

# Bioaccumulation and cross-ecosystem transfer of emerging contaminants with emergence of aquatic insects

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University of Zagreb

FACULTY OF SCIENCE  
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Marina Veseli

**BIOACCUMULATION AND CROSS-  
ECOSYSTEM TRANSFER OF EMERGING  
CONTAMINANTS WITH EMERGENCE OF  
AQUATIC INSECTS**

DOCTORAL THESIS

Zagreb, 2024



Sveučilište u Zagrebu

PRIRODOSLOVNO-MATEMATIČKI FAKULTET

BIOLOŠKI ODSJEK

Marina Veseli

**BIOAKUMULACIJA ONEČIŠĆUJUĆIH  
TVARI I NJIHOV PRIJENOS IZMEĐU  
EKOSUSTAVA PUTEM EMERGENCIJE  
VODENIH KUKACA**

DOKTORSKI RAD

Zagreb, 2024.

This doctoral thesis was made at the University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, under the supervision of Assoc. Prof. Ana Previšić, PhD and Marko Rožman, PhD (Institute Ruđer Bošković, Zagreb) as a part of the Doctoral programme of biology at the University of Zagreb, Faculty of Science, Department of Biology. Research was financed by Croatian Science Foundation as part of the project „ Effects of multiple stressors on freshwater biodiversity and ecosystem functioning “(PZS-2019-02-9479).



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*Dedicated to my Dad.*

*Who knew our local fishing and forest expeditions  
would end up with me writing a biology doctoral thesis.*

**Bioaccumulation and cross-ecosystem transfer of emerging contaminants with  
emergence of aquatic insects**

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Daily reports of freshwater ecosystem pollution worldwide raise concerns for aquatic communities underscoring the need for more detailed research. This study conducted in northwest Croatia investigated the presence, bioaccumulation, and trophic transfer of pharmaceuticals and endocrine disruptors in aquatic and riparian ecosystems. The findings suggest that fine scale differences in biological traits between taxa play a defining role in bioaccumulation and bioamplification patterns of pharmaceuticals and endocrine disruptors in aquatic insects. Additionally, this study sheds light on the transfer of contaminants in aquatic-terrestrial food webs, showing their presence across trophic levels of the studied food web. Notably, it reveals that the trophic transfer extends beyond a 3-meter distance from the river, indicating the need for further research into waterborne contaminants reach in riparian zones. Attempts to predict bioaccumulation using pharmacokinetic descriptors yielded, although novel, also limited results, highlighting the need for more detailed examination. (143 pages, 28 figures, 12+19 tables, 144 references, original in English)

**Keywords:** pharmaceuticals, endocrine disrupting compounds, emerging contaminants, aquatic insects, food webs

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**Bioakumulacija onečišćujućih tvari i njihov prijenos između ekosustava putem  
emergencije vodenih kukaca**

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Onečišćenje slatkovodnih ekosustava svakodnevna je pojava širom svijeta te izaziva zabrinutost za vodene zajednice. U ovom istraživanju, provedenom na nekoliko rijeka u sjeverozapadnoj Hrvatskoj, proučavana je prisutnost, bioakumulacija i trofički prijenos farmaceutika i endokrinih disruptora u vodenim i riparijskim ekosustavima. Rezultati ukazuju kako su precizne razlike u biološkim svojstvima između svojti ključne u određivanju obrazaca bioakumulacije i bioamplifikacije farmaceutika i endokrinih disruptora u vodenim kukcima. Nadalje, rezultati ovog istraživanja pužaju nove uvide u prijenos farmaceutika i endokrinih disruptora u hranidbenim mrežama na granici vode i kopna, potvrđujući prisutnost ovih spojeva na svim trofičkim razinama proučavane prehrambene mreže. Ova studija otkriva da se trofički prijenos ovih spojeva proteže i izvan udaljenosti od tri metra od rijeke, no potrebna su daljnja istraživanja kako bi se precizno odredio domet trofičkog prijenosa onečišćenja u riparijskoj zoni. Pokušaji predviđanja bioakumulacije korištenjem farmakokinetičkih deskriptora dali su, iako nove, ujedno i ograničene rezultate, naglašavajući potrebu za detaljnijim istraživanjem ove teme. (143 stranica, 28 slika, 12+19 tablica, 144 literaturnih navoda, jezik izvornika engleski)

Ključne riječi: farmaceutici, endokrini disruptori, novi onečišćivači, vodeni kukci, hranidbena mreža

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## **Abbreviations**

SPE - solid phase extraction

UPLC - ultra performance liquid chromatography

OPLS-DA - orthogonal partial least squares – discriminant analysis

ACN - acetonitrile

PhACs - pharmaceuticals

EDCs - endocrine disrupting compounds

ECs - emerging contaminants

ANOVA - analysis of variance

# 1. INTRODUCTION

The increasing presence of emerging contaminants (ECs) in global freshwater ecosystems is acknowledged as a rising threat. Every day, significant amounts of wastewater are released into freshwater environments, which may serve as the primary pathway for the introduction of different emerging contaminants such as pharmaceuticals (PhACs) and endocrine-disrupting compounds (EDCs) into surface waters. PhACs and EDCs are diverse groups of substances widely used in medical or personal care, as well as in food and other industries and they are frequently detected in natural freshwater environments contaminated by wastewater discharge (Tijani et al., 2013). Surface waters worldwide have been found to contain various pharmaceutical classes, including analgesics, anti-inflammatory drugs, antibiotics, antidepressants, antihistamines, and hormones (aus der Beek et al., 2016; de Solla et al., 2016; Huerta et al., 2016). Some PhACs, along with numerous other chemicals utilized for different purposes such as additives, preservatives, antiseptics, pesticides, and herbicides, have demonstrated endocrine-disrupting properties (Weber et al., 2014). Given the large number of these compounds and the complex nature of aquatic ecosystems, the ecological impacts of PhACs and EDCs on aquatic environments, as well as the specific effects of individual compounds and their combinations on aquatic biota, remains yet to be fully investigated (Ebele et al., 2017).

Contaminants present in freshwater can be readily absorbed and ingested by aquatic organisms, including aquatic insects, through aqueous and dietary exposure (Arnot and Gobas, 2006). These contaminants then can undergo processes such as excretion through digestion and respiration, metabolic transformation, or they can be retained within the organism (Mandarić et al., 2015). Exposure to PhACs and EDCs induces various metabolic and physiological changes in aquatic organisms, impacting their behaviour as well (Richmond et al., 2016). Contaminant bioaccumulation occurs when a substance is more retained in an organism than excreted and recent studies have confirmed the bioaccumulation of PhACs and EDCs in various aquatic insects (Lagešson et al., 2016; Previšić et al., 2021; Ruhí et al., 2016). Although biomagnification of some emerging contaminants like polychlorinated biphenyls (PCBs) and certain pesticides has been confirmed (Walters et al., 2016), uncertainties persist regarding the biomagnification potential of PhACs and EDCs. Nevertheless, it is possible that concentrations of some of them could increase with higher trophic positions, as research confirmed that



concentrations of flame retardant TBEP (EDC) are building up along higher food web levels (Ruhí et al., 2016). Contaminant concentration in organisms can also increase without additional exposure and in that case, the bioamplification is observed (Kraus et al., 2014b). Bioamplification typically occurs during significant developmental changes, often accompanied by weight loss and/or a decrease in the ability to eliminate contaminants from the organism proportionally (Daley et al., 2009). For example, bioamplification of certain metals (essential metals Cu, Zn, and Se, and non-essential metals Cd and Ag) has been observed during the metamorphosis of aquatic insects (Cetinić et al., 2021). Furthermore, studies have confirmed bioamplification of contaminants in aquatic insects belonging to both hemi- and holometabolous insects, including instances of polychlorinated biphenyl (PCB) in Ephemeroptera (Daley et al., 2011), organochlorine compounds and polybromodiphenyl ethers in Diptera and Trichoptera (Bartrons et al., 2007), and PhACs and EDCs in Trichoptera (Previšić et al., 2021).

Studies show that bioaccumulation and bioamplification patterns differ with respect to aquatic insect taxa. It is suggested that these differences are determined by ecological traits which affect contaminant availability as well as exposure of the organism to contaminants (Bartrons et al., 2007; Previšić et al., 2021). All Odonata species are predators throughout their life cycle, however the two suborders (Anisoptera and Zygoptera) differ in type of respiration as well as their habitat preferences and dispersal behaviour (Corbet, 1999), which could potentially cause differences in bioaccumulation patterns. Furthermore, type of insect metamorphosis (e.g. holometabolous Trichoptera and hemimetabolous Odonata) and feeding behaviour seem to play an important role in determining bioaccumulation and bioamplification patterns of metals (Cetinić et al., 2021), as well as of PhACs and EDCs (Previšić et al., 2021).

Life cycle of aquatic insects includes aquatic (larvae/nymphs and, in some orders, pupae) and terrestrial (imagines) life stages, hence aquatic insects are part of both aquatic and terrestrial food webs. In such way they connect aquatic and terrestrial ecosystems and represent an important inter-habitat linkage for energy and nutrient flow, but also for contaminant transfer to terrestrial environments (Daley et al., 2011; Kraus et al., 2014a). Adult aquatic insects like Diptera, Ephemeroptera and Trichoptera are an important food source for riparian predators (eg. spiders, bats, and birds) (Walters et al., 2008). Increased body burden of PhACs and EDCs measured in adult Trichoptera (Previšić et al., 2021) implies that their terrestrial predators could be exposed to mixtures of contaminants of aquatic origin possibly in even higher concentrations than trichopteran larvae in their polluted freshwater environment. Furthermore, presence of

PhACs and EDCs in riparian spiders that are mostly feeding on aquatic insect adults confirms consumption of the emerged aquatic insects as the source of contaminants for riparian predators and also their transfer from aquatic ecosystem (Previšić et al., 2021; Richmond et al., 2018). However, unlike for some other contaminants (e.g. PCBs (Raikow et al., 2011)), PHACs and EDCs fate in the riparian zone and knowledge on their lateral transport through food webs in the riparian zone is mostly unknown. Current data on this subject highlights the importance of trophic transfer of waterborne pollutants into terrestrial habitats. Emerging aquatic insects are the major food source of riparian spiders, i.e. their contribution in spider diets can be over 40% in riparian zones declining with distance from the stream/river (Walters et al., 2010). Thus, lateral extent of aquatic-terrestrial PhACs and EDCs transfer depends on both, their bioaccumulation in aquatic insects but also on the share that aquatic insects have in riparian spiders diets (Briers et al., 2005).

Apart from using biological and ecological traits of organisms, prediction of environmental fate of contaminants and bioaccumulation has also been related to physico-chemical properties of contaminants (e.g. octanol-water partition coefficient ( $\log K_{OW}$ ) and aqueous solubility ( $\log S$ ) (Du et al., 2014; Franke et al., 1994; Huerta et al., 2013), however, the extent of using physico-chemical properties of the compounds to realistically predict environmental fate and bioaccumulation of PhACs and EDCs it is yet to be discovered.

## 1.1. Research objectives and hypotheses

The main aim of this research for proposed doctoral thesis was to provide new information on behaviour and fate of waterborne pollutants, pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) on aquatic-terrestrial ecosystem interface.

Accordingly, the following specific goals were set:

- i. to establish bioaccumulation and bioamplification patterns of PhACs and EDCs at different taxonomic levels of aquatic insects related to fine scale differences in their biology;
- ii. to examine trophic transfer of PhACs and EDCs and their lateral extent in the riparian zone;
- iii. to assess the influence of contaminant physico-chemical descriptors and predictors related to pharmacokinetics on bioaccumulation and bioamplification of PhACs and EDCs in aquatic insects.

Hypotheses following the aims:

- i. Fine scale differences in biological traits influence contaminants uptake, bioaccumulation and bioamplification across metamorphosis comparing different aquatic insects.
- ii. PhAC and EDCs transfer from aquatic to terrestrial ecosystems with emerged aquatic insects as vectors and within aquatic and terrestrial food webs.
- iii. Prediction of bioaccumulation and bioamplification of PhACs and EDCs in aquatic insects is possible by means of linear models using their physico-chemical and pharmacokinetic properties.

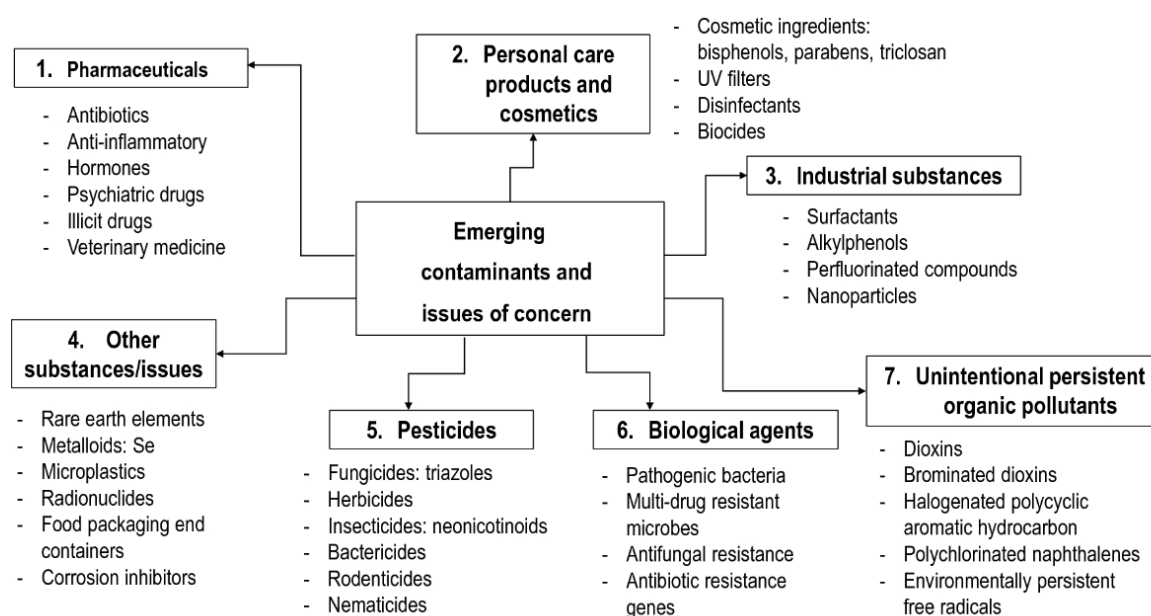
To achieve the above-stated aims, *in situ* research was conducted on selected rivers known to be impacted with wastewater effluents, ensuring that collected organisms were exposed to different contaminants originating from wastewater, including PhACs and EDCs. Quantification of contaminants in collected organisms allowed assessing how bioaccumulation and bioamplification patterns differ on different taxonomic levels of aquatic insects regarding their taxa-specific ecological and life history traits. Furthermore, contaminants measured in organisms belonging to different trophic levels of aquatic-terrestrial food webs were used to investigate trophic transfer of PhACs and EDCs in aquatic and terrestrial food webs.

Additionally, these were used to assess transfer of PhACs and EDCs across aquatic-terrestrial ecosystem boundary and the extent of the contaminants transfer in the riparian zone. Finally, to predict the bioaccumulation and bioamplification potential of PhACs and EDCs in aquatic insects based on molecular descriptors, thorough analyses were performed using linear correlation, linear regression, and OPLS-DA.

## 2. LITERATURE REVIEW

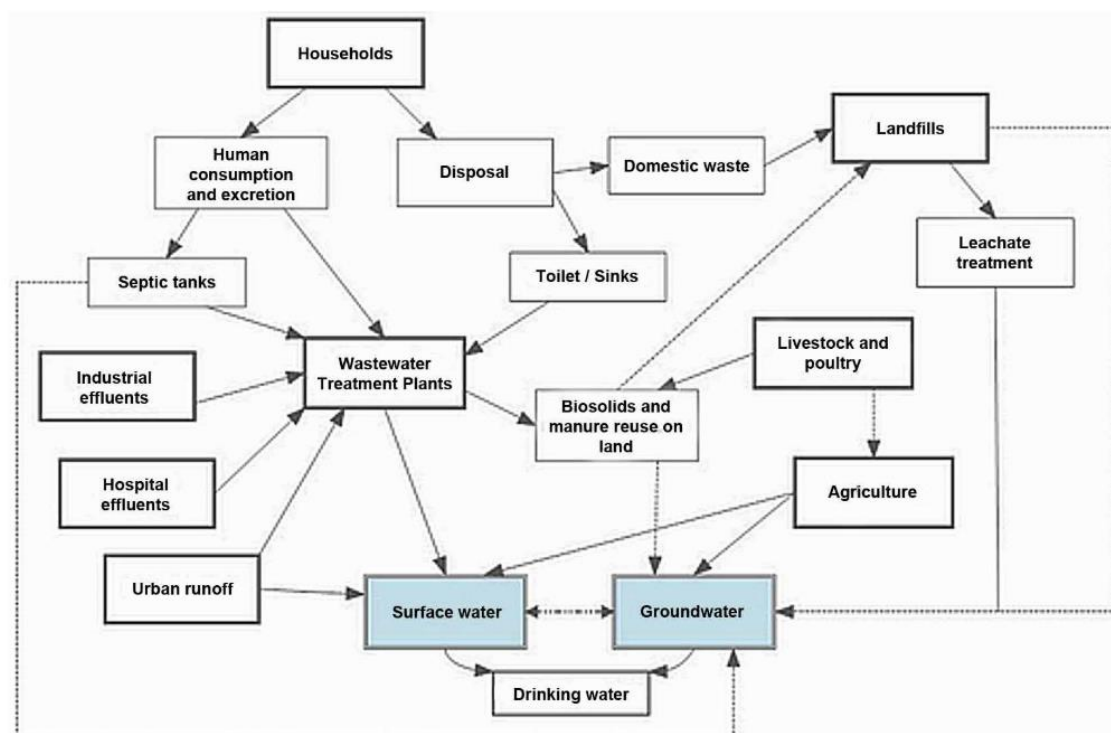
### 2.1. Presence of emerging contaminants in freshwaters

Emerging contaminants (ECs) is a term used to describe a various group of contaminants that are recognized as a growing threat for the environment but are still mostly unstudied. The definition of emerging contaminants as well as terminology and which contaminant groups should be included by the term is inconsistent in the literature. Even so, many share understanding that contaminants with unknown environmental fate and behaviour as well as unstudied ecological and toxicological effects, whose presence is confirmed in environment and are not part of regular monitoring programs at EU level are considered to be ‘emerging contaminants’ (Mandaric et al., 2015). Some contaminant groups considered to be emerging contaminants are pharmaceuticals, personal care products, various surfactants, persistent organic pollutants, biological agents (e.g. antifungal agents) and pesticides (Figure 1), and these contaminants are present in environment worldwide, often suspected to potentially have concerning effects on organisms (as endocrine disruptors, carcinogens, mutagens, etc.) (Morin-Crini et al., 2022).



**Figure 1.** Emerging contaminants present in the environment worldwide. (Source: Morin-Crini et al., 2022)

Most of the emerging contaminants are not newly created chemicals, yet they were just recently recognized as contaminants present in the environment proposing a potential risk to wildlife and human health (Stefanakis and Becker, 2015). Emerging contaminants presence in environment is occurring through various pathways, and it is confirmed that they are present in the soil, sea, air and both, groundwater and surface waters (Bayabil et al., 2022; Brumovsky et al., 2017; Enyoh et al., 2020; Stefanakis and Becker, 2015). Some of the sources of emerging contaminants are agricultural runoff, wastewater effluents (industrial, hospital, household), landfill leachates, livestock, etc. (Morin-Crini et al., 2022). Even though aquatic environments have natural ability to dilute the pollution, they are considered as the ‘pickers’ of anthropological contamination due to their constant exposure to various contaminant sources (Bashir et al., 2020). Figure 2 schematically shows pathways and types of emerging contaminant sources causing their occurrence in groundwater and surface waters, which also represents a threat for drinking water. The main contributor to the presence of the emerging contaminants in freshwater is wastewater, either untreated or treated, and coming from domestic discharges, hospital effluents and industrial wastewaters (Bai et al., 2018; Morin-Crini et al., 2022; Stefanakis and Becker, 2015).



**Figure 2.** Sources and pathways of emerging contaminants in freshwaters. (Source: Stefanakis and Becker, 2015)

### **2.1.1. Pharmaceuticals and endocrine disrupting compounds in the environment**

Pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) are very diverse groups of substances used for medical or personal care, as well as in food and manufacturing industry, and are often detected in natural freshwaters polluted with wastewater (Tijani et al., 2013). It is confirmed that the presence of pharmaceuticals in freshwaters is primarily connected to the municipal wastewater treatment plant effluents (Morin-Crini et al., 2022). Pharmaceuticals are used as prescription or over the counter drugs for human use and veterinary therapeutic drugs used to prevent or treat animal diseases. They are designed to remain in organisms after intake, in order to have the therapeutic effect they are designed to have, which also means they are very often excreted from organisms unchanged and can have potential to persist in the environment (Boxall et al., 2004; Huerta et al., 2016).

More than 600 individual PhACs or their metabolites are found worldwide in many natural habitats, including surface waters (aus der Beek et al., 2016). Endocrine disrupting compounds (often referred also as endocrine disrupting chemicals) is a functional group that includes contaminants of various chemical classes but with one common thing: they are suspected or proven to disrupt endocrine systems in humans and/or animals (Caliman and Gavrilescu, 2009; Huerta et al., 2016). Even some pharmaceuticals (e.g. anti-inflammatory drug naproxen) are proven to act as endocrine disruptors as they have hormonal side effects (Caliman and Gavrilescu, 2009; Sabir et al., 2018; Weber et al., 2014). Quan et al. (2005) reported that 38 000 different chemicals and heavy metals are suspected to have endocrine disrupting effects on organisms. Despite the growing number of studies investigating the presence and ecological effects of pharmaceuticals and endocrine disrupting compounds on aquatic ecosystems, due to large number and diversity of these compounds and complexity of aquatic ecosystems, specific impacts of individual compounds as well as their mixtures are yet to be discovered (Ebele et al., 2017).

## **2.2. Fate and behaviour of emerging contaminants in freshwater environment**

Contaminants and high nutrients concentrations present in freshwater, mostly due to wastewater pollution, disrupt balance of aquatic ecosystems and can negatively impact aquatic biota (Bashir et al., 2020; Previšić et al., 2020; Grgić et al., 2023). Most of the recognized emerging contaminants are not regulated, monitored and often unstudied in environmental conditions, which means their fate and behaviour after entering aquatic ecosystems is still mostly unknown (Stefanakis and Becker, 2015).

### **2.2.1. Emerging contaminants in aquatic ecosystems**

After entering aquatic ecosystems, processes that ECs undergo are determined by physico-chemical properties of the contaminants and environmental conditions of the surroundings (Caliman and Gavrilescu, 2009; Loffredo and Senesi, 2006). Their occurrence and persistence in the environment differ, however many of them are bioactive and bioaccumulative and as such, they represent a risk to aquatic life and ecosystems (Stefanakis and Becker, 2015).

Wastewater treatment plant systems are in the vast majority not designed to specifically remove emerging contaminants from municipal or industrial wastewater. Due to physico-chemical properties of many pharmaceuticals, conventional wastewater treatment processes are mostly not suitable for their removal (Ebele et al., 2017). Water solubility largely impacts behaviour and persistence of pharmaceuticals and endocrine disruptors in water. Polar and soluble compounds are more easily diluted and dispersed in aquatic environment, while non-polar compounds are more prone to bond to sediment and lipids when entering biota (Caliman and Gavrilescu, 2009).

Contaminants can undergo different degradation processes in the environment and in that way new compounds are formed. These new compounds can differ from the parent compound and have different physico-chemical properties, which can result in forming less harmful contaminants or more persistent ones (Boxall et al., 2004). Biodegradation is one of the processes emerging contaminants can go through in aquatic environment and it is usually driven by microbial and algal communities (Ebele et al., 2017; Samal et al., 2022). These processes are highly dependent on environmental conditions like temperature, as low temperature decreases biodegradation kinetics (Caliman and Gavrilescu, 2009). Due to continuous use and release to the environment, many pharmaceuticals and personal care



products are considered to be “pseudo-persistent” and as such they can be more prone to persist in the environment compared to, for example, some pesticides (Ebele et al., 2017). Furthermore, Samal et al., (2022) point out that “pharmacological active contaminants generated from pharmaceuticals and personal care products are persistent in aqueous media and show resistance to degradation”. It is also confirmed that biodegradation processes in conventional treatment plants with activated sludge have low efficiency in removal of endocrine disrupting compounds from wastewater (Caliman and Gavrilescu, 2009), which indicates even lower efficiency for biodegradation in natural freshwaters. Emerging contaminants can also undergo a photodegradation process in the environment and the extent and efficiency of photodegradation depends on the intensity of solar irradiation, water depth, seasonality and other (Ebele et al., 2017). Boreen et al. (2003) point out that photodegradation processes likely play a major role in determining the environmental fate of many pharmaceuticals. However, some pharmaceuticals, for example carbamazepine, show resistance to photodegradation in surface waters (Yamamoto et al., 2009).

### **2.2.2. Emerging contaminants in living organisms: bioconcentration, bioaccumulation, bioamplification and biotransformation**

Emerging contaminants present in aquatic environment can be absorbed and ingested by aquatic organisms with aqueous (respiration and/or body surface) and dietary (with food) exposure (Arnot and Gobas, 2006). Once in a living organism, contaminants can be retained in it, or metabolically transformed or excreted by digestion or respiration (Mandaric et al., 2015). Uptake of contaminants solely through respiration and through body surface is considered as bioconcentration and is preferably studied in controlled laboratory conditions (Arnot and Gobas, 2006, 2003). Current study was conducted *in situ* and bioconcentration was not further discussed. When contaminant uptake includes not only dermal and respiratory absorption, but also dietary absorption, bioaccumulation is observed (Arnot and Gobas, 2006).

Many emerging contaminants, including various pharmaceuticals and endocrine disrupting compounds, are confirmed to bioaccumulate in different aquatic organisms (Lageson et al., 2016; Meredith-Williams et al., 2012; Previšić et al., 2021; Ruhí et al., 2016; Wilkinson et al., 2018). Emerging contaminants in living organisms also undergo processes of elimination from the body with respiratory exchange, faecal egestion, metabolic biotransformation of the parent compound and growth dilution (Arnot and Gobas, 2006). When the uptake of a certain

contaminant is higher than the elimination rate through these processes, contaminant is retained in the organism. Bioaccumulation rates vary depending on the contaminant properties and organism metabolism. Most of the studies on the bioaccumulation of the emerging contaminants are based on fish, which are, according to Huerta et al. (2012), the most suitable organisms to monitor pollution in the aquatic environment. Thus, bioaccumulation of various contaminants has been widely confirmed in fish, even for pharmaceuticals (Garcia et al., 2012; Lagesson et al., 2016; Schwaiger et al., 2004; Valdés et al., 2014) and endocrine disruptors (Fan et al., 2019; Liu et al., 2012). However, fish are not always present in either polluted or non-polluted rivers, which increases the value of macroinvertebrate taxa (like aquatic insects), which are mostly present in all freshwaters, as model organisms for pollution and bioaccumulation studies (Rodriguez et al., 2018).

Literature review shows that bioaccumulation in aquatic invertebrates, and more precisely in aquatic insects, has been recently increasingly investigated (Haddad et al., 2018; Lagesson et al., 2016; Meredith-Williams et al., 2012; Richmond et al., 2018; Ruhí et al., 2016). Moreover, research shows that pharmaceuticals and endocrine disruptors can bioaccumulate not only in fish, but also in aquatic invertebrates. Bioaccumulation of pharmaceuticals has been detected in molluscs (Burket et al., 2019; de Solla et al., 2016; Wilkinson et al., 2018), aquatic isopods (Lagesson et al., 2016), amphipods and aquatic insects (Meredith-Williams et al., 2012). Moreover, bioaccumulation of different endocrine disrupting compounds has also been confirmed in various aquatic invertebrates (Burket et al., 2019; Huerta et al., 2015; Previšić et al., 2019; Ruhí et al., 2016).

Bioaccumulation of compounds in certain organisms depends on their life history and ecological traits like trophic position, food and habitat preferences, type of metamorphosis, etc. (Cetinić et al., 2021; Kraus et al., 2014a; Lagesson et al., 2016; Previšić et al., 2021). For example, Lagesson et al., (2016) pointed out that organisms with lower trophic positions in aquatic environment, especially those living on sediment (e.g. planarians), are the prime receivers of the pharmaceuticals. Habitat and food preferences directly affect availability and exposure to different contaminants. Burrowing predatory aquatic insects (e.g. some dragonfly nymphs (Anisoptera) and alderfly larvae (Megaloptera)) are more exposed to contaminants bonded to sediment, whereas filter-feeders like many caddisfly larvae ((Trichoptera, e.g. Hydropsychidae) are more exposed to contaminants present in the water column (Kraus et al., 2014b; Previšić et al., 2021). Differences in these traits are present not only on higher taxonomic levels (e.g. order level), but also on lower taxonomic levels. In fact, research

indicates that fine scale differences in biology could impact taxa related variations in availability, exposure and retention of contaminants in the body (Previšić et al., 2021).

In some organisms, the concentration of contaminants can increase even in the absence of additional exposure, primarily due to body mass loss, leading to the phenomenon known as bioamplification (Kraus et al., 2014b). Bioamplification typically occurs during life stages characterized by substantial developmental changes that result in weight loss and/or a diminished capacity to eliminate pollutants from the body proportionally (Daley et al., 2009). For instance, aquatic insect metamorphosis alters metal concentrations in the organism mostly by reducing their body burdens, however, some essential (Cu, Zn, Se) and non-essential metals (Cd, Ag) have shown an opposite trend (Cetinić et al., 2021), confirming bioamplification of metals. Moreover, the bioamplification of contaminants across metamorphosis has been observed in both hemimetabolous and holometabolous aquatic insects. Examples include polychlorinated biphenyls (PCBs) in Ephemeroptera (Daley et al., 2011), organochlorine compounds and polybrominated diphenyl ethers in Diptera and Trichoptera (Bartrons et al., 2007), and pharmaceuticals and endocrine disrupting compounds in Trichoptera (Previšić et al., 2021). Still, this matter is mostly unexplored, especially for emerging contaminants like pharmaceuticals and endocrine disrupting compounds, and further research is necessary to address how this affects aquatic organisms and generally the aquatic ecosystems.

Contaminants can undergo biotransformation in living organisms. Fu et al. (2020) pointed out that biotransformation plays a crucial role in determining bioaccumulation potential and toxicity of contaminants. Moreover, Luo et al. (2022) also confirmed biotransformation is playing a key role in determining whether or not biomagnification of organic pollutants occurs. Research on biotransformation of pharmaceuticals and endocrine disrupting compounds is very limited. However, it is confirmed that biotransformation affects bioaccumulation of pharmaceuticals (e.g., in amphipods (Fu et al., 2020; Miller et al., 2017)). Moreover, current knowledge on this matter indicates that there are significant species-specific differences in the processes included in biotransformation of pharmaceuticals between different aquatic macroinvertebrates (Cervený et al., 2021). Furthermore, metamorphosis impacts the contaminant concentrations in the organism due to significant changes in body chemistry and metabolism that occur during this period of aquatic insect life cycle (Kraus et al., 2014b). For example, during metamorphosis, protein catabolism is replaced with lipid catabolism (Späth et al., 2022). These changes in metabolism affect biotransformation of contaminants and consequently determine retention and excretion of the contaminants. For example, metal

concentrations are more likely to be decreased during the metamorphosis (Cetinić et al., 2021; Kraus et al., 2014b; Wesner et al., 2017), due to their storage in lysosomes which are excreted into the adult gut abdomen during metamorphosis (Kraus et al., 2014b). Other contaminants, like PCBs, are predominantly retained during metamorphosis and their concentrations in adults are increased compared to larvae (Bartrons et al., 2007). Current study predominately focused on bioaccumulation and bioamplification of contaminants, however, biotransformation was also discussed while analysing the results of the study.

### **2.2.3. Effects of emerging contaminants in living organisms**

The presence of emerging contaminants in aquatic environment alters the life cycles of aquatic organisms, leading to subsequent impacts on entire ecosystems through changes in the food webs, predator-prey interactions, and nutrient cycling (Huang et al., 2015; Previšić et al., 2021). It is confirmed that exposure to contaminants, like pharmaceuticals and endocrine disrupting compounds, affects behaviour, survival, and emergence of aquatic insects (Bose et al., 2022; Oetken et al., 2005). At the organism level, effects of the contaminants present in their environment can be direct and indirect (Fleege, 2020). Direct effects include direct toxic effects on an organism's physiology, behaviour, reproduction, or survival. These effects can be lethal or sublethal and result in changes in communities that can lead to severe impacts to the whole ecosystem (Dodds, 2002; Fleege, 2020). For example, it is confirmed that the exposure of larval, prepupal and pupal stages of aquatic insects to certain contaminants can alter the processes of metamorphosis by interfering with crucial hormonal signals (Grgić et al., 2023). Furthermore, research also shows that certain chemicals alter progression of metamorphosis, leading to deformities, malformations and lowered survival rates of adults (Oetken et al., 2005; Wesner et al., 2020, 2014).

An additional risk of emerging contaminants in freshwaters is that prolonged exposure, due to constant inflow of wastewater, which can result in chronic effects (Dodds, 2002). For example, prolonged exposure to certain mutagen substance increases the probability of mutation (Dodds, 2002). Indirect effects are defined as those that occur at higher organisational levels in the environment (i.e., population, community, ecosystem) which makes them even harder to study (Fleege, 2020). Moreover, research confirms that effects of emerging contaminants also depend on specific environmental conditions. For example, zinc toxicity for fish is confirmed to be greater at high temperatures and low conductivity water (Dodds, 2002).

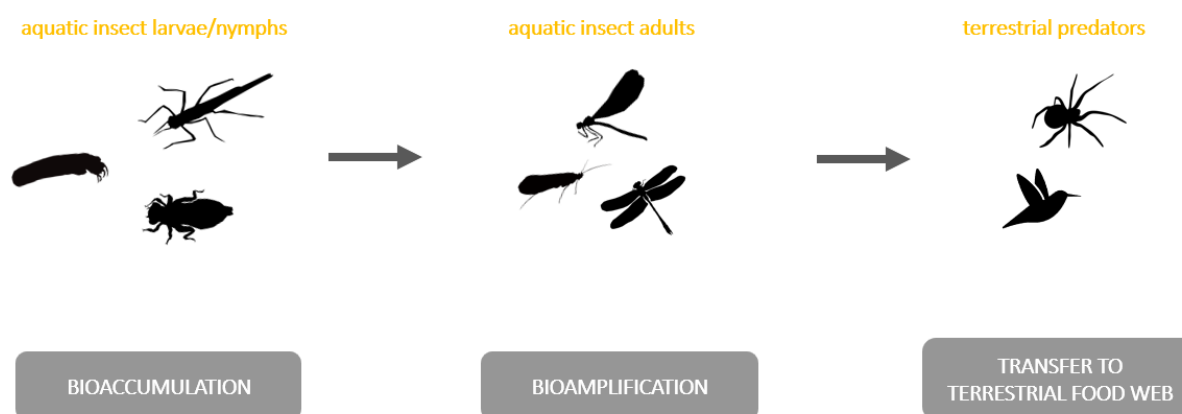
#### **2.2.4. Assessment of bioaccumulation and bioamplification of emerging contaminants using predictive approaches**

Predicting the bioaccumulation potential of emerging contaminants is novel and not very explored in studying bioaccumulation of emerging contaminants. According to literature, it usually includes using physico-chemical descriptors of the contaminants for various analyses, calculations, and models, in order to predict the fate of these contaminants in aquatic environment and aquatic organisms regarding their chemical properties (Arnot and Gobas, 2006, 2003; Du et al., 2014; Huerta et al., 2012; Stefanakis and Becker, 2015). Properties like polarity are directly connected to the tendency compound has to be in organic or aqueous phase and it is expressed as the descriptor octanol-water partition coefficient ( $K_{OW}$ ) (Stefanakis and Becker, 2015). More precisely, according to Arnot and Gobas (2006), “ $K_{OW}$  represents the lipophilicity and the hydrophobicity of a chemical and how it thermodynamically distributes, i.e., partitions, between aqueous and organic phases”. Using logarithm 10 of the octanol-water partition coefficient ( $\log K_{OW}$ ) has been identified as the appropriate measure for expressing the bioaccumulation potential of a chemical compound and  $\log K_{OW}$  greater than 5 is considered to indicate the compound has bioaccumulative potential (Arnot and Gobas, 2003). Predictors like  $\log K_{OW}$  and  $\log S$  (solubility) were used also in studies of predicting the bioaccumulative potential of pharmaceuticals and/or endocrine disrupting compounds (Du et al., 2014; Franke et al., 1994; Huerta et al., 2012). Furthermore, Caliman and Gavrilescu, (2009) pointed out that the hydrophobicity of a chemical substance will determine whether pharmaceuticals and endocrine disrupting compounds will bioaccumulate in the solid phase or not. However, initial research finding on predicting PhACs bioaccumulation in aquatic insects indicate that bioaccumulation cannot be readily predicted by basic physicochemical descriptors such as  $\log K_{OW}$ ,  $\log D$ ,  $\log S$  (Previšić et al., 2021). This is primarily because the predictive models are largely based on persistent organic pollutants, which may not accurately represent the behaviour of PhACs. As for bioamplification, it was confirmed that bioamplification of medium-chain chlorinated paraffins (SCCPs and MCCPs) (Liu et al., 2020) as well as persistent halogenated organic pollutants (Liu et al., 2018) in insects has also been related to  $\log K_{OW}$  values. However, data on pharmaceuticals and endocrine disrupting compounds are lacking, which highlights the need for additional and alternative approaches and models to be explored (e.g. OPLS-DA).

## 2.3. Cross-ecosystem transfer of emerging contaminants

### 2.3.1. Contaminant transfer across ecosystem boundary with emergent aquatic insects

Aquatic insects represent an important inter-habitat linkage between aquatic and terrestrial ecosystems as their life cycle includes aquatic (larvae/nymphs and, in some orders, pupae) and terrestrial (adults) life stages. Metamorphosis causes major changes in organisms' morphology, physiology and behaviour that allows an organism to inhabit different habitats in different stages of life cycle (Hodin, 2006; Rolff et al., 2019). In aquatic insects specifically, metamorphosis enables habitat switch from aquatic to terrestrial habitats. Emerging aquatic insects represent an important pathway for energy and nutrients transfer, but also for the transfer of bioaccumulated contaminants from aquatic to terrestrial environments (Bundschuh et al., 2022; Daley et al., 2011; Kraus et al., 2014a; Schulz and Bundschuh, 2020) (Figure 3).



**Figure 3.** Simplified scheme of the processes in aquatic-terrestrial transfer of the emerging contaminants with emergence of aquatic insects.

When adult aquatic insects are consumed by their terrestrial predators, contaminants built up during the aquatic phase of their life cycle can bioaccumulate in their predators (Previšić et al., 2021). Contaminants bioaccumulated during aquatic life stage can be retained or lost during metamorphosis (Bundschuh et al., 2022). Bioamplification across metamorphosis potentially represents an additional threat for contaminant transfer across ecosystem boundary, as the increase in concentration without additional exposure can result in terrestrial predators being exposed to even higher contaminant concentrations (Daley et al., 2011; Previšić et al., 2021). Bundschuh et al. (2022) point out that the “emergence-mediated contaminant transfer and thus the exposure risk for riparian insectivores partly depends on the impact of the same contaminants on aquatic life stages affecting their emergence success”. Furthermore, studies

show that pesticide contamination causes negative impacts on abundance of riparian spiders feeding on aquatic insects originating from a polluted stream (Graf et al., 2019). Adult caddisflies (Trichoptera), an important food source for riparian predators, show an increased body burden of pharmaceuticals and endocrine disrupting compounds, implying that terrestrial predators, such as riparian spiders, birds and bats are exposed to mixtures of contaminants of aquatic origin, which may affect their physiology and population dynamics (Previšić et al., 2021 and references therein).

