Use of periphyton and macroinvertebrates to assess pesticide contamination in agricultural streams

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Abstract

Streams in agricultural areas are susceptible to contamination by various compounds, including pesticides. Although spot water sampling is traditionally used for monitoring pesticide contamination of surface waters, this method may underestimate the presence and bioavailability of certain compounds. To better assess environmental exposure, this study investigates the potential of epilithic biofilm (periphyton) as a biomonitor of pesticide contamination. Biofilms were sampled from several streams in the province of Quebec (East Canada) subjected to "moderate" and "intense" agricultural pressure to determine pesticide accumulation of selected herbicides (atrazine, S-metolachlor, imazethapyr) and insecticides (chlorantraniliprole, clothianidin, thiamethoxam, chlorpyrifos) using an optimized QuEChERS extraction method. Benthic macroinvertebrates were also sampled at certain sites to assess their potential as indicators of pesticide exposure using the same approach as biofilms. High detection frequencies and elevated concentrations of atrazine, S-metolachlor, chlorantraniliprole and chlorpyrifos were observed in periphyton from areas under intense agricultural pressure. Pesticides were more frequently detected in water than in periphyton, except for chlorpyrifos. Relationships between concentrations in water and in periphyton followed a linear model for atrazine, S-metolachlor and chlorantraniliprole. This study highlights the potential of periphytic biofilms as complementary tools for pesticide monitoring, although further laboratory and *in situ* validation is needed. Macroinvertebrates also accumulated pesticides under intense agricultural pressure, particularly the insecticide chlorantraniliprole, raising concerns about potential risks to invertebrate communities. These findings illustrate the affinity of pesticides for different matrices (water versus periphyton versus invertebrates) and the need for monitoring programs not to rely solely on spot water measurements.

Keywords : Biomonitoring, contamination, macroinvertebrates, periphyton, pesticides

1. Introduction

Canada ranks as the eighth largest user of pesticides globally, consuming approximately 132,000 tonnes per year (Pest Management Regulatory Agency, Health Canada, 2023). Farmlands in Quebec (Canada) cover 5% of the province's total area (6.3 million hectares) with a majority of corn and soybean crops (Giroux, 2022; Massicotte et al., 2022). Agricultural activities are mainly concentrated in the most southern area of the province. Vegetable field crops and orchards are also present in this region. These agricultural productions rely on the extensive use of pesticides. In 2021, the total sales of pesticides in the province of Quebec were estimated at 3,600 tonnes of active ingredients, with herbicides accounting for 67 % of this total and insecticides for 8% (Gouvernement du Québec, 2023). For comparison, based on data from 2021, an average of 2.1 kg of pesticides are sold per hectare of farmland in Canada each year, compared to an average of 0.57 kg per hectare in Quebec.

To enhance productivity, agricultural activities involve the application of fertilizers and pesticides. These compounds may be transported from the terrestrial compartment to adjacent aquatic ecosystems, notably via leaching and surface runoff (Steffens et al., 2022), where they can exert adverse effects on the biota (Schürings et al., 2022). Pesticide contamination of surface waters and its potential threat to ecosystems led several organizations such as the Quebec government (Ministère de l'Environnement, de la Lutte contre les Changements Climatiques, de la Faune et des Parcs; MELCCFP) to monitor pesticide concentrations in agricultural streams. Indeed, the province of Quebec launched the "pesticide network" in 1992 where ten streams were selected for pesticide monitoring on a regular basis. Since 2012, a few additional streams are also monitored on a rotating basis. Certain pesticides such as the herbicide S-metolachlor, and the insecticides chlorantraniliprole, clothianidin and thiamethoxam, are frequently detected in Quebec streams (nearly 100% of the samples from certain streams, Giroux, 2022; MELCCFP, 2025a). The above-mentioned pesticides were reported with concentrations reaching up to 21 $\mu g \cdot L^{-1}$ for S-metolachlor, 0.94 $\mu g \cdot L^{-1}$ for chlorantraniliprole, 0.52 $\mu g \cdot L^{-1}$ for clothianidin, and 4.5 $\mu g \cdot L^{-1}$ for thiamethoxam during the 2015-2020 period. Concentrations measured in certain samples even exceed the province's criteria (chronic exposure) for the protection of aquatic life (CVAC, MELCCFP, 2019). For example, in 2020, clothianidin and thiamethoxam showed CVAC exceedance frequencies of 39% and 53%, respectively, in four watercourses included in the pesticide network (Giroux, 2022; MELCCFP, 2025b).

Traditionally, pesticide monitoring is based on spot water sampling. However, this method may not accurately account for the temporal variability in pesticide concentrations (Struger et al., 2017). Spot water sampling may therefore miss the presence of pesticides and thus lead to an underestimation of contamination. In order to accurately account for temporal variability, several water samples must thus be collected at a site over the year. In addition to being costly and time-consuming, this multi-sample approach does not provide information on the potential bioavailability of pesticides. To circumvent the limitations of spot sampling, passive samplers such as Polar Organic Chemical Integrative Samplers (POCIS) have been deployed *in situ* in several countries to monitor pesticide contamination over time in watercourses, including the American Midwest (Van Metre et al., 2017), Ontario in Canada (Metcalfe et al., 2019) and on a national scale in France (Mathon et al., 2022).

Periphytic biofilm (periphyton), a consortium of organisms composed of algae, bacteria, fungi and micro-meiofauna embedded in a three-dimensional matrix of extracellular polymeric substances (Flemming and Wingender, 2010; Wetzel, 1983), accumulate a wide range of inorganic contaminants (Bradac et al., 2010; Laderriere et al., 2021; Leguay et al., 2016) and organic contaminants such as pharmaceutical compounds (Fernandes et al., 2020) and pesticides (Izma et al., 2024b; Rheinheimer Dos Santos et al., 2020). Rheinheimer Dos Santos et al. (2023) demonstrated that biofilm sampling can offer an effective alternative for the detection of compounds that are sometimes not detected using POCIS. The accumulation of organic contaminants in biofilm is partly dependent on their physico-chemical properties, such as the octanol water partitioning coefficient (log K_{ow}) (Bonnineau et al., 2020). Izma et al. (2024b) showed that the log of soil adsorption coefficient (log K_{oc}), representing the adsorption of pesticides onto organic matter, is a strong predictor for the distribution and the affinity of pesticides in the biofilm compared to water. However, the relationship between pesticide concentrations in water and in biofilm is complex and not yet fully explored.

