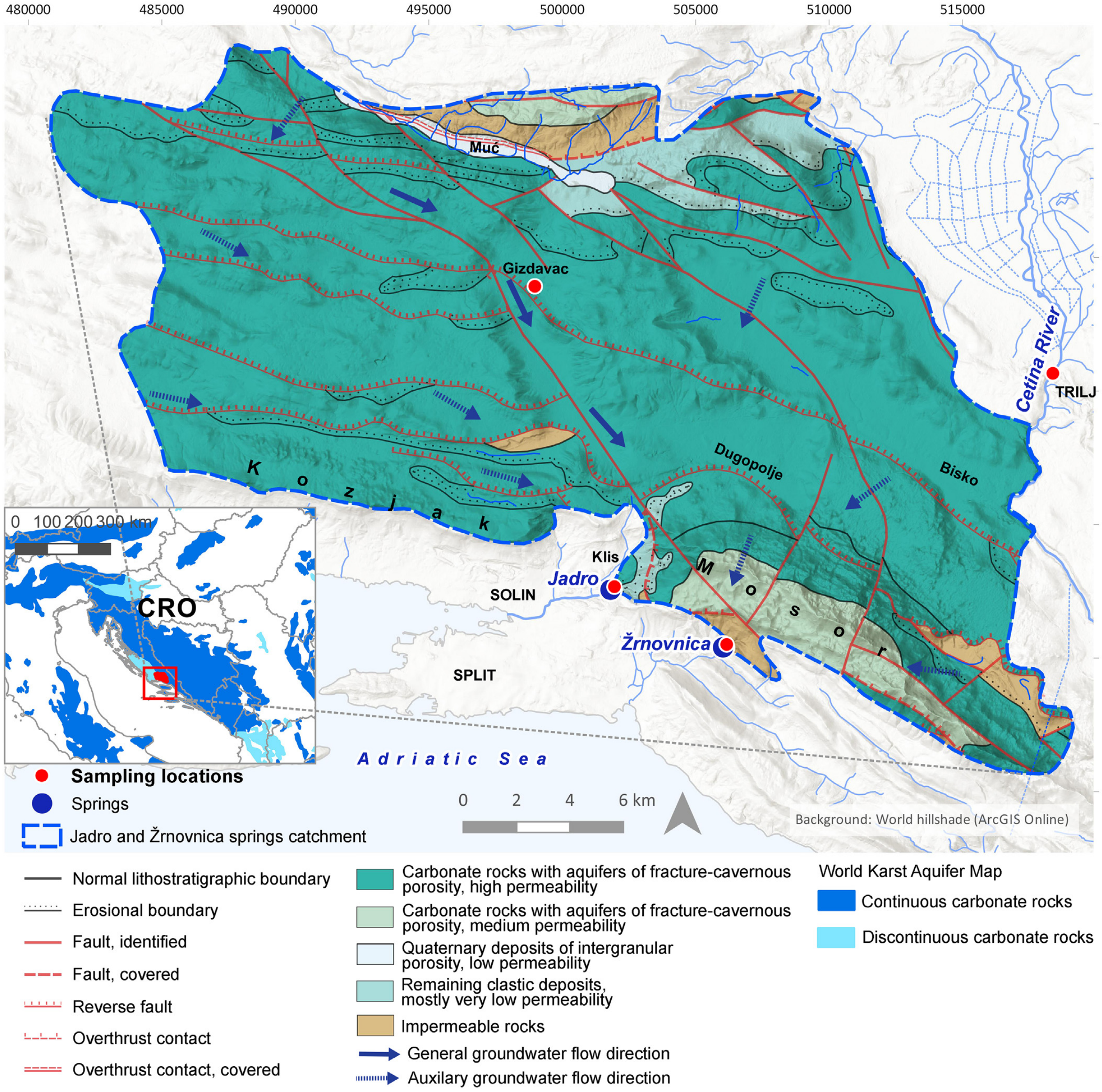

Fig. 1. Study area Jadro and Žrnovnica springs catchment (hydrogeological map modified from Biondić et al., 2003; World Karst Aquifer Map by BGR et al., 2017).

November 2020) were conducted at all four sampling locations as part of the Interreg Central Europe boDEREC-CE project. The Gizdavac borehole was not sampled in October 2019. In March and October 2019, samples were collected in pre-cleaned 1 L glass bottles (2 bottles per sampling point) provided by the National Laboratory Services UK (NLS UK), where analysis of the presence of 1518 ECs was done. The procedure from NLS UK did not require the cooling of the samples during shipping. For all other sampling campaigns, samples were collected in the pre-cleaned 60 mL amber-glass vials (2 vials per sampling point) given by the Vltava River Basin Authority Laboratory in the Czech Republic, where the presence of 102 ECs was analysed. Samples were transported in the field freezer and shipped in dry ice containers to the laboratory. A strict sampling procedure specified by the laboratories was followed during all sampling campaigns to minimise potential contamination. Spring water samples were collected directly, while water samples for the Cetina River were collected using a clean bucket from the middle of the stream. Prior to sampling at the Gizdavac borehole, the borehole was flushed with the three volumes of water and a grab sample was taken from the bucket.

Physicochemical parameters, i.e. water temperature and electrical conductivity (μS/cm) were measured in situ at all four sampling sites (Jadro spring, Žrnovnica spring, Cetina River, and Gizdavac borehole) using a WTW multi-parameter probe. At springs, measurements were made directly, while parameters for the Cetina River and Gizdavac borehole were measured in a bucket containing grabbed water sample.

2.3. Analytical methods

The analysis of ECs in surface and groundwater samples carried out in the course of the GeoTwinn project 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).

Samples collected within the boDEREC-CE project were analysed at Vltava laboratory following the EPA method 1694 (Axys Analytical Services, Ltd.) and valid procedures. The analysis was done with 1290 ultra-high-performance liquid chromatograph (UHPLC in electrospray ionisation ESI+ and ESI− modes) coupled with an Agilent 6495B Triple Quad Mass Spectrometer (MS/MS).

The detailed description of used analytical methods and limit of detection (LOD) for each detected analyte is available in Data in Brief under "Experimental design, materials, and methods" and Table 2.

2.4. Data processing and analysis tools

2.4.1. Physicochemical properties of detected emerging contaminants

The specific physicochemical properties of detected ECs were consulted in the publicly available NORMAN Substance Database (NORMAN Network, 2021a) and PubChem (National Center for Biotechnology Information, 2021). In the absence of experimental data, physicochemical properties were estimated using the EPI Suite™ 4.11 interface (US EPA, 2021a). The gathered information on physicochemical properties are the compound name, molecular formula, and weight, SMILES notation (Simplified Molecular Input Line Entry System), the logarithm of partition coefficient between octanol and water (log K_{OW}), organic carbon-water partitioning coefficient (log K_{OC}), acid dissociation constant (pK_a), and values of ECs solubility in water. In the EPI Suite™, the KOWIN v.1c68 model was used to estimate the log K_{OW}, which is one of the most important parameters for evaluating contaminants that have the potential to cause detrimental effects on the environment and biota. For obtaining log K_{OC} values KOCWIN v2.00 was utilized. The WSKOW v1.42 model was used to acquire predicted values of ECs solubility in water (in mg/L at 25 °C).

2.4.2. Principal coordinates analysis (PCoA)

Statistical analysis was performed in R (v. 1.4.1106) (R Core Team, 2020) using the vegan (Oksanen et al., 2019), dplyr (Wickham et al., 2020), and ggplot2 (Wickham, 2016) packages. The Bray-Curtis dissimilarity coefficient was applied to the Hellinger-transformed (Legendre and Gallagher, 2001), normalised ECs dataset.

To analyse the spatio-temporal (di)similarities of ECs content from different sampling sites and sampling months, the PERMANOVA test was carried out on the distance matrices calculated with the Bray-Curtis dissimilarity coefficient (Bray and Curtis, 1957), and lastly, visualised with the Principal Coordinate Analysis (PCoA).

A standardised environmental dataset (in situ measured water temperature and electrolytic conductivity) was used to analyse the link between environmental parameters and the distribution of ECs.

2.4.3. PBT ranking

PBT is a method utilized for prioritisation of organic compounds and hazard assessment, according to Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Commission regulation (EU), 2011). Compounds are scored according to their persistence (*P*), bioaccumulation (*B*) characteristics, and toxicity (*T*) (Pizzo et al., 2016a,b) as follows (Eq. (1)):

$$PBT = P^{0.4} \cdot B^{0.4} \cdot T^{0.2} \tag{1}$$

where component *T* has the lowest weight given the uncertainty of its assessment. *PBT* scores were calculated in Prometheus software (Pizzo et al., 2016b), using the SMILES notation of ECs molecules as input data. For each of the three properties, values are normalised between 0 and 1. A threshold of 0.5 for the total score is used to distinguish *non-PBT* (<0.5) and potentially *PBT* or *vPvB* (≥0.5) compounds (Pizzo et al., 2016b).

To account for the relationship between the measured concentration of ECs and their *PBT* properties, the weighted arithmetic mean (*PBTr*) of the individually assigned ranks was calculated, allowing prioritisation of ECs in the water environment of the Jadro and Žrnovnica catchment and at specific sampling sites. For each compound, *PBTr* values were calculated based on the following equation proposed by Babić et al. (2018) (Eq. (2)):

$$PBTr = \frac{\sum_{i=1}^{n} R_i w_i}{\sum_{i=1}^{n} w_i} \tag{2}$$

where R_i is the rank calculated by multiplying *PBT* score and measured concentration, and w_i is the weight. Since the *PBT* score and concentration are proposed to be equally relevant factors contributing to the potential risk for the aquatic organisms, all weights are set to 1.

2.4.4. PMT/vPvM assessment

The persistence (*P*), mobility (*M*), and toxicity (*T*) of all detected compounds were evaluated according to REACH guidelines for *PMT/vPvM* assessment (Arp and Hale, 2019). Detailed description of each criterion is given in Data in brief (under "Experimental design, materials, and methods"). Depending on which criteria are met, six categories are defined: *vPvM & PMT*, *vPvM*, *PMT*, *PM*, *potential PMT/vPvM*, and *not PMT/vPvM*. Although focused primarily on industrial compounds, we crosschecked the existing list of *PMT/vPvM* substances registered under REACH with the list of ECs detected in our study.

Existing experimental input data (half-lifes and results of biodegradability tests) for *P* criterion was searched in literature and PubChem database. The persistence was also predicted in QSAR Toolbox (OECD, 2021) and compared to the results of Prometheus software. ECs biodegradability was predicted with ready biodegradability model IRFMN 1.0.9 in VEGA QSAR (Benfenati et al., 2013) and BIOWIN models (1, 3, 4, and 5 models, v4.10) in EPI Suite™. The half-lifes were also searched in CompTox Chemicals Dashboard (US EPA, 2021b).

Data on mobility was obtained from the literature and PubChem database, or predicted with EPI Suite™ KOCWIN v2.00 model.

Primary sources for toxicity data were Classification and labelling inventory (ECHA, 2021) and EnviroTox database (HESI, 2021). VEGA QSAR software was used to predict the mutagenicity, carcinogenicity and toxicity. Cramer classification for each compound was done in Toxtree v3.1.0.1851 software (Ideaconsult Ltd., 2015).

2.4.5. Risk quotients – risk assessment

The risk quotient (*RQ*) approach regards detected environmental concentrations and chronic toxicity of organic compounds to non-target aquatic species and provides an opportunity for ERA based on screening and prioritisation of contaminants. To determine which compounds have the highest priority in terms of potential risk, two key indicators are employed - measured environmental concentration (*MEC*) and the lowest Predicted No-Effect Concentration (*PNEC*), as follows (Eq. (3)):

$$RQ = MEC/PNEC \tag{3}$$

The ecotoxicity data (lowest *PNEC* values presented in Table 6, Data in Brief) used for the analysis were gathered from the Ecotox database (NORMAN network, 2021b). These values are based on acute, chronic, or non-standard tests, and are intended as non-legally binding thresholds for protecting the receptors at risk in, or via, the aquatic environment (Dulio and von der Ohe, 2013). For the pharmaceutical ibuprofen, the lowest *PNEC* value of 1.65 μg/L was retrieved from published literature (Zhu et al., 2013), as it was not found in the NORMAN database.

The potential ecotoxicological risk of the target compound to the receiving aquatic ecosystems is classified into four levels, i.e. "negligible risk" ($RQ < 0.01$), "low risk" ($0.01 < RQ < 0.1$), "moderate risk" ($0.1 < RQ < 1$) and "high risk" ($RQ > 1$) (European Commission, 2003; Hernando et al., 2006; Česen et al., 2019).

The site-specific risk quotient RQ_{site} is by summing up the RQ values for all compounds detected at this particular site as seen in Eq. (4) (Köck-Schulmeyer et al., 2021):

$$RQ_{site} = \sum_{i=1}^{n} RQ_i \tag{4}$$

3. Results and discussion

3.1. The occurrence and physicochemical properties of ECs

The analytical information (detection frequency, maximal, minimal, and median concentrations, limits of quantification (LOQ), limits of detection (LOD), and corresponding CAS numbers) for the ECs detected in this study are listed in Table 2 (Data in Brief). Table 2 also includes locations of detection. Fig. 2 shows concentrations of detected ECs per sampling locations and contaminant group, as well as their detection frequency. Only four detected compounds (metformin, DEET, caffeine, and 1-H benzotriazole) exceeded 100 ng/L, the current EU drinking water limit for individual pesticides (Fig. 2).

During two GeoTwinn sampling campaigns in March 2019 and October 2019, ten different ECs out of 1518 monitored were detected in the Jadro spring. Four of them belonged to the pharmaceuticals group, three were agricultural compounds, and three were lifestyle products. Seven of them were detected in only one campaign, while artificial sweetener sucralose, the anticonvulsant carbamazepine, and the analgesic tramadol were detected in both campaigns. Concentration levels ranged from 0.3 ng/L (tramadol) to 55 ng/L (sucralose). Sucralose is a potential marker for tracing the sources of wastewater contamination (Van Stempvoort et al., 2011; Lapworth et al., 2012).

In five boDEREC-CE sampling campaigns, eleven out of 102 monitored ECs were detected at Jadro and Žrnovnica catchment. Out of eleven detected substances, seven belong to the pharmaceuticals group (analgesics/anti-inflammatory drugs in general), two substances can be classed as personal care products, one as the lifestyle product, and one as the industrial compound. The concentration levels ranged from 10.1 ng/L (valsartan in Jadro spring) to 372 ng/L (1H-benzotriazole in Cetina River). Seven ECs were detected in only one campaign, while industrial compound 1H-benzotriazole and antihyperglycemic drug metformin were detected in three campaigns, followed by analgesic/anti-inflammatory drug ibuprofen and insect repellent DEET detected in two campaigns. 1H-benzotriazole was the compound with the highest concentration detected in respect to all sampling campaigns (GeoTwinn and boDEREC-CE). The occurrence of 1H-benzotriazole in water samples at all sites discloses an anthropogenic impact, presumably because of the sewer networks and septic tanks (Seitz and Winzenbacher, 2017). As Loos et al. (2009) report, 1H-benzotriazole is ubiquitously detected even in remote areas, where rivers are characterised as pristine (with ECs detected in low concentrations of <50 ng/L). DEET was the most frequently detected EC (detection frequency of

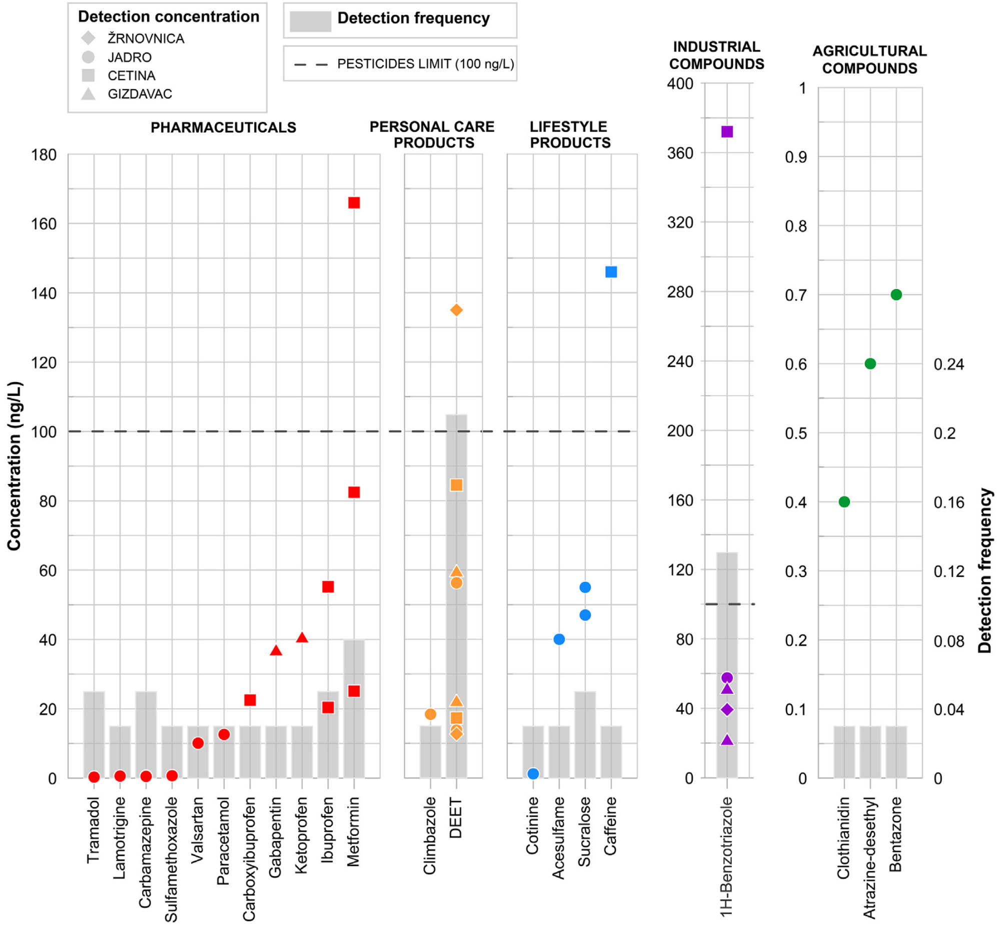

Fig. 2. Concentration and detection frequency of ECs in Jadro and Žrnovnica springs catchment.

0.21), with concentrations ranging from 12.7 ng/L to 135 ng/L both measured in Žrnovnica spring (Fig. 2), and average concentration in both surface water and groundwater of around 50 ng/L. A pan-European survey by Loos et al. (2010) on the occurrence of organic persistent compounds in groundwater also showed DEET and 1H-benzotriazole as one of the most frequently detected compounds with the frequency of 53% and 83.5% and maximum concentrations of 454 ng/L and 1032 ng/L, respectively. The average DEET concentration reported by Loos et al. (2010) in groundwater samples was significantly lower than in the case of Jadro and Žrnovnica catchment, and it amounted to 9 ng/L.

The spatial distribution of main ECs groups in water samples at four sampling locations within the Jadro and Žrnovnica catchment depicted the highest total concentrations of compounds measured in surface water of Cetina River (1004 ng/L), while groundwater samples at Žrnovnica spring had the lowest total ECs concentration (187 ng/L) (Fig. 3). Cetina and Jadro exhibited the most versatile array of compounds, with detection of all five previously mentioned ECs groups at Jadro spring, and Cetina River only lacking the agricultural group (Fig. 3). At Žrnovnica spring, only one compound per industrial and personal care group was identified.

Conducted Principal Coordinates Analysis (PCoA) showed 53.3% of the variance in data (29.6% variability in the first axis and 23.7% variability in the second axis) (Fig. 4) and confirmed how ECs content is subjected to sampling location and period.

The clustering of March 2020 data for Gizdavac, Jadro, and Žrnovnica indicated higher similarities in the compounds' distribution in groundwater. This can be explained by the detection of industrial compound 1H-benzotriazole at all those sampling sites. The only exception in March 2020 data is for Cetina River, as it encompassed lifestyle product caffeine and two pharmaceuticals, ibuprofen and metformin. However, those data correlated with March 2019 data of Jadro, October 2019 data of Jadro and Cetina, as well as with data of Gizdavac borehole sampled in September 2020. This clustering can be attributed to the detection of pharmaceuticals at those locations during mentioned periods. The high similarity of samples from March 2019 and October 2019 at Jadro spring is displayed as overlying points in Fig. 4. A seasonal variation can be observed as two distinct clusters, one for all sampling sites in November 2020 and the other determined for Cetina and Gizdavac and Jadro and Žrnovnica springs all for July 2020 data. The main reason behind the clustering of November and July data is the occurrence DEET.

The exchange of ECs substances from one environmental compartment to another strongly depends on their physicochemical properties like molecular structure, polarity, water solubility, and sorption. All ECs detected in this study share the property of being organic molecules. Table 3 (Data in Brief) encompasses the compound name, molecular formula and weight, $\log K_{OW}$, $\log K_{OC}$, pK_a, and the values of ECs solubility in water. Nevertheless, they do not represent a homogenous group of substances, as their structure (predominantly cyclic or polycyclic), and functionalities differ. Namely, oxygen and nitrogen are the main heteroatoms present in the majority of compounds, while sulphur and chlorine occur in a few of their structures. These structural characteristics directly influence the physical, chemical, or biological behaviour of detected compounds, and are important for the estimation and evaluation of their *PBT* potential according to the (Q)SAR methodology (Ortiz de García et al., 2013). Furthermore, the $\log K_{OW}$, $\log K_{OC}$, and pK_a parameters delineate the movement of compounds between environmental compartments, i.e. the hydrophobic phase (soil, sediment, and biota) and the water phase (Köck-Schulmeyer et al., 2021). pK_a affects the sorption behaviour and thus bioavailability and environmental mobility of the compounds (Ternes et al., 2004). Based on $\log K_{OW}$ values of ECs detected in this study (~ -1 to ~ 4 as seen in Table 3, Data in Brief), it can be assumed how the majority of

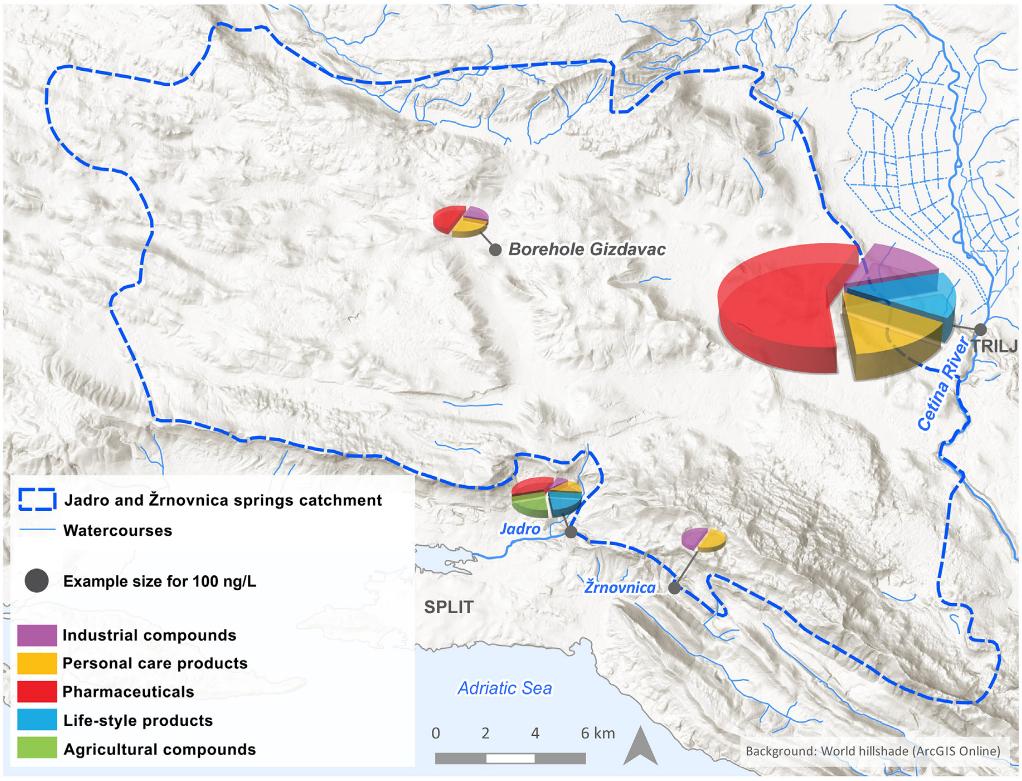

Fig. 3. Spatial distribution of main ECs groups in water samples at four sampling locations within Jadro and Žrnovnica springs catchment; circle sizes represent the total concentration of all detected ECs per site.

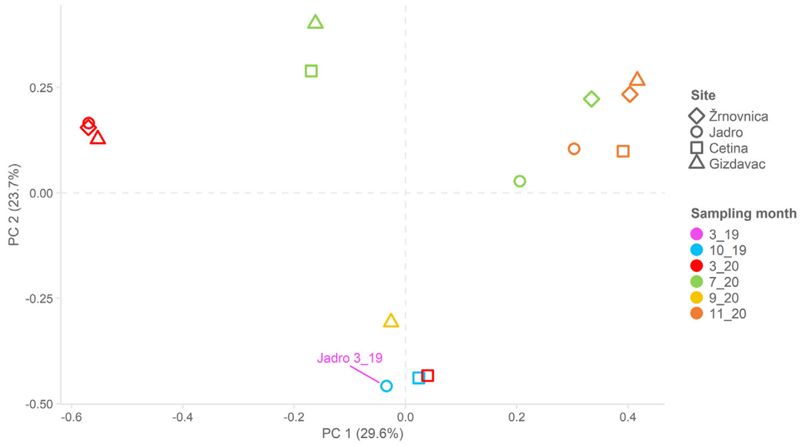

Fig. 4. Principal coordinates analysis (PCoA) done for concentrations of detected emerging contaminants per sampling locations and months. The percentage of variance is explained by each PC.

detected ECs substances are hydrophilic and will not likely bioaccumulate in aquatic species. Out of 21 detected ECs, analgesic/anti-inflammatory drugs ibuprofen, ketoprofen, antimycotic climbazole, anticonvulsant lamotrigine, analgesic tramadol, and antihypertensive agent valsartan have the highest log K_{OW} values (predicted or experimental values spanning from ~3 to ~4).

Upon reaching the aquatic environment, dynamics, concentration, and fate of ECs are subjected to different physical (dispersion and dilution), chemical, and biological processes like hydrolysis, photolysis, volatilisation, biodegradation, transformation, and sorption (Mandaric et al., 2015). Moreover, the variation in environmental conditions (saturated or unsaturated) also affects the attenuation of ECs in the aquifer (Lapworth et al., 2012). For instance, the sorption capacity is decreased with the increase of temperature, while biodegradation potential is increased (ten Hulscher and Cornelissen, 1996). However, the environmental data (measured water temperature and electrolytic conductivity, Table 4 of Data in Brief) used in this study did not explain a statistically significant proportion of the total variance that was included in the visualization (PERMANOVA, $p < 0.05$). Simply put, the slight differences in water temperature and electrolytic conductivity between the sampling campaigns, as well as low-frequency sampling data, did not allow finding statistically significant relationships between these environmental parameters and the occurrence of ECs.

3.2. PBT ranking

The in silico predicted values of *PBT* analysis are presented in Table 5 (Data in Brief), showing the ranking of all detected ECs. *PBT* scores are ranging from 0.158 to 0.433. According to Pizzo et al. (2016b), *PBT* values close to 1 are obtained for compounds of greatest concern, with *P, B,* and *T* assessments performed with good reliability. Thus, ECs detected in this study can be characterised as *non-PBT* or compounds of expected low concern, given their low *PBT* score values that are all under the threshold of 0.5. The reliability of the predicted *PBT* properties of all detected ECs was generally moderate to high for persistence, low to moderate for toxicity endpoint, and mainly high for bioaccumulation factor *B*.

Climbazole, carbamazepine, and valsartan detected at Jadro spring stand out with the highest *PBT* scores (0.433, 0.432, and 0.431, respectively) (Fig. 5). All three compounds are ranked as persistent to very persistent, although with medium reliability. Antimycotic preservative climbazole used globally in cosmetics has been frequently detected in wastewaters, water resources, and sediments denoting its poor dissipation potential and biodegradability in the natural environment and during conventional wastewater treatment (Chen et al., 2012; Chen and Ying, 2015; Liu et al., 2015). Similarly, widely reported carbamazepine is also resistant to natural attenuation and treatment due to low biodegradability and sorption capacity (Clara et al., 2004; Glassmeyer et al., 2005; Scheytt and Müller, 2011). Carbamazepine appeared at the highest *PBT* ranks among 313 organic contaminants found in sediment extracts from Sava River, Croatia (Babić et al., 2018). Carbamazepine transformation products are found in concentrations approximately 10 times higher than their corresponding parent compound (López-Serna et al., 2013) and are significantly more toxic (Donner et al., 2013), which is another concerning fact. Valsartan is one of the most frequently reported antihypertensive drugs in the environmental samples (Godoy et al., 2015), and its concentration of 10 ng/L detected at Jadro spring is far below the reported toxicity endpoints. The ecotoxicity study conducted by Bayer et al. (2014), showed no acute toxic valsartan effect on the aquatic test organisms at concentrations of up to 120 mg/L, while NOEC on the growth rate of algae was 85 mg/L.

Bioaccumulation factor *B* values are predicted in the range from 0.05 (artificial sweetener acesulfame) to 2.61 (anticonvulsant lamotrigine), all under the *B* threshold of 3.3 (log 2000 for bioaccumulative compounds, defined by Pizzo et al., 2016b). *B* value for climbazole is predicted to 1.81 (score 0.255), for carbamazepine to 1.26 (score 0.278) and for valsartan to 0.71 (score 0.242). There are only a few environmental studies on lamotrigine, which is substituting other widely used anticonvulsant drugs like carbamazepine (Ferrer and Thurman, 2012). Lamotrigine detected in wastewater, groundwater, and surface water might serve as an indicator of treated wastewater in raw water resources used for drinking water supply (Bollmann et al., 2016). In surface water, its frequencies are ranging from 47 to 97% (Young et al., 2014), while Ferrer and Thurman (2010) report how lamotrigine was found in 93% of 15 alluvial groundwater samples taken down gradient of wastewater treatment plants, in the mean concentration of 324 ng/L. Within our study, lamotrigine has been detected only once (October 2019) in Jadro spring at concentrations of 0.6 ng/L, considering how within two GeoTwinn sampling campaigns the limits of detection (LOD) were 1 ng/L, while within boDEREC-CE sampling they were 10 ng/L.

For the toxicity endpoint *T*, the threshold of 0.5 corresponds to 0.1 mg/L (−1 log unit), and substances with values above them are indicated as of possible concern (Pizzo et al., 2016b). The highest *T* scores in Jadro and Žrnovnica catchment have lamotrigine (0.658) and ketoprofen (0.519). In the risk-based ranking approach for prioritisation of pharmaceuticals inducing potential risk to ecological and human health, Dong et al. (2013) also assigned a high rank for lamotrigine because of its potential toxicity across a broad range of endpoints. Along with lamotrigine, another substance of possible concern is ketoprofen detected in groundwater

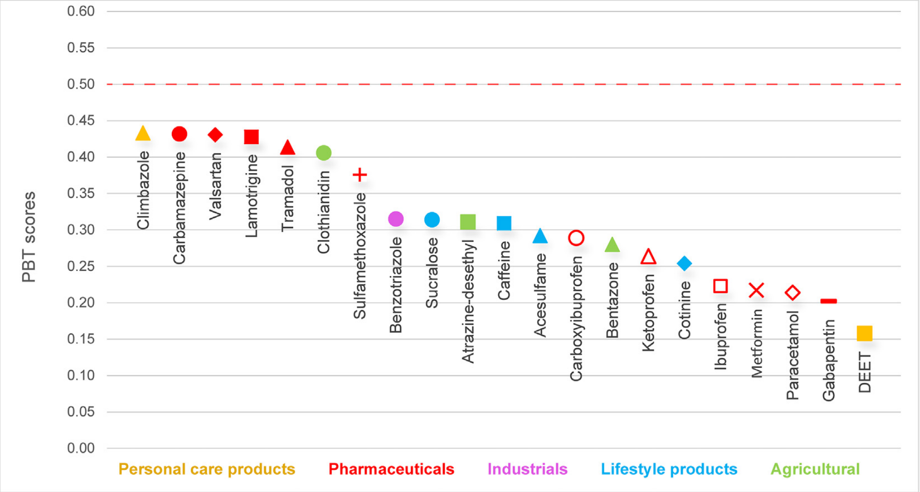

Fig. 5. In silico prediction of *PBT* values in Prometheus software; 0.5 threshold differentiates PBT substances from non-PBT substances.

samples at the Gizdavac borehole at a concentration of 40.8 ng/L. Ketoprofen was found in six out of 69 studies on ECs concentrations in groundwater and average concentrations of 611 ng/L (Lapworth et al., 2012), while Loos et al. (2010) report average concentrations of 26 ng/L in groundwater samples from the pan-European research. High acute ketoprofen toxicity to embryonic stages of zebrafish *Danio rerio* and detrimental effects on the growth and development of carp embryos were confirmed by Praskova et al. (2011, 2013).

The compound at the bottom of the overall *PBT* ranking with the score of 0.158 is DEET (Fig. 5), detected in both surface water and groundwater, and predicted as non-persistent. Its measured concentrations in Jadro and Žrnovnica catchment are several thousands to tens of thousands lower than the lowest reported NOEC values of 0.5 mg/L (Rao, 2003) and 24 mg/L (Harada et al., 2008). All the above implies a low DEET potential for adverse effects on environmental species.

It is to be noted how the Prometheus software failed to predict the persistence of metformin (CAS 657-24-9). Metformin is banned from use in any cosmetic products in the European Union (Regulation No 1223/2009) and is one of the substances proposed to be included in the future Watch List under the Water Framework Directive along with gabapentin and ibuprofen (Gomez Cortes et al., 2020). Furthermore, for valsartan, bentazone, and sucralose, toxicity could not be predicted.

The *PBTr* ranking results (Table 5, Data in Brief), based on *PBT* scores and concentration of ECs in water, denote 1H-benzotriazole and caffeine as ECs with the highest ranks i.e. *PBTr* values of 116.81 and 44.97, respectively (Fig. 6). 1H-benzotriazole, caffeine, and metformin were all detected in the highest concentrations within the Jadro and Žrnovnica catchment, which is one of the reasons why their *PBTr* scores are the highest. As Babić et al. (2018) conclude, the *PBTr* analysis accentuates those ECs having both high lipophilicity (high log K_{OW} value) and high concentration. Common to all sampling sites are the highest *PBTr* ranks of 1H-benzotriazole (Fig. 6), which corresponds to the clustering of March 2020 results for Jadro, Žrnovnica, and Gizdavac sites evidenced with PCoA analysis. Partitioning coefficient log K_{OW} of 1H-benzotriazole is 1.44 (Exp_logK_{OW}_EPISuite) and its experimental water solubility is 2.16e + 004 mg/L at 25 °C, while caffeine has log K_{OW} of −0.07 (Exp_logK_{OW}_EPISuite) and water solubility of 1.98e + 004 mg/L. This shows how 1H-benzotriazole and caffeine will be mainly present in the water phase, as they are both hydrophilic and highly water-soluble compounds. Metformin has an even higher predicted water solubility of 1e + 006 mg/L and the lowest predicted log K_{OW} value (−1.4) out of all detected compounds. At the bottom of the *PBTr* ranking are agricultural product clothianidin and pharmaceutical tramadol (Fig. 6), which are both far less water-soluble (predicted values of 5997 and 1151 mg/L, respectively).

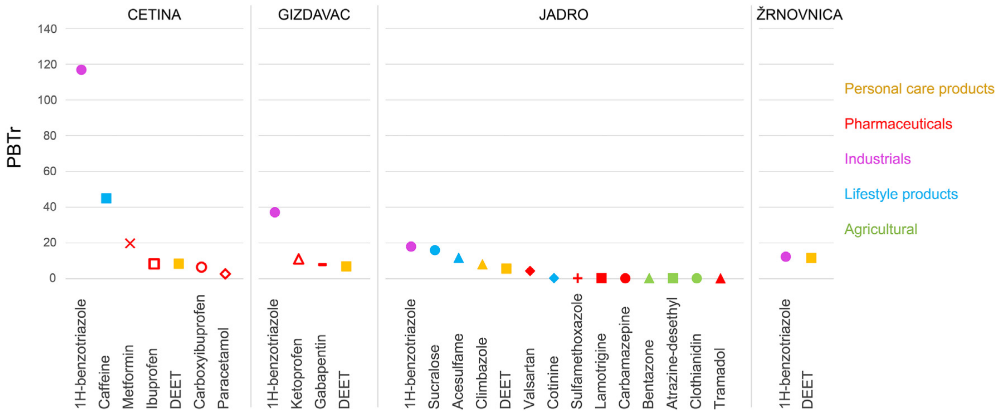

Fig. 6. *PBTr* ranking of detected ECs per sampling sites based on their environmental concentration and PBT score; different colours represent main groups of substances.