### **2.3.2. Trophic transfer of emerging contaminants in aquatic-terrestrial food web**

Various taxa show different trends in contaminant bioaccumulation and bioamplification, implying that ecological traits, through determining contaminant availability and exposure, as well as bioaccumulation, play a major role also in determining the transfer of contaminants through food webs (Bartrons et al., 2007; Previšić et al., 2021). Research shows that bioaccumulation rates for some emerging contaminants increase through food chains (e.g. perfluorinated compounds (PFCs)), while for others the rate decreases, mostly because the biotransformation rate of these compounds increases with higher food chain levels (e.g. plasticizers) (Mandaric et al., 2015). When contaminant concentration is increasing through the food chain, biomagnification is observed. The definition of biomagnification says it is the “process by which lipid normalized chemical concentrations (i.e. CB /lipid content) increase with trophic level in a food-chain” (Arnot and Gobas, 2003). Therefore, for the chemicals that are confirmed to be biomagnifying in the food web, the highest bioaccumulation factors are observed for the highest trophic level species (Arnot and Gobas, 2006). However, depending on the contaminant and the organisms, if higher trophic level organism has higher ability to transform and metabolize the substance, then trophic dilution of the contaminant within the food web can happen, in which case higher bioaccumulation factors are observed for lower level organisms (Arnot and Gobas, 2006). Biomagnification is very well studied for persistent organic pollutants (POPs) as they show great tendency for bioaccumulation in human and animal tissues and for biomagnification in food chains, which unfortunately had significant effects on human health and the environment (Mandaric et al., 2015). For many other emerging contaminants, like pharmaceuticals, little is known about how they are distributed in food chains and what is the extent of their trophic transfer in natural aquatic food web (Lagesson et al., 2016). However, some studies on bioaccumulation of pharmaceuticals in experimental aquatic trophic chains were able to conclude that lower trophic level organisms (like algae)

bioaccumulate pharmaceuticals more than organisms on higher trophic levels (e.g. water fleas and fish) (Ding et al., 2015; Vernouillet et al., 2010). Furthermore, Huerta et al. (2016) pointed out that biofilm in general has substantial capacity to bioaccumulate different emerging contaminants and therefore could play a major role in transferring pharmaceuticals and endocrine disrupting compounds to the higher trophic levels of aquatic food webs. Another study confirmed bioaccumulation of several pharmaceuticals in all levels of an experimental food chain and the results indicated that some persistent pharmaceuticals can reach up in top consumers through trophic transfer, as despite decreasing water concentrations of oxazepam, temporally increased concentrations were observed in perch (Lagesson et al., 2016). Moreover, Ruhí et al. (2016) confirmed accumulation of two pharmaceuticals (diclofenac and gemfibrozil) and endocrine disruptor TBEP (flame retardant) in water, biofilm and at least one macroinvertebrate taxon in the studied food web. Furthermore, Du et al. (2014) and Xie et al. (2015) confirmed bioaccumulation of pharmaceuticals in organisms of different trophic levels of the food web, and both pointed out that none of the investigated compounds showed biomagnification.

Knowledge on lateral transport through food webs in the riparian zone for pharmaceuticals and endocrine disrupting compounds is generally lacking. However, several studies displayed the importance of emerging aquatic insects as a prey for terrestrial predators like spiders, birds and bats (Schulz et al., 2015a), meaning that they could represent their main source of the waterborne contaminants. Briers et al. (2005) found out that adult aquatic insects contributed to spider diets with the share of 40% adjacent to the stream, while at 20 meters from the stream the share was only 1%. Furthermore, the transfer of polychlorinated biphenyls (PCBs) to spiders through consumption of emergent aquatic insects extends to the distance of five meters inland, while in insect predators (mostly social wasps) the transfer reached 30 meters inland (Raikow et al., 2011). Even though information on the transfer of pharmaceuticals and endocrine disrupting compounds across aquatic-terrestrial ecosystem boundary is very scarce, Previšić et al. (2021) and Richmond et al. (2018) confirmed accumulation of several compounds in aquatic insects and riparian spiders from the same study site. Schulz et al. (2015) pointed out that it is important to observe the abundance of emerging insects but also their seasonal timing as the crucial factor for the receiving ecosystem. However, due to diverse chemistry of emerging contaminants and unique characteristics of the food webs in nature (water column depth, dietary preference, primary production and organic matter, trophic structure, temperature, and varying degrees of benthic interaction with the sediment (Burkhard,

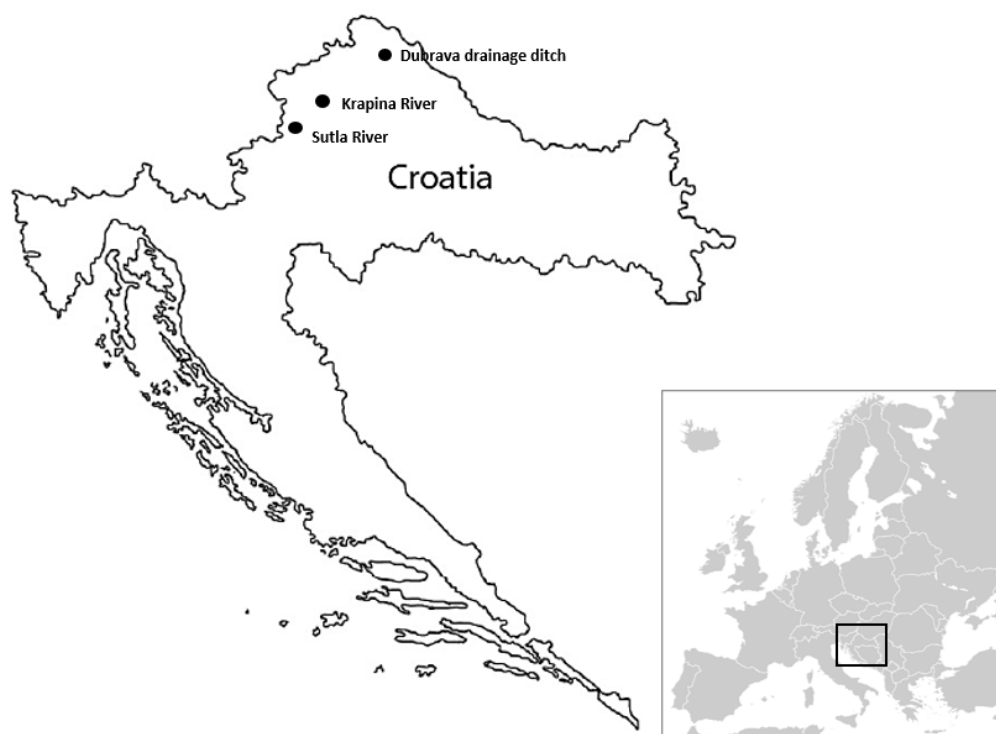


2003; Gobas and Maclean, 2003)), Arnot and Gobas (2006) stated that it is difficult to compare bioaccumulation of contaminants between two food webs.

### **3. MATERIAL AND METHODS**

#### **3.1. Study sites**

To conduct the planned research, three study sites located in north-west Croatia were selected: Krapina River, Drava River's Dubrava hydropower plant drainage ditch and Sutla River (Figure 4). Study sites were selected based on the availability of the organisms acquired for the research, such as aquatic insects (Trichoptera and Odonata) and diverse riparian aquatic-terrestrial food web. Additionally, sites were chosen considering the presence of wastewater effluent pollution, to ensure the presence of contaminants in water and the exposure of the organisms to these contaminants. Krapina and Sulta, tributaries of the Sava River, are medium sized lowland rivers with mostly natural watercourses. The study site at the Krapina River was near Kupljenovo, a settlement belonging to the town of Zaprešić, and the study site on the Sutla River was downstream the small town Klanjec. Both study sites are impacted with wastewater effluents, either with untreated or treated wastewater with secondary wastewater treatment systems. The Krapina River is a recipient of wastewater from multiple smaller and larger villages but also the city of Zabok, positioned approximately 15 km upstream from the study site. Likewise, the Sutla River receives wastewater from numerous smaller towns and villages. Notably, the closest one to the study site on the Sutla River is Klanjec, and its wastewater treatment plant, utilizing a secondary treatment system, is located approximately 500 meters upstream from the study site. Unlike the Krapina and Sutla rivers, the Dubrava drainage ditch is an artificial channel but according to the size and microhabitats present, it is very similar to a large lowland stream/small lowland river. Main source of pollution for the selected study site is untreated wastewater from the wastewater collector of the nearest town Prelog, which is drained through permeable soil into the Dubrava drainage ditch. At Krapina and Dubrava study sites collection of aquatic insects was conducted for examining contaminants in different aquatic life stages (chapter 4.1.), while in samples collected at Sutla River study site, contaminants were analysed in organisms from different trophic levels of aquatic-terrestrial food web (4.3.).



**Figure 4.** Study sites locations in NW Croatia: the Dubrava drainage ditch, the Krapina and Sutla rivers.

## 3.2. Research design and sampling

### 3.2.1. Sampling of aquatic insects for studying bioaccumulation and bioamplification of emerging contaminants

To study bioaccumulation and bioamplification patterns of emerging contaminants in aquatic insects, Odonata (dragonflies and damselflies) and Trichoptera (caddisflies) were sampled at study sites and from watercourses impacted with wastewater effluents. Both, Odonata and Trichoptera, were sampled two times within maximally 30 days in order to collect different life stages: aquatic larvae/nymphs and pupae (for Trichoptera) and terrestrial imagines. Moreover, two collections within maximally 30 days also had the aim to reduce variability in temporal dynamics of aquatic insect flux (Kato et al., 2003).

At Krapina study site, aquatic insects sampling included aquatic and terrestrial stages of the two Odonata suborders, Anisoptera (dragonflies) and Zygoptera (damselflies) (Figure 5, Table A1). More specifically, five different species were collected: *Gomphus vulgatissimus* (Linnaeus, 1758), *Orthetrum albistylum* (Selys, 1848), *Onychogomphus forcipatus* (Linnaeus, 1758), *Calopteryx splendens* (Harris, 1782) and *Platynemesis pennipes* (Pallas, 1771). Adult insects were collected in riparian zones sweeping riparian vegetation along the watercourse (up to three m laterally) using an entomological net. In order to avoid collecting specimens that have potentially dispersed from a different location and/or have fed as adults, we collected solely teneral adults, as particularly Anisoptera are known to disperse over relatively long distances (Corbet, 1999). Odonata nymphs were sampled with a D-net screening all present freshwater microhabitats. Screening stretch was approximately 100 meters long. In order to enable reliable species identification, but also to accurately reflect effects of bioamplification on bioaccumulated contaminants, we sampled nymphs belonging to final instars, that is within the size ranges listed for last instars for particular taxa (Brochard et al., 2012) (Table A1).

At Dubrava study site two collections were conducted in late spring within one month period and both collections included aquatic (larval and pupal) and terrestrial (adult) stages of the caddisfly *Silo nigricornis* (Pictet, 1834), Goeridae (Figure 6). This species is regarded as low dispersing species, rarely moving more than a few meters from the sites of their emergence (Sode and Wiberg-Larsen, 1993). Aquatic stages, i.e., *S. nigricornis* larvae and pupae, were sampled with a D-net screening of all present freshwater microhabitats in approximately 100 meters stretch of the drainage ditch. Adults were collected up to three meters laterally from the

stream using an insect net. Furthermore, to have more insights on how dietary and/or respiratory exposure impacts bioaccumulation in Trichoptera and Odonata, water samples were collected at both Krapina and Dubrava study sites while biofilm samples were collected only at Dubrava study site by brushing and scraping rocks from the stream. Upon transfer to laboratory and before further processing, larval samples of both Odonata and Trichoptera were left for 24 hours in river water to ensure gut clearance filled with water from respective sites. All taxa were separated according to species, and all samples, including water and biofilm samples, were freeze-dried, and stored at -80°C until further processing.



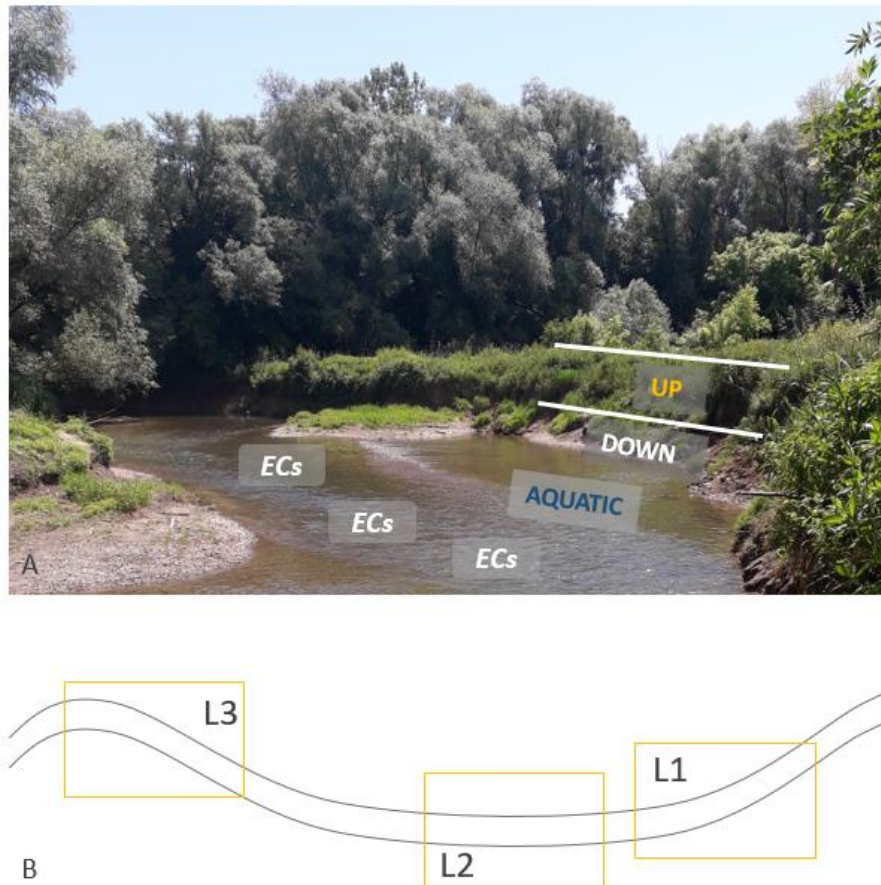
**Figure 5.** The Krapina River study site. A) Study site on the Krapina River; B) Adult damselfly (male) *Calopteryx splendens*.



**Figure 6.** The Dubrava study site. A) Drainage ditch – study site; B) Caddisfly *Silo nigricornis* larvae.

### **3.2.2. Sampling of aquatic and terrestrial invertebrates to study trophic transfer and cross-ecosystem transfer of emerging contaminants**

The study design for riparian food web and trophic transfer of emerging contaminants research included sampling of aquatic and terrestrial organisms belonging to different trophic levels as well as water, biofilm, macrophytes and soil samples. To collect as complete food web as possible, three sampling locations were selected, and they were positioned 50 meters apart on the same riverbank of the Sutla River (Figure 7B). Sampling was conducted two times in the span of one month and each sampling lasted two days, as 24-hour traps were also included in sampling. Aquatic samples at each sampling location included water, biofilm and macrophyte samples as well as aquatic insect samples. Biofilm was collected by brushing and scraping rocks from the stream and macrophytes were collected by hand. Aquatic insects were collected using D-net screening all present microhabitats. Soil samples were collected on the riverbank with digging on each sampling location. To inspect the extent of contaminants transfer into the riparian zone, each sampling location was divided in two transects on the riverbank: Down and Up. The transect Down included the stretch of the riverbank in immediate proximity of the river with a maximum distance of one meter. The transect Up was higher on the riverbank with distance from the river ranging from one to three meters (Figure 7A).



**Figure 7.** Design of the food web transfer study on the Sutla River. A) aquatic and two terrestrial transects on different distances from the river; B) three locations on the Sutla river where sampling was conducted.

On both transects, various terrestrial invertebrates were collected using several methods. Pit-fall traps were set for 24 hours on both transects to catch ground spiders (Araneae), isopods (Isopoda), millipedes (Diplopoda) and beetles (Coleoptera). Earthworms (Lumbricidae) were collected by digging on both transects of all three sample locations and net-hunting spiders were collected with insect net (Figure 8). Furthermore, Odonata adults were also collected with an insect net, whereas Trichoptera adults were collected by setting up UV lamp traps with ethanol during approximately two hours in the evening. List of all taxa collected in this study is presented in Table A2. After collection, all samples were transported to the laboratory. To empty their gut matter, aquatic insects were left in river water and earthworms on clean wet paper towels overnight. All samples were freeze dried (Freeze dryer Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH; Figure 9A) and stored at -80 degrees Celsius.





**Figure 8.** Invertebrates collected for the food web transfer study on the Sutla study site. A) Coleoptera and Diplopoda; B) Araneae; C) Odonata nymphs; D) Trichoptera adults caught using UV lamp trap.



### **3.3. Sample processing and contaminants quantification**

#### **3.3.1. Sample preparation**

All Odonata specimens from Krapina study site were identified to species level and pooled per species (from both collection dates) to create a composite sample (for Anisoptera 4-6 larvae and 1-3 adults, and for Zygoptera 6 – 15 larvae and 15 - 20 adults/per species). Freeze dried samples were homogenized and three analytical replicates of 50 mg freeze-dried insect tissue were created for each species. For *S. nigricornis* specimens from Dubrava study site, different life stages (larvae, pupae, imagines) were pooled per sampling dates to form a composite sample. Samples were freeze dried and homogenized. From each composite sample, an analytical triplicate of 50 mg freeze-dried insect tissue was created (corresponding to a mass of approximately 12 larvae and 20-22 pupae and imagines each). Biofilm samples from Dubrava study site were also freeze dried, homogenized, and weighed in replicates of 50 mg. All animal samples from Sutla study site were freeze dried and homogenized using bead mill homogenizer, whereas macrophytes and biofilm samples were homogenized using liquid nitrogen and shredding with mortar and pestle. Samples from Sutla study site were weighed in triplicates of 30 mg and criteria for making the composite samples were as described for samples from Krapina and Dubrava study sites. Water samples from all study sites were first filtered with 0.45 µm membrane syringe filters. Afterwards, triplicates per sample were formed, for Krapina and Dubrava study sites replicates of 250 ml, and for Sutla study site replicates of 155 ml.

#### **3.3.2. Sample extraction**

Extraction process for all biological samples was the same and it consisted of two parts. First part of the extraction process included sequence of dissolving the samples in the solvent acetonitrile (ACN; Fisher Scientific, UK) that was always kept in the freezer at -20 degrees Celsius. First step was adding 1.5 ml ACN and 30 µl of 1mg/mL internal standards (Table A3, Table A4) into 30 mg samples. Samples were then mixed in a vortex for 5 minutes, and after they were put in the freezer for 10 minutes and centrifuged on 20000 xg for 10 minutes (CENTRIC 200R, Tehnica, Slovenija). Supernatants were then isolated and kept in the freezer. Pellets were dissolved with additional 1.5 ml ACN and sonication (50%, 1 minute) was used for further extraction of compounds in samples. Samples were then again mixed for 5 minutes, put in the freezer for 10 minutes and centrifuged on 20000 xg for 10 minutes. Second

supernatant was isolated and for further processing pellet was not used. Both supernatants were dried in an evaporator (Figure 9B) for approximately 2.5 hours and stored in the freezer. Second part of extraction was purifying supernatants with solid phase extraction (SPE) using Oasis HLB columns (Waters Corporation, USA). The dried supernatants were dissolved in 1.5 ml of water (Fisher Scientific, UK) and mixed in vortex at medium speed for 20 min. Then samples were purified on Oasis HLB columns that were preconditioned by applying 1 ml of ACN and 1 ml of water (Figure 9C). The columns were placed on a vacuum extractor (Figure 9C). After applying the samples, every column was purified by applying 1 ml of water. Then the samples were washed from the column into a clean tube by adding 1.5 ml of ACN. The speed of the sample passing through the column was monitored to be about  $1 \mu\text{s}^{-1}$ . The purified samples were then dried on the evaporator (Figure 9B). Water samples were directly purified with the solid phase extraction (SPE) using Oasis HLB columns and the procedure was the same as with animal samples. All final extracts were evaporated to dryness and reconstituted with 0.3 mL of methanol/water (50:50, v/v) prior to mass spectrometric analysis.



**Figure 9.** Sample processing. A) freeze drying of samples; B) drying supernatants in evaporator; C) purifying samples with Oasis HLB columns.

### 3.3.3. Quantification of contaminants

Target analysis was performed using ultra-high performance liquid chromatography (Waters Acquity Ultra-Performance™, Waters, UK) coupled to quadrupole linear ion trap mass spectrometry (UPLC–QqLIT) (Applied Biosystems, USA). Mobile phases used for UPLC separation in positive mode were methanol (mobile phase A) and 0.1% formic acid in water

(mobile phase B), while in negative mode: acetonitrile (mobile phase A) and 5 mM ammonium acetate at pH 9 (mobile phase B). Instrument control, data acquisition, and data analysis were carried out using Analyst 1.5.1 software (Applied Biosystem). Details regarding instrument-dependent and scheduled MRM parameters are summarized in references Gros et al. (2012) and Jakimska et al. (2013). The list of compounds analysed in the samples from Krapina and Dubrava is shown in Table A3, while Table A4 shows the compounds analysed in the samples from Sutla.

For each compound, two MRM transitions between the precursor ion and the two most abundant fragment ions were monitored, with the exception of the isotopically labelled internal standards, which are unlikely to be present in the environmental matrix. Therefore, only one transition was monitored. The first transition is used for quantification, while the second is used to confirm the identity of the target compounds. In addition to monitoring the MRM transitions, other identification criteria were used for quantification: (i) the agreement of the UHPLC retention time of the compound in the standard with that in the samples (the retention time in the sample must be within  $\pm 1\%$  of the retention time of the compound in the standards) and (ii) the comparison between the relative abundances of the two selected analyte-MRM transitions in the sample with those in the standards. These relative abundances in the samples must be within  $\pm 30\%$  of the two MRM ratios in the analytical standards. Target compounds were quantified using an internal standard method with Analyst 1.5.1 software (Applied Biosystem). Detailed information regarding UPLC separation, ion source and MRM parameters is summarized in *Methods – additional information* in the Appendix. . For each individual compound, an internal standard of known concentration was added into the samples. That internal standard went through all stages of preparation and analysis like the target compound, which made it possible to compensate for possible compound losses during sample preparation (Ho et al., 2003). The concentration of the targeted compounds was achieved based on the ratio of signal areas of the target compound and the internal standard using a calibration curve.

### 3.4. Statistical analyses

#### 3.4.1. Contaminant concentrations analyses in Odonata and Trichoptera

Differences in total concentration of ECs (sum of quantified PhACs and EDCs), total concentration of PhACs, total concentration of EDCs and individual compounds concentrations quantified in Odonata samples in different life stages (nymphal [ny] and adult stage = imago [im]) were tested within the species/suborder/order using the Mann-Whitney U test (Table A7 and Table A8). The same test was also used to infer differences in ECs concentrations between Zygoptera and Anisoptera nymphs and adults (Table A9). Differences in concentrations of total ECs, total PhACs, total EDCs and individual compounds were tested among different life stages (larva [lv], pupa [pu] and imago [im]) of the Trichoptera species *Silo nigricornis*, using the Kruskal Wallis H test and Multiple comparisons *post hoc* test. The same tests were used also to compare differences in concentrations of total ECs and individual compounds among caddisfly, water, and biofilm samples from the Dubrava study site. Furthermore, nonparametric correlations of individual ECs concentrations among water, biofilm samples and Trichoptera samples were also calculated. The same analysis was performed also for individual contaminant concentrations in Odonata samples and water samples from the Krapina study site. All tests were conducted in SPSS ver. 27 (IBM).

#### 3.4.2. Calculation and analyses of bioaccumulation and bioamplification factors of contaminants

With the aim of comparing bioaccumulation of PhACs and EDCs among different taxa, bioaccumulation factors (BAFs) were calculated. BAFs were calculated by dividing concentrations of individual compounds in nymphs/larvae, at both suborder and order levels of Odonata and for *S. nigricornis* (Trichoptera) with concentrations quantified in water samples from the Krapina and Dubrava study sites, respectively (Arnot and Gobas, 2006; Ruhí et al., 2016; Sims et al., 2020). Given that certain compounds' concentrations were below the detection limit in water samples, BAF values were calculated for 15 compounds for Odonata samples, as shown in Figure 14. For Trichoptera samples, due to the same limitation as previously stated, BAF values were calculated for 13 compounds (Figure 15).

Bioamplification factors (BAMFs) were calculated to evaluate differential cross-ecosystem flux of PhACs and EDCs via aquatic insect emergence. BAMFs were calculated as the ratio of

concentrations of PhACs and EDCs between two consecutive life stages (Daley et al., 2011) and according to Daley (2013), bioamplification occurs when BAMF exceeds value of 1. For hemimetabolous Odonata one BAMF value was calculated, i.e. between adults and nymphs at the suborder and order level. Since Trichoptera are holometabolous, two BAMFs were calculated for each compound, i.e. between *S. nigricornis* pupae and larvae concentrations, and between adults and pupae concentrations. BAMF calculation was possible for 17 compounds in Odonata samples and 13 compounds that were quantified in *S. nigricornis* samples. As for statistical tests, for calculation of both factors, BAF and BAMF, all analytical replicates at the species level were included as input (all data for Odonata, and separately for Anisoptera and Zygoptera, respectively).

### **3.4.3. An assessment of applicability of existing approaches to predicting the bioaccumulation and bioamplification of PhACs and EDCs**

With the aim of assessing the relation of physico-chemical descriptors of PhACs and EDCs and bioaccumulation and bioamplification across metamorphosis in Odonata and Trichoptera, physico-chemical properties of individual ECs were compiled using National Institutes of Health (Maryland, USA) PubChem open chemistry database and DrugBank Online (University of Alberta, CA). This was also conducted with the aim of assessing the possibility to predict bioaccumulation potential of contaminants using these molecular descriptors. The most widely used descriptors: the octanol–water partition coefficient ( $\log K_{OW}$ ), and relative molecular mass ( $M_r$ ), aqueous solubility ( $\log S$ ), number of rotatable bonds and number of hydrogen bond donors and acceptors were used (Table S3) (Mamy et al., 2015 and references there in). Octanol–water distribution coefficient ( $\log D_{OW}$ ) and membrane-water distribution coefficient ( $\log D_{MW}$ ) were also considered and methods for estimating the distribution coefficients of studied PhACs and EDCs are summarized in the Appendix. The relationships between physico-chemical descriptors and  $\log$  BAFs as well as  $\log$  BAMFs (e.g. Liu et al., 2018, 2021) at the order (for Odonata and Trichoptera) and suborder level (for Odonata) were analysed by nonparametric correlations (Spearman's rank correlation) and linear regressions in SPSS 27 (IBM). Furthermore, orthogonal partial least squares – discriminant analysis (OPLS-DA) was employed to find descriptors responsible for bioaccumulation in aquatic insect tissues. For this purpose, OPLS-DA analysis was performed on a two-group data set; ECs quantified in both, nymphal/larval tissues, and water (assumed to be bioaccumulative) versus ECs only detected in water (assumed to be non bioaccumulative). For the OPLS-DA analysis, data matrix with

already mentioned descriptors was extended by absorption, distribution, metabolism, and excretion (ADME) properties of each compound. ADME descriptors (total of 28 properties) were calculated using the pkCSM platform available web interface (Pires et al., 2015). Final data matrix (provided in Supporting Material) contained following descriptors: log  $K_{OW}$ , log  $K_{MW}$ , log  $D_{OW}$ , log  $D_{MW}$ , water solubility, caco-2 cell permeability, intestinal absorption (human), skin permeability, P-glycoprotein substrate, P-glycoprotein I inhibitor, P-glycoprotein II inhibitor, CYP2D6 substrate, CYP3A4 substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, VDss (human), fraction unbound (human), BBB permeability, CNS permeability, total Clearance, renal OCT2 substrate, molecular mass, rotatable bonds, number of acceptors, number of donors, molecular surface area. OPLS-DA analysis was done in program R using the *ropls* package (Thévenot et al., 2015).

#### **3.4.4. Contaminant concentrations analyses regarding different trophic levels of a riparian food chain and the distance from the river**

Differences in total concentrations of ECs and individual compounds concentrations quantified in different biota samples belonging to aquatic and terrestrial food web collected at Sutla study site were tested using Kruskal-Wallis H test (SPSS ver. 27, IBM). To better visualize data on individual compounds, a concentration shade plot was made using Primer (Primer Version 7, PRIMER-e). Furthermore, concentrations of compounds quantified in the same taxa from both transects (Up and Down), were used to test differences in bioaccumulation at different distances from the river. For these analyses, compound concentrations in Lumbricidae, *Carabus*, Diplopoda and Araneae were used, as these groups were collected on both transects. Total ECs and individual compounds concentrations were tested between two transects using the Mann-Whitney U test (SPSS ver. 27, IBM). The same test was also used to analyse Up and Down transect differences in concentrations within the same taxa (Lumbricidae, *Carabus*, Diplopoda and Araneae separately). For Araneae, due to two families present on the site (Lysocidae and Tetragnathidae), additional tests were made observing concentrations quantified on family level. Lastly, aquatic insect concentrations were also observed separately as there were two suborders of Odonata present at the study site. Therefore, comparison of concentrations of total ECs, total parabens and individual compound concentrations that were measured in different life stages of Zygoptera and Anisoptera was conducted using the Mann-Whitney U test.

## **4. RESULTS**

### **4.1. Differences in bioaccumulation patterns of emerging contaminants on different taxonomic levels of aquatic insects**

#### **4.1.1. Emerging contaminants measured in Odonata and water samples from the Krapina river study site**

Odonata and water samples from the Krapina river study site were screened for a total of 143 contaminants, 119 PhACs and 24 EDCs. A total of 37 compounds was quantified in the Krapina water samples (25 PhACs and 12 EDCs; Table A5), while in aquatic and terrestrial stages of Odonata 20 compounds were measured (8 PhACs and 12 EDCs; Table 1).

Bioaccumulation patterns of measured PhACs and EDCs exhibited variations across taxa and life stages of aquatic insects in the present study. The total concentrations of ECs (sum of PhACs and EDCs) and EDCs separately, were significantly elevated in nymphal stages of Odonata (213% and 388% for ECs and EDCs, respectively) as well as on suborder level of Zygoptera (242% and 552% for ECs and EDCs, respectively; see Figure 10A&E; Mann-Whitney U test, Table A7). Whereas in Anisoptera, only the total concentration of EDCs showed significantly higher values in nymphal stages compared to adults (233% higher; see Figure 10 C, Mann-Whitney U test; Table A7). The suborder level (Anisoptera and Zygoptera) showed the slightly higher variability, with concentrations significantly differing in 11 compounds in Zygoptera (differences ranging from 41-100%) but only six in Anisoptera (differences ranging from 37-100%) (see Figure 10D&F; Mann-Whitney U test, Table A7). Correspondingly, the concentrations of individual ECs were positively correlated between life stages on order level as well as for Anisoptera (Spearman's rank correlation, Table 3). The same was also confirmed for Odonata level (Spearman's rank correlation, Table 2). On the other hand, for Zygoptera, which showed highest concentrations variability between life stages, there was no statistically significant correlation observed between nymphs and adults (Spearman's rank correlation, Table 4).

**Table 1.** Concentration of emerging contaminants measured aquatic (NY – nymphs) and terrestrial (IM – imagines) life stages of Odonata from the Krapina river – shown on order, suborder and species level, and their concentrations in water. Concentrations are shown as mean values and standard deviation in ngg<sup>-1</sup> of dry weight for Odonata samples and in ngL<sup>-1</sup> for water samples. (AZM – azithromycin, TIM – tilmicosin, GLC – glibenclamide, TIB – thiabendazole, CAZ – carbamazepine, KPF – ketoprofen, NPX – naproxen, SAA – salicylic acid; EDCs: 1HB – 1H-benzotriazole, CFN – caffeine, TBEP-tris(2-butoxyethyl)phosphate, TCPP – tris(1-chloro-2-propyl)phosphate, TCEP – tris(2-carboxyethyl)phosphine, PRG – progesterone, BPA – bisphenol-A, E3 – estriol, TCL – triclosan, MPB – methylparaben, EPB – ethylparaben, PBB – propylparaben; n.d. – not detected)

	Water	Odonata		Anisoptera		<i>Gomphus vulgatissimus</i>		<i>Onychogomphus forcipatus</i>		<i>Orthetrum albistylum</i>		Zygoptera		<i>Calopteryx splendens</i>		<i>Platynemis pennipes</i>	
		NY	IM	NY	IM	NY	IM	NY	IM	NY	IM	NY	IM	NY	IM	NY	IM
Ecs	2379.615 (988.293)	328.290 (139.385)	154.266 (80.835)	305.138 (190.877)	163.400 (105.618)	294.710 (103.458)	261.614 (144.958)	384.631 (341.968)	137.933	236.071 (35.962)	90.652 (15.101)	351.443 (60.372)	145.132 (50.421)	370.243 (80.411)	123.955 (44.363)	369.547 (38.173)	175.152 (23.574)
PhACs	1781.905 (900.571)	133.429 (161.188)	102.995 (76.634)	184.864 (20.480)	110.412 (100.334)	155.222 (97.136)	198.346 (149.577)	350.678 (338.193)	76.688	48.692 (16.170)	56.203 (13.485)	81.993 (25.435)	95.577 (47.843)	102.243 (38.560)	82.474 (48.546)	69.794 (8.146)	104.533 (27.604)
EDCs	597.710 (171.188)	193.070 (95.699)	49.759 (17.548)	118.828 (69.357)	51.079 (17.169)	137.985 (15.149)	60.594 (19.350)	32.522 (5.576)	59.450	185.975 (22.234)	33.193 (9.162)	267.313 (47.426)	48.438 (18.858)	265.610 (49.339)	40.324 (4.266)	298.120 (36.345)	69.640 (4.261)
AZM	0.847 (0.281)	1.401 (0.438)	1.369 (0.606)	1.088 (0.137)	1.737 (0.683)	1.117 (0.220)	2.417 (0.568)	1.074 (0.014)	1.795	1.074 (0.155)	1.000 (0.167)	1.714 (0.411)	1.000 (0.088)	1.925 (0.222)	1.055 (0.018)	1.257 (0.105)	0.979 (0.149)
TIM	n.d.	0.390 (0.075)	0.144 (0.230)	0.357 (0.064)	0.171 (0.280)	0.386 (0.091)	0.257 (0.445)	0.356 (0.045)	n.d.	0.330 (0.061)	0.257 (0.224)	0.422 (0.074)	0.116 (0.180)	0.464 (0.098)	0.102 (0.177)	0.376 (0.047)	n.d.
GLC	n.d.	0.402 (0.323)	0.517 (0.350)	0.141 (0.211)	0.494 (0.454)	n.d.	n.d.	n.d.	1.044	0.422 (0.021)	0.437 (0.022)	0.664 (0.150)	0.541 (0.230)	0.733 (0.101)	0.452 (0.417)	0.481 (0.039)	0.616 (0.012)
TIB	0.189 (0.219)	n.d.	0.358 (0.475)	n.d.	0.263 (0.474)	n.d.	0.790 (0.524)	n.d.	n.d.	n.d.	n.d.	n.d.	0.453 (0.485)	n.d.	0.218 (0.377)	n.d.	0.376 (0.180)
CAZ	5.319 (0.698)	0.011 (0.017)	n.d.	0.015 (0.022)	n.d.	0.0129 (0.016)	n.d.	n.d.	n.d.	0.032 (0.029)	n.d.	0.007 (0.012)	n.d.	n.d.	n.d.	0.020 (0.013)	n.d.
KPF	88.983 (29.391)	26.755 (23.103)	40.378 (25.737)	29.471 (31.974)	28.010 (20.084)	65.117 (28.045)	40.914 (34.787)	n.d.	19.603	23.296 (6.060)	23.514 (4.148)	24.039 (9.762)	52.746 (25.692)	31.154 (12.733)	43.272 (21.275)	18.822 (4.806)	78.664 (22.623)
NPX	86.789 (29.762)	23.866 (45.982)	0.000 (0.000)	38.958 (61.973)	n.d.	n.d.	n.d.	113.454 (53.218)	n.d.	3.419 (5.922)	n.d.	8.774 (11.826)	n.d.	9.419 (16.315)	n.d.	16.903 (8.835)	n.d.
SAA	8.688 (3.903)	24.567 (14.241)	22.705 (22.314)	15.379 (9.582)	29.016 (25.998)	21.287 (11.095)	26.448 (45.809)	17.573 (8.696)	43.034	7.279 (3.252)	17.566 (10.188)	33.754 (12.215)	16.395 (17.106)	39.796 (11.282)	15.451 (9.181)	20.097 (6.827)	8.477 (0.784)



	Water	Odonata		Anisoptera		<i>Gomphus vulgatissimus</i>		<i>Onychogomphus forcipatus</i>		<i>Orthetrum albistylum</i>		Zygoptera		<i>Calopteryx splendens</i>		<i>Platycnemis pennipes</i>	
		NY	IM	NY	IM	NY	IM	NY	IM	NY	IM	NY	IM	NY	IM	NY	IM
IHB	43.728 (23.943)	88.981 (53.238)	17.227 (14.130)	52.389 (46.924)	13.299 (15.743)	65.631 (44.639)	n.d.	n.d.	33.993	91.535 (11.670)	5.905 (1.276)	125.573 (28.429)	21.154 (11.905)	127.324 (28.265)	13.607 (4.119)	131.030 (28.510)	34.894 (4.185)
CFN	456.590 (124.873)	34.510 19.798	2.157 (4.963)	18.572 (13.829)	4.314 (6.470)	13.779 (7.927)	n.d.	6.695 (2.695)	12.941	35.243 (5.656)	n.d.	50.448 (8.378)	n.d.	56.293 (6.378)	n.d.	48.992 (11.149)	n.d.
TBEP	42.221 (8.905)	8.256 (14.563)	7.236 (14.538)	10.780 (20.236)	11.169 (19.635)	22.911 (35.468)	26.686 (31.585)	2.771 (4.799)	3.266	6.659 (3.815)	3.555 (1.649)	5.732 (5.180)	3.304 (5.368)	6.057 (5.197)	6.120 (9.173)	5.176 (5.391)	2.356 (3.263)
TCPP	33.900 (11.245)	40.475 (26.109)	4.761 (3.361)	18.889 (17.424)	5.004 (2.808)	20.339 (18.894)	3.085 (3.113)	1.891 (1.953)	5.583	34.438 (7.371)	6.344 (3.624)	62.060 (9.829)	4.518 (3.999)	57.953 (3.659)	4.177 (3.160)	72.687 (10.521)	7.282 (4.513)
TCEP	2.184 (0.751)	2.399 (2.406)	1.676 (1.609)	0.605 (1.222)	1.456 (1.792)	n.d.	n.d.	n.d.	3.667	1.816 (1.635)	0.701 (1.214)	4.193 (1.888)	1.897 (1.478)	2.906 (2.642)	1.884 (1.668)	5.247 (1.188)	2.694 (0.609)
PRG	n.d.	n.d.	0.092 (0.213)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.183 (0.279)	n.d.	n.d.	n.d.	0.550 (0.092)
BPA	6.188 (5.932)	8.263 (7.887)	11.773 (8.661)	7.866 (7.007)	12.212 (11.066)	12.086 (11.667)	22.605 (9.138)	4.083 (0.726)	n.d.	7.431 (3.356)	14.031 (3.953)	8.659 (9.096)	11.333 (6.041)	4.888 (4.255)	8.905 (4.123)	17.452 (9.902)	15.415 (3.834)
E3	n.d.	1.427 (3.627)	1.059 (1.841)	2.854 (4.835)	1.148 (2.213)	n.d.	3.443 (2.781)	8.561 (4.495)	n.d.	n.d.	n.d.	n.d.	0.971 (1.513)	n.d.	0.962 (1.665)	n.d.	n.d.
TCL	0.082 (0.137)	0.277 (0.478)	0.249 (0.221)	0.433 (0.642)	0.170 (0.235)	0.765 (1.174)	0.463 (0.159)	0.297 (0.137)	n.d.	0.237 (0.059)	0.046 (0.042)	0.121 (0.133)	0.328 (0.184)	0.037 (0.032)	0.190 (0.173)	0.233 (0.119)	0.364 (0.034)
MPB	3.698 (2.036)	6.473 (4.900)	2.783 (1.657)	4.859 (2.606)	1.801 (1.633)	1.932 (1.677)	3.649 (0.446)	7.036 (0.968)	n.d.	5.608 (1.616)	1.754 (0.691)	8.086 (6.195)	3.764 (0.998)	8.259 (7.315)	3.611 (0.121)	12.652 (1.171)	4.696 (0.198)
EPB	0.527 (0.150)	0.298 (0.446)	0.182 (0.201)	0.251 (0.385)	0.090 (0.139)	n.d.	n.d.	n.d.	n.d.	0.752 (0.162)	0.269 (0.068)	0.345 (0.519)	0.274 (0.217)	n.d.	0.247 (0.219)	1.035 (0.068)	0.453 (0.109)
PPB	0.493 (0.289)	1.712 (1.324)	0.565 (0.337)	1.330 (0.842)	0.417 (0.355)	0.543 (0.477)	0.663 (0.075)	1.189 (0.132)	n.d.	2.257 (0.588)	0.587 (0.320)	2.095 (1.639)	0.713 (0.257)	1.893 (1.650)	0.622 (0.264)	3.616 (0.269)	0.936 (0.050)

**Table 2.** Nonparametric correlations of individual ECs concentrations among water and Odonata samples calculated using Spearman's rank correlation. Significance is indicated in bold. (W – water, NY – nymphs, IM – imagines)

Correlations					
			W	NY	IM
Spearman's rho	W	Correlation	1.000	0.757**	0.807**
		Coefficient			
		Sig. (2-tailed)		<b>0.000</b>	<b>0.000</b>
	N	20	20	20	
	NY	Correlation	0.757**	1.000	0.550*
		Coefficient			
		Sig. (2-tailed)	<b>0.000</b>		<b>0.012</b>
	N	20	20	20	
	IM	Correlation	0.807**	0.550*	1.000
		Coefficient			
Sig. (2-tailed)		<b>0.000</b>	<b>0.012</b>		
N	20	20	20		
** Correlation is significant at the 0.01 level (2-tailed)					
* Correlation is significant at the 0.05 level (2-tailed)					

