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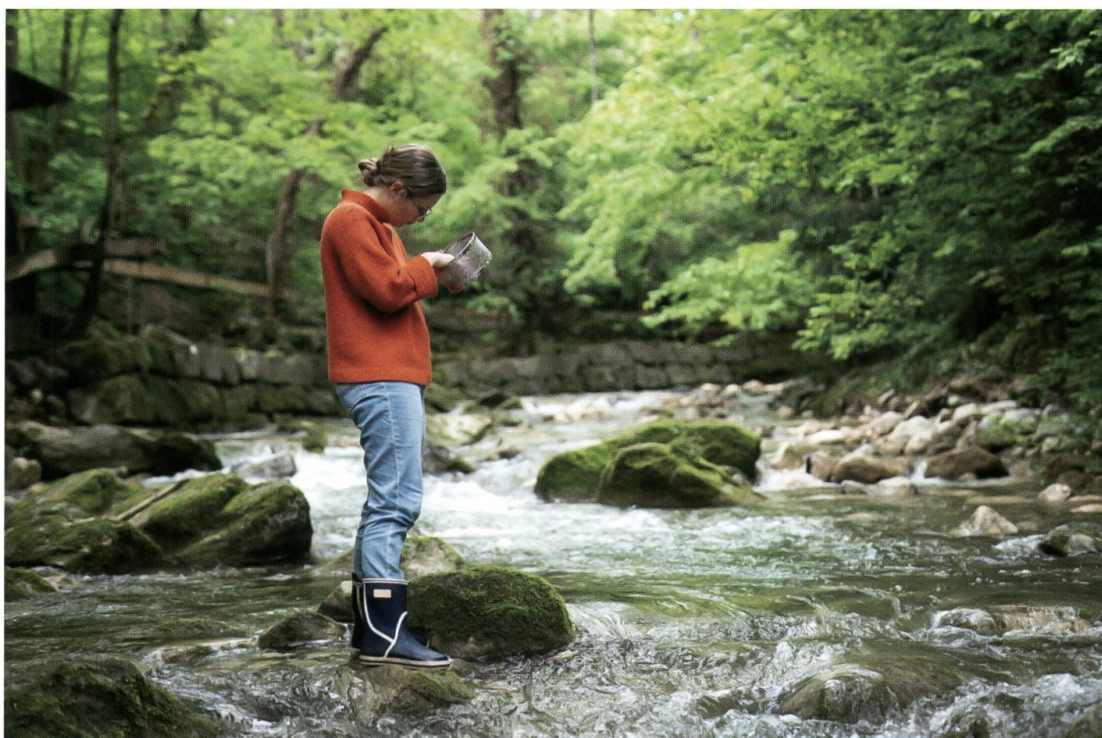
EVIDENCE OF NEONICOTINOID CONTAMINATION IN AQUATIC INVERTEBRATES : AN ASSESSMENT OF THE CURRENT STATE OF THE SEYON RIVER IN THE CANTON OF NEUCHÂTEL, SWITZERLAND

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Jeanne Käser sampling invertebrates in the Seyon River. Photograph by Jeremias Gisler.

Résumé

Depuis leur introduction dans les années 1990, les néonicotinoïdes sont rapidement devenus les pesticides les plus utilisés dans l'industrie phytosanitaire. Leurs effets néfastes sur les milieux naturels et la faune sont reconnus depuis longtemps mais sont encore peu étudiés. Ce travail analyse la présence et la prévalence de cinq néonicotinoïdes dans quatre différents taxons d'invertébrés aquatiques de la rivière du Seyon, en Suisse. Pour ce faire, deux méthodes ont été combinées : le protocole d'indice de la biodiversité IBCH pour l'échantillonnage, et la chromatographie liquide à haute performance couplée à la spectrométrie de masse en tandem (HPLC-MS/MS) pour l'analyse. Tous les échantillons analysés étaient contaminés par au moins un néonicotinoïde. Les néonicotinoïdes présents dans les échantillons avaient une concentration moyenne de 0,148 ng/g (SD = 27,8 %, n = 14) (valeur extrême exclue), illustrant l'omniprésence de ces pesticides dans l'environnement et l'exposition chronique à laquelle sont soumis les invertébrés. Deux des quatre néonicotinoïdes trouvés dans les échantillons avaient été interdits d'utilisation en extérieur neuf mois avant l'échantillonnage, ce qui indique leur grande persistance. Une valeur extrême de 7,375 ng/g, presque dix fois plus élevée que les autres, a été trouvée dans un échantillon, suggérant une forte contamination de certaines populations d'invertébrés. Ces résultats sont inquiétants et révèlent la nécessité de poursuivre les recherches sur le sujet. Les invertébrés aquatiques jouent un rôle clé dans la chaîne alimentaire et dans la qualité de l'eau de leurs habitats et sont particulièrement exposés aux grands dommages que causent les néonicotinoïdes dans les milieux naturels. Ce travail contribue à documenter cette exposition critique et propose des méthodes expérimentales qui pourront être utilisées dans de futures études.

Abstract

Since their introduction in the 1990s, neonicotinoids have rapidly become the most widely used pesticides in the crop protection industry. Their harmful effects on natural environments and wildlife have been recognised for a long time but are understudied. This work analyses the presence and prevalence of five neonicotinoids in four different taxa of aquatic invertebrates from the Seyon River, in Switzerland. To do this, two methods were combined: the Swiss Biodiversity Index (IBCH) protocol for biodiversity sampling and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for analysis. All the analysed samples were contaminated with at least one neonicotinoid. The neonicotinoids in the samples had an average concentration of 0.148 ng/g (standard deviation [SD] = 0.0411 ng/g [27.8 %], n = 14; extreme value excluded). This provides evidence of the ubiquity of these pesticides in the environment as well as the chronic exposure to which invertebrates are subjected. Two of the four neonicotinoids found in the samples had been banned from outdoor use 9 months prior to the sampling, indicating their high persistence. An extreme value of 7.375 ng/g, nearly tenfold higher than the others, was found in one sample, which suggests high contamination of some invertebrate populations. These results are worrisome and reveal the need for more research on the subject. Aquatic invertebrates play key roles in the food chain and in the water quality of their habitats and are particularly exposed to the great damage that neonicotinoids cause in natural environments. This work contributes to documenting this critical exposure and proposes experimental methods that could be used in future studies.

Zusammenfassung

Seit ihrer Einführung in den 1990er Jahren haben sich die Neonicotinoide schnell zu den am häufigsten verwendeten Pestiziden in der Pflanzenschutzindustrie entwickelt. Ihre schädlichen Auswirkungen auf die natürliche Umwelt und die Tierwelt sind seit langem bekannt, aber noch wenig erforscht. In dieser Arbeit wird das Vorhandensein und die Prävalenz von fünf Neonicotinoide in vier verschiedenen Taxa von wirbellosen Wassertieren aus dem Seyon in der Schweiz analysiert. Zu diesem Zweck wurden zwei Methoden kombiniert: das IBCH-Protokoll für die Probenahme und eine Hochleistungsflüssigkeitschromatographie-Massenspektrometrie (HPLC-MS/MS) für die Analyse. Alle untersuchten Proben waren mit mindestens einem Neonicotinoid kontaminiert. Die Neonicotinoide in den Proben hatten eine durchschnittliche Konzentration von 0,148 ng/g (SD = 27,8 %, n = 14) (Extremwert ausgeschlossen). Dies ist ein Beleg für die Allgegenwart dieser Pestizide in der Umwelt und die chronische Exposition, der die wirbellose Tiere ausgesetzt sind. Zwei der vier in den Proben gefundenen Neonicotinoide waren neun Monate vor der Probenahme für die Verwendung im Freien verboten worden, was auf ihre hohe Persistenz hinweist. In einer Probe wurde ein Extremwert von 7,375 ng/g gefunden, der fast

das Zehnfache der anderen Werte beträgt, was auf eine hohe Kontamination einiger Wirbellosenpopulationen schließen lässt. Diese Ergebnisse sind besorgniserregend und zeigen, dass weitere Forschungsarbeiten zu diesem Thema erforderlich sind. Wirbellose Wassertiere spielen eine Schlüsselrolle in der Nahrungskette und für die Wasserqualität ihrer Lebensräume und sind den großen Schäden, die Neonicotinoide in der Natur verursachen, besonders ausgesetzt. Diese Arbeit trägt dazu bei, diese kritische Exposition zu dokumentieren und schlägt experimentelle Methoden vor, die in zukünftigen Studien verwendet werden können.

Mots-Clés : néonicotinoïdes ; pesticides ; pollution des eaux ; invertébrés aquatiques ; écotoxicologie.

Keywords : neonicotinoids ; pesticides ; water pollution ; aquatic invertebrates ; ecotoxicology.

INTRODUCTION

Neonicotinoids have recently become a key topic in public discussions. In Switzerland, the topic is more relevant than ever with two popular federal initiatives voted in 2021 calling for the ban of synthetic pesticides and the protection of the purity of food and drinking water. After a partial ban introduced in 2012, three neonicotinoid molecules – imidacloprid, clothianidin, and thiamethoxam – were banned from outdoor use in 2018 in Switzerland (Office fédéral de l'agriculture [OFAG], 2018). Despite these bans, neonicotinoids remain the most widely used insecticides today, both in Switzerland and worldwide (SIMON-DELISO *et al.*, 2015). Scientific attention is also focussed on the following subject: in countless studies, researchers have shown that these insecticides act on the insect nervous system and are extraordinarily harmful to our environment (PISA *et al.*, 2017) and to human health (CIMINO *et al.*, 2017). Special attention has been drawn to the recent decline of pollinators linked to broad neonicotinoid use, especially of bees (European Food Safety Authority [EFSA], 2013; MITCHELL *et al.*, 2017). However, much less is known on the impact of neonicotinoids on the aquatic environment. This work provides a preliminary overview of the contamination of the invertebrate fauna of the Seyon River.

