Title:

Ardières-Morcille: a research catchment dedicated to the study of the transfer and impact of diffuse agricultural pollution in rivers

Abstract:

The Ardières catchment, (150 km²), in Beaujolais (France), belongs to the first European catchments where surface water contamination by pesticides has been highlighted in the late 1980s. Research on this site mainly aims at better understanding organic pesticides and trace elements hydrological pathways to watercourses, and at evaluating subsequent contamination and impact on aquatic ecosystems. Landscape as well as instream processes are studied in order to highlight catchment vulnerability to contaminants and possible mitigation actions. A consistent hydrological and chemical monitoring of the Ardières River and one of its tributaries, the Morcille River, has been taking place since 2002. It was supplemented by biological measurements on aquatic micro-organisms and macroinvertebrates, more particularly after 2005. The results show the importance of long-term study to account for the kinetics of contaminant transfer in hydro-biogeochemical systems. Physico-chemical, ecological and ecotoxicological measurements all showed spatial and temporal variability in water quality and a gradient of impact on community structures and ecological functions as a function of the pressure of human activity. Results allowed to develop indicators of toxic impacts and resilience of communities and provided avenues for action to improve water quality.

Key words: agricultural catchment, pesticides, trace metals, long-term monitoring, biological impacts, mitigation.

Main text:

Site description and main research findings

The Ardières catchment (150 km² outlet: 46° 6'N, 4° 43'E), and its sub-catchment Morcille (8 km²), are located about 70 km north of the city of Lyon in France. This site has been studied for more than 30 years by Irstea (formely Cemagref), and now INRAE (https://saam.inrae.fr/). It is representative of the North Beaujolais area and is a labelled site of the LTSER (Long term socio-ecological research) platform Zone Atelier Bassin du Rhône. Forest mainly covers the head of the catchment whereas vineyards occupy 45% of the total area (70% of the Morcille sub-catchment) with a significant use of pesticides to control fungal diseases and weeds (Figure 1). The climate is temperate with Mediterranean influence. Hydrological response of the watershed to rainfall is fast, mostly less than an hour at intermediate station S2 and a few hours downstream (S6) (Figure 1). The soil consists in sandy loams on a shallow Hercynian crystalline basement (porphyritic granite). Climate, soil and steep slopes, from 2% to more than 30%, are favourable to infiltration and sub-surface lateral flow. Surface runoff mainly occurs during thunderstorms when the intensity of the rain exceeds the infiltration capacity of the soil. Runoff concurs to dynamic hydrological conditions favourable to erosion and pesticide transfer to rivers. Research on this site aims to better identify (i) the determinism and spatiotemporal variability of pesticide transfers to watercourses and instream concentrations, (ii) their biological impacts on aquatic ecosystems, and (iii) possible ways of restoring water quality in feedback.

The methodological strategy consists in combining soil, hydrological, chemical and ecotoxicological sciences through "*in natura*" experiments at several scales for a range of substances. In addition, modelling and studies in controlled conditions aim to verify hypothesis from field experiments. In order to better characterise the contamination variability and impact in watercourses, complementary sampling strategies (manual, automatic, passive integrative samples, active biomonitoring) and a range of biological indicators have been implemented in the two rivers, Ardières and Morcille. Results have highlighted an upstream-downstream gradient of contamination (figure 2, A and B), toxicity and ecological impacts according to the increasing use of land by vines and human activities, (WWTP, urbanized areas), (Montuelle & al., 2010; Rabiet, Margoum, Gouy, Carluer, & Coquery, 2010). Long-term observation has especially made it possible to follow the decrease in the concentrations of certain pesticides after their ban, as well as the subsequent resilience of micro-organism communities. As an example, figure 2, B and C, shows that it respectively took four years and more than ten years after their ban, for the concentrations of diuron and norflurazon to drop below 0.1 µg/L in the Morcille River (diuron was banned in France at the end of 2008 and norflurazon in mid-2003). The concentration of the main metabolite of the latter (norflurazon desmethyl) still exceeded 1 µg/L after ten years. More generally, the results provide a range of concentration patterns of pesticides and trace element according to their physico-chemical characteristics, their origins, transfer routes and hydrometeorological conditions.