**Table 3.** Nonparametric correlations of individual ECs concentrations among water and Anisoptera samples calculated using Spearman's rank correlation. Significance is indicated in bold. (W – water, NY – nymphs, IM – imagines)

Correlations					
			W	NY	IM
Spearman's rho	W	Correlation	1.000	0.674**	0.810**
		Coefficient			
		Sig. (2-tailed)		<b>0.001</b>	<b>0.000</b>
		N	20	20	20
	NY	Correlation	0.674**	1.000	0.556*
		Coefficient			
		Sig. (2-tailed)	<b>0.001</b>		<b>0.011</b>
		N	20	20	20
	IM	Correlation	0.810**	0.556*	1.000
		Coefficient			
		Sig. (2-tailed)	<b>0.000</b>	<b>0.011</b>	
		N	20	20	20

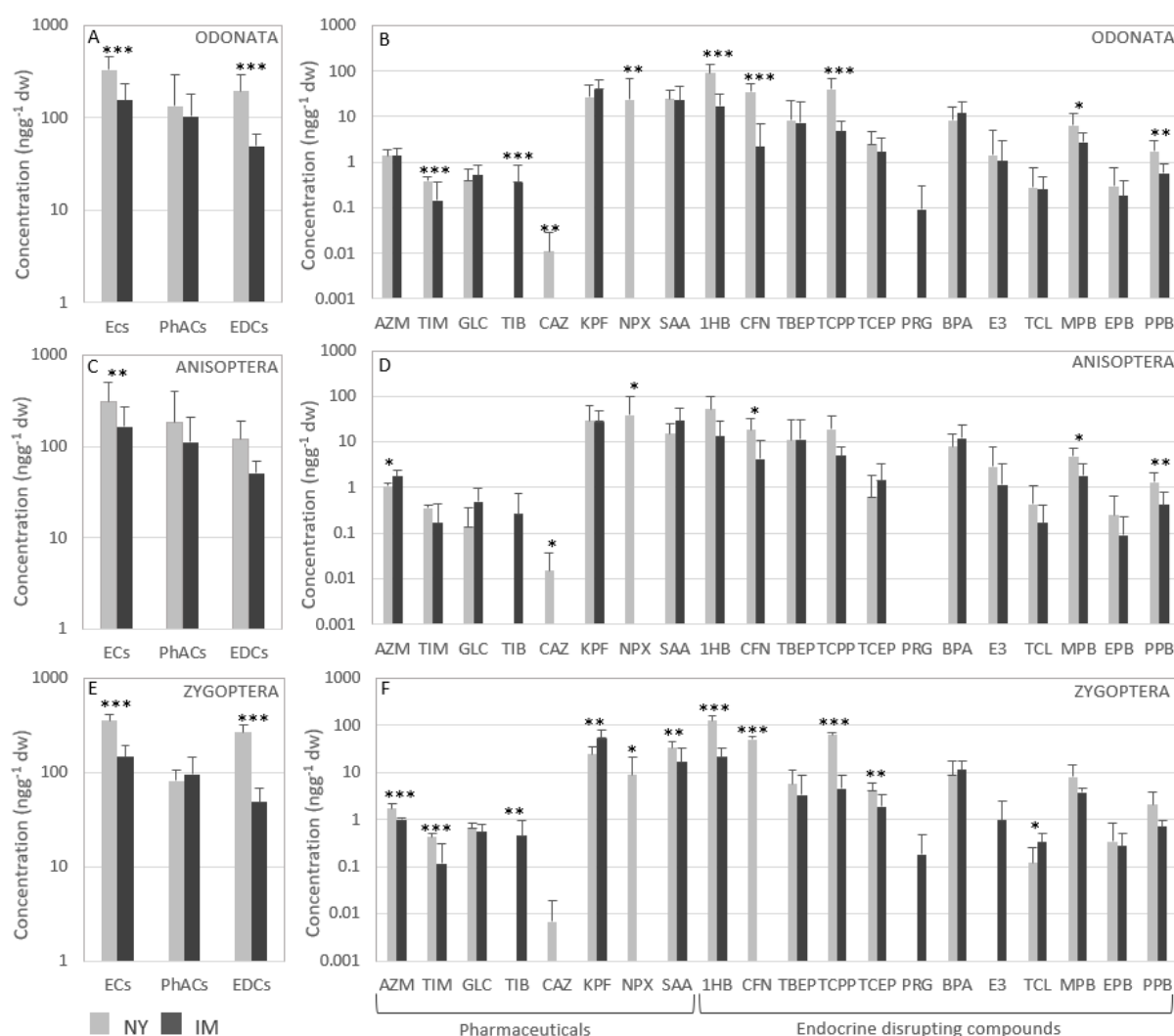
\*\* Correlation is significant at the 0.01 level (2-tailed)

\* Correlation is significant at the 0.05 level (2-tailed)

**Table 4.** Nonparametric correlations of individual ECs concentrations among water and Zygoptera samples calculated using Spearman's rank correlation. Significance is indicated in bold. (W – water, NY – nymphs, IM – imagines)

Correlations					
			W	NY	IM
Spearman's rho	W	Correlation Coefficient	1.000	0.528*	0.832**
		Sig. (2-tailed)		<b>0.017</b>	<b>0.000</b>
		N	20	20	20
	NY	Correlation Coefficient	0.528*	1.000	0.320
		Sig. (2-tailed)	<b>0.017</b>		0.169
		N	20	20	20
	IM	Correlation Coefficient	0.832**	0.320	1.000
		Sig. (2-tailed)	<b>0.000</b>	0.169	
		N	20	20	20
** Correlation is significant at the 0.01 level (2-tailed)					
* Correlation is significant at the 0.05 level (2-tailed)					

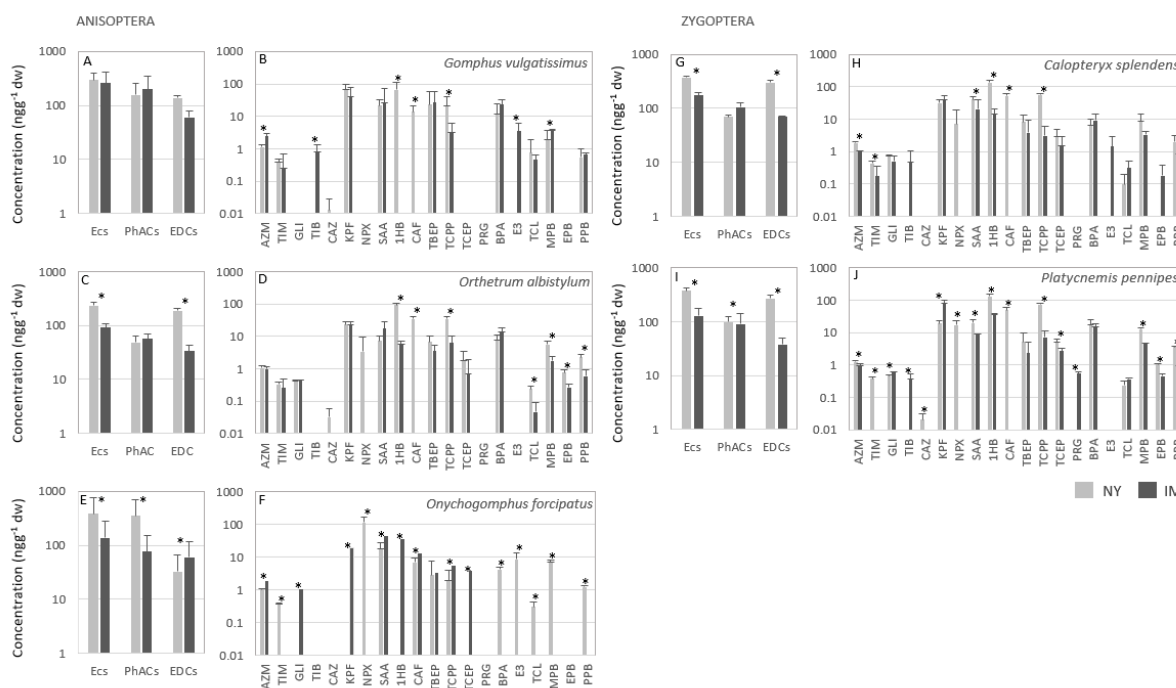
Zygoptera nymphs showed significantly higher concentrations in eight individual compounds compared to adults, including antibiotics (e.g., tilmicosin (TIM); 363% higher concentrations in nymphs), nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., salicylic acid (SAA); 206% higher concentration in nymphs), and organophosphorus flame retardants (OPFRs; e.g., TCPP 1374% higher concentration in nymphs; see Figure 10F; Mann-Whitney U test, Table A7). Concentrations of five individual compounds in Anisoptera nymphs similarly differed significantly from concentrations in adults, including preservatives (the methyl- and propylparaben (MPB and PPB), 270% and 319% higher concentrations in nymphs, respectively; see Figure 10D; Mann-Whitney U test, Table A7).



**Figure 10.** Total concentrations (A, C & E) of emerging contaminants (ECs: sum of PhACs & EDCs), pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) and individual compounds concentrations (B, D & F) in nymphs (NY) and adults (IM) of Odonata and separately on suborder taxonomic levels, in aquatic and terrestrial stages of Anisoptera and Zygoptera from the Krapina River – Kupljenovo, Croatia. Concentrations are shown as mean values (with standard error bars) in logarithmic scale in  $\text{ng g}^{-1}$  dry weight, significance is tested with the Mann-Whitney U test and listed in Table A7. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 1 caption.

In contrast, only one PhAC (antibiotic azithromycin (AZM)) showed a significantly higher concentration in the adult terrestrial stages of Anisoptera compared to aquatic nymphs (160% higher), and three individual compounds were significantly higher in adult Zygoptera compared to aquatic nymphs (e.g., triclosan (TCL), 272% higher; see Figure 10D&F; Mann-

Whitney U test, Table A7). None of the compounds exhibited significantly higher concentrations in terrestrial stages compared to nymphs on the order level (Figure 10B; Mann-Whitney U test, Table A7).

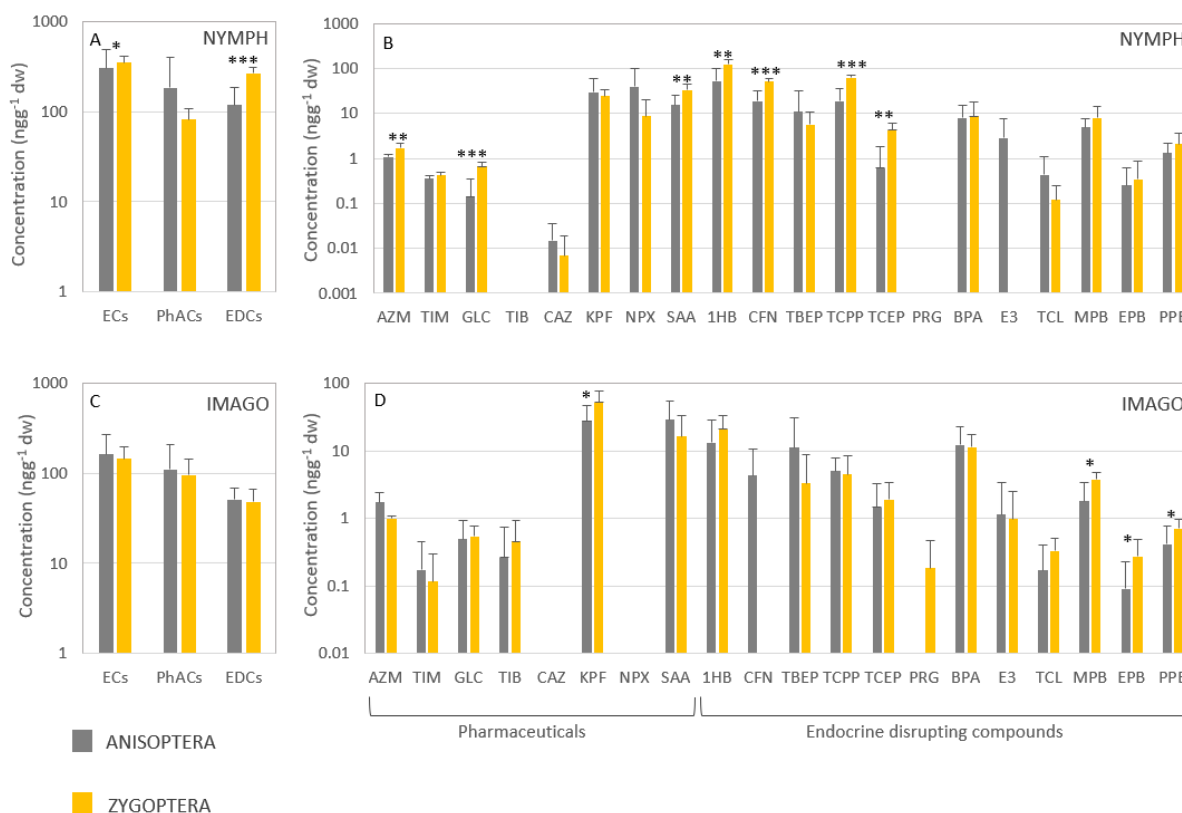


**Figure 11.** Total concentrations (A, C, E, G & I) of emerging contaminants (ECs: sum of PhACs & EDCs), pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) and individual compounds concentrations (B, D, F, H & J) in nymphs (NY) and adults (IM) stages of Odonata species level from the Krapina River – Kupljenovo, Croatia. Concentrations are shown as mean values (with standard error bars) in logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Mann-Whitney U test and listed in Table A8. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 1 caption.

On species level, some variability in bioaccumulation, but without clear patterns, was observed among species of both suborders concerning both total concentrations and concentrations of individual compounds (Figure 11B, D, F, H, J; Mann-Whitney U test, Table A8).

Comparison of the total concentrations of measured contaminants between the two suborders (Anisoptera and Zygoptera) reveals significantly higher values of total ECs and EDCs in zygopteran nymphs (115% and 225%, respectively; see Figure 12A; Mann-Whitney U test, Table A9). Accordingly, significant differences were observed for seven individual compounds, all exhibiting higher values in zygopteran nymphs (ranging from 158% to 693%;

see Figure 12B). In adults, total ECs concentrations (sum of PhACs & EDCs) showed no significant difference between the two suborders (Figure 12C; Mann-Whitney U test, Table A9), whereas in four individual compounds (the Nonsteroidal Anti-Inflammatory Drug (NSAID) ketoprofen (KPF) and the three parabens (MPB, EPB, PPB)), significantly higher values were measured in zygotpteran adults (ranging from 171% to 305%; see Figure 12D; Mann-Whitney U test, Table A9).



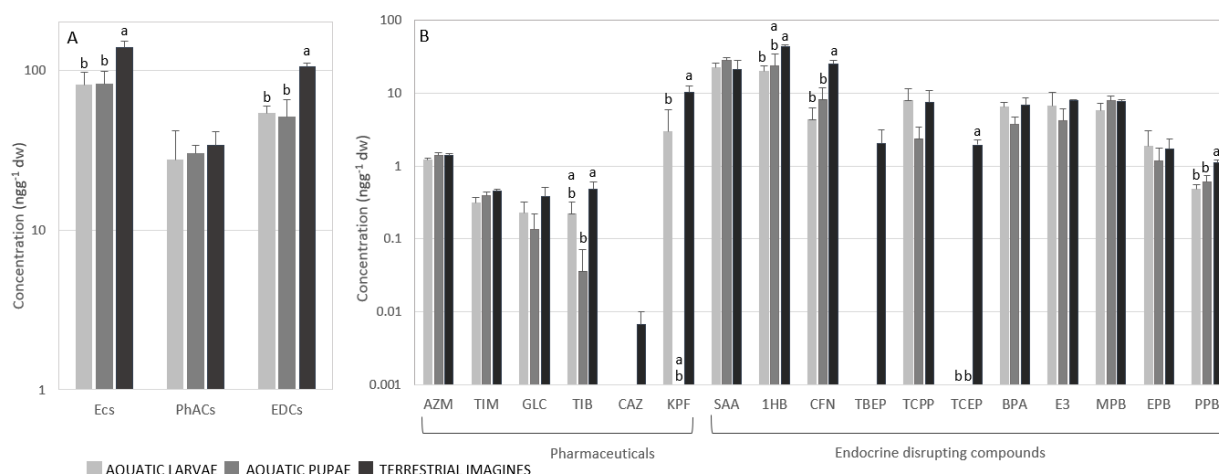
**Figure 12.** Total concentrations (A, C) of emerging contaminants (ECs: sum of PhACs & EDCs), pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) and individual compounds concentrations (B, D) in aquatic and terrestrial stages of Anisoptera and Zygoptera. Concentrations are shown as mean values (with standard error bars) in logarithmic scale in B) and D), significance is tested between suborders with the Mann-Whitney U test and listed in Table A9. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 1 caption.

#### 4.1.2. Emerging contaminants measured in Trichoptera, water and biofilm samples from the Dubrava drainage ditch

In the water samples collected at the Dubrava study site, a total of 28 compounds was measured, including 17 PhACs and 11 EDCs (Table A6). However, in biofilm samples, only five PhACs and five EDCs were quantified (Table A6). Across all life stages of the caddisfly *Silo nigricornis*, concentrations of 17 compounds were measured, including seven PhACs and 10 EDCs. Compounds with highest concentrations differed among all sample types, i.e. the highest concentration in water was measured for ibuprofen metabolite 2-hydroxyibuprofen ( $137 \text{ ngL}^{-1}$ , Table A6), whereas in biofilm samples norfluoxetine (metabolite of the antidepressant fluoxetine;  $267 \text{ ngg}^{-1}$ , Table A6) had the highest concentration. In all caddisfly samples, the highest value stands out for 1H-benzotriazole (1HB) reaching  $43 \text{ ngg}^{-1}$  in adults (Table 5).

Concentrations of individual PhACs and EDCs in *S. nigricornis* samples exhibit variations among different life stages. Conversely, the total concentration of all measured ECs as well as total EDCs concentration indicate a body burden increase with development, as their concentrations are significantly higher in terrestrial adult caddisflies compared to both pupae and larvae (Figure 13A; Kruskal-Wallis H test, Table A11, A12). Specifically, the total ECs concentration was 41% higher in adults than in pupae and larvae, while EDCs concentrations were 49% and 51% higher in adults compared to larvae and pupae, respectively. However, no significant differences were observed between aquatic stages (larvae vs pupae, see Figure 13A; Kruskal-Wallis H test, Table A10). Correspondingly, the concentrations of ECs demonstrated a strong positive correlation among all life stages (Spearman's rank correlation; Table 6).

However, for the majority of individual compounds, no significant differences were noted in concentrations between life stages. Nonetheless, some EDCs (e.g., CNS stimulant caffeine – CFN, 1HB, and preservative propylparaben – PPB; see Figure 13B; Kruskal-Wallis H test, Table A10) exhibited significantly higher concentrations in terrestrial adult stages compared to aquatic stages. For instance, the increase in concentrations in adults compared to larvae was 82% in CFN, 54% in 1HB, and 56% in PPB. Organophosphate flame-retardants, TBEP and TCEP, as well as carbamazepine (CAZ), were only measured above the limits of quantification in adults of *S. nigricornis* (Figure 13B; Kruskal-Wallis H test, Table A11).



**Figure 13.** Concentrations of (A) total concentrations of emerging contaminants (ECs) and (B) individual compounds in *Silo nigricornis* (Goeridae, Trichoptera) aquatic and terrestrial stages from the Dubrava drainage ditch, Croatia. Concentrations are shown as mean values (with standard error bars) in logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Kruskal-Wallis H test and listed in Table A10. Significance is shown with letters (a, b) and different letters depict significant differences ( $p > 0.05$ ). Full names of ECs are listed in Table 5 caption.



**Table 5.** Concentration of emerging contaminants measured in aquatic (LV – larvae; PU – pupae) and terrestrial (IM – imagines) life stages of *Silo nigricornis* (Goeridae, Trichoptera) from the Dubrava drainage ditch Croatia and their concentrations in water and biofilm samples. Concentrations are shown as mean values and standard deviation in ngg<sup>-1</sup> of dry weight for biofilm and caddisfly samples and in ngL<sup>-1</sup> for water samples. (AZM – azithromycin, TIM – tilmicosin, GLC – glibenclamide, TIB – thiabendazole, CAZ – carbamazepine, KPF – ketoprofen, SAA – salicylic acid, 1HB – 1H-benzotriazole, CFN – caffeine, TBEP – tris(2-butoxyethyl) phosphate, TCPP – tris(1-chloro-2-propyl) phosphate, TCEP – tris(2-carboxyethyl) phosphine hydrochloride, BPA – bisphenol-A, E3 – estriol, MPB – methylparaben, EPB – ethylparaben, PBB – propylparaben)

	AZM	TIM	GLC	TIB	CAZ	KPF	SAA	1HB	CAF	TBEP	TCPP	TCEP	BPA	E3	MPB	EPB	PBB	Total ECs
Water	0.971 (0.370)	0.000 (0.000)	0.000 (0.000)	0.010 (0.012)	1.790 (0.593)	14.460 (7.051)	11.923 (7.495)	8.423 (3.046)	55.044 (17.639)	9.937 (9.902)	12.688 (14.592)	2.426 (1.824)	1.194 (0.277)	0.000 (0.000)	2.891 (3.251)	0.746 (1.13)	0.123 (0.174)	122.627 (13.341)
Biofilm	2.114 (0.818)	0.914 (0.435)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.191 (2.062)	25.093 (5.447)	0.000 (0.000)	25.081 (3.000)	10.566 (2.066)	45.349 (27.186)	0.000 (0.000)	1.223 (2.119)	0.000 (0.000)	4.203 (1.880)	0.000 (0.000)	0.000 (0.000)	115.733 (12.906)
LV	1.190 (0.231)	0.310 (0.154)	0.223 (0.246)	0.218 (0.242)	0.000 (0.000)	2.980 (7.299)	22.521 (7.158)	20.035 (8.712)	4.305 (4.873)	0.000 (0.000)	7.944 (8.803)	0.000 (0.000)	6.519 (2.382)	6.684 (8.915)	5.813 (3.591)	1.867 (2.929)	0.483 (0.180)	81.091 (6.794)
PU	1.405 (0.282)	0.391 (0.123)	0.134 (0.207)	0.035 (0.087)	0.000 (0.000)	0.000 (0.000)	28.263 (5.167)	23.289 (27.018)	8.070 (8.868)	0.000 (0.000)	2.333 (2.738)	0.000 (0.000)	3.679 (2.461)	4.157 (4.705)	7.827 (3.076)	1.178 (1.378)	0.596 (0.339)	81.357 (8.360)
IM	1.380 (0.187)	0.450 (0.088)	0.377 (0.302)	0.474 (0.316)	0.007 (0.008)	10.163 (5.688)	20.936 (18.347)	43.179 (6.233)	24.902 (8.660)	2.002 (2.743)	7.372 (8.141)	1.943 (0.786)	6.919 (3.914)	7.866 (0.717)	7.628 (0.925)	1.700 (1.592)	1.103 (0.256)	138.399 (11.554)

**Table 6.** Nonparametric correlations of individual ECs concentrations among water and *S. nigricornis* samples calculated using Spearman's rank correlation. Significance is indicated in bold. (W – water, B – biofilm, LV – larvae, PU – pupae, IM - imagines)

			Correlations				
			W	B	LV	PU	IM
Spearman's rho	W	Correlation	1.000	0.646**	0.291	0.174	0.660**
		Coefficient					
		Sig. (2-tailed)		<b>0.009</b>	0.292	0.536	<b>0.007</b>
	B	N	15	15	15	15	15
		Correlation	0.646**	1.000	0.398	0.367	0.400
		Coefficient					
		Sig. (2-tailed)	<b>0.009</b>		0.142	0.179	0.139
	LV	N	15	15	15	15	15
		Correlation	0.291	0.398	1.000	0.868**	0.756**
		Coefficient					
		Sig. (2-tailed)	0.292	0.142		<b>0.000</b>	<b>0.001</b>
	PU	N	15	15	15	15	15
		Correlation	0.174	0.367	0.868**	1.000	0.674**
		Coefficient					
IM	IM	Sig. (2-tailed)	0.536	0.179	<b>0.000</b>		<b>0.006</b>
		N	15	15	15	15	15
		Correlation	0.660**	0.400	0.756**	0.674**	1.000
		Coefficient					
		Sig. (2-tailed)	<b>0.007</b>	0.139	<b>0.001</b>	<b>0.006</b>	
		N	15	15	15	15	15

\*\* Correlation is significant at the 0.01 level (2-tailed)

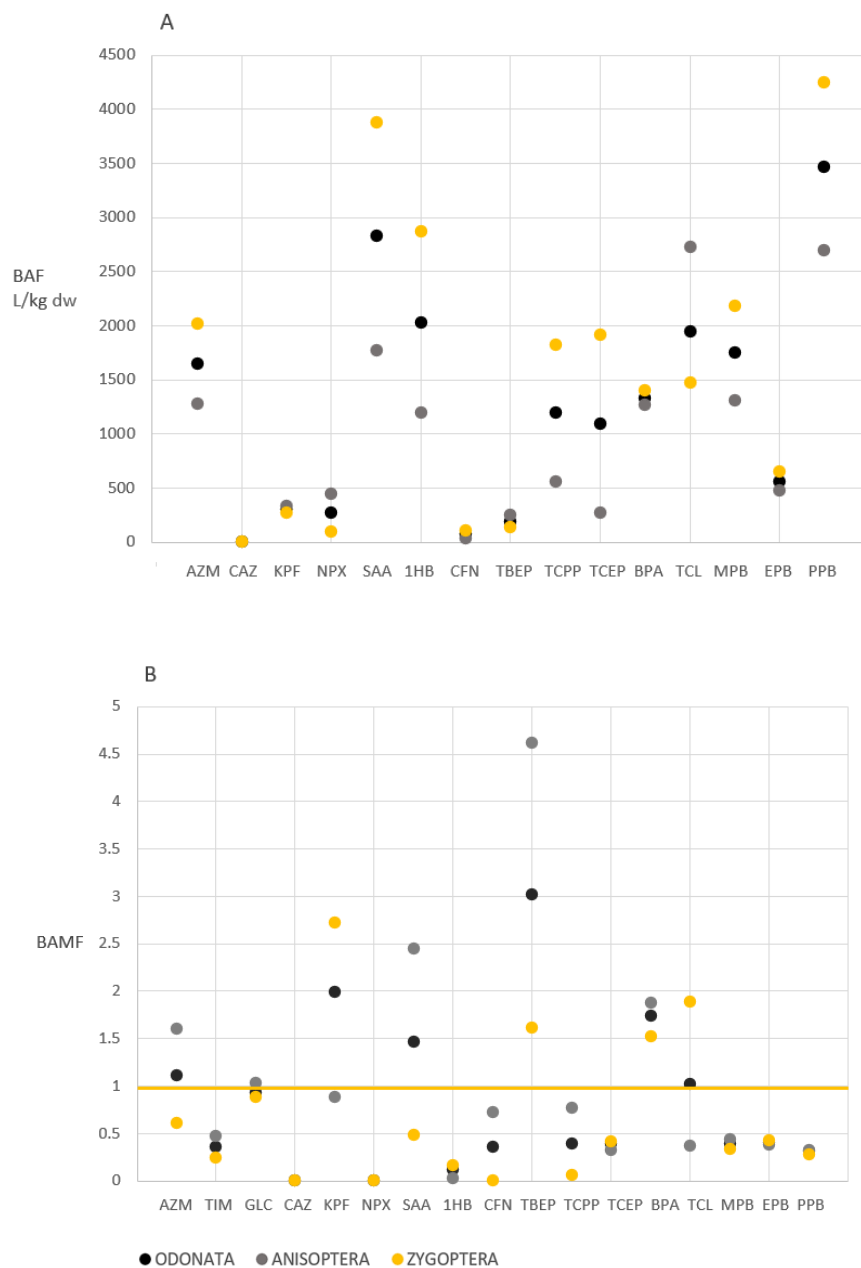
\* Correlation is significant at the 0.05 level (2-tailed)

Comparison of EC concentrations measured in different life stages of *S. nigricornis* with water and biofilm concentrations revealed that the highest total concentrations of ECs, as well as some individual PhACs and EDCs, were measured in *S. nigricornis* adults (see Table 5). Furthermore, concentrations of ECs showed a strong positive relationship between water and adult stages of *S. nigricornis* only (Spearman's rank correlation; Table 6).

#### **4.1.3. Bioaccumulation factors and bioamplification factors of measured emerging contaminants in Odonata and Trichoptera**

BAF values were calculated for 15 and BAMF values for 17 compounds detected in Odonata nymphs and water samples from the Krapina study site. In accordance with patterns observed for concentrations of individual compounds, both BAF and BAMF values also show variability between different taxonomic levels, especially between two suborders (Figure 14A). Comparing BAFs of ECs for aquatic nymphal stages of Zygoptera and Anisoptera, the following highest values stand out: propylparaben (PPB) and salicylic acid (SAA) for Zygoptera and propylparaben (PPB) and triclosan (TCL) for Anisoptera (Figure 14A). Generally, Zygoptera BAF values are higher for 10 out of 15 compounds, with five compounds having at least double the BAF values of Anisoptera. Moreover, organophosphorus flame retardants TCP and TCEP, have three times and almost seven times higher BAFs in Zygoptera compared to Anisoptera, respectively (Figure 14A).

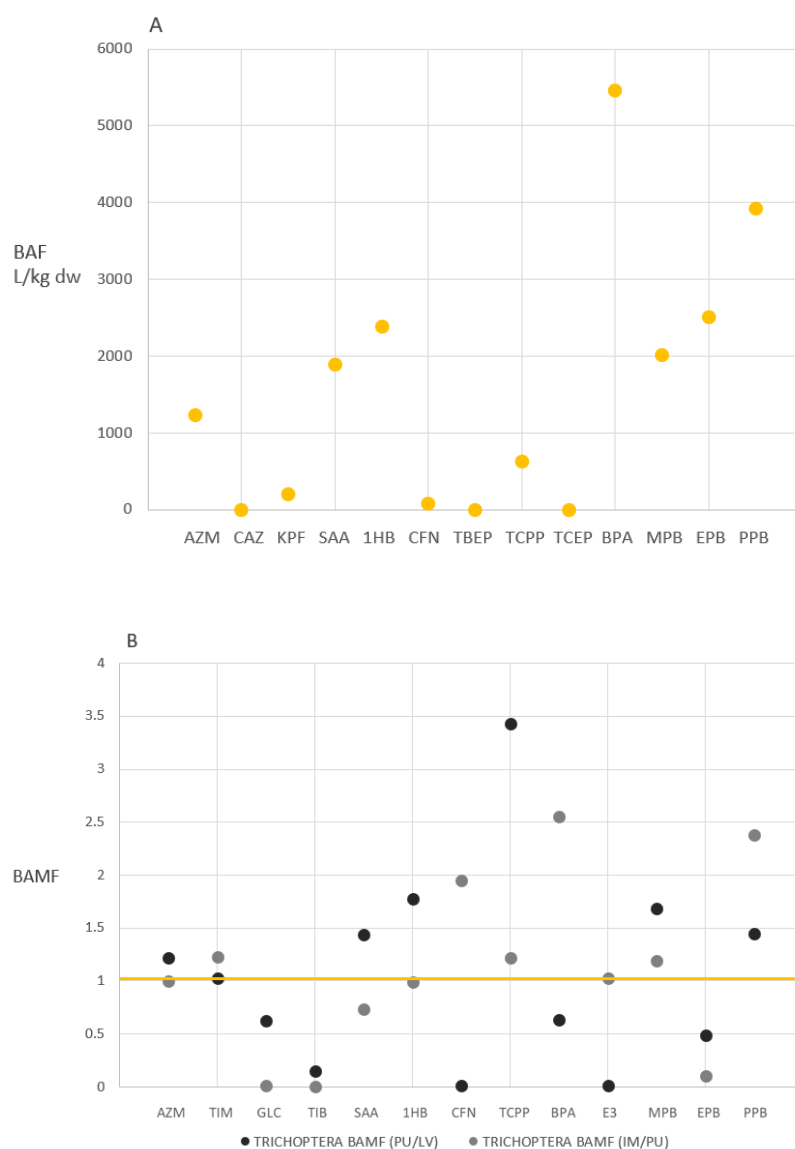
BAMF values patterns for Zygoptera and Anisoptera are not consistent (Figure 14B). Overall, seven compounds (41%) have BAMF values  $\geq 1$  for at least one suborder, whereas two compounds, TBEP and bisphenol-A show bioamplification in both, Anisoptera and Zygoptera (Figure 14B). Azitromycin (AZM), glibenclamide (GLI) and salicylic acid (SAA) BAMFs indicate bioamplification in Anisoptera solely. On the other hand, ketoprofen and triclosan have BAMF values  $\geq 1$  only in Zygoptera (Figure 14B).



**Figure 14.** Bioaccumulation factors (A; BAFs; L/kg dw) and bioamplification factors (B; BAMF) of emerging contaminants for aquatic stages of Odonata and each suborder separately (Anisoptera and Zygoptera). Yellow line (B) represents the BAMF value threshold (1) above which contaminant is considered to undergo bioamplification across metamorphosis. Full names of ECs are listed in Table 1 caption.

BAF values were calculated for 13 compounds measured in Trichoptera larvae and water samples from the Dubrava study site. From 13 calculated BAFs, the highest value stands out for bisphenol-A (BPA) and propylparaben (PPB) with values of 5461 and 3916, respectively (Figure 15A).

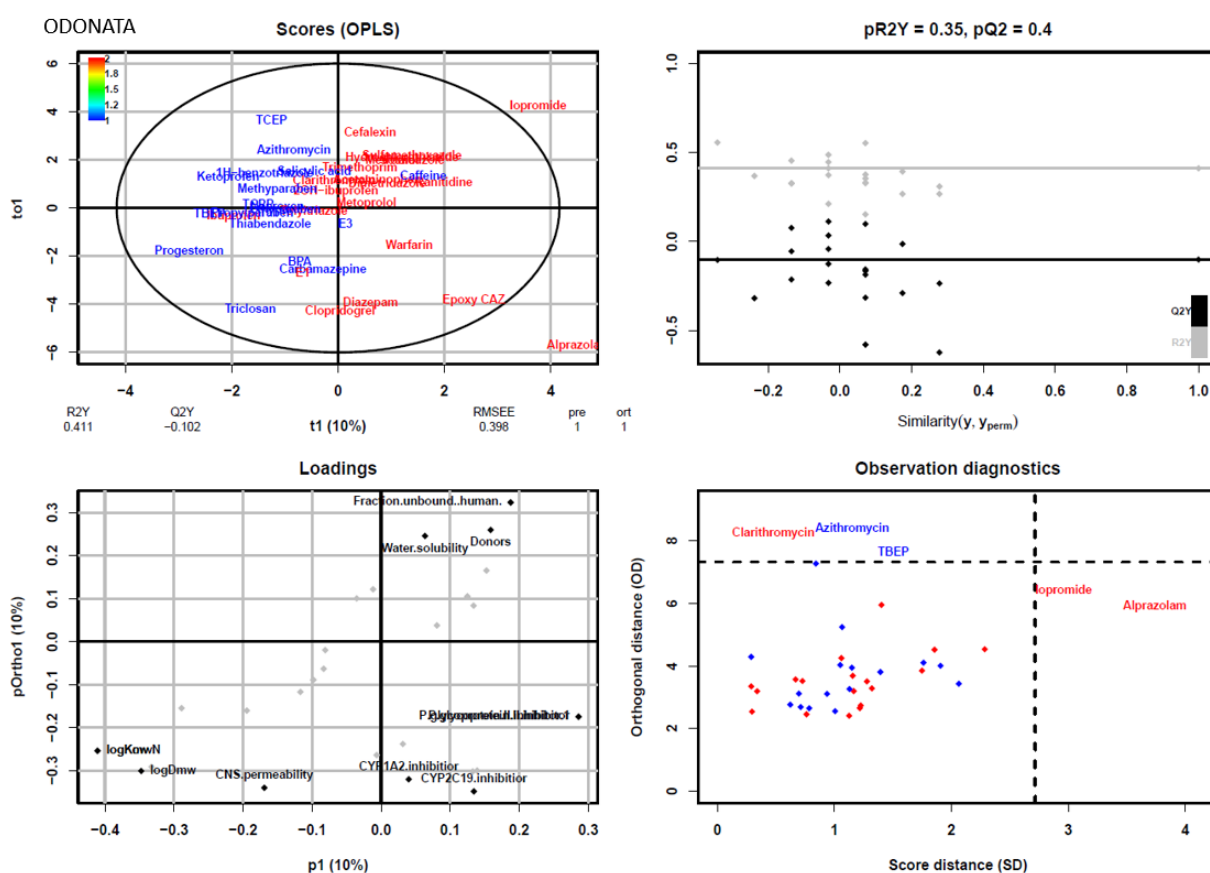
BAMF values indicate bioamplification for the majority of compounds (10 out of 13) during at least one stage of metamorphosis in *S. nigricornis*. In the initial stage of metamorphosis, transitioning from larvae to pupae, BAMFs indicate bioamplification for seven compounds (AZM, TIM, SAA, 1HB, TCPP, MPB, PPB; see Figure 15B). During the second metamorphosis stage, from pupae to adults, BAMFs show bioamplification of eight compounds (AZM, TIM, CFN, TCPP, BPA, E3, MPB, PPB; see Figure 15B). Moreover, for five compounds, including the antibiotics AZM and TIM, endocrine disruptors TCPP, and parabens (MPB and PPB), bioamplification was inferred (BAMFs  $\geq 1$ ) through both metamorphosis stages in *S. nigricornis*. Furthermore, TCPP and bisphenol-A (BPA) were the two compounds with the highest bioamplification potential, during the first and second metamorphosis stage, respectively (see Figure 15B).



**Figure 15.** Bioaccumulation factors (**A**; BAFs; L/kg dw) and bioamplification factors (**B**; BAMFs) of emerging contaminants for caddisfly *S. nigricornis* from the Dubrava study site. Yellow line (**B**) represents the BAMF value threshold (1) above which contaminant is considered to undergo bioamplification across metamorphosis. Full names of ECs are listed in Table 5 caption.

## 4.2. Relation of physico-chemical and pharmacokinetic descriptors of PhACs and EDCs and bioaccumulation and bioamplification in Odonata and Trichoptera

OPLS-DA classification model was computed to pinpoint specific descriptors of PhACs and EDCs influencing differential bioaccumulation behaviour in aquatic insects. However, OPLS-DA failed to expose group separation suggesting that no variation in the descriptor data matrix correlates with group membership, i.e. the ECs bioaccumulated in insect tissues could not be distinguished from those present only in the water based on the employed predictors in both Odonata and Trichoptera samples (Figure 16 and Figure 17).



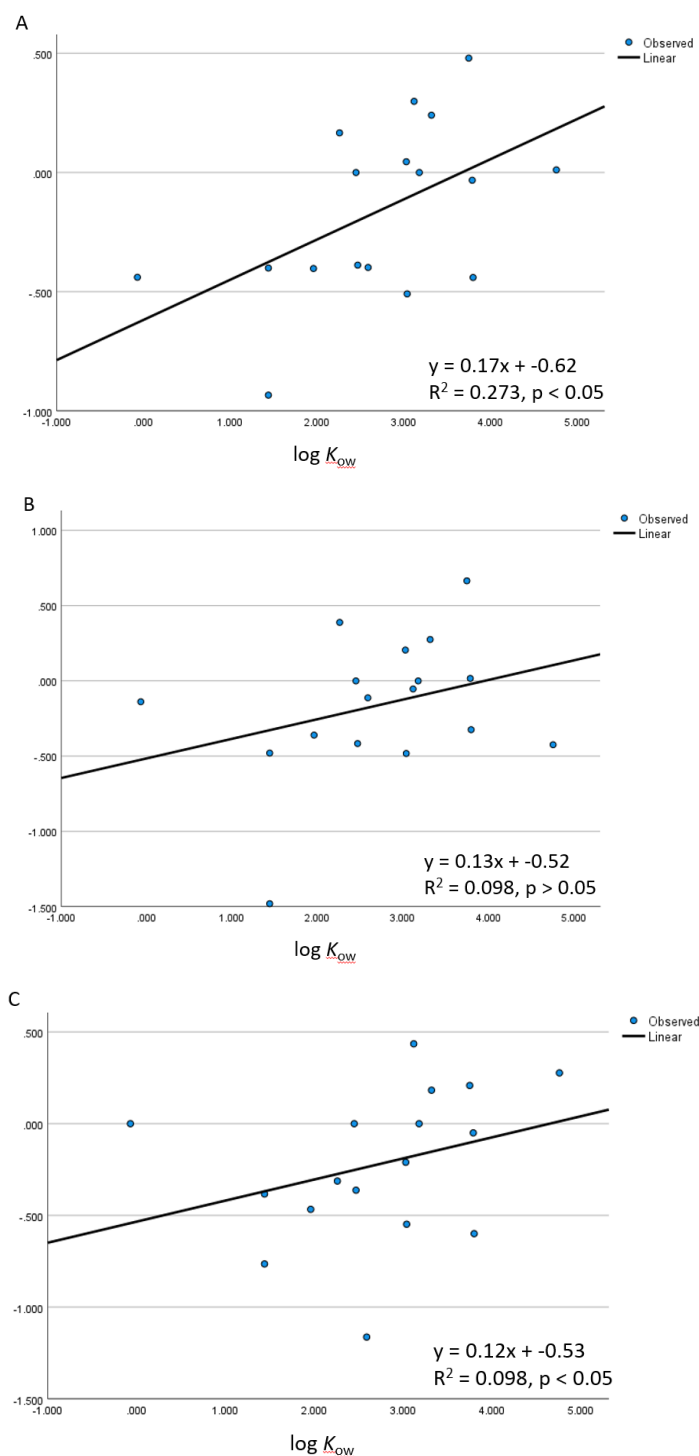
**Figure 16.** OPLS-DA analysis of bioaccumulative (value 1) vs. non-bioaccumulative (value 2) ECs recorded on the Krapina River study site. OPLS-DA fails to expose group separation (top left plot) as suggested by low variation of ECs explained by the model ( $R^2Y = 0.411$ ), poor prediction performance ( $Q^2Y = -0.102$ ) and  $p$ -value  $> 0.05$ .