Pesticides and neonicotinoids : a brief chronology

Alongside the development of organic chemistry at the end of the First World War,

pesticides such as dichlorodiphenyltrichloroethane (DDT) and other organochlorine molecules paved the way for the rise of synthetic pesticides in the early 1940s. About two decades later, following the work of Rachel Carson and the publication of her book *Silent Spring* in 1962, the scientific community began to document the toxicity of synthetic pesticides. The discussion around their effects on non-target wildlife marked the beginning of public awareness regarding the great harmfulness of synthetic pesticides to the environment and human health. Along with this debate, DDT was progressively banned from use in the 1970s. Despite this ban, the agrochemical industry began to develop an array of various crop-protection products (MCAFFEE, 2017).

Neonicotinoids entered the race in the late 1980s. Chemically, they are derivatives of nicotine, a natural molecule produced by tobacco to defend itself against pests. The mode of action of these insecticides targets nicotinic receptors in the insect central nervous system, which leads to overstimulation of these receptors and causes paralysis and eventual death of the organism (TOMIZAWA & CASIDA, 2011). In the 1990s, the first companies to develop these formulas on a larger scale were Bayer CropScience, Syngenta, and Sumitomo Chemical. These companies have kept their monopoly over the market until today, with active ingredients like acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam (BASS *et al.*, 2015). Since their discovery, neonicotinoids have become the most successful and fastest growing pesticides on the market in

record time. It is estimated that by 2015, they accounted for 25% of the total pesticides sold worldwide (BASS *et al.*, 2015).

Aquatic contamination

This work focusses on neonicotinoids present in the Seyon River, which flows between Villiers and Neuchâtel in north-west Switzerland (see fig. 1). According to preliminary studies, the Seyon is one of the most polluted rivers in Neuchâtel (AEBI, unpublished data). This high contamination can be explained by the ease by which neonicotinoids are propagated in natural environments during the treatment of the fields of Val-de-Ruz. Neonicotinoids are systemic, that is, they can propagate in the vascular system of plants; this characteristic enables crop treatment by seed coating, applying the product

to the surface of the seed before sowing it. As the plant germinates and grows, the neonicotinoid is distributed throughout the whole plant, which allows optimisation of the quantity of the product used and facilitates the treatment process, resulting in a more efficient way of fighting pests. This method was intended to allow a very deliberate and precise application – for example, to spare non-target wildlife. However, the seed coating method is less effective than thought at the time of its popularisation. Studies have shown that only about 5% – 20% of the coating appears to be absorbed by the seed (SUR & STORK, 2003). This leaves 80% – 95% of the applied neonicotinoid in the soil and subsurface water (GOULSON, 2013). Because of the high hydrophilicity of neonicotinoids, water is very easily contaminated with these residues. The water on the fields then collects in larger

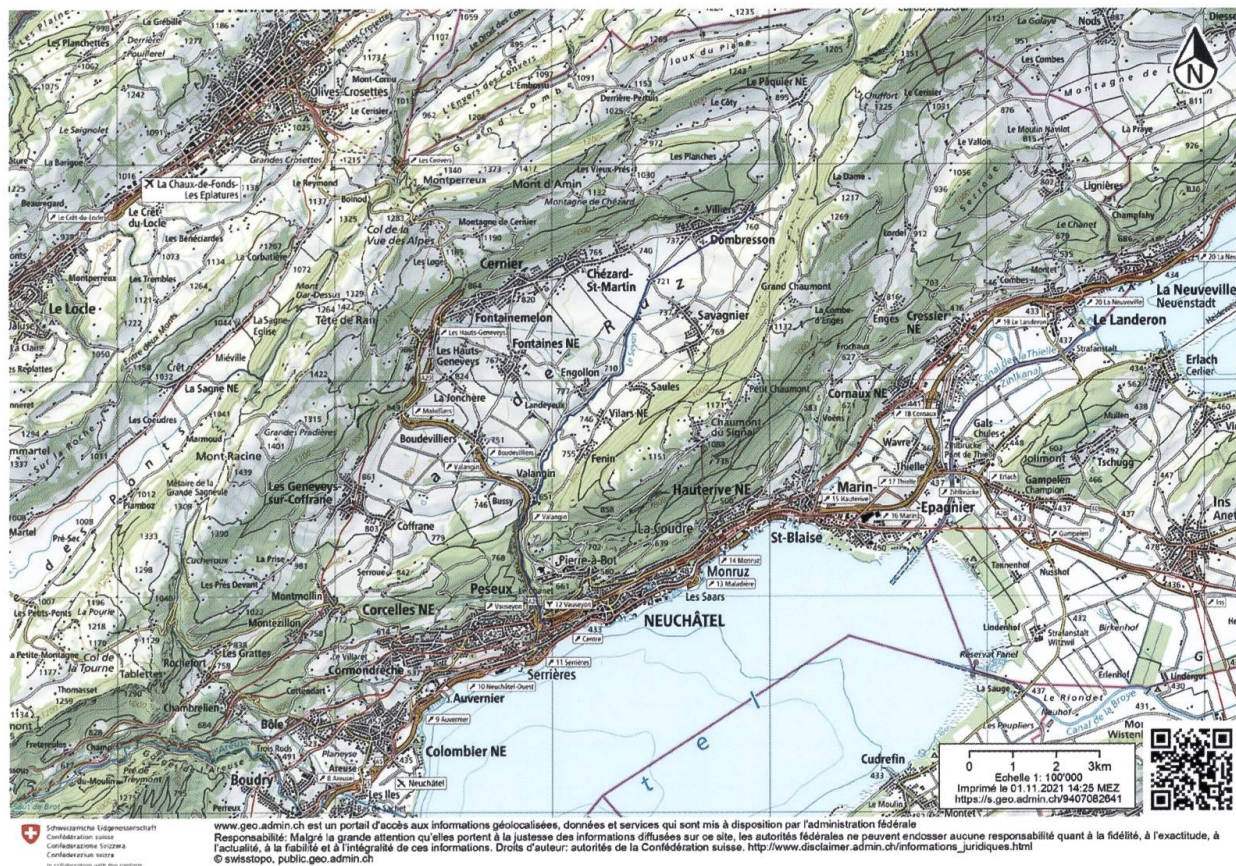


Figure 1. Map of the Seyon River (blue), running across the canton of Neuchâtel.

streams by running off on the surface or traveling underground, or infiltrates directly into the groundwater (BORSUAH *et al.*, 2020). In the case of the Seyon, the contaminated runoff water is easily and efficiently infiltrated due to the very porous and calcareous characteristics of the Val-de-Ruz region (Office fédéral de topographie [OFT] Swisstopo, n.d.). This factor, combined with the high agricultural density of the river's drainage area, makes the Seyon particularly prone to neonicotinoid contamination.

Today, neonicotinoids are everywhere in the environment – as vapours in the air, in the soil, or in the water – because of their properties and their extensive use (BONMATIN *et al.*, 2019; GOULSON, 2013). While at the beginning of their widespread use neonicotinoids were thought to have little influence on non-target biodiversity around crops, they are now known to have a considerable impact on non-target organisms, particularly invertebrates (PISA *et al.*, 2017). The chronic exposure to these substances that is mainly observed today in natural habitats results in cumulative effects for exposed invertebrates, including limited growth (HENRY *et al.*, 2012) as well as respiratory (LUKANCIC *et al.*, 2009), reproductive (CHARPENTIER *et al.*, 2014), and motor (GIROLAMI *et al.*, 2009) disorders. It is also known that chronic exposure to neonicotinoids at very low doses can be just as dangerous as acute exposure at high doses (VAN DER BRINK *et al.*, 2015). Once in natural environments, neonicotinoids have varying half-lives, depending on the compound, ecosystem, and across studies. For example, the half-lives range from 200 to more than 1000 days in the soil (GOULSON, 2013). These long half-lives increase the likelihood of persistence in surrounding ecosystems, including aquatic ecosystems. In water, the half-lives are shorter and range from a few days to 63 (MORRISSEY *et al.*, 2015), but ecotoxicologically relevant concentrations (0.1 – 0.2 µg/L) can be found a year after application (KANRAR *et al.*, 2006), depending on conditions like low temperatures and low pH (GUZSVÁNY *et al.*, 2006).

In this work, we evaluated the presence and prevalence of neonicotinoids in the

invertebrate fauna of the Seyon. To do so, we analysed the concentrations of different neonicotinoids in benthic macroinvertebrates (aquatic invertebrates > 0.5 mm), which play a key role in their environment as decomposers and as a food source. This research provides appropriate experimental methods to be applied in future studies. While our main goal was method development, the results can be used to hypothesise about the different factors involved in invertebrate contamination and the impact it has on their ecosystem. Finally, the results allow us to illustrate the current state of neonicotinoid contamination in the Seyon.

METHODS

Field methods

The following sampling method is based on the Swiss Biodiversity Index (IBCH) method (Office fédéral de l'environnement [OFEV], 2019).