3.3. PMT/vPvM assessment

Out of 21 detected, 4 substances were assessed as *vPvM & PMT*, 3 as *vPvM*, 1 as PMT, 1 as *PM*, and 8 as *potential PMT/vPvM* (Table 6, Data in Brief). Only 4 compounds not meeting the "*P*" criteria were assessed as *not PMT/vPvM*. Similarly to Huang et al. (2021), we found that drinking water resources i.e. karst springs had larger percentage of *PMT/vPvM* substances than surface water of Cetina River (Fig. 7). Karst springs also stood out with identified *vPvM*, *PMT*, and *PM* categories. In case of increased emission and elevated concentrations, those compounds will present a significant concern for the aquatic ecosystems and drinking water supply. It is important to emphasize that all compounds were identified as very mobile (log $K_{OC} < 3$), except for climbazole which was categorised as mobile (log $K_{OC} < 4$). Karstified aquifers with large fractures and conduits are generally considered having lower attenuation potential, due to rapid and concentrated groundwater flow, allowing contaminants to travel long distances and reach springs used for water supply in relatively short time. Furthermore, carboxyibuprofen and DEET were the only compounds assessed as non-toxic (*not T*). Still, carboxyibuprofen was predicted as potentially toxic with Developmental/Reproductive Toxicity library (PG) 1.1.0 and Fish Acute (LC50) Toxicity classification SarPy/IRFMN 1.0.2 models in VEGA QSAR. However, all substances detected in this study had concentrations in ng/L that are far below the limit values defined for toxicity criteria (<0.01 mg/L).

Climbazole, which had the highest predicted *PBT* score, was highlighted as *vPvM & PMT* compound, along with valsartan, sulfamethoxazole and frequently and ubiquitously detected 1-H benzotriazole (also having the highest *PBTr* score at all sites). Atrazine-desethyl, clothianidin, and lamotrigine were given *vPvM* category. Carbamazepine was evaluated as *PMT* and tramadol as PM substance. Apart from atrazine-desethyl having middle range *PBT* score, these ECs had also higher *PBT* scores compared to other detected ECs. Caffeine having similar *PBT* score to atrazine-desethyl was evaluated as not *PMT/vPvM* compound. Interestingly, DEET which was detected at all sites and given the lowest *PBT* score was assessed as potential *PMT/vPvM* substance.

3.4. Risk quotients – environmental risk assessment

Disclosed presence of various ECs groups in the Jadro and Žrnovnica catchment (Fig. 3) entails the need to examine related potential environmental risks. The preliminary ERA was performed to characterise the contamination extent and to estimate the incidence of adverse effects of ECs substances occurring in surface water and groundwater.

The calculated risk quotients *RQ* values were within the ranges of 0.0002 for valsartan to 0.12 for caffeine (Table 7, Data in Brief). The majority of detected ECs posed no environmental risk with *RQ* values predominantly below 0.01 (Fig. 8). The exceptions were carbamazepine (*RQ* of 0.01 and 0.012, detected in Jadro spring), ketoprofen (*RQ* of 0.019, detected in Gizdavac borehole), ibuprofen (*RQ* of 0.012 and 0.03, detected in Cetina River), climbazole (*RQ* of 0.035, detected in Jadro spring), and 1H-benzotriazole (*RQ* of 0.047, detected in Cetina River) posing low potential risk. Only caffeine (a *non PMT/vPvM* substance), detected in the Cetina River is characterised with moderate potential environmental risk due to its *MEC* of 146 ng/L.

The environmental risk of substances found in water resources is often assessed without considering the toxicity and effects of mixtures. Thus, in this study, a total risk quotient per each site RQ_{site} is derived. The acquired RQ_{site} values varied given the sampling season and detected compounds (Fig. 8).

Žrnovnica spring had the lowest RQ_{site} out of all analysed sampling sites, which ranged from 0.0001 in July 2020 to 0.005 in March 2020. Contrary, Jadro spring displayed the lowest RQ_{site} value of 0.007 in March 2020, and the highest RQ_{site} value of 0.035 in July 2020 driven by climbazole. Pharmaceutical ketoprofen accounted for the highest RQ_{site} value at the Gizdavac borehole in November 2020. Compared to other sampling sites, Cetina River showed the highest RQ_{site} value of 0.134 in March 2020, which is mostly driven by caffeine posing a moderate potential risk, and 1H-benzotriazole and ibuprofen, causing a low environmental risk. For Cetina River, the lowest RQ_{site} values are attributed to November 2020. When exhibiting the cumulative RQ_{site} values of all sampling campaigns per site, Cetina River (RQ_{site} of 0.22) comes across as the site with the highest potential environmental risk (Fig. 8). However, this does not imply how the risk is high, as the concentrations of detected ECs are still relatively low compared to other studies (Loos et al., 2009, 2010). For instance, the Sava River in the alluvial part of Croatia exhibited much greater total risk quotients of up to 20 per sampling site, which are attributed to different pollution sources (Köck-Schulmeyer et al., 2021).

The total risk quotient approach does not take into consideration the chemical characteristics of the detected contaminants nor the biology of the exposed organisms. Moreover, the applied ecotoxicological endpoints addressed only freshwater species (surface waters), given the scarcity of ecotoxicological testing on subterranean aquatic organisms (Castaño-Sánchez et al., 2020). Bearing in mind how stygofauna is susceptible to relatively slight variations in concentrations of contaminants (Castaño-Sánchez et al., 2020), the toxicity of occurring ECs and their mixtures on subterranean biota should be closely examined in future research.

In addition, the release of antibiotics into the environment consequently leads to bacteria resistance, which is an issue of arising substantial concern (Levy and Marshall, 2004). In water and sediments samples from the downstream part of Jadro River, which is under the influence of agricultural areas and municipal wastewaters, Maravić et al. (2016) identified a multidrug-resistant *Acinetobacter* spp. isolates with higher resistance rates compared to other environmental studies. Importantly, resistance to antibiotics is possible even at levels below the minimal inhibitory concentration (MIC) (Andersson and Hughes, 2014). It should be noted that each concentration of all ECs detected within this study (including the only detected antibiotic sulfamethoxazole identified at Jadro spring) is well below the corresponding lowest PNEC value, suggesting how currently there are no concerns regarding the promotion of antibiotic resistance in spring water. However, synergistic or antagonistic effects of antibiotics at sub-MIC levels, in tandem with other ECs, are still uncharted scientific territory. The potential risk of increased disease transmission due to occurrence of antibiotics in the aquatic environment highlights the need for more extensive studies, as it concerns human health not only on local but also global scales.

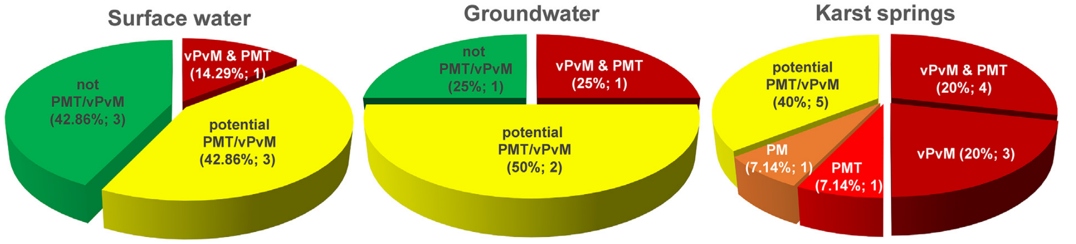

Fig. 7. The distribution of *PMT* categories assessed for ECs detected in Jadro and Žrnovnica springs catchment (the values in brackets represent percentage of each category per water resource and number of ECs per category).

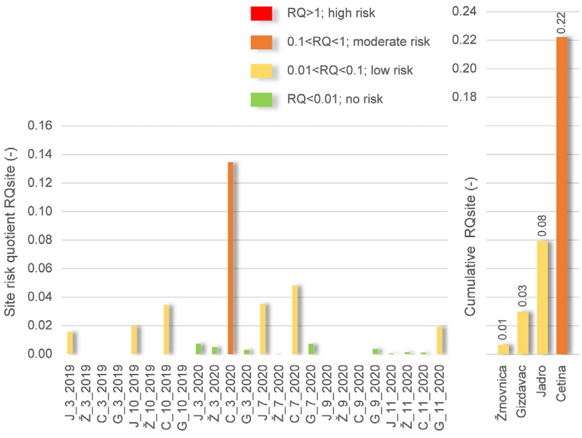

Fig. 8. Site risk quotient *RQ_{site}* calculated for each sampling site and campaign, and cumulative *RQ_{site}* for each sampling site (abbreviations meaning: J – Jadro, Ž – Žrnovnica, C – Cetina River, G – Gizdavac borehole).

4. Conclusion

An extensive array of natural and synthetic ECs, including their metabolites and transformation products, occur worldwide in karst aquifers imperilling dependent ecosystems and consequently human health. In this study, we assessed the occurrence of 21 ECs analysed for the first time in Croatian Dinaric karst, namely the catchment of Jadro and Žrnovnica springs used for water supply. Springs, surface water, and groundwater were sampled during seven campaigns. The ECs concentrations ranged from 0.3 ng/L (tramadol in Jadro spring) to 372 ng/L (1H-benzotriazole in Cetina River). There are no environmental limit values set for ECs in the current legislation, however we must emphasize how concentrations of metformin (pharmaceutical), DEET (personal care product), caffeine (life-style product), and 1-H benzotriazole (industrial compound) exceeded 100 ng/L, the current EU drinking water limit for individual pesticides. DEET was the most frequently detected ECs with an average concentration of around 50 ng/L in both surface water and groundwater, which is significantly higher value that the average concentration reported in the pan-European groundwater survey. Cetina River had the highest total ECs concentrations (1004 ng/L), and together with Jadro spring had the most versatile groups of ECs. Principal Coordinates Analysis demonstrated how ECs content was subject to sampling location and period.

Considering the paucity of research addressing ECs in karst aquifers at the global and national level, this study provides valuable findings on their potential environmental risk. The *PBT* prioritisation approach resulted in all ECs predicted as *non-PBT* or compounds of expected low concern, while *PBTr* ranking per site highlighted 1H-benzotriazole and caffeine as ECs with the highest ranks. The preliminary ERA also underlined caffeine as a compound posing moderate potential environmental risk (*RQ*). Cetina River stood out with the highest *PBTr* score and risk quotient *RQ_{site}*.

Notably, *PMT/vPvM* guidelines set by REACH showed how only 4 out of 21 detected ECs did not meet the *PMT/vPvM* criteria. Karst springs had larger percentage of *PMT/vPvM* substances than the surface water. Moreover, nearly all ECs were assessed as very mobile, which is especially worrisome as intrinsic karst properties could allow contaminants to travel long distances and reach drinking water supply resources in relatively short time.

The data obtained in this study can serve as a good foundation for future monitoring activities aimed at contaminants of concern. We stress the need for establishing regular monitoring of ECs, as it will acquire knowledge on their behaviour and fate, especially in still insufficiently explored karst aquatic environment, as well as set up the basis for regulatory changes directed towards prevention of their negative effects on ecosystems and human health. Lastly, we acknowledge how research aimed at understanding of long-term exposure to various ECs mixtures and their toxicity mechanisms across taxa, especially for stygobionts, is strongly needed.

CRediT authorship contribution statement

Ana Selak: Conceptualization, Methodology, Writing – original draft, Data curation, Visualization, Investigation. **Jasmina Lukač Reberski:** Writing – review & editing, Investigation, Supervision, Project administration. **Göran Klobučar:** Supervision, Methodology, Writing – review & editing. **Ivana Grčić:** Supervision.

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.

Acknowledgements

We would like to express our gratitude to MSc Lorena Selak from the Ruđer Bošković Institute for her technical support in R statistical analysis.

Funding

This work was supported by Interreg Central Europe boDEREC-CE project co-funded by the European Regional Development Fund and GeoTwinn project funded under H2020-EU.4.b., [grant number 809943].

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.scitotenv.2022.153827. These data include the Google map of the most important areas described in this article.

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Paper VI

Selak, A., Lukač Reberski, J., Klobučar, G., Grčić, I., 2022. Data on occurrence and ecotoxicological risk of emerging contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs. Data Br 42, 108157. https://doi.org/10.1016/j.dib.2022.108157

Data in Brief 42 (2022) 108157

Contents lists available at ScienceDirect

Data in Brief

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

Data Article

Data on occurrence and ecotoxicological risk of emerging contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs

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ARTICLE INFO

Article history:
Received 24 March 2022
Revised 1 April 2022
Accepted 4 April 2022
Available online 10 April 2022

Dataset link: Dataset on occurrence and ecotoxicological risk of emerging contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs (Original data)

Keywords:
Emerging contaminants
Persistence
Bioaccumulation
Toxicity
Mobility
Risk quotient (RQ)
Water
Karst

ABSTRACT

Karst catchments are valuable drinking water sources and fragile habitats to many endemic species. This dataset presents initial insights into the occurrence and ecotoxicological risk of 21 emerging contaminants (ECs) (including 11 pharmaceuticals, 4 lifestyle products, 2 personal care products, 3 agricultural and 1 industrial compound) detected in Dinaric karst catchment of Jadro and Žrnovnica springs in Croatia. Contaminants concentrations were determined with UHD Q-TOF LC/MS and UHP LC/MS in samples from two springs (Jadro and Žrnovnica), one river (Cetina), and a deep borehole (Gizdavac). Persistence (P), bioaccumulation (B), mobility (M) and toxicity (T) of detected ECs were assessed based on *in silico* strategy for PBT assessment and recently developed REACH PMT/vPvM guidelines. Risk quotients were calculated from PNEC values and measured contaminants' concentrations. In addition, physicochemical properties (estimated and existing experimental values of solubility in water, log K_{OW}, log K_{OC}, and pK_a) of detected substances and water (measured values of temperature and electrolytic conductivity) are provided. This dataset could be

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DOI of original article: 10.1016/j.scitotenv.2022.153827

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https://doi.org/10.1016/j.dib.2022.108157

useful for setting up the regular monitoring and improvement of existing water-related legislative, water safety plans, for modelling contaminant transport and identification of potential sources, and lastly for comparison with other studies conducted in karst aquifers.

The present dataset was interpreted and discussed in the article entitled "*Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer of Jadro and Žrnovnica springs*" by Selak et al. (2022).

Specifications Table

Subject	Environmental Science
Specific subject area	Ecotoxicological prioritization of emerging contaminants in water
Type of data	Tables Figure
How the data were acquired	Field monitoring and sampling; laboratory analysis (liquid chromatography-mass spectrometry); existing database research; use of EPI Suite^{TM} software; use of Prometheus software; use of PMT/vPvM guidelines; use of QSAR software; use of ToxTree software; use of risk quotient methodology
Data format	Raw Analyzed
Description of data collection	Data was obtained by analysing surface water and groundwater samples collected at 4 sampling points in the karst catchment of Jadro and Žrnovnica springs. Electrolytic conductivity and water temperature were observed at all sampling sites. PBT values of detected ECs were estimated *in silico*, PMT/vPvM categories were assessed according to REACH guidelines, while their environmental concentrations were used to determine the potential environmental risk that they pose.
Data source location	Institution: Croatian Geological Survey City/Town/Region: Split-Dalmatia County, Jadro and Žrnovnica catchment Country: Croatia Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: Jadro spring 43°32′34.6″N, 16°31′20.6″E; Žrnovnica spring 43°31′24.5″N, 16°34′28.4″E; Cetina River 43°37′02.9″N, 16°43′44.0″E; Gizdavac borehole 43°38′43.7″N, 16°29′07.6″E
Data accessibility	The dataset is hosted on a public repository. Repository name: Mendeley Data Data identification number: 10.17632/byk4tyh4jd.1 Direct URL to data: https://data.mendeley.com/datasets/byk4tyh4jd/1
Related research article	A. Selak, J. Lukač Reberski, G. Klobučar, I. Grčić Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer Sci Total Environ. 825 (2022) 153,827. https://doi.org/10.1016/j.scitotenv.2022.153827

Value of the Data

- The electrolytic conductivity and water temperature are parameters providing baseline knowledge on groundwater and surface water dynamics and character. They also serve as natural tracers for the determination of the relations between specific locations within the catchment area.
- The measured concentrations and main physicochemical properties of detected emerging contaminants provide initial insights into their occurrence in the karst catchment and allow their ecotoxicological characterisation.

- The persistence, bioaccumulation, mobility, and toxicity, estimated based on quantitative structure-activity relationships QSAR modelling and according to PBT and PMT/vPvM assessment guidelines, enable prioritization of emerging contaminant substances. Calculated PBTr values differentiate locations having no, low, medium, or high cumulative PBT rank.
- Risk quotient (RQ) values represent valuable findings on the potential environmental risks that detected emerging contaminants are posing. RQsite values enable the classification of sites based on cumulative risk quotient and point out sites with the highest potential environmental risk.
- The dataset has a multidisciplinary value, as it can be utilised by hydrogeologists, biologists, chemists, and other researchers dealing with water management or environmental issues, as well as national or regional authorities responsible for monitoring activities or practitioners like water suppliers.
- The dataset can be used as an input for modelling contaminant transport and identification of contamination sources, for prioritizing future (eco)toxicological and hydrochemical research, water safety plans, and lastly for comparison with other studies.

1. Data Description

The sampling locations chosen within the Dinaric karst catchment of Jadro and Žrnovnica springs (Croatia) are shown in Fig. 1, while their coordinates (in WGS84) are given in Table 1.

The dataset of six tables (Table 2 to 7) in XLSX format is deposited in Mendeley Data online repository [1]. Detection frequency, minimal, maximal, and median concentrations of detected

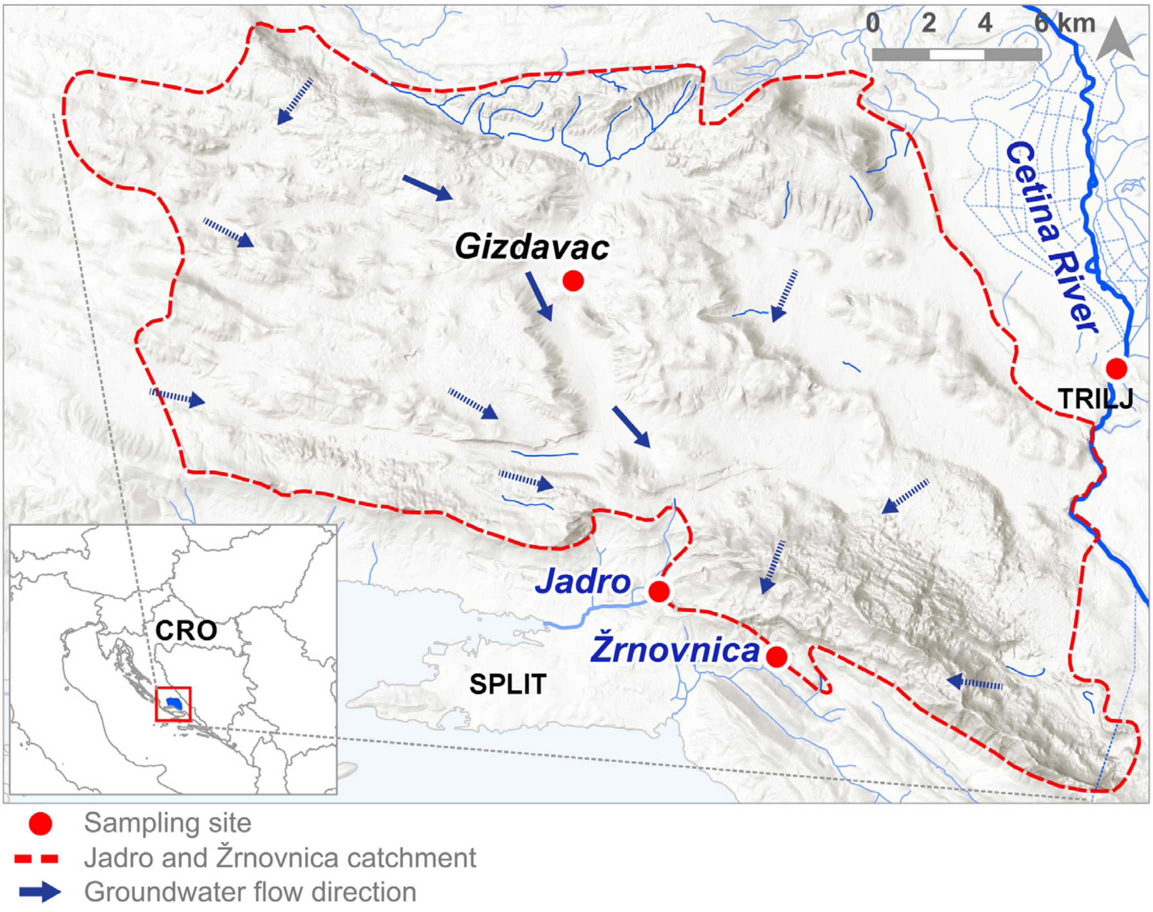

Fig. 1. Sampling locations within Jadro and Žrnovnica springs catchment.

Table 1
Sampling locations coordinates.

Sampling site	Sampling medium	Latitude (N)	Longitude (E)
Jadro	Spring water	43°32′34.6″	16°31′20.6″
Žrnovnica	Spring water	43°31′24.5″	16°34′28.4″
Cetina	River	43°37′02.4″	16°43′44.6″
Gizdavac	Borehole	43°38′43.7″	16°29′07.6″

emerging contaminants are shown in Table 2 along with their CAS number, description/use, substance group, limits of detections in two commissioned laboratories (National Laboratory Services, Starcross UK, and Plsen Laboratory Czech Republic), and detection location. Table 3 consists of two worksheets, one showing physicochemical properties of detected emerging contaminants and the other one used references. It includes the molecular formula and weight (g/mol) of each substance, their predicted and existing experimental logarithm of partition coefficient between octanol and water (log K_{OW}), organic carbon-water partitioning coefficient (log K_{OC}), acid dissociation constant (pK_a), and contaminants' solubility in water (mg/L at 25 °C). Physicochemical properties of water (temperature in the first worksheet and electrolytic conductivity in the second worksheet) measured at Jadro and Žrnovnica springs, Cetina River, and Gizdavac borehole, are given in Table 4. Table 5 presents persistence (P), bioaccumulation (B), toxicity (T) values of each contaminant with predicted reliability and scores in the first worksheet. The calculated PBTr values for each sampling site and detected compound are given in the second worksheet of Table 5. In Table 6 PMT/vPvM assessment is given. The first worksheet contains the summary of assessed PMT/vPvM categories. All steps of the PMT/vPvM assessment are given in the second worksheet, while the third one contains used references. Lastly, Table 7 includes the lowest predicted no-effect concentration values (ng/L), measured environmental concentrations (MEC, ng/L), and related risk quotients (RQ) calculated for each site and sampling campaign.

2. Experimental Design, Materials and Methods

2.1. Measurement of water physicochemical parameters

Physicochemical properties of water (temperature and electrolytic conductivity) were measured using a WTW multi-parameter probe. At Jadro and Žrnovnica springs, measurements were made directly, while at Cetina River parameters were measured in a bucket containing water grabbed from the middle of the river channel. At the Gizdavac borehole, physicochemical parameters were measured in a bucket containing the pumped third volume of groundwater [2].

2.2. Analytical methods

The analysis of emerging contaminants in surface and groundwater samples carried out in the course of the GeoTwinn project was done at UK National Laboratory Services at Star Cross 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). Sample extraction was done with Waters Oasis HLB SPE cartridges (200 mg) with an automated extraction system. Cartridges were conditioned with 6 mL of methanol followed by 6 mL of Ultra High Purity (UHP) water. The water sample (500 mL, flow rate 10 mL/min) was then loaded onto the cartridge. After loading, the cartridge was washed with 6 mL of UHP water and the sorbent dried fully with high purity nitrogen. The cartridges were then eluted twice, firstly with 6 mL of 0.1% formic acid in methanol:acetonitrile (1:1) and then with 6 mL of dichloromethane (DCM). The elutants are collected in separate vials. The DCM eluate was evaporated to incipient dryness under a gentle stream of nitrogen. Corresponding methanol:acetonitrile eluate is then transferred to the dry DCM vials and evaporated at 100 µL at 35–40 °C. 900 µL of UPH water is added to each of the vials containing the 100 µL extract. The sample is vortexed mixed, filtered, and transferred to a silanized screw-top vial ready for analysis. An isotopically labelled internal standard Carbutamide-d9 was added to each of the pre-conditioned SPE cartridges to assess instrument performance. Target compounds have been analysed using a blank sample and a standard with a concentration of 0.1 μg/L. The response factor obtained is used to create a calibration curve.

Samples collected within the boDEREC–CE project were analysed at the Vltava River Basin Authority laboratory following the EPA method 1694 (Axys Analytical Services, Ltd.) and valid procedures. Upon arrival at the laboratory, the samples were defrosted at a maximum temperature of 30 °C on the day of analysis. The analysis was done with 1290 ultra-high-performance liquid chromatograph (UHPLC in electrospray ionisation ESI+ and ESI- modes) coupled with an Agilent 6495B Triple Quad Mass Spectrometer (MS/MS). Sample preparation included centrifugation in headspace vials for 10 min at about 3500 rpm and weighting of 1.5 g of each sample in a 2 mL vial on the analytical balance. Subsequently, 1.5 μL of acetic acid was added to each sample. An isotope dilution was performed in the next step. Deuterated internal standards of carbamazepine-d_{10}, sulfamethoxazole-d_6, iopromide-d_3, iopamidol-d_3, erythromycin-$^{13}C_2$, ibuprofen-d_3, diclofenac-d_4, naproxen-d_3, chloramphenicol-d_5 and others were used. The separation was achieved with Waters Xbridge C18 analytical column (100 mm x 4.6 mm i.d., 3.5 μm particle size). Methanol and water with 0.02% acetic acid and 0.5 mM ammonium fluoride were used as the mobile phase additives at the flow rate of 0.5 mL/min. The injection volume was 0.050 mL. Each series of samples was verified by calibration control and by maintaining a clean environment, equipment, and agents. The performance of the analytical system was ensured by blank and spiked samples. The chemicals used for preparing calibration solutions had a certified purity of 99%. Calibration solutions were prepared from neat analytes or solutions with certified concentrations. Each fifth sample in a series was processed by the method of standard addition, which was used to control the effect of the matrix of the sample and to reset the actual recovery ratio of a specific analyte.

2.3. Physicochemical properties of emerging contaminants

Physicochemical properties of emerging contaminants were gathered from the publicly available NORMAN Substance Database [3] and PubChem [4]. In the absence of experimental data, those physicochemical properties were estimated using the EPI Suite^{TM} 4.11 interface [5].

2.4. PBT ranking

Persistence, bioaccumulation, toxicity (PBT) values of detected emerging contaminants were estimated in Prometheus software [6]. Given their persistence (*P*), toxicity (*T*), and bioaccumulation (*B*) characteristics compounds were scored following Eq. (1), defined by Pizzo et al. [6].

$$\mathrm{PBT} = \mathrm{P}^{0.4} \cdot \mathrm{B}^{0.4} \cdot \mathrm{T}^{0.2} \tag{1}$$

PBTr values were obtained based on estimated *PBT* values and measured concentrations of detected emerging contaminants, as proposed by Babić et al. [7] Eq. (2):

$$\mathrm{PBTr} = \frac{\sum_{i=1}^{n} R_i w_i}{\sum_{i=1}{}^{n} w_i} \tag{2}$$

where *Ri* is the rank calculated by multiplying PBT score and measured concentration, and *wi* is the weight. Given how PBT score and concentration are seen as factors equally relevant for the potential risk, all weights are set to 1.

2.5. PMT/vPvM assessment

The persistence (P), mobility (M), and toxicity (T) criteria of all detected compounds were evaluated according to REACH guidelines for PMT/vPvM assessment [8].

The criterion for persistency is the degradation half-live of ECs in water or sediment not shorter than 40 days, while very persistent substances (vP) are those having the degradation half-live longer than 180 days. The EC is assessed as potential p/vP substance if there is only screening data (results of inherent/readily biodegradable tests) or QSAR data that indicate potential persistency. A category of potential P/vP++ is given to those ECs for which sufficient weight of evidence indicates that P or vP criterion is met, but it is unclear which. In the absence of experimental data, QSAR Toolbox [9] was used to predict the persistence. To predict the ECs biodegradability, ready biodegradability model (IRFMN 1.0.9.) in VEGA QSAR [10] and BIOWIN models (1,3,4, and 5 models, v4.10) in EPI Suite^{TM} were utilized. The half-lifes were also searched in CompTox Chemicals Dashboard [11].

Experimental log K_{OC} values (within 4–9 pH range) as basis for the mobility criterion were obtained from the literature and PubChem database, or predicted with KOCWIN v2.00 model in EPI Suite^{TM}. The substances with log K_{OC} values of $\leq$4.0 are considered as mobile (M), while those having values of $\leq$3.0 are very mobile (vM).

The toxicity criterion (T) is fulfilled if EC has either: the long-term no-observed effect concentration (NOEC) or EC10 for freshwater organisms less than 0.01 mg/l; or if it is carcinogenic, germ cell mutagenic, or toxic for reproduction; or if there is other evidence of chronic toxicity such as specific target organ toxicity after repeated exposure. The ECHA Classification and labelling inventory [12] was searched for toxicity data. NOEC values were acquired from EnviroTox database [13]. In case no experimental data was available, models within VEGA QSAR software were used to predict the mutagenicity, carcinogenicity and toxicity. In case none of the above criteria is met, Cramer classification was done in Toxtree v3.1.0.1851 software [14] to differentiate potential toxic substances (pT) having Cramer Class III and non-toxic substances with assessed Cramer Class II or I.

Depending on which criteria are met, six categories are defined [8]

- *vPvM & PMT* (there is sufficient weight of evidence that the EC meets the vP, vM, and T criteria);
- *vPvM* (there is sufficient weight of evidence that the EC meets both the vP and vM criteria, but not the T criterion; this category is given also to substances suspected to be potential P/vP++ if they are detected in raw or drinking water);
- *PMT* (there is sufficient weight of evidence that the EC meets the P, M, and T criteria);
- *PM* (there is sufficient weight of evidence that the ECs meets both the P and M criteria, but does not meet the T criterion nor the vPvM criteria);
- *potential PMT/vPvM* (only screening or low-quality data is available for P, M or both, and that either a conclusion of “potential P/vP” and/or “potential M/vM” is obtained);
- and *not PMT/vPvM* (the “not P” or “not M” criteria was met with sufficient weight of evidence).

2.6. Risk quotients

Risk quotient (*RQ*) values were obtained by multiplying measured environmental concentrations of detected emerging contaminants (*MEC*) and the lowest Predicted No-Effect Concentration (*PNEC*) values gathered from the Ecotox database [15]. The lowest *PNEC* value of 1.65 µg/L for ibuprofen was retrieved from the published literature [16]. Four classes of potential ecotoxicological risk of the target compound to the receiving aquatic ecosystems are determined as follows: $RQ<0.01$ for negligible risk, $0.01<RQ<0.1$ for low risk, $0.1<RQ<1$ for moderate risk, and $RQ>1$ for high risk [17,18].

The sum of *RQ* values for all compounds detected at the particular sampling location indicates the site-specific risk quotient RQ_{site} [19]:

$$\mathrm{RQ_{site}} = \sum_{i=1}^{n} \mathrm{RQ_i} \tag{3}$$

3. Ethics Statements

Not relevant.

Funding

This work was supported by the Interreg Central Europe boDEREC–CE project co-funded by the European Regional Development Fund and Horizon 2020 GeoTwinn project under (Grant 809943).

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.

Data Availability

Dataset on occurrence and ecotoxicological risk of emerging contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs (Original data) (Mendeley Data).

CRediT Author Statement

Ana Selak: Conceptualization, Methodology, Data curation, Writing – original draft, Visualization, Investigation; **Jasmina Lukač Reberski:** Conceptualization, Supervision, Investigation, Writing – review & editing; **Göran Klobučar:** Supervision, Writing – review & editing; **Ivana Grčić:** Supervision.

Acknowledgments

We would like to express our gratitude to our colleagues from VIK Split for their support in conducting monitoring activities at Jadro and Žrnovnica springs.

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3. DISCUSSION

This PhD thesis represents a pioneering effort to comprehensively investigate the occurrence and fate of EOCs within the intricate and heterogeneous groundwater systems of the Dinaric karst region in Croatia. By addressing a notable void in the existing scientific literature, it makes substantial contributions to the academic discourse within this specialized field of research.

The hypotheses posited in the thesis will be elaborated upon in the subsequent sections, contextualized within the framework of the thesis results and findings, as well as existing research in the field.

Hypothesis #1: The occurrence and concentrations of EOCs are influenced by their physico-chemical properties and the prevailing hydrogeological and hydrological conditions within karst aquifers.

EOCs constitute a vast, heterogeneous and ever-increasing group of existing and newly synthesized chemical substances that are ubiquitously utilized and consistently emitted on a global scale (Rasheed et al., 2019). The physico-chemical attributes play a pivotal role in shaping the environmental fate and impact of EOCs, by influencing their physical, chemical, and biological behaviour (Bexfield et al., 2019). The majority of EOCs detected in Croatian Dinaric karst can be characterized as small heterocyclic aromatic compounds (molecular weight up to 870 g/mol for EOCs detected at regional and 440 g/mol at local scales) by having a ring structure containing at least one carbon atom and one other element (e.g. N, O, or S). Heterocycles are an important framework of about 70% of all pharmaceuticals and agricultural chemicals due to their important role in biochemical functions (Lamberth and Dinges, 2012). Large number of detected substances exhibit the ability to retain their chemical structure. Consequently, these EOCs are not readily biodegradable and are characterised as persistent chemicals (degradation half-lives in water >40 days or >120 in sediment) or very persistent compounds (degradation half-lives in water >60 days or >180 in sediment), as evidenced in Paper II, V, and VI. Similarly, Wielens Becker et al. (2021) and Montes et al. (2022) determined that the majority of EOCs identified in their studied water environments could be classified as persistent. Persistent compounds, may be stored in epikarst or aquifer matrix and slowly leach with the arrival of recharge waters (Morasch, 2013; Hillebrand et al., 2014; Doummar et al., 2018a,b; Dvory et al., 2018), while storm pulses may activate karst conduits and flush previously stored persistent compounds towards karst springs (Doummar et al., 2014).

Parameters like the octanol-water partitioning coefficient (log K_{OW}), solubility in water, and organic carbon-water partitioning coefficient (log K_{OC}) delineate the movement of compounds between environmental compartments, i.e. the hydrophobic phase (soil, sediment, and biota) and the water phase (Köck-Schulmeyer et al., 2021). The majority of EOCs detected in Croatian Dinaric karst groundwater exhibited a hydrophilic nature (log $K_{OW}<4$) and high mobility (log $K_{OC}<3$) (Paper II, V, and VI). This is particularly concerning, as the intrinsic properties of karst systems can facilitate the rapid transport of contaminants over long distances, potentially impacting drinking water supply resources in a relatively short timeframe. Among the few hydrophobic EOCs identified, were halogenated organic compounds such as bioaccumulative (Richardson and Ternes, 2014; Paper II), very persistent and very mobile perfluoroalkyl substances (PFAS) (Paper I and II), which have a worldwide industrial application. Nearly all of the PFAS recorded in the region were classified as substances of very high concern (SVHC) along with industrial compounds bisphenol S and melamine (Paper II). During the October 2019 campaign conducted at a regional scale in Croatian karst, the total PFAS sum concentrations recorded at six springs used for drinking water supply exceeded the groundwater quality standards of 4.4 ng/L proposed with COM/2022/540 (Paper I and II) but remained below the prescribed maximal allowed concentrations of total PFAS in drinking water (500 ng/L as per Regulation OG 64/2023).