**Table 7.** Spearman's rank correlation between bioaccumulation factors (log BAF) and bioamplification factors (log BAMF) calculated with Odonata contaminant concentrations and physico-chemical descriptors of ECs used: the octanol–water partition coefficient (log  $K_{OW}$ ), octanol–water distribution coefficient (log  $D_{OW}$ ), membrane-water distribution coefficient (log  $D_{MW}$ ), aqueous solubility (log  $S$ ), relative molecular mass (Mr), number of rotatable bonds and number of hydrogen bond donors and acceptors. Correlation is significant below the 0.05 level (bold).

		Log $K_{OW}$	Log $D_{MW}$	Log $D_{OW}$	Log $S$	Mr	Rotatable Bonds	Donors	Acceptors
<b>Log BAMF Odonata</b>	Correlation Coefficient	0.413	0.196	0.174	-0.210	0.269	0.192	0.040	-0.221
	Sig. (2-tailed)	0.099	0.450	0.505	0.452	0.297	0.460	0.878	0.393
	N	17	17	17	15	17	17	17	17
<b>Log BAMF Anisoptera</b>	Correlation Coefficient	-0.117	-0.053	0.201	-0.291	0.052	0.081	0.336	0.112
	Sig. (2-tailed)	0.679	0.839	0.440	0.258	0.844	0.756	0.187	0.669
	N	15	17	17	17	17	17	17	17
<b>Log BAMF Zygoptera</b>	Correlation Coefficient	-0.155	0.095	-0.064	-0.472	0.081	-0.292	0.214	-0.132
	Sig. (2-tailed)	0.582	0.718	0.806	0.056	0.757	0.256	0.410	0.615
	N	15	17	17	17	17	17	17	17
<b>Log BAF Odonata</b>	Correlation Coefficient	0.080	0.261	0.206	-0.057	-0.296	-0.058	0.386	-0.067
	Sig. (2-tailed)	0.776	0.348	0.462	0.840	0.283	0.838	0.156	0.812
	N	15	15	15	15	15	15	15	15
<b>Log BAF Anisoptera</b>	Correlation Coefficient	0.248	0.339	0.256	-0.211	-0.239	-0.056	0.358	-0.065
	Sig. (2-tailed)	0.372	0.216	0.358	0.451	0.390	0.843	0.190	0.818
	N	15	15	15	15	15	15	15	15
<b>Log BAF Zygoptera</b>	Correlation Coefficient	-0.238	0.032	-0.002	0.186	-0.350	0.011	0.407	0.142
	Sig. (2-tailed)	0.394	0.909	0.995	0.508	0.201	0.969	0.132	0.615
	N	15	15	15	15	15	15	15	15



**Figure 18.** Relationship (linear regressions) between bioamplification factor (BAMF) and octanol-water partition coefficient ( $\log K_{OW}$ ) for Odonata (**A**), Anisoptera (**B**) and Zygoptera (**C**).

More specifically, no statistically significant linear regressions or correlations between physico-chemical and pharmacokinetic descriptors and BAF and BAMF values were inferred

(Figure 18; Spearman's rank correlation, Table 7). The only exception was a positive relationship inferred between BAMF values in Odonata (order level solely) and log  $K_{ow}$  using linear regression (Figure 18A).

On the contrary, for Trichoptera, BAMFs from pupae to adults showed statistically significant positive correlations with log  $K_{ow}$  (Spearman's rank correlation; Table 8). Furthermore, Trichoptera BAFs showed positive correlations with the Mr (Spearman's rank correlation, Table 8). None of the linear regressions analyses showed any relationship between Trichoptera BAFs and BAMFs and descriptors.

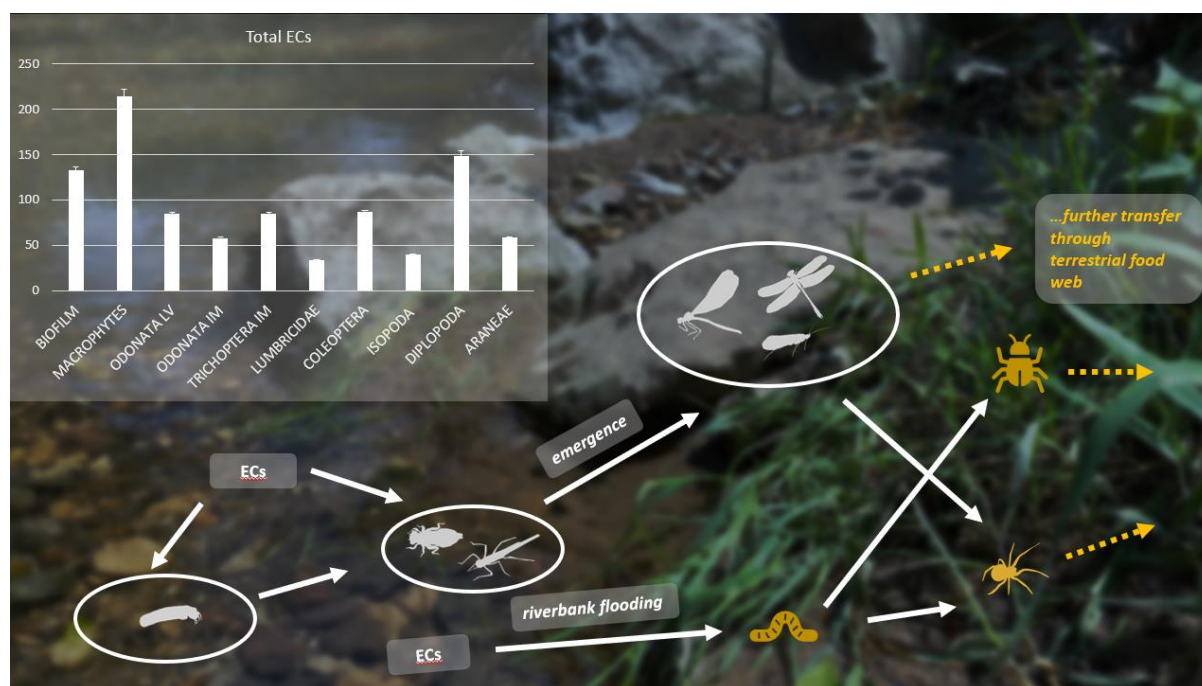
**Table 8.** Spearman's rank correlation between bioaccumulation factors (log BAF) and bioamplification factors (log BAMF) calculated with Trichoptera contaminant concentrations and physico-chemical descriptors of ECs used: the octanol–water partition coefficient (log  $K_{OW}$ ), octanol–water distribution coefficient (log  $D_{OW}$ ), membrane-water distribution coefficient (log  $D_{MW}$ ), aqueous solubility (log  $S$ ), relative molecular mass (Mr), number of rotatable bonds and number of hydrogen bond donors and acceptors. Correlation is significant at the 0.05 level (presented in bold).

		Log $K_{OW}$	Log $D_{MW}$	Log $D_{OW}$	Log $S$	Mr	Rotatable Bonds	Donors	Acceptors
<b>Log BAMF Trichoptera pu/lv</b>	Correlation Coefficient	-0.369	-0.055	0.091	0.187	-0.300	-0.055	-0.378	-0.132
	Sig. (2-tailed)	0.264	0.881	0.803	0.582	0.370	0.872	0.252	0.700
	N	11	10	10	11	11	11	11	11
<b>Log BAMF Trichoptera im/pu</b>	Correlation Coefficient	0.706	0.436	0.240	-0.409	0.555	0.207	0.391	-0.145
	Sig. (2-tailed)	<b>0.023</b>	0.180	0.478	0.212	0.077	0.542	0.235	0.671
	N	10	11	11	11	11	11	11	11
<b>Log BAF Trichoptera</b>	Correlation Coefficient	0.085	0.600	-0.098	-0.164	0.648	-0.399	-0.382	0.404
	Sig. (2-tailed)	0.828	0.067	0.788	0.651	<b>0.043</b>	0.253	0.276	0.247
	N	9	10	10	10	10	10	10	10

### 4.3. Food web transfer of bioaccumulated emerging contaminants on aquatic-terrestrial ecosystem boundary

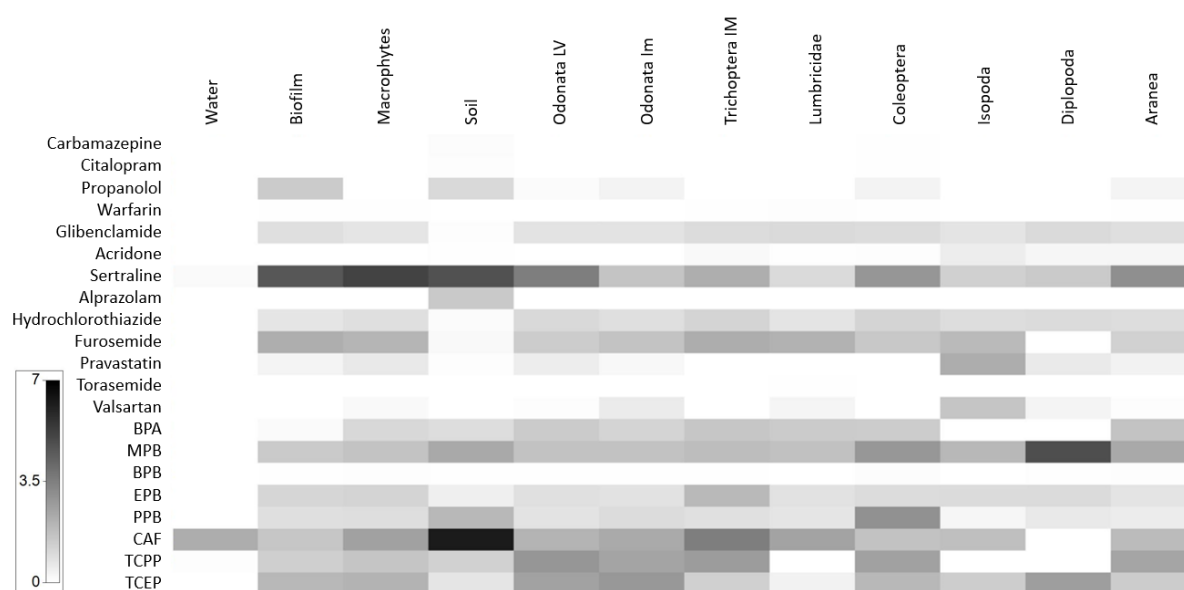
#### 4.3.1. Emerging contaminants measured at different levels of aquatic and terrestrial food web from the Sutla study site

Studied food web from the Sutla River site included following samples: biofilm, macrophytes, Odonata nymphs, Odonata and Trichoptera adults, Lumbricidae, Coleoptera (*Carabus* sp. adults, *Carabus* sp. larvae, *Pterostichus* sp. adults), Isopoda, Diplopoda and Araneae. In water, soil and biota samples from the Sutla river study site, a total of 21 compounds was quantified, including pharmaceuticals, parabens, and flame retardants (Table 9). Comparison of the total concentration of all quantified contaminants (total ECs) showed no significant differences between all biota samples from the Sutla River (Figure 19; Kruskal Wallis H test, Table A12). Certain individual compounds (glibenclamide, acridone, furosemide, pravastatin, valsartan, ethylparaben, propylparaben, TCP, TCEP ) significantly differed between biota samples (Kruskal Wallis H test, Table A12), however, pairwise comparison failed to reveal clear patterns in contaminant concentrations distribution in different samples (Multiple comparisons tests, Table A12). Highest total ECs concentration was measured in soil samples in the riparian zone of the Sutla river (Table 9).



**Figure 19.** Total ECs concentrations and possible pathways and trophic relations in ECs transfer through riparian food web. Concentrations are shown in  $\text{ngg}^{-1}$  dry weight and significance is tested with the Kruskal-Wallis H test; significance is listed in Table A12.

Out of 21 contaminants, seven compounds, including antidiabetic drug glibenclamide, antidepressant sertraline, diuretic hydrochlorothiazide, flame retardant TCEP and parabens (MPB, EPB and PPB), were measured in all groups of samples belonging to different trophic levels of the studied food web (Table 9, Figure 20). Highest concentration of  $512.219 \text{ ngg}^{-1}$  dw stands out for caffeine in soil. Furthermore, high concentrations of antidepressant sertraline: 98.983, 171.030 and  $115.857 \text{ ngg}^{-1}$  dw were measured in biofilm, macrophytes and soil, respectively (Table 9, Figure 20). Concentration of methylparaben (MPB) measured in Diplopoda samples with the value of  $124.107 \text{ ngg}^{-1}$  dw also stands out as one of the highest contaminant concentrations recorded in this study. In water samples highest concentrations of individual contaminants were recorded for caffeine and antidepressant sertraline ( $8391.046$  and  $155.295 \text{ ng/L}$ , respectively; Table 9).



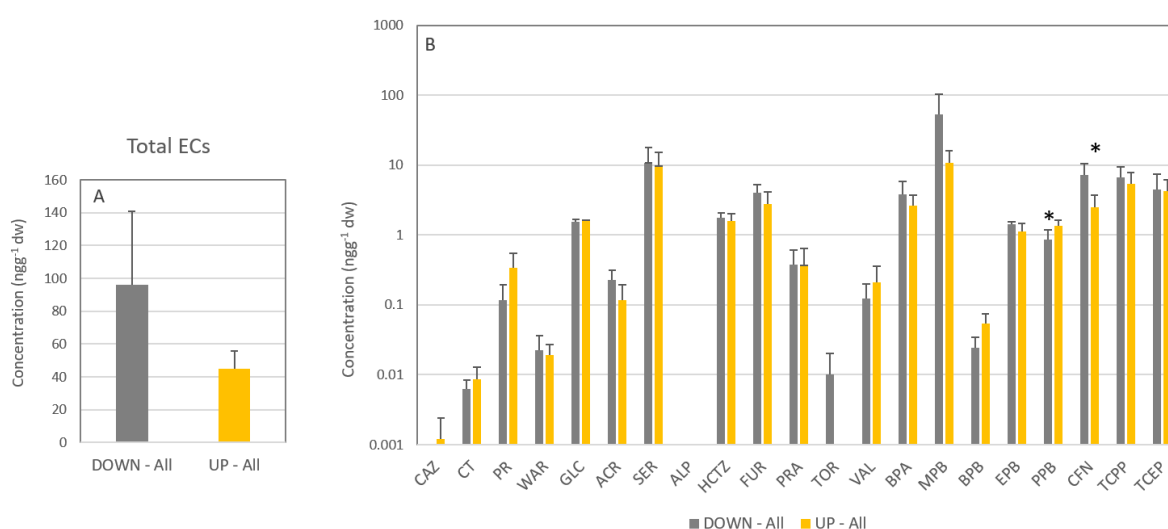
**Figure 20.** Data matrix of individual EC concentrations in samples from the Sutla River study site. White space denotes absence of individual ECs in the particular sample, whereas depth of grey scale is then linearly proportional to a  $\text{Log X}+1$  transformed concentration data (Primer Version 7, PRIMER-e). Full names of ECs are listed in Table 9 caption.

**Table 9.** Concentrations of 21 contaminants in samples from the Sutla River. Concentrations are shown as mean values and standard deviation in ngg-1 of dry weight for biofilm and caddisfly samples and in ngL-1 for water samples. (CAZ – carbamazepine, CT – citalopram, PR – propranolol, WAR – warfarin, GLC – glibenclamide, ACR – acridone, SER – sertraline, ALP – alprazolam, HCTZ – hydrochlorothiazide, FUR – furosemide, PRA – pravastatin, TOR – torasemide, VAL – valsartan, BPA - bisphenol-A, MPB – methylparaben, BPB – benzylparaben, EPB – ethylparaben, PPB – propylparaben, CFN - caffeine, TCPP - tris(1-chloro-2-propyl)phosphate, TCEP - tris(2-carboxyethyl)phosphine; W – water, B – biofilm, M – macrophytes, S – soil, O\_ny – Odonata nymphs, O\_im – Odonata imagines, T\_im – Trichoptera imagines, L - Lumbricidae, C – Coleoptera, I – Isopoda, D – Diplopoda, A – Araneae)

	CAZ	CT	PR	WAR	GLC	ACR	SER	ALP	HCTZ	FUR	PRA	TOR	VAL	BPA	MPB	BPB	EPB	PPB	CFN	TCPP	TCEP
W	4.396 (0.300)	0.000 (0.000)	0.000 (0.000)	0.024 (0.048)	0.258 (0.106)	0.076 (0.059)	155.30 (296.46)	0.009 (0.019)	4.131 (6.360)	0.924 (1.124)	0.000 (0.000)	0.000 (0.000)	3.657 (0.295)	5.353 (3.446)	1.808 (1.330)	0.002 (0.006)	0.563 (0.225)	0.425 (0.462)	8391.046 (1633.482)	45.452 (7.720)	2.790 (3.063)
B	0.000 (0.000)	0.003 (0.009)	3.145 (9.435)	0.017 (0.026)	1.367 (0.535)	0.000 (0.000)	98.983 (296.950)	0.000 (0.000)	1.064 (2.140)	8.070 (3.180)	0.342 (0.738)	0.000 (0.000)	0.000 (0.000)	0.134 (0.402)	3.257 (2.090)	0.039 (0.085)	2.109 (1.694)	1.412 (1.057)	3.773 (5.742)	2.740 (5.466)	5.931 (6.500)
M	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.017 (0.025)	1.056 (0.799)	0.000 (0.000)	171.030 (513.090)	0.000 (0.000)	1.397 (2.096)	6.579 (3.882)	0.810 (1.031)	0.000 (0.000)	0.168 (0.505)	1.912 (3.184)	4.128 (1.735)	0.052 (0.097)	2.254 (1.342)	1.462 (1.030)	11.988 (16.073)	3.922 (8.048)	6.945 (5.205)
S	0.087 (0.079)	0.068 (0.057)	1.816 (1.985)	0.000 (0.000)	0.049 (0.002)	0.025 (0.010)	115.857 (104.120)	3.382 (6.840)	0.113 (0.185)	0.163 (0.201)	0.060 (0.092)	0.011 (0.017)	0.000 (0.000)	1.515 (0.249)	9.593 (3.281)	0.016 (0.048)	0.537 (0.273)	6.009 (15.465)	512.219 (716.460)	2.490 (2.213)	1.047 (1.038)
O_ny	0.000 (0.000)	0.003 (0.010)	0.118 (0.410)	0.011 (0.027)	1.163 (0.551)	0.000 (0.000)	32.739 (65.305)	0.003 (0.010)	1.799 (1.915)	3.016 (3.758)	0.617 (1.217)	0.000 (0.000)	0.058 (0.202)	3.138 (6.199)	4.240 (1.561)	0.032 (0.042)	1.318 (0.776)	1.158 (1.085)	6.766 (8.427)	16.510 (20.861)	11.607 (5.094)
O_im	0.000 (0.000)	0.001 (0.001)	0.399 (1.197)	0.006 (0.017)	1.174 (0.848)	0.000 (0.000)	4.038 (12.113)	0.000 (0.000)	1.403 (2.108)	4.185 (4.794)	0.211 (0.633)	0.000 (0.000)	0.723 (2.119)	2.197 (4.599)	4.241 (1.865)	0.025 (0.048)	1.222 (0.676)	1.601 (0.560)	9.329 (9.196)	11.094 (11.671)	15.780 (11.519)
T_im	0.000 (0.000)	0.002 (0.008)	0.000 (0.000)	0.020 (0.034)	1.587 (0.163)	0.194 (1.188)	8.134 (18.619)	0.000 (0.000)	2.242 (2.044)	8.341 (2.803)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	3.774 (6.279)	5.061 (2.095)	0.037 (0.066)	5.825 (3.327)	1.313 (0.703)	33.149 (76.228)	14.063 (30.595)	2.627 (4.143)
L	0.002 (0.009)	0.006 (0.015)	0.000 (0.000)	0.046 (0.151)	1.725 (0.181)	0.024 (0.091)	1.721 (4.016)	0.000 (0.000)	1.088 (1.897)	7.238 (3.177)	0.000 (0.000)	0.030 (0.081)	0.304 (0.382)	3.124 (5.432)	4.459 (1.953)	0.001 (0.001)	1.203 (0.846)	1.011 (0.938)	11.390 (20.268)	0.000 (0.000)	0.427 (0.919)
C	0.026 (0.083)	0.020 (0.064)	0.402 (1.705)	0.019 (0.034)	1.576 (0.232)	0.058 (0.246)	16.514 (33.747)	0.000 (0.000)	2.210 (2.075)	3.503 (4.108)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	3.012 (4.651)	16.353 (24.375)	0.073 (0.101)	1.627 (0.647)	19.229 (58.847)	4.345 (6.691)	11.926 (22.837)	6.008 (6.082)
I	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.104 (0.845)	0.642 (0.384)	2.624 (4.410)	0.000 (0.000)	1.509 (2.085)	5.613 (5.153)	8.366 (7.531)	0.000 (0.000)	3.934 (5.394)	0.000 (0.000)	6.044 (2.539)	0.000 (0.000)	1.689 (0.832)	0.288 (0.119)	4.708 (8.980)	0.000 (0.000)	2.947 (5.500)
D	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.715 (0.173)	0.288 (0.142)	3.304 (8.094)	0.000 (0.000)	1.668 (2.780)	0.000 (0.000)	0.757 (0.844)	0.000 (0.000)	0.368 (0.661)	0.000 (0.000)	124.107 (136.88)	0.018 (0.045)	1.646 (0.624)	0.832 (1.073)	0.000 (0.000)	0.000 (0.00)	13.169 (4.354)
A	0.000 (0.000)	0.012 (0.019)	0.361 (0.791)	0.019 (0.033)	1.398 (0.612)	0.293 (0.403)	20.441 (33.078)	0.000 (0.000)	1.535 (2.290)	2.542 (3.625)	0.438 (1.277)	0.000 (0.000)	0.061 (0.114)	4.224 (5.915)	9.626 (12.560)	0.043 (0.055)	1.071 (0.690)	0.702 (0.551)	5.436 (5.997)	10.875 (12.470)	3.029 (2.874)

#### 4.3.2. Extent of the contaminants transfer into riparian zone through food web

Analyses of differences in contaminant concentrations regarding the distance from the river were calculated for samples belonging to the same taxa that were collected on both Down and Up transects (i.e. 0-1 meters and 1-3 meters distance from the waterline), meaning: Lumbricidae, *Carabus*, Diplopoda and Araneae (Table 10). While analysing the overall concentration of ECs in all samples collected from the Down transect compared to those from the Up transect (sum of contaminant concentrations), even though generally samples from the transect closer to the river exhibited elevated concentration of ECs, no statistically significant difference was observed, as determined by the Mann-Whitney U Test (Figure 21, Table A13). However, observing total concentrations of contaminants separately, propylparaben (PPB) and caffeine (CAF) differed significantly between Up and Down transect but in opposing trends: propylparaben (PPB) being higher in samples further from the river and caffeine being higher in samples closer to the river (Figure 21, Table A13).

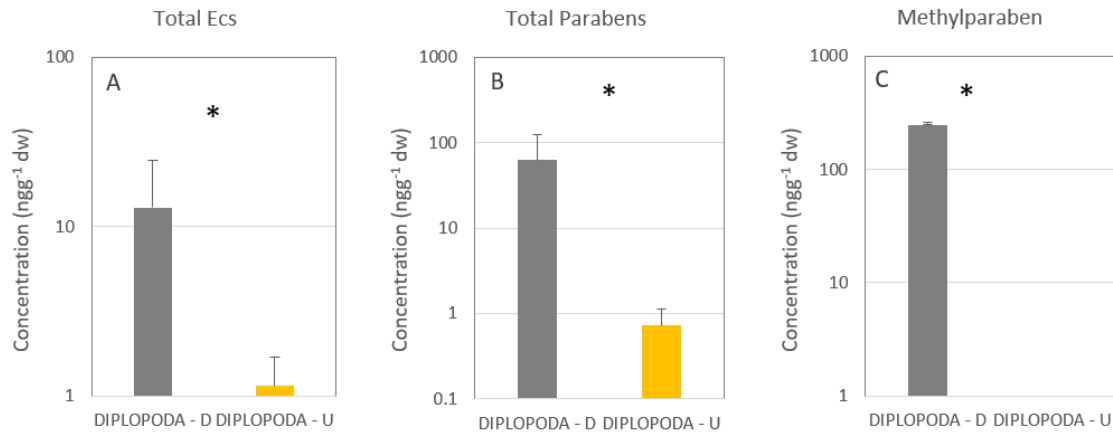


**Figure 21.** Total ECs concentration (A) and individual contaminant concentrations (B) measured in samples from two transects (Down - D and Up - U). Individual contaminant concentrations are shown as mean values (with standard error bars) in logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Mann-Whitney U test and significance is listed in Table A13. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)).

When testing observed differences in concentrations of individual compounds and total concentrations of compounds (e.g. total ECs and total parabens), within the same taxonomic groups from different transects (Up and Down), Diplopoda showed significantly higher total



ECs concentration and total parabens concentration in samples from transect Down versus samples from transect Up. The same pattern was observed also for methylparaben separately (Table A14, Figure 22).

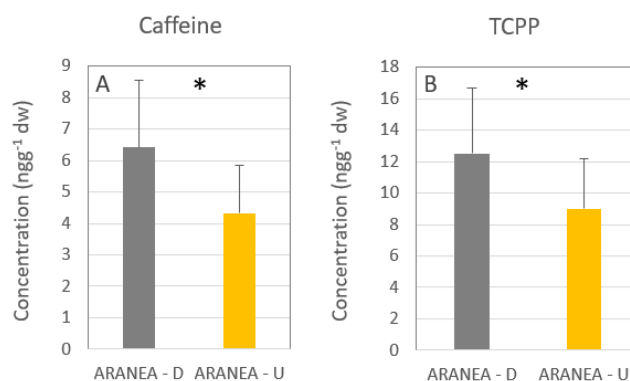


**Figure 22.** Concentration of total ECs (A), total concentration of parabens (B) and individual methylparaben (C) concentration in Diplopoda samples from two transects (Down -D and Up - U). Concentrations are shown as mean values (with standard error bars) in logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Mann-Whitney U test and listed in Table A14. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)).

**Table 10.** Total concentration of emerging contaminants (ECs), total paraben concentration and individual compounds concentrations recorded in taxa collected on both transects (Up - U and Down - D) on the Sutla study site (mean values and standard deviation, in ngL<sup>-1</sup> for water, in nng<sup>-1</sup> dw for all other samples). Full names of ECs are listed in Table 9 caption.

	CAZ	CT	PR	WAR	GLC	ACR	SER	ALP	HCTZ	FUR	PRA	TOR	VAL	BPA	MPB	BPB	EPB	PPB	CFN	TCPP	TCEP	Totals	Total parabens
LUMBRICIDAE - D	0.000 (0.000)	0.005 (0.015)	0.000 (0.000)	0.076 (0.193)	1.779 (0.190)	0.039 (0.118)	2.045 (4.714)	0.000 (0.000)	1.027 (2.048)	7.293 (3.005)	0.000 (0.000)	0.050 (0.102)	0.310 (0.431)	4.039 (6.146)	5.033 (2.386)	0.001 (0.002)	1.120 (0.830)	1.059 (1.237)	18.983 (23.595)	0.000 (0.000)	0.205 (0.615)	43.065 (25.595)	7.213 (4.351)
LUMBRICIDAE - U	0.006 (0.015)	0.007 (0.016)	0.000 (0.000)	0.000 (0.000)	1.643 (0.146)	0.000 (0.000)	1.234 (3.022)	0.000 (0.000)	1.181 (1.831)	7.154 (3.716)	0.000 (0.000)	0.000 (0.000)	0.295 (0.335)	1.752 (4.292)	3.598 (0.291)	0.001 (0.002)	1.326 (0.933)	0.939 (0.068)	0.000 (0.000)	0.000 (0.000)	0.759 (1.240)	19.895 (8.198)	5.864 (1.119)
CARABUS - D	0.000 (0.000)	0.007 (0.012)	0.000 (0.000)	0.019 (0.033)	1.612 (0.168)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.729 (2.995)	5.493 (4.991)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.648 (1.122)	4.333 (0.838)	0.042 (0.072)	1.330 (0.200)	2.002 (0.446)	3.598 (6.231)	7.453 (12.910)	1.486 (2.575)	29.751 (15.823)	7.706 (1.436)
CARABUS - U	0.000 (0.000)	0.000 (0.000)	0.804 (2.411)	0.025 (0.041)	1.503 (0.148)	0.000 (0.000)	9.813 (29.458)	0.000 (0.000)	2.020 (1.940)	4.198 (4.054)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	3.444 (4.914)	27.011 (31.683)	0.129 (0.114)	1.953 (0.470)	2.319 (0.663)	2.398 (4.762)	6.214 (12.333)	5.871 (4.735)	67.701 (35.642)	31.412 (32.534)
DIPLOPODA - D	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.763 (0.086)	0.342 (0.022)	0.000 (0.000)	0.000 (0.000)	2.209 (3.825)	0.000 (0.000)	1.088 (0.951)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	248.215 (25.155)	0.000 (0.000)	1.878 (0.807)	0.219 (0.379)	0.000 (0.000)	0.000 (0.000)	16.048 (4.533)	271.761 (25.267)	250.312 (25.279)
DIPLOPODA - U	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.668 (0.246)	0.234 (0.203)	6.609 (11.447)	0.000 (0.000)	1.127 (1.951)	0.000 (0.000)	0.427 (0.739)	0.000 (0.000)	0.736 (0.828)	0.000 (0.000)	0.000 (0.000)	0.036 (0.063)	1.413 (0.399)	1.444 (1.269)	0.000 (0.000)	0.000 (0.000)	10.290 (1.406)	23.985 (9.316)	2.894 (1.326)
ARANEAE - D	0.000 (0.000)	0.008 (0.017)	0.266 (0.529)	0.010 (0.031)	1.279 (0.734)	0.377 (0.472)	21.641 (30.630)	0.000 (0.000)	1.763 (2.307)	3.220 (3.867)	0.514 (1.543)	0.000 (0.000)	0.107 (0.143)	5.567 (6.834)	4.162 (0.740)	0.042 (0.059)	1.393 (0.544)	0.495 (0.600)	6.417 (6.152)	12.534 (12.936)	2.991 (3.165)	62.788 (38.425)	6.090 (1.076)
ARANEAE - U	0.000 (0.000)	0.018 (0.024)	0.533 (1.191)	0.036 (0.034)	1.613 (0.226)	0.142 (0.195)	18.281 (40.887)	0.000 (0.000)	1.279 (2.400)	1.778 (3.417)	0.352 (0.996)	0.000 (0.000)	0.009 (0.027)	2.714 (4.657)	15.773 (16.683)	0.045 (0.054)	0.708 (0.623)	0.934 (0.407)	4.332 (6.027)	9.009 (12.516)	3.071 (2.726)	52.893 (35.880)	17.460 (17.151)

Spiders (Araneae) also showed statistically significant difference in concentrations of contaminants between samples from transect closer to the river, Down, compared to samples from transect higher on the riverbank, Up. More precisely, TCP and caffeine concentrations are higher in spiders from transect Down compared to spiders from transect Up (Figure 23, Table A15).

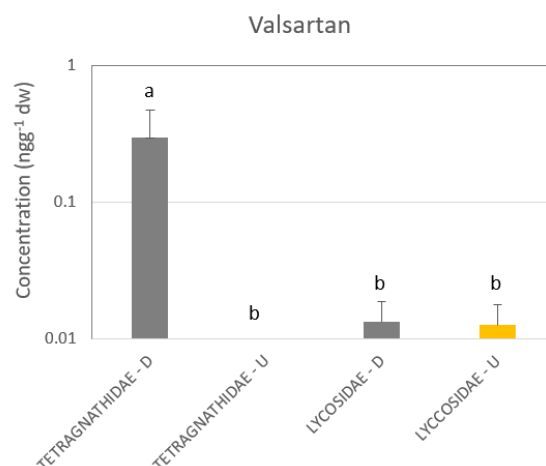


**Figure 23.** Concentrations of caffeine (A) and TCP (B) in Araneae from two transects (Down - D and Up - U). Concentrations are shown as mean values (with standard error bars) logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Mann-Whitney U test and listed in Table A14. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)).

Furthermore, spiders analysed in this study belong to two families: Lycosidae and Tetragnathidae (Table 11) and statistical analysis showed that Tetragnathidae, web hunting long jawed spiders, from transect closer to the river (Down), had significantly higher concentration of pharmaceutical valsartan (VAL) compared to the same spider family from transect Up (Figure 24, Table A16, Table A17). However, in Lycosidae, ground hunting wolf spiders, no difference in concentration of valsartan between the two transects was observed (Figure 24, Table A17).

**Table 11.** Total concentration of emerging contaminants (ECs), total paraben concentration and individual compounds concentrations recorded in two spider families: Tetragnathidae and Lycosidae, collected on both transects (Up - U and Down - D) on the Sutla study site (mean values and standard deviation,  $\text{ngg}^{-1}$  dry weight). Significance is tested with Kruskal-Wallis H test (Table A16). Full names of ECs are listed in Table 9 caption.

	CAZ	CT	PR	WAR	GLC	ACR	SER	ALP	HCTZ	FUR	PRA	TOR	VAL	BPA	MPB	BPB	EPB	PPB	CFN	TCPP	TCEP	Totals	Total parabens
TETRAGNATHIDAE - D	0.000 (0.000)	0.013 (0.023)	0.384 (0.666)	0.000 (0.000)	1.124 (0.980)	0.356 (0.027)	36.979 (17.158)	0.000 (0.000)	2.559 (2.219)	4.394 (3.811)	0.000 (0.000)	0.000 (0.000)	0.295 (0.004)	11.527 (2.550)	3.366 (0.314)	0.033 (0.057)	1.221 (0.544)	0.630 (0.549)	8.362 (7.293)	13.600 (13.345)	0.000 (0.000)	84.844 (21.583)	5.251 (1.175)
TETRAGNATHIDAE - U	0.000 (0.000)	0.022 (0.031)	0.000 (0.000)	0.032 (0.045)	1.520 (0.135)	0.356 (0.017)	0.000 (0.000)	0.000 (0.000)	2.929 (4.142)	0.000 (0.000)	1.409 (1.993)	0.000 (0.000)	0.000 (0.000)	6.411 (9.066)	3.666 (0.514)	0.063 (0.089)	0.000 (0.000)	1.205 (0.222)	6.461 (9.138)	13.041 (18.443)	0.000 (0.000)	37.114 (31.084)	4.934 (0.648)
LYCOSIDAE - D	0.000 (0.000)	0.006 (0.015)	0.207 (0.507)	0.015 (0.038)	1.357 (0.675)	0.388 (0.597)	13.972 (34.225)	0.000 (0.000)	1.364 (2.444)	2.633 (4.109)	0.771 (1.890)	0.000 (0.000)	0.013 (0.032)	2.587 (6.337)	4.560 (0.517)	0.047 (0.065)	1.479 (0.572)	0.428 (0.663)	5.444 (6.000)	12.001 (13.982)	4.487 (2.823)	51.760 (41.691)	6.513 (0.656)
LYCCOSIDAE - U	0.000 (0.000)	0.015 (0.025)	0.888 (1.538)	0.038 (0.035)	1.675 (0.280)	0.000 (0.000)	30.468 (52.772)	0.000 (0.000)	0.729 (1.785)	2.371 (3.829)	0.000 (0.000)	0.000 (0.000)	0.013 (0.031)	1.482 (2.577)	19.809 (17.647)	0.039 (0.048)	0.944 (0.621)	0.844 (0.428)	3.622 (5.634)	7.665 (11.942)	4.094 (2.318)	58.152 (38.453)	21.636 (18.113)



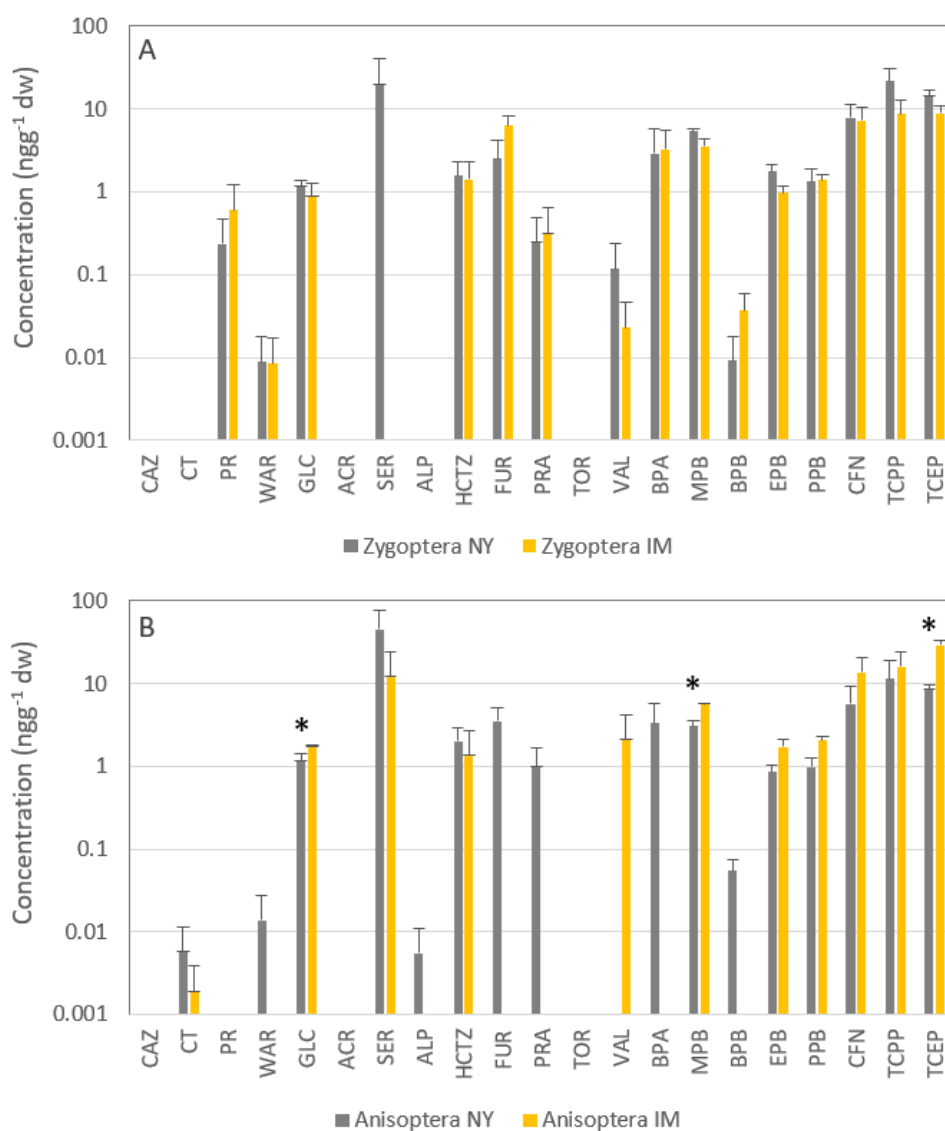
**Figure 24.** Concentrations (shown in logarithmic scale) of Valsartan in Tetragnathidae and Lycosidae from two transects (Down - D and Up - U). Concentrations are shown as mean values (with standard error bars) in logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Kruskal-Wallis H test and listed in Table A17. Significance is shown with letters (a, b) and different letters depict significant differences ( $p > 0.05$ ).