The periphyton is at the base of aquatic and terrestrial food webs and represents an essential dietary resource for meiofauna and macroinvertebrates that are primary consumers (Battin et al., 2016; Ijzerman et al., 2023; Rhea et al., 2006). The accumulation of pesticides in the periphyton can thus be a potential exposure pathway for organisms that feed on this key resource. Benthic macroinvertebrates, which are primary consumers of periphyton in streams, occupy a pivotal position at the interface between the water column, sediments and the biofilm. These organisms play an important role in the aquatic food web and mediate the transfer of energy (Yan et al., 2024) and contaminants between aquatic and terrestrial ecosystems (Raikow et al., 2011). Furthermore, they are extensively used as sensitive indicators of ecological conditions in freshwater environments (Huang and Gergel, 2023). Owing to their trophic position, macroinvertebrates may be exposed to pesticides via the water column as well as via their diet. Along this trophic pathway, the effects of pesticides may arise through direct toxicity or indirectly through alterations in periphyton biomass or nutritional quality, which can subsequently influence macroinvertebrate growth and survival (Ijzerman et al., 2023; Izma et al., 2024a). Despite their ecological importance, very few studies have quantified pesticide occurrence in macroinvertebrates under natural field conditions.

In this field study, the accumulation of several pesticides was investigated in biofilm and macroinvertebrate samples from agricultural streams. The herbicide atrazine and the insecticides chlorpyrifos, clothianidin and thiamethoxam were selected because of their frequent detection in agricultural streams of Quebec over several years and because the concentrations of some of these pesticides exceeded the criteria for the protection of aquatic life. S-metolachlor, chlorantraniliprole and imazethapyr were also investigated in this study for their heavy use in corn/soybean crops and orchards, as well as for their frequent detection in surface waters of the study area (Giroux, 2022, 2017). The objectives of this study were (1) to characterize the levels of pesticide contamination in periphytic biofilms from streams under contrasting agricultural pressure, hypothesizing that contamination is higher in streams with intense agricultural activity and higher in the biofilm than in water; (2) to assess whether the log K_{ow} and log K_{oc} of pesticides influence their bioaccumulation, and (3) to measure pesticide concentrations in benthic macroinvertebrates and to evaluate their accumulation patterns compared to biofilms. In addition, (4) we explored the relationship between pesticide concentrations in water and biofilms. Based on the literature, we hypothesized that bioaccumulation may follow a non-linear relationship. This study is one of the few studies investigating the use of periphytic biofilm as a biomonitor of pesticide exposure using data collected from the natural environment, and to attempt to model relationships between pesticide concentrations in water and in biofilm.

2. Materials and methods

2.1. Sampling sites

Samples were collected at 25 sites in the province of Quebec (Canada) (Figure 1). Fourteen sites, located in the regions of Montérégie, Centre-du-Québec and the southern portion of the Laurentides, were included in the “pesticide network” managed by the provincial government

(MELCCFP). Agricultural activities are widespread in Montérégie and in the northern portion of Centre-du-Québec (see Table S. 1). The Laurentides region is predominantly forested, but the sampling sites were located in the southern part of the region, where vegetable farms, orchards and corn/soybean crops occupy a substantial area (Giroux, 2022, 2017). We therefore considered these sites to be subjected to "intense" agricultural pressure. Biofilm was also sampled at 11 sites in the regions of Centre-du-Québec, Capitale-Nationale and Chaudière-Appalaches, where agricultural pressure is also present, but to a lesser extent (Minville, 2007) (Table S. 2). These agricultural sites were therefore categorized under "moderate" agricultural pressure. This study was conducted over five consecutive summers from 2019 to 2023. For the years 2019 and 2020, a single sampling campaign was conducted in early August. For 2021, 2022 and 2023, most sites were sampled on a bimonthly basis, in June, July and August. These periods were selected as they correspond to agricultural application seasons, which are associated with a higher potential for pesticide transfer to aquatic environments through runoff. Sampling was carried out after a few days of dry weather to avoid flood events, which may impair biofilm and macroinvertebrate collection.

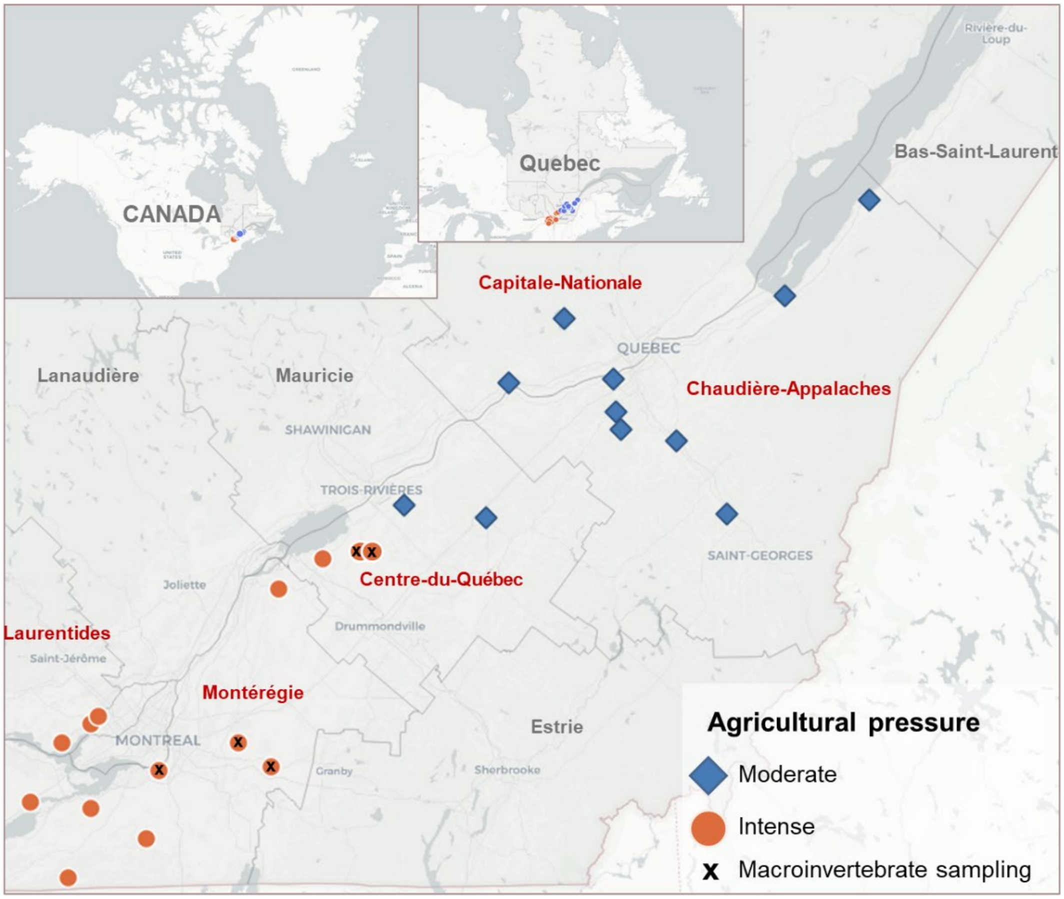

Figure 1: Study area and location of sampling sites in the province of Quebec (Canada). Orange circles correspond to sites included in the Quebec government "pesticide network" and were considered in this study to be under "intense" agricultural pressure. Blue diamonds indicate sites situated in areas where agricultural pressure is "moderate". Circles marked with "x" are sites where macroinvertebrates were sampled. This map was generated using QGIS 3.40.2.