In Paper III, the postulation that persistent EOCs will be detected in groundwater during the baseflow period was confirmed. Contrarily to springs, groundwater from deep borehole Gizdavac exhibited the highest total EOCs concentrations under low flow conditions, underscoring the vulnerability of karst aquifers (Paper III). Elevated concentrations of very mobile and potentially persistent insect repellent DEET (Paper V), used in personal care products, were observed in groundwater (from the Gizdavac borehole) during the baseflow period, suggesting a constrained biodegradation process within the aquifer, i.e. limited attenuation capacity (Paper III). High susceptibility of groundwater resources to surface contamination is corroborated also by the fact that during six concurrent campaigns, groundwater from the borehole exhibited both higher total EOCs concentration and a greater number of detections above limits of detection (LOD) than springs Jadro and Žrnovnica (Paper III). In addition, persistent and very mobile industrial compound 1H-benzotriazole was detected in groundwater during baseflow conditions, but also in both groundwater and mentioned springs during spring runoff episode. The aquifers' vulnerability is further underscored by the detection of the pharmaceutical gabapentin, which regardless of its fast biodegradation was found in deep aquifer parts (borehole) presumably due to its high mobility and the oligotrophic nature of this karst system (Paper III).

Apart from the physico-chemical properties of the compounds, the structural characteristics of the aquifers, temporal fluctuations in karst springs' discharge, and existing pollution sources govern the transport, concentrations, and potential storage of EOCs (Paper I and III). Given the complex and heterogeneous nature of karst aquifers, the sampling sites observed across the Croatian karst exhibit distinct local hydrogeological and geomorphological characteristics, dictating ground cover and land use, as well as the leaching and transport of contaminants from the surface (Paper II). In extensively karstified environments like the Dinaric karst of high fracture-cavernous porosity, EOCs can enter the aquifers with minimal to no filtration via ponors or large fractures, due to a general lack of protective soil layers, which are confined to karst poljes (Paper III). Doummar et al. (2014) found how rain events triggered the rapid infiltration of EOCs, like wastewater indicator carbamazepine, via ponor into their karst aquifer. For Jadro and Žrnovnica springs aquifer, a conceptual transport model was made illustrating the groundwater flow direction between ponors and springs, proven with tracer tests (Paper III). At springs, the highest total EOCs concentrations were recorded following autumn recharge events. This suggests that surface contamination infiltrates directly through ponors and highly karstified areas or some EOCs may persist in the fracture-porous epikarst zone, later being pushed to the springs by freshly infiltrated water (Paper III). As hypothesized in Paper I and Paper III, EOCs were not detected during peak discharge presumably due to a significant dilution process. Finding a direct link between spring discharge and EOCs occurrence proves challenging due to significant temporal variations in Dinaric karst springs' discharge and groundwater flow path heterogeneity (Paper I). According to Dvory et al. (2018), the EOCs concentrations can vary influenced by the fast and slow flow mechanism in karst. For instance, insect repellent DEET outflowed in lower concentrations during baseflow at Jadro and Žrnovnica springs in the summer. Contrarily, in late autumn when the discharge episode commenced, DEET was detected at approximately five times higher total concentration recorded at the catchment scale (Paper III). Seasonal variations observed in DEET concentrations reflect its usage as an insect repellent and the compound's mobility (Paper III). In their case, Sorensen et al. (2015) attribute such occurrence patterns to diffuse sources of contamination and contaminant pulses related to recharge processes.

The research conducted at a regional scale found lower EOCs concentrations in Croatian karst springs compared to other karst groundwater studies, likely due to the high discharge rates of

Dinaric springs and a significant proportion of natural areas within the catchments. Despite recorded low concentrations, the high discharges resulted in considerable EOCs mass fluxes ranging from 10 to 10^6 ng/s, suggesting substantial pollution loads and vulnerability of Croatian karst (Paper I and III). The absence of a consistent spatial pattern in total concentrations, the number of compounds, and EOCs types indicates the highly variable nature of karst aquifers, driven by local hydrogeological conditions and varying anthropogenic influences (Paper I and II). The notable differences in the detected compounds and their concentrations observed at the same locations between the two sampling campaigns, with no clear link to discharge or season, highlight the combined effects of discharge fluctuations, seasonal land use practices changes, and the localized responses of karst springs to recharge (Paper I and II).

EOCs were also observed in the Dinaric karst surface water resources (Paper I and III). Despite being surrounded by natural areas, Vransko Lake - partially fed by karst springs and lake *vruljas* and serving the water supply needs of Cres island - contained seven agricultural, five industrial (PFAS), and one lifestyle compound (Paper I). Notably, Vransko Lake was the only site within the Dinaric karst where the industrial compound perfluoro nonanoic acid (PFNA) was detected in this regional-scale study. However, its concentration of 0.3 ng/L was below the European surface water average, which stands at 2 ng/L (Loos et al., 2009) (Paper II). The concentrations of agricultural compounds atrazine-desethyl and simazine found in Vransko Lake were much lower than those observed by Loos et al. (2007) in Lake Maggiore (Italy), which unlike Vransko Lake is surrounded by more urban areas with a denser population. The number of EOCs detected in Vransko Lake remained consistent across both sampling campaigns, yet there was a significant seasonal variation in the ΣEOCs concentrations, with levels recorded at 20.7 ng/L in spring and 4.9 ng/L in autumn, and the lake's water level being 0.4 m lower in October. Similar trends of higher EOCs concentrations in spring compared to autumn were reported by Malnes et al. (2022) but for Swedish lakes impacted by urban wastewater, while non-urban lakes exhibited relatively stable concentrations without marked seasonal differences. It is important to note that the results of only two sampling campaigns conducted at Vransko Lake are insufficient for drawing conclusions regarding the occurrence of EOCs. Thus, further research involving more frequent sampling is needed.

In Cetina River peak EOCs concentrations (ng/L) coincided with the river's lowest discharge levels observed in the summer period (Paper III). Similarly, Mandarić et al. (2019) noted the elevated pharmaceuticals concentration during the low-flow summer period in the Evortas River, which is fed by numerous karst springs. Malnes et al. (2022) also documented elevated

EOCs concentrations in Swedish rivers during low discharge conditions, where reduced dilution was noted, supported by a notable negative correlation between concentration and discharge. Kasprzyk-Hordern et al. (2008) conclude also that dilution is the main parameter influencing the concentrations of pharmaceuticals and personal care products in Welsh rivers, with concentration being elevated during dry weather conditions. European rivers exhibited higher contamination levels, with an average detection frequency of 61%, compared to groundwater, which had an average detection frequency of 25% (Loos et al., 2009, 2010). As expected, Cetina River exhibited the highest total concentration of EOCs among all sampled water resource types, with a value of 1056 ng/L (all sampling campaigns included). Additionally, the highest mass flux of EOCs at the local scale was observed in the Cetina River, specifically for the pharmaceutical metformin, with a recorded value of 1013 g/day.

EOCs offer valuable insights into contaminant storage potential and water residence time in karst aquifers. Hillebrand et al. (2014) found a robust correlation between calcium ions and the herbicide atrazine banned 20 years ago in Germany, attributing this to the gradual release of atrazine from the karst rock matrix into the groundwater. The Ca^{2+}-HCO_3^- hydrogeochemical facies is characteristic of Dinaric karst water resources (Paper IV). At the local scale, a strong positive correlation was observed between the EOCs detection number and Ca^{2+} levels in the Jadro spring samples (Paper IV). This confirms the persistence of EOCs within the epikarst and aquifer matrix.

All the above-mentioned findings validate hypothesis #1 that in karst aquifers EOCs occurrence and concentrations are strongly shaped by the prevailing hydrogeological and hydrological conditions.

Implication of results:

Groundwater represents a crucial source of drinking water, yet it faces increasing contamination pressures resulting from anthropogenic activities (Campanale et al., 2022). Additionally, climate changes are anticipated to lead to a further deterioration in both the quality and quantity of groundwater (Lukač Reberski et al. 2020). Supplying around 9.2% of the global population with water renders karst aquifers a resource of paramount importance (Stevanović, 2019). Their intrinsic vulnerability to contamination arises from the pronounced heterogeneity and anisotropy observed in structural and hydraulic properties (Moreno-Gómez-et al., 2024). The Dinaric karst aquifers serve as the primary source of drinking water for almost half of Croatia’s territory. They are characterized by an extremely high degree of karstification, well-developed conduit networks, and high spring discharge systems. All these properties make karst aquifers highly productive (Padilla and Vesper, 2018), yet the direct and rapid infiltration through ponors, fractures, and conduits within karstic terrain constrains natural attenuation processes, rendering karst aquifers especially vulnerable to anthropogenic contaminants like EOCs (Hartmann et al., 2021). Due to their persistence, EOCs may pose a significant and long-term risk to the drinking water supply, necessitating proactive protection measures.

The systematic identification and quantification of EOCs within this thesis offer first insights into EOCs in Croatian Dinaric karst water resources (springs, river, and groundwater) at regional and local scale and facilitate a comprehensive assessment of associated risks to the environment and human health, laying the groundwork for appropriate future monitoring activities. The thesis explored the intricate relationship between hydrogeological and hydrological conditions and the occurrence of EOCs within the karst catchment, with a focus on the Jadro and Žrnovnica springs catchment. This enhanced scientific understanding of the factors influencing EOCs transport and behaviour within complex groundwater systems, which could benefit other regions with similar geological features. Understanding the dynamics of EOCs in karst aquifers, i.e. contaminant pathways, potential storage, and vulnerable areas, enables more accurate risk assessments and monitoring programs tailored to specific conditions of karst aquifers. This knowledge is essential for informing the management and protection of valuable karst groundwater resources in Croatia.

Limitations:

The limitations of this thesis are multifaceted, encompassing data constraints, methodological challenges, variability in detection limits between selected laboratories, and restrictions imposed by the Covid-19 pandemic. This research is a preliminary investigation that includes a limited number of analysed EOCs that may be present in the karst water environment. The intricate nature of karst aquifers further complicates the establishment of direct correlations between hydrogeological parameters and the occurrence of EOCs. A significant limitation in both regional and local research is the paucity of laboratories equipped with suitable analytical instrumentation for EOCs analysis. Hale et al. (2022) identify several key gaps in currently employed analytical methods, including the lack of reference standards for target analysis and the challenges associated with quantifying unknown compounds with non-target analysis. The absence of standardized methods for detecting and quantifying EOCs presents a challenge for the comparison of results obtained across different laboratories and through diverse testing methodologies. Moreover, the extremely high costs associated with analysis, sample preservation, and transport to selected laboratories further restricted the sampling regime. Consequently, the limited number of samples, coupled with low detection frequencies, hampered the assessment of the statistical significance of seasonal variability in EOCs occurrence. Given the highly variable nature of karst aquifers, the limited number of samples may not fully depict the overall contamination scenario. The variability in limits of detection (LODs) across laboratories also affects the results. For example, the National Laboratory Services (UK) exhibited a significantly higher LOD for 1H-benzotriazole (5000 ng/L) compared to the Vltava River Basin Authority laboratory (CZ) (7 ng/L). Consequently, detectable levels of 1H-benzotriazole were found only in the Jadro and Žrnovnica catchment, highlighting the necessity of considering the analytical capabilities of laboratories when conducting environmental monitoring and assessment of EOCs in karst aquifers. The regular monthly field surveys for years 2020 and 2021 were disrupted due to the unprecedented Covid-19 pandemic.

A large number of detected EOCs lack experimental physico-chemical properties. Consequently, properties such as solubility in water, log K_{OW}, and log K_{OC} values had to be estimated using the *in silico* tools. Another significant hindrance is the scarcity of existing studies on EOCs in karst aquifers, which limits the ability to draw comprehensive parallels and comparisons with the results obtained in this thesis.

Despite all these constraints, the findings of this thesis provide a valuable contribution to the understanding of EOCs in karst aquifers and offer a foundation for future research to build upon with more extensive resources and optimized methodologies.

Future research:

This thesis underscores a crucial need for high-frequency sampling and in-depth event analysis to better understand the behaviour and dynamics of EOCs under varying hydrological conditions within karst aquifers. Such efforts could shed light on the potential existence of background levels of certain EOCs, indicating their persistence in aquifers, and provide essential data for future risk assessment. Focus should be placed on investigating the natural attenuation of EOCs in the Dinaric karst and developing targeted remediation strategies for these unique aquifer systems. The concurrent use of hydrochemical markers, tracer tests, and EOCs as novel tracers of various pollution sources could offer enhanced clarity in discerning the intricate interplay of dominant factors shaping hydrochemical patterns within karst aquifers subjected to various anthropogenic pressures.

Hypothesis #2: The vast array of EOCs, originating from diverse anthropogenic activities, infiltrate Dinaric karst groundwater.

The widespread use of EOCs is one of the factors influencing their occurrence in the environment. The exceptionally high discharge rates of many Croatian karst springs, substantial karstification and interconnected nature of aquifers that facilitate the rapid transport of contaminants from various sources, coupled with the extensive catchment areas, render the identification of specific EOCs sources particularly challenging. The region is characterized by low population density, limited industrial activity, extensive agriculture, and predominant natural land cover within catchments. Most inhabitants reside along the coast and downstream of the springs' catchment zones. Highly heterogeneous natural characteristics of karst aquifers along with varying anthropogenic activities, and management practices contribute to the absence of distinct spatial patterns between concentrations and the number of EOCs detected at coastal and continental areas of Croatian Dinaric karst (Paper I). Out of 65 different EOCs detected at the regional scale, 26 were pharmaceuticals, 26 agricultural compounds, 10 industrial substances, and 3 personal care/lifestyle compounds (Paper I). The highest total concentrations of measured EOCs, representing the sum of all sampling campaigns, were recorded at Golubinka (886.8 ng/L), Prud (498 ng/L), Rakonek (306.5 ng/L), and Kupica (197 ng/L) springs (Paper I). At the local scale, out of 22 detected EOCs, 12 were pharmaceuticals, 4 lifestyle and 2 personal care products, 3 agricultural compounds, and 1 industrial substance (Paper III and V). Pharmaceuticals and agricultural compounds being the most frequently detected EOCs in Croatian karst is in line with the findings of other karst groundwater studies (Lukač Reberski et al., 2022). However, certain compounds, such as pharmaceuticals carbamazepine, sulfamethoxazole, sotalol, and oxazepam, the pesticide desethylatrazine, as well as artificial sweeteners sucralose and acesulfame, exhibited higher detection frequencies compared to karst groundwater worldwide and other groundwater types (Paper I). Carbamazepine was also the most frequently detected EOC found in European groundwater (Bunting et al., 2021) and karst groundwater worldwide (Lukač Reberski et al., 2022), and is considered an indicator of wastewater pollution in aquifers (Doummar et al., 2014; Gasser et al., 2014; Dvory et al., 2018) along with artificial sweeteners (Zirlewagen et al., 2016; Doummar & Aoun, 2018a,b). Most frequently found pharmaceuticals were antihypertensives, antiepileptics, and antibiotics (Paper I). According to the Annual report on drug utilisation of the Agency for medicinal products and medical devices of Croatia, these three pharmaceutical groups ranked among the top 50 therapeutic medicine groups in terms of consumption in 2019.

Bexfield et al. (2019) observed that pharmaceuticals with relatively high usage rates are among the most frequently detected in the USA groundwater. Besides carbamazepine, pharmaceuticals that were among the 20 most frequently detected EOCs in Croatian karst, include lamotrigine, hydrochlorothiazide, tramadol, sulfamethoxazole, chlorothiazide, and clarithromycin (in descending order of detection frequency) (Paper I). Notably, only tramadol was listed among the top 50 most utilized medicines per defined daily dose per 1000 inhabitants per day in Croatia in 2019. This disparity may be elucidated by the fact that, except for lamotrigine, all aforementioned pharmaceuticals are also employed in veterinary medicine. This might clarify the observed weak correlation, as documented in Paper I, between the number of detected pharmaceuticals and the proportion of urban land use within the studied springs' catchments.

Furthermore, the number of detected agricultural products was slightly higher than the values reported in other karst studies (Paper I). Desethylatrazine, clothianidin, desiopropylatrazine, azoxystrobin, boscalid, bentazone, and chloridazon-desphenyl-methyl were agricultural compounds among the 20 most frequently detected EOCs in Croatian Dinaric karst (Paper I). According to the Statistical Office of the European Commission Eurostat, Croatia recorded over one and a half million pesticide sales in 2019, representing only half the average pesticide consumption in the European Union. However, pesticides like desethylatrazine and chloridazon-desphenyl-methyl (Paper I) exhibited higher detection frequency (67% and 14%, respectively) in Croatian karst groundwater compared to a pan-European groundwater survey by Loos et al. (2010). In New Zealand's groundwater, Moreau et al. (2019) identified pesticide degradates chloridazon-desphenyl-methyl at 47% and desethylatrazine at 30% of investigated sites. Desethylatrazine is a metabolite of the pesticide atrazine that was not included in Annex I of the Directive 91/414/EEC leading to the withdrawal of authorisations for plant protection products containing this substance. Desethylatrazine was found to be the third most frequently detected (7.92 %) EOC in groundwater of England and Wales (Manamsa et al., 2016), and second most quantified (41.4 %) EOCs in France (Lopez et al., 2015).

The elevated detection frequencies of specific agricultural and pharmaceutical EOCs indicate that diffuse sources such as agricultural runoff and point sources like domestic wastewater effluents containing pharmaceuticals used by the local population, play a critical role in contaminant occurrence. Similar to the findings of Zemann et al. (2015) and Richards et al. (2023), a statistically significant correlation (R=0.90) was identified between the NO_3^- concentrations and the number of EOCs in Croatian karst water at the local scale, in particular, at the Cetina River (Paper IV). Nitrates are indicative of anthropogenic contamination from wastewater or agriculture (Dogden et al., 2017). This positive correlation validates the EOCs sources map and conceptual model presented in Paper III. Although both Jadro and Žrnovnica springs are situated upstream of the main urban area of Split city, identified contamination sources include potentially leaking septic tanks and sewage networks without wastewater treatment, agricultural activities in upstream karst poljes, industrial activities, unsanitary landfills and illegal waste dump sites. Loborec et al. (2015) also identified those activities as potential sources of anthropogenic contamination when constructing a hazards map of the catchment area. The widespread detection of insect repellent DEET and industrial compound 1H-benzotriazole - two most frequently detected EOCs at the local scale and among the top 20 most abundant EOCs identified in global karst resources (Lukač Reberski et al., 2022) - suggests their potential as markers of anthropogenic contamination. Some authors related the occurrence of DEET in karst groundwater to the leakage of septic tanks (Katz et al., 2008) or sewage networks (Sorensen et al., 2015). Like DEET, 1H-benzotriazole occurred in all sampled water types, including groundwater, springs, and river. This finding aligns with the observation of Loos et al. (2009), who reported the presence of 1H-benzotriazole even in remote areas considered pristine. Persistent 1H-benzotriazole was identified in groundwater sampled from a deep borehole Gizdavac (Paper III), a sparsely populated area that includes some industrial facilities. This compound is usually utilized as anticorrosive in the metalworking industry and as a dishwashing agent in the food processing industry, both present in borehole vicinity. The occurrence of acute and neuropathic pain reliever drugs ketoprofen and gabapentin has also been detected in groundwater collected from the aforementioned borehole. This contamination likely originates from the wastewater effluents of an upstream nursing home (Paper III).

Moreover, the concurrent presence of pharmaceuticals like paracetamol, ibuprofen, and ibuprofen-carboxy alongside the peak mass flux of the very mobile antihyperglycemic drug metformin (the most frequently detected pharmaceutical at the local scale) in the karstic Cetina River strongly indicates the leakage from sewer systems or septic tanks in the adjacent settlements and presumably health center situated near the river sampling location (Paper III). This is also corroborated with the strong positive, statistically significant correlation (R=0.89) observed between metformin and NO_3^- ions in Cetina River (Paper IV). Nitrates were associated with certain EOCs, including herbicides (Hillebrand et al., 2014), X-ray contrast media (Zemann et al., 2015), and pharmaceutical carbamazepine (Doummar et al., 2014). Metformin ranked near the top of the lists of the 50 most used and most sold medicines in 2019 and 2020 in Croatia (Agency for medicinal products and medical devices of Croatia, 2019 and 2020).

The highest EOCs concentrations in Croatian Dinaric karst were that of personal care product/lifestyle compound groups, namely artificial sweeteners sucralose (440 ng/L) and acesulfame (150 ng/L) identified in spring water at the regional scale, insect repellent DEET (135 ng/L) in spring water at local scale, and 1H-benzotriazole recorded at 375 ng/L in a karst river (Paper V, VI). Concentrations of acesulfame and sucralose in the same order of magnitude were observed in other karst regions (Doummar & Aoun, 2018b), with acesulfame proven as a powerful marker of domestic wastewater pollution of groundwater (Buerge et al., 2009; Zirlewagen et al., 2016). Both compounds when ingested are excreted largely unchanged (Renwick et al., 1986; Roberts et al., 2000). Additionally, they are characterized by great water solubility and high mobility (Paper II and V) making their environmental fate a critical concern for both the aquatic ecosystem and groundwater quality. Both compounds can persist in the karst aquifer matrix during low flow periods as proven by Doummar & Aoun (2018b). According to Robertson et al. (2016), sucralose can be used for constraining groundwater age, indicating recharge after 2003. This is explained by the introduction of sucralose as an active substance (E955) in food in the European Union, following the amendment to Directive 94/35/EC (no longer valid and repealed by Regulation No 1333/2008) in January 2004. Artificial sweeteners are also utilized in livestock feed (Ma et al., 2017), however, both sucralose and acesulfame are not included among the additives approved for livestock feed within the European Union, as per Regulation (EC) No 1831/2003. Thus, their presence in manure is unlikely as indicated by Zirlewagen et al. (2016) for their case in Germany's karst groundwater. Personal care products/lifestyle compounds group exhibited a moderate, statistically significant positive correlation with urban land cover, with R^2=0.34 (Paper I). This was the strongest correlation identified between land use in the Croatian karst region and the EOCs group. The observed spatial variability in concentrations of artificial sweeteners in the karst groundwater of Croatia can be attributed to differences in consumption patterns, population density, wastewater treatment processes, and the types of wastewater effluents infiltrating the groundwater (e.g., septic tanks, sewer systems, or treated wastewater). Acesulfame was detected exclusively during the March campaign, whereas sucralose was identified more frequently in the October campaign (Paper I). Similarly, Marazuela et al. (2023) observed a peak in acesulfame concentrations during March campaigns in most years (2014-2022) within their alluvial groundwater, with negligible concentrations in the autumn months. They attribute such acesulfame concentration patterns to temperature-governed biodegradation. To gain insights into potential seasonal variability in artificial sweetener loads and concentrations in karst aquifers, it is proposed to conduct more frequent sampling in future studies, similar to the approach of Marazuela et al. (2023), with simultaneous collection of wastewater samples where possible. Furthermore, the analysis of transformation products like sulfamic acid, is advised, as recent research has demonstrated the biodegradation of acesulfame (Castronovo et al., 2017).

Implication of results:

A spatial analysis was conducted to pinpoint potential sources of EOCs within karst aquifers at both regional and local levels, correlating EOCs occurrence with particular land use types and elucidating distribution patterns and potential contamination pathways. The conducted research showed that even in regions with sparse population, low levels of development and urbanization like the majority of the karst region in Croatia, significant sources of pollutants exist, potentially affecting water quality. This underscores the region's vulnerability to pollution. The findings under hypothesis #2 contribute to data-driven policy making by informing the establishment of regulations aimed at minimizing EOCs emissions to water resources from unsustainable land use practices such as septic tanks effluents, untreated wastewater discharge, and intensive use of agricultural products.

Limitations:

The limited borehole data impeded a comprehensive assessment of EOCs contamination in the groundwater of the Jadro and Žrnovnica springs catchment. In particular, the inoperable state of a deep borehole in Dugopolje further restricted the investigation of potential EOCs sources within the catchment such as present medicine laboratory (Paper III). The high costs of borehole pumping for sampling purposes constrained the frequency of groundwater sampling at Gizdavac borehole.

Future research:

Findings from the regional-level study indicate the need for future monitoring of the four Dinaric karst springs - Golubinka, Prud, Rakonek and Kupica - which exhibited the highest number of detected EOCs and the highest total concentrations in both campaigns (Paper I). Further research at their individual catchment levels is necessary to gain better insights into potential contamination sources and to define appropriate water supply protection measures. The most prevalent EOCs in Croatia were carbamazepine, lamotrigine, sucralose, desethylatrazine, hydrochlorothiazide, perfluorobutanesulfonic acid (PFBS), acesulfame, cotinine, and bentazone (descending order of detection frequency), warranting their inclusion in future research and monitoring.

At the local scale of Jadro and Žrnovnica springs, the ubiquitous presence of the most frequently detected EOCs, DEET and 1H-benzotriazole across sampling sites suggests limited attenuation of persistent and mobile compounds within the karst aquifer. Thus, the occurrence of these compounds should be investigated more closely through high-frequency sampling. Continued research will enhance our understanding of EOCs in karst aquifers and support the development of effective strategies for managing and protecting these critical water resources.

To reinforce the protection of karst groundwater resources, initiating the implementation of a Voluntary Groundwater Watch List (GWWL) (Lapworth et al., 2019) tailored to unique Dinaric karst characteristics is suggested. Out of the substances proposed for the Voluntary GWWL, four were detected in the regional scale study, namely clopidol (≤4.7 ng/L), sulfadiazine (≤19 ng/L), sotalol (≤0.5 ng/L) and clarithromycin (≤0.2 ng/L) (Paper I and II). The list should be revised and expanded to include the most frequently detected EOCs within this thesis and those that were prioritized under hypothesis #3.

Hypothesis #3: The occurrence of EOCs within karst water resources poses potential detrimental effects on ecosystem and human health.

Amidst growing concerns regarding the potential human health implications of EOCs present in the water resources used for water supply, there are currently no guidelines at the EU or national level (Croatia) that specify the evaluation of indirect human exposure. As discussed in Chapter 1.5, the Watch list of substances and compounds of concern for water intended for human consumption includes guidance values for only two compounds: nonylphenol (300 ng/l) and 17-beta-estradiol (1 ng/l). Within this research, nonylphenol was not analyzed, whereas 17-beta-estradiol was assessed on a local scale in four sampling campaigns and on a regional scale in two sampling campaigns, with no detections exceeding the LODs of 0.3 ng/L and 200 ng/L, respectively.

The typical treatment of drinking water in Croatian karst is disinfection with NaClO. While conventional water treatment methods ensure microbiological safety, they are ineffective in removing EOCs (Huerta-Fontela et al., 2011) and can result in the formation of genotoxic disinfection by-products like those of carbamazepine (Han et al., 2018), the most frequently detected pharmaceutical in the regional-scale research (Paper I). Similar to the studies conducted by Schriks et al. (2010), Zainab et al. (2020), and Sengar & Vijayanandan (2022), this thesis compares the maximum concentrations of EOCs measured at regional and local scales in Croatian karst springs used for water supply with existing or derived drinking water guideline levels to assess the potential risk to consumers. For most compounds, no significant risk to human health is presumed. However, the pharmaceuticals sulfadiazine and possibly carcinogenic hydrochlorothiazide were detected at a maximum concentration above the guideline values, indicating a potential risk to human health at lifelong exposure (Paper III). Sulfadiazine was evaluated as PMT/vPvM, while hydrochlorothiazide as a potential PMT/vPvM compound (Paper II). Both substances exhibit very low log Kow (< 0) and low log K_{OC} (< 3) values, indicating their hydrophilic nature, high mobility, and lack of bioaccumulation potential (Paper II). Moreover, the assessment of human health risks showed how another five EOCs: industrial compound PFOA, pharmaceuticals 10,11-dihydroxycarbazepine, clopidol, sulfamethoxazole, and REACH-registered tramadol should be examined more closely and monitored in drinking water resources, given their maximal concentrations close to guideline values or structural alerts for carcinogenicity or genotoxicity (Paper II).

At the local-scale research, the analysis found how current environmental levels of EOCs in Jadro and Žrnovnica springs do not pose a potential adverse risk to human health. The highest, although negligible risk quotient values were determined for infants of age 0-3 months for EOCs like lifestyle compound cotinine, and pharmaceuticals valsartan, carbamazepine recorded in Jadro spring and personal care product DEET found in Žrnovnica spring. Previous research by Sharma et al. (2019), Kibuye et al. (2019), and Sengar and Vijayanandan (2022) reported higher risk quotient values in children compared to adults, which supports the notion of increased susceptibility to EOCs in the younger population (Paper III).

The List of classification by the International Agency for Research on Cancer (IARC) was consulted to identify EOCs that might pose a carcinogenic hazard to humans. EOCs herbicide 2,4-dichlorophenoxyacetic acid (4.3 ng/L), medicines phenobarbital (1.8 ng/L), hydrochlorothiazide (up to 11 ng/L), griseofulvin (0.1 ng/L), oxazepam (0.3 ng/L), and industrial compound PFOA (0.6 ng/L) detected in springs at regional scale are evaluated by IARC as possible human carcinogens. At the local scale, only medicine sulfamethoxazole detected in Jadro spring water samples (0.7 ng/L) is included on the List of classification by the IARC, as "not classifiable as to its carcinogenicity to humans" (classification used for compounds with unknown carcinogen potential due to gaps in animal and human studies). While most detected EOCs in Dinaric karst springs at current environmental concentrations do not individually present a significant risk to human health, the synergistic and long-term effects of their mixtures are not yet understood and should be in focus of future research.

To prioritize compounds of concern that should be part of future monitoring activities and to establish appropriate regulatory thresholds for EOCs detected in Croatian karst drinking water resources, *in silico* tools based on quantitative structure-activity relationships were used in PBT/vPvB (persistence, bioaccumulation, and toxicity/very persistent and very bioaccumulative) and PMT/vPvM (persistent, mobile, and toxic/very persistent and very mobile) analyses. Substances identified as PBT/vPvB or PMT/vPvM have the same level of concern (Neumann and Schliebner, 2019). At the regional-scale research, 7 out of 65 EOCs were classified as PBT/vPvB, while only 2 out of 65 EOCs were not classified as PMT/vPvM. In the local-scale research, none of the detected EOCs were evaluated as PBT/vPvB, while only 4 out of 21 substances were not assessed as PMT/vPvM (Paper II, V, and VI). According to Chirsir et al. (2024), substances assessed as PBT/vPvB or PMT/vPvM have the potential for long-lasting and long-range exposure when released to the environment in substantial quantities, with PMT/vPvM substances most likely to accumulate in water resources and PBT/vPvB substances being first monitored in humans and other living organisms. Worryingly, 60 out of 65 EOCs detected at the regional scale and 20 out of 21 EOCs detected at the local scale were categorised as very mobile compounds (Paper II, V, and VI). Mobile EOCs can bypass protective soil and epikarst layers via concentrated infiltration through ponors and be rapidly transported through interconnected fractures and conduits within highly karstified aquifers. This indicates that EOCs are not subject to natural attenuation processes (e.g. filtration and degradation) that are typical in other geological formations (Paper II). Notably, karst springs Jadro and Žrnovnica demonstrated a higher percentage of EOCs assessed as PMT/vPvM compared to Cetina River, corroborating the elevated risk associated with EOCs occurrence in karst springs in contrast to surface waters.

Implication of results:

The research of EOCs detected in Croatian Dinaric karst was extended to assess environmental risks and potential impacts associated with these compounds on water ecosystems and human health. By prioritizing substances of concern, this assessment guides future research and aids in developing targeted measures to mitigate the adverse effects of the most harmful contaminants, thereby protecting both the environment and public health. Around 30% of EOCs detected in the Dinaric karst at the regional scale (Paper II) and half of the compounds identified at the local scale (Paper V) are registered under REACH (Regulation (EC) No 1907/2006), which requires manufacturers, importers, and downstream users to evaluate and manage the risks these chemicals pose to human health and the environment throughout their lifecycle. More than half of agricultural EOCs identified, both at regional and local scale are not approved for use in plant protection products according to the EU Pesticide database (2023, 2024). Among these substances, the majority have been banned in recent years, while a few were prohibited as long as two decades ago. Being assessed as potentially persistent/persistent/very persistent and very mobile compounds (Paper II and V), reinforces the need for stricter control of active pesticide substances use. The efficient management of the chemically heterogeneous group of predominantly persistent and very mobile contaminants, which exhibit high fluxes in Dinaric karst springs characterized by seasonally varying discharges and intricate groundwater flow through networks of conduits and enlarged fractures, is a significant challenge. Thus, stronger collaboration between the scientific community and the water management sector is needed to facilitate adequate prevention, early detection of PMT/vPvM substances in karst water resources and timely responses to potential pollution events through exchange of knowledge and experience. This thesis serves as an early warning, highlighting areas of concern and identifying gaps in current water-related legislation and management. It provides a robust basis for enhancing existing policies and points out that urgent prevention and control measures are needed at both national (Croatia) and regional (Dinaric karst) levels to achieve the European Commission zero pollution vision for 2050, which among others seeks to reduce water pollution to levels no longer harmful to associated ecosystems and human health. The dataset covering detected EOCs and their properties acquired with Papers II, V, and VI, has a multidisciplinary value, as it can be utilised by hydrogeologists, biologists, chemists, and other researchers dealing with water management or environmental issues.

Limitations:

Given the serious lack of experimental data for most of the detected EOCs, *in silico* (computational) QSAR (quantitative structure-activity relationships) tools were used to assess their persistence, bioaccumulation, mobility, and toxicity. In order to estimate the physicochemical properties of compounds, *in silico* tools use molecular descriptors such as SMILES (Simplified molecular-input line-entry system) derived from chemical structures. Caution should be exercised when interpreting the results of such simulations. In particular, models may lead to prioritization of EOCs that are similar to well-characterized compounds based on whose physico-chemical properties are the models trained (Muir et al., 2019), while simulated properties could be under or over-predicted (Arp & Hale, 2022).

It is important to acknowledge the limitations inherent in current human health risk assessment methodologies, such as those employed in this thesis. Specifically, these methodologies do not account for the combined impacts of EOCs mixtures or potential chronic effects, a concern highlighted in recent studies. Despite low environmental concentrations, lifetime exposure to certain EOCs or a mixture of EOCs may result in adverse toxic effects. Due to the limited number and frequency of samples, which may not fully capture the extent and variability of EOCs, the associated human health risks could be underestimated. Consequently, the obtained prioritization and human health risks findings can serve as valuable guidance for future research and decision-making processes aimed at the management and protection of karstic water ecosystems and human health. There is a strong need for a multilevel approach that includes targeted, long-term monitoring, advancements in (waste)water treatment technologies, and a regulatory framework aligned with the latest scientific findings.

Moreover, as highlighted in Papers III and V, the absence of ecotoxicological data and standardized protocols for stygobiotic species, especially concerning the vast and heterogeneous group of EOCs, prevented the assessment of the environmental risk of EOCs to subterranean species.

Future research:

Establishing routine monitoring protocols for prioritized EOCs in vulnerable Dinaric karst aquifers is crucial due to the projected increase in contamination mass loads in coming years worldwide. EOCs prioritized at the regional level should be further investigated at the individual aquifers level to gain a comprehensive understanding of their sources, trends, and fate. The remarkable biodiversity of the Croatian karst region is globally recognized, with nearly 70% of the species identified in Croatia being endemic (Gottstein et al., 2002). For example, two cave-dwelling bivalves *Congeria jalzici* and *Congeria kusceri* are found in the subterranean Dinaric karst of Croatia, with the latter identified in the Vilina cave-Ombla spring cave system (Bilandžija et al., 2014), one of the sampling sites in this thesis. The only known representative of the freshwater sponges among stygobionts in the world was discovered in the catchment of rivers Dobra, Tounjčica and Zagorska Mrežnica (*Eunapius subterraneus*

subterraneus, Sket & Velikonja, 1984), and Rudnica cave (*Eunapius subterraneus mollisparpanis*, Sket & Velikonja 1984). Moreover, the Jadro spring is a habitat for the endemic species *Proteus anguinus* and is the *locus typicus* for at least three subterranean freshwater gastropod species: C*ostellina turrita, Kerkia jadertina*, and *Iglica elongata* (Kuščer, 1933). Stygofauna are particularly sensitive to even slight variations in contaminants concentrations (Castaño-Sánchez et al., 2020), and their responses to anthropogenic persistent contaminants may differ from their surface counterparts (Di Lorenzo et al., 2019). Consequently, future research should closely examine the toxicity of EOCs and their mixtures on subterranean biota. To protect the endemic subterranean species in the Croatian karst aquifers, it is essential to develop specific guidelines for the ecotoxicological testing of groundwater species and to establish an appropriate regulatory framework.

The current drinking water monitoring suite should be aligned with the thesis findings on EOCs occurrence, as this alignment is necessary for safeguarding water quality and public health in the Dinaric karst region. Industrial compound PFOA and pharmaceuticals sulfadiazine, hydrochlorothiazide, 10,11-dihydroxycarbazepine, clopidol, sulfamethoxazole, and tramadol warrant additional monitoring in karst springs used in water supply and further research of associated human health risks. The short or long-term, dose additive or synergistic effects of EOCs mixtures on human health remain unexplored and insights are essential for establishing safe future guideline values for EOCs mixtures. Moreover, in-depth research focusing on efficient EOCs removal from drinking water in Dinaric karst is necessary.