Sampling. Three benthic macroinvertebrate samplings were carried out for this work in October 2019 (2, 7, and 16 October 2019). They were carried out in the Seyon River, near the Prés Maréchaux in Vauseyon (see fig. 2). Survey sites were different for each of the three sampling days to maximise the diversity of the samples. Five sampling sites were selected per sampling day to maximise the number of species and to obtain the most accurate invertebrate representation and distribution in the samples. The selected sites had to be as diverse as possible: they mainly differed in substrate type, current, and vegetation.

Samples were collected by using the kick-sampling method, which consists of catching benthic fauna in a net by lifting the bottom of the riverbed with foot work on a square foot area. Large organic debris and rocks were removed, the water was filtered out, and the remaining material was placed in Falcon tubes and submerged in ethanol (70%).

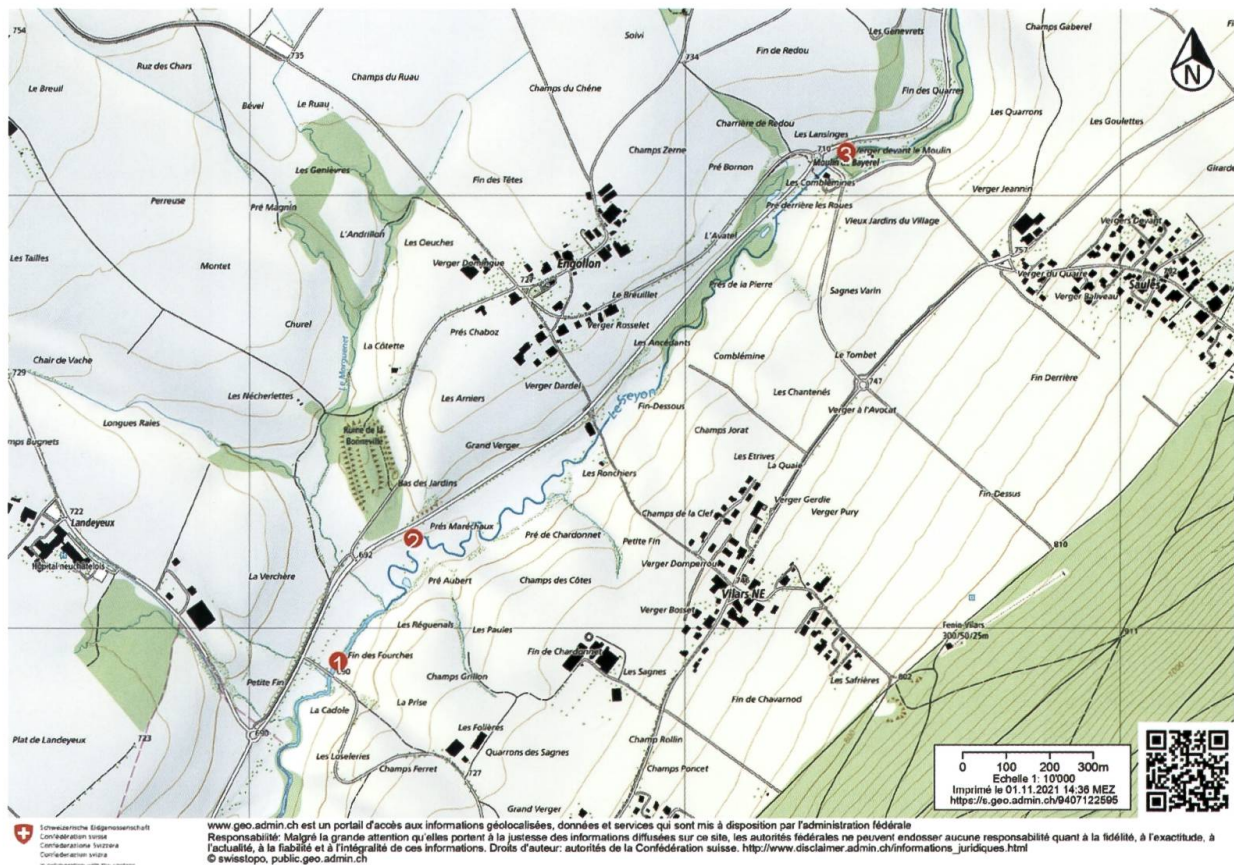


Figure 2. Map of the three sampling sites, between Engollon and Vilars (1 : sampled 2 October 2019 ; 2 : sampled 7 October 2019 ; 3 : sampled 16 October 2019).

Sorting. All organisms in the samples were separated from the remaining organic waste and sorted – they were always kept in alcohol to prevent desiccation. Organisms were sorted into morpho-groups, that is, into approximate and temporary groups based on their appearance, without formal determination. The factors of morpho-group sorting are the invertebrate's size, the symmetry, the number of legs, or the presence of gills.

Identification. Preservation varied according to the organisms and their exposure to light or heat. The sampling and the determination of the organisms should be conducted not more than 2 weeks apart. In this time, the samples should be kept protected from direct light

exposure to minimise putrefaction or other degradation. The identification was carried out with a binocular magnifying glass and a Petri dish covered with ethanol. According to the different states of preservation, the identification was carried out up to the genus or the taxonomic family. Only the taxa of morpho-groups that had more than 30 representatives for each sampling day were determined. After identification, the samples were sorted according to their sampling day and taxon and returned to Eppendorf tubes. A census of the collected organisms was also carried out. A precise count was made only for those organisms whose taxa were abundant (≥ 30 representatives) and thus relevant to use in our chemical analysis, whereas the other counts

were rounded off to the dozen, to be used in a simplified IBCH analysis (see Chapter 4). For some samples with more than 100 individuals, the number was approximated. This rough census was crucial in determining the number of invertebrates present in the final analysed samples. The number of invertebrates was also used to determine a biodiversity index (IBCH) of the analysed river reach to estimate the degree of pollution of the water in which these invertebrates live.

Sample processing

The neonicotinoid concentrations were determined by using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). The objective was to measure the concentrations of five different neonicotinoids in the samples. Four taxa were selected for analysis based on their abundance in the collected water samples and their diversity, considering factors like food web position, tolerance to neonicotinoids and morphology. The selected taxa were (order, family, genus): Amphipoda, Gammaridae, *Gammarus* and *Echinogammarus*; Ephemeroptera Baetidae *Centroptilum* (mayflies); Trichoptera, Hydropsychidae, *Hydropsyche* (caddisflies); and Arynchobdellida, Arhynchobdellidae, *Erpobdella* (leeches) (fig. 3). The HPLC-MS/MS protocol was adapted from the steps described in the methodological paper written for neonicotinoid contamination in honey samples by KAMMOUN *et al.* (2019). The sample processing had to be adapted to the studied invertebrates.

Drying. The invertebrates selected for analysis were arranged in Petri dishes separated by taxa and sampling day. These Petri dishes were placed to dry lid open for 2 days in a fume hood ventilating at low intensity. Each petri dish was labelled as follows: ABC# (ABC being the first three letters of the taxon in the dish [amp, eph, tri, or arh], # being the number of the sampling day [day 1, 2 or 3]).

Grinding. The samples were ground one by one under liquid nitrogen using a mortar and pestle. They were ground until the nitrogen had completely dissipated, and a very fine powder was obtained. The powder was then carefully transferred into 15 mL tubes and weighed. Aliquots between 1 and 100 mg were used for analysis. The mass of one of the samples was smaller than 0.001 g and it had to be excluded from the analysis. These steps were repeated for each sample, after disinfection of all utensils.

Extraction. Five microlitres of acetonitrile and 20 μ L of internal standard solution containing labelled forms of each neonicotinoid were pipetted into each sample tube. The tubes were then mixed with the Polytron for 3 minutes and centrifuged at 4000 g for 5 minutes. As much supernatant as possible was then pipetted into the sample's corresponding ST1 (Salt Tube 1: 15 mL Falcon tube, filled with 3.25 g of extraction salts). Five millilitres of Mili-Q water were added, and the tube was shaken vigorously for 1 minute. The tubes were again centrifuged for 5 minutes. The upper phase in each tube was pipetted into the corresponding ST2s (Salt Tube 2: 15 mL Falcon tube, filled with 150 mg of MgSO_4 ,



Figure 3. The four selected taxa : *Gammarus*, *Centroptilum*, *Hydropsyche*, and *Erpobdella* (from left to right).

100 mg of primary-secondary amine [PSA], and 100 mg of octyldecylsilane [C18]). The tubes were shaken vigorously for 30 seconds and then centrifuged. The supernatants from the tubes were carefully collected and deposited into 13×100 mm glass tubes. All glass tubes were then left in a Speedvac vacuum for 5 hours to evaporate to dryness. Five hundred microlitres of H_2O :methanol 75%:25% (v/v) were added to each tube and vortexed for 20 seconds. The tubes were finally put in an ultrasonic bath for 1 minute. The contents of the glass tubes were transferred to Eppendorf tubes, centrifuged for 2 minutes at maximum speed, and filtered through polytetrafluoroethylene (PTFE) hydrophilic filters into an HPLC flask with a 250 μ L conical insert.

Analysis

Analysis of the samples was carried out by Dr Gaéтан Glauser at the Neuchâtel platform of analytical chemistry, at the Faculty of Sciences of Neuchâtel. It is based on HPLC-MS/MS analysis. This process allows separation of the different neonicotinoids dissolved in the samples and their identification and quantification. For this work, 10 samples of invertebrate pools were analysed.