2.2. Water physico-chemistry

For each sampling event from 2021 to 2023, pH, conductivity and water temperature were recorded *in situ* (Thermo Scientific™ Orion™ Star A325, ROSS Ultra pH/TAC triode and Orion™ Duraprobe™ 4-cell conductivity probe). Concentrations of pesticides, ammonia, nitrites/nitrates and total phosphorus in water were provided by the MELCCFP via the Water Atlas (https://www.environnement.gouv.qc.ca/eau/atlas/index.htm) for all sampling campaigns conducted between 2019 and 2023. Data from a limited number of pesticides were included in this study: atrazine, S-metolachlor, imazethapyr, chlorantraniliprole, clothianidin, thiamethoxam and chlorpyrifos. These compounds were selected mainly based on their status as pesticides of interest monitored by the Quebec government, and because their concentrations had already been observed to exceed water quality guidelines.

2.3. Biofilm sampling

From 2019 to 2023, periphytic biofilm was collected in summer, from 10 to 20 submerged rocky substrates (cobbles and pebbles), at depths ranging from 5 to 20 cm. Substrates were randomly selected from a riffle/run and unshaded section of the stream. Substrates were gently removed from the water and the periphyton was collected using a toothbrush. A clean toothbrush was used at each site and for each sampling event. Toothbrushes were cleaned with detergent and hot water, rinsed with demineralized water, then rinsed and soaked in 70% ethanol overnight, and finally rinsed with Milli-Q ultra-pure water and dried in a fume hood. The collected periphyton was suspended in 30 ml of site water in a single 50 mL polypropylene tube to form one composite sample per site and sampling date (Lavoie et al., 2008). The composite sample was then manually homogenized on site, was divided to fill glass vials that were immediately frozen on dry ice. Once at the laboratory, the samples were stored at -80 °C before being freeze-dried. A total of 107 composite periphyton samples were generated across all sites and sampling dates/years.

2.4. Macroinvertebrates sampling

Benthic macroinvertebrates were sampled during the field campaigns of 2021, 2022 and 2023 (see Figure 1). A targeted sampling of the macroinvertebrate groups Trichoptera, Chironomidae, Ephemeroptera, and Gammaridae was conducted by hand-picking using forceps (Evans-White et al., 2001) previously rinsed with 70% ethanol and Milli-Q ultra-pure water. Although hand-picking is not suitable for assessing macroinvertebrate community structure, it is highly effective for targeting specific rock-dwelling taxa (Yanai and Dorchin, 2025) and for collecting sufficient biomass for pesticide accumulation analyses, which was the primary objective of this study. At each sampling date, individuals were pooled according to their taxonomic group, rinsed three times using site water and then frozen on dry ice in glass vials. Invertebrate samples were then stored at -80 °C before being freeze-dried. Due to the low biomass of samples at each sampling event, individuals from the same macroinvertebrate group were pooled per site to obtain one sample per year.

2.5. Pesticide extraction and quantification

Freeze-dried periphyton and macroinvertebrate samples were ground into a homogeneous powder using a spatula (previously rinsed with 70% ethanol). For each composite sample collected in the field, three technical replicates were performed to account for variability in the periphyton matrix and macroinvertebrate samples. The pesticide extraction method for the periphyton was modified and adapted from the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) presented in Liu et al. (2019). In brief, 50 mg of freeze-dried periphyton was weighed into 1.5 mL microtubes (Sarstedt), followed by the addition of 300 µL of MiliQ ultra-pure water and 10 µL of a recovery standard (100 $\mu g \cdot L^{-1}$, metolachlor-D6 50:50 ultra-pure water/acetonitrile). After one hour at 4 °C, 500 µL of acetonitrile were added. Then, 50 mg of NaCl (Fisher Chemical™) were added to control the polarity, which enhances the partitioning of the analytes into the organic phase and improves their overall recovery. The samples were vortexed for 5 sec and then placed in an ultrasonic bath for 30 min at room temperature, with the bath temperature not exceeding 25 °C to prevent thermal degradation of the pesticides. Subsequently, 100 mg of anhydrous $MgSO_4$ (Fisher Chemical™) were added to enhance the ionic strength and the migration of pesticides into the acetonitrile organic phase. The samples were vortexed for 5 sec and centrifuged at 6000 rpm for 5 min at room temperature, and 450 µL of the top fraction were recovered and transferred into a clean Eppendorf tube. Then, 10 mg of silica gel (SiliaFlash® G60, 60-200 µm, 60 Å, SiliCycle) were added for clean-up. This was followed by vortexing for 5 sec and centrifuging at 6000 rpm for 5 min at room temperature. Finally, 150 µL of the supernatant were transferred into a 2 mL amber glass vial (Calibre Scientific) and evaporated to dryness under nitrogen flow at 25 °C. The residue was then reconstituted into 150 µL of the internal standard Atrazine-D5/Thiamethoxame-D3 (10 $\mu g \cdot L^{-1}$, 50:50 ultra-pure water/acetonitrile) and transferred into screw-top vials for analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS; QTRAP® 5500 ; AB SCIEX).

Internal standard atrazine-D5 was used for atrazine, S-metolachlor, clothianidin, imazethapyr and chlorpyrifos quantification, whereas thiamethoxam-D3 was used for the quantification of thiamethoxam and chlorantraniliprole. Metolachlor-D6 was used as an extraction standard to evaluate the recovery rate of pesticides in complex environmental samples. During each extraction session, controls were added to samples: three procedural blanks with only solvents, one matrix control (non-contaminated reference periphyton) and one spiked matrix control (non-contaminated reference periphyton + 10 µL of a 100 $\mu g \cdot L^{-1}$ mix solution of pesticides). Reference periphyton refers to periphyton collected from a site with little anthropogenic impact and cultivated in the laboratory for a few months then freeze-dried and analyzed to ensure the absence of pesticides. Details on the extraction method validation are documented in supplementary material

S. 2. In general, the recovery rates of pesticides as well as extraction standard metolachlor-D6 ranged from 60% to 130%.

2.6. Bioconcentration factors

We calculated bioconcentration factors (BCFs) for the different pesticides at each site. These BCFs were calculated only when concentrations in water and in periphyton samples were above detection limits. They were calculated as described in Arnot & Gobas (2006) and in Beecraft & Rooney (2021) where:

BCF= (ppb pesticides in periphyton) / (ppb pesticides in surface water)

With ppb pesticides in periphyton in $\mu g \cdot kg^{-1}$ and ppb pesticides in surface water in $\mu g \cdot L^{-1}$.