4. CONCLUSION

Through systematic and rigorous investigation, this thesis has yielded novel and valuable findings that advance understanding of the occurrence and eco(toxico)logical implications of EOCs in vulnerable and complex Dinaric karst systems. The significance of these contributions transcends local and regional boundaries, particularly considering the scarcity of global research addressing EOCs in karst aquifers. Thesis underscores how continued research and stringent EOCs monitoring efforts are imperative to safeguard water quality and protect both environmental and human health in karst regions worldwide. The proposed directions for future research stem from the findings of this thesis and aim to advance understanding of EOCs behaviour in karst environments.

Based on research conducted at a regional scale (17 karst springs and one karst lake), the following conclusions were drawn:

- During two sampling campaigns conducted in March and October 2019 at a regional scale, 65 EOCs were identified, with 277 detections across 35 samples. Of these 35 samples, only one showed no detection of EOCs. The identified contaminants included 26 pharmaceuticals, 26 agricultural compounds, 10 industrial substances, and 3 personal care/lifestyle compounds (Paper I).
- Pharmaceuticals and agricultural compounds were detected most frequently like in other karst studies. Lifestyle compounds exhibited the highest concentrations that reached up to 440 ng/L for the artificial sweetener sucralose detected in the Golubinka spring, in comparison to the 100 ng/L limit set for individual pesticides in drinking water (Directive 2020/2184) and groundwater (COM (2022) 540) and 250 ng/L for total pharmaceuticals in groundwater (COM (2022) 540). Both detected artificial sweeteners, sucralose and acesulfame, were proven good markers of wastewater pollution in previous research.
- At two karst springs in March and six springs in October 2019, the detected perfluoroalkyl substances (PFAS) exceeded the groundwater quality standards threshold proposed with COM(2022)540 (Paper I).
- The highest total EOCs concentration per sampling location was recorded at Golubinka spring, with a measurement of 886.8 ng/L (Paper I).
- EOCs detected in Croatian karst springs exhibited lower concentrations compared to other karst groundwater studies. This finding is attributed to the combined effect of

dilution, due to the exceptionally high discharge of the "classical" (Dinaric) karst springs, and the predominance of natural areas within catchments. The considerable EOCs mass fluxes, ranging from 10 to 10^6 ng/s indicate the vulnerability of the Croatian karst (Paper I).

- The conducted spatial analysis revealed no consistent pattern in the total concentration or number of detected EOCs and no strong correlation between EOCs and land use, due to varying anthropogenic influences and the highly variable nature of karst aquifers (hourly/daily timescales) dictated by local hydrogeological conditions. The strongest observed, but moderate correlation (R^2=0.34) was found between urban land use and personal care products/lifestyle compounds group, followed by a weaker correlation between urban land use and pharmaceuticals (R^2=0.28). This weaker correlation might be explained by the fact that many of the detected pharmaceuticals can also be used in agriculture as veterinary medicines. Notably, the proportion of agricultural land use in the catchments showed a statistically insignificant correlation with the number of detected compounds or their concentrations. The reasons behind this are the extensive size of the studied catchments, the intricacy of contaminants transport in karst, and the long-range atmospheric deposition of pesticides. Further research at individual catchments is necessary to better elucidate the relationship between land use and EOCs in Croatian Dinaric karst, and the transport and attenuation of EOCs (Paper I).
- This thesis represents the first study to prioritize the diverse chemical array of EOCs detected in Croatian karst groundwater resources used for drinking water supply based on their persistence, bioaccumulation potential, toxicity, and mobility and to assess the potential environmental and health risk to consumers (Paper II).
- Among EOCs detected at regional scale, 22 compounds are registered under REACH Regulation (No 1907/2006), seven are included on the Candidate List of substances of very high concern (SVHC), ten are unauthorized pesticides (EU Pesticide Database, 2023, 2024), and four pharmaceuticals are listed on Voluntary Groundwater Watch List (Paper II).
- Perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), perfluoro octanoic acid (PFOA), perfluorononanoic acid (PFNA), fungicides boscalid and azoxystrobin had the highest calculated PBT (persistent, bioaccumulative, and toxic) scores above the threshold of 0.5, and therefore

were characterized as potentially PBT or vPvB (very persistent and very bioaccumulative) compounds (Paper II).

- Most of EOCs detected during regional-scale research met PMT/vPvM (persistent, mobile, and toxic/very persistent and very mobile) criteria, with 64 compounds assessed as potentially persistent to very persistent, 60 out of 65 assessed as very mobile, 35 characterized as toxic, and 30 as potentially toxic. However, it is important to note that the detection of only 65 EOCs out of 740 analysed does not imply the absence of environmental pollution risks. Slight temporal and more pronounced spatial variations in the occurrence of different PMT/vPvM categories were observed between two sampling campaigns (March and October 2019), though no clear pattern emerged (Paper II).
- For only 16 of 65 EOCs detected during regional-scale research, drinking water guideline values were reported in previous research. The comparison of maximum detected EOCs concentrations with the calculated provisional or existing statutory drinking water guideline values showed how most substances occurring in Croatian karst drinking water resources individually do not pose significant risk to human health. However, pharmaceuticals sulfadiazine and hydrochlorothiazide are suspected to pose a potential health risk at lifelong exposure (Paper II), while industrial compound PFOA and pharmaceuticals 10,11-dihydroxycarbazepine, clopidol, sulfamethoxazole, and tramadol warrant additional research and monitoring (Paper II).

Based on research conducted at the local scale of Jadro and Žrnovnica springs catchment (including two springs, one karstic river, and one deep borehole) the following conclusions were drawn:

- Jadro and Žrnovnica springs aquifer exhibited a Ca^{2+}-HCO_3^- hydrogeochemical facies typical for coastal Dinaric karst aquifers, which are characterised by highly karstified structures, a predominance of limestone, and seaspray influence (Paper IV).
- Fluctuations in electrical conductivity observed at the Jadro and Žrnovnica springs indicate a highly karstified system that is significantly vulnerable to potential anthropogenic contamination, due to rapid infiltration pathways (Paper III).

- Along with rapid hydrodynamic responses, sharp spikes observed in chemographs of Jadro and Žrnovnica springs confirm a highly karstified and inherently vulnerable system (Paper IV).
- Stable isotope signatures verified that Jadro and Žrnovnica springs share a common catchment area. Additionally, they revealed intercatchment groundwater flow originating from the Cetina River (Paper IV).
- Conventional indicators of anthropogenic pollution, namely NO_3^-, Cl^-, and SO_4^{2-} remained below their respective maximum allowable concentrations in drinking water (Jadro and Žrnovnica springs), while detection of EOCs across sampling sites (Jadro and Žrnovnica springs, groundwater from Gizdavac borehole, and Cetina River) under varying hydrological conditions reflects the anthropogenic impact on water resources within the studied catchment (Paper IV).
- In total 22 EOCs were identified at concentrations ranging between 0.3 ng/L (tramadol detected at Jadro spring) to 372 ng/L (1H-benzotriazole in Cetina River) at the local-scale research conducted between 2019 and 2021 (Paper III, V and VI).
- Personal care product DEET emerged as the most frequently detected EOC in the local-scale research, with an average concentration of ~50 ng/L, which is higher than the average concentration reported in the pan-European groundwater survey. DEET was detected across all sampling sites (Paper V and VI).
- Pharmaceutical metformin, personal care product DEET, life-style compound caffeine, and industrial compound 1-H benzotriazole exceeded the EU drinking water limit for individual pesticides set at 100 ng/L (Paper V and VI).
- Among all sampling sites, Cetina River exhibited the highest total EOCs concentrations (1056.4 ng/L). Together with Jadro spring, Cetina River had the most versatile groups of EOCs (Paper III, V and VI).
- The results of the Principal Coordinates Analysis showed how EOCs content was influenced by both sampling location (Jadro and Žrnovnica springs, groundwater from Gizdavac borehole, and Cetina River) and sampling period (Paper V).
- The seasonal variation observed in markers of wastewater contamination DEET and 1H-benzotriazole, coupled with their widespread occurrence across sampling sites in local-scale research, suggest limited attenuation of persistent and mobile compounds within the karst aquifer (Paper III).

- At local-scale research, EOCs were not detected during peak high-flow owing to substantial dilution processes (Paper III). The highest total concentrations at Jadro and Žrnovnica springs were measured following autumn recharge events, which suggests that surface contamination either infiltrates directly through ponors and highly karstified areas or persists in the fracture-porous epikarst and aquifer matrix, later being pushed to the springs by freshly infiltrated water (Paper III). Contrarily to springs, Cetina River had the highest total EOCs concentration measured along the lowest discharge. Similarly, groundwater from Gizdavac borehole exhibited the highest total EOCs concentration during the baseflow period, indicating site-specific contamination sources (Paper III).
- The occurrence of biodegradable yet highly mobile EOC gabapentin in groundwater from Gizdavac borehole indicated the oligotrophic nature of the investigated system (Paper III).
- Quantitative estimation of human health exposure suggested that consumption of water containing EOCs concentrations recorded in Jadro and Žrnovnica springs is unlikely to pose health risks. However, the applied human health risk assessment method did not consider the potential long-term and synergistic effects of EOCs mixtures, which should be investigated in future studies (Paper III).
- EOCs identified at local-scale research were assessed as non-persistent-bioaccumulative-toxic (non-PBT), while ranking per site (PBTr) highlighted industrial compound 1H-benzotriazole and life-style compound caffeine recorded in Cetina River as EOCs having the highest risk ranks. The Cetina River stood out with the highest PBTr score (Paper V and VI).
- The preliminary environmental risk assessment (ERA), conducted at local-scale research, underlined caffeine as a compound posing moderate potential environmental risk (RQ), with Cetina River having the highest risk quotient RQ_{site} (Paper V and VI).
- The persistence (P), mobility (M), and toxicity (T) of all detected compounds were evaluated according to REACH guidelines for PMT/vPvM assessment (vP- very persistent, vM- very mobile). The analysis showed how only 4 out of 21 compounds

(results of October and December 2021 sampling campaigns were not included in the analysis[1]) were not assessed as PMT/vPvM compounds (Paper V and VI).

- In the context of human health safety, as well as the protection and management of karst springs Jadro and Žrnovnica, it is worrisome that both springs exhibited a larger percentage of PMT/vPvM substances than the Cetina River (Paper V). Nearly all examined EOCs were identified as very mobile compounds, which is particularly warring given the intrinsic properties of karst systems that facilitate rapid and long-distance contaminant transport.
- In the Cetina River, a significant strong positive correlation was observed between pharmaceutical metformin and NO_3^- concentration. Additionally, there was a significant strong positive correlation between total EOCs concentration, EOCs number, and NO_3^- ion. These findings suggest potential contamination originating from wastewater or agricultural activities (Paper IV).
- The EOCs detection rate determined in the Jadro spring samples exhibited a strong, statistically significant positive correlation with calcium ion (Ca^{2+}) content. This finding indicates the potential persistence of EOCs like DEET within the epikarst and aquifer matrix (Paper IV).

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[1] Paper V and associated paper VI were already under review when the laboratory provided results for the October and December 2021 sampling campaigns.

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6. BIOGRAPHY

Ana Selak born on the 25th of December 1988 in Zagreb, Croatia, pursued dual undergraduate studies: Geological Engineering at the Faculty of Mining, Geology and Petroleum Engineering and Landscape Architecture at the Faculty of Agriculture, obtaining Bachelor's degrees in 2011 and 2012, respectively. Thereafter, she pursued graduate studies in both fields, earning Master's degrees in 2013 and 2015. Noteworthy, she received two Dean's Awards for academic excellence during her graduate studies. Her professional journey commenced in 2016 at Oikon Ltd. Institute of Applied Ecology as a geologist and landscape architect, followed by roles at the Croatian Geological Survey, where she progressively advanced from Senior project associate, Expert Project Advisor and to later Assistant at the Department of Hydrogeology and Engineering Geology in 2022. She enrolled in the Postgraduate Doctoral Study of Environmental Engineering at the Faculty of Geotechnical Engineering in 2018/2019. Throughout her career, she received recognition for her contributions, including two Annual Awards for Young Researchers from the Croatian Geological Survey in 2020 and 2023, and in 2024, she was honoured with the Annual Award for Young Researchers by The Association of University Teachers and other Scientists in Zagreb.

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7. APPENDICES

APPENDIX 1: Supplementry Materials for the Paper I

Lukač Reberski, J., Selak, A., Lapworth, D.J., Maurice, L.D., Terzić, J., Civil, W., Stroj, A., 2023. Emerging organic contaminants in springs of the highly karstified Dinaric region. J Hydrol 621, 129583. https://doi.org/10.1016/j.jhydrol.2023.129583

Table S1 List of compounds analysed in National Laboratory Services

Compound name	CAS No.	Description / use	LOD (µg/L)
1,2,5,6,9,10-Hexabromocyclododecane (HBCDD/HBCD)	3194-55-6	Brominated flame retardant	0.002
1,4,5,6,7,7-Hexachloro-5-norbornene-2,3-dicarboxylic acid	115-28-6	Reactive flame retardent	0.01
10,11-Dihydroxycarbamazepine or 10,11-Dihydroxycarbazepine	58955-93-4	Antiepileptic metabolite	0.005
10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine	35079-97-1	Anticonvulsant	0.005
17-alpha-19-Nortestosterone or Nandrolone	4409-34-1	Veterinary drug	Q-na
17-Methyltestosterone	58-18-4	Veterinary drug; Equine drug; Anabolic	0.001
2,3,6-TBA / 2,3,6-Trichlorobenzoic acid	50-31-7	Pesticide; Herbicide	0.04
2,4,5-T / 2,4,5-Trichlorophenoxyacetic acid	93-76-5	Pesticide; Herbicide	0.005
2,4,5-TP / Silvex (Fenoprop)	93-72-1	Pesticide; Herbicide	0.005
2,4-D / 2,4-Dichlorophenoxyacetic acid	94-75-7	Pesticide; Herbicide	0.005
2,4-DB / 2,4-Dichlorophenoxybutyric acid	94-82-6	Pesticide; Herbicide	0.1
2,6-Dichlorobenzamide	2008-58-4	Pesticide; Dichlobenil metabolite	0.001
2-Ethylhexldiphenyl phosphate	1241-94-7	Plasticiser	0.01
2-Phenoxypropionic acid	940-31-8	Intermediate; Pesticides	0.01
3-Hydroxycarbofuran	16655-82-6	Pesticide; Acaricide; Insecticide; Veterinary drug	0.001
4-Chloro-2-methylphenol	1570-64-5	Pesticide; Herbicide	0.02
4-Chlorophenoxyacetic acid / CPA / 4-CPA	122-88-3	Pesticide; Herbicide; Plant growth regulator	0.02
4-Cyclohexylphenol	1131-30-8	Disinfectant	Q-na
4-Phenoxybutyric acid	6303-58-8	Intermediate	0.01
4-tert-Butylphenyl diphenyl phosphate	56803-37-3	Flame retardant plasticiser	Q-na
5-hydroxymebendazole	60254-95-7	Veterinary drug	0.001
Acephate	30560-19-1	Pesticide; Insecticide	0.1
Acesulfame (Acesulfame-K)	33665-90-6	Artificial Sweetener (K salt)	0.05
Acetaminophen (Paracetemol)	103-90-2	Eterinary drug; Equine drug; Analgesic	0.005
Acetamiprid	135410-20-7	Neonicotinoid Insecticide	0.001
Acetazolamide	59-66-5	Pesticide; Equine drug; Diuretic	0.005
Acetochlor	34256-82-1	Pesticide; Herbicide	0.005
Acibenzolar-S-methyl (BTH)	135158-54-2	Pesticide; Fungicide; Veterinary drug	0.005
Aflatoxin B1	1162-65-8	Veterinary drug; Biomolecule	0.001
Aflatoxin B2	7220-81-7	Veterinary drug; Biomolecule	0.001
Aflatoxin G1	1165-39-5	Veterinary drug; Biomolecule	0.001
Aflatoxin G2	7241-98-7	Veterinary drug; Biomolecule	0.001
Alachlor	15972-60-8	Pesticide; Herbicide	0.005

Compound name	CAS No.	Description / use	LOD (µg/L)
Alanycarb	83130-01-2	Pesticide; Insecticide	L-na
Albendazole	54965-21-8	Pesticide; Veterinary drug; Anthelmintic	Q-na
Aldicarb	116-06-3	Pesticide; Insecticide	0.1
Aldicarb sulfoxide (Aldicarb sulphoxide)	1646-87-3	Pesticide; Insecticide metabolite	0.005
Aldicarb-sulfone (Aldoxycarb)	1646-88-4	Pesticide; Insecticide metabolite	0.005
Alfuzosin	81403-80-7	Antihypertensive	Q-na
Alizapride	59338-93-1	Antihistamine	0.001
Alverine	150-59-4	Equine drug; Parasympatholytic	Q-na
Ametryn (Ametrex)	834-12-8	Pesticide; Herbicide; Veterinary drug	0.001
Amicarbazone	129909-90-6	Pesticide; Herbicide	0.005
Amidosulfuron	120923-37-7	Pesticide; Herbicide	0.001
Amiloride	2609-46-3	Equine drug; Diuretic	Q-na
Aminocarb	2032-59-9	Pesticide; Insecticide; Veterinary drug	0.001
Aminocyclopyrachlor	858956-08-8	Pesticide; Herbicide	0.1
Amino-mebendazole	52329-60-9	Veterinary drug	0.005
Amiodarone	1951-25-3	Equine drug; Antiarrhythmic	Q-na
Amisulbrom	348635-87-0	Pesticide; Fungicide	0.005
Amisulpride	71675-85-9	Equine drug; Neuroleptic	0.001
Amitraz	33089-61-1	Pesticide; Acaricide; Insecticide	L-na
Amitriptyline	50-48-6	Equine drug; Antidepressant	0.005
Amlodipine	88150-42-9	Equine drug; Antihypertensive	Q-na
Amobarbital	57-43-2	Equine drug; Hypnotic	0.005
Ampicillin	69-53-4	Veterinary drug; Antibiotic	Q-na
Anastrozole	120511-73-1	Equine drug; Cytostatic; Aromatase inhibitor	Q-na
Anhydrotetracycline	13803-65-1	Gene expression	Q-na
Anilazine	101-05-3	Pesticide; Fungicide; Veterinary drug	0.001
Aniracetam	72432-10-1	Nootropic	0.001
Aripiprazole	129722-12-9	Antipsychotic drug	0.001
Aspirin (Acetylasalicylic acid)	50-78-2	Veterinary drug; Analgesic	Q-na
Asulam	3337-71-1	Pesticide; Veterinary drug; Antibiotic	0.005
Atazanavir	198904-31-3	HIV protease inhibitor	0.001
Atenolol	29122-68-7	Equine drug; Beta-Blocker	0.001
Atorvastatin	134523-00-5	Antihyperlipemic	Q-na
Atraton	1610-17-9	Pesticide; Herbicide	0.001
Atrazine	1912-24-9	Pesticide; Herbicide; Veterinary drug	0.001
Atrazine-desethyl (Desethylatrazine)	6190-65-4	Pesticide; Herbicide; Degradation	0.001
Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	Pesticide; Herbicide; Degradation	0.001
Avermectin B1a (Abamectin B1a)	65195-55-3	Pesticide; Veterinary drug	0.1
Avermectin B1b (Abamectin B1b)	65195-56-4	Pesticide; Veterinary drug	0.1
Azinphos-ethyl (Guthion ethyl)	2642-71-9	Pesticide; Insecticide	0.001
Azinphos-methyl (Guthion)	86-50-0	Pesticide; Insecticide	0.01
Azithromycin	83905-01-5	Veterinary drug; Antibiotic	0.05
Azoxystrobin	131860-33-8	Pesticide; Fungicide; Veterinary drug	0.001
Beclomethasone dipropionate	5534-09-8	Veterinary drug; Glucocorticoid	Q-na

Compound name	CAS No.	Description / use	LOD (µg/L)
Benalaxyl	71626-11-4	Pesticide; Fungicide; Veterinary drug	0.01
Benazolin	3813-05-6	Pesticide; Herbicide	0.04
Bendiocarb	22781-23-3	Pesticide; Insecticide; Veterinary drug	0.001
Benfuracarb	82560-54-1	Pesticide; Insecticide; Veterinary drug	L-na
Bentazone	25057-89-0	Pesticide; Herbicide; Veterinary drug	0.001
Benthiavalicarb-isopropyl	177406-68-7	Pesticide; Fungicide	Q-na
Bentranil	1022-46-4	Pesticide; Herbicide	0.005
Benzoximate	29104-30-1	Pesticide; Insecticide	0.01
Benzoylecgonine	519-09-5	Addictive drug; metabolite of cocaine	0.001
Benzydamine	642-72-8	Equine drug; Analgesic	Q-na
Betamethasone	378-44-9	Veterinary drug; Equine drug; Corticoid	0.001
Betamethasone valerate	2152-44-5	Corticoid	Q-na
Bezafibrate	41859-67-0	Anticholesteremic	0.005
Bicalutamide	90357-06-5	Antiandrogen medication	0.001
Bifenazate	149877-41-8	Pesticide; Acaricide	0.005
Bifenox	42576-02-3	Pesticide; Herbicide; Veterinary drug	Q-na
Bisoprolol	66722-44-9	Equine drug; Beta-Blocker	0.001
Bisphenol S	80-08-1	Endocrine disruptor	0.005
Bitertanol	55179-31-2	Pesticide; Fungicide; Veterinary drug	0.005
Boldenone (Dehydrotestosterone)	846-48-0	Veterinary drug; Equine drug; Biomolecule	0.001
Boscalid (Nicobifen)	188425-85-6	Pesticide; Herbicide; Veterinary drug	0.005
Brodifacoum	66052-95-7	Pesticide; Rodenticide	Q-na
Bromacil	314-40-9	Pesticide; Herbicide; Veterinary drug	0.005
Bromadiolone	28772-56-7	Pesticide; Rodenticide	0.005
Bromazepam	1812-30-2	Benzodiazepine; Anti-Anxiety drug	0.001
Brombuterol	41937-02-4	Veterinary drug	0.001
Bromoxynil	1689-84-5	Pesticide; Herbicide	0.001
Bromuconazole (I)	116255-48-2	Pesticide; Fungicide; Bacteriocide; Veterinary drug	0.001
Bromuconazole (II)	116255-48-2	Pesticide; Fungicide; Bacteriocide; Veterinary drug	0.001
Bumetanide	28395-03-1	Equine drug; Diuretic	Q-na
Bupirimate	41483-43-6	Pesticide; Fungicide; Veterinary drug	0.001
Buprenorphine	52485-79-7	Designer drug; Equine drug; Opioid	0.005
Buprofezin	69327-76-0	Pesticide; Insecticide; Veterinary drug	0.002
Buspirone	36505-84-7	Equine drug; Tranquilizer	0.001
Butabarbital (Secubarbital)	125-40-6	Equine drug; Hypnotic	0.005
Butafenacil	134605-64-4	Pesticide; Herbicide	0.01
Butalbital	77-26-9	Equine drug; Hypnotic	0.005
Butoxycarboxim	34681-23-7	Pesticide; Insecticide; Veterinary drug	0.001
Candesartan	139481-59-7	Equine drug; Angiotensin antagonist	Q-na
Carbamazepine	298-46-4	Equine drug; Anticonvulsant	0.001
Carbaryl	63-25-2	Pesticide; Insecticide; Veterinary drug	0.001
Carbendazim (Azole)	10605-21-7	Pesticide; Chemotherapeutic	0.005
Carbetamide	16118-49-3	Pesticide; Herbicide; Veterinary drug	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Carbofuran	1563-66-2	Pesticide; Insecticide; Veterinary drug	0.002
Carbophenothion	786-19-6	Pesticide; Veterinary drug	0.01
Carboxin	5234-68-4	Pesticide; Fungicide	0.001
Carfentrazone ethyl	128639-02-1	Pesticide; Herbicide	0.005
Carisoprodol	78-44-4	Equine drug; Muscle relaxant	0.001
Carvedilol	72956-09-3	Equine drug; Beta-Blocker	Q-na
CBD / Cannabidiol	13956-29-1	Designer drug; Equine drug; Psychedelic	Q-na
Cefapirin	21593-23-7	Veterinary drug; Antibiotic	L-na
Cefazolin	25953-19-9	Veterinary drug; Antibiotic	Q-na
Cefoperazone	62893-19-0	Veterinary drug; Antibiotic	Q-na
Cefotaxime	63527-52-6	Antibiotic	Q-na
Ceftiofur	80370-57-6	Veterinary drug; Antibiotic	L-na
Celecoxib	169590-42-5	Equine drug; Antiphlogistic	Q-na
Celiprolol	56980-93-9	Equine drug; Beta-Blocker	0.001
Cetirizine	83881-51-0	Equine drug; Antihistamine	0.1
Cetylpyridinium	7773-52-6	Preservative; Antiseptic; Disinfectant	Q-na
Chloramphenicol	56-75-7	Veterinary drug; Chemotherapeutic	0.005
Chlorantraniliprole	500008-45-7	Pesticide; Insecticide	0.001
Chlorbrombuterol (Bromoclenbuterol)	37153-52-9	Veterinary drug	0.001
Chlorfenvinphos	470-90-6	Pesticide; Insecticide; Acaricide; Veterinary drug	0.001
Chlorfluazuron	71422-67-8	Pesticide; Insecticide	0.005
Chloridazon (PAC)	1698-60-8	Pesticide; Herbicide	0.001
Chloridazon-desphenyl	6339-19-1	Pesticide	0.1
Chloridazon-desphenyl-methyl	17254-80-7	Pesticide; Herbicide	0.001
Chlorimuron-ethyl	90982-32-4	Pesticide; Herbicide	0.001
Chlorothiazide	58-94-6	Equine drug; Diuretic	0.001
Chloroxuron	1982-47-4	Pesticide; Herbicide; Veterinary drug	0.001
Chlorpheniramine	132-22-9	Equine drug; Antihistamine	0.005
Chlorpromazine	50-53-3	Veterinary drug; Equine drug; Neuroleptic	0.001
Chlorpropham (Chloropropham)	101-21-3	Pesticide; Herbicide; Veterinary drug	Q-na
Chlorpyrifos	2921-88-2	Pesticide; Insecticide; Veterinary drug	0.01
Chlorpyrifos Methyl	5598-13-0	Pesticide; Insecticide; Veterinary drug	0.1
Chlorsulfuron	64902-72-3	Pesticide; Herbicide	0.001
Chlortetracycline	57-62-5	Veterinary drug; Antibiotic	Q-na
Chlortoluron (Chlorotoluron)	15545-48-9	Pesticide; Herbicide	0.002
Cimaterol	54239-37-1	Veterinary drug; Equine drug; Sympathomimetic	Q-na
Cimbuterol	54239-39-3	Veterinary drug	0.005
Cimetidine	51481-61-9	H2-Blocker	0.005
Cinnarizine	298-57-7	Equine drug; Antihistamine	Q-na
Ciprofloxacin	85721-33-1	Antibiotic	0.005
Citalopram	59729-33-8	Equine drug; Antidepressant	0.001
Clarithromycin	81103-11-9	Veterinary drug; Antibiotic	0.001
Clenbuterol	37148-27-9	Veterinary drug; Equine drug; Bronchodilator	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Clenbuterolhydroxymethyl	38339-18-3	Veterinary drug	0.001
Clencyclohexerol	157877-79-7	Veterinary drug	0.005
Clenpenterol	38339-21-8	Veterinary drug	0.001
Clenproperol	38339-11-6	Veterinary drug	0.005
Clethodim	99129-21-2	Pesticide; Herbicide	0.005
Climbazole	38083-17-9	Pesticide; Fungicide; Veterinary drug	0.001
Clobazam (Urbadan)	22316-47-8	Veterinary drug; Equine drug; Tranquilizer	0.001
Clobetasol propionate	25122-46-7	Veterinary drug; Corticoid	Q-na
Clofentezine	74115-24-5	Pesticide; Fungicide; Veterinary drug	0.1
Clomazone	81777-89-1	Pesticide; Herbicide; Veterinary drug	0.001
Clonazepam	1622-61-3	Anticonvulsant; Antiepileptic	0.001
Clonidine	4205-90-7	Equine drug; Antihypertensive	0.001
Clopidogrel	113665-84-2	Antiplatelet agent	0.001
Clopidol	2971-90-6	Veterinary drug; Coccidiostatic	0.001
Clopyralid	1702-17-6	Pesticide; Herbicide	>0.1
Clorsulon	60200-06-8	Veterinary drug; Chemotherapeutic	0.005
Clothianidin	210880-92-5	Pesticide; Veterinary drug	0.001
Clotrimazole	23593-75-1	Pesticide; Antimycotic	Q-na
Cloxacillin	61-72-3	Veterinary drug; Antibiotic	Q-na
Clozapine	5786-21-0	Equine drug; Neuroleptic	0.001
Cocaine	50-36-2	Stimulant; Drug of Abuse	0.001
Codeine	76-57-3	Opiate used to treat pain	0.001
Cotinine	486-56-6	Veterinary drug; Equine drug; Stimulant	0.005
Coumaphos	56-72-4	Pesticide; Insecticide; Veterinary drug	0.001
Cyanazine (Fortrol)	21725-46-2	Pesticide; Herbicide	0.005
Cyazofamid	120116-88-3	Pesticide; Fungicide	0.005
Cyclizine	82-92-8	Equine drug; Antihistamine	Q-na
Cyclophosphamide	50-18-0	Antineoplastic	0.001
Cycluron	2163-69-1	Pesticide; Herbicide; Veterinary drug	0.001
Cyflufenamid	180409-60-3	Pesticide; Fungicide	0.001
Cyhalofop-butyl	122008-85-9	Pesticide; Veterinary drug	Q-na
Cymoxanil (Curzate)	57966-95-7	Pesticide; Fungicide; Veterinary drug	0.005
Cyproconazole	113096-99-4	Pesticide; Fungicide; Veterinary drug	0.001
Cyprodinil	121552-61-2	Pesticide; Fungicide; Veterinary drug	0.001
Cyproterone acetate	427-51-0	Antiandrogen	Q-na
Cyromazine	66215-27-8	Pesticide; Insecticide; Veterinary drug	0.001
Dalapon	75-99-0	Pesticide; Herbicide	Q-na
Daminozide	1596-84-5	Pesticide; Herbicide	Q-na
Dapson	80-08-0	Equine drug; Veterinary drug; Chemotherapeutic	0.001
Demeclocycline	127-33-3	Veterinary drug; Antibiotic	0.1
Demeton-S-methyl	919-86-8	Pesticide; Insecticide	Q-na
Demeton-S-methylsulfone	17040-19-6	Pesticide; Insecticide	Q-na
Demeton-S-methylsulfoxide	301-12-2	Pesticide; Insecticide	Q-na
Deoxynivalenol (Vomitoxin) (DON)	51481-10-8	Veterinary drug	0.01

Compound name	CAS No.	Description / use	LOD (µg/L)
Desmetryn	1014-69-3	Pesticide; Herbicide; Veterinary drug	0.001
Desthio-Prothioconazole	120983-64-4	Pesticide; Fungicide	0.001
Dexamethasone	50-02-2	Veterinary drug; Equine drug; Corticoid	0.001
Dextrorphan (Levorphanol - d form)	125-73-5	Equine drug; Potent analgesic; Potemt antitussive	0.001
Diazepam	439-14-5	Benzodiazepine; Treat anxiety; Veterinary drug	0.001
Diazinon (Dimpylate)	333-41-5	Pesticide; Insecticide	0.01
Dicamba	1918-00-9	Pesticide; Herbicide	0.04
Dichlobutrazol (Diclobutrazol)	75736-33-3	Pesticide; Fungicide; Veterinary drug	0.001
Dichlormid	37764-25-3	Pesticide; Herbicide	0.005
Dichlorprop	120-36-5	Pesticide; Herbicide	0.005
Dichlorvos	62-73-7	Pesticide; Insecticide; Veterinary drug	0.005
Diclofenac	15307-86-5	Nonsteroidal anti-inflammatory drug	0.004
Dicloxacillin	3116-76-5	Veterinary drug; Antibiotic	Q-na
Dicrotophos (Bidrin)	141-66-2	Pesticide; Insecticide; Veterinary drug	0.001
Dienestrol	84-17-3	Veterinary drug; Estrogen	Q-na
Diethofencarb	87130-20-9	Pesticide; Herbicide; Veterinary drug	0.002
Diethylstilbestrol	56-53-1	Veterinary drug; Estrogen	L-na
Difenacoum	56073-07-5	Pesticide; Rodenticide	Q-na
Difenconazole	119446-68-3	Pesticide; Fungicide; Veterinary drug	0.005
Difloxacin	98106-17-3	Veterinary drug; Gyrase inhibitor	0.005
Diflubenzuron	35367-38-5	Pesticide; Insecticide	0.001
Digoxin	20830-75-5	Equine drug; Cardiotonic	0.1
Dihexyl Phthalate (DnHP)	84-75-3	Plasticizer	0.01
Dihydromorphine	509-60-4	Equine drug; Potent analgesic	0.005
Diltiazem	42399-41-7	Equine drug; Ca antagonist	0.001
Dimefox	115-26-4	Pesticide; Insecticide	0.001
Dimethenamid (SAN 582H)	87674-68-8	Pesticide; Herbicide	0.001
Dimethoate	60-51-5	Pesticide; Insecticide	0.001
Dimethomorph	110488-70-5	Pesticide; Fungicide	0.001
Dimetilan	644-64-4	Pesticide; Insecticide	0.001
Dimetridazole	551-92-8	Nitroimidazole; Veterinary drug	0.01
Dimoxystrobin	149961-52-4	Pesticide; Fungicide; Veterinary drug	0.001
Diniconazole	83657-24-3	Pesticide; Fungicide; Veterinary drug	0.01
Dinotefuran	165252-70-0	Pesticide; Insecticide	0.001
Diofenolan	63837-33-2	Pesticide	Q-na
Dioxacarb	6988-21-2	Pesticides; Insecticide; Veterinary drug	0.001
Diphenhydramine	58-73-1	Equine drug; Antihistamine	0.01
Dipyridamol	58-32-2	Equine drug; Coronary dilator	Q-na
Dithianone	3347-22-6	Pesticide; Fungicide	Q-na
Diuron	330-54-1	Pesticide; Herbicide	0.001
Dodine	112-65-2	Pesticide; Fungicide	Q-na
Domiphen	13900-14-6	Antiseptic	Q-na
Doramectin	117704-25-3	Veterinary drug; Treatment of parasites	0.1