#### 4.3.3. Emerging contaminants measured in aquatic insects from Sutla River

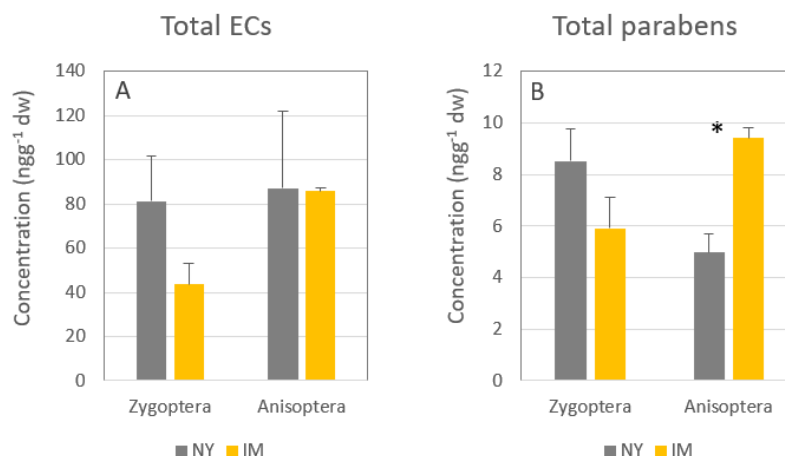
At the Sutla River study site, aquatic insects Trichoptera and Odonata were collected. For Trichoptera, only adults (taxa list available in Table A2) were collected and in their tissues 15 contaminants were quantified, with highest concentrations recorded for caffeine, flame retardant TCPP and diuretic drug furosemide (33.15, 14.06, 8.34 ngg<sup>-1</sup>, respectively; Table 9). For Odonata, contaminants were quantified in both nymphs and adults belonging to two suborders: Zygoptera and Anisoptera. Out of the total of 21 contaminants measured in Sutla River samples, in Odonata samples 18 contaminants were quantified (Table 12). The Mann-Whitney U test did not reveal any significant differences in concentrations across different life stages (Table A18). However, notable variations were observed at the Odonata suborder level, both between taxa (Zygoptera and Anisoptera) and life stages (nymphs and adults). Specifically, when comparing concentrations in different life stages of Zygoptera and Anisoptera, Anisoptera adults exhibited significantly higher concentrations of glibenclamide (GLI), methylparaben (MPB) and TCEP as well as total parabens compared to Anisoptera nymphs. In contrast, concentrations of individual contaminants and total parabens in different life stages of Zygoptera showed no significant differences (Mann Whitney U test, Table A18).

**Table 12.** Individual ECs concentrations measured in Anisoptera and Zygoptera from Sutla study site (mean values and standard deviation,  $\text{ngg}^{-1}$  dry weight). Full names of ECs are listed in Table 9 caption.

	CAZ	CT	PR	WAR	GLC	ACR	SER	ALP	HCTZ	FUR	PRA	TOR	VAL	BPA	MPB	BPB	EPB	PPB	CFN	TCP	TCEP
Zygoptera NY	0.000 (0.000)	0.000 (0.000)	0.237 (0.580)	0.009 (0.022)	1.161 (0.585)	0.000 (0.000)	20.149 (49.356)	0.000 (0.000)	1.581 (1.738)	2.519 (3.934)	0.244 (0.599)	0.000 (0.000)	0.117 (0.286)	2.922 (7.156)	5.409 (0.994)	0.009 (0.022)	1.776 (0.780)	1.327 (1.409)	7.814 (8.623)	21.634 (23.709)	14.484 (5.454)
Zygoptera IM	0.000 (0.000)	0.000 (0.000)	0.599 (1.467)	0.009 (0.021)	0.892 (0.927)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.425 (2.211)	6.278 (4.583)	0.317 (0.776)	0.000 (0.000)	0.023 (0.056)	3.296 (5.431)	3.528 (1.922)	0.037 (0.057)	0.973 (0.539)	1.382 (0.516)	7.205 (7.897)	8.644 (10.743)	8.930 (4.410)
Anisoptera NY	0.000 (0.000)	0.006 (0.014)	0.000 (0.000)	0.014 (0.033)	1.166 (0.572)	0.000 (0.000)	45.329 (81.031)	0.006 (0.013)	2.017 (2.222)	3.513 (3.873)	0.989 (1.602)	0.000 (0.000)	0.000 (0.000)	3.354 (5.764)	3.072 (1.046)	0.054 (0.048)	0.860 (0.461)	0.989 (0.732)	5.717 (8.902)	11.386 (18.223)	8.731 (2.739)
Anisoptera IM	0.000 (0.000)	0.002 (0.003)	0.000 (0.000)	0.000 (0.000)	1.738 (0.117)	0.000 (0.000)	12.113 (20.981)	0.000 (0.000)	1.359 (2.354)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	2.124 (3.678)	0.000 (0.000)	5.666 (0.326)	0.000 (0.000)	1.719 (0.740)	2.040 (0.390)	13.578 (11.905)	15.996 (14.221)	29.480 (7.742)



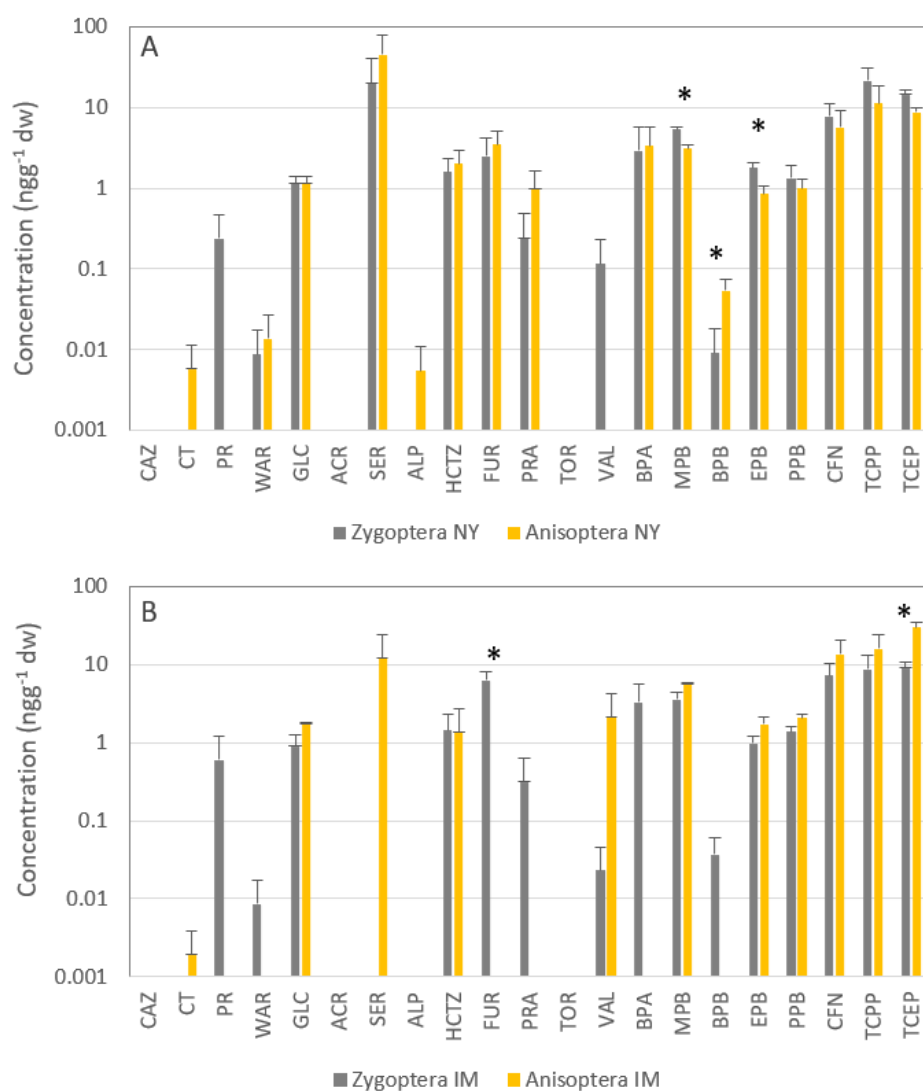
**Figure 25.** Individual compounds concentrations in nymphs (NY) and adults (IM) of Zygoptera (A) and Anisoptera (B) aquatic and terrestrial life stages. Concentrations are shown as mean values (with standard error bars) logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Mann-Whitney U test and listed in Table A18. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 9 caption.



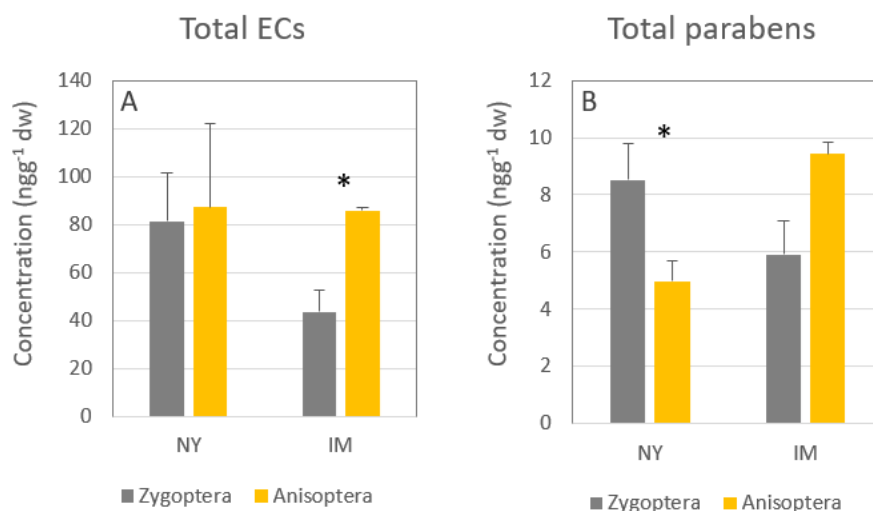
**Figure 26.** Total ECs concentration (A) and total parabens concentrations (sum of MPB, BPB, EPB and PPB) (B) in nymphs (NY) and adults (IM) of Anisoptera and Zygoptera. Concentrations are shown as mean values (with standard error bars) logarithmic scale in ngg<sup>-1</sup> dry weight, significance is tested with the Mann-Whitney U test and listed in Table A18. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 9 caption.

Comparison between taxa showed that concentrations of individual parabens (MPB, BPB and EPB), as well as concentration of total parabens (PPB included) statistically differed between nymphs of Zygoptera and Anisoptera, with Zygoptera having higher concentrations of all these ECs, except BPB (Figure 27, Table A19). When comparing concentrations of contaminants quantified in adults, two contaminants show significant difference between Zygoptera and Anisoptera, furosemide being higher in Zygoptera (not present in Anisoptera) and TCEP being higher in Anisoptera. Furthermore, total ECs concentration differed between Zygoptera and Anisoptera adults being higher in Anisoptera (Figure 28, Table A19).





**Figure 27.** Individual compounds concentrations in nymphs (NY) (**A**) and adults (IM) (**B**) of Anisoptera and Zygoptera. Concentrations are shown as mean values (with standard error bars) logarithmic scale in ngg-1 dry weight, significance is tested with the Mann-Whitney U test and listed in Table A19. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 9 caption.



**Figure 28.** Total ECs concentration (**A**) and total parabens concentration (sum of MPB, BPB, EPB AND PPB) (**B**) in nymphs (NY) and adults (IM) of Anisoptera and Zygoptera. Concentrations are shown as mean values (with standard error bars) logarithmic scale in ngg-1 dry weight, significance is tested with the Mann-Whitney U test and listed in Table A19. Significant difference between tested groups is shown with asterisk ( $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Full names of ECs are listed in Table 9 caption.

## **5. DISCUSSION**

### **5.1. The role of taxonomic resolution and ecological and life history traits of aquatic insects in determining bioaccumulation and bioamplification patterns of PhACs and EDCs**

The present study confirmed the presence of PhACs and EDCs of aquatic origin in all stages of aquatic insects inhabiting both aquatic and terrestrial habitats at all studied watercourses, the Krapina and Sutla rivers and the Dubrava drainage ditch. More importantly, the results suggest that differences in fate and behaviour of bioaccumulated PhACs and EDCs at the aquatic-terrestrial ecosystem boundary depend on aquatic insect taxa and/or life history traits. In accordance with the previous findings (Previšić et al., 2021; Ruhí et al., 2016), both hemimetabolous Odonata and holometabolous Trichoptera showed generally higher concentrations of ECs in aquatic nymphal/larval stages as opposed to adults. However, considerable differences in both bioaccumulation and bioamplification patterns were observed between the two Odonata suborders (Anisoptera and Zygoptera), emphasising the importance of the taxonomic level and differences in ecological traits when studying this subject. In addition, the results on contaminants bioaccumulation in two aquatic insect orders with different metamorphosis, Odonata and Trichoptera, not only indicated different bioaccumulation patterns in relation to different life history traits, but also displayed the importance of the pupal stage in holometabolous insects for determining the final contaminant concentration in the adults.

#### **5.1.1. Ecological traits and preferences influence trophic and respiratory exposure to contaminants in aquatic insects**

Comparing bioaccumulation for detected PhACs and EDCs at both Odonata order and suborder level observed in this study, it is apparent that Zygoptera have overall highest concentrations of ECs, and consequently highest BAF values. Although Anisoptera and Zygoptera belong to the same order, they are very different. Therefore, nymphal ecological traits and dietary and habitat preferences could explain differences between the two Odonata suborders recorded in the study from the Krapina study site. These traits and preferences directly determine available routes for contaminant exposure in macroinvertebrates (Ducrot et al., 2005; Sidney et al.,

2016). All Odonata species observed in this study (Anisoptera - *Gomphus vulgatissimus*, *Onychogomphus forcipatus*, *Orthetrum albistylum*; Zygoptera - *Calopteryx splendens*, *Platycnemis pennipes*; as listed in Table 1) are predators (Dijkstra et al., 2022). Nevertheless, the nymphs of the two suborders occupy different trophic positions. Anisoptera nymphs can prey, not only on other organisms, but also on Zygoptera and/or smaller Anisoptera, whereas Zygoptera do not show the same feeding behaviour (Johansson, 1991). That difference in trophic position could also add up to the differences in bioaccumulation of ECs on suborder level. Furthermore, habitat preferences also differ between taxa, and that is also confirmed to affect contaminant availability (Mayer-Pinto et al., 2016). Anisopteran nymphs of the genera *Gomphus* and *Orthetrum* like to burrow themselves in fine substrates. This behaviour potentially increases their exposure to contaminants adsorbed on sediment particles (Simon et al., 2019). On the other hand, zygopteran nymphs *Calopteryx splendens* and *Platycnemis pennipes* prefer mostly phytal, however they can also be found in microhabitats with predominant particulate organic matter (POM) and *P. pennipes* can also be found on lithal (Dijkstra et al., 2024). Moreover, nymphs of the two suborders also have considerable differences in respiration organs, with zygopteran nymphs having external sets of gills at the abdomen tip, and anisopteran nymphs having internal rectal gills (Corbet and Brooks, 2008). It has been recorded that different types of respiration in aquatic invertebrates affect exposure to dissolved contaminants in water (Baird and Van den Brink, 2007). For example, plastron breathing in Hemiptera (e.g. *Notonecta glauca*) can reduce availability of contaminants, compared to biota with gills and breathing oxygen from water (Meredith-Williams et al., 2012). Furthermore, it has been confirmed that Anisoptera nymphs can be air-breathing, at least in specific conditions such as hypoxia, as in their imaginal respiratory systems are already developed during last stages of nymphal development (de Pennart and Matthews, 2020; Gaino et al., 2007; Kriska, 2013; Ubhi and Matthews, 2018). Moreover, certain aeshnid nymphs (family Aeshnidae) can also use their nymphal rectal gills for breathing air outside of water (de Pennart and Matthews, 2020). Therefore, nymphs with the ability to breathe air instead of using gills and filtrating oxygen dissolved in water, could result in considerably lower exposure and uptake levels of contaminants in polluted aquatic environments.

In contrast to the Odonata, the results from the Dubrava study site show a general increase of the total ECs concentration, as well as concentrations of certain individual contaminants along the life cycle of the caddisfly *Silo nigricornis*. This species was recorded in a very high population density (Previšić et al., 2007)) at the sampling site influenced by the untreated urban

wastewaters and agricultural runoff, even though it is classified as sensitive to pesticide pollution according to the SPEAR indicator (Liess, 2005). Larvae of this species primarily feed as grazers, feeding on biofilm, which is often highlighted as a sink for various contaminants (Bonnineau et al., 2020). Similar as in Odonata, trophic exposure could explain some patterns observed in *S. nigricornis* in the current study, with larval concentrations of individual ECs very similar to the biofilm (e.g. KPF, SAA, MPB, etc.). However, no significant positive correlation was found between ECs concentrations in biofilm and larval *S. nigricornis* samples. The different feeding behaviour has an impact on trace metals accumulation in aquatic macroinvertebrates (Pastorino et al., 2020), as food preferences define spatial distribution and feeding behaviour of individuals. Furthermore, feeding habits of fish have been confirmed to influence bioaccumulation of certain endocrine disrupting compounds as well (Fan et al., 2019). Our results, therefore, support the assertion that the observed differences in bioaccumulation patterns are organism/taxa-specific (Chang et al., 2019; Previšić et al., 2021, 2019).

Knowledge on species ecological traits has been successfully integrated into freshwater ecological assessment systems, however, those developed using autecological information on species level and are not applicable on higher taxonomic levels (Schmidt-Kloiber and Nijboer, 2004). Similarly, higher taxonomic categories are potentially composed of ecologically diverse groups whose contribution to “the dark side of subsidies” (Walters et al., 2008) could differ considerably. Existing data on the flow of PhACs and EDCs flux are obtained either at the coarse taxonomic resolution, e.g. insect orders (Bartrons et al., 2007; Park et al., 2009) or at the genus or species level (de Solla et al., 2016; Ruhí et al., 2015), without establishing potential hierarchical patterns and their casualties. Although all Odonata species are predators throughout their life cycle, the two suborders differ in their trophic position, habitat preferences, dispersal behaviour and type of respiration, which may influence different bioaccumulation and bioamplification patterns (Corbet, 1999).

### **5.1.2. Bioaccumulation potential of the PhACs and EDCs and possible effects on aquatic insects**

All bioaccumulation factors (BAFs) of PhACs and EDCs detected in this study, for both Odonata and *S. nigricornis* (Trichoptera), are below the threshold of 5000 L/kg wet weight (ww), suggesting that none of the measured compounds are highly bioaccumulative in observed

aquatic insect larvae and nymphs (Arnot and Gobas, 2006; Borgå, 2013). Even though BAF values were expressed on dry weight (dw) basis, they are still comparable, as values based on dry weight (dw) are 3-10 times higher than those based on wet weight (ww) (Karlsson et al., 2002). Nevertheless, some studies have shown that certain compounds can be significantly bioaccumulative in aquatic insects, e.g. hydroxyzine in Zygoptera (Lagesson et al., 2016) and azithromycin in Trichoptera (Grabicova et al., 2015), having BAF values over 5000 L/kg wet basis.

According to calculated bioaccumulation factors for contaminants measured in *S. nigricornis* samples from the Dubrava study site, endocrine disrupting compound bisphenol-A (BPA), which is often used for the production of plastics, showed the highest potential for bioaccumulation with a value of 5461 L/kg dry weight. Although this value is not indicating that BPA can be considered as highly bioaccumulative in caddisflies (Arnot and Gobas, 2006; Borgå, 2013), it still confirms that BPA is prone to bioaccumulate in caddisflies more likely than other compounds present at the site. BPA also shows the highest bioamplification across metamorphosis from pupae to adults, implying that it is also susceptible to considerably bioamplify during development of Trichoptera. This finding is also in line with data on bioamplification of BPA in Odonata. Even though information on impacts of BPA on aquatic insects is limited, it was observed to cause reduced adult emergence and moulting malformities in holometabolous Noctuidae butterfly (Kontogiannatos et al., 2015).

When observing individual pharmaceuticals, concentrations of thiabendazole and ketoprofen both show increase in concentrations through *S. nigricornis* development with significantly higher concentrations in adults compared to pupae. Information on exact effects of the ECs measured in this study on aquatic insects like caddisflies is mostly lacking, however, research on other aquatic organisms and terrestrial insects (Kontogiannatos et al., 2015; Prášková et al., 2013; Qiao et al., 2022), indicates most of these contaminants could be toxic and cause developmental alterations in aquatic insects too. Concentrations of thiabendazole (also commonly used in agriculture as fungicide (EFSA, 2014)) measured in this study are generally very low (maximum 0.474 ngg-1 dry weight) and according to EFSA pesticide risk assessment (2014), this contaminant is considered to have low level of risk to aquatic organisms. However, studies show that NSAID ketoprofen causes growth and development delay in fish embryos and larvae, with effects recorded even at low water concentrations (0.003 mg/l) (Prášková et al., 2013). EDCs measured in this study with significantly higher concentrations in adults (bioamplification during the second metamorphosis stage) are also shown to be toxic (1H-

benzotriazole (1HB) in Seeland et al. (2012); PPB in Medkova et al. (2023) or cause developmental (TCEP in Qiao et al. (2022)) and behavioural changes in various aquatic organisms (CFN in Fraker and Smith (2004)). All previously mentioned effects, coupled with the additional increase in their concentrations throughout their life cycle, as presented in this study, could cause population level changes with increased mortalities.

### **5.1.3. Life history traits determine cross-ecosystem transfer of PhACs and EDCs with emergence of aquatic insects**

Differences in the type of metamorphosis (hemimetabolous or holometabolous aquatic insects) as well as biological differences in the life stages of aquatic insect are observed and recognised as one of the factors determining accumulation of contaminants in aquatic insects as well as their cross-ecosystem transfer (Bundschuh et al., 2022; Kraus et al., 2014b; Previšić et al., 2021). The current study confirms bioamplification across the metamorphosis of some PhACs and EDCs in both Odonata and Trichoptera, in agreement with Previšić et al. (2021). However, here we show considerable differences at different taxonomic levels, i.e. inconsistent patterns between Anisoptera and Zygoptera (i.e. at the suborder level) and among species. The differences between taxa are most likely also attributable to specific life history traits (Previšić et al., 2021). Furthermore, despite the relatively uniform pattern observed in the majority of ECs in *S. nigricornis*, patterns of some contaminant's concentrations (eg. CFN, 1HB and PPB) suggest potential life stage-specific bioaccumulation. This means that the exposure and availability of contaminants, but also differences in metabolic functioning, biotransformation and excretion of these contaminants are influenced by biological traits of a certain life stage of aquatic insects.

For example, when discussing changes in contaminant concentrations during and after insect metamorphosis, it is important to observe the excretion through exuviae. This has been shown to be a pathway of elimination greatly responsible for the decrease in contaminant concentration during metamorphosis of the mosquito *Culex pipiens* Linnaeus, 1758 for the pharmaceutical ivermectin (Lorente et al., 2023). The development of Odonata nymphs involves an individually specific, variable number of moults (ecdyses) – up to 30 moults over a period of three months to 10 years depending on the species (Corbet and Brooks, 2008). Recent research indicates that ecdysis is a valuable pathway for contaminant elimination in Odonata (Liu et al., 2021), thus the number of moults most likely determines bioaccumulation

rates, and subsequently bioamplification. For example, *Gomphus vulgatissimus* (Anisoptera) collected in this study has a total of 15 stadia – periods between two moults (Corbet, 2002). On the other hand, the zygopterans *Platycnemis pennipes* and *Calopteryx splendens*, which were also used in this study, have 11 and 13 stadia, respectively (Corbet, 2002). The lower overall contaminant bioaccumulation observed in Anisoptera could therefore be related to differences in moulting, which also leads to lower bioamplification and explains observed variations between taxa. However, it must also be considered that differences in the number of moults also depend on developmental environmental conditions and often differences are observed also within the same taxa (Corbet and Brooks, 2008).

It is very likely that PhACs and EDCs are partially excreted from the organisms through exuviae in caddisflies as well. However, caddisflies have a lower, fixed number of moults (five for most families) (Waringer and Graf, 2011). Therefore, their importance as an excretory pathway for contaminants could be minor compared to hemimetabolous insects. However, even taking this factor into account, our study documented an overall increase in the total concentrations of ECs, along with an increase in the number of individual PhACs and EDCs (TIB, KPF, CFN, TCEP, PPB), in emergent adults when compared to pupae.

The bioaccumulation of contaminants in insect pupae is generally very poorly studied. The presence of some persistent organic pollutants (e.g., polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)) has been reported in pupae of both terrestrial (Huang et al., 2020; Luo et al., 2022) and aquatic insects (Bartrons et al., 2007). Only recently, however, some studies have confirmed that certain pharmaceutical compounds, like ivermectin, also bioaccumulate in aquatic insects pupae (Lorente et al., 2023). Significant developmental changes occurring during pupation could strongly influence bioaccumulation and bioamplification processes and eventually leading to a higher retention of contaminant ending in higher concentrations in the adults, as observed in this study for certain contaminants in both Odonata and Trichoptera. Luo et al. (2022) described biotransformation of contaminants that occurs during insect metamorphosis as an essential regulatory process for the change in contaminant concentrations between life stages. Data on biotransformation of contaminants in aquatic organisms are only available for a few compounds and show considerable differences between fish and aquatic insects, e.g. biotransformation of temazepam in perch and dragonfly nymphs (Cervený et al., 2021). In addition, differences in the biotransformation of polybrominated diphenyl ethers (PBDEs) have been hypothesised to influence differences in bioamplification rates between Diptera and



Trichoptera (Bartrons et al., 2007). Finally, differential metabolic efficiency to biotransform PhACs and EDCs in different Odonata suborders cannot be excluded as a reason for the observed differences in bioaccumulation and bioamplification patterns of PhACs and EDCs in Odonata as well.

Although bioamplification has not been extensively studied, it has been shown that many contaminants can undergo bioamplification in aquatic insects (PCBs in Ephemeroptera in Kraus et al. (2014a); metals, e.g. Cd in Trichoptera and Odonata in Cetinić et al. (2021); organochlorine compounds (OCs) and polybromodiphenyl ethers (PBDEs) in Trichoptera and Diptera in Bartrons et al. (2007)), including PhACs and EDCs in Odonata and Trichoptera (Previšić et al., 2021). Bioamplification of PhACs and EDCs was also confirmed in this study for several compounds in both, Odonata and Trichoptera. The increase in concentrations across metamorphosis may be mainly the result of the reduction in body mass. This could explain the differences in concentration between nymphs/larvae and adults, as well as the differences in bioamplification observed between orders and Odonata suborder levels. Very large differences in mass loss have been reported for different insect taxa (e.g. 90% loss in Lepidoptera, 20% loss in Ephemeroptera, (Kraus et al., 2014b)), but there is no data on the body mass loss during metamorphosis of Odonata species included in the current study. However, considering the observed variability, data from the literature might provide only limited information, especially if obtained from unimpacted sites.

However, in the experiments with the caddisfly *Micropterna nycterobia* (McLachlan, 1875) under conditions with multiple stressors, considerable variability in mass loss was observed during metamorphosis in relation to environmental conditions (e.g. increased water temperature and pollution with PhACs & EDCs compared to controls) and sex (Kokotović et al., 2024). The loss of body mass in Trichoptera is due to the cessation of feeding in pupal and adult stages, with additional energy expenditure for flying in the adult stage (Huryn and Wallace, 2000). This allows certain PhACs and EDCs to concentrate in the caddisfly tissues. The bioamplification factors calculated in this study indicate that concentration enrichment in holometabolous Trichoptera is happening during both, development from larvae to pupae and from pupae to adults (i.e., first and second stage of metamorphosis in caddisflies, respectively). In this study, greater increase occurred during the second part of caddisflies metamorphosis, from pupal to adult stage, which is the opposite of what Cetinić et al. (2021) observed for metals, where most metals showed a decrease in concentrations, which was more pronounced during the transition from larval to pupal stage.

## 5.2. Predicting bioaccumulation patterns of PhACs and EDCs by assessing the relation of contaminant properties and bioaccumulation processes

After entering natural waters, contaminants tend to undergo varying degrees of biodegradation, solubility, adsorption, persistence and mobility varies (Stasinakis, 2012). In their study, Heynen et al. (2016) demonstrated that the prompt response of the dragonfly *Aeshna grandis* (Linnaeus, 1758) (Anisoptera) to variations in water concentrations of the pharmaceutical oxazepam was due to processes involving the adsorption of the compound on the body's surface, rather than genuine uptake and metabolic elimination. This result emphasises the importance of the properties of the contaminant in defining bioaccumulation patterns in biota. The vast majority of compounds measured in the aquatic insect samples from the Dubrava and Krapina study sites have low or low to moderate potential for bioconcentration (contaminant uptake via body surface and respiration, excluding dietary exposure) according to models based on their log  $K_{ow}$  values (PubChem Database, Arnot and Gobas, 2006; Borgå, 2013). This is consistent with the results of this study, as in general none of the compounds showed a high bioaccumulation potential. Wilkinson et al. (2018) found a positive relationship between the log  $K_{ow}$  values of the contaminants and the log BCF (bioconcentration factors) in their study of various emerging contaminants (including pharmaceuticals), with compounds with log  $K_{ow}$  value above 5 having the highest log BCF and log BAF values. In addition, Lagesson et al. (2016) attempted to predict the bioaccumulation potential of pharmaceuticals in aquatic organisms by calculating the predicted BAF values using their log  $K_{ow}$  values and following the regression described in Arnot and Gobas (2006). Their analyses revealed large discrepancies between the predicted BAF values using log  $K_{ow}$  and the ones calculated from the results of the study, and they concluded that bioaccumulation differs significantly among organisms due to their trophic position and habitat use and that the prediction of bioaccumulation is therefore very limited. In this current study Trichoptera BAFs showed positive correlations with the molecular mass (Mr) (Spearman's rank correlation, Table 8). Molecular size is related to absorption and membrane permeation and Arnot et al. (2010) in their review of the influence of molecular size descriptors (including MW) on bioconcentration (and bioaccumulation) stated that "molecular size influences solubility and diffusivity in water and organic phases (membranes), and larger molecules may have slower uptake rates" leading to their higher bioaccumulation potential. The results of this study therefore confirm the relationship between molecular size and bioaccumulation, but further research is needed to obtain more precise information. On the other hand, the results of the orthogonal partial least

squares – discriminant analysis (OPLS-DA) using the ADME descriptors of the measured compounds and the information on contaminant bioaccumulation potential (quantified in water and nymphs/larvae versus quantified only in water), could not provide clear separation between bioaccumulating and non-bioaccumulating contaminants. The results also did not indicate which descriptors are responsible for bioaccumulation in aquatic insect tissues (Figure 16, 17). Consequently, this study shows that none of the physico-chemical and pharmacokinetic descriptors could be used to accurately predict bioaccumulation in Odonata and Trichoptera, suggesting that the used descriptors poorly reflect the underlying biochemistry of bioaccumulation in these organisms. In line with Previšić et al. (2021), this study further suggests that previous models mainly established on persistent organic pollutants may not be easily applied to predict bioaccumulation and bioaccumulation potential of other different compounds in aquatic environment (Ismail et al., 2014; Puckowski et al., 2016).

This study confirmed a positive relationship between log  $K_{ow}$  and bioamplification factor values in Odonata (linear regressions, Table 7), but only at the order level. Log  $K_{ow}$  also showed statistically significant positive correlations (Spearman's rank correlation, Table 6) with the bioamplification factor values in Trichoptera, but only for the second metamorphosis stage (pupa to imago). The confirmed positive relationships between BAMF values and log  $K_{ow}$ , are consistent with observations for persistent organic pollutants (e.g. PCBs), where bioamplification has been shown to be dependent on log  $K_{ow}$  with higher BAMFs of more hydrophobic chemicals (Daley et al., 2012; Kraus, 2019). However, not all BAMF values calculated for all different taxa levels or metamorphosis stages (in Trichoptera) were positively related to log  $K_{ow}$ . To accurately predict such a process, which involves complex biochemical changes during insect metamorphosis, the analysis of a larger dataset and probably more complex models are required.

### **5.3. Food web transfer of waterborne emerging contaminants and the extent of the transfer into riparian zone**

The current study confirmed the presence of seven ECs in all organism groups in the analysed aquatic-terrestrial food web from the Sutla River study site. The presence of three pharmaceuticals (including antidiabetic drug glibenclamide, antidepressant sertraline and diuretic hydrochlorothiazide) and four endocrine disruptors (flame retardant TCEP and parabens (MPB, EPB and PPB)) confirms that these waterborne emerging contaminants undergo trophic transfer to the riparian food webs, corroborating the research findings of several other studies (Du et al., 2014; Lagesson et al., 2016; Previšić et al., 2021; Richmond et al., 2018; Ruhí et al., 2016; Xie et al., 2015).

Although determining the exact trophic level and relationships was beyond the scope of this study, based on the literature and information on feeding preferences and trophic levels of the invertebrates collected in this study, we can assume that investigated aquatic-terrestrial food web contains organisms belonging to different trophic positions. For example, biofilm and macrophytes are aquatic primary producers. In addition, the Trichoptera species collected in this study (Table A2) belong to different trophic groups, some are predators (e.g. *Rhyacophila dorsalis*), while others are partially filter feeders - predators - grazers (e.g. Hydropsychidae, 50-30-20%; Graf et al., 2023). Only adult Trichoptera were collected in this study, but they all feed exclusively as larvae (Waringer and Graf, 2011), which means that their contaminant concentrations depend only on the uptake during aquatic phase. Due to the small sample size, the Trichoptera were observed at the order level, which limits the identification of their trophic level. However, as they are an important food source for terrestrial predators, understanding their contaminant body burden is crucial for the study of contaminant flux in the terrestrial food web (Huryn and Wallace, 2000). On the contrary, all Odonata species are predators, suggesting that the Odonata nymphs collected in this study play the most important predatory role in the aquatic segment of the studied food web (Corbet and Brooks, 2008). The adult Odonata observed in this study are also terrestrial predators. However, sampling included predominantly teneral adults with the aim of reducing the impact of their terrestrial predation on their contaminants concentrations. Consequently, the concentrations of ECs measured in adult Odonata are primarily the result of bioaccumulation during the aquatic (nymphal) stages. Furthermore, Lumbricidae function as detritivores (Steffan et al., 2017) and Diplopoda (Coleman et al., 2004) and Isopoda (Zimmer, 2002) as mostly saprophages. Although their

food preferences may vary, e.g. some diplopods can also feed on soil invertebrates (Coleman et al., 2004), in this studied food web they are considered as predominantly decomposers. Hence, their contaminant concentration is most likely primarily influenced by soil contamination. Coleoptera collected in this study belong to the Carabidae family (ground beetles), are predators (Weseloh and Hare, 2009) as well as all spiders (Araneae), including families Tetragnathidae (long jawed web hunting spiders ) and Lycosidae (ground wolf spiders) collected within this study. As such, their contaminant concentration is mostly dependent on their dietary exposure (Graf et al., 2020). Ground beetles in our food web could represent a link to decomposers they can feed on and their contamination uptake. Ground wolf spiders similarly hunt their prey on the ground and their food preferences depend on the invertebrate/insect community in their environment. In contrast, long jawed web hunting spiders, consume insects caught in their web, and in riparian zone that can primarily include aquatic insects, at least during some seasons (Huryn and Wallace, 2000; Schulz et al., 2015b). The difference in ECs concentration between these two spider families thus shows how their different food uptake caused by different catching technique affects their contaminants uptake. In particular, the concentrations observed in Tetragnathidae show the extent to which waterborne ECs can transfer through the food web in the riparian zone.

The highest total concentration of contaminants, comparing all samples investigated in this study, was measured in soil samples (especially high concentrations of caffeine and antidepressant sertraline). Schulz et al. (2015) pointed out the great importance of flooding for the dynamics of energy, nutrient, and contamination flux between aquatic and terrestrial ecosystems, in both directions, from aquatic to terrestrial, and vice versa. The high soil contaminant concentrations observed in this study are likely the result of riverbank flooding, suggesting that flooding may play an important role in determining bioaccumulation patterns for organisms in the detritivores food chain. This is consistent with the observed significantly higher concentrations of total parabens and methylparaben in Diplopoda (millipedes) from the transect closer to the river (Down) compared to samples collected in the Up transect, as their habitat and feeding preferences are closely associated with soil and soil organisms (Coleman et al., 2004). Furthermore, this study recorded high concentrations of antidepressant sertraline not only in soil, but also in biofilm and macrophyte samples. This result confirms the previous assertion that pharmaceuticals have the greatest potential for bioaccumulation in organisms at lower trophic levels, such as algae (Ding et al., 2015; Huerta et al., 2016; Vernouillet et al., 2010).

Although the total concentrations of contaminants in all samples do not differ significantly with respect to distance from the river, some individual compounds show significant differences in concentrations between samples collected closer to the river (0-1 m) and those collected higher on the riverbank (1-3 m). However, opposite trends are observed, as propylparaben is recorded to be higher in samples collected further from the river, whereas caffeine is higher in samples collected closer to the river. This suggests that further research with a bigger set of data is required to obtain a clearer picture of the extent of trophic transfer of PhACs and EDCs in riparian zone. Nevertheless, an interesting pattern was observed in spiders from different transects. At the order level (Araneae), spiders from transects closer to the river showed a higher bioaccumulation potential for flame retardant TCPP and caffeine, as their concentrations were significantly higher in samples collected closer to the river. Looking at the recorded contaminant concentrations at the family level also reveals an interesting pattern for valsartan. Tetragnathidae, web hunting long-jawed spiders, had significantly higher concentrations of valsartan in samples closer to the river, compared to the spiders belonging to the same family collected higher on the riverbank. However, the other spider family, Lycosidae, showed no significant differences in contaminants concentrations regarding the distance from the river. This suggests that web hunting spiders, like Tetragnathidae, are more susceptible to contaminant flux generated by aquatic insects. This is consistent with the fact that riparian Tetragnathidae communities are recognised to be entirely dependent on emergent aquatic insects as a food source (Krell et al., 2015; Schulz et al., 2015b). Furthermore, Walters et al. (2008) pointed out that tetragnathid spiders “deserve greater consideration as sentinels of aquatic contamination” due to the fact that they are relatively sedentary and specialise in consuming aquatic insects.

Aquatic insects in this food web study confirmed the bioaccumulation of various PhACs and EDCs originating from the polluted Sutla River. More specifically, 15 and 18 compounds were quantified in Trichoptera and Odonata samples, respectively. It is important to monitor these contaminants not only in relation to aquatic insects, but to the entire ecosystem, as studied suggest that aquatic insects are the main food source (and consequently possibly a source of contamination) for their riparian predators (Schulz et al., 2015b). One of the highest contaminant concentrations in adult Trichoptera was recorded for flame retardant TCPP (Table 9, 14 ngg<sup>-1</sup> dry weight) and the same compound shows significantly increased concentration in tetragnathid spiders closer to the river. This could indicate that adult caddisflies are a major part of the diet of tetragnathid spiders inhabiting the transect closer to the river.

The concentrations of contaminants recorded in Odonata are primarily the result of their predation in the aquatic food web. When comparing the bioaccumulation of the same contaminants measured at two different study sites for two *in situ* studies, the Krapina and the Sutla, different patterns are observed. In the Sutla River adult Anisoptera showed statistically higher concentrations of several compounds (glibenclamide, methylparaben, TCEP, total parabens) compared to Anisoptera nymphs. Additionally, TCEP levels were higher in adult Anisoptera compared to adult Zygoptera. In contrast, TCEP concentrations in Odonata samples from the Krapina River study site showed opposite patterns in Anisoptera and Zygoptera. Zygoptera nymphs from the Krapina River showed significantly higher concentrations of TCEP compared to Zygoptera adults and Anisoptera nymphs. In addition, the adult Anisoptera from the Sutla River had statistically higher concentrations of total parabens compared to the nymphs. However, in the Krapina River study site, Anisoptera nymphs generally had higher concentrations of individual parabens (methylparaben and propylparaben) compared to adults, representing an opposite trend. These contrasting results are further evidence that the taxonomic level of observed organisms determines the bioaccumulation patterns but also confirm the statements of Arnot and Gobas (2006) that the bioaccumulation patterns of contaminants are difficult to compare in different food webs in nature. This means that the structure and relationships in the aquatic food web in the Krapina River differ from that of the Sutla River, which most likely influences the bioaccumulation process in Zygoptera and Anisoptera and causes the observed deviations.

## 6. CONCLUSIONS

### **i. Differences in biology and taxonomic level determine bioaccumulation and bioamplification patterns.**

The current study showed that taxonomic level and underlying biological traits are important when determining the resolution at which the rates of bioaccumulation and bioamplification of PhACs and EDCs in aquatic insects are assessed, evaluated, and predicted. Findings on contaminants bioaccumulation in two different aquatic insect orders, hemimetabolous Odonata and holometabolous Trichoptera, indicated different bioaccumulation patterns regarding different life history traits, and displayed the importance of the pupal stage in holometabolous insects in determining final contaminant concentration in adults. That is, exposure and availability of contaminants, but also differences in metabolic functioning, biotransformation and excretion of these contaminants are affected by biological traits of a certain aquatic insect's life stage. Furthermore, results of this study confirmed that contaminants uptake could be driven by fine scale differences between taxa which points out the importance of the taxonomical resolution when studying this subject.