PRELIMINARY ANALYSIS OF THE BIODIVERSITY OF THE SEYON

The IBCH, computed for a given river section, is an evaluation of the water quality of said river according to the biodiversity of its aquatic macroinvertebrate wildlife. An adapted version of the IBCH was used to interpret my sampling results. The IBCH value depends on two factors: the faunistic group (FG) and the variety class (VC). The FG is evaluated based on the presence of specific taxa in the studied sample. The taxa have a variable affinity for polluted waters. Depending on the invertebrate taxa present in the samples, a grade from 1 to 9 is given to the stream. The VC is evaluated

based on the diversity of the invertebrates in the sample. A grade from 1 to 14 is given to the sample accordingly. The IBCH is then computed with the formula $IBCH = (FG + VC) - 1$, resulting in a grade from 1 (worst) to 14 (best) (OFEV, 2019).

The IBCH is usually determined with an exhaustive list of each taxon represented in each sample, as well as an exact count of the number of individuals of each taxon. In this work, only a basic census was done during sample sorting, but an IBCH grade can still be approximated rather precisely. It allows a more comprehensive understanding of the water quality of the Seyon and puts the results in perspective.

RESULTS

The IBCH obtained with the preliminary analysis was a 6 on a scale of 1 to 14. This value indicates poor water quality (HWI between 5 and 8). Such a low value, while not solely due to the presence of neonicotinoids in the water, underlines the importance of conducting chemical analyses to investigate a river's contamination with neonicotinoids. This value is also consistent with previous results on the water quality of the Seyon. The HPLC-MS/MS analysis provided the concentrations in ng/g invertebrate of five different neonicotinoids in my 10 samples. The five substances were thiamethoxam, clothianidin, imidacloprid, acetamiprid, and thiacloprid (table 1). As replicate measures were not done for my samples, an error range cannot be calculated or estimated precisely. For error ranges of comparable studies, several papers with similar methods are available in literature (KAMMOUN *et al.*, 2019; SCHLÄPPI *et al.*, 2020).

Thiamethoxam was not found in any sample. Clothianidin was detected in six samples and all four studied taxa were contaminated, with concentrations ranging from 0.028 to 0.140 ng/g. Imidacloprid was detected in four

Table 1. Raw results of the high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) analysis (concentrations of neonicotinoids, ng/g).

amp1	0,000	0,028	0,000	0,000	0,019
amp2	0,000	0,000	0,000	0,000	0,000
amp3	0,000	0,140	0,197	0,026	0,000
eph1	0,000	0,000	0,000	0,000	0,000
eph2	0,000	0,144	0,806	0,000	0,000
eph3	0,000	0,000	0,000	0,000	0,000
tri2	0,000	0,134	0,000	0,000	0,000
tri3	0,000	0,133	7,375	0,000	0,000
ary1	0,000	0,000	0,000	0,000	0,054
ary2+3	0,000	0,034	0,257	0,020	0,085

samples and is represented in all four taxa. The three lowest concentrations ranged from 0.197 to 0.806 ng/g, but one sample was well above this range, with a concentration of 7.375 ng/g. This last value will not be considered in the following graphs and statistics because it would overwhelm all other data. However, it will be given special attention during the discussion of the results. Acetamiprid was present in two samples, with concentrations of 0.020 and 0.026 ng/g. Thiacloprid was detected in three samples, with concentrations ranging from 0.019 to 0.085 ng/g.

Discussion and limitations of the methods

One of the objectives of this work was to establish a methodological test for future studies on the same subject.

Method adaptations

Sampling. The IBCH sampling needed little adaptation and allowed a diverse sampling of the invertebrate fauna and is adapted to a work like ours. The Seyon was an apt sampling site choice for this initial study, thanks to its proximity but

also regarding the results of preliminary studies, which highlighted its strong contamination.

The sampling for this work was carried out during the month of October. A sampling in April would have been optimum to avoid extreme temperatures or floods that would disturb the usual wildlife. Sampling shortly after neonicotinoid field treatments or sowing of treated seeds would also be more representative of the maximum doses experienced by the aquatic organisms. In our case, the Neuchâtel Wildlife Service forbid us from taking samples between November and May to protect the aquatic fauna.

Between the sampling and the analysis, the invertebrate samples were stored in an ethanol solution to limit putrefaction. This storage method might allow interaction between the neonicotinoids and ethanol, thereby reducing the amounts of pesticides recovered for the analysis. Thus, an unknown fraction of neonicotinoids could have already been extracted from the samples before their processing, meaning that our data are actually conservative. For a future study, to prevent this involuntary extraction, the invertebrates should rather be frozen and stored at a low temperature until their analysis.

Sample processing. To maintain the diversity of the samples, we were careful to select four taxa for analysis that were as diverse as possible, both in sensitivity and development. These differences are to be considered during the discussion of the results.

Analysis. The chemical protocol used for this work had never been used on invertebrates. An objective of this work was to see whether it was adaptable to organisms other than fish. Filtration and chemical treatments prior to analysis were very effective with invertebrates. The adaptations made were adequate and allowed for pure samples. The HPLC-MS/MS methods used have been verified to be accurate and precise in a previous study on invertebrates (KAMMOUN *et al.*, 2019; SCHLÄPPI *et al.*, 2020). The analytical method is therefore very suitable for invertebrates. These values could be used to determine the effects of the neonicotinoids on the contaminated organisms themselves, but usually the values used to determine this are environmental concentrations like the median lethal dose (LC_{50}), not internal ones. However, our results could be used to determine the dynamics of neonicotinoid contamination in a specific environment – for example, how the contamination varies following the food web. This point will be considered in the discussion of the results.

Limitations

The main limitation of this work is the number of samples. Only three sampling sessions were carried out due to limited time, resources, and the scope of this work (a high school project). The three samplings were very close in time, but at irregular intervals (5 and 9 days), and were all carried out in the same river stretch. The results of our analysis, while being good first indicators of the situation, are therefore not representative of the overall state of the waters and invertebrates of the Seyon. For these methods to be valuable and for the results obtained to be significant,

it would first be necessary to carry out a much more exhaustive sampling in space (different locations) and time. A primary focus of a study investigating the influence of geographic, taxonomic, or seasonal factors on contamination should also be chosen and the sampling should be tailored accordingly. This approach would allow for a more precise study.

Another limitation of these methods is that they only consider the initial form of neonicotinoids prior to any degradation. Metabolites of neonicotinoids derived from their degradation, particularly those of imidacloprid, could be just as dangerous to invertebrates as the original substance (TOMIZAWA & CASIDA, 2011). Although these metabolites are rarely considered in neonicotinoid analytical methods used in research, their significance must be recognised.

DISCUSSION

The analyses were carried out on sets of invertebrates, the number of which varies between 22 and 280, depending on their mass. The exact meaning of the results is difficult to understand: the environment studied in this work has been considered to only a limited extent in previous studies and there are not much data available in the literature. Moreover, our limited sampling does not allow us to generate complete hypotheses on the contamination of the subjects studied. However, we have made several observations on the contamination of the section of river and its invertebrates by comparing the different concentrations detected in the samples. The results could ultimately be used to illustrate ground truths of neonicotinoid contamination elsewhere.

The ubiquity of neonicotinoids

Overall, each sample analysed was contaminated by at least one neonicotinoid (fig. 4), demonstrating the ubiquity of these pesticides

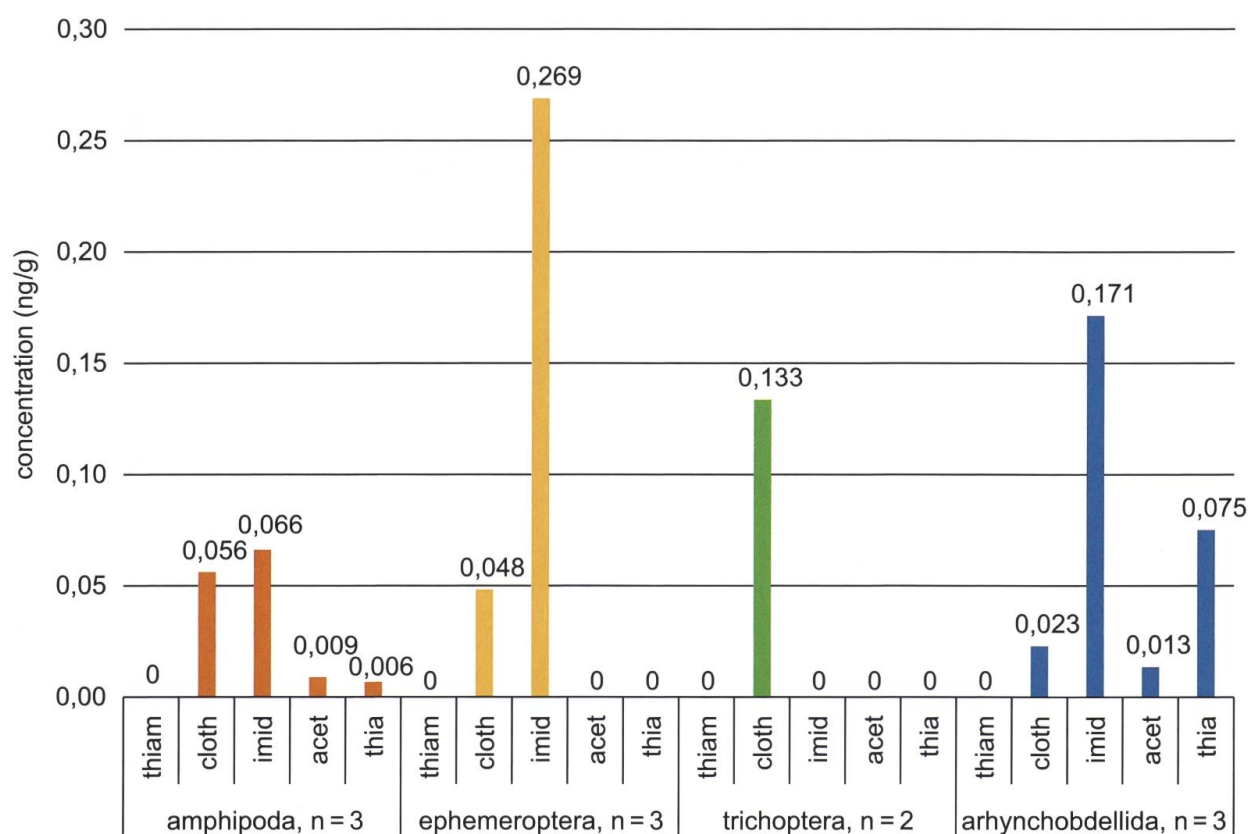


Figure 4. Average concentrations of neonicotinoids per taxon, all days (n) combined. The taxa are called by their order name.