2.7. Data analyses

Data analyses were performed in R/Rstudio (v4.3.3, R Core Team (2024)). Boxplots of environmental variables between the two categories of sites were produced using *ggplot2* (Wickham (2016), R version: 3.5.3) and the non-parametric Wilcoxon-Mann-Whitney test was used to assess differences in water physico-chemistry between sites belonging to the two levels of agricultural pressure.

Given the heterogeneity of the periphyton matrix, the pesticide concentration value for each sample corresponds to the average concentration calculated from the value of three extraction replicates (pseudoreplicates). The detection frequencies in the water and in the periphyton were calculated for each of the two agricultural pressure categories using data from all years and all sites. This approach allowed for concentration comparisons between sites with moderate and intense agricultural pressure. Differences in pesticide detection frequency between both agricultural pressures for each pesticide in periphyton samples were investigated using the non-parametric Wilcoxon-Mann-Whitney test. Spearman's correlation coefficient (rho) was determined using the "cor.test" function to investigate if there were significant correlations (ranks) between pesticide detection in water and in periphyton samples as a function of log K_{ow} and log K_{oc}. Values of log K_{ow} and log K_{oc} were obtained from the National Center for Biotechnology Information (2025).

The relationship between pesticide concentrations in water and in periphyton samples was investigated only for sites with "intense" agricultural pressure (orange circles, Figure 1) because pesticide concentrations in water were not available for the sites from "moderate" agricultural pressure (not included in the government pesticide monitoring program). The relationship was modelled by selecting only those measurements where concentrations were above the limit of detection (LOD) in both matrices. To model robust relationships between pesticide concentrations in water and in periphyton, we focused on compounds that were frequently detected (atrazine, S-metolachlor and chlorantraniliprole). Linear models imply that pesticide accumulation in the periphyton is directly proportional to water concentrations. However, various processes may lead to non-linear accumulation patterns (Chaumet et al., 2019), such as binding site saturation at high concentration. We therefore explored linear relationships as well as generalized additive models (GAMs) using log10 transformed data. Linear relationships were modelled using the function: *log_{10} concentration in periphyton ~ log_{10} concentration in water* and generalized additive models (GAM) were modelled using the function: *log_{10} concentration in periphyton ~ s(log_{10} concentration in water), family = Gamma (link = "identity")*. The s() function was used to "smooth" the relationship, thereby rendering it more flexible and allowing for capturing potential non-linear relationships without any preconceived notions regarding the nature of such relationships. The Akaike information criterion (AIC) (Akaike, 1974) was used to compare the relative adjustment quality of linear and generalized additive models.

3. Results and discussion

3.1. Physico-chemical conditions

The sites from the two agricultural pressure categories exhibit distinct physical and chemical characteristics (Figure S. 1). In particular, total nitrogen concentrations were more than three times higher at "intense pressure" sites (3.09 ± 1.49 mg·L^{-1} *versus* 0.95 ± 0.70 mg·L^{-1}; Wilcoxon test, p-value < 0.001) and total phosphorus concentrations were more than six times higher (0.25 ± 0.29 mg·L^{-1} *versus* 0.04 ± 0.03 mg·L^{-1}, Wilcoxon test, p-value < 0.001) than at "moderate pressure" sites. The use of fertilizers in agriculture is recognized as a major source of nitrogen and phosphorus in streams, altering the chemical parameters and the ecological functioning of aquatic ecosystems (Chambers et al., 2008; Smith, 2003). Conductivity was also substantially elevated under intense agricultural pressure compared to moderate agricultural pressure (830 ± 288 µS·cm^{-2} *versus* 282 ± 162 µS·cm^{-2}; Wilcoxon test, $p < 0.001$). Additionally, pH values were slightly but significantly higher at sites under intense agricultural pressure (8.4 ± 0.4 *versus* 8.0 ± 0.6; $p < 0.05$). These findings are consistent with Atwell and Bouldin (2022) who showed that increases in pH and conductivity with agricultural intensity are likely partly driven by underlying geological features influencing the establishment of intensive agriculture in these regions. Indeed, agricultural activities are well developed in the Southern Quebec (Montérégie) region, which is characterized by marine deposit soils considered well suited to agriculture (Lajoie, 1975). Elevated conductivity may also come from salts used in agricultural (Kaushal et al., 2018) and photosynthesis (high periphytic biomass) may also increase pH (Francoeur et al., 2006).

3.2. Detection frequencies in water and in periphyton

Pesticide detection between moderate and intense agricultural pressure

The selected pesticides were above detection limits in the water and in the periphyton at least once across our sites and our sampling periods. Certain pesticides were detected in periphyton samples from sites with "moderate" agricultural pressure (Table 1), which is not surprising as agricultural activities are present in the watershed. In particular, chlorpyrifos and thiamethoxam were detected in 5% of periphyton samples collected at sites under "moderate" pressure. The presence of these pesticides may be related to their use in forestry and agriculture, two activities taking place in proximity to the sampling sites.

Table 1: Minimum, maximum and median concentrations (ppb) and frequencies of pesticide detection (%) in periphyton and water samples when measured concentrations were above the limit of detection (LOD, matrix specific). Concentrations of pesticides in the water are only available from sites under "intense" agricultural pressure included in the "pesticide network" of the Quebec ministry of the environment. Values in bold font with an asterisk correspond to a significant difference in pesticide detection between intense and moderate agricultural pressure. Statistics are outlined in Table 2.

		Periphyton (n = 107)					Water (n = 107)				
Agricultural pressure	Pesticides	LOD	Min	Max	Median	Detection (%)	LOD	Min	Max	Median	Detection (%)
Intense	Atrazine	7.1	7.3	220	9.4	**10***	0.010	0.010	2.40	0.024	73
	S-metolachlor	5.2	5.2	370	11	**53***	0.010	0.010	8.20	0.180	81
	Imazethapyr	6.4	8.2	8.2	8.2	1	0.007	0.008	0.270	0.029	65
	Chlorantraniliprole	2.9	3.2	131	6.9	**41***	0.002	0.002	2.200	0.034	92
	Chlorpyrifos	3.2	3.4	16	4.6	14	0.010	0.018	0.020	0.018	1
	Clothianidin	4.2	7.5	8.6	8.1	2	0.005	0.005	0.06	0.010	34
	Thiamethoxam	3.4	4.1	7.8	5.1	4	0.002	0.002	0.300	0.017	36

		Periphyton (n=22)					Water (n=0)				
Agricultural pressure	Pesticides	LOD	Min	Max	Median	Detection (%)	LOD	Min	Max	Median	Detection (%)
Moderate	Atrazine	7.1	<LOD	<LOD	<LOD	0	0.010				
	S-metolachlor	5.2	<LOD	<LOD	<LOD	0	0.010				
	Imazethapyr	6.4	<LOD	<LOD	<LOD	0	0.007				
	Chlorantraniliprole	2.9	<LOD	<LOD	<LOD	0	0.002				
	Chlorpyrifos	3.2	7.6	7.6	7.6	5	0.010				
	Clothianidin	4.2	<LOD	<LOD	<LOD	0	0.005				
	Thiamethoxam	3.4	3.4	3.4	3.4	5	0.002				