Compound name	CAS No.	Description / use	LOD (µg/L)
Dothiepin	113-53-1	Equine drug; Antidepressant	Q-na
Doxazosin	74191-85-8	Antihypertensive	Q-na
Doxycycline	564-25-0	Veterinary drug; Antibiotic	Q-na
DXM / Dextromethorphan (Methorphan)	125-71-3	Designer drug; Equine drug; Antitussive	0.001
Emamectin B1a	121124-29-6	Pesticide; Veterinary drug	0.005
Emamectin B1b	121424-52-0	Pesticide; Veterinary drug	L-na
Endothal	145-73-3	Pesticide; Herbicide	Q-na
Enoxacin	74011-58-8	Gyrase inhibitor	0.005
Enrofloxacin	93106-60-6	Pesticide; Veterinary drug; Antibacterial	0.005
Epoxiconazole (BAS 480F)	135319-73-2	Pesticide; Fungicide; Veterinary drug	0.001
Eprinomectin B1a (component of Eprinex)	133305-88-1	Veterinary drug	0.1
Eprinomectin B1b (component of Eprinex)	133305-89-2	Veterinary drug	Q-na
Erythromycin	114-07-8	Veterinary drug; Antibiotic	0.005
Estazolam	29975-16-4	Sedative; Hypnotic	0.001
Estrone	53-16-7	Veterinary drug; Equine drug; Estrogen	Q-na
Etaconazole	71245-23-3	Pesticide; Fungicide	0.001
Ethinylestradiol	57-63-6	Veterinary drug; Equine drug; Estrogen	Q-na
Ethiofencarb	29973-13-5	Pesticide; Insecticide; Veterinary drug	0.001
Ethiolate	2941-55-1	Pesticide; Herbicide	0.05
Ethion	563-12-2	Pesticide; Insecticide; Acaricide; Veterinary drug	0.005
Ethiprole	181587-01-9	Pesticide; Insecticide	0.001
Ethirimol	23947-60-6	Pesticide; Fungicide	0.001
Ethofumesate	26225-79-6	Pesticide; Herbicide; Veterinary drug	0.005
Ethoprop (Ethoprophos)	13194-48-4	Pesticide; Insecticide; Veterinary drug	0.001
Ethylmorphine	76-58-4	Equine drug; Potent antitussive	0.005
Etoxazole	153233-91-1	Pesticide; Acaricide	0.005
Famoxadone	131807-57-3	Pesticide; Fungicide; Veterinary drug	0.1
Febantel	58306-30-2	Pesticide; Veterinary drug; Anthelmintic	0.001
Felodipine	72509-76-3	Equine drug; Ca Antagonist	Q-na
Fenamidone	161326-34-7	Pesticide; Fungicide; Veterinary drug	0.002
Fenamiphos	22224-92-6	Pesticide; Insecticide; Veterinary drug	0.001
Fenarimol	60168-88-9	Pesticide; Fungicide	0.005
Fenazaquin	120928-09-8	Pesticide; Insecticide; Veterinary drug	0.001
Fenbendazole	43210-67-9	Pesticide; Veterinary drug; Anthelmintic	0.001
Fenbendazol-sulfone (Oxfendazole sulfone)	54029-20-8	Veterinary drug	0.005
Fenbuconazole	119611-00-6	Pesticide; Fungicide; Veterinary drug; Antimycotic	0.005
Fenchlorphos (Ronnel)	299-84-3	Pesticide; Insecticide	Q-na
Fenhexamid	126833-17-8	Pesticide; Veterinary drug	0.005
Fenitrothion	122-14-5	Pesticide; Insecticide; Veterinary drug	Q-na
Fenobucarb (BPMC)	3766-81-2	Pesticide; Insecticide; Veterinary drug	0.001
Fenofibrate	49562-28-9	Anticholesteremic	Q-na
Fenoterol (Th 1165a)	13392-18-2	Veterinary drug; Equine drug; Bronchodilator	0.005
Fenoxycarb	79127-80-3	Pesticide; Insecticide; Veterinary drug	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Fenpropimorph (Ro 14-3169)	67564-91-4	Pesticide; Fungicide; Morpholine	0.001
Fenpyroximate	134098-61-6	Pesticide; Acaricide	0.005
Fentanyl	437-38-7	Potent opioid analgesic; Equine drug	0.001
Fenthion	55-38-9	Pesticide; Insecticide; Veterinary drug	0.01
Fenthion sulfoxide (Mesulfenfos)	3761-41-9	Pesticide; Insecticide; Metabolite of Fenthion	0.001
Fenuron (N,N-Dimethyl-N-phenylurea)	101-42-8	Pesticide; Herbicide	0.002
Fexofenadine	83799-24-0	Equine drug; Antihistaminic	0.001
Finasteride	98319-26-7	Reductase inhibitor	Q-na
Fipronil	120068-37-3	Pesticide; Acaricide; Insecticide; Veterinary drug	0.001
Fipronil Sulfide	120067-83-6	Pesticide; metabolite	0.001
Fipronil sulfon (M & B 46136)	120068-36-2	Pesticide; metabolite	0.001
Flamprop-isopropyl	52756-22-6	Pesticide; Herbicide	0.001
Flamprop-methyl	52756-25-9	Pesticide; Herbicide	0.001
Flazasulfuron	104040-78-0	Pesticide; Herbicide	0.005
Flecainide	54143-55-4	Equine drug; Antiarrhythmic	0.001
Flocoumafen	90035-08-8	Pesticide; Rodenticide	Q-na
Flonicamid	158062-67-0	Pesticide; Insecticide	0.005
Florasulam	145701-23-1	Pesticide; Herbicide	0.005
Florfenicol	73231-34-2	Veterinary drug; Aquaculture	0.005
Fluazifop	69335-91-7	Pesticide; Herbicide	0.005
Fluazinam (Shirlan)	79622-59-6	Pesticide; Fungicide; Veterinary drug	0.001
Flubendazole	31430-15-6	Pesticide; Veterinary drug; Anthelmintic	0.005
Flubendiamide	272451-65-7	Pesticide; Insecticide	0.001
Flucarbazone sodium	181274-17-9	Pesticide; Herbicide	0.005
Flucofuron	370-50-3	Pesticide; Insecticide	Q-na
Fluconazole(I) (Diflucan)	86386-73-4	Pesticide; Antimycotic	0.001
Fludioxonil	131341-86-1	Pesticide; Fungicide; Veterinary drug	0.001
Flufenacet (Fluthiamide) (BAY FOE 5043)	142459-58-3	Pesticide; Herbicide	0.001
Flufenoxuron	101463-69-8	Pesticide; Insecticide; Veterinary drug	0.005
Flumequine	42835-25-6	Pesticide; Veterinary drug; Chemotherapeutic	0.001
Flumethasone (Flumetasone)	2135-17-3	Veterinary drug; Equine drug; Corticoid	Q-na
Flumioxazin	103361-09-7	Pesticide; Herbicide	0.01
Flunitrazepam	1622-62-4	Veterinary drug; Equine drug; Benzodiazepine	0.001
Flunixin	38677-85-9	Veterinary drug; Equine drug; Analgesic	0.001
Fluometuron	2164-17-2	Pesticide; Herbicide	0.001
Fluopicolid	239110-15-7	Pesticide; Fungicide	0.001
Fluoxastrobin	361377-29-9	Pesticide; Veterinary drug	0.001
Fluoxetine (Prozac)	54910-89-3	Antidepressant; Equine drug	0.005
Fluphenazine enanthate	2746-81-8	Antipsychotic drug	0.005
Fluquinconazole	136426-54-5	Pesticide; Fungicide; Veterinary drug	0.005
Flurazepam	17617-23-1	Equine drug; Benzodiazepine	0.001
Fluroxypyr	69377-81-7	Pesticide; Herbicide	0.02
Flurtamone	96525-23-4	Pesticide; Herbicide; Veterinary drug	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Flusilazol	85509-19-9	Pesticide; Veterinary drug	0.001
Fluticasone 17-propionate	80474-14-2	Equine drug; Local corticoid	Q-na
Flutolanil	66332-96-5	Pesticide; Veterinary drug	0.001
Flutriafol	76674-21-0	Pesticide; Fungicide	0.001
Fluvoxamine	54739-18-3	Equine drug; Antidepressant	Q-na
Fomesafen	72178-02-0	Pesticide; Herbicide	0.001
Fonofos (Dyfonate)	944-22-9	Pesticide; Insecticide	0.005
Forchlorfenuron	68157-60-8	Plant growth regulator	0.002
Formetanate	22259-30-9	Pesticide; Insecticide	0.005
Fosthiazate	98886-44-3	Pesticide; Nematicide	0.001
Fuberidazole	3878-19-1	Pesticide; Fungicide; Veterinary drug	0.001
Furalaxyl	57646-30-7	Pesticide; Fungicide	0.001
Furathiocarb	65907-30-4	Pesticide; Insecticide; Veterinary drug	0.001
Furosemide	54-31-9	Equine drug; Diuretic	0.01
Fusidic acid	1859-24-0	Mycotoxin	Q-na
Gabapentin	60142-96-3	Equine drug; Anticonvulsant	0.01
Gatifloxacin (Tequin) (Zymar)	112811-59-3	Veterinary drug	0.001
Gliclazide	21187-98-4	Antidiabetic	0.001
Griseofulvin	126-07-8	Pesticide; Mycotoxin; Antimycotic	0.001
Guaifenesin	93-14-1	Equine drug; Expectorant; Muscle relaxant	0.001
Halofenozide	112226-61-6	Pesticide; Insecticide	0.01
Haloperidol	52-86-8	Veterinary drug; Equine drug; Neuroleptic	Q-na
Haloxyfop-methyl	69806-40-2	Pesticide; Herbicide; Veterinary drug	0.001
Heptafluorobutyric acid (HFBA)	375-22-4	Organofluorine compound	0.001
Hexaconazole	79983-71-4	Pesticide; Fungicide; Veterinary drug	0.001
Hexaflumuron	86479-06-3	Pesticide; Insecticide	0.1
Hexazinone	51235-04-2	Pesticide; Herbicide; Veterinary drug	0.005
Hexestrol	84-16-2	Estrogen; Antineoplastic (hormonal)	Q-na
Hexobarbital	56-29-1	Equine drug; Anesthetic	0.005
Hexythiazox	78587-05-0	Pesticide; Fungicide; Veterinary drug	0.005
HMMNI / Hydroxydimetridazole (Dimetridazol-OH)	936-05-0	Veterinary drug	L-na
Hydramethylnon	67485-29-4	Pesticide; Insecticide	L-na
Hydrochlorothiazide	58-93-5	Equine drug; Diuretic	0.001
Hydrocodone	125-29-1	Equine drug; Opioid	0.001
Hydrocortisone (Cortisol)	50-23-7	Veterinary drug; Equine drug; Corticoid	Q-na
Hydrocortisone acetate	50-03-3	Corticoid	Q-na
Hydroxy-Ipronidazole	35175-14-5	Veterinary drug	L-na
Hydroxymetronidazole	4812-40-2	Veterinary drug	L-na
Hydroxyzine	68-88-2	Equine drug; Tranquilizer	Q-na
Ibuprofen	15687-27-1	Nonsteroidal anti-inflammatory; Analgesic; Veterinary drug	0.001
Imazalil (Enilconazole)	35554-44-0	Pesticide; Herbicide; Veterinary drug; Antimycotic	0.001
Imazamethabenz-methyl	81405-85-8	Pesticide; Herbicide	0.001
Imazamox	114311-32-9	Pesticide; Herbicide	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Imazapic (Imazameth)	104098-48-8	Pesticide; Herbicide	0.005
Imazapyr	81334-34-1	Pesticide; Herbicide	0.02
Imibenconazole	86598-92-7	Pesticide; Fungicide	0.1
Imidacloprid	138261-41-3	Pesticide; Insecticide; Veterinary drug	0.001
Indapamide	26807-65-8	Equine drug; Diuretic	Q-na
Indaziflam	950782-86-2	Pesticide; Herbicide	0.001
Indoxacarb	173584-44-6	Pesticide; Insecticide	0.001
Iodofenphos	18181-70-9	Pesticide; Insecticide	Q-na
Iohexol	66108-95-0	X-ray Contrast	0.005
Ioxynil	1689-83-4	Pesticide; Herbicide	0.001
Ipconazole	125225-28-7	Pesticide; Fungicide	0.01
Iprodione (Glycophen)	36734-19-7	Pesticide; Fungicide	0.001
Ipronidazole	14885-29-1	Veterinary drug; Chemotherapeutic	0.005
Iprovalicarb	140923-17-7	Pesticide; Veterinary drug	0.002
Irbesartan	138402-11-6	Equine drug	0.005
Irgarol (Cybutryne)	28159-98-0	Pesticide; Algicide; Antifouling	0.005
Isocarbophos	24353-61-5	Pesticide; Acaricide; Insecticide	0.1
Isoprocarb	2631-40-5	Pesticide; Insecticide; Veterinary drug	0.001
Isoproturon	34123-59-6	Pesticide; Herbicide; Veterinary drug	0.001
Isopyrazam	881685-58-1	Pesticide; Fungicide	0.005
Isopyrin	3615-24-5	Veterinary drug; Equine drug; Analgesic	L-na
Isoxsuprine (Isolait)	395-28-8	Veterinary drug; Equine drug; Vasodilator	0.001
Ivermectin B1a	71827-03-7	Pesticide; Veterinary drug; Anthelmintic	0.1
Ivermectin B1b	70209-81-3	Pesticide; Veterinary drug; Anthelmintic	0.1
Josamycin	16846-24-5	Veterinary drug; Antibiotic	0.005
Ketoconazole	65277-42-1	Pesticide; Antimycotic	0.001
Ketoprofen	22071-15-4	Nonsteroidal anti-inflammatory; Veterinary drug; Equine drug	0.001
Ketorolac	74103-06-3	Equine drug; Antiphlogistic	0.005
Kresoxim-methyl	143390-89-0	Pesticide; Fungicide; Veterinary drug	0.005
Labetalol (Laberalol)	36894-69-6	Pesticide; Veterinary drug; Equine drug; Antihypertensive	Q-na
Lamotrigine	84057-84-1	Equine drug; Anticonvulsant	0.001
Lansoprazole	103577-45-3	Antiulcerative; Anti-infective	Q-na
Lasalocid A-sodium	25999-20-6	Veterinary drug	Q-na
Latanoprost	130209-82-4	Prostaglandin analogue	Q-na
Lenacil	2164-08-1	Pesticide; Herbicide; Veterinary drug	0.005
Leucomalachite green	129-73-7	Dye; Antimicrobial	0.001
Levamisole	14769-73-4	Veterinary drug; Antirheumatic	0.001
Lidocaine (Diocaine)	137-58-6	Anesthetic; Antiarrhythmic	0.001
Lincomycin	154-21-2	Veterinary drug; Antibiotic	0.001
Linuron	330-55-2	Pesticide; Herbicide; Veterinary drug	0.001
Lisinopril	76547-98-3	Equine drug; Antihypertensive	Q-na
Lomefloxacin	98079-51-7	Gyrase inhibitor	0.005
Loperamide	53179-11-6	Equine drug; Antidiabetic	Q-na

Compound name	CAS No.	Description / use	LOD (µg/L)
Loratadine	79794-75-5	Equine drug; Antihistamine	Q-na
Lorazepam	846-49-1	Equine drug; Benzodiazepine	0.005
Losartan	114798-26-4	Equine drug; Angiotensin antagonist	0.001
Lufenuron	103055-07-8	Pesticide; Insecticide	0.1
Mabuterol	56341-08-3	Veterinary drug; Equine drug; Sympathomimetic	0.001
Malachite Green (Basic Green)	2437-29-8	Dye; Antimicrobial	Q-na
Malathion	121-75-5	Pesticide; Insecticide; Veterinary drug	0.001
Mandipropamid	374726-62-2	Pesticide; Fungicide	0.001
Mapenterol (Methylmabuterol)	95656-68-1	Veterinary drug	0.001
Marbofloxacin	115550-35-1	Veterinary drug; Antibiotic	0.005
MCPA (MCP)	94-74-6	Pesticide; Herbicide	0.01
MCPB (2,4-MCPB)	94-81-5	Pesticide; Herbicide	0.005
MCPP / Mecoprop	7085-19-0	Pesticide; Herbicide	0.005
Mebendazole	31431-39-7	Pesticide; Veterinary drug; Anthelmintic	0.001
Mebeverine	630-20-3	Equine drug; Antispamotic	Q-na
Medroxyprogesterone	520-85-4	Veterinary drug; Equine drug; Progestin	0.001
Medroxyprogesterone acetate	71-58-9	Veterinary drug; Gestagen	Q-na
Mefenacet (Rancho)	73250-68-7	Pesticide; Herbicide	0.001
Mefenamic acid	61-68-7	Veterinary drug; Equine drug; Antiphlogistic	L-na
Megestrol acetate	595-33-5	Veterinary drug; Estrogen	0.001
Melatonin	73-31-4	Hormone; Sedative	0.005
Melengestrol acetate	2919-66-6	Veterinary drug; Progestin	0.001
Meloxicam	71125-38-7	Veterinary drug; Equine drug; Antiphlogistic	L-na
Mepanipyrim	110235-47-7	Pesticide; Fungicide; Veterinary drug	0.001
Meprobamate	57-53-4	Equine drug; Hypnotic	0.005
Mepronil	55814-41-0	Pesticide; Fungicide	0.001
Mesotrione	104206-82-8	Pesticide; Herbicide	0.01
Metaflumizone	139968-49-3	Pesticide; Insecticide	0.05
Metalaxyl	57837-19-1	Pesticide; Fungicide; Veterinary drug	0.001
Metamitron	41394-05-2	Pesticide; Herbicide; Veterinary drug	0.001
Metaxalone	1665-48-1	Equine drug; Muscle relaxant	0.005
Metazachlor	67129-08-2	Pesticide; Herbicide; Veterinary drug	0.001
Metconazole	125116-23-6	Pesticide; Fungicide; Veterinary drug	0.001
Metformin	657-24-9	Antidiabetic	0.1
Methabenzthiazuron	18691-97-9	Pesticide; Herbicide	0.001
Methadone	76-99-3	Equine drug; Opioid	0.005
Methamidophos (Metamidophos)	10265-92-6	Pesticide; Insecticide; Veterinary drug	0.1
Methaqualone	72-44-6	Sedatine; Hypnotic	0.001
Methiocarb (Mercaptodimethur)	2032-65-7	Pesticide; Insecticide	0.001
Methomyl	16752-77-5	Pesticide; Insecticide; Veterinary drug	0.005
Methoprotryne	841-06-5	Pesticide; Herbicide; Veterinary drug	0.001
Methoxyfenozide (Intrepid)	161050-58-4	Pesticide; Insecticide	0.005
Methylprednisolone	83-43-2	Veterinary drug; Equine drug; Corticoid	0.005
Metobromuron	3060-89-7	Pesticide; Herbicide; Veterinary drug	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Metoclopramide	364-62-5	Equine drug; Antiemetic	Q-na
Metolachlor	51218-45-2	Pesticide; Herbicide; Veterinary drug	0.001
Metoprolol	37350-58-6	Veterinary drug; Equine drug; Beta-Blocker	Q-na
Metoxuron	19937-59-8	Pesticide; Herbicide	0.001
Metrafenone	220899-03-6	Pesticide	0.001
Metribuzin	21087-64-9	Pesticide; Herbicide; Veterinary drug	0.002
Metribuzin-desamino	35045-02-4	Pesticide	0.001
Metribuzin-diketo	56507-37-0	Pesticide	0.001
Metronidazole	443-48-1	Antibiotic; Antiprotozoal medication	L-na
Metsulfuron-methyl	74223-64-6	Pesticide; Herbicide	0.01
Mevinphos (Phosdrin)	7786-34-7	Pesticide; Insecticide	0.005
Mexacarbate (Zectran)	315-18-4	Pesticide; Insecticide	0.001
Miconazole	22916-47-8	Pesticide; Antimycotic	0.001
Midazolam	59467-70-8	Sedative drug	0.001
Minocyclin	10118-90-8	Veterinary drug; Antibiotic	Q-na
Mirtazapine	61337-67-5	Equine drug; Antidepressant	Q-na
Modafinile	68693-11-8	Equine drug; Sympathomimetic	0.001
Molinate	2212-67-1	Pesticide; Herbicide; Veterinary drug	0.005
Monensin	17090-79-8	Veterinary drug; Antiamebic	0.005
Monocrotophos (Azodrin)	6923-22-4	Pesticide; Insecticide; Veterinary drug	0.001
Monolinuron	1746-81-2	Pesticide; Herbicide; Algaecide; Veterinary drug	0.001
Monuron	150-68-5	Pesticide; Herbicide	0.001
Morphine	57-27-2	Equine drug; Opioid	0.001
Moxidectin (Cydectin)	113507-06-5	Pesticide; Veterinary drug	0.005
Moxonidine	75438-57-2	Antihypertensive	Q-na
Myclobutanil (systhane)	88671-89-0	Pesticide; Fungicide; Veterinary drug	0.001
Nafcillin	147-52-4	Veterinary drug; Antibiotic	Q-na
Naloxone	465-65-6	Equine drug; Opioid antagonist	0.001
Naltrexone	16590-41-3	Equine drug; Opioid antagonist	0.001
Nandrolone or 17-alpha-19-Nortestosterone	434-22-0	Veterinary drug; Equine drug; Anabolic	0.005
Napropamide	15299-99-7	Pesticide; Herbicide; Veterinary drug	0.001
Naproxen	22204-53-1	Veterinary drug; Equine drug; Analgesic	0.01
Nebivolol	99200-09-6	Equine drug; Beta-Blocker	Q-na
Neburon (Phosphoramidothioic acid)	555-37-3	Pesticide; Herbicide	0.001
Niclosamide	50-65-7	Pesticide; Molluscicide	0.005
Nicosulfuron	111991-09-4	Pesticide; Herbicide	0.001
Nifedipine	21829-25-4	Equine drug; Ca antagonist	Q-na
Nitenpyram	150824-47-8	Pesticide; Insecticide	0.001
Nitrazepam	146-22-5	Hypnotic drug; Drug of Abuse	0.001
Nitrofurantoin	67-20-9	Veterinary drug; Chemotherapeutic	Q-na
Norcodeine	467-15-2	Analgesic	0.001
Norethisterone	68-22-4	Veterinary drug; Progestin	Q-na
Norfloxacin	70458-96-7	Veterinary drug; Chemotherapeutic	0.005
Norfluoxetine	56161-73-0	Equine drug; Fluoxetine metabolite	Q-na

Compound name	CAS No.	Description / use	LOD (µg/L)
Normorphine	466-97-7	Analgesic	0.005
Novaluron	116714-46-6	Pesticide; Insecticide	0.1
Nuarimol	63284-71-9	Pesticide; Fungicide; Veterinary drug	0.005
Ochratoxin A	303-47-9	Veterinary drug; Mycotoxin; Biomolecule	0.005
Ofloxacin	82419-36-1	Veterinary drug; Chemotherapeutic	0.005
Omeprazole	73590-58-6	Antiulcerative	Q-na
Omethoate	1113-02-6	Pesticide; Insecticide; Veterinary drug	0.001
OMPA / Schradan	152-16-9	Pesticide	0.001
Orlistat	96829-58-2	Antiobesity agent	Q-na
Oxacillin	66-79-5	Veterinary drug; Antibiotic	0.1
Oxadiazon	19666-30-9	Pesticide; Herbicide; Veterinary drug	0.005
Oxadixyl	77732-09-3	Pesticide; Fungicide; Veterinary drug	0.005
Oxamyl	23135-22-0	Pesticide; Insecticide; Veterinary drug	0.1
Oxazepam	604-75-1	Anxiety drug; Veterinary drug; Equine drug	0.001
Oxcarbazepine	28721-07-5	Equine drug; Anticonvulsant	0.005
Oxfendazole	53716-50-0	Pesticide; Veterinary drug; Anthelmintic	0.001
Oxibendazole	20559-55-1	Pesticide; Veterinary drug; Anthelmintic	0.001
Oxolinic acid	14698-29-4	Pesticide; Veterinary drug; Gyrase inhibitor	0.001
Oxprenolol	6452-71-7	Equine drug; Beta-Blocker	Q-na
Oxybutynine	5633-20-5	Antispamotic	Q-na
Oxycodone	76-42-6	Equine drug; Opioid	0.001
Oxytetracycline	79-57-2	Pesticide; Veterinary drug; Antibiotic	Q-na
Paclobutrazol	76738-62-0	Pesticide; Veterinary drug	0.002
Pantoprazole	102625-70-7	Proton pump inhibitor; Ulcus remedy	Q-na
Parathion-ethyl	56-38-2	Pesticide; Insecticide	Q-na
Parathion-methyl	298-00-0	Pesticide; Insecticide	Q-na
Paroxetine	61869-08-7	Equine drug; Antidepressant	Q-na
Penconazole	66246-88-6	Pesticide; Fungicide; Veterinary drug	0.001
Pencycuron (Monceren)	66063-05-6	Pesticide; Fungicide	0.01
Pendimethalin (Penoxalin)	40487-42-1	Pesticide; Herbicide	0.005
Penicillin G (Benzylpenicillin)	61-33-6	Antibiotic; Therapeutic Agent; Natural Product	0.1
Penicillin V	87-08-1	Antibiotic; Therapeutic Agent; Natural Product	Q-na
Penthiopyrad	183675-82-3	Pesticide: Fungicide	0.001
Pentobarbital	76-74-4	Equine drug; Anesthetic; Hypnotic	0.005
Perfluoro Decanoic Acid	335-76-2	Surfactant	0.01
Perfluoro Dodecanoic Acid	307-55-1	Surfactant	0.1
Perfluoro Heptanoic Acid	375-85-9	Surfactant	0.005
Perfluoro Hexanoic Acid	307-24-4	Surfactant	0.005
Perfluoro Nonanoic Acid	375-95-1	Surfactant	0.005
Perfluoro Octanoic Acid	335-67-1	Surfactant	0.005
Perfluoro Pentanoic Acid	2706-90-3	Surfactant	0.005
Perfluoro Tetradecanoic Acid	376-06-7	Surfactant	0.1
Perfluoro Undecanoic Acid	2058-94-8	Surfactant	0.1

Compound name	CAS No.	Description / use	LOD (µg/L)
Perfluorobutane sulfonate	375-73-5	Surfactant	0.005
Perfluoroctylsulfonamide (PFOSA)	754-91-6	Surfactant; breaks down to form PFOS	0.005
Perfluorohexane sulfonate	108427-53-8	Surfactant	0.005
Perfluorooctane sulfonate (PFOS)	1763-23-1	Surfactant; Fire fighting foams	0.01
Pethoxamid	106700-29-2	Pesticide; Herbicide	0.001
Phenacetin	62-44-2	Analgesic; Antipyretic	Q-na
Phenmedipham	13684-63-4	Pesticide; Herbicide; Veterinary drug	Q-na
Phenobarbital	50-06-6	Equine drug; Hypnotic; Anticonvulsant	0.005
Phenoxyacetic acid	122-59-8	Pesticide; Fungicide	0.02
Phenoxymethylpenicillin	87-08-1	Veterinary drug; Antibiotic	Q-na
Phenytoin	57-41-0	Equine drug; Anticonvulsant	0.01
Phorate (Isothioate)	298-02-2	Pesticide; Insecticide	0.1
Picaridin (Bayrepel)	119515-38-7	Insect repellent	0.001
Picloram	1918-02-1	Pesticide; Herbicide	>0.1
Picoxystrobin	117428-22-5	Pesticide; Fungicide; Veterinary drug	0.005
Pinoxaden	243973-20-8	Pesticide; Herbicide	0.001
Pirimicarb	23103-98-2	Pesticide; Insecticide; Veterinary drug	0.001
Pirimiphos-ethyl (Pirimifos-ethyl)	23505-41-1	Pesticide; Insecticide	0.001
Pirimiphos-methyl (Pirimifos-methyl)	29232-93-7	Pesticide; Insecticide	0.001
Piroxicam	36322-90-4	Veterinary drug; Equine drug; Antiphlogistic	0.001
Prazepam	2955-38-6	Benzodiazepine derivative drug; Anxiolytic	0.001
Praziquantel	55268-74-1	Pesticide; Veterinary drug; Anthelmintic	0.001
Prednisolone	50-24-8	Veterinary drug; Equine drug; Corticoid	0.005
Primidone	125-33-7	Equine drug; Anticonvulsant	0.005
Prochloraz	67747-09-5	Pesticide; Fungicide; Veterinary drug	0.001
Procyclidine	77-37-2	Equine drug; Antiparkisonian	Q-na
Profenofos	41198-08-7	Pesticide; Insecticide; Veterinary drug	0.001
Progesterone	57-83-0	Pesticide; Veterinary drug; Progestin	Q-na
Promecarb	2631-37-0	Pesticide; Insecticide; Veterinary drug	0.001
Prometon	1610-18-0	Pesticide; Herbicide; Veterinary drug	0.001
Prometryn	7287-19-6	Pesticide; Herbicide; Veterinary drug	0.001
Propachlor	1918-16-7	Pesticide; Herbicide; Veterinary drug	Q-na
Propamocarb	24579-73-5	Pesticide; Fungicide; Veterinary drug	0.001
Propargite	2312-35-8	Pesticide; Veterinary drug	0.1
Propazine	139-40-2	Pesticide; Herbicide; Veterinary drug	0.001
Propetamphos (Tsar)	31218-83-4	Pesticide; Insecticide	0.005
Propham	122-42-9	Pesticide; Herbicide; Veterinary drug	0.1
Propiconazole	60207-90-1	Pesticide; Fungicide; Veterinary drug	0.001
Propionylpromazine	3568-24-9	Veterinary drug; Equine drug; Tranquilizer	0.001
Propoxur (baygon)	114-26-1	Pesticide; Insecticide	0.002
Propoxycarbazone	145026-81-9	Pesticide; Herbicide	0.005
Propoxyphene (Dextropropoxyphene)	469-62-5	Equine drug; Potent analgesic	Q-na
Propranolol	525-66-6	Veterinary drug; Equine drug; Beta-Blocker	0.005
Propyzamide (Pronamide)	23950-58-5	Pesticide; Herbicide	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Proquinazid	189278-12-4	Pesticide; Veterinary drug	0.001
Prosulfuron	94125-34-5	Pesticide; Herbicide	0.001
Prothioconazole	178928-70-6	Pesticide; Veterinary drug	0.005
Pymetrozine	123312-89-0	Pesticide; Insecticide; Veterinary drug	0.001
Pyracarbolid	24691-76-7	Pesticide; Fungicide	0.001
Pyraclostrobin	175013-18-0	Pesticide; Fungicide	0.001
Pyridaben	96489-71-3	Pesticide; Acaricide; Insecticide	0.005
Pyrimethanil	53112-28-0	Pesticide; Fungicide; Veterinary drug	0.001
Pyriproxyfen (Pyriproxifen)	95737-68-1	Pesticide; Insecticide; Veterinary drug	0.001
Pyroxsulam	422556-08-9	Pesticide; Herbicide	0.005
Quinidine	56-54-2	Pesticide; Equine drug; Antiarrhythmic	0.005
Quinine	130-95-0	Pesticide; Equine drug; Antimalarial	Q-na
Quinoxyfen	124495-18-7	Pesticide; Fungicide; Veterinary drug	0.001
Quizalofop-p-ethyl	100646-51-3	Pesticide; Herbicide; Veterinary drug	0.001
Ractopamine	97825-25-7	Veterinary drug; Equine drug; Anticholesteremic	L-na
Ranitidine	66357-35-5	H2-Blocker	0.001
Rifabutin	72559-06-9	Antibiotic	0.005
Rifaximin	80621-81-4	Veterinary drug; Antibiotic	0.005
Rimsulfuron	122931-48-0	Pesticide; Herbicide	0.001
Risperidone	106266-06-2	Equine drug; Neuroleptic	Q-na
Ronidazole	7681-76-7	Pesticide; Veterinary drug; Antiamebic	L-na
Ropinirole	91374-21-9	Antiparkinsonian	Q-na
Rotenone	83-79-4	Pesticide; Piscicide; Insecticide	0.005
Roxithromycin	80214-83-1	Veterinary drug; Antibiotic	0.005
Saccharin	81-07-2	Pharmaceutical aid; Artificial Sweetener	0.001
Salbutamol (Albuterol)	18559-94-9	Pesticide; Veterinary drug; Equine drug; Bronchodilator	0.005
Salmeterol	89365-50-4	Veterinary drug; Equine drug; Bronchodilator	Q-na
Sarafloxacin	98105-99-8	Veterinary drug; Antibacterial	0.005
Scopolamine	51-34-3	Equine drug; Parasympatholytic	Q-na
Secobarbital	76-73-3	Equine drug; Hypnotic	0.005
Sertraline	79617-96-2	Equine drug; Antidepressant	0.001
Siduron	1982-49-6	Pesticide; Herbicide	0.002
Sildenafil	139755-83-2	Equine drug; Vasodilator	Q-na
Silthiofam	175217-20-6	Pesticide; Veterinary drug	0.001
Simazine	122-34-9	Pesticide; Herbicide; Veterinary drug	0.001
Simetryn	1014-70-6	Pesticide; Herbicide	0.001
Simvastatin	79902-63-9	Cholesterol synthesis inhibitor	0.005
Sotalol	3930-20-9	Equine drug; Beta-Blocker	0.005
Spectinomycin	1695-77-8	Veterinary drug; Antibiotic	Q-na
Spinetoram (J&L)	187166-40-1	Pesticide; Insecticide	0.001
Spinosad	168316-95-8	Pesticide; Insecticide	0.001
Spiramycin I	24916-50-5	Veterinary drug; Antibiotic	Q-na
Spirodiclofen	148477-71-8	Pesticide; Acaricide	0.005

Compound name	CAS No.	Description / use	LOD (µg/L)
Spiromesifen	283594-90-1	Pesticide; Insecticide	0.1
Spirotetramat	203313-25-1	Pesticide; Insecticide	0.001
Spiroxamine	118134-30-8	Pesticide; Fungicide	0.001
Stanozolol	10418-03-8	Veterinary drug; Equine drug; Anabolic	0.001
Sucralose	56038-13-2	Artificial sweetner	0.01
Sulcofuron	24019-05-4	Pesticide; Insecticide	Q-na
Sulcotrione	99105-77-8	Pesticide; Herbicide	0.001
Sulfachloropyridazine	80-32-0	Pesticide; Veterinary drug; Chemotherapeutic	0.005
Sulfadiazine (Silvadene)	68-35-9	Veterinary drug; Chemotherapeutic	0.005
Sulfadimethoxine	122-11-2	Pesticide; Veterinary drug; Chemotherapeutic	0.005
Sulfamerazine	127-79-7	Veterinary drug; Antibiotic	0.005
Sulfamethazine	57-68-1	Antibacterial	0.005
Sulfamethizole	144-82-1	Pesticide; Veterinary drug; Chemotherapeutic	0.005
Sulfamethoxazole	723-46-6	Veterinary drug; Antibiotic	0.005
Sulfanilamide	63-74-1	Veterinary drug; Antibiotic; Chemotherapeutic	0.01
Sulfaquinoxaline	59-40-5	Pesticide; Rodenticide; Veterinary drug	0.005
Sulfathiazole	72-14-0	Pesticide; Veterinary drug; Chemotherapeutic	0.005
Sulfentrazone	122836-35-5	Pesticide; Herbicide	0.005
Tadalafil	171596-29-5	Vasodilator; Erection enhancement	0.005
Tamoxifen	10540-29-1	Equine drug; Antiestrogen	Q-na
Tebuconazole (Terbuconazole)	107534-96-3	Pesticide; Fungicide	0.001
Tebufenozide	112410-23-8	Pesticide; Insecticide	0.005
Tebufenpyrad	119168-77-3	Pesticide; Veterinary drug	0.005
Tebuthiuron	34014-18-1	Pesticide; Herbicide	0.001
Teflubenzuron	83121-18-0	Pesticide; Insecticide; Veterinary drug	0.1
Telmisartan	144701-48-4	Antihypertonic	0.001
Temephos (Abate)	3383-96-8	Pesticide; Insecticide; Veterinary drug	0.005
Terbinafine	91161-71-6	Antifungal drug	0.001
Terbumeton	33693-04-8	Pesticide; Herbicide; Veterinary drug	0.001
Terbutaline	23031-25-6	Pesticide; Veterinary drug; Equine drug; Bronchodilator	L-na
Terbuthylazine (TERBA)	5915-41-3	Pesticide; Herbicide; Veterinary drug	0.005
Terbutryn	886-50-0	Pesticide; Herbicide	0.001
Testosterone	58-22-0	Veterinary drug; Equine drug; Androgen	0.001
Tetraconazole	112281-77-3	Pesticide; Veterinary drug	0.001
Tetracycline	60-54-8	Pesticide; Veterinary drug; Antibiotic	Q-na
Theophylline	58-55-9	Equine drug; Vasodilator; Bronchodilator	Q-na
Thiabendazole	148-79-8	Pesticide; Veterinary drug; Anthelmintic	0.001
Thiacloprid	111988-49-9	Pesticide; Insecticide; Veterinary drug	0.001
Thiamethoxam	153719-23-4	Pesticide; Insecticide; Veterinary drug	0.001
Thiamphenicol	15318-45-3	Veterinary drug; Chemotherapeutic	0.005
Thidiazuron	51707-55-2	Pesticide; Plant growth regulator	0.001
Thifensulfuron-methyl	792777-27-3	Pesticide; Herbicide; Veterinary drug	0.1
Thiobencarb	28249-77-6	Pesticide; Herbicide	0.001

Compound name	CAS No.	Description / use	LOD (µg/L)
Thiodicarb	59669-26-0	Pesticide; Insecticide; Veterinary drug	0.001
Thiofanox	39196-18-4	Pesticide; Insecticide; Veterinary drug	0.1
Thiofanox-sulfoxide	39184-27-5	Pesticide; Acaricide/Insecticide; Veterinary drug	0.005
Thiophanate-methyl	23564-05-8	Pesticide; Fungicide; Veterinary drug	0.1
Thioridazine	50-52-2	Equine drug; Neuroleptic	0.001
Thiram	137-26-8	Pesticide; Fungicide	Q-na
Tiamulin	55297-95-5	Veterinary drug; Chemotherapeutic	Q-na
Tilmicosin	108050-54-0	Veterinary drug	0.005
Timolol	26839-75-8	Equine drug; Beta-Blocker	Q-na
Tolbutamide	64-77-7	Antidiabetic	0.001
Tolfenamic acid	13710-19-5	Veterinary drug; Equine drug; Antiphlogistic	L-na
Toltrazuril	69004-03-1	Veterinary drug; Coccidiostatic	0.001
Toltrazuril Sulfon	69004-04-2	Veterinary drug; metabolite of Toltrazuril	0.005
Tonalide (Fixolide)	1506-02-1	Veterinary drug	0.005
Topiramate	97240-79-4	Anticonvulsant	0.005
Tramadol	27203-92-5	Equine drug; Potent analgesic	0.001
Trazodone	19794-93-5	Equine drug; Antidepressant	Q-na
Trenbolone or Hexestrol	10161-33-8	Veterinary drug; Equine drug; Anabolic	0.001
Triadimefon	43121-43-3	Pesticide; Fungicide; Veterinary drug	0.001
Triadimenol	55219-65-3	Pesticide; Fungicide; Veterinary drug	0.005
Triallate	2303-17-5	Pesticide; Herbicide	0.001
Triazophos	24017-47-8	Pesticide; Insecticide; Veterinary drug	0.001
Tribenuron-methyl	101200-48-0	Pesticide; Herbicide	0.005
Trichlorfon (Metrifonate)	52-68-6	Pesticide; Insecticide; Veterinary drug	0.001
Triclabendazole	68786-66-3	Pesticide; Veterinary drug; Anthelmintic	0.005
Triclabendazole sulfoxide	100648-13-3	Veterinary drug	0.005
Triclocarban	101-20-2	Pesticide; Disinfectant	0.001
Triclopyr	55335-06-3	Pesticide; Herbicide	0.01
Triclosan	3380-34-5	Antiseptic; Bactericide; Disinfectant	0.001
Tricyclazole	41814-78-2	Pesticide; Fungicide; Veterinary drug	0.001
Tridemorph	24602-86-6	Pesticide; Fungicide	0.001
Trietazine	1912-26-1	Pesticide; Herbicide	0.005
Trifloxystrobin	141517-21-7	Pesticide; Fungicide; Veterinary drug	0.001
Triflumizol	68694-11-1	Pesticide; Fungicide; Veterinary drug	0.001
Triflumuron	64628-44-0	Pesticide; Insecticide	0.005
Triflusulfuron-methyl	126535-15-7	Pesticide; Herbicide	Q-na
Triforine	26644-46-2	Pesticide; Fungicide	0.005
Trimethoprim	738-70-5	Veterinary drug; Antibiotic	0.001
Trimipramine	739-71-9	Equine drug; Antidepressant	0.001
Trinexapac	104273-73-6	Pesticide; Plant growth regulator	0.1
Trinexapac-ethyl	95266-40-3	Pesticide; Plant growth regulator	0.005
Triphenyl phosphate (TPPA)	115-86-6	Flame retardant; Plasticizer	0.01
Triticonazole	131983-72-7	Pesticide; Fungicide; Veterinary drug	0.1
Trixylenyl phosphate	25155-23-1	Fire retardant	0.1

Compound name	CAS No.	Description / use	LOD (µg/L)
Tulobuterol	41570-61-0	Veterinary drug; Equine drug; Bronchodilator	0.001
Tylosin	1401-69-0	Veterinary drug; Antibiotic	0.005
Valsartan	137862-53-4	Antihypertensive	0.005
Vamidothion	2265-23-2	Pesticide; Insecticide	0.001
Vedaprofen	71109-09-6	Veterinary drug; Equine drug	L-na
Venlafaxine	93413-69-5	Equine drug; Antidepressant	0.005
Verapamil	52-53-9	Equine drug; Ca antagonist	Q-na
Virginiamycin M1 (Mikamycin A)	21411-53-0	Veterinary drug; Antibiotic	0.001
Warfarin	81-81-2	Pesticide; Anticoagulant; Rodenticide	0.001
Zearalanone (Zanone)	5975-78-0	Veterinary drug; Mycotoxin	0.005
Zeranol	26538-44-3	Veterinary drug; Equine drug; Estrogen	0.005
Zilpaterol	117827-79-9	Veterinary drug; Equine drug; Beta agonist/repartitioning agent	0.005
Zoxamide	156052-68-5	Pesticide; Veterinary drug	0.001

Abbrevations: *LOD* – limits of detection; *Q-na* - quantitation is not yet available (presence or absence); *L-na* - LoD not evaluated, quantitation available.