in our environment: in one way or another, each organism is contaminated by phytosanitary products, albeit to a variable degree. This observation highlights the ease of propagation of neonicotinoids. They flow easily from cultivated areas to various springs/streams/water bodies, from which they infiltrate the entire surface water network of the region. The Seyon is especially exposed to neonicotinoid contamination because it flows through an intensely agricultural area with calcareous soil.

Chronic exposure

Because of the variable weight of the analysed aliquots, the limit of quantification of the method also varied slightly depending on the samples, but it was estimated to be up to 0.05 ng/g. Several concentrations found in the

samples are below this limit, meaning that they could be due to neonicotinoid exchanges between invertebrates in the samples, possibly facilitated by the conservation alcohol.

Overall (extreme sample tri3 excluded), the rest of the concentrations are quite low and do not exceed 0.806 ng/g. These results match the values obtained in a 2016 study of pesticide concentrations in aquatic invertebrates in the Danube River, with insecticide concentrations ranging from 0.1 to 0.53 ng/g (SHAHID *et al.*, 2018). However, the contamination of aquatic invertebrates illustrated in these samples remains minimal compared with, for example, contamination of pollinators. In 2016, a study conducted on honeybee contamination rates shows concentrations of 53 ng/g for imidacloprid and 32 ng/g for acetamiprid

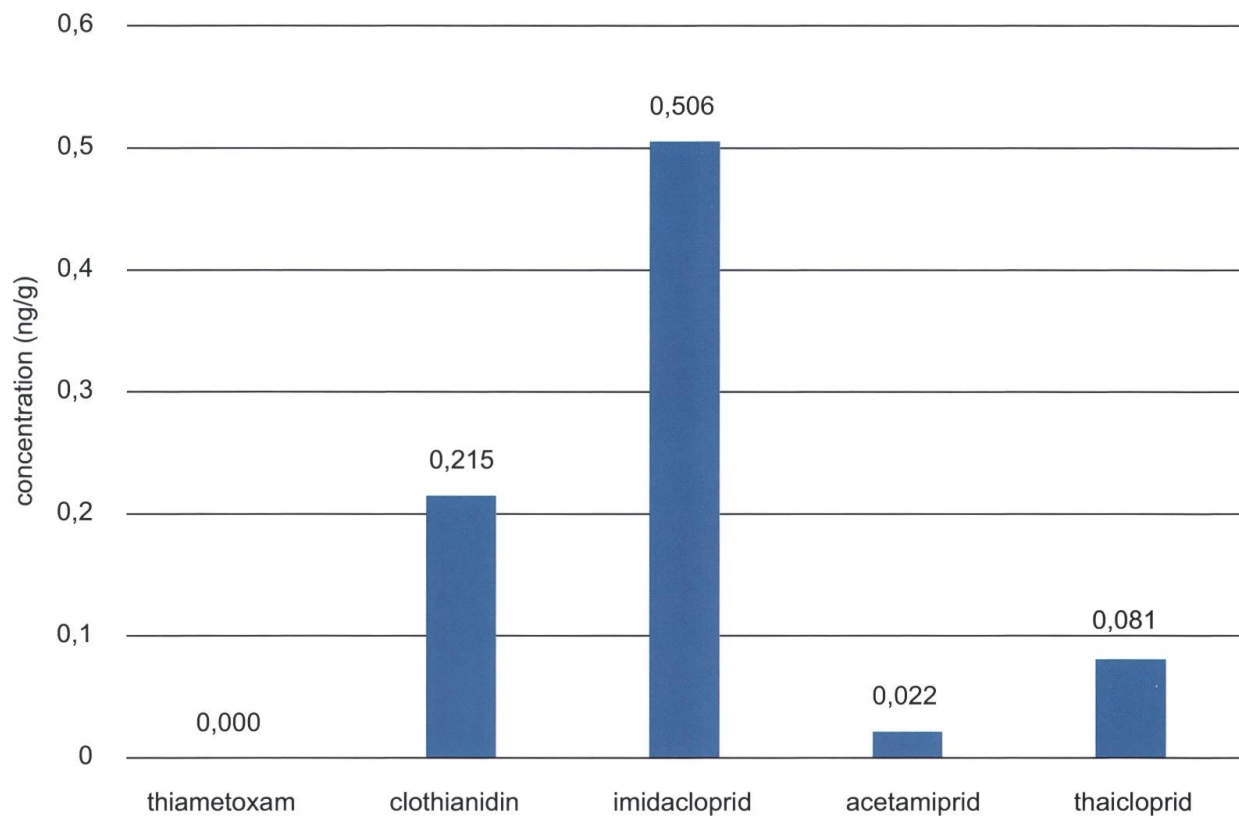


Figure 5. Average concentration per substance, all days and taxa combined (number of samples = 10).

(CALATAYUD-VERNICH *et al.*, 2016). By comparison, the aquatic invertebrate exposure to neonicotinoids is clearly lower. As mentioned in the introduction, such low exposure, if chronic, may still have an impact on invertebrate populations.

High persistence

The concentrations of five different neonicotinoids in each of the samples were obtained, and it is interesting to compare the concentrations of these different substances. Of the five neonicotinoids analysed, three substances have been banned from outdoor use in Switzerland since January 2019 by the OFAG: thiamethoxam, clothianidin, and imidacloprid. Thiamethoxam is no longer present in the analysed invertebrates, but clothianidin and imidacloprid are the two

most concentrated substances in the samples (fig. 5), showing the high persistence of these two substances in our environment. However, clothianidin and imidacloprid are considered fast-degrading substances in water (PEÑA *et al.*, 2011): their presence in invertebrates 9 months after their ban proves that neonicotinoids can be stored for a long time in the soil, where their half-life is extended, before gradually diffusing into aquatic environments. Imidacloprid, for example, can have a persistence of up to 1000 days in crop soils (BONMATIN *et al.*, 2015). The use of these three neonicotinoids is still allowed in permanent, strictly closed greenhouses. In addition to their high remanence, their presence in the samples could also indicate that neonicotinoids applied indoors could reach natural ecosystems despite the restrictions, by drainage or wastewater. A 2010 study

conducted on several stream draining areas with greenhouses in Sweden found imidacloprid concentrations substantially higher than in other outdoor cultivation sites, indicating probable leaking (KREUGER *et al.*, 2010).

Bioaccumulation and biomagnification

Even with our restricted sampling, comparing the samples according to the four taxa analysed could be an interesting illustration of how neonicotinoids are distributed in an ecosystem. The taxa will be referred to with the name of their order: Amphipoda, Ephemeroptera, Trichoptera and Arynchobdellida.

Figure 6 is a comparative table of the arithmetic means of the concentrations of all neonicotinoids present in the different taxa. However, this ranking is not very representative of the real situation: to discuss the effective neonicotinoid contamination of the organisms, the diversity of compounds in

the samples must also be considered. Indeed, neonicotinoids are known for their synergic effects, which emerge when several of these molecules are mixed. This property has been recognised by scientific research (MALONEY *et al.*, 2017) and agrochemical manufacturers, for example, Bayer CropScience AG. It is important to take this into account in this discussion.