As expected, the frequency of pesticide detection in periphyton was significantly higher for sites with “intense” agricultural pressure compared to sites experiencing “moderate” agricultural pressure (Wilcoxon test, $W = 134.5$, p-value < 0.01, Table 2). S-metolachlor, chlorantraniliprole and atrazine were the most frequently detected pesticides, with detection frequencies of 53%, 41% and 10%, respectively, compared to no detection in the samples from “moderate” agricultural pressure (Wilcoxon test, p-values < 0.05, Table 1). Atrazine was widely used in Quebec until 2018, after which its use in agriculture required an agronomic prescription (Gouvernement du Québec, 2018). Even if atrazine sales decreased by almost 93% from 2017 to 2022 (MELCCFP, 2024a), this herbicide remains frequently detected in agricultural streams in Quebec. In France where atrazine has been banned since 2003, the substance is still detected in stream waters, as a likely consequence of its persistence in soils and remobilization potential (Bernard et al., 2023). Chlorantraniliprole is an insecticide from the diamide family, included in several formulations used in field vegetable farming and in orchard production (Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec (MAPAQ) et al., 2010) as a replacement for the neonicotinoid insecticides clothianidin and thiamethoxam, which are common in corn seed treatments and have been under agronomic prescription in Quebec since 2018 (Gouvernement du Québec, 2018). However, the substitution of those two insecticides by other compounds such as chlorantraniliprole does not necessarily improve the health of aquatic and terrestrial environments. This insecticide is frequently detected in surface waters, typically in the range of 0.9-2 $\mu g \cdot L^{-1}$ in the province of Quebec (Giroux, 2022; MELCCFP, 2024b), up to 8 $\mu g \cdot L^{-1}$ in other Canadian provinces,), and up to 10.2 $\mu g \cdot L^{-1}$ in the United States of America (Stinson et al., 2022). Although studies assessing chlorantraniliprole toxicity are scarce, effects on non-target species of aquatic invertebrates have been observed (USEPA, 2008). Lavtižar et al., (2015) showed that chlorantraniliprole was highly toxic to *Daphnia magna*, with acute and chronic LC_{50} of 9.4 and 3.7 $\mu g \cdot L^{-1}$, respectively. Rodrigues et al., (2015) showed that 3 $\mu g \cdot L^{-1}$ of chlorantraniliprole, a concentration observed under environmental conditions, affected the growth of *Chironomus riparius* larvae (full life cycle test, 28 days). As another example, Sanford et al., (2021) determined a LC_{50} of 2.9 $\mu g \cdot L^{-1}$ for the mayfly larvae (*Neocloeon triangulifer*). The fact that chlorantraniliprole accumulated in several of our periphyton samples raises concerns, as this biological matrix is a key food source for aquatic invertebrates.

The presence of certain compounds in the biofilm differed depending on the type of cultivated crops. For example, chlorantraniliprole was more frequently detected in periphyton from streams in orchard areas (69%) than in corn/soybean areas (29%). In parallel, clothianidin and thiamethoxam were more frequently detected in periphyton from areas where orchards are intensively cultivated (respectively 6% and 3%) than in corn/soybean zones (respectively 0% and 4%) (Table S. 3). Pesticide concentrations in the periphyton samples revealed a contamination footprint that seemed to be linked to the type of land use (type of farming), as also observed in Bernard et al. (2019, 2023).

Table 2: W-statistic and p-value associated with the Wilcoxon Mann-Whitney test used to compare pesticide detection frequencies between samples from the "moderate" agricultural pressure area and those from the "intense" agricultural pressure area.

Pesticide	W-statistic	p-value
Atrazine	104	**< 0.05***
S-metolachlor	138	**< 0.001*****
Imazethapyr	82.5	> 0.05
Chlorantraniliprole	126	**< 0.01****
Chlorpyrifos	86.5	> 0.05
Clothianidin	82.5	> 0.05
Thiamethoxam	80	> 0.05
All pesticides	134.5	**< 0.01****

Pesticide detection in water versus periphyton

In general, pesticide detection frequencies were higher in water than in periphyton. This result is consistent with the study by Ijzerman et al., (2024) who observed higher frequencies of detection in water than in periphyton samples for the herbicides atrazine, metolachlor, and imazethapyr, and for the insecticides clothianidin, thiamethoxam and chlorantraniliprole. In our study, the neonicotinoids clothianidin and thiamethoxam were detected in ≥ 30% of the water samples, whereas they were rarely detected in the periphyton samples (< 5%). Similarly, imazethapyr was detected in very few periphyton samples. In contrast, chlorpyrifos was almost exclusively quantified in the periphyton matrix (14% in the periphyton *versus* 1% in the water).

The difference in detection frequencies between water and biofilm samples may be due to the intrinsic properties of pesticides. In particular, the log octanol-water partition coefficient (log K_{ow}) is a constant often used to reflect the affinity of a compound for lipids and its hydrophobicity and, therefore, its ability to accumulate in organisms (Mackay and Fraser, 2000). Chlorpyrifos has a high log K_{ow} of 4.96 and a low solubility in water of 2 $mg \cdot L^{-1}$ in contrast to imazethapyr (log K_{ow} = 1.49 and solubility = 1.4 $g \cdot L^{-1}$). Spearman's correlations showed a significant positive correlation between the frequency of pesticide detection in the periphyton and log K_{ow} (rho = 0.37, p-value < 0.001). In parallel, the log of soil adsorption coefficient (log K_{oc}) of chlorpyrifos is elevated (log K_{oc} = 4.00 compared with 1.12 for imazethapyr) (National Center for Biotechnology Information, 2025), underscoring the greater affinity of chlorpyrifos for organic matter than imazethapyr. Indeed, our results showed a positive correlation between the frequency of pesticide detection in the periphyton and log K_{oc} (Spearman's correlation, rho = 0.33, p-value < 0.001). In our study, both log K_{ow} and log K_{oc} were good predictors of pesticide detection in water and in periphyton samples, which is consistent with previous studies (Ijzerman et al., 2024; Izma et al., 2024b). Compounds with high log K_{oc} such as chlorpyrifos may bind preferentially with organic-rich matrices, such as periphyton, which is composed of microorganisms and extracellular substances, whereas compounds with a lower affinity for organic matter, such as imazethapyr, tend to remain in the water column rather than accumulating within the periphyton matrix. These results suggest that differences in partition coefficients may explain the selective affinity of chlorpyrifos and imazethapyr for periphyton.