Table S2 EOCs analysis results

March_2019			
Sample Name	Substance	CAS#	Conc. (µg/l)
Kupica	Atrazine	1912-24-9	0,0003
Kupica	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0011
Kupica	Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	0.0001
Kupica	Carbamazepine	298-46-4	0.0004
Kupica	Chloridazon-desphenyl-methyl	17254-80-7	0.0001
Kupica	Clarithromycin	81103-11-9	0.0002
Kupica	Cotinine	486-56-6	0.0006
Kupica	Lamotrigine	84057-84-1	0.0007
Kupica	Propiconazole	60207-90-1	0.0017
Kupica	Sulfamethoxazole	723-46-6	0.0007
Kupica	Triphenyl phosphate (TPPA)	115-86-6	0.09
Kupica	Tramadol	27203-92-5	0.0005
Kupica	Valsartan	137862-53-4	0.009
Kupica	Hydrochlorothiazide	58-93-5	0.001
Kupica	Perfluoro Pentanoic Acid	2706-90-3	0.0008
Kupica	Perfluorooctane sulfonate (PFOS)	1763-23-1	0.0002
Zagorska Mrežnica	Lamotrigine	84057-84-1	0.0001
Zagorska Mrežnica	Sulfadiazine (Silvadene)	68-35-9	0.0002
Tonković	Carbamazepine	298-46-4	0.0007
Tonković	Triphenyl phosphate (TPPA)	115-86-6	0.03
Tonković	Perfluoro Pentanoic Acid	2706-90-3	0.0007

October_2019			
Sample Name	Substance	Cas#	Conc. (µg/l)
Kupica	Atrazine	1912-24-9	0.0004
Kupica	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0014
Kupica	Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	0.0001
Kupica	Carbamazepine	298-46-4	0.0013
Kupica	Lamotrigine	84057-84-1	0.0014
Kupica	Metformin	657-24-9	0.012
Kupica	Metribuzin-desamino	35045-02-4	0.0001
Kupica	Prednisolone	50-24-8	0.0017
Kupica	Sulfamethoxazole	723-46-6	0.0008
Kupica	Telmisartan	144701-48-4	0.0001
Kupica	Tramadol	27203-92-5	0.0009
Kupica	Perfluorobutane sulfonate	375-73-5	0.0024
Kupica	Perfluorohexane sulfonate	108427-53-8	0.0077
Kupica	Perfluorooctane sulfonate (PFOS)	1763-23-1	0.0002
Kupica	Sucralose	56038-13-2	0.056
Zagorska Mrežnica	Atrazine	1912-24-9	0.0002
Zagorska Mrežnica	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0009
Zagorska Mrežnica	Carbamazepine	298-46-4	0.0002
Zagorska Mrežnica	Lamotrigine	84057-84-1	0.0003
Zagorska Mrežnica	Melamine	108-78-1	0.02
Zagorska Mrežnica	Tramadol	27203-92-5	0.0003

March_2019			
Sample Name	Substance	CAS#	Conc. (µg/l)
Ombla	Carbamazepine	298-46-4	0.0006
Ombla	Lamotrigine	84057-84-1	0.0014
Ombla	Sulfadiazine (Silvadene)	68-35-9	0.0005
Ombla	Sulfamethoxazole	723-46-6	0.0019
Ombla	Tramadol	27203-92-5	0.0001
Ombla	Chlorothiazide	58-94-6	0.0003
Ombla	Hydrochlorothiazide	58-93-5	0.0097
Ombla	Irbesartan	138402-11-6	0.0003
Ombla	Sucralose	56038-13-2	0.07
Prud	Carbamazepine	298-46-4	0.0021
Prud	Clarithromycin	81103-11-9	0.0001
Prud	Clothianidin	210880-92-5	0.0002
Prud	Griseofulvin	126-07-8	0.0001
Prud	Lamotrigine	84057-84-1	0.0018
Prud	Oxazepam	604-75-1	0.0001
Prud	Oxcarbazepine	28721-07-5	0.0003
Prud	Sulfadiazine (Silvadene)	68-35-9	0.0015
Prud	Sulfamethoxazole	723-46-6	0.0023
Prud	Tramadol	27203-92-5	0.0004
Prud	Acesulfame (Acesulfame-K)	33665-90-6	0.07
Prud	Chlorothiazide	58-94-6	0.0007
Prud	Hydrochlorothiazide	58-93-5	0.01
Prud	Perfluoro Pentanoic Acid	2706-90-3	0.0006
Prud	Perfluorobutane sulfonate	375-73-5	0.0042
Prud	Phenobarbital	50-06-6	0.0018
Prud	Sucralose	56038-13-2	0.17
Opačac	Carbamazepine	298-46-4	0.0005
Opačac	Lamotrigine	84057-84-1	0.0001
Čikola	Emamectin B1b	121424-52-0	0.082
Čikola	Bromoxynil	1689-84-5	0.0002
Miljacka	Carbamazepine	298-46-4	0.0003
Miljacka	Lamotrigine	84057-84-1	0.0002
Miljacka	Tramadol	27203-92-5	0.0005
Miljacka	Acesulfame (Acesulfame-K)	33665-90-6	0.016
Golubinika	Boscalid (Nicobifen)	188425-85-6	0.0004
Golubinika	Carbamazepine	298-46-4	0.0085
Golubinika	Chlorantraniliprole	500008-45-7	0.0014
Golubinika	Chloridazon-desphenyl-methyl	17254-80-7	0.0005
Golubinika	Clarithromycin	81103-11-9	0.0001

October_2019			
Sample Name	Substance	Cas#	Conc. (µg/l)
Zagorska Mrežnica	Hydrochlorothiazide	58-93-5	0.0009
Zagorska Mrežnica	Perfluorobutane sulfonate	375-73-5	0.0056
Zagorska Mrežnica	Sucralose	56038-13-2	0.025
Tonković	Atrazine	1912-24-9	0.0004
Tonković	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.001
Tonković	Carbamazepine	298-46-4	0.0007
Tonković	Lamotrigine	84057-84-1	0.0004
Tonković	Hydrochlorothiazide	58-93-5	0.0012
Tonković	Sucralose	56038-13-2	0.057
Ombla	10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine	35079-97-1	0.0019
Ombla	Carbamazepine	298-46-4	0.0005
Ombla	Lamotrigine	84057-84-1	0.0007
Ombla	Metformin	657-24-9	0.012
Ombla	Sulfamethoxazole	723-46-6	0.0037
Ombla	Hydrochlorothiazide	58-93-5	0.0038
Ombla	Perfluorobutane sulfonate	375-73-5	0.0028
Ombla	Sucralose	56038-13-2	0.035
Prud	10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine	35079-97-1	0.0029
Prud	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0012
Prud	Boscalid (Nicobifen)	188425-85-6	0.0002
Prud	Carbamazepine	298-46-4	0.0039
Prud	Lamotrigine	84057-84-1	0.0026
Prud	Metalaxyl	57837-19-1	0.0003
Prud	Oxazepam	604-75-1	0.0004
Prud	Sulfadiazine (Silvadene)	68-35-9	0.019
Prud	Sulfamethoxazole	723-46-6	0.0033
Prud	Sulfanilamide	63-74-1	0.0015
Prud	Tramadol	27203-92-5	0.0006
Prud	Chlorothiazide	58-94-6	0.0007
Prud	Clothianidin	210880-92-5	0.0006
Prud	Hydrochlorothiazide	58-93-5	0.011
Prud	Perfluorobutane sulfonate (PFBS)	375-73-5	0.0036
Prud	Sucralose	56038-13-2	0.18
Opačac	Acetaminophen (Paracetemol)	103-90-2	0.0015
Opačac	Carbamazepine	298-46-4	0.0003
Opačac	Lamotrigine	84057-84-1	0.0002
Opačac	Morphine	57-27-2	0.0001
Opačac	Tramadol	27203-92-5	0.0001
Opačac	Hydrochlorothiazide	58-93-5	0.0011

March_2019			
Sample Name	Substance	CAS#	Conc. (µg/l)
Golubinika	Clopidol	2971-90-6	0.0001
Golubinika	Clothianidin	210880-92-5	0.0004
Golubinika	Cotinine	486-56-6	0.0018
Golubinika	Lamotrigine	84057-84-1	0.002
Golubinika	Metribuzin-desamino	35045-02-4	0.0001
Golubinika	Sulfamethoxazole	723-46-6	0.0028
Golubinika	Thiamethoxam	153719-23-4	0.0001
Golubinika	Tramadol	27203-92-5	0.0024
Golubinika	Venlafaxine	93413-69-5	0.0002
Golubinika	Acesulfame (Acesulfame-K)	33665-90-6	0.15
Golubinika	Bentazone	25057-89-0	0.0054
Golubinika	Chlorothiazide	58-94-6	0.0004
Golubinika	Hydrochlorothiazide	58-93-5	0.0077
Golubinika	Perfluorobutane sulfonate (PFBS)	375-73-5	0.003
Golubinika	Saccharin	81-07-2	0.008
Golubinika	Sucralose	56038-13-2	0.19
Zvir	Carbamazepine	298-46-4	0.0008
Zvir	Chloridazon (PAC)	1698-60-8	0.0001
Zvir	Clopidol	2971-90-6	0.0001
Zvir	Lamotrigine	84057-84-1	0.001
Zvir	Tramadol	27203-92-5	0.0006
Zvir	Hydrochlorothiazide	58-93-5	0.0012
Zvir	Perfluorobutane sulfonate (PFBS)	375-73-5	0.0015
Zvir	Sucralose	56038-13-2	0.05
Sveti Ivan	Carbamazepine	298-46-4	0.0001
Sveti Ivan	Imidacloprid	138261-41-3	0.0005
Sveti Ivan	Lamotrigine	84057-84-1	0.0003
Sveti Ivan	Tramadol	27203-92-5	0.0003
Sveti Ivan	Chlorothiazide	58-94-6	0.0002
Sveti Ivan	Hydrochlorothiazide	58-93-5	0.003
Rakonek	Azoxystrobin	131860-33-8	0.0001
Rakonek	Boscalid (Nicobifen)	188425-85-6	0.0004
Rakonek	Carbamazepine	298-46-4	0.001
Rakonek	Chloridazon-desphenyl-methyl	17254-80-7	0.0009
Rakonek	Clarithromycin	81103-11-9	0.0001
Rakonek	Clopidol	2971-90-6	0.0047
Rakonek	Diuron	330-54-1	0.0001
Rakonek	Lamotrigine	84057-84-1	0.0012
Rakonek	Oxazepam	604-75-1	0.0003

October_2019			
Sample Name	Substance	Cas#	Conc. (µg/l)
Opačac	Sucralose	56038-13-2	0.018
Čikola	Melamine	108-78-1	0.02
Čikola	Perfluorobutane sulfonate (PFBS)	375-73-5	0.018
Miljacka	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.001
Miljacka	Azoxystrobin	131860-33-8	0.0005
Miljacka	Carbamazepine	298-46-4	0.0006
Miljacka	Lamotrigine	84057-84-1	0.0004
Miljacka	Sulfamethoxazole	723-46-6	0.0007
Miljacka	Tramadol	27203-92-5	0.0005
Miljacka	Venlafaxine	93413-69-5	0.0001
Miljacka	Sucralose	56038-13-2	0.044
Golubinka	10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine	35079-97-1	0.0054
Golubinka	Atrazine	1912-24-9	0.0003
Golubinka	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0007
Golubinka	Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	0.0001
Golubinka	Carbamazepine	298-46-4	0.012
Golubinka	Chloridazon-desphenyl-methyl	17254-80-7	0.0003
Golubinka	Lamotrigine	84057-84-1	0.0076
Golubinka	Oxcarbazepine	28721-07-5	0.0012
Golubinka	Telmisartan	144701-48-4	0.0002
Golubinka	Tramadol	27203-92-5	0.0032
Golubinka	Venlafaxine	93413-69-5	0.0002
Golubinka	Bentazone	25057-89-0	0.0056
Golubinka	Clothianidin	210880-92-5	0.001
Golubinka	Hydrochlorothiazide	58-93-5	0.0094
Golubinka	Perfluorobutane sulfonate (PFBS)	375-73-5	0.0055
Golubinka	Saccharin	81-07-2	0.0088
Golubinka	Sucralose	56038-13-2	0.44
Zvir	Carbamazepine	298-46-4	0.0015
Zvir	Diazepam	439-14-5	0.0001
Zvir	Lamotrigine	84057-84-1	0.0017
Zvir	Tramadol	27203-92-5	0.0008
Zvir	Clothianidin	210880-92-5	0.0001
Zvir	Perfluorobutane sulfonate (PFBS)	108427-53-8	0.0022
Zvir	Sucralose	56038-13-2	0.036
Sveti Ivan	Carbamazepine	298-46-4	0.0004
Sveti Ivan	Lamotrigine	84057-84-1	0.0003
Sveti Ivan	Sotalol	3930-20-9	0.0005
Sveti Ivan	Hydrochlorothiazide	58-93-5	0.0025

March_2019			
Sample Name	Substance	CAS#	Conc. (µg/l)
Rakonek	Terbutryn	886-50-0	0.0008
Rakonek	Tramadol	27203-92-5	0.0013
Rakonek	Venlafaxine	93413-69-5	0.0001
Rakonek	Acesulfame (Acesulfame-K)	33665-90-6	0.095
Rakonek	Bentazone	25057-89-0	0.005
Rakonek	Hydrochlorothiazide	58-93-5	0.0033
Rakonek	Perfluorobutane sulfonate (PFBS)	375-73-5	0.0065
Rakonek	Perfluorohexane sulfonate	108427-53-8	0.011
Rakonek	Perfluorooctane sulfonate (PFOS)	1763-23-1	0.0003
Rakonek	Sucralose	56038-13-2	0.09
Vransko lake	Azoxystrobin	131860-33-8	0.0002
Vransko lake	Boscalid (Nicobifen)	188425-85-6	0.0005
Vransko lake	Cotinine	486-56-6	0.012
Vransko lake	2,4-D / 2,4-Dichlorophenoxyacetic acid	94-75-7	0.0043
Vransko lake	Bisphenol S	80-08-1	0.0015
Vransko lake	Perfluoro Heptanoic Acid	375-85-9	0.0005
Vransko lake	Perfluoro Nonanoic Acid	375-95-1	0.0003
Vransko lake	Perfluoro Octanoic Acid (PFOA)	335-67-1	0.0006
Vransko lake	Perfluoro Pentanoic Acid	2706-90-3	0.0008
Novljanska Žrnovnica	Azoxystrobin	131860-33-8	0.0002
Novljanska Žrnovnica	Chloridazon-desphenyl	6339-19-1	0.0001
Koreničko vrelo	Propranolol	525-66-6	0.0002
Slunjčica	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0006
Slunjčica	Carbamazepine	298-46-4	0.0001

October_2019			
Sample Name	Substance	Cas#	Conc. (µg/l)
Sveti Ivan	Sucralose	56038-13-2	0.044
Rakonek	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0004
Rakonek	Azoxystrobin	131860-33-8	0.0004
Rakonek	Carbamazepine	298-46-4	0.0015
Rakonek	Chloridazon-desphenyl-methyl	17254-80-7	0.0009
Rakonek	Clopidol	2971-90-6	0.0023
Rakonek	Lamotrigine	84057-84-1	0.0012
Rakonek	Metalaxyl	57837-19-1	0.0008
Rakonek	Metolachlor	51218-45-2	0.0006
Rakonek	Sulfamethoxazole	723-46-6	0.0007
Rakonek	Tramadol	27203-92-5	0.0011
Rakonek	Bentazone	25057-89-0	0.0036
Rakonek	Clothianidin	210880-92-5	0.0003
Rakonek	Perfluorobutane sulfonate	375-73-5	0.0042
Rakonek	Perfluorohexane sulfonate	108427-53-8	0.0087
Rakonek	Perfluorooctane sulfonate (PFOS)	1763-23-1	0.0001
Rakonek	Sucralose	56038-13-2	0.059
Vransko lake	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0016
Vransko lake	Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	0.0004
Vransko lake	Azoxystrobin	131860-33-8	0.0002
Vransko lake	Boscalid (Nicobifen)	188425-85-6	0.0006
Vransko lake	Dimethomorph	110488-70-5	0.0009
Vransko lake	Simazine	122-34-9	0.0002
Vransko lake	Perfluoro Heptanoic Acid	375-85-9	0.0005
Koreničko vrelo	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0005
Slunjčica	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.0011
Bistrac	Atrazine	1912-24-9	0.0034
Bistrac	Atrazine-desethyl (Desethylatrazine)	6190-65-4	0.013
Bistrac	Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	0.0006
Bistrac	Carbamazepine	298-46-4	0.0004
Bistrac	Lamotrigine	84057-84-1	0.0018
Bistrac	Metribuzin-desamino	35045-02-4	0.0001
Bistrac	Simazine	122-34-9	0.0003
Bistrac	Sulfamethoxazole	723-46-6	0.0015
Bistrac	Terbuthylazine	5915-41-3	0.0005
Bistrac	Tramadol	27203-92-5	0.0014
Bistrac	Chlorothiazide	58-94-6	0.0003
Bistrac	Clothianidin	210880-92-5	0.0004
Bistrac	Hydrochlorothiazide	58-93-5	0.0025
Bistrac	Perfluorobutane sulfonate	375-73-5	0.068

March_2019			
Sample Name	Substance	CAS#	Conc. (µg/l)

October_2019			
Sample Name	Substance	Cas#	Conc. (µg/l)
Bistrac	Perfluorohexane sulfonate	108427-53-8	0.0028
Bistrac	Sucralose	56038-13-2	0.1

APPENDIX 2: Supplementry Materials for the Paper II

Selak, A., Lukač Reberski, J., Klobučar, G., 2023. Assessing the persistence, mobility and toxicity of emerging organic contaminants in Croatian karst springs used for drinking water supply. Sci Total Environ 903, 166240. https://doi.org/10.1016/j.scitotenv.2023.166240

Table S1: Detected EOCs list

SUBSTANCE	CAS No.	Use group	REACH regulation (No 1907/2006)	Candidate List of substances of very high concern (SVHC)	Active pesticide substances status under Reg. (EC) No 1107/2009
10,11-Dihydroxycarbamazepine	35079-97-1	pharmaceutical	NO	NO	not applicable
2,4-Dichlorophenoxyacetic acid	94-75-7	agricultural	NO	NO	approved
Acesulfame (Acesulfame-K)	33665-90-6	lifestyle product	NO	NO	not applicable
Acetaminophen (Paracetamol)	103-90-2	pharmaceutical	**YES**	NO	not applicable
Atrazine	1912-24-9	agricultural	**YES**	NO	**not approved**
Atrazine-desethyl (Desethylatrazine)	6190-65-4	agricultural	NO	NO	not included
Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	agricultural	NO	NO	not included
Azoxystrobin	131860-33-8	agricultural	NO	NO	approved
Bentazone	25057-89-0	agricultural	NO	NO	approved
Bisphenol S	80-09-1	industrial	**YES**	**YES**	not applicable
Boscalid (Nicobifen)	188425-85-6	agricultural	NO	NO	approved
Bromoxynil	1689-84-5	agricultural	NO	NO	**not approved**
Carbamazepine	298-46-4	pharmaceutical	NO	NO	not applicable
Chlorantraniliprole	500008-45-7	agricultural	NO	NO	approved
Chloridazon (PAC)	1698-60-8	agricultural	NO	NO	**not approved**
Chloridazon-desphenyl	6339-19-1	agricultural	NO	NO	not included
Chloridazon-desphenyl-methyl	17254-80-7	agricultural	NO	NO	not included
Chlorothiazide	58-94-6	pharmaceutical	NO	NO	not applicable
Clarithromycin	81103-11-9	pharmaceutical	NO	NO	not applicable
Clopidol	2971-90-6	pharmaceutical	NO	NO	not applicable
Clothianidin	210880-92-5	agricultural	**YES**	NO	**not approved**
Cotinine	486-56-6	lifestyle product	NO	NO	not applicable
Diazepam	439-14-5	pharmaceutical	NO	NO	not applicable
Dimethomorph	110488-70-5	agricultural	registration no longer valid	NO	approved
Diuron	330-54-1	agricultural	**YES**	NO	**not approved**
Emamectin B1b	121424-52-0	agricultural	NO	NO	approved
Griseofulvin	126-07-8	pharmaceutical	NO	NO	not applicable
Hydrochlorothiazide	58-93-5	pharmaceutical	NO	NO	not applicable
Imidacloprid	138261-41-3	agricultural	**YES**	NO	**not approved**
Irbesartan	138402-11-6	pharmaceutical	NO	NO	not applicable
Lamotrigine	84057-84-1	pharmaceutical	NO	NO	not applicable
Melamine	108-78-1	industrial	**YES**	**YES**	not applicable

SUBSTANCE	CAS No.	Use group	REACH regulation (No 1907/2006)	Candidate List of substances of very high concern (SVHC)	Active pesticide substances status under Reg. (EC) No 1107/2009
Metalaxyl	57837-19-1	agricultural	NO	NO	approved
Metformin	657-24-9	pharmaceutical	NO	NO	not applicable
Metolachlor	51218-45-2	agricultural	NO	NO	approved
Metribuzin-desamino	35045-02-4	agricultural	NO	NO	not included
Morphine	57-27-2	pharmaceutical	**YES**	NO	not applicable
Oxazepam	604-75-1	pharmaceutical	NO	NO	not applicable
Oxcarbazepine	28721-07-5	pharmaceutical	NO	NO	not applicable
Perfluoro Heptanoic Acid (PFHpA)	375-85-9	industrial	NO	**YES**	not applicable
Perfluoro Nonanoic Acid (PFNA)	375-95-1	industrial	NO	**YES**	not applicable
Perfluoro Octanoic Acid (PFOA)	335-67-1	industrial	NO	**YES**	not applicable
Perfluoro Pentanoic Acid (PFPeA)	2706-90-3	industrial	NO	NO	not applicable
Perfluorobutane sulfonate (PFBS)	375-73-5	industrial	**YES**	**YES**	not applicable
Perfluorohexane sulfonate (PFHxS)	108427-53-8	industrial	NO	**YES**	not applicable
Perfluorooctane sulfonate (PFOS)	1763-23-1	industrial	NO	NO	not applicable
Phenobarbital	50-06-6	pharmaceutical	**YES**	NO	not applicable
Prednisolone	50-24-8	pharmaceutical	**YES**	NO	not applicable
Propiconazole	60207-90-1	agricultural	**YES**	NO	**not approved**
Propranolol	525-66-6	pharmaceutical	NO	NO	not applicable
Saccharin	81-07-2	lifestyle product	**YES**	NO	not applicable
Simazine	122-34-9	agricultural	**YES**	NO	**not approved**
Sotalol	3930-20-9	pharmaceutical	NO	NO	not applicable
Sucralose	56038-13-2	lifestyle product	**YES**	NO	not applicable
Sulfadiazine (Silvadene)	68-35-9	pharmaceutical	**YES**	NO	not applicable
Sulfamethoxazole	723-46-6	pharmaceutical	NO	NO	not applicable
Sulfanilamide	63-74-1	pharmaceutical	**YES**	NO	not applicable
Telmisartan	144701-48-4	pharmaceutical	NO	NO	not applicable
Terbuthylazine	5915-41-3	agricultural	NO	NO	approved
Terbutryn	886-50-0	agricultural	NO	NO	**not approved**
Thiamethoxam	153719-23-4	agricultural	**YES**	NO	**not approved**
Tramadol	27203-92-5	pharmaceutical	**YES**	NO	not applicable
Triphenyl phosphate (TPPA)	115-86-6	industrial	**YES**	NO	not applicable
Valsartan	137862-53-4	pharmaceutical	**YES**	NO	not applicable
Venlafaxine	93413-69-5	pharmaceutical	**YES**	NO	not applicable

Table S2: PBT analysis results

Substance	**LogP**	**P**	**B**	**T**	**Score P**	**Score B**	**Score T**	**PBT**	**PB**
Perfluorooctane sulfonate (PFOS)	6.28	P/vP	3.73	-	0.785	0.812	0.5	0.727	0.798
Perfluorohexane sulfonate (PFHxS)	2.84	P/vP	3.53	-	0.785	0.724	0.5	0.694	0.754
Perfluoro Heptanoic Acid (PFHpA)	3.32	P/vP	3.42	-	0.785	0.681	0.5	0.678	0.731
Perfluoro Octanoic Acid (PFOA)	3.81	P/vP	3.12	-	0.785	0.56	0.5	0.626	0.663
Perfluoro Nonanoic Acid (PFNA)	7.27	P/vP	2.22	-	0.785	0.383	0.5	0.538	0.548
Boscalid (Nicobifen)	4.21	P/vP	2.32	0.046	0.712	0.396	0.553	0.535	0.531
Azoxystrobin	2.5	P/vP	1.49	0.00013	0.712	0.299	0.745	0.508	0.461
Propiconazole	3.72	P/vP	2.06	0.045	0.8	0.293	0.546	0.496	0.484
Perfluorobutane sulfonate	2.41	P/vP	1.38	-	0.785	0.288	0.5	0.48	0.476
Diazepam	2.82	P/vP	1.57	0.087	0.712	0.306	0.509	0.475	0.467
Oxazepam	2.24	P/vP	1.28	0.083	0.712	0.28	0.511	0.459	0.446
Thiamethoxam	1.32	vP	0.59	-	0.854	0.236	0.5	0.458	0.449
Imidacloprid	1.16	vP	1.03	0.585	0.854	0.261	0.4	0.457	0.472
Carbamazepine	2.45	P/vP	1.26	0.817	0.712	0.278	0.383	0.445	0.432
Griseofulvin	1.63	P/vP	0.47	0.01	0.712	0.23	0.644	0.444	0.405
Clothianidin	0.66	vP	0.41	10	0.854	0.228	0.291	0.441	0.406
Perfluoro Pentanoic Acid	3.4	P/vP	1.51	24	0.785	0.3	0.272	0.433	0.485
Oxcarbazepine	3.01	P/vP	1.02	0.337	0.712	0.261	0.429	0.431	0.431
Venlafaxine	3.15	nP/P	1.53	0.17	0.584	0.302	0.464	0.428	0.42
Hydrochlorothiazide	-0.07	P/vP	0.42	-	0.712	0.228	0.5	0.421	0.403
Chloridazon (PAC)	1.14	P/vP	0.57	1.7	0.8	0.235	0.35	0.415	0.434
Valsartan	2.74	P/vP	0.71	-	0.712	0.242	0.5	0.415	0.431
Chloridazon-desphenyl	-1.95	P/vP	0.13	-	0.712	0.218	0.5	0.413	0.394
Sotalol	0.24	P/vP	0.9	1.38	0.712	0.253	0.359	0.41	0.424
10,11-Dihydroxycarbamazepine	1.7	P/vP	0.81	1.25	0.712	0.248	0.363	0.408	0.42
Tramadol	2.57	nP/P	1.31	0.236	0.584	0.282	0.45	0.406	0.414
Telmisartan	8.76	nP/P	0.98	-	0.571	0.258	0.5	0.404	0.384
Dimethomorph	2.68	nP/P	1.76	-	0.595	0.246	0.5	0.403	0.382
Sulfamethoxazole	0.89	P/vP	0.38	4	0.712	0.227	0.29	0.402	0.376
Lamotrigine	2.4	nP	2.61	0.008	0.359	0.412	0.658	0.384	0.428
Triphenyl phosphate (TPPA)	4.59	nP	2.48	0.063	0.333	0.372	0.542	0.384	0.352
Metribuzin-desamino	1.95	nP/P	0.8	0.543	0.571	0.247	0.404	0.381	0.376
Metolachlor	3.02	P/vP	1.29	0.543	0.8	0.18	0.376	0.379	0.38
Irbesartan	4.8	nP	1.9	-	0.359	0.342	0.5	0.376	0.35
Chloridazon-desphenyl-methyl	-1.73	P/vP	0.35	14	0.712	0.225	0.283	0.374	0.401
Simazine	2.18	vP	1.11	0.425	0.854	0.15	0.392	0.364	0.358
Chlorantraniliprole	2.62	nP	1.47	-	0.359	0.296	0.5	0.355	0.326
Emamectin B1b	5.44	nP	1.43	-	0.359	0.292	0.5	0.353	0.324
Terbutryn	3.74	nP/P	1.19	-	0.571	0.168	0.5	0.341	0.31
Atrazine	2.61	vP	0.9	0.993	1	0.109	0.337	0.332	0.33
Terbuthylazine	3.21	P/vP	0.85	0.633	0.712	0.124	0.5	0.33	0.297
Morphine	0.89	nP	1.15	0.132	0.333	0.27	0.477	0.329	0.3
Clarithromycin	3.16	nP	0.66	-	0.359	0.24	0.5	0.326	0.293
Chlorothiazide	0.2	nP	0.39	0.095	0.359	0.227	0.503	0.32	0.285

Substance	LogP	P	B	T	Score P	Score B	Score T	PBT	PB
Bisphenol S	1.9	nP	0.65	0.652	0.359	0.239	0.394	0.311	0.293
Diuron	2.68	P/vP	1.15	0.935	0.712	0.136	0.312	0.311	0.311
Propranolol	3.48	nP	1.89	1.22	0.359	0.267	0.308	0.309	0.309
Bromoxynil	2.89	P/vP	0.83	0.765	0.712	0.123	0.326	0.302	0.296
Atrazine-desethyl	1.51	vP	0.4	0.51	0.854	0.103	0.368	0.296	0.31
Atrazine-desisopropyl	1.15	vP	0.28	0.88	0.854	0.096	0.33	0.295	0.286
Acesulfame (Acesulfame-K)	-0.68	nP	0.05	1.72	0.359	0.215	0.349	0.278	0.291
Sucralose	-1	nP	0.08	-	0.359	0.216	0.5	0.278	0.313
Prednisolone	1.62	nP	1.06	0.101	0.359	0.154	0.499	0.273	0.235
Melamine	-1.37	vP	0.58	3.85	0.854	0.082	0.291	0.269	0.264
Sulfanilamide	-0.62	nP/P	0.27	1.88	0.571	0.096	0.322	0.249	0.234
Acetaminophen (Paracetamol)	0.46	nP/P	0.39	54	0.571	0.102	0.133	0.242	0.214
Bentazone	2.34	nP	1.32	-	0.333	0.175	0.5	0.241	0.279
Metalaxyl	1.68	nP	0.85	0.195	0.333	0.124	0.454	0.239	0.203
Clopidol	0.79	nP	0.47	-	0.359	0.107	0.5	0.236	0.196
Cotinine	0.07	nP	1.06	2.13	0.359	0.155	0.341	0.235	0.253
Metformin	-0.6	-	0.14	425	0.5	0.089	0.24	0.211	0.217
Phenobarbital	1.47	nP	1	32	0.333	0.149	0.145	0.204	0.223
Sulfadiazine (Silvadene)	-0.09	nP	0.18	4.04	0.359	0.091	0.289	0.199	0.181
2,4-Dichlorophenoxyacetic acid	2.81	nP/P	-0.5	17	0.595	0.053	0.215	0.185	0.178
Saccharin	0.91	nP	0.18	1088	0.333	0.091	0.159	0.171	0.174

Abbrevations: *P* - persistence; *B* - bioaccumulation; *T* – toxicity; *vP* - very persistent; *P* – persistent; *nP* - not persistent.