To consider the diversity of substances present in each taxa, a second table can be designed, for which each taxon's average concentration is multiplied by the number of compounds found in each taxon. The table represents the neat concentrations and diversity of neonicotinoids, and thus the ranking of the taxa's contamination changes considerably (fig. 7). Arhynchobdellida are now the most affected by neonicotinoids, as they are contaminated by four different compounds at high concentrations. They are followed by Ephemeroptera, contaminated by only two compounds but with a high concentration

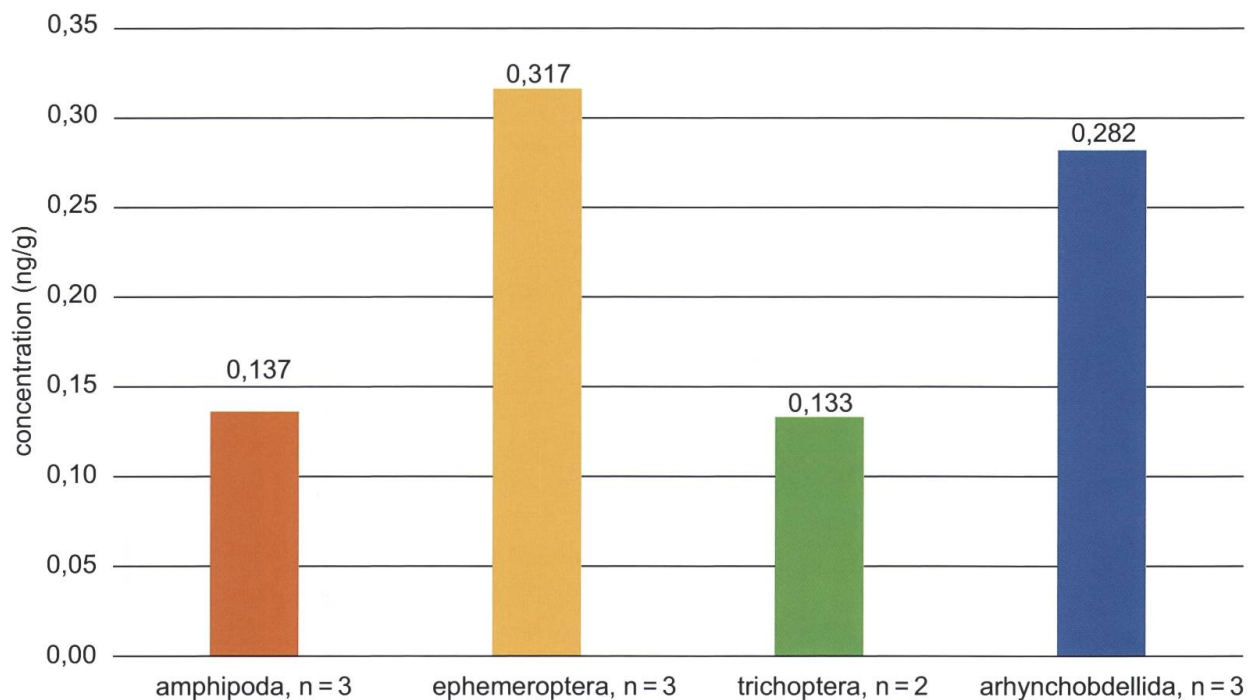


Figure 6. Average concentrations per taxa, all days (n) and substances combined.

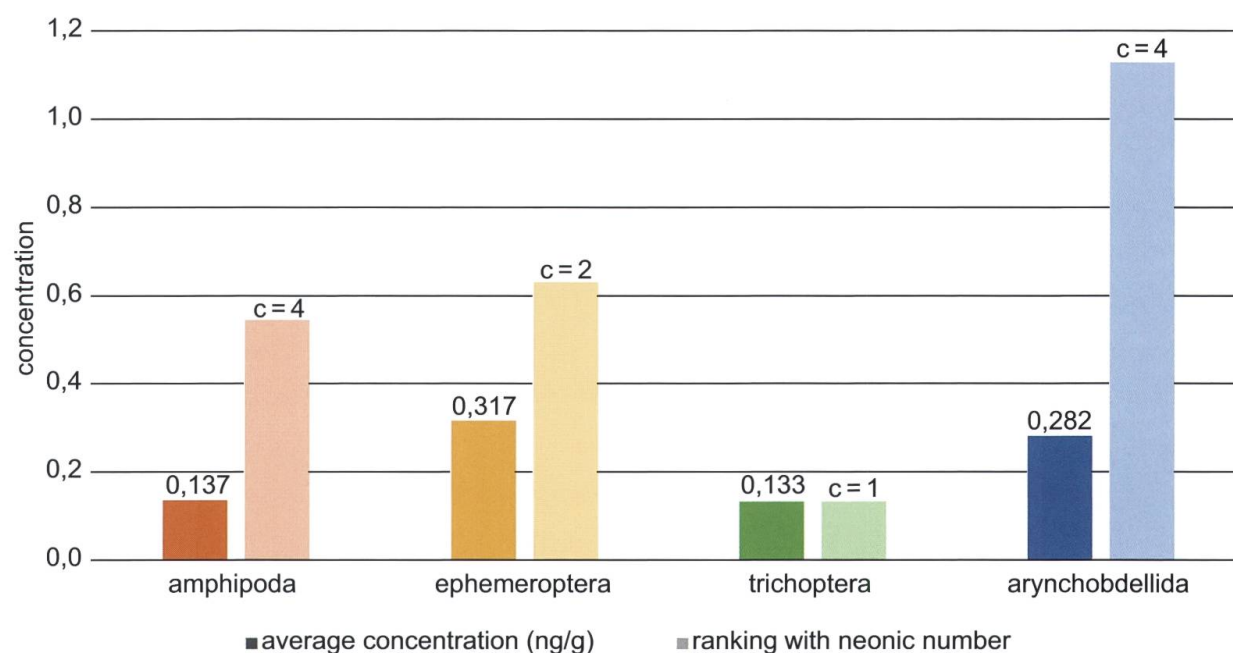


Figure 7. Ranking of the taxa according to their average neonicotinoid concentrations and number of substances.

of imidacloprid. Amphipoda are contaminated by four different compounds but at low concentrations. Finally, Trichoptera are contaminated by only one compound at a low concentration (the extreme value tri3 is not considered in these calculations).

Bioaccumulation. One hypothesis to explain such differences in contamination is the bioaccumulation capacity of each taxon. The bioaccumulation of an organism is its capacity of progressive absorption of a substance present in its environment. This capacity can vary from taxon to taxon and even from one individual to another, depending on age, health, or genetic reasons. The different capacities of neonicotinoid absorption of the analysed taxa could explain why the same concentrations are not found in each taxon.

Biomagnification. Another factor of contamination could be variations in diet. The taxon most affected by neonicotinoids in the samples is carnivorous. The next two

most affected taxa are detritivores and the least affected one is algivorous-detritivore/carnivore. This alignment of different diets could indicate a biomagnification phenomenon. Biomagnification refers to the increase in concentrations of certain substances at each stage of the food web; a predator eating its prey also absorbs the toxins that they have accumulated over their lifetime, which gradually accumulate in the predator's fatty tissues. Neonicotinoids have been documented to show biomagnifying properties (BERLIOZ-BARBIER *et al.*, 2014; TENNEKES *et al.*, 2011). Arhynchobdellids exclusively eat living invertebrates, which themselves store various neonicotinoids in varying concentrations. During their digestion, neonicotinoids that were in their prey accumulate in leeches and add to those that the leeches have absorbed environmentally. Ephemeroptera and Amphipoda are detritus feeders and consume organic waste from debris of decaying plants or other invertebrates. Remnants of accumulated neonicotinoids may still be

present in the carcasses of the invertebrates on which they feed. Ephemeroptera and Amphipoda are thus subject to the same biomagnification phenomenon as leeches, but on a smaller scale. Finally, Trichoptera are for the most part algivorous, so they are less susceptible to biomagnification.

Extreme value

The sample analysis shows an extreme imidacloprid concentration of 7.375 ng/g in a Trichoptera sample (tri3), a value almost 10 times higher than the second highest value. Because all samples were analysed simultaneously in the same machine and the concentrations of the other four neonicotinoids in the sample were normal, this value is experimentally valid. It should, however, be taken with caution, as it cannot be repeated for confirmation.

For the discussion of this result, it is important to highlight that the samples analysed consist of pools of, on average, a few dozen invertebrates. Sample tri3 represents a pool of about 76 individuals of the taxon Hydropsychidae Hydropsyche, collected on 16 October 2019. This high concentration is therefore not an isolated case of a single individual but the average of a small population of Hydropsyche, all collected on the same day within a few metres. The other Trichoptera sample obtained on 7 October 2019 had an imidacloprid concentration close to zero. In view of the time lag between the two Trichoptera samples, it is probable that the extreme value in sample tri3 could have been caused by a sudden acute exposure to imidacloprid, for example, by spilling substances from the stream. However, the other taxa analysed on the same day did not show a comparable change in the imidacloprid contamination, and even a regression in the concentrations in the case of Ephemeroptera.

This value could also indicate extreme contamination at the scale of the entire Trichoptera populations. The 76 Trichoptera

in this sample are indicative of at least a portion of the population with a very high imidacloprid concentration. This hypothesis is alarming as it would indicate a much higher population contamination than previously suspected. Neonicotinoid levels in some individuals would exceed many of the threshold concentrations allowed for aquatic ecosystem health. It is also possible that this is the case for the other taxa analysed. Our sampling would then have captured only the less contaminated part of the population (the majority), and the highly contaminated portion would have escaped sampling (except for sample tri3).

Louise Barbe also observed a similar peak imidacloprid concentration when evaluating neonicotinoid contamination of fish in Switzerland, conducted in parallel to this work. There was an imidacloprid concentration of 14.184 ng/g in a sample of chub liver caught in the Jura on 27 August 2019. This peak is just as extreme compared with other fish as the extreme from this study compared with other invertebrates. It must be also noted that Barbe's sampling is much more extensive than the invertebrates, with a few hundred samples. The extreme contamination of this fish is therefore more likely to be an isolated case than in the Trichoptera sample. These irregularities in imidacloprid concentration are observed in two distinct but linked food chain levels, namely algivorous-detritivore invertebrates and fish, a predator. Trichoptera are preyed upon by a variety of fish, and occasionally by the chub tested by Barbe. A biomagnification phenomenon is again possible to explain such a peak in fish. With the biomagnification hypothesis, one can explain the extreme fish sample by speculating that it had, some time before the sample was taken, eaten a population of aquatic invertebrates (possibly Trichoptera), which itself was contaminated due to high exposure to imidacloprid. This also explains why the concentration in fish was higher than the concentration found in insects. These two extreme values in parallel studies indicate that such concentration

peaks are probably observable at all levels of the food web in an aquatic environment.