In parallel, the Pollution-Induced Community Tolerance (PICT) approach was applied to a series of biological samples (natural periphytic microbial communities) taken along the Morcille River after the ban on diuron at the end of 2008. Results show that the tolerance levels to diuron (EC_{50} values derived from toxicity tests on photosynthesis) significantly decreased in the most contaminated station (S3) from 2009 to 2011 to finally drop to the same values as the lightly contaminated reference station (S1) (Pesce, Margoum, & Foulquier, 2016). These results, combined with structural and functional measurements, (including the microbial capacity to degrade diuron in surface sediments; Pesce, Margoum, Rouard, Foulquier, & Martin Laurent, 2013) demonstrated the ability of microbial communities to recover after diuron ban.

In this model watershed, data have also been acquired to contribute to the development and calibration of biomonitoring tools using sentinel species (notably the *Gammarus* crustacean) to define indicators of bioavailable contamination and toxicity (Besse & al., 2013; Coulaud & al., 2011; Lopes & al., 2020). All these data make it possible to better define restoration trajectories of aquatic ecosystems.

Jointly, experimental works on the Morcille catchment aim at evaluating pesticide transfer processes from the vineyard to watercourses and the effect of landscape features (hedge, grassland) to mitigate runoff and pesticide. As an example, an experimental vegetative buffer strip (6.3 m long, 4 m wide and slope of 25 %) was set-up between a vineyard field (2800 m²) upslope and the Morcille stream downslope (figure 3, A) in the St Joseph site (figure 1) in 2004. Both natural and artificial simulated runoff events were studied and the results demonstrated the grassed strip could drastically reduce surface runoff as well as pesticide concentration in runoff and infiltrated water collected by a series of lysimeters set 50 cm deep below the strip surface (Figure 3, A, B) (Boivin &

al., 2007). These data were used to calibrate a model of attenuation of runoff within a grassed strip and a tool to help with buffer strip sizing (Carluer, Lauvernet, Noll, & Muñoz Carpena, 2016).

Current studies on this site aim to achieve a more comprehensive understanding of processes at catchment scale, by considering more compartments (soil, water, sediment, biota), contaminants (metabolites, pharmaceuticals, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, hormones, resistance genes) and impacts (molecular, structural and functional indicators).

Main instrumentation and data

<u>- St Joseph experimental site</u> (figure 1): the flow rates of surface runoff entering and leaving the grassed strip were measured owing to a Venturi flume (E 1253AZ and E 1253 AX, Hydrologic, respectively) equipped with pressure sensors (Hydrologic Alphée 3020-1) connected to a data logger (Campbell CR10X) for seven natural and five artificial runoff events. Cumulated infiltration volumes in the four lysimeters have been manually measured after one natural and five simulated runoff events. Average concentrations of pesticides in runoff and infiltrated water into the lysimeters were measured in cumulated samples from automatic samplers and manual sampling respectively. In addition, punctual data on the level of the water table and associated pesticide concentrations in the shallow groundwater under the grassed strip are also available. Only the dissolved fraction has been analysed so far on this site, but an ongoing project now addresses the transport of the particulate fraction.

<u>- Hydrological monitoring at the catchment scale:</u>

S1 to S6 refer to the studied stations (figure 1).

The water levels have been continuously evaluated since 2002 in the Morcille River in S2 and since 2011 in the Ardières River in S6, using respectively a pressure sensor (Druck PDCR 1830) and a radar sensor (Vega CS475) linked to data logger (Campbell CR10X and CR800 respectively). Water flow have been calculated owing to rating curves. Data are available at the variable time step of their acquisition or averaged over the hour, day or month. In addition, rainfall has been continuously measured at the station Calvaire (46°09'41.8"N 4°39'18.8"E) since 1992, owing to a tipping counter 0.1 L polycarbonate UGT, linked to a data logger (D2E, Alcyr).