The results from this study suggest that analyses of periphyton pesticide concentrations provide complementary information to traditional water chemistry monitoring. The present study is consistent with recent studies carried out in other countries (United States of America: Mahler et al. (2020), Switzerland: Desiante et al. (2021), Brazil: Fernandes et al. (2023, 2020); Rheinheimer Dos Santos et al. (2023, 2020), France : Chaumet et al. (2023) and in Canada (Ontario : Ijzerman et al. (2024); Rooney et al. (2020)), highlighting the potential for periphyton to be used as a biomonitor for a more complete and integrated assessment of pesticide contamination in aquatic ecosystems.

3.3. Bioconcentration factors (BCFs) in periphyton

In this study, we also investigated the bioconcentration factors (BCFs) of pesticides in the periphyton. Periphyton bioconcentrated all pesticides with BCFs ranging between 36 (imazethapyr) and 362 (chlorpyrifos) (Table 3). The BCFs of atrazine and S-metolachlor were similar to those reported by Rooney et al. (2020) (242.4 ± 49.2 for atrazine) and Ijzerman et al. (2024) (120.5 ± 82 for atrazine and 97.2 ± 39 for S-metolachlor). However, in general, BCFs showed high variability between sites for the same compound, suggesting that the composition of the periphyton itself may influence the bioconcentration of organic compounds. The EPS matrix, which provides adsorption sites for organic and inorganic compounds (Chaumet et al., 2019), varies in nature and quantity depending on the periphyton composition and environmental conditions. Several other environmental factors (e.g., flow velocity, water temperature, light intensity) may also play a role in pesticide exposure and bioaccumulation (Chaumet et al. 2020; Guasch et al. 2016), partly causing this variability in BCFs for a single compound.

Table 3: Number of samples in which pesticides were detected in both the water and the periphyton and bioconcentration factors (BCFs) (n = 107).

Pesticide	Number of detections	BCF (mean ± sd) from this study
Atrazine	11	240 ± 141
S-metolachlor	53	104 ± 203
Imazethapyr	1	82
Chlorantraniliprole	42	154 ± 118
Chlorpyrifos	1	362
Clothianidin	1	143
Thiamethoxam	4	190 ± 190

3.4. Relationship between pesticides in the water and in the periphyton

In the present study, linear and non-linear (generalized additive models, GAM) models were used to investigate the relationship between concentrations of pesticides in water and in periphyton samples (Figure 2, Table 4). The available data allowed these relationships to be modelled only for the pesticides most frequently detected in the two matrices, i.e., atrazine, S-metolachlor and chlorantraniliprole (Figure 2). In addition, the relationship between water and periphyton concentrations was also modelled for the three pesticides together based on their relatively similar log K_{ow} (2.7 for atrazine, 3.1 for S-metolachlor and 2.9 for chlorantraniliprole), thus increasing the number of data points for modelling.

Linear regressions and GAM models were all significant, where pesticide concentrations in the water significantly influenced pesticide concentrations in the periphyton, with 47% to 71% of the variance explained (Figure 2, Table 4). For the three pesticides tested individually, and for the three pesticides pooled together, the GAM models showed lower AIC and higher R^2 than the linear regression models. However, most data points appeared to cluster in the linear portion of the plot ; thus, the relationship between concentrations of pesticides in water and in periphyton was well defined by the linear regression model. Only chlorantraniliprole and the combined data set for the three pesticides presented slight deviations from the linear regression model at the lowest and highest concentrations. Despite a paucity of studies exploring the potential for periphyton to accumulate pesticides as a function of pesticide concentrations in the water under natural conditions, this relationship has already been investigated by several authors under laboratory conditions. Chaumet et al. (2019) showed that the accumulation of diuron in the periphyton was likely non-linear and that it can be explained by simple Langmuir isotherm models, which may offer a more accurate representation of accumulation, as these models consider the potential saturation on sorption sites (Zhang et al., 2018). At low concentrations, contaminants may be transferred from ambient water to the periphyton by adsorption on EPS and diffusion through the matrix. This linear uptake may be followed by the saturation of adsorption sites (which may not have been the case in the present study), as well as the potential metabolism of the pesticide by periphyton microorganisms, as observed by Beecraft & Rooney (2021) in their study with glyphosate. In the context of our field conditions, pesticide concentrations were generally lower and spanning a shorter range of concentrations than under laboratory conditions. In contradiction to our hypothesis, this situation only enabled us to observe linear relationships.