Impacts on invertebrates

Neonicotinoid concentrations that are too high can have disastrous effects on the contaminated environment (PISA *et al.*, 2017). In general, these effects are measured in relation to environmental concentrations of substances, not in relation to concentrations within organisms as measured in this work. The LC_{50} and half maximal effective concentration (EC_{50}) for specific invertebrates are used for analysis.

Ephemeroptera and Trichoptera are considered to be insect orders that are highly sensitive to neonicotinoids (MORRISSEY *et al.*, 2015), with very low LC_{50} values. In Amphipoda, the Gammaridae family is more resistant, and the impact of neonicotinoids on leeches has been poorly studied. The minimum EC_{50} values of three of the four taxa analysed (Trichoptera, Ephemeroptera, and Gammaridae, all neonicotinoids combined) found in a 2018 study are below the 10 $\mu\text{g/L}$ threshold (RABY *et al.*, 2018). These values are below the neonicotinoid concentration of the Seyon, estimated at 9.79 $\mu\text{g/L}$ by Alex Aebi in preliminary research (AEBI, unpublished data). Moreover, these EC_{50} values were calculated for acute exposures, whereas invertebrates in the Seyon are subject to mostly chronic exposures; it has been shown that chronic exposure to the same substances decreases the LC_{50} and EC_{50} of organisms considerably, with chronic LC_{50} values between 3 and up to 800 times lower than acute LC_{50} values, going from an exposure time of 24 to only 96 hours (VAN DER BRINK *et al.*, 2015). Thus, it is very likely that the average EC_{50} values for Trichoptera, Ephemeroptera, and Gammaridae in the Seyon were reached and their populations were impacted by the presence of neonicotinoids in their environment.

Such contamination in aquatic invertebrates can have disastrous consequences for the whole environment. Detritus-feeding and

algorivorous benthic macroinvertebrates, such as Ephemeroptera, Trichoptera, and Gammaridae, are extremely important players in the recycling of organic matter that is deposited in waterways. This recycling is crucial for the diet of many aquatic organisms, but also to maintain the quality of the water itself (COVICH *et al.*, 1999). Macroinvertebrates also form the basis of the food web in their environment and thus are necessary for the survival of other invertebrates and fish. If the exposure to neonicotinoids is too high, whether acute or chronic, it can reduce the benthic invertebrate population – either by causing the death of individuals or by affecting their reproduction (SÁNCHEZ-BAYO & GOYKA, 2006; HAYASAKA *et al.*, 2012). The gradual disappearance of these populations disrupts the entire food web balance of their environment and lowers the quality of that same environment (SÁNCHEZ-BAYO *et al.*, 2016).

CONCLUSION

This work allowed us to develop and test a method adapted to the analysis of neonicotinoid contamination of a river and the aquatic invertebrates living there. Using these methods, samples were collected and analysed to study the presence and prevalence of neonicotinoids in aquatic invertebrates. By interpreting the results obtained, we were able to produce pilot observations of the situation of the Seyon and of four common taxa of its fauna.

The quality of the results validated the IBCH and HPLC-MS/MS methods used. The adapted methods proved to be efficient, accurate, and adapted to the analysis of aquatic invertebrates. These methods can therefore be used in subsequent research. However, for such methods to be truly valuable, they must be applied to sample a wider range than what we have sampled. A weekly follow-up of a particular site over the long term, or sampling more sites, to analyse the geographical factors that influence the contamination of aquatic invertebrates, could be considered. Finally, the choice of taxa analysed should also be modified: research

should either focus on a single taxon to obtain targeted results or broaden the analyses to as many taxa as possible, to obtain overarching results that would cover the entire invertebrate fauna of the environment. In this way, meaningful and statistically supported hypotheses could be reasonably advanced.

All samples analysed showed contamination with at least one neonicotinoid, demonstrating the ubiquity of these substances in the researched environment. Four of the five neonicotinoids analysed were represented in the samples. Two of these four substances have been banned from use since early 2019 (imidacloprid and clothianidin). The time lag between this ban and the sampling illustrates the persistence of these substances in the Seyon as well as in the organisms living there. The concentrations obtained ranged from 0.0275 to 0.806 ng/g, which indicate chronic exposure to neonicotinoids. Chronic exposure is still scarcely considered in pesticide toxicity research, even though this is the most common type of exposure in our environment. In addition to these low concentrations, an extreme imidacloprid concentration of 7.375 ng/g was found in sample tri3. A particularly worrisome hypothesis could be that this extreme value is a proxy for extreme contamination of entire Trichoptera populations. Evidence of extreme neonicotinoid contamination in other populations of the ecosystem, such as Barbe's fish, suggest a systemic-scale phenomenon. To examine this further, combined studies on all different levels of the ecosystem would be appropriate: it would give a complete overview of the situation and would allow links to be made between the data found. This is in part studied at the University of Neuchâtel, where studies around neonicotinoids are carried out for various wildlife, such as bees (MITCHELL *et al.*, 2017), ants (SCHLÄPPI, 2020), fishes (BARBE, unpublished), and birds (HUMANN-GUILLEMINOT *et al.*, 2019).

The aquatic environment and its fauna constitute a compartment of our ecosystem that has been considered little by the scientific studies to

date but deserves greater attention. The results obtained with my limited sampling confirm a need for further research on the subject, the result of which would be interesting to discuss and mobilise in future assessments of the health of our streams. In general, studies assessing invertebrate contamination in their natural environments and not in laboratories are sorely lacking. These studies, representative of the actual state of the environment, are crucial for the regulation of neonicotinoid use. A greater diversity of this kind of research would allow us to assemble a coherent and exhaustive inventory of the presence of neonicotinoids around us.

Finally, all research about neonicotinoids allows us to question our use of these substances. In Switzerland, it is a debate at the heart of public attention, with proposals for radical measures on the issue, such as the initiative 'For a Switzerland free of synthetic pesticides' launched two years ago, on 24 April 2019, which aims to ban the use of all synthetic pesticides in Switzerland.

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BIBLIOGRAPHY

- BASS, C., DENHOLM, I., WILLIAMSON, M. S. & NAUEN, R. 2015. The global status of insect resistance to neonicotinoid insecticides. *Pesticide Biochemistry and Physiology* 121: 78–87. <https://doi.org/10.1016/j.pestbp.2015.04.004>
- BERLIOZ-BARBIER, A., VAUCHEZ, A., WIEST, L., BAUDOT, R., VUILLET, E. & CREN-OLIVÉ, C. 2014. Multi-residue analysis of emerging pollutants in sediment using QuEChERS-based extraction followed by LC-MS/MS analysis. *Analytical and Bioanalytical Chemistry* 406: 1259–1266. <https://dx.doi.org/10.1007/s00216-013-7450-8>
- BONMATIN, J.-M., NOOME, D. A., MORENO, H., MITCHELL, E. A. D., GLAUSER, G., SOUMANA, O. S., BIJLEVELD VAN LEXMOND, M. & SÁNCHEZ-BAYO, F. 2019. A survey and risk assessment of neonicotinoids in water, soil and sediments of Belize. *Environmental Pollution* 249: 949–958. <https://doi.org/10.1016/j.envpol.2019.03.099>
- BORSUAH, J. F., MESSER, T. L., SNOW, D. D., COMFORT, S. D. & MITTELSTET, A. R. 2020. Literature review: Global neonicotinoid insecticide occurrence in aquatic environments. *Water* 12(12): 3388. <https://doi.org/10.3390/w12123388>
- CALATAYUD-VERNICH, P., CALATAYUD, F., SIMÓ, E., SUAREZ-VARELA, M. M. & PICÓ, Y. 2016. Influence of pesticide use in fruit orchards during blooming on honeybee mortality in 4 experimental apiaries. *The Science of the Total Environment* 541: 33–41. <https://doi.org/10.1016/j.scitotenv.2015.08.131>
- CARSON, R. 1962. *Silent spring*. Houghton Mifflin. Boston.
- CHARPENTIER, G., LOUAT, F., BONMATIN, J.-M., MARCHAND, P. A., VANIER, F., LOCKER, D. & DECOVILLE, M. 2014. Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model *Drosophila melanogaster*. *Environmental Science & Technology* 48(7): 4096–4102. <https://doi.org/10.1021/es405331c>
- CIMINO, A. M., BOYLES, A. L., THAYER, K. A. & PERRY, M. J. 2017. Effects of neonicotinoid pesticide exposure on human health: A systematic review. *Environmental Health Perspectives* 125(2): 155–162. <https://doi.org/10.1289/EHP515>
- COVICH, A. P., PALMER, M. A. & CROWL, T. A. 1999. The role of benthic invertebrate species in freshwater ecosystems: Zoobenthic species influence energy flows and nutrient cycling. *BioScience* 49(2): 119–127. <https://doi.org/10.2307/1313537>
- EUROPEAN FOOD SAFETY AUTHORITY. 2015. The 2013 European Union report on pesticide residues in food. *EFSA Journal* 13(3): 4038. <https://doi.org/10.2903/j.efsa.2015.4038>
- GIROLAMI, V., MAZZON, L., SQUARTINI, A., MORI, N., MARZARO, M., DI BERNARDO, A., GREATTI, M., GIORIO, C. & TAPPARO, A. 2009. Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: A novel way of intoxication for bees. *Journal of Economic Entomology* 102(5): 1808–1815. <https://doi.org/10.1603/029.102.0511>
- GOULSON, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology* 50(4): 977–987. <https://doi.org/10.1111/1365-2664.12111>
- GUZSVÁNY, V., CSANÁDI, J. & GAÁL, F. 2006. NMR study of the influence of pH on the persistence of some neonicotinoids in water. *Acta Chimica Slovenica* 53: 52–57.
- HAYASAKA, D., KORENAGA, T., SUZUKI, K., SAITO, F., SÁNCHEZ-BAYO, F. & GOKA, K. 2012. Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicology and Environmental Safety* 80: 355–362. <https://doi.org/10.1016/j.ecoenv.2012.04.004>
- HENRY, M., BÉGUIN, M., REQUIER, F., ROLLIN, O., ODOUX, J.-F., AUPINEL, P., APTEL, J., TCHAMITCHIAN, S. & DECOURTYE, A. 2012. A common pesticide decreases foraging