### **ii. Cross-ecosystem flux of waterborne pharmaceuticals and endocrine disrupting compounds is driven by emergent aquatic insects and trophic transfer of contaminants depends on composition of the food web.**

Emergence of aquatic insects accumulating PhACs and EDCs during the aquatic phase, represents a pathway for these compounds to be further transferred from aquatic to terrestrial food webs. Due to the typically small size of adult aquatic insects, elevated rates of prey consumption in riparian predators may result in significantly higher exposures to PhACs and EDCs compared to the concentrations present in the insect's aquatic environment. Consequently, a comprehensive understanding of these processes linking the two ecosystems merit further attention. Results of this study confirmed the presence of pharmaceuticals and endocrine disrupting compounds on all levels of the observed food web, which confirms the trophic transfer of these contaminants on aquatic-terrestrial ecosystem boundary. Furthermore, results indicate that the transfer is occurring through different pathways and the amount and resolution of the data in this study seems to be limiting factor in drawing strong conclusions about which pathway is primarily responsible for the transfer of certain compounds. Opposite



trends observed for bioaccumulation of contaminants in aquatic food webs from different study sites, additionally indicates that bioaccumulation trends and patterns established for one food web are most likely not applicable for the other food webs. However, confirming the presence of the contaminants originating from the wastewater effluents in all trophic levels of aquatic-terrestrial food web further proves that, even though transfer of these contaminants is still mostly unexplored, it seems necessary to start monitor the uptake and effects these contaminants have on aquatic biota. This especially refers to communities and organisms that show the highest potential for bioaccumulation of pharmaceuticals and endocrine disruptors, e.g. biofilm, macrophytes. Furthermore, monitoring the bioaccumulation in aquatic insects is also essential, as they represent the main trophic connection for the transfer of pharmaceuticals and endocrine disruptors between ecosystems. In general, further research on this matter could provide valuable information on exposure routes, persistence, effects, and accumulation potential in biota of both, aquatic and terrestrial food webs, as well as consequently defining mitigation strategies.

**iii. Using contaminant physico-chemical descriptors and predictors related to pharmacokinetics for underlying the prediction of bioaccumulation and bioamplification of pharmaceuticals and endocrine disrupting compounds is very limited.**

The results of this study indicate a notable limitation in the efficacy of physico-chemical and pharmacokinetic descriptors when it comes to accurately predicting bioaccumulation in Odonata and Trichoptera. The results suggest that the descriptors used in this study inadequately capture the underlying biochemistry of bioaccumulation in these organisms as none of the descriptors demonstrated a high level of success in precise predictions. This highlights the complexity of the bioaccumulation process in Odonata and Trichoptera and indicates that additional factors or descriptors may be necessary for a more comprehensive understanding. Further research, using a more extensive dataset, could provide clearer insights into the potential utility of physico-chemical descriptors in predicting the bioaccumulation potential of pharmaceuticals and endocrine disrupting compounds.

## 7. LITERATURE

- Arnot, J.A., Arnot, M.I., Mackay, D., Couillard, Y., Macdonald, D., Bonnell, M., Doyle, P., 2010. Molecular Size Cutoff Criteria for Screening Bioaccumulation Potential : Fact or Fiction ? 6, 210–224. <https://doi.org/10.1897/IEAM>
- Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ. Rev.* 14, 257–297. <https://doi.org/10.1139/A06-005>
- Arnot, J.A., Gobas, F.A.P.C., 2003. A Generic QSAR for Assessing the Bioaccumulation Potential of Organic Chemicals in Aquatic Food Webs. *QSAR Comb. Sci.* 22, 337–345.
- aus der Beek, T., Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A., 2016. Pharmaceuticals in the environment-Global occurrences and perspectives. *Environ. Toxicol. Chem.* 35, 823–835. <https://doi.org/10.1002/etc.3339>
- Bai, X., Lutz, A., Carroll, R., Keteles, K., Dahlin, K., Nguyen, D., 2018. Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. *Chemosphere* 200, 133–142. <https://doi.org/10.1016/j.chemosphere.2018.02.106>
- Baird, D.J., Van den Brink, P.J., 2007. Using biological traits to predict species sensitivity to toxic substances. *Ecotoxicol. Environ. Saf.* 67, 296–301. <https://doi.org/10.1016/j.ecoenv.2006.07.001>
- Bartrons, M., Grimalt, J.O., Catalan, J., 2007. Concentration changes of organochlorine compounds and polybromodiphenyl ethers during metamorphosis of aquatic insects. *Environ. Sci. Technol.* 41, 6137–6141. <https://doi.org/10.1021/es0703271>
- Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A., Dar, S.A., 2020. Concerns and threats of contamination on aquatic ecosystems, in: *Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation*. pp. 1–26. [https://doi.org/10.1007/978-3-030-35691-0\\_1](https://doi.org/10.1007/978-3-030-35691-0_1)
- Bayabil, H.K., Teshome, F.T., Li, Y.C., 2022. Emerging Contaminants in Soil and Water. *Emerg. Contam.* 10, 1–8. <https://doi.org/10.3389/fenvs.2022.873499>
- Bonnineau, C., Artigas, J., Chaumet, B., Dabrin, A., Faburé, J., Ferrari, B.J.D., Lebrun, J.D., Margoum, C., Mazzella, N., Miège, C., Morin, S., Uher, E., Babut, M., Pesce, S., 2020. Role of Biofilms in Contaminant Bioaccumulation and Trophic Transfer in Aquatic

- Ecosystems: Current State of Knowledge and Future Challenges Chloé, in: *Reviews of Environmental Contamination and Toxicology*. pp. 115–153.  
[https://doi.org/10.1007/398\\_2019\\_39](https://doi.org/10.1007/398_2019_39)
- Boreen, A.L., Arnold, W.A., McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment : A review. *Aquat. Sci. Rev.* 65, 320–341.  
<https://doi.org/10.1007/s00027-003-0672-7>
- Borgå, K., 2013. Ecotoxicology: Bioaccumulation. *Ref. Modul. Earth Syst. Environ. Sci.* 6–8.  
<https://doi.org/10.1016/b978-0-12-409548-9.00765-x>
- Bose, A.P.H., McCallum, E.S., Avramović, M., Bertram, M.G., Blom, E.L., Cervený, D., Grønlund, S.N., Leander, J., Lundberg, P., Martin, J.M., Michelangeli, M., Persson, L., Brodin, T., 2022. Pharmaceutical pollution disrupts the behavior and predator-prey interactions of two widespread aquatic insects. *iScience* 25.  
<https://doi.org/10.1016/j.isci.2022.105672>
- Boxall, A.B.A., Sinclair, C.J., Fenner, K., Kolpin, D., Maund, S.J., 2004. Peer Reviewed: When Synthetic Chemicals Degrade in the Environment. *Environ. Sci. Technol.* 38, 368A–375A.
- Briers, R.A., Cariss, H.M., Geoghegan, R., Gee, J.H.R., 2005. The lateral extent of the subsidy from an upland stream to riparian lycosid spiders. *Ecography (Cop.)*. 28, 165–170. <https://doi.org/10.1111/j.0906-7590.2005.04020.x>
- Brochard, C., Groenendijk, D., van der Ploeg, E., Termaat, T., 2012. *Fotogids Larvenhuidjes van Libellen 2012*.
- Brumovsky, M., Bečanová, J., Kohoutek, J., Borghini, M., Nizzetto, L., 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean \*. *Environ. Pollut.* 229, 976–983. <https://doi.org/10.1016/j.envpol.2017.07.082>
- Bundschuh, M., Pietz, S., Roodt, A.P., Kraus, J.M., 2022. Contaminant fluxes across ecosystems mediated by aquatic insects. *Curr. Opin. Insect Sci.*  
<https://doi.org/10.1016/j.cois.2022.100885>
- Burket, S.R., White, M., Ramirez, A.J., Stanley, J.K., Banks, K.E., Waller, W.T., Chambliss, C.K., Brooks, B.W., 2019. *Corbicula fluminea* rapidly accumulate pharmaceuticals from an effluent dependent urban stream. *Chemosphere* 224, 873–883.

- <https://doi.org/10.1016/j.chemosphere.2019.03.014>
- Burkhard, L.P., 2003. Factors influencing the design of bioaccumulation factor and biota-sediment accumulation factor field studies. *Environ. Toxicol. Chem.* 22, 351–360.  
[https://doi.org/10.1897/1551-5028\(2003\)022<0351:FITDOB>2.0.CO;2](https://doi.org/10.1897/1551-5028(2003)022<0351:FITDOB>2.0.CO;2)
- Caliman, F.A., Gavrilescu, M., 2009. Pharmaceuticals , Personal Care Products and Endocrine Disrupting Agents in the Environment – A Review. *Clean Soil Air Water* 37, 277–303. <https://doi.org/10.1002/clen.200900038>
- Cervený, D., Fick, J., Klaminder, J., McCallum, E.S., Bertram, M.G., Castillo, N.A., Brodin, T., 2021. Water temperature affects the biotransformation and accumulation of a psychoactive pharmaceutical and its metabolite in aquatic organisms. *Environ. Int.* 155, 106705. <https://doi.org/10.1016/j.envint.2021.106705>
- Cetinić, K.A., Previšić, A., Rožman, M., 2021. Holo- and hemimetabolism of aquatic insects: Implications for a differential cross-ecosystem flux of metals. *Environ. Pollut.* 277. <https://doi.org/10.1016/j.envpol.2021.116798>
- Chang, E.D., Hogstrand, C., Miller, T.H., Owen, S.F., Bury, N.R., 2019. The Use of Molecular Descriptors to Model Pharmaceutical Uptake by a Fish Primary Gill Cell Culture Epithelium. *Environ. Sci. Technol.* 53, 1576–1584.  
<https://doi.org/10.1021/acs.est.8b04394>
- Coleman, D.C., Crossley, D.A.J., Hendrix, P.F., 2004. Secondary Production : Activities of Heterotrophic Organisms — The Soil Fauna, in: *Fundamentals of Soil Ecology* (Second Edition). pp. 79–185. <https://doi.org/10.1016/B978-0-12-179726-3.50005-8>
- Corbet, P., Brooks, S., 2008. Dragonflies. Collins New Naturalist Library series, Book 106. London: Harper Collins, 480 p.
- Corbet, P.S., 2002. Stadia and growth ratios of Odonata: A review. *Int. J. Odonatol.* 5, 45–73. <https://doi.org/10.1080/13887890.2002.9748176>
- Corbet, P.S., 1999. Dragonflies: Behaviour and Ecology of Odonata. Cornell University Press. New York, United States 1999.
- Daley, J.M., 2013. Bioamplification as a bioaccumulation mechanism. Electronic Theses and Dissertations. 4758. University of Windsor Scholarship, Windsor, Ontario, Canada. <https://scholar.uwindsor.ca/etd/4758>.

- Daley, J.M., Corkum, L.D., Drouillard, K.G., 2011. Aquatic to terrestrial transfer of sediment associated persistent organic pollutants is enhanced by bioamplification processes. *Environ. Toxicol. Chem.* 30, 2167–2174. <https://doi.org/10.1002/etc.608>
- Daley, J.M., Leadley, T.A., Drouillard, K.G., 2009. Evidence for bioamplification of nine polychlorinated biphenyl (PCB) congeners in yellow perch (*Perca flavescens*) eggs during incubation. *Chemosphere* 75, 1500–1505. <https://doi.org/10.1016/j.chemosphere.2009.02.013>
- Daley, J.M., Leadley, T.A., Pitcher, T.E., Drouillard, K.G., 2012. Bioamplification and the selective depletion of persistent organic pollutants in Chinook salmon larvae. *Environ. Sci. Technol.* 46, 2420–2426. <https://doi.org/10.1021/es204003a>
- de Pennart, A., Matthews, P.G.D., 2020. The bimodal gas exchange strategies of dragonfly nymphs across development. *J. Insect Physiol.* 120, 103982. <https://doi.org/10.1016/j.jinsphys.2019.103982>
- de Solla, S.R., Gilroy, A.M., Klinck, J.S., King, L.E., McInnis, R., Struger, J., Backus, S.M., Gillis, P.L., 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel *Lasmigona costata* in a river receiving wastewater effluent. *Chemosphere* 146, 486–496. <https://doi.org/10.1016/j.chemosphere.2015.12.022>
- Dijkstra, K.-D., Wildermuth, H., Martens, A., 2024. Dataset “Odonata”. [www.freshwaterecology.info](http://www.freshwaterecology.info) - the taxa and autecology database for freshwater organisms [WWW Document]. version 8.0 (accessed 07.02.2024).
- Dijkstra, K.-D., Wildermuth, H., Martens, A., 2022. Dataset “Odonata” [WWW Document]. [Www.Freshwaterecology.Info](http://Www.Freshwaterecology.Info).
- Ding, J., Lu, G., Li, S., Nie, Y., Liu, J., 2015. Biological fate and effects of propranolol in an experimental aquatic food chain. *Sci. Total Environ.* 532, 31–39. <https://doi.org/10.1016/j.scitotenv.2015.06.002>
- Dodds, W.K., 2002. Effects of Toxic Chemicals and Other Pollutants on Aquatic Ecosystems, in: *Freshwater Ecology, Concepts and Environmental Applications*. pp. 267–293.
- Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A., Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., Brooks, B.W., 2014. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-

- dependent wadeable stream. *Philos. Trans. R. Soc. B Biol. Sci.* 369.  
<https://doi.org/10.1098/rstb.2014.0058>
- Ducrot, V., Usseglio-Polatera, P., Péry, A.R.R., Mouthon, J., Lafont, M., Roger, M.C., Garric, J., Férard, J.F., 2005. Using aquatic macroinvertebrate species traits to build test batteries for sediment toxicity assessment: Accounting for the diversity of potential biological responses to toxicants. *Environ. Toxicol. Chem.* 24, 2306–2315.  
<https://doi.org/10.1897/04-559R.1>
- Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg. Contam.* 3, 1–16.  
<https://doi.org/10.1016/j.emcon.2016.12.004>
- EFSA, 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance thiabendazole. *EFSA J.* 12. <https://doi.org/10.2903/j.efsa.2014.3880>
- Enyoh, C.E., Verla, A.W., Qingyue, W., Ohiagu, F.O., Chowdhury, A.H., Enyoh, E.C., Chowdhury, T., Verla, E.N., Chinwendu, U.P., 2020. An Overview of Emerging Pollutants in Air: Method of Analysis and Potential Public Health Concern from Human Environmental Exposure. *Trends Environ. Anal. Chem.* 28, e00107.  
<https://doi.org/10.1016/j.teac.2020.e00107>
- Fan, J.J., Wang, S., Tang, J.P., Zhao, J.L., Wang, L., Wang, J.X., Liu, S.L., Li, F., Long, S.X., Yang, Y., 2019. Bioaccumulation of endocrine disrupting compounds in fish with different feeding habits along the largest subtropical river, China. *Environ. Pollut.* 247, 999–1008. <https://doi.org/10.1016/j.envpol.2019.01.113>
- Fleeger, J.W., 2020. How Do Indirect Effects of Contaminants Inform Ecotoxicology? A Review. *Processes* 8, 1659.
- Fraker, S.L., Smith, G.R., 2004. Direct and interactive effects of ecologically relevant concentrations of organic wastewater contaminants on *Rana pipiens* tadpoles. *Environ. Toxicol.* 19, 250–256. <https://doi.org/10.1002/tox.20017>
- Franke, C., Studinger, G., Berger, G., Böhling, S., Bruckmann, U., Cohors-Fresenborg, D., Jöhncke, U., 1994. The Assessment of Bioaccumulation. *Chemosphere* 29, 1501–1514.  
[https://doi.org/10.1007/10503050\\_4](https://doi.org/10.1007/10503050_4)
- Fu, Q., Fedrizzi, D., Kosfeld, V., Schlechtriem, C., Ganz, V., Derrer, S., Rentsch, D.,

- Hollender, J., 2020. Biotransformation Changes Bioaccumulation and Toxicity of Diclofenac in Aquatic Organisms. *Environ. Sci. Technol.* 54, 4400–4408.  
<https://doi.org/10.1021/acs.est.9b07127>
- Gaino, E., Piersanti, S., Rebora, M., 2007. Ultrastructural organization of the larval spiracles in *Libellula depressa* L. (Anisoptera: Libellulidae). *Odonatologica* 36, 373–379.
- Garcia, S.N., Foster, M., Constantine, L.A., Huggett, D.B., 2012. Field and laboratory fish tissue accumulation of the anti-convulsant drug carbamazepine. *Ecotoxicol. Environ. Saf.* 84, 207–211. <https://doi.org/10.1016/j.ecoenv.2012.07.013>
- Gobas, F.A.P.C., Maclean, L.G., 2003. Sediment-water distribution of organic contaminants in aquatic ecosystems: The role of organic carbon mineralization. *Environ. Sci. Technol.* 37, 735–741. <https://doi.org/10.1021/es020925+>
- Grabicova, K., Grabic, R., Blaha, M., Kumar, V., Cervený, D., Fedorova, G., Randak, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. *Water Res.* 72, 145–153.  
<https://doi.org/10.1016/j.watres.2014.09.018>
- Graf, N., Battes, K.P., Cimpean, M., Dittrich, P., Entling, M.H., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Schäfer, R.B., 2019. Do agricultural pesticides in streams influence riparian spiders? *Sci. Total Environ.* 660, 126–135.  
<https://doi.org/10.1016/j.scitotenv.2018.12.370>
- Graf, N., Battes, K.P., Cimpean, M., Entling, M.H., Frisch, K., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Zubrod, J.P., Schäfer, R.B., 2020. Relationship between agricultural pesticides and the diet of riparian spiders in the field. *Environ. Sci. Eur.* 32, 1–12. <https://doi.org/10.1186/s12302-019-0282-1>
- Graf, W., Murphy, J., Dahl, J., Zamora-Muñoz, C., López-Rodríguez, M., Schmidt-Kloiber, A., 2023. Dataset “Trichoptera” [WWW Document]. URL [www.freshwaterecology.info](http://www.freshwaterecology.info) - the taxa and autecology database for freshwater organisms
- Grgić, I., Cetinić, K.A., Karačić, Z., Previšić, A., Rožman, M., 2023. Fate and effects of microplastics in combination with pharmaceuticals and endocrine disruptors in freshwaters: Insights from a microcosm experiment. *Sci. Total Environ.* 859.  
<https://doi.org/10.1016/j.scitotenv.2022.160387>

- Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. *J. Chromatogr. A* 1248, 104–121. <https://doi.org/10.1016/j.chroma.2012.05.084>
- Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Burket, S.R., Kristofco, L.A., Corrales, J., Rasmussen, J.B., Chambliss, C.K., Luers, M., Rogers, C., Brooks, B.W., 2018. Spatio-temporal bioaccumulation and trophic transfer of ionizable pharmaceuticals in a semi-arid urban river influenced by snowmelt. *J. Hazard. Mater.* 359, 231–240. <https://doi.org/10.1016/j.jhazmat.2018.07.063>
- Heynen, M., Fick, J., Jonsson, M., Klaminder, J., Brodin, T., 2016. Effect of bioconcentration and trophic transfer on realized exposure to oxazepam in 2 predators, the dragonfly larvae (*Aeshna grandis*) and the Eurasian perch (*Perca fluviatilis*). *Environ. Toxicol. Chem.* 35, 930–937. <https://doi.org/10.1002/etc.3368>
- Ho, C.S., Lam, C.W., Chan, M.H.M., Cheung, R.C.K., Law, L.K., Lit, L.C.W., Ng, K.F., Suen, M.W.M., Tai, H.L., 2003. Electrospray Ionisation Mass Spectrometry: Principles and Clinical Applications. *Clin Biochem Rev* 24, 3–12. <https://doi.org/10.1002/9781118307816.ch34>
- Hodin, J., 2006. Expanding networks: Signaling components in and a hypothesis for the evolution of metamorphosis. *Integr. Comp. Biol.* 46, 719–742. <https://doi.org/10.1093/icb/icl038>
- Huang, L.-Q., Luo, X.-J., Qi, X.-M., Mai, B.X., 2020. Sex-Specific Bioamplification of Halogenated Organic Pollutants during Silkworm (*Bombyx mori*) Metamorphosis and Their Adverse Effects on Silkworm Development. *Environ. Sci. Technol.* 54, 8167–8176. <https://doi.org/10.1021/acs.est.9b07585>
- Huang, Q., Wang, H., Lewis, M.A., 2015. The impact of environmental toxins on predator-prey dynamics. *J. Theor. Biol.* 378, 12–30. <https://doi.org/10.1016/j.jtbi.2015.04.019>
- Huerta, B., Jakimska, A., Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2013. Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* 1288, 63–72. <https://doi.org/10.1016/j.chroma.2013.03.001>



- Huerta, B., Jakimska, A., Llorca, M., Ruhí, A., Margoutidis, G., Acuña, V., Sabater, S., Rodríguez-Mozaz, S., Barceló, D., 2015. Development of an extraction and purification method for the determination of multi-class pharmaceuticals and endocrine disruptors in freshwater invertebrates. *Talanta* 132, 373–381.  
<https://doi.org/10.1016/j.talanta.2014.09.017>
- Huerta, B., Rodríguez-Mozaz, S., Barceló, D., 2012. Pharmaceuticals in biota in the aquatic environment: Analytical methods and environmental implications. *Anal. Bioanal. Chem.* 404, 2611–2624. <https://doi.org/10.1007/s00216-012-6144-y>
- Huerta, B., Rodríguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., Sabater, S., Barceló, D., 2016. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. *Sci. Total Environ.* 540, 241–249. <https://doi.org/10.1016/j.scitotenv.2015.05.049>
- Huryn, A.D., Wallace, J.B., 2000. LIFE HISTORY AND PRODUCTION OF STREAM INSECTS. *Annu. Rev. Entomol.* 83–110.  
<https://doi.org/10.1146/annurev.ento.51.110104.151107>
- Ismail, N.S., Müller, C.E., Morgan, R.R., Luthy, R.G., 2014. Uptake of contaminants of emerging concern by the bivalves *Anodonta californiensis* and *Corbicula fluminea*. *Environ. Sci. Technol.* 48, 9211–9219. <https://doi.org/10.1021/es5011576>
- Jakimska, A., Huerta, B., Bargańska, Z., Kot-Wasik, A., Rodríguez-Mozaz, S., Barceló, D., 2013. Development of a liquid chromatography-tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers. *J. Chromatogr. A* 1306, 44–58.  
<https://doi.org/10.1016/j.chroma.2013.07.050>
- Johansson, F., 1991. Foraging modes in an assemblage of odonate larvae - effects of prey and interference. *Hydrobiologia* 209, 79–87. <https://doi.org/10.1007/BF00006721>
- Karlsson, S., Meili, M., Bergström, U., 2002. Bioaccumulation factors in A critical review 67.
- Kato, C., Iwata, T., Nakano, S., Kishi, D., 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. *Oikos* 103, 113–120.  
<https://doi.org/10.1034/j.1600-0706.2003.12477.x>

- Kokotović, I., Veseli, M., Ložek, F., Karačić, Z., Rožman, M., Previšić, A., 2024. Pharmaceuticals and endocrine disrupting compounds modulate adverse effects of climate change on resource quality in freshwater food webs. *Sci. Total Environ.* 912. <https://doi.org/10.1016/j.scitotenv.2023.168751>
- Kontogiannatos, D., Swevers, L., Zakasis, G., Kourti, A., 2015. The molecular and physiological impact of bisphenol A in *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Ecotoxicology* 24, 356–367. <https://doi.org/10.1007/s10646-014-1384-6>
- Kraus, J.M., 2019. Contaminants in linked aquatic–terrestrial ecosystems: Predicting effects of aquatic pollution on adult aquatic insects and terrestrial insectivores. *Freshw. Sci.* 38, 919–927. <https://doi.org/10.1086/705997>
- Kraus, J.M., Schmidt, T.S., Walters, D.M., Wanty, R.B., Zuellig, R.E., Wolf, R.E., 2014a. Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. *Ecol. Appl.* 24, 235–243. <https://doi.org/10.1890/13-0252.1>
- Kraus, J.M., Walters, D.M., Wesner, J.S., Stricker, C.A., Schmidt, T.S., Zuellig, R.E., 2014b. Metamorphosis alters contaminants and chemical tracers In insects: Implications for food webs. *Environ. Sci. Technol.* 48, 10957–10965. <https://doi.org/10.1021/es502970b>
- Krell, B., Röder, N., Link, M., Gergs, R., Entling, M.H., Schäfer, R.B., 2015. Aquatic prey subsidies to riparian spiders in a stream with different land use types. *Limnologia* 51, 1–7. <https://doi.org/10.1016/j.limno.2014.10.001>
- Kriska, G., 2013. Freshwater Invertebrates in Central Europe, in: *Freshwater Invertebrates in Central Europe*. pp. 194–209. <https://doi.org/10.1007/978-3-7091-1547-3>
- Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., Klaminder, J., 2016. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web - Insights from a field experiment. *Sci. Total Environ.* 568, 208–215. <https://doi.org/10.1016/j.scitotenv.2016.05.206>
- Liess, M., 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environ. Toxicol. Chem.* 24, 954–965. <https://doi.org/10.1897/03-652.1>
- Liu, J., Wang, R., Huang, B., Lin, C., Zhou, J., Pan, X., 2012. Biological effects and bioaccumulation of steroidal and phenolic endocrine disrupting chemicals in high-back crucian carp exposed to wastewater treatment plant effluents. *Environ. Pollut.* 162, 325–

331. <https://doi.org/10.1016/j.envpol.2011.11.036>
- Liu, Y., Luo, X., Zeng, Y., Wang, Q., Tu, W., Yang, C., Mai, B., 2020. Trophic Magnification of Short- And Medium-Chain Chlorinated Paraffins in Terrestrial Food Webs and Their Bioamplification in Insects and Amphibians during Metamorphosis. *Environ. Sci. Technol.* 54, 11282–11291. <https://doi.org/10.1021/acs.est.0c03096>
- Liu, Y., Luo, X.J., Huang, L.Q., Yu, L.H., Mai, B.X., 2018. Bioaccumulation of Persistent Halogenated Organic Pollutants in Insects: Common Alterations to the Pollutant Pattern for Different Insects during Metamorphosis. *Environ. Sci. Technol.* 52, 5145–5153. <https://doi.org/10.1021/acs.est.8b00616>
- Liu, Y.E., Luo, X.J., Liu, Y., Zeng, Y.H., Mai, B.X., 2021. Bioaccumulation of legacy and emerging organophosphorus flame retardants and plasticizers in insects during metamorphosis. *J. Hazard. Mater.* 406, 124688. <https://doi.org/10.1016/j.jhazmat.2020.124688>
- Loffredo, E., Senesi, N., 2006. The role of humic substances in the fate of anthropogenic organic pollutants in soil with emphasis on endocrine disruptor compounds. *Soil Water Pollut. Monit. Prot. Remediat.* 3–23. <https://doi.org/10.1007/978-1-4020-4728-2>
- Lorente, C.J., Mesa, L., Montalto, L., Gutiérrez, M.F., Miró, M. V., Lifschitz, A., 2023. Ivermectin bioaccumulation and transfer through developmental stages in *Culex pipiens* (Diptera: Culicidae). *Chemosphere* 322, 2013–2015. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2023.138106>
- Luo, X.J., Huang, L.Q., Guan, K.L., Qi, X.M., Zeng, Y.H., Mai, B.X., 2022. Bioamplification and Biomagnification of Polycyclic Aromatic Hydrocarbons and Halogenated Organic Pollutants in Moths from an Electronic Waste Recycling Site. *Environ. Toxicol. Chem.* 41, 2395–2403. <https://doi.org/10.1002/etc.5432>
- Mamy, L., Patureau, D., Barriuso, E., Bedos, C., Bessac, F., Louchart, X., Martin-Laurent, F., Miege, C., Benoit, P., 2015. Prediction of the fate of organic compounds in the environment from their molecular properties: A review. *Crit. Rev. Environ. Sci. Technol.* 45, 1277–1377. <https://doi.org/10.1080/10643389.2014.955627>
- Mandaric, L., Celic, M., Marce, J.L., Petrovic, M., 2015. Introduction on Emerging Contaminants in Rivers and Their Environmental Risk, in: *Emerging Contaminants in*

- River Ecosystems. The Handbook of Environmental Chemistry. pp. 1–12.  
<https://doi.org/10.1007/698>
- Mayer-Pinto, M., Matias, M.G., Coleman, R.A., 2016. The interplay between habitat structure and chemical contaminants on biotic responses of benthic organisms. *PeerJ* 2016, 1–19. <https://doi.org/10.7717/peerj.1985>
- Medkova, D., Hollerova, A., Riesova, B., Blahova, J., Hodkovicova, N., Marsalek, P., Doubkova, V., Weiserova, Z., Mares, J., Faldyna, M., Tichy, F., Svobodova, Z., Lakdawala, P., 2023. Pesticides and Parabens Contaminating Aquatic Environment: Acute and Sub-Chronic Toxicity towards Early-Life Stages of Freshwater Fish and Amphibians.
- Meredith-Williams, M., Carter, L.J., Fussell, R., Raffaelli, D., Ashauer, R., Boxall, A.B.A., 2012. Uptake and depuration of pharmaceuticals in aquatic invertebrates. *Environ. Pollut.* 165, 250–258. <https://doi.org/10.1016/j.envpol.2011.11.029>
- Miller, T.H., Bury, N.R., Owen, S.F., Barron, L.P., 2017. Uptake, biotransformation and elimination of selected pharmaceuticals in a freshwater invertebrate measured using liquid chromatography tandem mass spectrometry. *Chemosphere* 183, 389–400. <https://doi.org/10.1016/j.chemosphere.2017.05.083>
- Morin-Crini, N., Lichtfouse, E., Liu, G., Balaram, V., Rita, A., Ribeiro, L., Lu, Z., Stock, F., Carmona, E., Ribau, M., Picos-corrales, L.A., Morin-crini, N., Lichtfouse, E., Liu, G., Balaram, V., Rita, A., Ribeiro, L., 2022. Worldwide cases of water pollution by emerging contaminants : a review. *Environ. Chem. Lett.*
- Oetken, M., Nentwig, G., Löffler, D., Ternes, T., Oehlmann, J., 2005. Effects of pharmaceuticals on aquatic invertebrates. Part I. The antiepileptic drug carbamazepine. *Arch. Environ. Contam. Toxicol.* 49, 353–361. <https://doi.org/10.1007/s00244-004-0211-0>
- Pastorino, P., Zaccaroni, A., Doretto, A., Falasco, E., Silvi, M., Dondo, A., Elia, A.C., Prearo, M., Bona, F., 2020. Functional feeding groups of aquatic insects influence trace element accumulation: Findings for filterers, scrapers and predators from the po basin. *Biology (Basel)*. 9, 1–15. <https://doi.org/10.3390/biology9090288>
- Prášková, E., Štěpánová, S., Chromcová, L., Plhalová, L., Voslářová, E., Pištěková, V.,

- Prokeš, M., Svobodová, Z., 2013. The effects of subchronic exposure to ketoprofen on early developmental stages of common carp. *Acta Vet. Brno* 82, 343–347.  
<https://doi.org/10.2754/avb201382030343>
- Previšić, A., Mihaljević, Z., Kerovec, M., 2007. Caddisfly (Insecta: Trichoptera) fauna of altered and man-made habitats in the Drava River, NW Croatia. *Nat. Croat.* 16, 181–187.
- Previšić, A., Rožman, M., Mor, J.R., Acuña, V., Serra-Compte, A., Petrović, M., Sabater, S., 2019. Aquatic macroinvertebrates under stress: Bioaccumulation of emerging contaminants and metabolomics implications. *Sci. Total Environ.* 135333.  
<https://doi.org/10.1016/j.scitotenv.2019.135333>
- Previšić, A., Vilenica, M., Vučković, N., Petrović, M., Rožman, M., 2021. Aquatic Insects Transfer Pharmaceuticals and Endocrine Disruptors from Aquatic to Terrestrial Ecosystems. *Environ. Sci. Technol.* 55, 3736–3746.  
<https://doi.org/10.1021/acs.est.0c07609>
- Puckowski, A., Mioduszevska, K., Łukaszewicz, P., Borecka, M., Caban, M., Maszkowska, J., Stepnowski, P., 2016. Bioaccumulation and analytics of pharmaceutical residues in the environment: A review. *J. Pharm. Biomed. Anal.* 127, 232–255.  
<https://doi.org/10.1016/j.jpba.2016.02.049>
- Qiao, Y., Liu, D., Feng, C., Liu, N., Wang, J., Yan, Z., Bai, Y., 2022. Ecological risk assessment for tris(2-chloroethyl) phosphate to freshwater organisms. *Front. Environ. Sci.* 10, 1–11. <https://doi.org/10.3389/fenvs.2022.963918>
- Quan, C.S., Liu, Q., Tian, W.J., Kikuchi, J., Fan, S.D., 2005. Biodegradation of an endocrine-disrupting chemical, di-2-ethylhexyl phthalate, by *Bacillus subtilis* No. 66. *Appl. Microb. Cell Physiol.* 66, 702–710. <https://doi.org/10.1007/s00253-004-1683-6>
- Raikow, D.F., Walters, D.M., Fritz, K.M., Mills, M.A., 2011. The distance that contaminated aquatic subsidies extend into lake riparian zones. *Ecol. Appl.* 21, 983–990.  
<https://doi.org/10.1890/09-1504.1>
- Richmond, E.K., Rosi-Marshall, E.J., Lee, S.S., Thompson, R.M., Grace, M.R., 2016. Antidepressants in stream ecosystems: Influence of selective serotonin reuptake inhibitors (SSRIs) on algal production and insect emergence. *Freshw. Sci.* 35, 845–855.

<https://doi.org/10.1086/687841>

- Richmond, E.K., Rosi, E.J., Walters, D.M., Fick, J., Hamilton, S.K., Brodin, T., Sundelin, A., Grace, M.R., 2018. A diverse suite of pharmaceuticals contaminates stream and riparian food webs. *Nat. Commun.* 9, 1–9. <https://doi.org/10.1038/s41467-018-06822-w>
- Rodriguez, P., Méndez-Fernández, L., Pardo, I., Costas, N., Martinez-Madrid, M., 2018. Baseline tissue levels of trace metals and metalloids to approach ecological threshold concentrations in aquatic macroinvertebrates. *Ecol. Indic.* 91, 395–409. <https://doi.org/10.1016/j.ecolind.2018.04.004>
- Rolff, J., Johnston, P.R., Reynolds, S., 2019. Complete metamorphosis of insects. *Philos. Trans. R. Soc. B Biol. Sci.* <https://doi.org/10.1098/rstb.2019.0063>
- Ruhí, A., Acuña, V., Barceló, D., Huerta, B., Mor, J.R., Rodríguez-Mozaz, S., Sabater, S., 2016. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. *Sci. Total Environ.* 540, 250–259. <https://doi.org/10.1016/j.scitotenv.2015.06.009>
- Sabir, S., Akhtar, M.F., Saleem, A., 2018. Endocrine disruption as an adverse effect of non-endocrine targeting pharmaceuticals. *Environ. Sci. Pollut. Res.* 26, 1277–1286.
- Samal, K., Mahapatra, S., Hibzur Ali, M., 2022. Pharmaceutical wastewater as Emerging Contaminants (EC): Treatment technologies, impact on environment and human health. *Energy Nexus* 6, 100076. <https://doi.org/10.1016/j.nexus.2022.100076>
- Schulz, R., Bundschuh, M., 2020. Pathways of Contaminant Transport Across the Aquatic-Terrestrial Interface: Implications for Terrestrial Consumers, Ecosystems, and Management, in: *Contaminants and Ecological Subsidies*. pp. 35–57. [https://doi.org/10.1007/978-3-030-49480-3\\_3](https://doi.org/10.1007/978-3-030-49480-3_3)
- Schulz, R., Bundschuh, M., Gergs, R., Brühl, C.A., Diehl, D., Entling, M.H., Fahse, L., Frör, O., Jungkunst, H.F., Lorke, A., Schäfer, R.B., Schaumann, G.E., Schwenk, K., 2015a. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. *Sci. Total Environ.* 538, 246–261. <https://doi.org/10.1016/j.scitotenv.2015.08.038>
- Schulz, R., Bundschuh, M., Gergs, R., Brühl, C.A., Diehl, D., Entling, M.H., Fahse, L., Frör, O., Jungkunst, H.F., Lorke, A., Schäfer, R.B., Schaumann, G.E., Schwenk, K., 2015b. Review on environmental alterations propagating from aquatic to terrestrial ecosystems.

- Sci. Total Environ. 538, 246–261. <https://doi.org/10.1016/j.scitotenv.2015.08.038>
- Schwaiger, J., Ferling, H., Mallow, U., Wintermayr, H., Negele, R.D., 2004. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: Histopathological alterations and bioaccumulation in rainbow trout. *Aquat. Toxicol.* 68, 141–150. <https://doi.org/10.1016/j.aquatox.2004.03.014>
- Seeland, A., Oetken, M., Kiss, A., Fries, E., Oehlmann, J., 2012. Acute and chronic toxicity of benzotriazoles to aquatic organisms. *Environ. Sci. Pollut. Res.* 19, 1781–1790. <https://doi.org/10.1007/s11356-011-0705-z>
- Sidney, L.A., Diepens, N.J., Guo, X., Koelmans, A.A., 2016. Trait-based modelling of bioaccumulation by freshwater benthic invertebrates. *Aquat. Toxicol.* 176, 88–96. <https://doi.org/10.1016/j.aquatox.2016.04.017>
- Simon, E., Tóthmérész, B., Kis, O., Jakab, T., Szalay, P.É., Vincze, A., Baranyai, E., Harangi, S., Miskolczi, M., Dévai, G., 2019. Environmental-friendly contamination assessment of habitats based on the trace element content of dragonfly exuviae. *Water (Switzerland)* 11. <https://doi.org/10.3390/w11112200>
- Sims, J.L., Burket, S.R., Franco, M.E., Lovin, L.M., Scarlett, K.R., Steenbeek, R., Chambliss, C.K., Ashcroft, C., Luers, M., Lavado, R., Brooks, B.W., 2020. Pharmaceutical uptake kinetics in rainbow trout: In situ bioaccumulation in an effluent-dominated river influenced by snowmelt. *Sci. Total Environ.* 736. <https://doi.org/10.1016/j.scitotenv.2020.139603>
- Sode, A., Wiberg-Larsen, P., 1993. Dispersal of adult Trichoptera at a Danish forest brook. *Freshw. Biol.* 30, 439–446. <https://doi.org/10.1111/j.1365-2427.1993.tb00827.x>
- Späth, J., Brodin, T., McCallum, E., Cervený, D., Fick, J., Nording, M.L., 2022. Metabolomics reveals changes in metabolite profiles due to growth and metamorphosis during the ontogeny of the northern damselfly. *J. Insect Physiol.* 136. <https://doi.org/10.1016/j.jinsphys.2021.104341>
- Stasinakis, A.S., 2012. Review on the fate of emerging contaminants during sludge anaerobic digestion. *Bioresour. Technol.* 121, 432–440. <https://doi.org/10.1016/j.biortech.2012.06.074>
- Stefanakis, A.I., Becker, J., 2015. A Review of Emerging Contaminants in Water:

- Classification, Sources, and Potential Risks, in: *Impact of Water Pollution on Human Health and Environmental Sustainability*. pp. 55–80. <https://doi.org/10.4018/978-1-4666-9559-7.ch003>
- Steffan, S.A., Chikaraishi, Y., Dharampal, P.S., Pauli, J.N., Guédot, C., Ohkouchi, N., 2017. Unpacking brown food-webs : Animal trophic identity reflects rampant microbivory. *Ecol. Evol.* 7, 3532–3541. <https://doi.org/10.1002/ece3.2951>
- Thévenot, E.A., Roux, A., Xu, Y., Ezan, E., Junot, C., 2015. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. *J. Proteome Res.* 14, 3322–3335. <https://doi.org/10.1021/acs.jproteome.5b00354>
- Tijani, J.O., Fatoba, O.O., Petrik, L.F., 2013. A review of pharmaceuticals and endocrine-disrupting compounds: Sources, effects, removal, and detections. *Water. Air. Soil Pollut.* 224. <https://doi.org/10.1007/s11270-013-1770-3>
- Ubhi, R., Matthews, P.G.D., 2018. The transition from water to air in aeshnid dragonflies is associated with a change in ventilatory responses to hypoxia and hypercapnia. *J. Insect Physiol.* 106, 172–178. <https://doi.org/10.1016/j.jinsphys.2017.09.010>
- Valdés, M.E., Amé, M.V., Bistoni, M. de los A., Wunderlin, D.A., 2014. Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquía River basin (Córdoba, Argentina). *Sci. Total Environ.* 472, 389–396. <https://doi.org/10.1016/j.scitotenv.2013.10.124>
- Vernouillet, G., Eullaffroy, P., Lajeunesse, A., Blaise, C., Gagné, F., Juneau, P., 2010. Toxic effects and bioaccumulation of carbamazepine evaluated by biomarkers measured in organisms of different trophic levels. *Chemosphere* 80, 1062–1068. <https://doi.org/10.1016/j.chemosphere.2010.05.010>
- Walters, D.M., Fritz, K.M., Otter, R.R., 2008. The dark side of subsidies: Adult stream insects export organic contaminants to riparian predators. *Ecol. Appl.* 18, 1835–1841. <https://doi.org/10.1890/08-0354.1>
- Walters, D.M., Jardine, T.D., Cade, B.S., Kidd, K.A., Muir, D.C.G., Leipzig-Scott, P., 2016. Trophic Magnification of Organic Chemicals: A Global Synthesis. *Environ. Sci.*



- Technol. 50, 4650–4658. <https://doi.org/10.1021/acs.est.6b00201>
- Walters, D.M., Mills, M.A., Fritz, K.M., Raikow, D.F., 2010. Spider-mediated flux of PCBs from contaminated sediments to terrestrial ecosystems and potential risks to arachnivorous birds. *Environ. Sci. Technol.* 44, 2849–2856. <https://doi.org/10.1021/es9023139>
- Waringer, J.A., Graf, W., 2011. *Atlas of Central European Trichoptera Larvae*. Erik Mauch Verlag.
- Weber, F.-A., Aus Der Beek, T., Carius, A., Grüttner, G., Hickmann, S., Ebert, I., Hein, A., Küster, A., Rose, J., Koch-Jugl, J., Stolzenberg, H.-C., 2014. Pharmaceuticals in the environment – the global perspective. *Environ. Toxicol. Chem.* 35, 823–835.
- Weseloh, R.M., Hare, J.D., 2009. Predation / Predatory Insects, in: *Encyclopedia of INSECTS (Second Edition)*. Elsevier Inc., pp. 837–839. <https://doi.org/10.1016/B978-0-12-374144-8.00219-8>
- Wesner, J., Kraus, J.M., Henry, B., Kerby, J., 2020. Metamorphosis and the Impact of Contaminants on Ecological Subsidies, in: *Contaminants and Ecological Subsidies*. pp. 111–125. [https://doi.org/10.1007/978-3-030-49480-3\\_6](https://doi.org/10.1007/978-3-030-49480-3_6)
- Wesner, J.S., Kraus, J.M., Schmidt, T.S., Walters, D.M., Clements, W.H., 2014. Metamorphosis enhances the effects of metal exposure on the mayfly, *Centroptilum triangulifer*. *Environ. Sci. Technol.* 48, 10415–10422. <https://doi.org/10.1021/es501914y>
- Wesner, J.S., Walters, D.M., Schmidt, T.S., Kraus, J.M., Stricker, C.A., Clements, W.H., Wolf, R.E., 2017. Metamorphosis Affects Metal Concentrations and Isotopic Signatures in a Mayfly (*Baetis tricaudatus*): Implications for the Aquatic-Terrestrial Transfer of Metals. *Environ. Sci. Technol.* 51, 2438–2446. <https://doi.org/10.1021/acs.est.6b05471>
- Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. *Environ. Pollut.* 234, 864–875. <https://doi.org/10.1016/j.envpol.2017.11.090>
- Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., Chen, W., 2015. Occurrence, bioaccumulation, and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. *Chemosphere* 138, 140–147.