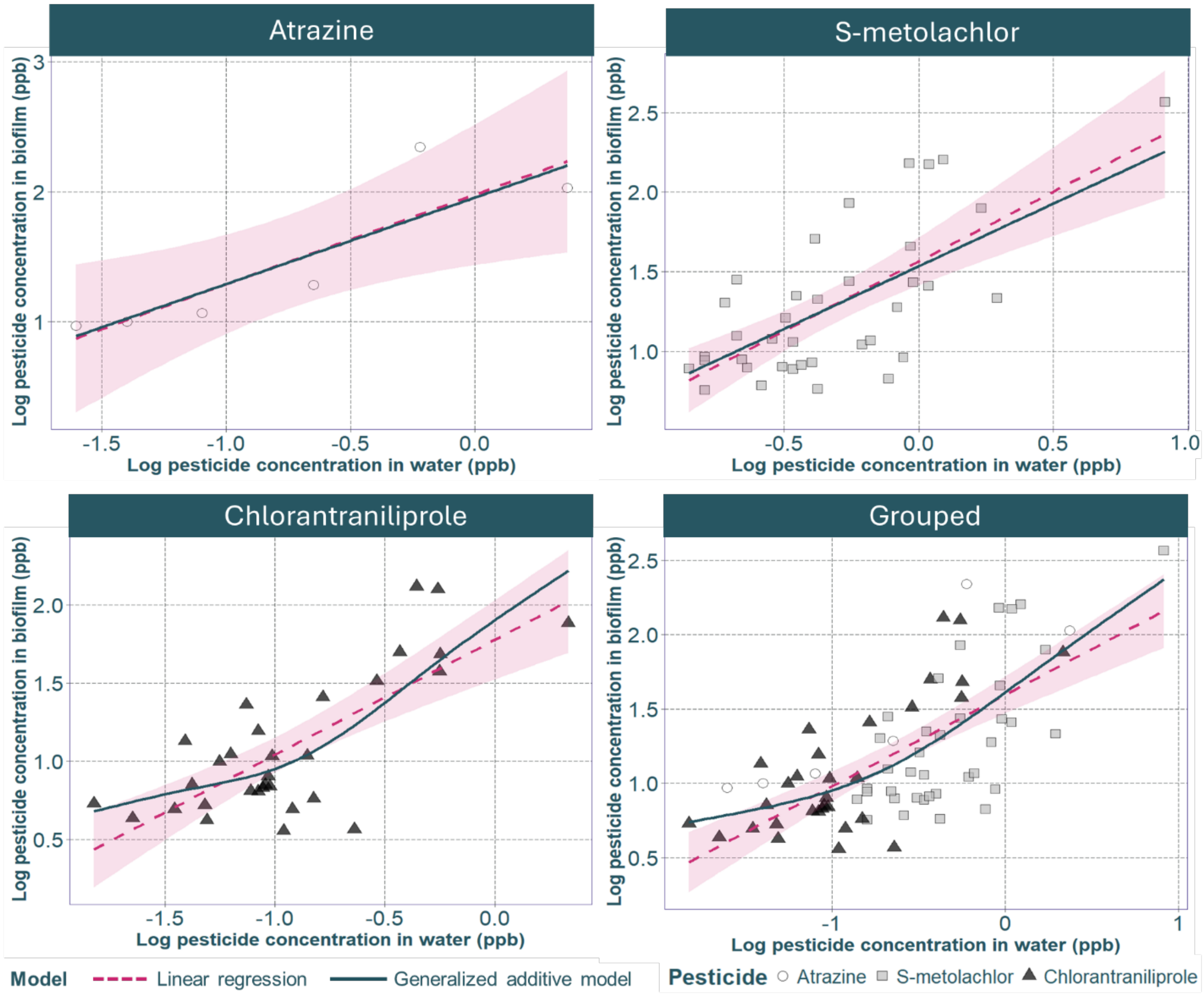

Figure 2: Linear regression (dashed pink line) and generalized linear models (solid blue line) of the relationship between concentrations in water and in biofilm for atrazine, S-metolachlor and chlorantraniliprole separately and grouped. The pink shaded area corresponds to the 95% confidence interval of the linear regression.

Table 4: Results for linear regressions and generalized additive models (GAM, family = Gamma, link = "identity") on the log concentrations of the pesticides atrazine, S-metolachlor and chlorantraniliprole in the biofilm as a function of their log concentration in the water. The regressions were performed for each compound individually and for the three compounds (grouped).

Pesticides	Model	Intercept	Standard deviation intercept	Coefficient	Standard error coefficient	R-squared/deviance explained	p-value	AIC
Atrazine	Linear regression	2.0	0.19	0.69	0.19	0.71	**< 0.05***	6.9
	GAM	1.5	0.12			0.82	**< 0.05***	4.6
S-metolachlor	Linear regression	1.6	0.07	0.88	0.15	0.47	**< 0.001*****	29
	GAM	1.3	0.05			0.46	**< 0.001*****	23
Chlorantraniliprole	Linear regression	1.8	0.12	0.74	0.12	0.56	**< 0.001*****	18
	GAM	1.1	0.05			0.59	**< 0.001*****	12
Grouped	Linear regression	1.6	0.06	0.62	0.08	0.47	**< 0.001*****	59
	GAM	1.2	0.04			0.51	**< 0.001*****	40

In a multi-contaminant context, as it is generally the case under *in situ* conditions, the transfer of organic compounds to the periphyton matrix may also be affected by the co-presence of other contaminants (El Azzi et al., 2018). Furthermore, the biofilm is a dynamic matrix from which organic compounds may be released or in which they may be degraded (Desiante et al., 2021). Although these interactions may lead to non-linear relationships between pesticide concentrations in water and periphyton, our field data suggest that a linear regression may well describe this relationship for a limited range of concentrations. To better define this relationship, it would be interesting to generate new relationships, including the results from other *in situ* studies, and to compare these relationships with laboratory data.

The linear relationship between pesticide concentrations in water and those accumulated in the biofilm is an interesting prospect for applications in biomonitoring, as a complement to water sampling. This relationship may allow for the estimation of pesticide concentrations in water from those accumulated in the biofilm. This approach would be particularly relevant for compounds whose quantification in water seems to be underestimated compared to measurements from biofilm samples, such as it was the case for chlorpyrifos in the present study. Furthermore, measurements in periphytic biofilm reflect the exposure of aquatic organisms to pesticides and also reflect the proportion potentially available to consumers. Periphyton may thus represent a complementary and ecologically relevant tool for assessing the contamination status of aquatic ecosystems.

Surface water quality monitoring programs are largely based on spot water sampling, which provides limited information on time-integrated contaminant exposure. To overcome this limitation, passive samplers such as POCIS have recently been adopted in France to complement conventional water sampling (Mathon et al., 2022). However, the integration of passive sampling into environmental monitoring programs remains relatively recent and limited. Biofilm-based sampling could potentially have a similar integrative role, acting as a natural sampler of contaminants. For example, Rheinheimer Dos Santos et al. (2023) demonstrated that biofilm sampling can be an effective alternative for detecting compounds that are sometimes not detected by POCIS. More broadly, water sampling, biofilm sampling, and standardized passive samplers (e.g., POCIS; diffusive gradients in thin films, DGT) each present distinct advantages and limitations. For instance, Bernard (2018) discussed the applicability of passive samplers, such as POCIS, as a complement to water sampling in monitoring programs. POCIS offers a better time-integrating approach than grab water samples but comes with logistical constraints and higher costs associated with their deployment (two visits: installation and retrieval). In contrast, periphyton sampling on natural substrates requires only a single visit and involves lower logistical constraints. Izma et al. (2025) further emphasized that when the objective is to assess temporal trends in pesticide occurrence, passive sampling devices such as DGTs should be considered due to their high sensitivity across a wide range of pesticides and their capacity for time integration. However, concerns remain regarding the extrapolation of concentrations from DGT measurements, and such approaches lack biological relevance. Because the biofilm occupies a central position at the base of aquatic and terrestrial food webs, it can be used to study the link between chemical exposure and biological compartments. Therefore, when the objective is to better understand the environmental fate of pesticides and their potential risks to the biota, biofilm-based sampling should be considered as a complementary component to water-quality monitoring programs.

Before large-scale application, further studies are needed, including improvement of temporal and spatial resolution, and a better understanding of the relationships between biofilm characteristics and other water-quality parameters. As already mentioned by Izma et al. (2025), standardization of the periphyton can be challenging, as its growth and composition are variable across sites. Sampling is generally carried out when the water level is low, if possible, at least 24 hours after rainfall and in non-stagnant waters. Several studies explored the application of using artificial substrates as standardized surfaces during sampling. One of the advantages of this method is that this allows for sampling in watercourses where substrates are lacking, and for standardization of substrate types for periphyton growth and composition, allowing better inter-site comparisons (Ijzerman et al., 2024). The use of artificial substrates helps to better control the effect of biofilm maturity/thickness, which can influence pesticide accumulation in the biofilm. Indeed, as periphyton matures, extracellular polymeric substances (EPS) production increases, forming a thicker barrier, limiting the transfer of pesticides in the periphyton matrix (Ivorra et al., 2000; Tlili et al., 2011), as seen by Khadra et al.(2018) with the limiting penetration of glyphosate in older periphyton. In our study, the age of periphytic communities sampled was not determined. However, research on the use of biofilm as an integrator of metal contamination has highlighted an almost "universal" relationship between free metal concentrations in water and in biofilm (Cd, Pb and Zn) (Malbezin et al., 2025). Although metals and pesticides exhibit fundamentally different behaviours in aquatic environments, these results suggest that periphyton has strong potential as a tool for monitoring aquatic environments at broad spatial scales, despite inherent environmental and compositional variability.