Table S3: PMT/vPvM analysis results

SUBSTANCE	CAS No.	PMT/vPvM assessment	Persistence (P)	Mobility (M)	Toxicity (T)
10,11-Dihydroxycarbamazepine	35079-97-1	vPvM	vP	vM	potential T
2,4-Dichlorophenoxyacetic acid	94-75-7	PM	P	vM	potential T
Acesulfame (Acesulfame-K)	33665-90-6	Potential PMT/vPvM	potential P/vP	vM	potential T
Acetaminophen (Paracetamol)	103-90-2	Potential PMT/vPvM	potential P/vP	vM	T
Atrazine	1912-24-9	PMT & vPvM	potential P/vP++	vM	T
Atrazine-desethyl (Desethylatrazine)	6190-65-4	vPvM	potential P/vP++	vM	potential T
Atrazine-desisopropyl (Deisopropylatrazine)	1007-28-9	PMT & vPvM	potential P/vP++	vM	T
Azoxystrobin	131860-33-8	Potential PMT/vPvM	potential P/vP	M	T
Bentazone	25057-89-0	Potential PMT/vPvM	potential P/vP	vM	potential T
Bisphenol S	80-09-1	Potential PMT/vPvM	potential P/vP	vM	T
Boscalid (Nicobifen)	188425-85-6	PMT & vPvM	potential P/vP++	vM	T
Bromoxynil	1689-84-5	vPvM	potential P/vP++	vM	potential T
Carbamazepine	298-46-4	PMT	P	vM	T
Chlorantraniliprole	500008-45-7	Potential PMT/vPvM	potential P/vP	vM	potential T
Chloridazon (PAC)	1698-60-8	PM	P	vM	potential T
Chloridazon-desphenyl	6339-19-1	vPvM	potential P/vP++	vM	potential T

SUBSTANCE	CAS No.	PMT/vPvM assessment	Persistence (P)	Mobility (M)	Toxicity (T)
Chloridazon-desphenyl-methyl	17254-80-7	vPvM	potential P/vP++	vM	potential T
Chlorothiazide	58-94-6	Potential PMT/vPvM	potential P/vP	vM	potential T
Clarithromycin	81103-11-9	PMT & vPvM	potential P/vP++	vM	T
Clopidol	2971-90-6	Potential PMT/vPvM	potential P/vP	vM	potential T
Clothianidin	210880-92-5	vPvM	potential P/vP++	vM	potential T
Cotinine	486-56-6	Potential PMT/vPvM	potential P/vP	vM	potential T
Diazepam	439-14-5	PMT & vPvM	potential P/vP++	vM	T
Dimethomorph	110488-70-5	Potential PMT/vPvM	potential P/vP	vM	T
Diuron	330-54-1	PMT & vPvM	potential P/vP++	vM	T
Emamectin B1b	121424-52-0	vPvM	potential P/vP++	vM	potential T
Griseofulvin	126-07-8	Potential PMT/vPvM	potential P/vP	vM	T
Hydrochlorothiazide	58-93-5	Potential PMT/vPvM	potential P/vP	vM	T
Imidacloprid	138261-41-3	Potential PMT/vPvM	potential P/vP	vM	Potential T
Irbesartan	138402-11-6	Potential PMT/vPvM	potential P/vP	M	T
Lamotrigine	84057-84-1	PMT & vPvM	potential P/vP++	vM	T
Melamine	108-78-1	PMT & vPvM	vP	vM	T
Metalaxyl	57837-19-1	vPvM	potential P/vP++	vM	potential T
Metformin	657-24-9	Potential PMT/vPvM	potential P/vP	vM	potential T
Metolachlor	51218-45-2	PMT & vPvM	vP	vM	T
Metribuzin-desamino	35045-02-4	vPvM	vP	vM	potential T
Morphine	57-27-2	Potential PMT/vPvM	potential P/vP	vM	T
Oxazepam	604-75-1	PMT & vPvM	vP	vM	T
Oxcarbazepine	28721-07-5	Potential PMT/vPvM	potential P/vP	vM	potential T
Perfluoro Heptanoic Acid (PFHpA)	375-85-9	vPvM	vP	vM	potential T
Perfluoro Nonanoic Acid (PFNA)	375-95-1	PMT & vPvM	vP	vM	T
Perfluoro Octanoic Acid (PFOA)	335-67-1	PMT & vPvM	vP	vM	T
Perfluoro Pentanoic Acid (PFPeA)	2706-90-3	vPvM	potential P/vP++	vM	potential T
Perfluorobutane sulfonate (PFBS)	375-73-5	vPvM	vP	vM	potential T
Perfluorohexane sulfonate (PFHxS)	108427-53-8	vPvM	vP	vM	potential T
Perfluorooctane sulfonate (PFOS)	1763-23-1	PMT & vPvM	vP	vM	T
Phenobarbital	50-06-6	PMT & vPvM	potential P/vP++	vM	T
Prednisolone	50-24-8	Potential PMT/vPvM	potential P/vP	vM	T
Propiconazole	60207-90-1	PMT	vP	M	T
Propranolol	525-66-6	Potential PMT/vPvM	potential P/vP	vM	potential T
Saccharin	81-07-2	PMT & vPvM	potential P/vP++	vM	T
Simazine	122-34-9	PMT & vPvM	potential P/vP++	vM	T
Sotalol	3930-20-9	Potential PMT/vPvM	potential P/vP	vM	potential T
Sucralose	56038-13-2	Potential PMT/vPvM	potential P/vP	vM	potential T
Sulfadiazine (Silvadene)	68-35-9	PMT & vPvM	potential P/vP++	vM	T
Sulfamethoxazole	723-46-6	PMT & vPvM	potential P/vP++	vM	T
Sulfanilamide	63-74-1	Potential PMT/vPvM	potential P/vP	vM	potential T
Telmisartan	144701-48-4	Not PMT/vPvM	potential P/vP	not M	T

SUBSTANCE	CAS No.	PMT/vPvM assessment	Persistence (P)	Mobility (M)	Toxicity (T)
Terbuthylazine	5915-41-3	PMT & vPvM	potential P/vP++	vM	T
Terbutryn	886-50-0	Potential PMT/vPvM	potential P/vP	vM	T
Thiamethoxam	153719-23-4	vPvM	potential P/vP++	vM	Potential T
Tramadol	27203-92-5	PM	P	vM	Potential T
Triphenyl phosphate (TPPA)	115-86-6	Not PMT/vPvM	not P	M	T
Valsartan	137862-53-4	PMT & vPvM	potential P/vP++	vM	T
Venlafaxine	93413-69-5	PMT & vPvM	potential P/vP++	vM	T

Abbrevations: *vP* - very persistent; *P* – persistent; *potential P/vP* - potential persistent or potential very persistent; *not P* - not persistent; *vM* - very mobile; *M* – mobile; *not M* - not mobile; *T* – toxic; *potential T* - potential toxic; *PM* - persistent and mobile; *PMT* - persistent, mobile and toxic; *vPvM* - very persistent and very mobile.

Table S4: Human exposure analysis results

Locations	EOC	MAX conc. (µg/l)	Toxtree	TDI or ADI or RfD (µg/kg bw)	GLV (µg/l)	TTC for non-threshold chemical (µg/l)	pGLV (µg/l)	BQ	Daily intake* (µg/kg bw/day)	Inernational Agency for Research on Cancer (IARC) List of classification	Gentoxicity *In vivo* Micronucleus activity (IRFMN) 1.0.1	Genotoxicity QSAR ISS profiler
Golubinka spring	10,11-Dihydroxycarbazepine	0.0054	Cramer Class III	not used in analysis	-	0.01	-	0.54	1.80E-05	not included/classified	non-genotoxic	no alert found
Opačac spring	Acetaminophen (Paracetemol)	0.0015	Cramer Class I	not used in analysis	-	0.01	-	0.15	5.00E-06	3 - Not classifiable as to its carcinogenicity to humans	non-genotoxic	structural alert for genotoxic carcinogenicity
Miljacka spring	Azoxystrobin	0.0005	Cramer Class III	not used in analysis	-	0.01	-	0.05	1.67E-06	not included/classified	non-genotoxic	no alert found
Golubinka spring	Chlorantraniliprole	0.0014	Cramer Class III	not used in analysis	-	0.01	-	0.14	4.67E-06	not included/classified	non-genotoxic	structural alert for non-genotoxic carcinogenicity
Zvir spring	Chloridazon (PAC)	0.0001	Cramer Class III	not used in analysis	-	0.01	-	0.01	3.33E-07	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Novljanska spring	Chloridazon-desphenyl	0.0001	Cramer Class III	not used in analysis	-	0.01	-	0.01	3.33E-07	not included/classified	not possible to predict	structural alert for genotoxic carcinogenicity
Rakonek spring	Chloridazon-desphenyl-methyl	0.0009	Cramer Class III	not used in analysis	-	0.01	-	0.09	3.00E-06	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Prud spring	Chlorothiazide	0.0007	Cramer Class III	not used in analysis	-	0.01	-	0.07	2.33E-06	not included/classified	not possible to predict	structural alert for non-genotoxic carcinogenicity
Rakonek spring	Clopidol	0.0047	Cramer Class III	not used in analysis	-	0.01	-	0.47	1.57E-05	not included/classified	non-genotoxic	structural alert for non-genotoxic carcinogenicity
Zvir spring	Diazepam	0.0001	Cramer Class III	not used in analysis	-	0.01	-	0.01	3.33E-07	3 - Not classifiable as to its carcinogenicity to humans	genotoxic	no alert found
Rakonek spring	Diuron	0.0001	Cramer Class III	not used in analysis	-	0.01	-	0.01	3.33E-07	not included/classified	non-genotoxic	no alert found
Prud spring	Griseofulvin	0.0001	Cramer Class III	not used in analysis	-	0.01	-	0.01	3.33E-07	2B - Possibly carcinogenic to humans	non-genotoxic	structural alert for genotoxic carcinogenicity
Prud spring	Hydrochlorothiazide	0.011	Cramer Class III	not used in analysis	-	0.01	-	1.10	3.67E-05	2B - Possibly carcinogenic to humans	non-genotoxic	structural alert for non-genotoxic carcinogenicity

Locations	EOC	MAX conc. (µg/l)	Toxtree	TDI or ADI or RfD (µg/kg bw)	GLV (µg/l)	TTC for non-threshold chemical (µg/l)	pGLV (µg/l)	BQ	Daily intake* (µg/kg bw/day)	Inernational Agency for Research on Cancer (IARC) List of classification	Gentoxicity *In vivo* Micronucleus activity (IRFMN) 1.0.1	Genotoxicity QSAR ISS profiler
Sv. Ivan spring	Imidacloprid	0.0005	Cramer Class III	not used in analysis	-	0.01	-	0.05	1.67E-06	not included/classified	non-genotoxic	no alert found
Rakonek spring	Metalaxyl	0.0008	Cramer Class I	not used in analysis	-	0.01	-	0.08	2.67E-06	not included/classified	non-genotoxic	no alert found
Opačac spring	Morphine	0.0001	Cramer Class III	not used in analysis	-	0.01	-	0.01	3.33E-07	not included/classified	genotoxic	no alert found
Prud spring	Oxazepam	0.0004	Cramer Class III	not used in analysis	-	0.01	-	0.04	1.33E-06	2B - Possibly carcinogenic to humans	non-genotoxic	no alert found
Golubinka spring	Oxcarbazepine	0.0012	Cramer Class III	not used in analysis	-	0.01	-	0.12	4.00E-06	not included/classified	non-genotoxic	no alert found
Vransko Lake	Perfluoro Nonanoic Acid (PFNA)	0.0003	Cramer Class III	not used in analysis	-	0.01	-	0.03	1.00E-06	not included/classified	non-genotoxic	structural alert for non-genotoxic carcinogenicity
Kupica spring	Prednisolone	0.0017	Cramer Class III	not used in analysis	-	0.01	-	0.17	5.67E-06	not included/classified	genotoxic	structural alert for genotoxic carcinogenicity
Vrelo Koreničko spring	Propranolol	0.0002	Cramer Class III	not used in analysis	-	0.01	-	0.02	6.67E-07	not included/classified	genotoxic	no alert found
Sv. Ivan spring	Sotalol	0.0005	Cramer Class III	not used in analysis	-	0.01	-	0.05	1.67E-06	not included/classified	non-genotoxic	no alert found
Prud spring	Sulfadiazine (Silvadene)	0.019	Cramer Class III	not used in analysis	-	0.01	-	1.90	6.33E-05	not included/classified	genotoxic	structural alert for both genotoxic and nongenotoxic carcinogenicity
Ombla spring	Sulfamethoxazole	0.0037	Cramer Class III	not used in analysis	-	0.01	-	0.37	1.23E-05	3 - Not classifiable as to its carcinogenicity to humans	non-genotoxic	structural alert for both genotoxic and non-genotoxic carcinogenicity

Locations	EOC	MAX conc. (µg/l)	Toxtree	TDI or ADI or RfD (µg/kg bw)	GLV (µg/l)	TTC for non-threshold chemical (µg/l)	pGLV (µg/l)	BQ	Daily intake* (µg/kg bw/day)	Inernational Agency for Research on Cancer (IARC) List of classification	Gentoxicity *In vivo* Micronucleus activity (IRFMN) 1.0.1	Genotoxicity QSAR ISS profiler
Prud spring	Sulfanilamide	0.0015	Cramer Class III	not used in analysis	-	0.01	-	0.15	5.00E-06	not included/classified	non-genotoxic	structural alert for both genotoxic and nongenotoxic carcinogenicity
Golubinka spring	Telmisartan	0.0002	Cramer Class III	not used in analysis	-	0.01	-	0.02	6.67E-07	not included/classified	genotoxic	structural alert for non-genotoxic carcinogenicity
Rakonek spring	Terbutryn	0.0008	Cramer Class III	not used in analysis	-	0.01	-	0.08	2.67E-06	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Golubinka spring	Tramadol	0.0032	Cramer Class III	not used in analysis	-	0.01	-	0.32	1.07E-05	not included/classified	non-genotoxic	no alert found
Golubinka spring	Venlafaxine	0.0002	Cramer Class I	not used in analysis	-	0.01	-	0.02	6.67E-07	not included/classified	non-genotoxic	no alert found
Vransko Lake	Boscalid (Nicobifen)	0.0006	Cramer Class III	not used in analysis	-	0.01		0.06	2.00E-06	not included/classified	non-genotoxic	no alert found
Vransko Lake	Perfluoro Octanoic Acid (PFOA)	0.0006	Cramer Class III	0.0006 (EFSA, 2020)	-	-	0.002	0.33	2.00E-06	2B - Possibly carcinogenic to humans	genotoxic	structural alert for non-genotoxic carcinogenicity
Golubinka spring	Saccharin	0.0088	Cramer Class III	5000 (Joint FAO/WHO, 1993)	-	-	15000	5.87 E-07	2.93E-05	3 - Not classifiable as to its carcinogenicity to humans	non-genotoxic	no alert found
Golubinka spring	Acesulfame (Acesulfame-K)	0.15	Cramer Class III	9000 (SCF, 2000a)	-	-	27000	5.56 E-06	5.00E-04	not included/classified	genotoxic	structural alert for genotoxic carcinogenicity
Golubinka spring	Sucralose	0.44	Cramer Class III	15000 (SCF, 2000b)	-	-	45000	9.78 E-06	1.47E-03	not included/classified	not possible to predict	structural alert for genotoxic carcinogenicity
Golubinka spring	Carbamazepine	0.012	Cramer Class III	not used in analysis	0.3 (Dieter , 2014)	-	-	0.04	4.00E-05	not included/classified	non-genotoxic	no alert found
Golubinka spring	Lamotrigine	0.0076	Cramer Class III	not used in analysis	0.3 (UBA, 2019)	-	-	0.03	2.53E-05	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Rakonek spring	Perfluorooctane sulfonate (PFOS)	0.0003	Cramer Class III	0.0006 (EFSA, 2020)	-	-	0.002	0.17	1.00E-06	not included/classified	non-genotoxic	no alert found
Kupica spring	Clarithromycin	0.0002	Cramer Class III	0.2 (Citron and Appleman, 2001)	-	-	0.60	3.33 E-04	6.67E-07	not included/classified	non-genotoxic	no alert found
Čikola spring	Emamectin B1b	0.082	Cramer Class III	0.5 (EFSA, 2012)	-	-	1.50	0.05	2.73E-04	not included/classified	non-genotoxic	no alert found
Vransko Lake	Cotinine	0.012	Cramer Class III	0.8 (EFSA, 2022)	-	-	2.40	0.01	4.00E-05	not included/classified	genotoxic	no alert found

Locations	EOC	MAX conc. (µg/l)	Toxtree	TDI or ADI or RfD (µg/kg bw)	GLV (µg/l)	TTC for non-threshold chemical (µg/l)	pGLV (µg/l)	BQ	Daily intake* (µg/kg bw/day)	Inernational Agency for Research on Cancer (IARC) List of classification	Gentoxicity *In vivo* Micronucleus activity (IRFMN) 1.0.1	Genotoxicity QSAR ISS profiler
Ombla spring	Irbesartan	0.0003	Cramer Class III	2.143 (RXList, 2023)	-	-	6.43	4.67 E-05	1.00E-06	not included/classified	non-genotoxic	no alert found
Čikola spring	Bromoxynil	0.0002	Cramer Class III	3 (EFSA, 2017)	-	-	9	2.22 E-05	6.67E-07	not included/classified	non-genotoxic	no alert found
Vransko Lake	Bisphenol S	0.0015	Cramer Class III	7 (EFSA, 2020)	-	-	21	7.14 E-05	5.00E-06	not included/classified	genotoxic	no alert found
Golubinka spring	Thiamethoxam	0.0001	Cramer Class III	26 (EU Pesticide database, 2023)	-	-	78	1.28 E-06	3.33E-07	not included/classified	genotoxic	no alert found
Vransko Lake	Dimethomorph	0.0009	Cramer Class III	50 (EFSA, 2006)	-	-	150	6.00 E-06	3.00E-06	not included/classified	non-genotoxic	no alert found
Kupica spring	Propiconazole	0.0017	Cramer Class III	70 (APVMA, 2022)	-	-	210	8.10 E-06	5.67E-06	not included/classified	non-genotoxic	no alert found
Bistrac spring Golubinka spring Kupica spring	Metribuzin-desamino	0.0001	Cramer Class III	73.2 (Dodor, 2008)	-	-	219.60	4.55 E-07	3.33E-07	not included/classified	non-genotoxic	no alert found
Golubinka spring	Bentazone	0.0056	Cramer Class III	90 (EFSA, 2019)	-	-	270	2.07 E-05	1.87E-05	not included/classified	non-genotoxic	no alert found
Golubinka spring	Clothianidin	0.001	Cramer Class III	97 (EU 528/2012)	-	-	291	3.44 E-06	3.33E-06	not included/classified	genotoxic	no alert found
Čikola spring Zagorska Mrežnica spring	Melamine	0.02	Cramer Class III	200 (EFSA, 2010)	-	-	600	3.33 E-05	6.67E-05	not included/classified	non-genotoxic	no alert found
Kupica spring	Triphenyl phosphate (TPPA)	0.09	Cramer Class III	70000 (Sutton et al., 1960)	-	-	210000	4.29 E-07	3.00E-04	not included/classified	not possible to predict	no alert found
Vransko Lake	2,4-D / 2,4-Dichlorophenoxyacetic acid	0.0043	Cramer Class III	not used in analysis	30 (WHO, 2022)	-	-	1.43 E-04	1.43E-05	2B - Possibly carcinogenic to humans	non-genotoxic	no alert found
Bistrac spring	Atrazine	0.0034	Cramer Class III	not used in analysis	100 (WHO, 2022)	-	-	3.40 E-05	1.13E-05	3 - Not classifiable as to its carcinogenicity to humans	genotoxic	structural alert for genotoxic carcinogenicity
Bistrac spring	Atrazine-desethyl (Desethylatrazine)	0.013	Cramer Class III	not used in analysis	100 (WHO, 2022)	-	-	1.30 E-04	4.33E-05	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity

Locations	EOC	MAX conc. (µg/l)	Toxtree	TDI or ADI or RfD (µg/kg bw)	GLV (µg/l)	TTC for non-threshold chemical (µg/l)	pGLV (µg/l)	BQ	Daily intake* (µg/kg bw/day)	Inernational Agency for Research on Cancer (IARC) List of classification	Gentoxicity *In vivo* Micronucleus activity (IRFMN) 1.0.1	Genotoxicity QSAR ISS profiler
Bistrac spring	Atrazine-desisopropyl (Deisopropylatrazine)	0.0006	Cramer Class III	not used in analysis	100 (WHO, 2022)	-	-	6.00E-06	2.00E-06	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Rakonek spring	Metolachlor	0.0006	Cramer Class III	not used in analysis	10 (WHO, 2022)	-	-	6.00E-05	2.00E-06	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Vransko Lake	Perfluoro Heptanoic Acid (PFHpA)	0.0005	Cramer Class III	not used in analysis	0.3 (UBA, 2019)	-	-	1.67E-03	1.67E-06	not included/classified	non-genotoxic	structural alert for non-genotoxic carcinogenicity
Vransko Lake Kupica spring	Perfluoro Pentanoic Acid (PFPeA)	0.0008	Cramer Class III	not used in analysis	3 (UBA, 2019)	-	-	2.67E-04	2.67E-06	not included/classified	non-genotoxic	no alert found
Bistrac spring	Perfluorobutane sulfonate (PFBS)	0.068	Cramer Class III	not used in analysis	3 (Dieter, 2011)	-	-	0.02	2.27E-04	not included/classified	non-genotoxic	no alert found
Rakonek spring	Perfluorohexane sulfonate (PFHxS)	0.011	Cramer Class III	not used in analysis	0.3 (Dieter, 2011)	-	-	0.04	3.67E-05	not included/classified	non-genotoxic	no alert found
Bistrac spring	Simazine	0.0003	Cramer Class III	not used in analysis	2 (WHO, 2022)	-	-	1.50E-04	1.00E-06	3 - Not classifiable as to its carcinogenicity to humans	non-genotoxic	structural alert for genotoxic carcinogenicity
Bistrac spring	Terbuthylazine	0.0005	Cramer Class III	not used in analysis	7 (WHO, 2022)	-	-	7.14E-05	1.67E-06	not included/classified	non-genotoxic	structural alert for genotoxic carcinogenicity
Kupica spring	Valsartan	0.009	Cramer Class III	not used in analysis	0.3 (UBA, 2019)	-	-	0.03	3.00E-05	not included/classified	non-genotoxic	no alert found
Ombla spring Kupica spring	Metformin	0.012	Cramer Class III	not used in analysis	1 (UBA, 2019)	-	-	0.01	4.00E-05	not included/classified	not possible to predict	no alert found
Prud spring	Phenobarbital	0.0018	Cramer Class III	not used in analysis	0.3 (Dieter, 2014)		-	0.01	6.00E-06	2B - Possibly carcinogenic to humans	genotoxic	no alert found

* 2L for person of 60 kg and 10% drinking water allocation.

Mentioned references can be found in Paper II. Abbrevations: TDI – tolerable daily intake; ADI - acceptable daily intake; RfD – reference dose; *GLV* - drinking water guideline value; *TTC* – threshold of toxicological concern; *pGLV* – provisional drinking water guideline value; *BQ* – benchmark quotient.

Table S4: Human exposure analysis results – EOCs intake

Sampling location	Substance	Daily intake* DWI_{bw} 3.2019 (µg/kg bw/day)	Daily intake* DWI_{bw} 10.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 3.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 10.2019 (µg/kg bw/day)
Bistrac spring	Atrazine		1.13E-05		6.57E-04
	Atrazine-desethyl (Desethylatrazine)		4.33E-05		
	Atrazine-desisopropyl (Deisopropylatrazine)		2.00E-06		
	Carbamazepine		1.33E-06		
	Chlorothiazide		1.00E-06		
	Clothianidin		1.33E-06		
	Hydrochlorothiazide		8.33E-06		
	Lamotrigine		6.00E-06		
	Metribuzin-desamino		3.33E-07		
	Perfluorobutane sulfonate		2.27E-04		
	Perfluorohexane sulfonate		9.33E-06		
	Simazine		1.00E-06		
	Sucralose		3.33E-04		
	Sulfamethoxazole		5.00E-06		
	Terbuthylazine		1.67E-06		
	Tramadol		4.67E-06		
Čikola spring	Bromoxynil	6.67E-07		2.74E-04	1.27E-04
	Emamectin B1b	2.73E-04			
	Melamine		6.67E-05		
	Perfluorobutane sulfonate		6.00E-05		
Golubinka spring	10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine		1.80E-05	1.28E-03	1.67E-03
	Acesulfame (Acesulfame-K)	5.00E-04			
	Atrazine		1.00E-06		
	Atrazine-desethyl (Desethylatrazine)		2.33E-06		
	Atrazine-desisopropyl (Deisopropylatrazine)		3.33E-07		
	Bentazone	1.80E-05	1.87E-05		
	Boscalid (Nicobifen)	1.33E-06			
	Carbamazepine	2.83E-05	4.00E-05		
	Chlorantraniliprole	4.67E-06			
	Chloridazon-desphenyl-methyl	1.67E-06	1.00E-06		
	Chlorothiazide	1.33E-06			
	Clarithromycin	3.33E-07			
	Clopidol	3.33E-07			
	Clothianidin	1.33E-06	3.33E-06		
	Cotinine	6.00E-06			
	Hydrochlorothiazide	2.57E-05	3.13E-05		
	Lamotrigine	6.67E-06	2.53E-05		
	Metribuzin-desamino	3.33E-07			
	Oxcarbazepine		4.00E-06		
	Perfluorobutane sulfonate	1.00E-05	1.83E-05		
	Saccharin	2.67E-05	2.93E-05		
	Sucralose	6.33E-04	1.47E-03		
	Sulfamethoxazole	9.33E-06			
	Telmisartan		6.67E-07		
	Thiamethoxam	3.33E-07			
	Tramadol	8.00E-06	1.07E-05		
	Venlafaxine	6.67E-07	6.67E-07		
Kupica spring	Atrazine	1.00E-06	1.33E-06	3.58E-04	2.88E-04
	Atrazine-desethyl (Desethylatrazine)	3.67E-06	4.67E-06		
	Atrazine-desisopropyl (Deisopropylatrazine)	3.33E-07	3.33E-07		
	Carbamazepine	1.33E-06	4.33E-06		
	Chloridazon-desphenyl-methyl	3.33E-07			

Sampling location	Substance	Daily intake* DWI_{bw} 3.2019 (µg/kg bw/day)	Daily intake* DWI_{bw} 10.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 3.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 10.2019 (µg/kg bw/day)
	Clarithromycin	6.67E-07			
	Cotinine	2.00E-06			
	Hydrochlorothiazide	3.33E-06			
	Lamotrigine	2.33E-06	4.67E-06		
	Metformin		4.00E-05		
	Metribuzin-desamino		3.33E-07		
	Perfluoro Pentanoic Acid	2.67E-06			
	Perfluorobutane sulfonate		8.00E-06		
	Perfluorohexane sulfonate		2.57E-05		
	Perfluorooctane sulfonate (PFOS)	6.67E-07	6.67E-07		
	Prednisolone		5.67E-06		
	Propiconazole	5.67E-06			
	Sucralose		1.87E-04		
	Sulfamethoxazole	2.33E-06	2.67E-06		
	Telmisartan		3.33E-07		
	Tramadol	1.67E-06	3.00E-06		
	Triphenyl phosphate (TPPA)	3.00E-04			
	Valsartan	3.00E-05			
Miljacka spring	Acesulfame (Acesulfame-K)	5.33E-05		5.67E-05	1.59E-04
	Atrazine-desethyl (Desethylatrazine)		3.33E-06		
	Azoxystrobin		1.67E-06		
	Carbamazepine	1.00E-06	2.00E-06		
	Lamotrigine	6.67E-07	1.33E-06		
	Sucralose		1.47E-04		
	Sulfamethoxazole		2.33E-06		
	Tramadol	1.67E-06	1.67E-06		
	Venlafaxine		3.33E-07		
Novljanska spring	Azoxystrobin	6.67E-07		1.00E-06	
	Chloridazon-desphenyl	3.33E-07			
Ombla spring	10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine		6.33E-06	2.83E-04	2.01E-04
	Carbamazepine	2.00E-06	1.67E-06		
	Chlorothiazide	1.00E-06			
	Hydrochlorothiazide	3.23E-05	1.27E-05		
	Irbesartan	1.00E-06			
	Lamotrigine	4.67E-06	2.33E-06		
	Metformin		4.00E-05		
	Perfluorobutane sulfonate		9.33E-06		
	Sucralose	2.33E-04	1.17E-04		
	Sulfadiazine (Silvadene)	1.67E-06			
	Sulfamethoxazole	6.33E-06	1.23E-05		
	Tramadol	3.33E-07			
Opačac spring	Acetaminophen (Paracetemol)		5.00E-06	2.00E-06	7.10E-05
	Carbamazepine	1.67E-06	1.00E-06		
	Hydrochlorothiazide		3.67E-06		
	Lamotrigine	3.33E-07	6.67E-07		
	Morphine		3.33E-07		
	Sucralose		6.00E-05		
	Tramadol		3.33E-07		
Prud spring	10,11-Dihydroxycarbazepine or 10,11-Dihydroxycarbamazepine		9.67E-06	8.87E-04	7.73E-04
	Acesulfame (Acesulfame-K)	2.33E-04			
	Atrazine-desethyl (Desethylatrazine)		4.00E-06		
	Boscalid (Nicobifen)		6.67E-07		
	Carbamazepine	7.00E-06	1.30E-05		
	Chlorothiazide	2.33E-06	2.33E-06		
	Clarithromycin	3.33E-07			
	Clothianidin	6.67E-07	2.00E-06		
	Griseofulvin	3.33E-07			

Sampling location	Substance	Daily intake* DWI$_{bw}$ 3.2019 (µg/kg bw/day)	Daily intake* DWI$_{bw}$ 10.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 3.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 10.2019 (µg/kg bw/day)
	Hydrochlorothiazide	3.33E-05	3.67E-05		
	Lamotrigine	6.00E-06	8.67E-06		
	Metalaxyl		1.00E-06		
	Oxazepam	3.33E-07	1.33E-06		
	Oxcarbazepine	1.00E-06			
	Perfluoro Pentanoic Acid	2.00E-06			
	Perfluorobutane sulfonate	1.40E-05	1.20E-05		
	Phenobarbital	6.00E-06			
	Sucralose	5.67E-04	6.00E-04		
	Sulfadiazine (Silvadene)	5.00E-06	6.33E-05		
	Sulfamethoxazole	7.67E-06	1.10E-05		
	Sulfanilamide		5.00E-06		
	Tramadol	1.33E-06	2.00E-06		
Rakonek spring	Acesulfame (Acesulfame-K)	3.17E-04		7.40E-04	2.81E-04
	Azoxystrobin	3.33E-07	1.33E-06		
	Bentazone	1.67E-05	1.20E-05		
	Boscalid (Nicobifen)	1.33E-06			
	Carbamazepine	3.33E-06	5.00E-06		
	Chloridazon-desphenyl-methyl	3.00E-06	3.00E-06		
	Clarithromycin	3.33E-07			
	Clopidol	1.57E-05	7.67E-06		
	Diuron	3.33E-07			
	Hydrochlorothiazide	1.10E-05			
	Lamotrigine	4.00E-06	4.00E-06		
	Metalaxyl		2.67E-06		
	Metolachlor		2.00E-06		
	Oxazepam	1.00E-06			
	Perfluorobutane sulfonate	2.17E-05	1.40E-05		
	Perfluorohexane sulfonate	3.67E-05	2.90E-05		
	Perfluorooctane sulfonate (PFOS)	1.00E-06	3.33E-07		
	Sucralose	3.00E-04	1.97E-04		
	Terbutryn	2.67E-06			
	Tramadol	4.33E-06	3.67E-06		
	Venlafaxine	3.33E-07			
Slunjčica spring	Atrazine-desethyl (Desethylatrazine)	2.00E-06	3.67E-06	2.33E-06	3.67E-06
	Carbamazepine	3.33E-07			
Sv. Ivan spring	Carbamazepine	3.33E-07	1.33E-06	1.47E-05	1.59E-04
	Chlorothiazide	6.67E-07			
	Hydrochlorothiazide	1.00E-05	8.33E-06		
	Imidacloprid	1.67E-06			
	Lamotrigine	1.00E-06	1.00E-06		
	Sotalol		1.67E-06		
	Sucralose		1.47E-04		
	Tramadol	1.00E-06			
Tonković spring	Atrazine		1.33E-06	1.05E-04	2.02E-04
	Atrazine-desethyl (Desethylatrazine)		3.33E-06		
	Carbamazepine	2.33E-06	2.33E-06		
	Hydrochlorothiazide		4.00E-06		
	Lamotrigine		1.33E-06		
	Perfluoro Pentanoic Acid	2.33E-06			
	Sucralose		1.90E-04		
	Triphenyl phosphate (TPPA)	1.00E-04			
Vransko Lake	2,4-D / 2,4-Dichlorophenoxyacetic acid	1.43E-05		6.90E-05	1.47E-05
	Atrazine-desethyl (Desethylatrazine)		5.33E-06		
	Atrazine-desisopropyl (Deisopropylatrazine)		1.33E-06		
	Azoxystrobin	6.67E-07	6.67E-07		
	Bisphenol S	5.00E-06			
	Boscalid (Nicobifen)	1.67E-06	2.00E-06		

Sampling location	Substance	Daily intake* DWI_{bw} 3.2019 (µg/kg bw/day)	Daily intake* DWI_{bw} 10.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 3.2019 (µg/kg bw/day)	Exposure to EOCs sum per location* 10.2019 (µg/kg bw/day)
	Cotinine	4.00E-05			
	Dimethomorph		3.00E-06		
	Perfluoro Heptanoic Acid	1.67E-06	1.67E-06		
	Perfluoro Nonanoic Acid	1.00E-06			
	Perfluoro Octanoic Acid (PFOA)	2.00E-06			
	Perfluoro Pentanoic Acid	2.67E-06			
	Simazine		6.67E-07		
Vrelo Koreničko spring	Atrazine-desethyl (Desethylatrazine)		1.67E-06	6.67E-07	1.67E-06
	Propranolol	6.67E-07			
Zagorska Mrežnica spring	Atrazine		6.67E-07	1.00E-06	1.78E-04
	Atrazine-desethyl (Desethylatrazine)		3.00E-06		
	Carbamazepine		6.67E-07		
	Hydrochlorothiazide		3.00E-06		
	Lamotrigine	3.33E-07	1.00E-06		
	Melamine		6.67E-05		
	Perfluorobutane sulfonate		1.87E-05		
	Sucralose		8.33E-05		
	Sulfadiazine (Silvadene)	6.67E-07			
	Tramadol		1.00E-06		
Zvir spring	Carbamazepine	2.67E-06	5.00E-06	1.84E-04	1.41E-04
	Chloridazon (PAC)	3.33E-07			
	Clopidol	3.33E-07			
	Clothianidin		3.33E-07		
	Diazepam		3.33E-07		
	Hydrochlorothiazide	4.00E-06			
	Lamotrigine	3.33E-06	5.67E-06		
	Perfluorobutane sulfonate	5.00E-06			
	Perfluorohexane sulfonate		7.33E-06		
	Sucralose	1.67E-04	1.20E-04		
	Tramadol	2.00E-06	2.67E-06		

* 2L for person of 60 kg and 10% drinking water allocation.

APPENDIX 3: Supplementry Materials for the Paper III

Selak, A., Lukač Reberski, J., Boljat, I., Terzić, J., 2024. Characterizing occurrence of emerging organic contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs, Croatia. Emerg Contam 10, 100327. https://doi.org/10.1016/j.emcon.2024.100327

Table S1 Potential sources of EOCs detected in the study area

Contaminant group	Emerging organic contaminant	Potential sources	Location of detection
Industrial compound	Benzotriazole	industrial effluents, wastewater from dishwashing	all sampling locations
Agricultural compound	Atrazine-desethyl (Desethylatrazine)	agricultural activities in karst *poljes* Muć, Bisko, Dugopolje	Jadro spring
	Bentazone		Jadro spring
	Clothianidin		Jadro spring
Lifestyle compound	Acesulfame (Acesulfame-K)	wastewater effluents, leaking septic tanks, disposed waste	Jadro spring
	Caffeine		Cetina River
	Cotinine		Jadro spring
	Sucralose		Jadro spring
Personal care products	Climbazole	wastewater effluents, leaking septic tanks	Jadro spring
	DEET		all sampling locations
Pharmaceuticals	Carbamazepine	wastewater effluents from the settlement and health centar, leaking septic tanks, disposed waste	Jadro spring
	Diclofenac		Cetina River
	Gabapentin	wastewater effluents from nursing home in Muć *polje*	Gizdavac borehole
	Ibuprofen	wastewater effluents from the settlement and health centar, leaking septic tanks, disposed waste	Cetina River
	Ibuprofen-carboxy		Cetina River
	Ketoprofen	wastewater effluents from nursing home in Muć *polje*	Gizdavac borehole
	Lamotrigine	wastewater effluents from the settlement and health centar, leaking septic tanks, disposed waste	Jadro spring
	Metformin		Cetina River
	Paracetamol		Cetina River
	Sulfamethoxazole		Jadro spring
	Tramadol		Jadro spring
	Valsartan		Jadro spring

Table S2 Concentration (ng/L) and mass fluxes (g/day) of most frequently detected emerging organic contaminants in spring and river water samples (LOD values in grey are indicated with < and provided by Czech laboratory). Springs' discharge data provided by Croatian Meteorological and Hydrological Service. River discharge data provided by Croatian Waters.