<https://doi.org/10.1016/j.chemosphere.2015.05.086>

Yamamoto, H., Nakamura, Yudai, Moriguchi, S., Nakamura, Yuki, Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., Sekizawa, J., 2009. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment : Laboratory photolysis , biodegradation , and sorption experiments. *Water Res.* 43, 351–362.  
<https://doi.org/10.1016/j.watres.2008.10.039>

Zimmer, M., 2002. Nutrition in terrestrial isopods ( Isopoda : Oniscidea ) : an evolutionary-ecological approach. *Biol. Rev.* 77, 455–493.

## 8. APPENDIX

### *Methods – additional information*

#### **Details regarding UPLC separation, ion source and MRM parameters**

Target analysis was performed using an ultra-performance liquid chromatography (UPLC) system (Waters Milford, MA, USA) coupled to a hybrid quadrupole linear ion trap mass spectrometer Qtrap 5500 (Applied Biosystems, Foster City, CA, USA).

#### **UPLC separation settings:**

Target analysis was performed using an ultra-performance liquid chromatography (UPLC) system (Waters Milford, MA, USA) coupled to a hybrid quadrupole linear ion trap mass spectrometer Qtrap 5500 (Applied Biosystems, Foster City, CA, USA). Following chromatographic columns were used:

- PhACs positive ion mode (PIM) and EDCs PIM - Waters Acquity HSS T3 (50 mm × 2.1 mm i.d., 1.8 µm particle size),
- PhACs negative ion mode (NIM) and EDCs NIM – Waters Acquity BEH C18 (50 mm × 2.1 mm i.d., 1.7 µm particle size).

The sample volume injected was 0.05 mL for all analyses.

For the separation of PhACs in PIM methanol (solvent A) and 10 mM ammonium formate at pH 3.2 (solvent B) at the 0.5 ml/min flow rate were used. The gradient elution was: 0 - 4.5 min, 5 – 95% A; 4.5 – 6 min, 100% A; 6 - 6.7 min, 5% A.

For the separation of PhACs in NIM acetonitrile (solvent A) and 10 mM ammonium acetate at pH 8 (solvent B) at the 0.6 ml/min flow rate were used. The gradient elution was: 0 - 1.5 min, 5 – 60% A; 1.5 – 2 min, 60-100% A; 2 - 3 min, 100% A; 3.2 -3.7 min, 5% A.

For the separation of EDCs in PIM methanol (solvent A) and 10 mM ammonium formate at pH 3.2 (solvent B) at the 0.4 ml/min flow rate were used. The gradient elution was: 0–3 min, 30 – 100% A; 3 – 4.75 min, 100% A; 4.75 – 5.75 min, 100-30% A; 5.75–7 min, 30% A.

For the separation of EDCs in NIM methanol (solvent A) and water at pH 9 solvent (B) at the flow rate 0.4 ml/min were used. The gradient elution was: 0 – 4 min, 30–100% A; 4–5 min, 100% A; 5–6 min, 100-30% A; 6–7.5 min, 30% A.

### **Ion source settings**

**Positive ion mode:** curtain gas 30, nitrogen collision gas medium; source temperature was 600 C; ion spray voltage was 5 kV; ion source gases 1 and 2 were 60 and 40, respectively.

**Negative ion mode:** curtain gas 30, nitrogen collision gas medium; source temperature was 650 C; ion spray voltage was –3.5 kV; ion source gases 1 and 2 were 60 and 70, respectively.

### **Scheduled MRM parameters**

Employed scheduled MRM parameters can be found in references (Gros et al., 2012; Huerta et al., 2015).

### **Methods for estimating the distribution coefficients**

As suggested by Armitage et al. (2013), octanol–water distribution coefficient ( $\log D_{OW}$ ) at pH 8 (mean value at the sampling site) was estimated as:

$$\log D_{OW} = \log (x_N \times K_{OW} + x_I \times K'_{OW})$$

where  $x_N$  is the fraction of EC in neutral form,  $x_I$  is the fraction of EC in ionized form at given pH,  $K_{OW}$  is the octanol–water partition constant for neutral EC and  $K'_{OW}$  is the octanol–water partition constant of EC ionized form.  $K'_{OW}$  was predicted by scaling down the octanol–water partition coefficient of the neutral form by 3.1 log units according to the ref (Armitage et al., 2013) i.e.  $\log K'_{OW} = \log K_{OW} - 3.1$ .

Similarly, membrane – water distribution coefficient ( $\log D_{MW}$ ) at pH 8 (mean value at the sampling site) was estimated as:

$$\log D_{MW} = \log (x_N \times K_{MW} + x_I \times K'_{MW})$$

where  $x_N$  is the fraction of EC in neutral form,  $x_I$  is the fraction of EC in ionized form at given pH.  $K_{MW}$  is the membrane–water partition constant for neutral EC predicted from  $K_{OW}$  using following equation:

$$\log K_{\text{MW}} = 1.01\log K_{\text{OW}} + 0.12 \text{ (Armitage et al., 2013).}$$

$K_{\text{MW}}^{\text{I}}$ , the membrane–water partition constant of ionic EC form, was predicted by scaling down the membrane–water partition coefficient  $\log K_{\text{MW}}^{\text{I}} = \log K_{\text{MW}} - \Delta_{\text{MW}}$ . Scaling factors ( $\Delta_{\text{MW}}$ ) were taken from Armitage (2013) and summarized in Table S2.

Scaling factors for correcting partition coefficients of the ionized form of ECs.

<b>Compound Class</b>	<b><math>\Delta_{\text{MW}}</math></b>
<i>Acids</i>	
Phenolics	0.75
Carboxylic acids	2.00
Sulfonic acids	2.00
Other	2.00
<i>Bases</i>	
Primary amine	0.30
Secondary amine	0.50
Tertiary amine	1.25
Other	1.25

**Table A1.** Information on size ranges for last instars of the nymphs belonging to taxa collected at the Krapina study site (Brochard et al., 2012).

Odonata nymphs	
Anisoptera	<i>Gomphus vulgatissimus</i> (28-32 mm)
	<i>Onychogomphus forcipatus</i> (22-26 mm)
	<i>Orthetrum albistylum</i> (20-25 mm)
Zygoptera	<i>Calopteryx splendens</i> (30-37 mm)
	<i>Platynemesis pennipes</i> (20-22 mm)

**Table A2.** List of taxa collected for food web study on the Sutla River. Samples are collected on three transects and processed and studied as composite samples. (NY - nymphs, IM - imagines, LV - larvae; D - Down, U - Up)

Sample type / Taxon group	Method	Taxa (specific)	Transect
water	direct sample		
biofilm	scraping		
macrophytes	picking		
soil	digging		
Zygoptera NY	D-net	<i>Calopteryx splendens</i> (Harris, 1782)	
Anisoptera NY	D-net	<i>Onychogomphus forcipatus</i> (Linnaeus, 1758)	
Zygoptera IM	entomological net	<i>Platynemesis pennipes</i> (Pallas, 1771)	
		<i>Calopteryx splendens</i> (Harris, 1782)	
Anisoptera IM	entomological net	<i>Onychogomphus forcipatus</i> (Linnaeus, 1758)	
Trichoptera IM	UV light trap	<i>Rhyacophila dorsalis</i> (Curtis, 1834)	
		<i>Polycentropus irroratus</i> (Curtis, 1835)	
		<i>Hydropsyche</i> spp.	
		<i>Cheumatopsyche lepida</i> (Pictet, 1834)	
		<i>Athripsodes cinereus</i> (Curtis, 1834)	
		<i>Mystacides longicornis</i> (Linnaeus, 1758)	
		<i>Ceraclea dissimilis</i> (Stephens, 1836)	
		Hydroptilidae non det.	
		Psychomyiidae non det.	
Oligochaeta	digging	Lumbricidae	D
Oligochaeta	digging	Lumbricidae	U
Coleoptera	pitfall traps	<i>Carabus</i> sp.	D
Coleoptera	pitfall traps	<i>Carabus</i> sp.	U
Coleoptera	pitfall traps	<i>Carabus</i> sp. LV	U
Coleoptera	pitfall traps	<i>Pterostichus</i> sp.	U
Isopoda	pitfall traps	Isopoda terrestrial	D

Sample type / Taxon group	Method	Taxa (specific)	Transect
Diplopoda	pitfall traps	Diplopoda	D
Diplopoda	pitfall traps	Diplopoda	U
Araneae	net	Tetragnathidae	D
Araneae	net	Tetragnathidae	U
Araneae	pitfall traps	Lycosidae	D
Araneae	pitfall traps	Lycosidae	U

**Table A3.** List compounds screened in the *in situ* samples from Krapina and Dubrava study sites and internal standards (IS) used for analysis.

1. E2	E2 D2
2. Benzylparaben	Methylparaben D4
3. BPA	BPA D4
4. Diethylstibesterol	E2 D2
5. E1-3G	E1 D4
6. E1-3S	E1 D4
7. E2-17G	E2 D2
8. E3	E2 D2
9. EE2	EE2 D4
10. E1	E1 D4
11. Ethylparaben	Methylparaben D4
12. Methylparaben	Methylparaben D4
13. Octylphenol	Octylphenol D17
14. Propylparaben	Methylparaben D4
15. Triclosan	BPA D4
16. Nonylphenol	Octylphenol D17
17. Triclosan	BPA D4
18. 1H-benzotriazole	1H-Benzotriazole D4
19. Caffeine	Caffeine D3
20. Progesterone	Progesterone D8
21. TBEP	Triphenyl phosphate D15
22. TCPP	Triphenyl phosphate D15
23. TCEP	Triphenyl phosphate D15
24. Tolyltriazole	1H-Benzotriazole D4
25. Levonorgestrel	1H-Benzotriazole D4
26. Acetaminophen	Acetaminophen D4
27. Ibuprofen	Ibuprofen D3
28. Indomethacine	Indomethacine D4
29. Diclofenac	Ibuprofen D3
30. Piroxicam	Ibuprofen D3
31. Meloxicam	Ibuprofen D3
32. Bezafibrate	Bezafibrate D6
33. Gemfibrozil	Gemfibrozil D6
34. Hydrochlorothiazide	Hydrochlorothiazide D2
35. Furosemide	Furosemide D5
36. Losartan	Valsartan D8
37. Dexamethasone	Dexamethasone D4
38. Ketoprofen	Ibuprofen D3
39. Naproxen	Ibuprofen D3
40. Salicylic Acid	Acetaminophen D4
41. Atorvastatin	Gemfibrozil D6

42. Fluvastatin	Gemfibrozil D6
43. Irbesartan	Valsartan D8
44. Pravastatin	Gemfibrozil D6
45. Torasemide	Furosemide D5
46. Valsartan	Valsartan D8
47. Tenoxicam	Ibuprofen D3
48. 1OH-Ibuprofen	Ibuprofen D3
49. Carboxy Ibuprofen	Ibuprofen D3
50. 2OH Ibuprofen	Ibuprofen D3
51. 3OH Acetaminophen	Acetaminophen D4
52. 4OH Diclofenac	Ibuprofen D3
53. 5OH Diclofenac	Ibuprofen D3
54. Diclofenac acyl glucuronide	Ibuprofen D3
55. Naproxen A	Ibuprofen D3
56. Clopidogrel	Warfarin D5
57. Tamsulosin	Azaperone D4
58. Warfarin	Warfarin D5
59. Glibenclamide	Glibenclamide D3
60. Acridone	Carbamazepine D10
61. Albendazole	Ronidazole D3
62. Thiabendazole	Ronidazole D3
63. Levamislo	Ronidazole D3
64. Dimetridazole	Ronidazole D3
65. Ronidazole	Ronidazole D3
66. Xylazine	Xylazine D6
67. Carazolol	Atenolol D4
68. Azaperone	Azaperone D4
69. Azaperol	Azaperone D4
70. Salbutamol	Atenolol D4
71. Dilitiazem	Carbamazepine D10
72. Olanzapine	Carbamazepine D10
73. Propylphenazone	Amlodipine D4
74. Epoxy Carbamazepine	Carbamazepine D10
75. 2-OH Carbamazepine	Carbamazepine D10
76. Metronidazole	Amlodipine D4
77. Loratadine	Cimetidine D3
78. Paroxetine	Fluoxetine D5
79. Sertraline	Fluoxetine D5
80. Codeine	Carbamazepine D10
81. Oxycodone	Carbamazepine D10
82. Metoprolol	Atenolol D4
83. Lorazepam	Diazepam D5
84. Trazodone	Fluoxetine D5 1
85. Iopromide	Cimetidine D3
86. Norfluoxetine	Fluoxetine D5
87. Diazepam	Diazepam D5
88. Alprazolam	Diazepam D5
89. Phenazone	Amlodipine D4
90. Desloratadine	Cimetidine D3
91. Ranitidine	Atenolol D4
92. Famotidine	Atenolol D4
93. Fluoxetine	Fluoxetine D5
94. Carbamazepine	Carbamazepine D10
95. Citalopram	Citalopram D4
96. Venlafaxine	Venlafaxine D6
97. Atenelol	Atenolol D4
98. Sotalol	Atenolol D4
99. Propanolol	Atenolol D4



100.Nadolol	Atenolol D4
101.Amlodipine	Amlodipine D4
102.Verapamil	Verapamil D6
103.Norverapamil	Verapamil D6
104.O-Desmethyl-Venlafaxine	Venlafaxine D6
105.Metoprolol Acid	Atenolol D4
106.N-Desmethyl-Venlafaxine	Venlafaxine D6
107.Tiamulin	Clarithromycin D1
108.Tilmicosin	Tilmicosin D1
109.Lincomycin	Lincomycin D1
110.Sulfamethoxazole	Sulfamethox D1
111.Trimethoprim	Trimethoprim D1
112.Tetracyclin	Tetracyclin D1
113.Oxytetracyclin	Tetracyclin D1
114.Doxycycline	Doxycycline D1
115.Chlortetracycline	Tetracyclin D1
116.Ciprofloxacin	Ciprofloxacin D1
117.Ofloxacin	Ofloxacin D1
118.Enrofloxacin	Ofloxacin D1
119.Marbofloxacin	Marbofloxacin D2
120.Ceftiofur	Clindamycin D1
121.Amoxicillin	Amoxicillin D1
122.Flubendazole	Clarithromycin D1
123.Flunixin	Flunixin D1
124.Sulfamethazine	Sulfamethazine D1
125.Florfenicol	Clindamycin D1
126.Ampicillin	Norfloxacin D1
127.PenicillinV	Norfloxacin D1
128.Cefalexin	Metronidazole D1
129.Norfloxacin	Norfloxacin D1
130.Pipemidic Acid	Ciproflox D1
131.Azithromycin	Azithromycin D1
132.Clarithromycin	Clarithromycin D1
133.Clindamycin	Clindamycin D1
134.Sulfadiazine	Sulfadiazine D1
135.Sulfapyridine	Sulfapyridine D
136.Metronidazole	Metronidazole D1
137.Metronidazole-OH	Metronidazole-OH D1
138.Erythromycin	Azithromycin D1
139.Tylosin	Azithromycin D1
140.Spiramycin	Azithromycin D1
141.Roxithromycin	Azithromycin D1
142.Sulfadimethoxine	Sulfamethox D1
143.Sulfisoxazole	Sulfamethox D1

**Table A4.** List compounds screened in the *in situ* samples from Sutla study sites and internal standards (IS) used for analysis.

1. Acetaminophen	Acetaminophen D4
2. Ibuprofen	Ibuprofen D3
3. Indomethacine	Indomethacine D4
4. Diclofenac	Diclofenac D4
5. Piroxicam	Meloxicam D3
6. Meloxicam	Meloxicam D3
7. Bezafibrate	Bezafibrate D6
8. Gemfibrozil	Gemfibrozil D6
9. Hydrochlorothiazide	Hydrochlorothiazide D2
10. Furosemide	Furosemide D5
11. Dexamethasone	Dexamethasone D4
12. Ketoprofen	Ketoprofen D3
13. Naproxen	Naproxen D3
14. Salicylic Acid	Salicylic Acid D6
15. Atorvastatin	Gemfibrozil D6
16. Fluvastatin	Gemfibrozil D6
17. Irbesartan	Valsartan D8
18. Tenoxicam	Meloxicam D3
19. Naproxen	Naproxen D3
20. Chloramphenicol	Indomethacine D4
21. 1OH-Ibuprofen	Ibuprofen D3
22. 3OH-Acetaminophen	Acetaminophen D4
23. 4OH-Diclofenac	Diclofenac D4
24. Diclofenac acyl glucuronide	Diclofenac D4
25. Ketoprofen	Ketoprofen D3
26. Phenazone	Phenazone D3
27. Desloratadine	Cimetidine D3
28. Ranitidine	Cimetidine D3
29. Famotidine	Cimetidine D3
30. Cimetidine	Cimetidine D3
31. Fluoxetine	Fluoxetine D5
32. Carbamazepine	Carbamazepine D10
33. Citalopram	Citalopram D4
34. Venlafaxine	Venlafaxine D6
35. Atenolol	Atenolol D7
36. Sotalol	Atenolol D7
37. Propanolol	Atenolol D7
38. Nadolol	Atenolol D7
39. Amlodipine	Amlodipine D4
40. Verapamil	Verapamil D6
41. Norverapamil	Verapamil D6
42. Clopidogrel	Sulfamethoxazole D4
43. Tamsulosin	Sulfamethoxazole D4
44. Warfarin	Warfarin D5
45. Glibenclamide	Glibenclamide D3
46. Iopromide 1	Sulfamethoxazole D4
47. Acridone	Carbamazepine D10
48. Albendazole	Ronidazole D3
49. Levamisole	Ronidazole D3
50. Dimetridazole	Ronidazole D3
51. Ronidazole	Ronidazole D3
52. Xylazine	Xylazine D6
53. Carazolol	Atenolol D7
54. Azaperone	Azaperone D4

55. Azaperol	Azaperone D4
56. Salbutamol	Atenolol D7
57. Diltiazem	Carbamazepine D10
58. Olanzapine	Carbamazepine D10
59. propyphenazone	Phenazone D3
60. Epoxy-Carbamazepine	Carbamazepine D10
61. 2-OH-Carbamazepine	Carbamazepine D10
62. Cefalexin	Sulfamethoxazole D4
63. Erythromycin	Erythromycin 13C D3
64. Azithromycin	Azithromycin D3
65. Tetracycline	Sulfamethoxazole D4
66. Ofloxacin	Ofloxacin D3
67. Ciprofloxacin	Ofloxacin D3
68. Clarithromycin	Azithromycin D3
69. Sulfamethoxazole	Sulfamethoxazole D4
70. Trimetoprim	Sulfamethoxazole D4
71. Metronidazole	Ronidazole D3
72. Metronidazole-OH	Ronidazole D3
73. Loratadine	Clmetidine D3
74. Paroxetine	Fluoxetine D5
75. Sertraline	Sertraline D3
76. Codeine	Carbamazepine D10
77. Oxycodone	Carbamazepine D10
78. Metoprolol	AtenololD4
79. Norfluoxetine	Fluoxetine D5
80. Diazepam	Diazepam D5
81. Alprazolam	Diazepam D5
82. Lorazepam	Diazepam D5
83. Tradozone	Fluoxetine D5
84. O-Desmethyl-Venlafaxine	Venlafaxine D6
85. N-Acetyl-Sulfamethoxazole	Sulfamethoxazole D4
86. Sildenafil	Diazepam D5
87. Metoprolol Acid	Atenolol D4
88. N-Desmethyl-Venlafaxine	Venlafaxine D6
89. 4-Nitrososulfamethoxazole	Sulfamethoxazole D4
90. Desamino-Sulfamethoxazole	Sulfamethoxazole D4
91. Diphenylamine	Carbamazepine D10
92. Benzylparaben	Methylparaben D4
93. BPA	BPA D4
94. Diethylstibesterol	E1 D4
95. E1-3G	E1 D4
96. E1-3S	E1 D4
97. E2-17G	E2 D2
98. E3	E2D2
99. EE2	EE2 D4
100.E	E1 D4
101.Ethylparaben	Methylparaben D4
102.Methylparaben	Methylparaben D4
103.Octylphenol	Octylphenol D17
104.Propylparaben	Methylparaben D4
105.Triclosan	Triclosan D3
106.Nonylphenol	Nonylphenol D4
107.1H-benzotriazole	1H- benzotriazole D4
108.Caffeine	Caffeine D3
109.Progesterone	Progesterone D8
110.TBEP	Triphenyl phosphate D15
111.TCPP	Triphenyl phosphate D15
112.TCEP	Triphenyl phosphate D15

113.Tolyltriazole	1H- benzotriazole D4
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**Table A5.** Total concentrations (ngL<sup>-1</sup>) of emerging contaminants (ECs), pharmaceuticals (PhACs) and endocrine disrupting and individual compounds concentrations (ngL<sup>-1</sup>) measured in water samples from the Krapina River shown as mean value  $\pm$  standard deviation.

Water samples			
Total	2379,615 $\pm$ 988,293	Dimetridazole	5.167 $\pm$ 1.461
PhACs	1781.905 $\pm$ 900.571	Acetaminophen	126.037 $\pm$ 41.982
EDCs	597.710 $\pm$ 171.188	Ibuprofen	177.877 $\pm$ 12.609
Azithromycin	0.847 $\pm$ 0.281	2OH-ibuprofen	1126.689 $\pm$ 689.369
Clarithromycin	3.598 $\pm$ 1.743	Hydrochlorothiazide	35.615 $\pm$ 23.155
Cefalexin	7.547 $\pm$ 2.982	Ketoprofen	88.983 $\pm$ 29.391
Metronidazole	2.751 $\pm$ 1.733	Naproxen	86.789 $\pm$ 29.762
Sulfamethoxazole	6.320 $\pm$ 3.390	Salicylic acid	8.688 $\pm$ 3.903
Trimethoprim	12.312 $\pm$ 5.247	1H-benzotriazole	43.728 $\pm$ 23.943
Clopidogrel	0.076 $\pm$ 0.042	Caffeine	456.590 $\pm$ 124.873
Warfarin	0.419 $\pm$ 0.188	TBEP	42.221 $\pm$ 8.905
Thiabendazole	0.189 $\pm$ 0.219	TCPP	33.900 $\pm$ 11.245
epoxy CBZ	0.690 $\pm$ 0.207	TCEP	2.184 $\pm$ 0.751
Metoprolol	0.369 $\pm$ 0.181	Tolyltriazole	7.028 $\pm$ 4.804
Iopromide	75.822 $\pm$ 102.647	BPA	6.188 $\pm$ 5.932
Diazepam	0.294 $\pm$ 0.207	Triclosan	0.082 $\pm$ 0.137
Alprazolam	0.309 $\pm$ 0.144	E1	1.071 $\pm$ 0.458
Ranitidine	1.485 $\pm$ 0.704	Methylparaben	3.698 $\pm$ 2.036
Carbamazepine	5.319 $\pm$ 0.698	Ethylparaben	0.527 $\pm$ 0.150
Sotalol	7.714 $\pm$ 3.445	Propylparaben	0.493 $\pm$ 0.289

**Table A6.** Concentration of emerging contaminants measured in water and biofilm samples from the Dubrava drainage ditch. Mean values and standard deviation in ngg<sup>-1</sup> of dry weight are shown for biofilm and ngL<sup>-1</sup> for water samples. (BQL – below quantification limit)

	<b>Water</b>	<b>Biofilm</b>
Azithromycin	0.971 ± 0.370	2.114 ± 0.818
Clarithromycin	0.072 ± 0.090	0.000 ± 0.000
Sulfamethoxazole	0.589 ± 0.682	0.000 ± 0.000
Tilmicosin	0.000 ± 0.00	0.914 ± 0.435
Trimethoprim	0.712 ± 0.881	0.000 ± 0.000
Thiabendazole	BQL	0.000 ± 0.000
Azaperol	0.117 ± 0.141	0.000 ± 0.000
Epoxy Carbamazepine	0.186 ± 0.075	0.000 ± 0.000
Norfluoxetine	0.000 ± 0.000	267.172 ± 235.534
Ranitidine	0.033 ± 0.039	0.000 ± 0.000
Carbamazepine	1.790 ± 0.593	0.000 ± 0.000
Sotalol	0.177 ± 0.131	0.000 ± 0.000
Acetaminophen	60.789 ± 18.879	0.000 ± 0.000
Ibuprofen	11.548 ± 13.949	0.000 ± 0.000
2-Hydroxyibuprofen	137.219 ± 69.527	0.000 ± 0.000
Hydrochlorothiazide	1.384 ± 1.679	0.000 ± 0.000
Ketoprofen	14.460 ± 7.051	1.191 ± 2.062
Valsartan	8.196 ± 4.756	0.000 ± 0.000
Naproxen	3.270 ± 3.819	0.000 ± 0.000
Salicylic acid	11.923 ± 7.496	25.093 ± 25.093
1H-benzotriazole	8.423 ± 3.046	0.000 ± 0.000
Caffeine	55.044 ± 17.639	25.081 ± 10.566
TBEP	9.937 ± 9.902	10.566 ± 2.066
TCPP	12.688 ± 14.592	45.349 ± 27.186
TCEP	2.426 ± 1.824	0.000 ± 0.000
Tolyltriazole	1.869 ± 1.579	0.000 ± 0.000
BPA	1.194 ± 0.277	1.223 ± 2.119
Triclosan	BQL	0.000 ± 0.000
E1	0.114 ± 0.034	0.000 ± 0.000
Methyparaben	2.891 ± 3.252	4.203 ± 1.881
Ethyparaben	0.746 ± 1.130	0.000 ± 0.000
Propylparaben	0.123 ± 0.174	0.000 ± 0.000

**Table A7.** Significance of differences of total concentrations and concentrations of individual compounds (pharmaceuticals [PhACs] and endocrine disrupting compounds [EDCs]) between Odonata life stages on order and suborder level (Odonata, Anisoptera, Zygoptera); according to the Mann-Whitney U tests. Significant values ( $p < 0.05$ ) are shown in bold. Full names of compounds and abbreviations are listed in Table 1.

	Odonata nymphs-adults		Anisoptera nymphs-adults		Zygoptera nymphs-adults	
Totals	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>
ECs	25	<b>0.000</b>	10	<b>0.007</b>	0	<b>0.000</b>
PhACs	152	0.752	32	0.452	37	0.757
EDCs	46	0.000	23	0.122	0	<b>0.000</b>
Individual						
compounds	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>
AZM	126	0.254	18	<b>0.046</b>	0	<b>0.000</b>
TIM	53	<b>0.000</b>	22	0.096	5	<b>0.001</b>
GLC	131	0.321	20	0.053	31	0.401
TIB	81	<b>0.001</b>	27	0.067	13.5	<b>0.005</b>
CAZ	99	<b>0.004</b>	22.5	<b>0.029</b>	27	0.067
KPF	103	0.062	39	0.894	8	<b>0.004</b>
NPX	90	<b>0.002</b>	22.5	<b>0.029</b>	22.5	<b>0.029</b>
SAA	134	0.375	29	0.309	11	<b>0.009</b>
IHB	55.5	<b>0.001</b>	25.5	0.176	0	<b>0.000</b>
CFN	15	<b>0.000</b>	15	<b>0.022</b>	0	<b>0.000</b>
TBEP	134.5	0.383	39	0.894	24	0.143
TCPP	45.5	<b>0.000</b>	24.5	0.157	0	<b>0.000</b>
TCEP	134	0.353	29.5	0.246	10.5	<b>0.008</b>
PRG	135	0.075	33	1.000	30	0.353
BPA	122.5	0.210	40.5	0.507	27	0.067
E3	143	0.429	37	0.713	30	0.067
TCL	138	0.444	26	0.196	15.5	<b>0.026</b>
MPB	92	<b>0.026</b>	14.5	<b>0.021</b>	27	0.232
EPB	161	0.972	36	0.636	36	0.671
PPB	75	<b>0.006</b>	8.5	<b>0.005</b>	27	0.232

**Table A8.** Significance of differences of total concentrations and concentrations of individual compounds (pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs)) between life stages (nymphs and adults) on Odonata species level (*Gomphus vulgatissimus*, *Orthetrum albistylum*, *Onychogomphus forcipatus*, *Calopteryx splendens*, *Platycnemis pennipes*); according to the Mann-Whitney U tests. Significant values ( $p < 0.05$ ) are shown in bold. Full names of compounds and abbreviations are listed in Table 1.

	<i>Gomphus vulgatissimus</i>		<i>Orthetrum albistylum</i>		<i>Onychogomphus forcipatus</i>		<i>Calopteryx splendens</i>		<i>Platycnemis pennipes</i>	
Totals	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>
ECs	3	0.5127	0	<b>0.0495</b>	0	<b>0.0369</b>	0	<b>0.0495</b>	0	<b>0.0495</b>
PhACs	4	0.8273	3	0.5127	0	<b>0.0369</b>	2	0.2752	0	<b>0.0495</b>
EDCs	0	<b>0.0495</b>	0	<b>0.0495</b>	0	<b>0.0369</b>	0	<b>0.0495</b>	0	<b>0.0495</b>
Individual compounds	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>	Mann-Whitney U	<i>p-value</i>
AZM	0	<b>0.0495</b>	3	0.5127	0	<b>0.0369</b>	0	<b>0.0495</b>	0	<b>0.0495</b>
TIM	3	0.5066	4	0.8273	0	<b>0.0369</b>	0	<b>0.0463</b>	0	<b>0.0369</b>
GLC	4.5	1.0000	2	0.2752	0	<b>0.0253</b>	3	0.5127	0	<b>0.0495</b>
TIB	0	<b>0.0369</b>	4.5	1.0000	4.5	1.0000	3	0.3173	0	<b>0.0369</b>
CAZ	1.5	0.1213	1.5	0.1213	4.5	1.0000	4.5	1.0000	0	<b>0.0369</b>
KPF	2	0.2752	4	0.8273	0	<b>0.0253</b>	3	0.5127	0	<b>0.0495</b>
NPX	4.5	1.0000	3	0.3173	0	<b>0.0369</b>	3	0.3173	0	<b>0.0369</b>
SAA	3	0.5066	1	0.1266	0	<b>0.0369</b>	0	<b>0.0495</b>	0	<b>0.0495</b>
IHB	0	<b>0.0369</b>	0	<b>0.0495</b>	0	<b>0.0253</b>	0	<b>0.0495</b>	0	<b>0.0495</b>
CFN	0	<b>0.0369</b>	0	<b>0.0369</b>	0	<b>0.0369</b>	0	<b>0.0369</b>	0	<b>0.0369</b>
TBEP	4	0.8273	2	0.2752	3	0.4795	3	0.5127	3.5	0.6579
TCPP	0	<b>0.0495</b>	0	<b>0.0495</b>	0	<b>0.0369</b>	0	<b>0.0495</b>	0	<b>0.0495</b>
TCEP	4.5	1.0000	2	0.2463	0	<b>0.0253</b>	2.5	0.3758	0	<b>0.0495</b>
PRG	4.5	1.0000	4.5	1.0000	4.5	1.0000	4.5	1.0000	0	<b>0.0369</b>

	<i>Gomphus vulgatissimus</i>		<i>Orthetrum albistylum</i>		<i>Onychogomphus forcipatus</i>		<i>Calopteryx splendens</i>		<i>Platynemis pennipes</i>	
BPA	2	0.2752	1	0.1266	0	<b>0.0369</b>	2	0.2752	3	0.5127
E3	0	<b>0.0369</b>	4.5	1.0000	0	<b>0.0369</b>	3	0.3173	4.5	1.0000
TCL	3	0.5127	0	<b>0.0495</b>	0	<b>0.0369</b>	2.5	0.3758	2	<b>0.2752</b>
MPB	0	<b>0.0495</b>	0	<b>0.0495</b>	0	<b>0.0369</b>	3	0.5127	0	<b>0.0495</b>
EPB	4.5	1.0000	0	<b>0.0495</b>	4.5	1.0000	1.5	0.1213	0	<b>0.0495</b>
PPB	3	0.5127	0	<b>0.0495</b>	0	<b>0.0369</b>	3	0.5127	0	<b>0.0495</b>



**Table A9.** Significance of differences of total concentrations and concentrations of individual compounds (pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs)) between suborder level (Anisoptera and Zygoptera) in nymphs and imagines of Odonata according to the Mann-Whitney U tests. Significant values ( $p < 0.05$ ) are shown in bold.

NYMPHS:

	Mann-Whitney U	<i>p-value</i>
Total ECS	17.000	<b>0.038</b>
Total PhAC	29.000	0.309
Total EDC	0.000	<b>0.000</b>
Azithromycin	4.000	<b>0.001</b>
Tilmicosin	21.000	0.084
Glibenclamide	0.000	<b>0.000</b>
Thiabendazole	40.500	1.000
Carbamazepine	34.000	0.514
Ketoprofen	40.000	0.965
Naproxen	35.500	0.628
Salicylic acid	8.000	<b>0.004</b>
1H-benzotriazole	7.000	<b>0.003</b>
Caffeine	1.000	<b>0.000</b>
TBEP	38.000	0.825
TCPP	0.000	<b>0.000</b>
TCEP	6.500	<b>0.002</b>
Progesterone	40.500	1.000
BPA	39.000	0.894
E3	27.000	0.067
Triclosan	21.500	0.092
Methyparaben	25.500	0.183
Ethyparaben	36.000	0.636
Propylparaben	27.500	0.249

ADULTS:

	Mann-Whitney U	<i>p-value</i>
Total ECs	37.000	0.757
Total PhAC	36.000	0.690

Total EDC	38.000	0.825
Azithromycin	14.000	0.019
Tilmicosin	38.000	0.793
Glibenclamide	34.500	0.594
Thiabendazole	27.000	0.203
Carbamazepine	40.500	1.000
Ketoprofen	13.000	<b>0.015</b>
Naproxen	40.500	1.000
Salicylic acid	28.000	0.268
1H-benzotriazole	27.000	0.231
Caffeine	27.000	0.065
TBEP	21.000	0.084
TCPP	40.000	0.965
TCEP	37.500	0.781
Progesterone	27.000	0.067
BPA	40.000	0.965
E3	40.000	0.958
Triclosan	26.000	0.196
Methyparaben	13.000	<b>0.015</b>
Ethyparaben	19.000	<b>0.043</b>
Propylparaben	17.000	<b>0.038</b>

**Table A10.** Significance of differences of total concentration of emerging contaminants (ECs) and concentrations of individual compounds (pharmaceuticals and endocrine disrupting compounds) between life stages (larvae, pupae and adults) of the caddisfly *Silo nigricornis* using Kruskal Wallis H test. Significant values ( $p < 0.05$ ) indicated in bold. Full names of compounds and abbreviations are listed in Table 5.

	ECs	AZM	TIM	GLC	TIB	CAZ	KPF	SAA	1HB	CAF	TBEP	TCPP	TCEP	BPA	E3	MPB	EPB	PPB
Kruskal-Wallis H test	8.456	4.643	4.082	3.417	7.552	6.733	8.019	0.855	7.094	12.040	6.733	0.894	16.12 9	3.895	1.860	1.170	1.130	9.696
df	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>p-value</i>	<b>0.015</b>	0.098	0.130	0.181	<b>0.023</b>	<b>0.035</b>	<b>0.018</b>	0.652	<b>0.029</b>	<b>0.002</b>	<b>0.035</b>	0.639	<b>0.000</b>	0.143	0.395	0.557	0.568	<b>0.008</b>

**Table A11.** Significance of pairwise comparisons in total concentration of emerging contaminants (ECs) and concentrations of individual compounds (pharmaceuticals and endocrine disrupting compounds) between life stages (larvae, pupae and adults) of the caddisfly *Silo nigricornis* using Multiple comparisons tests. Significant values ( $p < 0.05$ ) indicated in bold. LV - larvae, PU - pupae, IM - imagines.

	Ecs	Thiabendazole	Carbamazepine	Ketoprofen	1H-benzotriazole	Caffeine	TBEP	TCEP	Propylparaben
	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>	<i>p-value</i> <sup>a</sup>
LV - PU	1.000	0.640	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LV - IM	<b>0.028</b>	<b>0.018</b>	0.074	<b>0.019</b>	<b>0.038</b>	<b>0.004</b>	0.074	<b>0.002</b>	<b>0.012</b>
PU - IM	<b>0.045</b>	0.401	0.074	0.138	0.118	<b>0.019</b>	0.074	<b>0.002</b>	<b>0.039</b>

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**Table A12.** Significance of differences of total concentration of emerging contaminants (ECs) and individual contaminant concentrations between all taxa groups collected on the Sutla study site tested using Kruskal Wallis H test and significance of differences of the pairwise comparison between taxa groups tested with Multiple comparisons tests. Significant values ( $p < 0.05$ ) are shown in bol. (Biofilm - 1; Macrophytes - 2; Odonata nymphs - 3; Odonata adults - 4; Trichoptera adults - 5; Lumbricidae - 6; Coleoptera - 7; Isopoda - 18; Diplopoda - 9; Araneae - 10).

Hypothesis Test Summary				
	Null Hypothesis	Test	<i>p-value</i> <sup>a,b</sup>	Decision
1	The distribution of Carbamazepine is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.575	Retain the null hypothesis.
2	The distribution of Citalopram is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.593	Retain the null hypothesis.
3	The distribution of Propanolol is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.471	Retain the null hypothesis.
4	The distribution of Warfarin is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.740	Retain the null hypothesis.
5	The distribution of Glibenclamide is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.004	Reject the null hypothesis.
6	The distribution of Acridone is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
7	The distribution of Sertraline is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.896	Retain the null hypothesis.
8	The distribution of Alprazolam is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.534	Retain the null hypothesis.
9	The distribution of Hydrochlorothiazide is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.891	Retain the null hypothesis.
10	The distribution of Furosemide is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
11	The distribution of Pravastatin is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
12	The distribution of Torasemide is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.149	Retain the null hypothesis.
13	The distribution of Valsartan is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.006	Reject the null hypothesis.
14	The distribution of BPA is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.410	Retain the null hypothesis.
15	The distribution of Methylparaben is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.061	Retain the null hypothesis.
16	The distribution of Benzylparaben is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.313	Retain the null hypothesis.
17	The distribution of Ethylparaben is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
18	The distribution of Propylparaben is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.