In Quebec, the Eastern Canadian Diatom Index (IDEC) is a biomonitoring tool used by the provincial governmental in routine monitoring to evaluate stream biological integrity. This evaluation is based on the analysis of diatom communities present within the periphyton. In this context, the periphyton sampled as part of IDEC-based monitoring could also be used to determine the concentrations of accumulated contaminants, as the sampling method is similar, with only the sample preservation method differing.

3.5. Exploratory study of pesticide accumulation in macroinvertebrates

Macroinvertebrates were sampled at various locations (orange circles marked with an X; Figure 1) in 2021, 2022 and 2023. The specimens collected were larvae of the families Trichoptera, Chironomidae and Ephemeridae. Adult Gammaridae (order Amphipoda) were also sampled. Unfortunately, comparing pesticide bioaccumulation in macroinvertebrates between sites under moderate and intense agricultural pressure was not possible, particularly due to the insufficient quantities of macroinvertebrates sampled under "moderate" pressure.

Detection frequencies and concentrations of pesticides measured in water, periphyton and macroinvertebrates are presented when data were available for all three matrices (Table 5, n = 12). The pesticides most frequently detected in macroinvertebrates were chlorantraniliprole (75%) and chlorpyrifos (25%), but atrazine and S-metolachlor were those detected with the highest concentrations (68.9 ppb for both, in 8% and 17% of the samples, respectively). Several studies have shown that macroinvertebrates can accumulate xenobiotics such as pesticides under laboratory conditions (Izma et al., 2024a; Roodt et al., 2022). However, Lauper et al. (2022) caged gammarids in a natural environment and demonstrated that experimental approaches tend to underestimate pesticide bioaccumulation compared to *in situ* measurements.

Not all pesticides investigated in this study were detected in macroinvertebrate samples. Specifically, imazethapyr was detected in 100% of the water samples but was not detected in macroinvertebrates (nor in periphyton), which can be explained by the greater affinity for water than for both matrices (see section 3.2). Similarly, thiamethoxam was detected in periphyton and water samples but not in macroinvertebrate samples. Concurrently, the insecticide chlorpyrifos was not detected in the water nor in periphyton, yet it was detected in 25% of the macroinvertebrate samples (based on data available for the three matrices: water, periphyton and macroinvertebrates, n = 12). This finding is consistent with the results from a laboratory study conducted by Lundqvist et al. (2012) which demonstrated an accumulation of chlorpyrifos in the aquatic snail *Theodoxus fluviatilis*. The authors of the study also noted that the transfer of chlorpyrifos from periphyton to snails was minimal (less than 2%) and that bioaccumulation of this compound in the invertebrate was likely to occur through a direct route (water). Results from pesticide accumulation in periphyton and macroinvertebrates suggest that the physicochemical properties of pesticides influence their environmental distribution, as previously demonstrated in other studies (Ijzerman et al., 2024).

The present study is one of the rare to assess pesticide bioaccumulation in macroinvertebrates collected from natural streams. Although exploratory, these results may be important for better understanding and assessing the effects of pesticides on different trophic levels. Further laboratory studies on trophic transfer are necessary to determine the uptake routes of pesticides in benthic macroinvertebrates.

Table 5: Minimum, maximum and median concentrations (ppb) and detection frequencies (%) for the pesticides monitored in macroinvertebrate, periphyton and water samples (orange circles with X; Figure 1). The limit of detection of pesticides in macroinvertebrate samples was the same as for periphyton samples (see Table 1).

	Macroinvertebrates (n=12)				Periphyton (n=12)				Water (n=12)			
Pesticide	Min	Max	Median	Detection (%)	Min	Max	Median	Detection (%)	Min	Max	Median	Detection (%)
Atrazine	69	69	69	8	81	81	81	8	0.013	1.195	0.023	100
S-metolachlor	6.5	69	38	17	5.3	13	10	75	0.043	1.025	0.156	100
Imazetapyr	< LOD	< LOD	< LOD	0	< LOD	< LOD	< LOD	0	0.011	0.070	0.037	100
Chlorantraniliprole	4.1	9.3	5.9	75	3.1	9.0	5.1	50	0.015	0.075	0.031	100
Chlorpyrifos	4.0	7.1	5.0	25	< LOD	< LOD	< LOD	0	< LOD	< LOD	< LOD	0
Clothianidin	< LOD	< LOD	< LOD	0	< LOD	< LOD	< LOD	0	0.006	0.014	0.010	75
Thiamethoxam	< LOD	< LOD	< LOD	0	< LOD	< LOD	< LOD	0	0.003	0.038	0.005	67

4. Conclusion

The current study is one of the few to report the accumulation of pesticides in periphyton and in macroinvertebrates collected in the field, and to investigate the relationship between bioaccumulated concentrations and ambient water concentrations. The results highlighted the accumulation of several pesticides of interest in the periphyton matrix. In particular, periphyton analysis allowed for the quantification of compounds such as chlorpyrifos, which were rarely detected in the water column, thus providing additional information to traditional water measurements. Periphyton is ubiquitous and easy to sample, enhancing its potential as a biomonitoring tool for complementing spot water sampling. However, before using periphyton as a pesticide exposure indicator in biomonitoring programs, the relationship between pesticide concentrations in the water and those in this complex biological matrix must be further investigated, for example, with a wider range of pesticide concentrations. The results from this study also indicated an accumulation of several pesticides in macroinvertebrates, particularly chlorantraniliprole, an insecticide used as a replacement for neonicotinoids. Its accumulation in macroinvertebrates is of concern due to its potential toxicity to the organisms themselves and to higher trophic consumers. Additional trophic transfer studies under laboratory conditions are needed to better characterize the uptake pathways of pesticides into macroinvertebrates. Based on the results from this study, periphyton-based pesticide accumulation has emerged as a promising tool in biomonitoring. With further research, model refinement (additional samples), standardization as well as temporal and spatial validation, this approach may be integrated into existing water-quality monitoring frameworks, in complement to existing chemical and biological assessments.

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7. Author contributions

All authors significantly contributed to this paper. LM was in charge of conceptualization, methodology, investigation, formal analysis and writing-original draft. SM was involved in methodology and editing. JMG was involved in investigation, formal analysis and editing. SM was involved in project administration and writing-review and editing. JC was involved in project administration and writing-review and editing. IL was in charge of project administration, conceptualization, funding acquisition, writing-review and editing.

8. Conflict of interests

The authors declare that they have no conflict of interest.

9. Competing Interests

None.

10. Data availability statements

The authors declare that data supporting the findings of this study are available within the paper and in Supplementary Information. Should any raw data files be needed, they are available from corresponding authors upon request.

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