Name		Jadro spring							
Sampling Date		21.3.2019	16.10.2019	3.3.2020	13.7.2020	22.9.2020	11.11.2020	13.10.2021	15.12.2021
Discharge (m^3/s)		7.85	4.35	9.6	4.67	4.4	6.48	7.37	29.92
1H-benzotriazole	g/day	-	-	47.7	-	-	-	-	-
	ng/L	<7.0	<7.0	57.5	<7.0	<7.0	<7.0	<7.0	<7.0
DEET	g/day	-	-	-	5.57	-	31.52	-	-
	ng/L	<3.0	<3.0	<3.0	13.8	<3.0	56.3	<3.0	<3.0
Name		Žrnovnica spring							
Sampling Date		21.3.2019.	16.10.2019	3.3.2020	13.7.2020	22.9.2020	11.11.2020	13.10.2021	15.12.2021
Discharge (m^3/s)		-	0.39	1.57	0.34	0.3	0.68	0.72	4.77
1H-benzotriazole	g/day	Not sampled	-	5.3	-	-	-	-	-
	ng/L	Not sampled	<7.0	39.3	<7.0	<7.0	<7.0	<7.0	<7.0
DEET	g/day	Not sampled	-	-	0.4	-	7.9	-	-
	ng/L	Not sampled	<3.0	<3.0	12.7	<3.0	135	<3.0	<3.0
Name		Cetina River							
Sampling Date		21.3.2019.	16.10.2019	5.3.2020	13.7.2020	22.9.2020	10.11.2020	11.10.2021	14.12.2021
Discharge (m^3/s)		-	70.6	78.5	30.6	32.6	63.9	63.3	181
1H-benzotriazole	g/day	Not sampled	-	-	983.5	-	-	-	-
	ng/L	Not sampled	<7.0	<7.0	372	<7.0	<7.0	<7.0	<7.0
DEET	g/day	Not sampled	-	-	45.7	-	466.5	-	-
	ng/L	Not sampled	<3.0	<3.0	17.3	<3.0	84.5	<3.0	<3.0
Metformin	g/day	Not sampled	1012.6	559.6	-	-	138.6	129.1	-
	ng/L	Not sampled	166	82.5	<7.0	<7.0	25.1	23.6	<7.0

Table S3 Indirect human exposure to detected emerging organic contaminants

Location	EOC	Concentration (ng/L)	ADI or MTD (μg/kg bw)	Drinking water equivalent levels (DWEL) (μg/l)									
				0-3 m	3-6 m	6-12 m	1-2 y	2-3 y	3-6 y	6-11 y	11-16 y	16-18 y	adults ≥18 y
ŽR	DEET	135	100	507.2	657.9	829.8	1470.6	1731.9	1706.2	2419.4	3070.2	3354.5	3063.7
JD	1H-benzotriazole	57.5	295	1496.4	1940.8	2447.9	4338.2	5109.2	5033.2	7137.1	9057	9895.8	9038
JD	DEET	56.3	100	507.2	657.9	829.8	1470.6	1731.9	1706.2	2419.4	3070.2	3354.5	3063.7
JD	sucralose	55	15000	76087	98684.2	124470.3	220588.2	259789.2	255926.7	362903.2	460526.3	503178	459558.8
JD	acesulfame	40	9000	45652.2	59210.5	74682.2	132352.9	155873.5	153556	217741.9	276315.8	301906.8	275735.3
ŽR	1H-benzotriazole	39.3	295	1496.4	1940.8	2447.9	4338.2	5109.2	5033.2	7137.1	9057	9895.8	9038
JD	climbazole	18.4	150	760.9	986.8	1244.7	2205.9	2597.9	2559.3	3629	4605.3	5031.8	4595.6
JD	Valsartan	10.1	5.33	27	35.1	44.2	78.4	92.3	90.9	129	163.6	178.8	163.3
JD	cotinine	1.2	0.28	1.4	1.8	2.3	4.1	4.8	4.8	6.8	8.6	9.4	8.6
JD	Sulfamethoxazole	0.7	130	659.4	855.3	1078.7	1911.8	2251.5	2218	3145.2	3991.2	4360.9	3982.8
JD	Bentazone	0.7	90	456.5	592.1	746.8	1323.5	1558.7	1535.6	2177.4	2763.2	3019.1	2757.4
JD	Lamotrigine	0.6	0.5	2.5	3.3	4.1	7.4	8.7	8.5	12.1	15.4	16.8	15.3
JD	Carbamazepine	0.6	0.34	1.7	2.2	2.8	5	5.9	5.8	8.2	10.4	11.4	10.4
JD	Atrazine-desethyl	0.6	20	101.4	131.6	166	294.1	346.4	341.2	483.9	614	670.9	612.7
JD	clothianidin	0.4	97	492	638.2	804.9	1426.5	1680	1655	2346.8	2978.1	3253.9	2971.8
JD	Tramadol	0.3	8.3	42.1	54.6	68.9	122.1	143.8	141.6	200.8	254.8	278.4	254.3

Location	EOC	Risk quotient									
		0-3 m	3-6 m	6-12 m	1-2 y	2-3 y	3-6 y	6-11 y	11-16 y	16-18 y	adults ≥18 y
ŽR	DEET	0.0002661	0.0002052	0.0001627	0.0000918	7.795E-05	7.912E-05	0.0000558	4.397E-05	4.024E-05	4.406E-05
JD	1H-benzotriazole	3.843E-05	2.963E-05	2.349E-05	1.325E-05	1.125E-05	1.142E-05	8.056E-06	6.349E-06	5.811E-06	6.362E-06
JD	DEET	0.000111	8.558E-05	6.785E-05	3.828E-05	3.251E-05	3.3E-05	2.327E-05	1.834E-05	1.678E-05	1.838E-05
JD	sucralose	7.229E-07	5.573E-07	4.419E-07	2.493E-07	2.117E-07	2.149E-07	1.516E-07	1.194E-07	1.093E-07	1.197E-07

JD	acesulfame	8.762E-07	6.756E-07	5.356E-07	3.022E-07	2.566E-07	2.605E-07	1.837E-07	1.448E-07	1.325E-07	1.451E-07
ŽR	1H-benzotriazole	2.626E-05	2.025E-05	1.605E-05	9.059E-06	7.692E-06	7.808E-06	5.506E-06	4.339E-06	3.971E-06	4.348E-06
JD	climbazole	2.418E-05	1.865E-05	1.478E-05	8.341E-06	7.083E-06	7.19E-06	5.07E-06	3.995E-06	3.657E-06	4.004E-06
JD	Valsartan	0.0003736	0.000288	0.0002284	0.0001289	0.0001094	0.0001111	7.832E-05	6.172E-05	5.649E-05	6.185E-05
JD	cotinine	0.0008449	0.0006514	0.0005165	0.0002914	0.0002475	0.0002512	0.0001771	0.0001396	0.0001278	0.0001399
JD	Sulfamethoxazole	1.062E-06	8.185E-07	6.489E-07	3.662E-07	3.109E-07	3.156E-07	2.226E-07	1.754E-07	1.605E-07	1.758E-07
JD	Bentazone	1.533E-06	1.182E-06	9.373E-07	5.289E-07	4.491E-07	4.559E-07	3.215E-07	2.533E-07	2.319E-07	2.539E-07
JD	Lamotrigine	0.0002366	0.0001824	0.0001446	0.0000816	6.929E-05	7.033E-05	0.0000496	3.909E-05	3.577E-05	3.917E-05
JD	Carbamazepine	0.0003479	0.0002682	0.0002127	0.00012	0.0001019	0.0001034	7.294E-05	5.748E-05	5.261E-05	0.0000576
JD	Atrazine-desethyl	5.914E-06	4.56E-06	3.615E-06	2.04E-06	1.732E-06	1.758E-06	1.24E-06	9.771E-07	8.943E-07	9.792E-07
JD	clothianidin	8.13E-07	6.268E-07	4.97E-07	2.804E-07	2.381E-07	2.417E-07	1.704E-07	1.343E-07	1.229E-07	1.346E-07
JD	Tramadol	7.126E-06	5.494E-06	4.356E-06	2.458E-06	2.087E-06	2.118E-06	1.494E-06	1.177E-06	1.077E-06	1.18E-06

Abbrevations: *EOC* – emerging organic contaminant; *JD* – Jadro spring; *ŽR* – Žrnovnica spring; *ADI* - acceptable daily intake; *MTD* – maximum tolerated dose.

APPENDIX 4: Supplementry Materials for the Paper IV

Selak, A., Lukač Reberski, J., Briški, M., Selak, L., 2024. Hydrochemical characterization of Dinaric karst catchment in relation to emerging organic contaminants. Geol Croat 77(2), 145-158. https://doi.org/10.4154/gc.2024.10

Figure S1 Correlation matrix of physicochemical parameters for Jadro (A), Žrnovnica (B), Gizdavac (C), and Cetina (D) (correlation coefficients order with AOE algorithm - the angular order of the eigenvectors). Values marked with X are not significant.

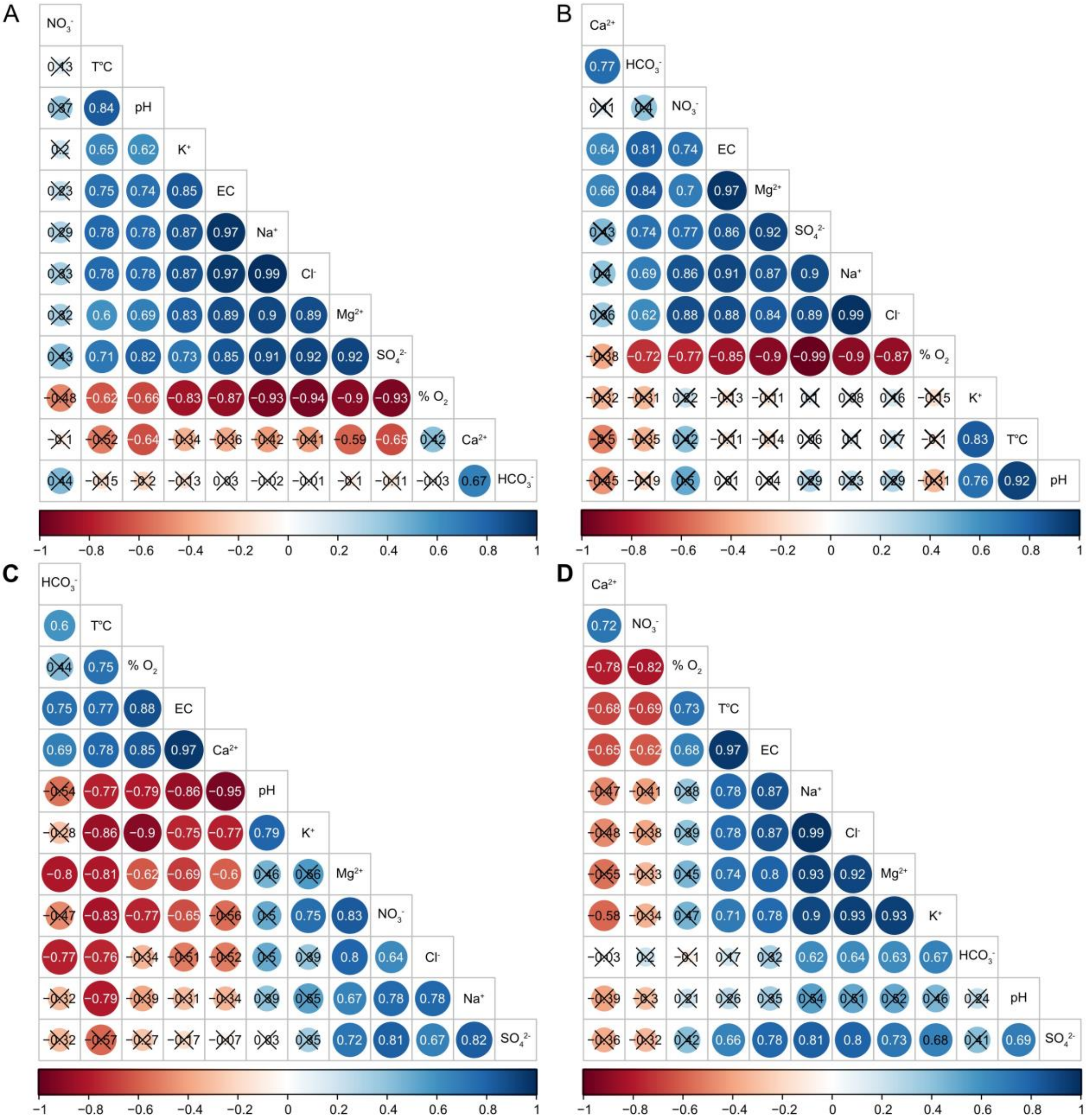

Table S1 Results of Shapiro-Wilk test (values in bold indicate normally distributed data i.e. p-value>0.05) (data October 2019 – October 2022)

	Jadro		Žrnovnica		Gizdavac		Cetina	
Parameter	W	p-value	W	p-value	W	p-value	W	p-value
Temperature	0.96	**0.35**	0.98	**0.76**	0.90	**0.40**	0.96	**0.42**
pH	0.89	0.01	0.92	0.04	0.89	**0.34**	0.96	**0.49**
Electrical conductivity	0.98	**0.83**	0.97	**0.52**	0.93	**0.61**	0.89	0.01
Na^+	0.86	2.36E-03	0.85	1.41E-03	0.89	**0.30**	0.84	8.02E-04
K^+	0.97	**0.73**	0.84	1.03E-03	0.80	**0.06**	0.87	2.93E-03
Mg^{2+}	0.92	4.75E-02	0.94	**0.14**	0.71	0.01	0.99	**1.00**
Ca^{2+}	0.97	**0.62**	0.90	0.02	0.90	**0.36**	0.87	3.01E-03
Cl^-	0.84	7.73E-04	0.82	4.68E-04	0.78	0.04	0.85	1.25E-03
HCO_3^-	0.95	**0.28**	0.97	**0.50**	0.95	**0.77**	0.81	2.28E-04
NO_3^-	0.76	3.74E-05	0.79	1.02E-04	0.79	**0.05**	0.78	7.93E-05
SO_4^{2-}	0.96	**0.40**	0.97	**0.54**	0.87	**0.23**	0.92	0.04
$\delta^{18}O$	0.96	**0.48**	0.96	**0.43**	0.83	**0.11**	0.86	2.40E-03
$\delta^{2}H$	0.97	**0.71**	0.94	**0.16**	0.86	**0.18**	0.92	**0.05**
O_2 (%)	0.76	4.48E-05	0.93	**0.07**	0.95	**0.77**	0.91	0.02
	Jadro		Žrnovnica		Gizdavac		Cetina	

Table S2 Results of Kruskal-Wallis post hoc Dunn test (values in bold indicate significant difference i.e. p.adjusted<0.05) (data October 2019 – October 2022)

Comparison	P.adjusted						
	Ca^{2+}	**Mg^{2+}**	**Na^+**	**Cl-**	**HCO_3^-**	**SO_4^{2-}**	**NO_3^-**
Cetina - Gizdavac	**1.72E-08**	1	**1.28E-04**	**1.94E-03**	**8.49E-08**	**5.06E-06**	**5.11E-06**
Cetina - Jadro	**5.14E-12**	1	0.34	0.34	**2.17E-08**	0.13	**1.44E-07**
Cetina - Žrnovnica	**5.40E-03**	0.73	0.3	0.37	**6.85E-03**	**4.60E-05**	0.8
Gizdavac - Jadro	0.71	1	**0.01**	0.09	0.24	**2.64E-03**	0.13
Gizdavac - Žrnovnica	**5.63E-04**	0.42	**0.01**	0.08	**1.40E-03**	0.17	**2.64E-03**
Jadro - Žrnovnica	**7.63E-04**	**0.04**	1	1	0.05	0.18	**6.21E-03**

Table S3 Main statistical descriptors of saturation indices (SI) calculated with PHREEQC for Jadro, Žrnovnica, Gizdavac, and Cetina samples (data October 2019 – October 2022)

Site	Statistics	Calcite ($CaCO_3$)	Dolomite ($CaMg(CO_3)_2$)	Anhydrite ($CaSO_4$)	Gypsum ($CaSO_4 \cdot 2H_2O$)
Jadro spring	Min	-0.06	-1.22	-3.41	-2.97
	Max	0.71	0.65	-2.60	-2.16
	Mean	0.17	-0.51	-2.89	-2.45
	Median	0.16	-0.49	-2.87	-2.43
Žrnovnica spring	Min	-0.02	-1.19	-3.49	-3.05
	Max	1.16	1.49	-2.76	-2.32
	Mean	0.43	-0.07	-3.04	-2.60
	Median	0.39	-0.10	-3.03	-2.59
Cetina River	Min	0.37	-0.32	-3.04	-2.55
	Max	1.11	1.50	-2.56	-2.13
	Mean	0.79	0.77	-2.82	-2.36
	Median	0.82	0.77	-2.84	-2.37
Gizdavac borehole	Min	0.17	-0.93	-3.47	-3.03
	Max	0.45	0.04	-3.13	-2.68
	Mean	0.27	-0.43	-3.31	-2.86
	Median	0.26	-0.42	-3.31	-2.86

APPENDIX 5: Supplementry Materials for the Paper VI

Selak, A., Lukač Reberski, J., Klobučar, G., Grčić, I., 2022. Data on occurrence and ecotoxicological risk of emerging contaminants in Dinaric karst catchment of Jadro and Žrnovnica springs. Data Br 42, 108157. https://doi.org/10.1016/j.dib.2022.108157

Table S1 Sampling locations coordinates

Sampling site	Sampling medium	Latitude (N)	Longitude (E)
Jadro	spring water	43°32'34.6"	16°31'20.6"
Žrnovnica	spring water	43°31'24.5"	16°34'28.4"
Cetina	river	43°37'02.4"	16°43'44.6"
Gizdavac	borehole	43°38'43.7"	16°29'07.6"

Table S2 EOCs concentration, detection frequency and LOD

EOC	CAS number	Group	Description/use	Detection frequency	Max. conc. (ng/L)	Min. conc. (ng/L)	Median conc. (ng/L)	Single event detection (ng/L)	LOD (ng/L) CZ lab	LOD (ng/L) NLS lab	Detection location
DEET	134-62-3	Personal care	Insect repellent	0.21	135	12.7	39.4	-	3	10	all sampling sites
1H-Benzotriazole	95-14-7	Industrial	Corrosion inhibitor, dishwashing agent	0.13	372	22.3	51.7	-	7	5000	all sampling sites
Metformin	657-24-9	Pharmaceutical	Antidiabetic and antihyperglycemic	0.08	166	25.1	82.5	-	7	100	Cetina River
Ibuprofen	15687-27-1	Pharmaceutical	Analgesic/anti-inflammatory	0.05	55.2	20.4	37.8	-	7	1	Cetina River
Carbamazepine	298-46-4	Pharmaceutical	Anticonvulsant; veterinary drug	0.05	0.6	0.5	0.55	-	3	1	Jadro spring
Tramadol	27203-92-5	Pharmaceutical	Potent analgesic; veterinary drug	0.05	0.3	0.3	0.3	-	3	1	Jadro spring
Sucralose	56038-13-2	Lifestyle product	Artificial sweetener	0.05	55	47	51	-	300	10	Jadro spring
Caffeine	58-08-2	Lifestyle product	Stimulant	0.03	-	-	-	146	30	10	Cetina River
Ketoprofen	22071-15-4	Pharmaceutical	Analgesic/anti-inflammatory	0.03	-	-	-	40.8	3	1	Gizdavac borehole
Gabapentin	60142-96-3	Pharmaceutical	Antiepileptic/analgesic	0.03	-	-	-	37.1	3	10	Gizdavac borehole
Carboxyibuprofen	15935-54-3	Pharmaceutical	Ibuprofen derivate	0.03	-	-	-	22.5	7	-	Cetina River
Climbazole	38083-17-9	Personal care	Preservative, anti-aging and antimycotic	0.03	-	-	-	18.4	3	1	Jadro spring
Paracetamol	103-90-2	Pharmaceutical	Analgesic/anti-inflammatory	0.03	-	-	-	12.6	3	5	Cetina River
Valsartan	137862-53-4	Pharmaceutical	Antihypertensive agent, angiotensin receptor antagonist	0.03	-	-	-	10.1	3	5	Jadro spring
Atrazine-desethyl	6190-65-4	Agricultural	Pesticide; herbicide; degradation product	0.03	-	-	-	0.6	-	1	Jadro spring
Sulfamethoxazole	723-46-6	Pharmaceutical	Antibiotic; veterinary drug	0.03	-	-	-	0.7	3	5	Jadro spring
Clothianidin	210880-92-5	Agricultural	Pesticide; veterinary drug	0.03	-	-	-	0.4	-	1	Jadro spring
Cotinine	486-56-6	Lifestyle product	Nicotine metabolite	0.03	-	-	-	1.2	7	5	Jadro spring
Lamotrigine	84057-84-1	Pharmaceutical	Anticonvulsant; veterinary drug	0.03	-	-	-	0.6	3	1	Jadro spring

EOC	CAS number	Group	Description/use	Detection frequency	Max. conc. (ng/L)	Min. conc. (ng/L)	Median conc. (ng/L)	Single event detection (ng/L)	LOD (ng/L) CZ lab	LOD (ng/L) NLS lab	Detection location
Acesulfame	33665-90-6	Lifestyle product	Artificial sweetener (K salt)	0.03	-	-	-	40	15	50	Jadro spring
Bentazone	25057-89-0	Agricultural	Pesticide; herbicide; veterinary drug	0.03	-	-	-	0.7	-	1	Jadro spring

Abbrevations: *EOC* – emerging organic contaminant; *LOD* – limits of detection.

Table S3 EOCs physico-chemical properties

EOC	Molecular formula	MW (g/mol)	log K_{ow} experimental	log K_{ow} (KOWIN v1.68)	log K_{ow} (Prometheus)	log K_{OC}	pKa	Solubility (mg/L) experimental	Solubility (mg/L at 25°C) (WSKOW v1.42)
Acesulfame	$C_4H_5NO_4S$	163.15	N/A	-1.33	-0.68	min log K_{OC} = 0.25 (anion) [1] (calculated based on equation)	3.02 (acidic) [1] (calculated with ChemAxon)	N/A	9.10E+05
Atrazine-desethyl	$C_6H_{10}ClN_5$	187.63	1.51	1.78	1.51	min log K_{OC} = 1.38 [2] (experimental)	1.0 [16] (estimated)	3200 at 22°C	2593
1H-benzotriazole	$C_6H_5N_3$	119.1	1.44	1.17	1.44	min log K_{OC} = 1.69 (neutral) [1] (calculated based on equation)	9.04 (acidic) [1] (calculated with ChemAxon)	1.98e+004 at 25°C	5957
Bentazone	$C_{10}H_{12}N_2O_3S$	240.28	2.34	1.67	2.34	min log K_{OC} = 1.11 [3]	2.86 [17] (experimental)	500 at 20°C	268.6
Caffeine	$C_8H_{10}N_4O_2$	194.2	-0.07	0.16	0.29	min log K_{OC} = 1.85 [4]	10.4 (basic) [18, 19]	2.16e+004 at 25°C	2632
Carbamazepine	C15H12N2O	236.27	2.45	2.45	2.45	min log K_{OC} = 2.0 (neutral) [5] (experimental)	14.0 [5]	112 at 25°C	17.66
Carboxyibuprofen	$C_{13}H_{16}O_4$	236.3	N/A	1.97	2.45	min log K_{OC} = 1.25 [6]	3.97 [20] (estimated with Marvin Sketch 5.5.0©)	N/A	1453
Climbazole	$C_{15}H_{17}ClN_2O_2$	292.8	N/A	3.76	2.71	min log K_{OC} = 3.5 (ionizable compound) [7]	7.51 (basic) [21]	N/A	8.281
Clothianidin	$C_6H_8ClN_5O_2S$	249.68	0.7	0.64	0.66	min log K_{OC} = 1.92 [8]	11.09 [22]	N/A	5997
Cotinine	$C_{10}H_{12}N_2O$	176.21	0.07	0.34	0.07	min log K_{OC} = 2.11 [9]	4.79 (basic) [23] (calculated with ChemAxon)	N/A	9.99E+05
DEET	$C_{12}H_{17}NO$	191.3	2.18	2.26	2.18	min log K_{OC} = 1.64 [10]	0.4 [24]	N/A	666

EOC	Molecular formula	MW (g/mol)	log K_{ow} experimental	log K_{ow} (KOWIN v1.68)	log K_{ow} (Prometheus)	log K_{OC}	pKa	Solubility (mg/L) experimental	Solubility (mg/L at 25°C) (WSKOW v1.42)
Gabapentin	$C_9H_{17}NO_2$	292.8	-1.1	-1.37	-1.1	min log K_{OC} = 0.36 (zwitterion) [1] (calculated based on equation)	4.63 (acidic); 9.91 (basic) [1] (calculated with ChemAxon)	N/A	4491
Ibuprofen	$C_{13}H_{18}O_2$	206.3	3.97	3.79	3.97	min log K_{OC} = 2.14 [5]	4.52 (acidic) [25]	21 at 25°C	41.05
Ketoprofen	$C_{16}H_{14}O_3$	254.3	3.12	3	3.12	min log K_{OC} = 0.2 [11] (calculated)	4.45 (acidic) [26, 27]	51 at 22°C	120.4
Lamotrigine	$C_9H_7Cl_2N_5$	256.09	2.57	0.99	2.4	min log K_{OC} = 2.1 [12] (calculated)	5.7 [28]	N/A	139.7
Metformin	$C_4H_{11}N_5$	129.2	N/A	-1.4	-0.6	min log K_{OC} = 1.08 [13]	12.33 [13]	N/A	1.00E+06
Paracetamol	$C_8H_9NO_2$	151.2	0.46	0.27	0.46	min log K_{OC} = 2.94 (ionizable compound) [14]	9.38 [29] (experimental)	1.4e+004 at 25°C	4190
Sucralose	$C_{12}H_{19}Cl_3O_8$	397.6	N/A	-1	-1	min log K_{OC} = -0.72 [15] (calculated with EPI Suite^{TM} KOCWIN v2.00)	11.8 [30] (calculated with ChemAxon)	N/A	2.28E+04
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28	0.89	0.48	0.89	min log K_{OC} = 1.09 (anion) [1] (calculated based on equation)	6.16 (acidic) [1] (calculated with ChemAxon)	379 at 25°C	3942
Tramadol	$C_{16}H_{25}NO_2$	263.37	2.51; 2.63	3.01	2.57	min log K_{OC} = 2.79 (ionizable compound) [14]	9.41 [31]	N/A	1151
Valsartan	$C_{24}H_{29}N_5O_3$	435.5	N/A	3.65	2.74	min log K_{OC} = 1.88 (anion) [1] (calculated based on equation)	4.35; 5.86 (acidic) [1] (calculated with ChemAxon)	N/A	1.406

Abbrevations: *EOC* – emerging organic contaminant; *MW* – molecular weight; *log* K_{OW} - octanol-water partition coefficient; *log* K_{OC} - organic carbon-water partition coefficient.

Mentioned references can be found in Paper VI.

Table S4 Measured physico-chemical properties of water

Water temperature

Sampling date	March 2019	October 2019	March 2020	July 2020	September 2020	November 2020
Sampling site	Water temperature (°C)					
Jadro	12.7	13.3	12.5	13	13.14	12.9
Žrnovnica	-	12.7	12.5	13.3	12.6	12.7
Cetina	-	14.6	9.4	15.4	16.9	11.7
Gizdavac	-	-	13.65	16.5	15	14.1

Electrical conductivity

Sampling date	March 2019	October 2019	March 2020	July 2020	September 2020	November 2020
Sampling site	Electrical conductivity (µS/cm)					
Jadro	426	366	439	495	536	448
Žrnovnica	-	340	412	422	499	408
Cetina	-	410	369	494	633	406
Gizdavac	-	-	500	494	509	522

Table S5 PBT values predicted with Prometheus software

SUBSTANCE	LogP	Persistance (P)	Bioaccumulation (B)	Toxicity (T)	P score	B score	T score	PB	PBT
Climbazole	2.710	P/vP	1.810	0.193	0.712	0.255	0.461	0.426	0.433
Carbamazepine	2.450	P/vP	1.260	0.817	0.712	0.278	0.383	0.445	0.432
Valsartan	2.740	P/vP	0.710	-	0.712	0.242	0.500	0.415	0.431
Lamotrigine	2.400	nP	2.610	0.008	0.359	0.412	0.658	0.384	0.428
Tramadol	2.570	nP/P	1.310	0.236	0.584	0.282	0.450	0.406	0.414
Clothianidin	0.660	vP	0.410	10.000	0.854	0.228	0.291	0.441	0.406
Sulfamethoxazole	0.890	P/vP	0.380	4.000	0.712	0.227	0.290	0.402	0.376
1H-benzotriazole	1.44	nP	0.5	0.271	0.359	0.232	0.442	0.288	0.314
Sucralose	-1	nP	0.08	-	0.359	0.216	0.5	0.278	0.313
Atrazine-desethyl	1.51	vP	0.4	0.51	0.854	0.103	0.368	0.296	0.310
Caffeine	0.29	vP	0.77	10	0.854	0.128	0.231	0.331	0.308
Acesulfame	-0.68	nP	0.05	1.72	0.359	0.215	0.349	0.278	0.291
Carboxyibuprofen	2.45	nP/P	0.65	0.393	0.584	0.120	0.409	0.264	0.288
Bentazone	2.34	nP	1.32	-	0.333	0.175	0.5	0.241	0.279
Ketoprofen	3.12	nP	1	0.075	0.333	0.148	0.519	0.222	0.263
Cotinine	0.07	nP	1.06	2.13	0.359	0.155	0.341	0.235	0.253
Ibuprofen	3.970	nP	0.430	0.210	0.333	0.104	0.449	0.186	0.222
Metformin	-0.6	-	0.14	425	0.500	0.089	0.240	0.211	0.217
Paracetamol	0.46	nP/P	0.39	54	0.571	0.102	0.133	0.242	0.214
Gabapentin	-1.1	nP	0.3	1.99	0.333	0.097	0.319	0.180	0.202
DEET	2.18	nP	0.38	7.31	0.333	0.068	0.194	0.150	0.158

Abbrevations: *vP* - very persistent; *P* – persistent; *nP* - not persistent.

Table S5 Calculated PBTr values

	Sampling site			
Substance	Cetina	Gizdavac	Jadro	Žrnovnica
Climbazole	-	-	7.97	-
Carbamazepine	-	-	0.24	-
Valsartan	-	-	4.35	-
Lamotrigine	-	-	0.26	-
Tramadol	-	-	0.12	-
Clothianidin	-	-	0.16	-
Sulfamethoxazole	-	-	0.26	-
Ibuprofen	8.39	-	-	-
1H-Benzotriazole	116.81	37	18.06	12.34
Sucralose	-	-	15.96	-
Atrazine-desethy	-	-	0.19	-
Caffeine	44.97	-	-	-
Acesulfame	-	-	11.64	-
Carboxyibuprofen	6.48	-	-	-
Bentazone	-	-	0.2	-
Ketoprofen	-	10.73	-	-
Cotinine	-	-	0.3	-
Metformin	19.79	-	-	-
Paracetamol	2.7	-	-	-
Gabapentin	-	7.49	-	-
DEET	8.04	6.49	5.54	11.67

Table S6 PMT/vPvM analysis

SUBSTANCE	PMT/vPvM assessment	Persistence (P)	Mobility (M)	Toxicity (T)	Detection in Jadro and Žrnovnica springs catchment			
					Jadro spring	Žrnovnica spring	Cetina River	Gizdavac borehole
Climbazole	vPvM & PMT	potential P/vP++	M	T	+			
Sulfamethoxazole	vPvM & PMT	potential P/vP++	vM	T	+			
Valsartan	vPvM & PMT	potential P/vP++	vM	T	+			
1H-benzotriazole	vPvM & PMT	potential P/vP++	vM	T	+	+	+	+
Atrazine-desethyl	vPvM	potential P/vP++	vM	potential T	+			
Clothianidin	vPvM	potential P/vP++	vM	potential T	+			
Lamotrigine	vPvM	potential P/vP++	vM	Potential T	+			
Carbamazepine	PMT	P	vM	T	+			
Tramadol	PM	P	vM	Potential T	+			
Acesulfame	Potential PMT/vPvM	potential P/ vP	vM	potential T	+			
Bentazone	Potential PMT/vPvM	potential P/ vP	vM	potential T	+			
Cotinine	Potential PMT/vPvM	potential P/ vP	vM	potential T	+			

SUBSTANCE	PMT/vPvM assessment	Persistence (P)	Mobility (M)	Toxicity (T)	Detection in Jadro and Žrnovnica springs catchment			
					Jadro spring	Žrnovnica spring	Cetina River	Gizdavac borehole
DEET	Potential PMT/vPvM	potential P/vP	vM	Not T	+	+	+	+
Ketoprofen	potential PMT/vPvM	potential P/vP	vM	T				+
Metformin	Potential PMT/vPvM	potential P/vP	vM	potential T			+	
Paracetamol	Potential PMT/vPvM	potential P/vP	vM	T			+	
Sucralose	Potential PMT/vPvM	potential P/ vP	vM	potential T	+			
Caffeine	not PMT/vPvM	not P	vM	potential T			+	
Carboxyibuprofen	not PMT/vPvM	not P	vM	Not T			+	
Gabapentin	not PMT/vPvM	not P	vM	Potential T				+
Ibuprofen	not PMT/vPvM	not P	vM	T			+	

Abbrevations: *vP* - very persistent; *P* – persistent; *potential P/vP* - potential persistent or potential very persistent; *not P* - not persistent; *vM* - very mobile; *M* – mobile; *not M* - not mobile; *T* – toxic; *potential T* - potential toxic; *PM* - persistent and mobile; *PMT* - persistent, mobile and toxic; *vPvM* - very persistent and very mobile.

Table S7 Risk quotient (RQ)

Substance	Lowest PNEC (ng/L)	Indicator species	Site_Date	Measured environmental concentration (MEC) (ng/L)	Risk quotient RQ
1H-Benzotriazole	7770	*Selenastrum capricornutum*	Ž_3_2020	39.3	0.01
			J_3_2020	57.5	0.01
			G_3_2020	22.3	0.003
			G_7_2020	51.7	0.01
			C_7_2020	372	0.05
DEET	88000	n.r.	Ž_7_2020	12.7	0.0001
			Ž_11_2020	135	0.0015
			J_7_2020	13.8	0.0002
			J_11_2020	56.3	0.0006
			G_3_2020	22.4	0.0003
			G_7_2020	59.8	0.0007
			C_7_2020	17.3	0.0002
			C_11_2020	84.5	0.001
Metformin	156000	n.r.	C_10_2019	166	0.0011
			C_3_2020	82.5	0.0005
			C_11_2020	25.1	0.0002
Ibuprofen	1650	n.r.	C_10_2019	55.2	0.033
			C_3_2020	20.4	0.012
Carbamazepine	50	*Daphnia magna*	J_3_2019	0.5	0.01
			J_10_2019	0.6	0.01
Tramadol	8650	*Selenastrum capricornutum*	J_3_2019	0.3	0.00003
			J_10_2019	0.3	0.00003
Sucralose	29700	*Selenastrum capricornutum*	J_3_2019	47	0.002
			J_3_2019	55	0.002

Substance	Lowest PNEC (ng/L)	Indicator species	Site_Date	Measured environmental concentration (MEC) (ng/L)	Risk quotient RQ
Caffeine	1200	*Daphnia magna*	C_3_2020	146	0.12
Ketoprofen	2100	*Pimephales promelas*	G_11_2020	40.8	0.02
Gabapentin	10000	n.r.	G_9_2020	37.1	0.004
Carboxyibuprofen	5600	*Pimephales promelas*	C_10_2019	22.5	0.004
Climbazole	520	n.r.	J_7_2020	18.4	0.04
Acetaminophen	134000	n.r.	C_10_2019	12.6	0.00009
Valsartan	560000	*Daphnia magna*	J_11_2020	10.1	0.00002
Deethylatrazine	600	*Hyalella azteca*	J_3_2019	0.6	0.001
Sulfamethoxazole	600	n.r.	J_3_2019	0.7	0.001
Clothianidin	2230	*Pimephales promelas*	J_3_2019	0.4	0.0002
Cotinine	10000	*Lemna gibba*	J_10_2019	1.2	0.00012
Lamotrigine	10000	*Desmodesmus subspicatus*	J_10_2019	0.6	0.00006
Acesulfame	72400	*Daphnia magna*	J_10_2019	40	0.0006
Bentazone	100	n.r.	J_10_2019	0.7	0.007

Abbrevations: *PNEC* – predicted no effect concentration; *n.r.* - not reported.