19	The distribution of Caffeine is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.624	Retain the null hypothesis.
20	The distribution of TCPP is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.038	Reject the null hypothesis.
21	The distribution of TCEP is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.
22	The distribution of Total ECS is the same across categories of All groups.	Independent-Samples Kruskal-Wallis Test	.257	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

#### Pairwise Comparisons of All groups

##### Glibenclamide

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
3-2	16.000	13.804	1.159	.246	1.000
3-1	21.222	13.804	1.537	.124	1.000
3-8	-25.467	16.663	-1.528	.126	1.000
3-7	-29.111	11.666	-2.495	<b>.013</b>	.566
3-10	-29.952	12.315	-2.432	<b>.015</b>	.675
3-4	-31.667	13.804	-2.294	<b>.022</b>	.981
3-5	-33.485	13.067	-2.563	<b>.010</b>	.468
3-9	-51.667	15.652	-3.301	<b>.001</b>	.043
3-6	-52.067	12.124	-4.294	<b>.000</b>	.001
2-1	5.222	14.757	.354	.723	1.000
2-8	-9.467	17.461	-.542	.588	1.000
2-7	-13.111	12.780	-1.026	.305	1.000
2-10	-13.952	13.375	-1.043	.297	1.000
2-4	-15.667	14.757	-1.062	.288	1.000
2-5	-17.485	14.070	-1.243	.214	1.000
2-9	-35.667	16.499	-2.162	<b>.031</b>	1.000
2-6	-36.067	13.199	-2.733	<b>.006</b>	.283
1-8	-4.244	17.461	-.243	.808	1.000
1-7	-7.889	12.780	-.617	.537	1.000
1-10	-8.730	13.375	-.653	.514	1.000
1-4	-10.444	14.757	-.708	.479	1.000
1-5	-12.263	14.070	-.872	.383	1.000
1-9	-30.444	16.499	-1.845	.065	1.000
1-6	-30.844	13.199	-2.337	<b>.019</b>	.875
8-7	3.644	15.825	.230	.818	1.000
8-10	-4.486	16.309	-.275	.783	1.000

8-4	6.200	17.461	.355	.723	1.000
8-5	8.018	16.884	.475	.635	1.000
8-9	-26.200	18.956	-1.382	.167	1.000
8-6	26.600	16.166	1.645	.100	1.000
7-10	-.841	11.155	-.075	.940	1.000
7-4	2.556	12.780	.200	.842	1.000
7-5	4.374	11.980	.365	.715	1.000
7-9	-22.556	14.757	-1.528	.126	1.000
7-6	22.956	10.944	2.098	<b>.036</b>	1.000
10-4	1.714	13.375	.128	.898	1.000
10-5	3.532	12.613	.280	.779	1.000
10-9	21.714	15.275	1.422	.155	1.000
10-6	22.114	11.633	1.901	.057	1.000
4-5	-1.818	14.070	-.129	.897	1.000
4-9	-20.000	16.499	-1.212	.225	1.000
4-6	-20.400	13.199	-1.546	.122	1.000
5-9	-18.182	15.888	-1.144	.252	1.000
5-6	-18.582	12.427	-1.495	.135	1.000
9-6	.400	15.122	.026	.979	1.000

Acridone

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
1-2	.000	11.072	.000	1.000	1.000
1-3	.000	10.357	.000	1.000	1.000
1-4	.000	11.072	.000	1.000	1.000
1-6	-3.367	9.903	-.340	.734	1.000
1-7	-3.583	9.589	-.374	.709	1.000
1-5	-28.273	10.557	-2.678	<b>.007</b>	.333
1-10	-31.286	10.035	-3.118	<b>.002</b>	.082
1-9	-40.917	12.379	-3.305	<b>.001</b>	.043
1-8	-58.900	13.101	-4.496	<b>.000</b>	.000
2-3	.000	10.357	.000	1.000	1.000
2-4	.000	11.072	.000	1.000	1.000
2-6	-3.367	9.903	-.340	.734	1.000
2-7	-3.583	9.589	-.374	.709	1.000
2-5	-28.273	10.557	-2.678	<b>.007</b>	.333
2-10	-31.286	10.035	-3.118	<b>.002</b>	.082
2-9	-40.917	12.379	-3.305	<b>.001</b>	.043

2-8	-58.900	13.101	-4.496	<b>.000</b>	.000
3-4	.000	10.357	.000	1.000	1.000
3-6	-3.367	9.097	-.370	.711	1.000
3-7	-3.583	8.753	-.409	.682	1.000
3-5	-28.273	9.804	-2.884	<b>.004</b>	.177
3-10	-31.286	9.240	-3.386	<b>.001</b>	.032
3-9	-40.917	11.744	-3.484	<b>.000</b>	.022
3-8	-58.900	12.502	-4.711	<b>.000</b>	.000
4-6	-3.367	9.903	-.340	.734	1.000
4-7	-3.583	9.589	-.374	.709	1.000
4-5	-28.273	10.557	-2.678	<b>.007</b>	.333
4-10	-31.286	10.035	-3.118	<b>.002</b>	.082
4-9	-40.917	12.379	-3.305	<b>.001</b>	.043
4-8	-58.900	13.101	-4.496	<b>.000</b>	.000
6-7	-.217	8.211	-.026	.979	1.000
6-5	24.906	9.323	2.671	<b>.008</b>	.340
6-10	-27.919	8.728	-3.199	<b>.001</b>	.062
6-9	-37.550	11.345	-3.310	<b>.001</b>	.042
6-8	-55.533	12.129	-4.579	<b>.000</b>	.000
7-5	24.689	8.989	2.747	<b>.006</b>	.271
7-10	-27.702	8.370	-3.310	<b>.001</b>	.042
7-9	-37.333	11.072	-3.372	<b>.001</b>	.034
7-8	-55.317	11.873	-4.659	<b>.000</b>	.000
5-10	-3.013	9.463	-.318	.750	1.000
5-9	-12.644	11.920	-1.061	.289	1.000
5-8	-30.627	12.668	-2.418	<b>.016</b>	.703
10-9	9.631	11.461	.840	.401	1.000
10-8	27.614	12.237	2.257	<b>.024</b>	1.000
9-8	17.983	14.222	1.264	.206	1.000

Furosemide

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
9-10	-15.176	15.205	-.998	.318	1.000
9-3	17.167	16.010	1.072	.284	1.000
9-7	22.889	15.095	1.516	.129	1.000
9-4	32.000	16.876	1.896	.058	1.000
9-8	36.000	19.390	1.857	.063	1.000
9-2	47.222	16.876	2.798	<b>.005</b>	.231



9-6	50.667	15.468	3.276	<b>.001</b>	.047
9-5	60.857	15.625	3.895	<b>.000</b>	.004
9-1	61.333	16.876	3.634	<b>.000</b>	.013
10-3	1.990	12.073	.165	.869	1.000
10-7	7.712	10.829	.712	.476	1.000
10-4	16.824	13.200	1.275	.202	1.000
10-8	20.824	16.290	1.278	.201	1.000
10-2	32.046	13.200	2.428	<b>.015</b>	.684
10-6	35.490	11.343	3.129	<b>.002</b>	.079
10-5	45.681	11.556	3.953	<b>.000</b>	.003
10-1	46.157	13.200	3.497	<b>.000</b>	.021
3-7	-5.722	11.933	-.480	.632	1.000
3-4	-14.833	14.120	-1.051	.293	1.000
3-8	-18.833	17.044	-1.105	.269	1.000
3-2	30.056	14.120	2.129	<b>.033</b>	1.000
3-6	-33.500	12.402	-2.701	<b>.007</b>	.311
3-5	-43.690	12.597	-3.468	<b>.001</b>	.024
3-1	44.167	14.120	3.128	<b>.002</b>	.079
7-4	9.111	13.072	.697	.486	1.000
7-8	-13.111	16.187	-.810	.418	1.000
7-2	24.333	13.072	1.861	.063	1.000
7-6	27.778	11.195	2.481	<b>.013</b>	.589
7-5	37.968	11.411	3.327	<b>.001</b>	.039
7-1	38.444	13.072	2.941	<b>.003</b>	.147
4-8	-4.000	17.860	-.224	.823	1.000
4-2	15.222	15.095	1.008	.313	1.000
4-6	-18.667	13.501	-1.383	.167	1.000
4-5	-28.857	13.681	-2.109	<b>.035</b>	1.000
4-1	29.333	15.095	1.943	.052	1.000
8-2	11.222	17.860	.628	.530	1.000
8-6	14.667	16.535	.887	.375	1.000
8-5	24.857	16.682	1.490	.136	1.000
8-1	25.333	17.860	1.418	.156	1.000
2-6	-3.444	13.501	-.255	.799	1.000
2-5	-13.635	13.681	-.997	.319	1.000
2-1	14.111	15.095	.935	.350	1.000
6-5	10.190	11.899	.856	.392	1.000
6-1	10.667	13.501	.790	.429	1.000

5-1	.476	13.681	.035	.972	1.000
Pravastatin					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
7-1	11.778	8.759	1.345	.179	1.000
6-8	-50.000	11.079	-4.513	<b>.000</b>	.000
7-3	14.333	7.996	1.793	.073	1.000
5-1	11.778	9.166	1.285	.199	1.000
6-1	11.778	9.046	1.302	.193	1.000
7-8	-50.000	10.846	-4.610	<b>.000</b>	.000
6-7	.000	7.500	.000	1.000	1.000
5-7	.000	7.645	.000	1.000	1.000
5-10	-7.294	7.743	-.942	.346	1.000
5-2	24.111	9.166	2.630	<b>.009</b>	.384
5-9	-26.167	10.469	-2.500	<b>.012</b>	.560
5-8	-50.000	11.177	-4.473	<b>.000</b>	.000
6-2	24.111	9.046	2.665	<b>.008</b>	.346
7-2	24.111	8.759	2.753	<b>.006</b>	.266
5-6	.000	7.973	.000	1.000	1.000
7-4	6.333	8.759	.723	.470	1.000
6-4	6.333	9.046	.700	.484	1.000
5-4	6.333	9.166	.691	.490	1.000
5-3	14.333	8.440	1.698	.089	1.000
6-10	-7.294	7.600	-.960	.337	1.000
6-3	14.333	8.309	1.725	.085	1.000
6-9	-26.167	10.363	-2.525	<b>.012</b>	.521
7-10	-7.294	7.256	-1.005	.315	1.000
7-9	-26.167	10.114	-2.587	<b>.010</b>	.435
4-10	-.961	8.844	-.109	.913	1.000
4-1	5.444	10.114	.538	.590	1.000
4-3	8.000	9.460	.846	.398	1.000
4-2	17.778	10.114	1.758	.079	1.000
4-9	-19.833	11.307	-1.754	.079	1.000
4-8	-43.667	11.967	-3.649	<b>.000</b>	.012
10-1	4.484	8.844	.507	.612	1.000
10-3	7.039	8.089	.870	.384	1.000
10-2	16.817	8.844	1.901	.057	1.000
10-9	18.873	10.188	1.852	.064	1.000

10-8	42.706	10.915	3.913	<b>.000</b>	.004
1-3	-2.556	9.460	-.270	.787	1.000
1-2	-12.333	10.114	-1.219	.223	1.000
1-9	-14.389	11.307	-1.273	.203	1.000
1-8	-38.222	11.967	-3.194	<b>.001</b>	.063
3-2	9.778	9.460	1.034	.301	1.000
3-9	-11.833	10.727	-1.103	.270	1.000
3-8	-35.667	11.420	-3.123	<b>.002</b>	.081
2-9	-2.056	11.307	-.182	.856	1.000
2-8	-25.889	11.967	-2.163	<b>.031</b>	1.000
9-8	23.833	12.991	1.835	.067	1.000

Valsartan

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
1-5	.000	9.361	.000	1.000	1.000
1-7	.000	8.945	.000	1.000	1.000
1-3	-4.958	9.661	-.513	.608	1.000
1-2	-6.944	10.328	-.672	.501	1.000
1-4	-12.667	10.328	-1.226	.220	1.000
1-10	-14.735	9.032	-1.631	.103	1.000
1-9	-20.167	11.548	-1.746	.081	1.000
1-8	-26.400	12.221	-2.160	<b>.031</b>	1.000
1-6	-26.700	9.238	-2.890	<b>.004</b>	.173
7-4	12.667	8.945	1.416	.157	1.000
5-7	.000	7.808	.000	1.000	1.000
5-2	6.944	9.361	.742	.458	1.000
5-6	-26.700	8.142	-3.279	<b>.001</b>	.047
7-2	6.944	8.945	.776	.438	1.000
5-10	-14.735	7.907	-1.863	.062	1.000
5-9	-20.167	10.691	-1.886	.059	1.000
5-8	-26.400	11.415	-2.313	<b>.021</b>	.933
7-6	26.700	7.660	3.486	<b>.000</b>	.022
5-3	4.958	8.619	.575	.565	1.000
7-3	4.958	8.165	.607	.544	1.000
7-10	-14.735	7.410	-1.989	<b>.047</b>	1.000
7-9	-20.167	10.328	-1.953	.051	1.000
7-8	-26.400	11.076	-2.384	<b>.017</b>	.772
5-4	12.667	9.361	1.353	.176	1.000

3-2	1.986	9.661	.206	.837	1.000
3-4	-7.708	9.661	-.798	.425	1.000
3-10	-9.777	8.261	-1.184	.237	1.000
3-9	-15.208	10.955	-1.388	.165	1.000
3-8	-21.442	11.662	-1.839	.066	1.000
3-6	-21.742	8.486	-2.562	<b>.010</b>	.468
2-4	-5.722	10.328	-.554	.580	1.000
2-10	-7.791	9.032	-.863	.388	1.000
2-9	-13.222	11.548	-1.145	.252	1.000
2-8	-19.456	12.221	-1.592	.111	1.000
2-6	-19.756	9.238	-2.139	<b>.032</b>	1.000
4-10	-2.069	9.032	-.229	.819	1.000
4-9	-7.500	11.548	-.649	.516	1.000
4-8	-13.733	12.221	-1.124	.261	1.000
4-6	-14.033	9.238	-1.519	.129	1.000
10-9	5.431	10.404	.522	.602	1.000
10-8	11.665	11.147	1.046	.295	1.000
10-6	11.965	7.761	1.542	.123	1.000
9-8	6.233	13.267	.470	.638	1.000
9-6	6.533	10.583	.617	.537	1.000
8-6	.300	11.314	.027	.979	1.000

Ethylparaben

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
10-4	5.448	13.621	.400	.689	1.000
10-6	6.059	11.705	.518	.605	1.000
10-3	7.434	12.458	.597	.551	1.000
10-9	21.225	15.690	1.353	.176	1.000
10-7	22.920	11.175	2.051	<b>.040</b>	1.000
10-8	24.559	16.810	1.461	.144	1.000
10-1	25.392	13.621	1.864	.062	1.000
10-2	35.503	13.621	2.607	<b>.009</b>	.412
10-5	68.630	11.925	5.755	<b>.000</b>	.000
4-6	-.611	13.932	-.044	.965	1.000
4-3	1.986	14.570	.136	.892	1.000
4-9	-15.778	17.415	-.906	.365	1.000
4-7	-17.472	13.489	-1.295	.195	1.000
4-8	-19.111	18.430	-1.037	.300	1.000

4-1	19.944	15.576	1.280	.200	1.000
4-2	30.056	15.576	1.930	.054	1.000
4-5	-63.183	14.117	-4.476	<b>.000</b>	.000
6-3	1.375	12.797	.107	.914	1.000
6-9	-15.167	15.961	-.950	.342	1.000
6-7	-16.861	11.552	-1.460	.144	1.000
6-8	-18.500	17.063	-1.084	.278	1.000
6-1	19.333	13.932	1.388	.165	1.000
6-2	29.444	13.932	2.113	<b>.035</b>	1.000
6-5	62.571	12.279	5.096	<b>.000</b>	.000
3-9	-13.792	16.521	-.835	.404	1.000
3-7	-15.486	12.314	-1.258	.209	1.000
3-8	-17.125	17.588	-.974	.330	1.000
3-1	17.958	14.570	1.233	.218	1.000
3-2	28.069	14.570	1.927	.054	1.000
3-5	-61.196	12.999	-4.708	<b>.000</b>	.000
9-7	1.694	15.576	.109	.913	1.000
9-8	3.333	20.008	.167	.868	1.000
9-1	4.167	17.415	.239	.811	1.000
9-2	14.278	17.415	.820	.412	1.000
9-5	47.405	16.123	2.940	<b>.003</b>	.148
7-8	-1.639	16.704	-.098	.922	1.000
7-1	2.472	13.489	.183	.855	1.000
7-2	12.583	13.489	.933	.351	1.000
7-5	45.710	11.774	3.882	<b>.000</b>	.005
8-1	.833	18.430	.045	.964	1.000
8-2	10.944	18.430	.594	.553	1.000
8-5	44.071	17.214	2.560	<b>.010</b>	.471
1-2	-10.111	15.576	-.649	.516	1.000
1-5	-43.238	14.117	-3.063	<b>.002</b>	.099
2-5	-33.127	14.117	-2.347	<b>.019</b>	.853

Propylparaben

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
8-10	-11.647	16.777	-.694	.488	1.000
8-9	-14.833	19.968	-.743	.458	1.000
8-6	19.267	17.029	1.131	.258	1.000
8-3	29.000	17.553	1.652	.099	1.000

8-1	33.444	18.393	1.818	.069	1.000
8-5	37.357	17.180	2.174	<b>.030</b>	1.000
8-2	39.444	18.393	2.144	<b>.032</b>	1.000
8-4	45.444	18.393	2.471	<b>.013</b>	.607
8-7	59.944	16.670	3.596	<b>.000</b>	.015
10-9	3.186	15.659	.203	.839	1.000
10-6	7.620	11.682	.652	.514	1.000
10-3	17.353	12.433	1.396	.163	1.000
10-1	21.797	13.594	1.603	.109	1.000
10-5	25.710	11.901	2.160	<b>.031</b>	1.000
10-2	27.797	13.594	2.045	<b>.041</b>	1.000
10-4	33.797	13.594	2.486	<b>.013</b>	.581
10-7	48.297	11.153	4.331	<b>.000</b>	.001
9-6	4.433	15.929	.278	.781	1.000
9-3	14.167	16.488	.859	.390	1.000
9-1	18.611	17.380	1.071	.284	1.000
9-5	22.524	16.091	1.400	.162	1.000
9-2	24.611	17.380	1.416	.157	1.000
9-4	30.611	17.380	1.761	.078	1.000
9-7	45.111	15.545	2.902	.004	.167
6-3	9.733	12.772	.762	.446	1.000
6-1	14.178	13.904	1.020	.308	1.000
6-5	18.090	12.254	1.476	.140	1.000
6-2	20.178	13.904	1.451	.147	1.000
6-4	26.178	13.904	1.883	.060	1.000
6-7	-40.678	11.529	-3.528	.000	.019
3-1	4.444	14.541	.306	.760	1.000
3-5	-8.357	12.973	-.644	.519	1.000
3-2	10.444	14.541	.718	.473	1.000
3-4	-16.444	14.541	-1.131	.258	1.000
3-7	-30.944	12.290	-2.518	.012	.531
1-5	-3.913	14.089	-.278	.781	1.000
1-2	-6.000	15.545	-.386	.700	1.000
1-4	-12.000	15.545	-.772	.440	1.000
1-7	-26.500	13.463	-1.968	.049	1.000
5-2	2.087	14.089	.148	.882	1.000
5-4	8.087	14.089	.574	.566	1.000

5-7	-22.587	11.751	-1.922	.055	1.000
2-4	-6.000	15.545	-.386	.700	1.000
2-7	-20.500	13.463	-1.523	.128	1.000
4-7	-14.500	13.463	-1.077	.281	1.000

TCPP

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
9-4	28.556	13.651	2.092	<b>.036</b>	1.000
6-5	15.071	9.625	1.566	.117	1.000
8-5	15.071	13.494	1.117	.264	1.000
9-5	15.071	12.639	1.192	.233	1.000
6-1	9.667	10.921	.885	.376	1.000
8-1	9.667	14.447	.669	.503	1.000
8-4	28.556	14.447	1.977	<b>.048</b>	1.000
9-1	9.667	13.651	.708	.479	1.000
8-7	19.722	13.094	1.506	.132	1.000
6-8	.000	13.375	.000	1.000	1.000
6-9	.000	12.512	.000	1.000	1.000
6-2	10.889	10.921	.997	.319	1.000
6-7	-19.722	9.055	-2.178	<b>.029</b>	1.000
6-10	-25.471	9.176	-2.776	<b>.006</b>	.248
8-2	10.889	14.447	.754	.451	1.000
9-2	10.889	13.651	.798	.425	1.000
9-7	19.722	12.210	1.615	.106	1.000
9-3	27.167	12.951	2.098	<b>.036</b>	1.000
8-9	.000	15.684	.000	1.000	1.000
6-3	27.167	10.032	2.708	<b>.007</b>	.305
8-10	-25.471	13.177	-1.933	.053	1.000
8-3	27.167	13.787	1.970	<b>.049</b>	1.000
9-10	-25.471	12.300	-2.071	<b>.038</b>	1.000
6-4	28.556	10.921	2.615	<b>.009</b>	.402
1-2	-1.222	12.210	-.100	.920	1.000
1-5	-5.405	11.066	-.488	.625	1.000
1-7	-10.056	10.574	-.951	.342	1.000
1-10	-15.804	10.677	-1.480	.139	1.000
1-3	-17.500	11.422	-1.532	.125	1.000
1-4	-18.889	12.210	-1.547	.122	1.000
2-5	-4.183	11.066	-.378	.705	1.000

2-7	-8.833	10.574	-.835	.404	1.000
2-10	-14.582	10.677	-1.366	.172	1.000
2-3	-16.278	11.422	-1.425	.154	1.000
2-4	-17.667	12.210	-1.447	.148	1.000
5-7	-4.651	9.230	-.504	.614	1.000
5-10	-10.399	9.348	-1.112	.266	1.000
5-3	12.095	10.190	1.187	.235	1.000
5-4	13.484	11.066	1.218	.223	1.000
7-10	-5.748	8.760	-.656	.512	1.000
7-3	7.444	9.653	.771	.441	1.000
7-4	8.833	10.574	.835	.404	1.000
10-3	1.696	9.766	.174	.862	1.000
10-4	3.085	10.677	.289	.773	1.000
3-4	-1.389	11.422	-.122	.903	1.000

TCEP

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
6-5	13.583	12.015	1.131	.258	1.000
6-8	-15.433	16.696	-.924	.355	1.000
6-10	-19.010	11.453	-1.660	.097	1.000
6-7	-32.833	11.303	-2.905	<b>.004</b>	.165
6-1	36.611	13.632	2.686	<b>.007</b>	.326
6-2	40.889	13.632	2.999	<b>.003</b>	.122
6-3	62.417	12.522	4.985	<b>.000</b>	.000
6-4	65.556	13.632	4.809	<b>.000</b>	.000
6-9	-68.500	15.618	-4.386	<b>.000</b>	.001
5-8	-1.850	16.844	-.110	.913	1.000
5-10	-5.426	11.669	-.465	.642	1.000
5-7	-19.250	11.521	-1.671	.095	1.000
5-1	23.028	13.814	1.667	.096	1.000
5-2	27.306	13.814	1.977	<b>.048</b>	1.000
5-3	48.833	12.719	3.839	<b>.000</b>	.006
5-4	51.972	13.814	3.762	<b>.000</b>	.008
5-9	-54.917	15.776	-3.481	<b>.000</b>	.022
8-10	-3.576	16.449	-.217	.828	1.000
8-7	17.400	16.344	1.065	.287	1.000
8-1	21.178	18.034	1.174	.240	1.000
8-2	25.456	18.034	1.412	.158	1.000



8-3	46.983	17.210	2.730	<b>.006</b>	.285
8-4	50.122	18.034	2.779	<b>.005</b>	.245
8-9	-53.067	19.578	-2.711	<b>.007</b>	.302
10-7	13.824	10.935	1.264	.206	1.000
10-1	17.601	13.328	1.321	.187	1.000
10-2	21.879	13.328	1.642	.101	1.000
10-3	43.407	12.190	3.561	<b>.000</b>	.017
10-4	46.546	13.328	3.492	<b>.000</b>	.022
10-9	49.490	15.353	3.224	<b>.001</b>	.057
7-1	3.778	13.199	.286	.775	1.000
7-2	8.056	13.199	.610	.542	1.000
7-3	29.583	12.049	2.455	<b>.014</b>	.634
7-4	32.722	13.199	2.479	<b>.013</b>	.593
7-9	-35.667	15.241	-2.340	<b>.019</b>	.867
1-2	-4.278	15.241	-.281	.779	1.000
1-3	-25.806	14.257	-1.810	.070	1.000
1-4	-28.944	15.241	-1.899	.058	1.000
1-9	-31.889	17.040	-1.871	.061	1.000
2-3	-21.528	14.257	-1.510	.131	1.000
2-4	-24.667	15.241	-1.618	.106	1.000
2-9	-27.611	17.040	-1.620	.105	1.000
3-4	-3.139	14.257	-.220	.826	1.000
3-9	-6.083	16.166	-.376	.707	1.000
4-9	-2.944	17.040	-.173	.863	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**Table A13.** Significance of differences of total concentration of ECs and concentrations of individual compounds of all collected samples between Up and Down transects; according to the Mann-Whitney U tests. Significant values ( $p < 0.05$ ) are shown in bold.

Hypothesis Test Summary				
	Null Hypothesis	Test	<i>p-value</i> . <sup>a,b</sup>	Decision
1	The distribution of Carbamazepine is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.307	Retain the null hypothesis.
2	The distribution of Citalopram is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.783	Retain the null hypothesis.
3	The distribution of Propanolol is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.895	Retain the null hypothesis.
4	The distribution of Warfarin is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.552	Retain the null hypothesis.
5	The distribution of Glibenclamide is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.110	Retain the null hypothesis.
6	The distribution of Acridone is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.058	Retain the null hypothesis.
7	The distribution of Sertraline is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.613	Retain the null hypothesis.
8	The distribution of Hydrochlorothiazide is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.964	Retain the null hypothesis.
9	The distribution of Furosemide is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.434	Retain the null hypothesis.
10	The distribution of Pravastatin is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.551	Retain the null hypothesis.
11	The distribution of Torasemide is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.137	Retain the null hypothesis.

12	The distribution of Valsartan is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.556	Retain the null hypothesis.
13	The distribution of BPA is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.590	Retain the null hypothesis.
14	The distribution of Methylparaben is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.786	Retain the null hypothesis.
15	The distribution of Benzylparaben is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.071	Retain the null hypothesis.
16	The distribution of Ethylparaben is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.741	Retain the null hypothesis.
17	The distribution of Propylparaben is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	<b>0.013</b>	Reject the null hypothesis.
18	The distribution of Caffeine is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	<b>0.050</b>	Reject the null hypothesis.
19	The distribution of TCPP is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.748	Retain the null hypothesis.
20	The distribution of TCEP is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.188	Retain the null hypothesis.
21	The distribution of Total ECS is the same across categories of Group all.	Independent-Samples Mann-Whitney U Test	0.244	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

**Table A14.** Significance of differences of total concentration of ECs and concentrations of individual compounds in Diplopoda samples between Up and Down transects; according to the Mann-Whitney U tests. Significant values ( $p < 0.05$ ) are shown in bold.

	Mann-Whitney U	<i>p-value</i> <sup>a,b</sup>
Carbamazepine	4.5	1.000
Citalopram	4.5	1.000
Propanolol	4.5	1.000
Warfarin	4.5	1.000
Glibenclamide	3.0	0.513
Acridone	3.0	0.513
Sertraline	3.0	0.317
Hydrochlorothiazide	4.0	0.796
Furosemide	4.5	1.000
Pravastatin	2.0	0.246
Torasemide	4.5	1.000
Valsartan	1.5	0.121
BPA	4.5	1.000
Methylparaben	0.0	<b>0.037</b>
Benzylparaben	3.0	0.317
Ethylparaben	2.0	0.275
Propylparaben	2.0	0.246
Caffein	4.5	1.000
TCPP	4.5	1.000
TCEP	1.0	0.127
Total ECS	0.0	<b>0.050</b>
Total parabens	0.0	<b>0.050</b>

**Table A15.** Significance of differences of total concentration of ECs and concentrations of individual compounds in Araneae samples between Up and Down transects; according to the Mann-Whitney U tests. Significant values ( $p < 0.05$ ) are shown in bold.

	Mann-Whitney U	<i>p-value</i> <sup>a,b</sup>
Carbamazepine	24.0	1.000
Citalopram	18.5	0.374
Propanolol	20.0	0.472
Warfarin	12.0	0.052
Glibenclamide	23.0	0.897
Acridone	13.5	0.158
Sertraline	13.0	0.098
Hydrochlorothiazide	28.0	0.368

Furosemide	27.0	0.311
Pravastatin	27.0	0.122
Torsemide	36.0	1.000
Valsartan	23.5	0.136
BPA	24.0	0.196
Methylparaben	18.0	0.083
Benzylparaben	33.0	0.755
Ethylparaben	28.0	0.44
Propylparaben	31.0	0.623
Caffein	15.0	<b>0.029</b>
TCPP	14.0	<b>0.022</b>
TCEP	22.0	0.163
Total ECS	17.0	0.068
Total parabens	29.0	0.501

**Table A16.** Significance of differences of total concentration of emerging contaminants (ECs) between all taxa groups collected on the Sutla study site tested using Kruskal Wallis H test. Significant values ( $p < 0.05$ ) indicated in bold.

Hypothesis Test Summary				
	Null Hypothesis	Test	<i>p-value</i> <sup>a,b</sup>	Decision
1	The distribution of Carbamazepine is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	1.000	Retain the null hypothesis.
2	The distribution of Citalopram is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.711	Retain the null hypothesis.
3	The distribution of Propanolol is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.776	Retain the null hypothesis.
4	The distribution of Warfarin is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.391	Retain the null hypothesis.
5	The distribution of Glibenclamide is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.944	Retain the null hypothesis.

6	The distribution of Acridone is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.275	Retain the null hypothesis.
7	The distribution of Sertraline is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.211	Retain the null hypothesis.
8	The distribution of Hydrochlorothiazide is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.613	Retain the null hypothesis.
9	The distribution of Furosemide is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.653	Retain the null hypothesis.
10	The distribution of Pravastatin is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.313	Retain the null hypothesis.
11	The distribution of Torasemide is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	1.000	Retain the null hypothesis.
12	The distribution of Valsartan is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	<b>0.012</b>	Reject the null hypothesis.
13	The distribution of BPA is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.124	Retain the null hypothesis.
14	The distribution of Methylparaben is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	<b>0.036</b>	Reject the null hypothesis.
15	The distribution of Benzylparaben is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.948	Retain the null hypothesis.
16	The distribution of Ethylparaben is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.092	Retain the null hypothesis.
17	The distribution of Propylparaben is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.341	Retain the null hypothesis.
18	The distribution of Caffeine is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.523	Retain the null hypothesis.

19	The distribution of TCPP is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.868	Retain the null hypothesis.
20	The distribution of TCEP is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.056	Retain the null hypothesis.
21	The distribution of Total ECS is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.225	Retain the null hypothesis.
22	The distribution of Total ECS w/o CFN is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.162	Retain the null hypothesis.
23	The distribution of Total parabens is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.118	Retain the null hypothesis.
24	The distribution of Flame retardants is the same across categories of Group spiders.	Independent-Samples Kruskal-Wallis H Test	0.865	Retain the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

**Table A17.** Significance of pairwise comparisons of valsartan concentration between spider families from two transects using Multiple comparisons tests. Significant values ( $p < 0.05$ ) indicated in bold. (Tetragnathidae Down – 1, Tetragnathidae Up – 2, Lycosidae Down – 3, Lycosidae – Up).

Pairwise Comparisons of Group spiders					
Sample 1- Sample 2	Test Statistic	Std. Error	Std. Test Statistic	<i>p-value</i>	Adj. Sig. <sup>a</sup>
2-4	-1.083	3.323	-0.326	0.744	1.000
2-3	-1.250	3.323	-0.376	0.707	1.000
2-1	9.500	3.715	2.557	<b>0.011</b>	0.063
4-3	0.167	2.350	0.071	0.943	1.000
4-1	8.417	2.878	2.925	<b>0.003</b>	0.021
3-1	8.250	2.878	2.867	<b>0.004</b>	0.025

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

**Table A18.** Significance of differences of total concentration of ECs and concentrations of individual compounds in Odonata, Anisoptera and Zygoptera between nymphs and adults; according to the Mann-Whitney U tests. Significant p values are shown in bold.

Test Statistics <sup>a</sup>	Mann-Whitney U	ODONATA	ANISOPTERA	ZYGOPTERA
		<i>p-value</i>	<i>p-value</i>	<i>p-value</i>
Carbamazepine	54.0	1.000	1.000	1.000
Citalopram	53.0	0.889	0.724	1.000
Propanolol	52.0	0.780	1.000	0.902
Warfarin	50.0	0.641	0.480	0.902
Glibenclamide	33.0	0.134	<b>0.020</b>	0.747
Acridone	54.0	1.000	1.000	1.000
Sertraline	45.0	0.351	0.759	0.317
Alprazolam	49.5	0.386	0.480	1.000
Hydrochlorothiazide	52.0	0.875	0.777	1.000
Furosemide	40.0	0.283	0.167	0.068
Pravastatin	46.0	0.407	0.289	0.902
Torsemide	54.0	1.000	1.000	1.000
Valsartan	46.5	0.381	0.157	0.902
BPA	53.0	0.929	0.289	0.391
Methylparaben	51.0	0.831	0.020	0.055
Benzylparaben	49.0	0.684	0.090	0.216
Ethylparaben	52.0	0.887	0.071	0.055



Propylparaben	39.0	0.286	0.071	0.873
Caffein	47.0	0.591	0.258	0.798
TCP	50.0	0.759	0.572	0.442
TCEP	41.0	0.356	<b>0.020</b>	0.150
Total ECS	45.0	0.522	0.439	0.200
Total parabens	46.0	0.570	<b>0.020</b>	0.200

**Table A19.** Significance of differences of total concentration of ECs and concentrations of individual compounds in nymphs and adults between Anisoptera and Zygoptera; according to the Mann-Whitney U tests. Significant p values are shown in bold.

Test Statistics <sup>a</sup>	Mann-Whitney U	NYMPHS	IMAGINES
		Asymp. Sig. (2-tailed)	Asymp. Sig. (2-tailed)
Carbamazepine	18.0	1.000	1.000
Citalopram	15.0	0.317	0.157
Propanolol	15.0	0.317	0.480
Warfarin	17.5	0.902	0.480
Glibenclamide	13.5	0.470	0.300
Acridone	18.0	1.000	1.000
Sertraline	15.0	0.528	0.157
Alprazolam	15.0	0.317	1.000
Hydrochlorothiazide	13.5	0.442	1.000
Furosemide	16.0	0.721	0.043
Pravastatin	14.0	0.400	0.480

Torasemide	18.0	1.000	1.000
Valsartan	15.0	0.317	0.480
BPA	16.0	0.674	0.167
Methylparaben	2.0	<b>0.010</b>	0.121
Benzylparaben	7.0	<b>0.050</b>	0.167
Ethylparaben	4.0	<b>0.025</b>	0.197
Propylparaben	15.0	0.631	0.121
Caffein	16.0	0.721	0.225
TCPP	12.0	0.284	0.345
TCEP	6.0	0.055	<b>0.020</b>
Total ECs	18.0	1.000	<b>0.020</b>
Total parabens	5.0	<b>0.037</b>	0.071

## 9. CURRICULUM VITAE

### PERSONAL INFORMATION

Name and surname: Marina Veseli

Date of birth: April 13, 1993

Home address: Budina 5, Šenkovec, 40000 Čakovec, Croatia

Phone number: +385994100150

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### EDUCATION

2020 - : University of Zagreb, Faculty of Science, Department of Biology, Doctoral programme of Biology

2015 - 2018: University of Zagreb, Faculty of Science, Department of Biology, Graduate programme of Ecology and Nature Protection, module Freshwater

2012 - 2015: University of Zagreb, Faculty of Science, Department of Biology, Undergraduate programme of Biology

### WORKING EXPERIENCE

#### Research assistant

2020 -

Department of biology, Faculty of Science, University of Zagreb

Main research subject: patterns of bioaccumulation of pharmaceuticals and other pollutants from wastewater in freshwater organisms. Mastered procedures for extraction (SPE), measurement (UPLC chromatography) and quantification (Analyst Software) of pollutants in biological samples and in water, as well as comprehensive statistical data processing. Planning and conducting field research, conducting practical classes in environmental subjects.

#### Engineer

2019 - 2020

Hrvatske vode, Glavni vodnogospodarski laboratorij, Zagreb

Field sampling of benthic macroinvertebrate and determination of benthic macroinvertebrates for the purposes of the water quality monitoring.

**Expert associate**

2018 - 2019

Ires ekologija d.o.o. za zaštitu  
prirode i okoliša, Zagreb

Writing professional documentation for environmental  
impact assessment, conducting field surveys of flora and  
fauna inventory, work in the QGIS program.

**PROJECTS****2023 PATHways of Chemicals Into Freshwaters and their ecological ImpaCts (PACIFIC) -  
LilExStream mesocosm experiment**

- part of the project led by Dr Michelle Jackson from the University of Oxford, UK
- stay in Oxford and south-west England for three months
- participation in the planning and implementation of an experiment with 64 mesocosms at four  
wastewater treatment plant locations in south-west England
- collaboration with a team of scientists from the University of Oxford

**2020 - 2023 Effects of multiple stressors on freshwater biodiversity and ecosystem  
functioning (MUSE**

- project led by Dr Ana Previšić, University of Zagreb, Faculty of Science, Department of  
Biology
- conducting research for the purposes of the doctoral dissertation

**MEMBERSHIP**

2021 - 2023 **Society for Environmental Toxicology and Chemistry (SETAC)**

2021 - 2023 **Student Advisory Council (SAC) of the Society for Environmental Toxicology  
and Chemistry (SETAC)**, social media representative

2019 - 2024 Croatian association of freshwater ecologists (HUSEk)

**TRAINING AND COURSES**

2022 advanced one month training in laboratory work in sample preparation and mass  
spectrometry analysis at the **Catalan Institute for Water Research (ICRA)**, Girona, Spain

2022 completed a course in using the graphic design tool GIMP (University Computing Centre  
of the University of Zagreb - Sreće)

2022 completed a course in using INKSCAPE graphic design tools (University Computing Centre of the University of Zagreb - Srce)

## PRIZES AND SCHOLARSHIPS

2022 scholarship from the **British Scholarship Trust** (for the research on Aquatic community response to multiple stressors exposure)

2021 financial support for the project Impacts of Pollution and Climate Change on Freshwater Invertebrates (M. Veseli, A. Brigić, W. Graf, A. Previšić), Foundation of the Croatian Academy of Sciences and Arts

## RESEARCH PAPERS

1. Kokotović, Iva; **Veseli, Marina**; Ložek, Filip; Karačić, Zrinka; Rožman, Marko; Previšić Ana (2024) Pharmaceuticals and endocrine disrupting compounds modulate adverse effects of climate change on resource quality in freshwater food webs // Science of the total environment, 912, 168751 DOI: <https://doi.org/10.1016/j.scitotenv.2023.168751>
2. **Veseli, Marina**; Rožman, Marko; Vilenica, Marina; Petrović, Mira; Previšić, Ana (2022) Bioaccumulation and bioamplification of pharmaceuticals and endocrine disruptors in aquatic insects // Science of the total environment, 838, 2; 156208, 9. DOI: 10.1016/j.scitotenv.2022.156208
3. Borza, Péter; Csányi, Béla; Đanić, Vjeran; Kenderov, Lyubomir; Kladarić, Lidija; Lešťáková, Margita; Muc, Tjaša; Němejcová, Denisa; Očadlík, Miroslav; Paunović, Momir; Rotar, Bernarda; Szekeres, József; **Veseli, Marina**; Zorić, Katarina (2021) Peracarid crustaceans in the River Danube and its tributaries: results of the 4th Joint Danube Survey // BioInvasions records, 10, 3; str. 623-628. DOI: 10.3391/bir.2021.10.3.12
4. Mijošek, Tatjana; Filipović Marijić, Vlatka; Dragun, Zrinka; Ivanković, Dušica; Krasnići, Nesrete; Redžović, Zuzana; **Veseli, Marina**; Gottstein, Sanja; Lajtner, Jasna; Sertić Perić, Mirela; Matoničkin Kepčija, Renata; Erk, Marijana (2020) Thallium accumulation in different organisms from karst and lowland rivers of Croatia under wastewater impact. // Environmental chemistry

## **PARTICIPATIONS**

- actively participated at four international meetings: Symposium for European Freshwater Sciences 2021 (online), SETAC Annual Meeting in 2022 (Copenhagen, Denmark) and 2023 (Dublin, Ireland), 17th International Symposium on Trichoptera – IST 2022 (Lunz am See, Austria)
  - actively participated at five national meetings: Symposium of doctoral students of PMF in 2021 and 2022, Environmental Science School (Institute Ruđer Bošković) in 2021, Symposium of the Croatian Entomological Society in 2022, 4th Symposium on Freshwater Biology (SOBS) in 2023
  - Joint Danube Survey (JDS4) – joint survey of the Danube in 2019
-