- success and survival in honey bees. *Science* 336(6079): 348–350. <https://doi.org/10.1126/science.1215039>
- HUMANN-GUILLEMINOT, S., CLÉMENT, S., DESPRAT, J., BINKOWSKI, Ł. J., GLAUSER, G. & HELFENSTEIN, F. 2019. A large-scale survey of house sparrows feathers reveals ubiquitous presence of neonicotinoids in farmlands. *The Science of the Total Environment* 660: 1091–1097. <https://doi.org/10.1016/j.scitotenv.2019.01.068>
- KAMMOUN, S., MULHAUSER, B., AEBI, A., MITCHELL, E. A. D. & GLAUSER, G. 2019. Ultra-trace level determination of honey as a tool for assessing environmental contamination. *Environnemental Pollution* 247: 964–972. <https://doi.org/10.1016/j.envpol.2019.02.004>
- KANRAR, B., GHOSH, T., PRAMANIK, S. K., DUTTA, S., BHATTACHARYYA, A. & DHURI, A. V. 2006. Degradation dynamics and persistence of imidacloprid in a rice ecosystem under West Bengal climatic conditions. *Bulletin of Environmental Contamination and Toxicology* 77(5): 631–637. <https://doi.org/10.1007/s00128-006-1109-5>
- KREUGER, J. & GRAAF, S. 2010. *Pesticides in surface water in areas with open ground and greenhouse horticultural crops in Sweden 2008*. Swedish University of Agricultural Sciences. Uppsala. <https://pub.epsilon.slu.se/5413/>
- LUKANCIC, S., ZIBRAT, U., MEZEK, T., JEREBIC, A., SIMCIC, T. & BRANCELJ, A. 2010. Effects of exposing two non-target crustacean species, *Asellus aquaticus* L., and *Gammarus fossarum* Koch., to atrazine and imidacloprid. *Bulletin of Environmental Contamination and Toxicology* 84(1): 85–90. <https://doi.org/10.1007/s00128-009-9854-x>
- MALONEY, E. M., MORRISSEY, C. A., HEADLEY, J. V., PERU, K. M. & LIBER, K. 2017. Cumulative toxicity of neonicotinoid insecticide mixtures to *Chironomus dilutus* under acute exposure scenarios. *Environmental Toxicology and Chemistry* 36(11): 3091–3101. <https://doi.org/10.1002/etc.3878>
- MCAFEE, A. 2017. A brief history of pesticides. *American Bee Journal* 157: 781–783.
- MITCHELL, E. A. D., MULHAUSER, B., MULOT, M., MUTABAZI, A., GLAUSER, G. & AEBI, A. 2017. A worldwide survey of neonicotinoids in honey. *Science* 358(6359): 109–111. <https://doi.org/10.1126/science.aan3684>
- MORRISSEY, C. A., MINEAU, P., DEVRIES, J. H., SANCHEZ-BAYO, F., LIESS, M., CAVALLARO, M. C. & LIBER, K. 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environnement International* 74: 291–303. <https://doi.org/10.1016/j.envint.2014.10.024>
- PEÑA, A., RODRÍGUEZ-LIÉBANA, J. A., & MINGORANCE, M. D. 2011. Persistence of two neonicotinoid insecticides in wastewater, and in aqueous solutions of surfactants and dissolved organic matter. *Chemosphere*, 84(4), 464–470. <https://doi.org/10.1016/j.chemosphere.2011.03.039>
- PISA, L., GOULSON, D., YANG, E.-C., GIBBONS, D., SÁNCHEZ-BAYO, F., MITCHELL, E., AEBI, A., VAN DER SLUIJS, J., MACQUARRIE, C. J. K., GIORIO, C., LONG, E. Y., MCFIELD, M., BIJLEVELD VAN LEXMOND, M. & BONMATIN, J.-M. 2021. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: Impacts on organisms and ecosystems. *Environmental Science and Pollution Research International* 28(10): 11749–11797. <https://doi.org/10.1007/s11356-017-0341-3>
- RABY, M., NOWIERSKI, M., PERLOV, D., ZHAO, X., HAO, C., POIRIER, D. G. & SIBLEY, P. K. 2018. Acute toxicity of 6 neonicotinoid insecticides to freshwater invertebrates. *Environmental Toxicology and Chemistry* 37(5): 1430–1445. <https://doi.org/10.1002/etc.4088>
- SANCHEZ-BAYO, F. & GOKA, K. 2014. Pesticide residues and bees - A risk assessment. *PLoS One* 9(4): e94482. <https://doi.org/10.1371/journal.pone.0094482>

- SCHLÄPPI, D., KETTLER, N., STRAUB, L., GLAUSER, G. & NEUMANN, P. 2020. Long-term effects of neonicotinoid insecticides on ants. *Communications Biology* 3(1): 335. <https://doi.org/10.1038/s42003-020-1066-2>
- SHAHID, N., BECKER, J. M., KRAUSS, M., BRACK, W. & LIESS, M. 2018. Pesticide body burden of the crustacean *Gammarus pulex* as a measure of toxic pressure in agricultural streams. *Environmental Science & Technology* 52(14): 7823–7832. <https://doi.org/10.1021/acs.est.8b01751>
- SIMON-DELISO, N., AMARAL-ROGERS, V., BELZUNCES, L. P., BONMATIN, J. M., CHAGNON, M., DOWNS, C., FURLAN, L., GIBBONS, D. W., GIORIO, C., GIROLAMI, V., GOULSON, D., KREUTZWEISER, D. P., KRUPKE, C. H., LIESS, M., LONG, E., MCFIELD, M., MINEAU, P., MITCHELL, E. A. D., MORRISSEY, C. A., ... WIEMERS, M. 2015. Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research International* 22(1): 5–34. <https://doi.org/10.1007/s11356-014-3470-y>
- SUR, R. & STORK, A. 2003. Uptake, translocation and metabolism of imidacloprid in plants. *Bulletin of Insectology* 56: 35–40.
- TENNEKES, H. A. & SANCHEZ-BAYO, F. P. 2011. Time-dependent toxicity of neonicotinoids and other toxicants: Implications for a new approach to risk assessment. *Journal of Environmental & Analytical Toxicology* 01(S4). <https://doi.org/10.4172/2161-0525.S4-001>
- TOMIZAWA, M. & CASIDA, J. E. 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action. *Annual Review of Pharmacology and Toxicology* 45: 247–268. <https://doi.org/10.1146/annurev.pharmtox.45.120403.095930>
- TOMIZAWA, M. & CASIDA, J. E. 2011. Neonicotinoid insecticides: Highlights of a symposium on strategic molecular designs. *Journal of Agricultural and Food Chemistry* 59(7): 2883–2886. <https://doi.org/10.1021/jf103856c>
- VAN DEN BRINK, P. J., VAN SMEDEN, J. M., BEKELE, R. S., DIERICK, W., DE GELDER, D. M., NOTEBOOM, M. & ROESSINK, I. 2016. Acute and chronic toxicity of neonicotinoids to nymphs of a mayfly species and some notes on seasonal differences. *Environmental Toxicology and Chemistry* 35(1): 128–133. <https://doi.org/10.1002/etc.3152>

WEBOGRAPHY

- BAYER CROPSCIENCE AG. n.d. *Synergistic insecticide mixtures patent*. Retrieved February 3, 2020, from <https://patents.google.com/patent/US20090215760A1/en>
- OFFICE FÉDÉRAL DE L'AGRICULTURE. 2018. *Décision de portée générale sur l'interdiction d'utiliser certains produits phytosanitaires (4924)*. Retrieved February 25, 2020, from <https://www.admin.ch/opc/fr/federal-gazette/2018/4924.pdf>
- OFFICE FÉDÉRAL DE L'ENVIRONNEMENT. 2019. *Méthodes d'analyse et d'appréciation des cours d'eau en Suisse: Macrozoobenthos*. <https://www.bafu.admin.ch/bafu/fr/home/themen/thema-wasser/wasser--publikationen/publikationen-wasser/methoden-zur-untersuchung-und-beurteilung-der-fliessgewaesser-makrozoobenthos-stufe-f.html>
- OFFICE FEDERAL DE TOPOGRAPHIE SWISSTOPO. n.d. *Map of Switzerland*. Retrieved November 1, 2021, from map.geo.admin.ch
- PERLA. n.d. *Homepage*. Retrieved March 13, 2020, from <http://www.perla.developpement-durable.gouv.fr/index.php>