<u>- Chemical monitoring:</u>

Chemical data in all stations cover suspended particulate matter loads, major and trace element and pesticide concentrations. Major elements, trace elements (16 since 2007) and organic pesticide (8 since 2004 and more than 20 since 2009) concentrations were acquired by manual grab or by passive sampling (DGT, silicone-based and POCIS) (Assoumani, Lissalde, Margoum, Mazzella & Coquery, 2013) in all stations. Fractional or cumulative automatic sampling based on time or flow intervals were performed during specific monitoring campaigns between 2007 and 2013 in S2 and between 2011 and 2016 in S6. This made it possible to sample the flood events at short time intervals (less than 15 min) and to acquire event-scale concentration profiles. Analyses mainly concerned the dissolved phase, but some contaminants were also analysed on sediment, suspended particulate matter, biofilm and crustacean Gammarus sp.

Most analyses were performed in the INRAE-Riverly laboratory which is part of the French National Reference Laboratories for the monitoring of aquatic environments.

<u>- Biological monitoring:</u>

Microbial monitoring (natural periphytic, surface sediment and leaf-litter associated microbial communities) has been carried out during targeted campaigns of few months since 2005 in all stations. Global parameters, structures (microbial biodiversity, taxonomy of diatoms, etc.) and functions (decomposition of dissolved organic matter and plant litter, capacity for biodegradation of pesticides, photosynthesis, etc.) have been measured by combining molecular approaches (DNA fingerprinting and sequencing), microscopy analyses (diatom morphology and taxonomy), potential activity measurements (photosynthesis by pulse-amplitude modulation, selected extracellular enzymatic activities by fluorometric assays, production/consumption of CO_2, NO_2 or CH_4 by gas chromatography, pesticide biodegradation by radiorespirometry) and effective activity measurements (*in situ* decomposition of natural or artificial cellulose-based substrates). Punctual data is available on macroinvertebrates (diversity, biological traits) at S1, S2 and S3.

In addition, neurotoxicity (AChE, standard protocol AFNOR XP T90-722-1), impacts on feeding activity (standard protocol AFNOR XP T90-722-3), moulting cycle, and reproduction markers (standard protocol AFNOR XP T90-722-2) measured on caged crustacean Gammarus sp. (active biomonitoring, standard protocol AFNOR XP T90-721) since 2010 at S4, S5 and S6, allowed to depict spatial gradients and temporal variability of contamination-induced ecotoxicological effects in the Ardières River.

Data availability:

Hydrological and chemical chronicles are publically available on DBOH database: https://doi.org/10.17180/obs.saam

The other data, associated metadata, and calculation tools are available upon request to the corresponding author. The metadata and contact details of the scientist responsible for each dataset are available at:

https://metazabr.irstea.fr/geonetwork/srv/fre/catalog.search?node=srv#/search?any=ardieres

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Figure legends:

Figure 1. Ardières-Morcille catchment location and land use, experimental site of St Joseph and studied stations

Figure 2. Decrease in diuron and its main metabolite, DCPMU, concentrations estimated owing to grab samplers, after the ban on diuron, at upstream station S1 (A) and downstream station S2 (B) and comparative decrease in norflurazon and norflurazon desmethyl concentrations into grab samplers at S2 (C). LQ in (A) refers to Limit of Quantification of diuron and DCPMU, NFZ to Norflurazon and NFZD to Norflurazon Desmethyl

Figure 3. Hydrograph of runoff upstream and downstream of a grassed buffer strip (B) after a natural high frequency return rainfall event on the experimental site of St Joseph and average concentrations of diuron in runoff and infiltrated water (lysimeters A, B, C and D) (A)