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Fate of the herbicide glyphosate and its metabolite AMPA in soils and their transfer to surface waters : A multi-scale approach in the Lavaux vineyards, western Switzerland

Silwan Daouk

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Faculté des géosciences
et de l'environnement

Institut des sciences de la Terre

Fate of the herbicide glyphosate and its metabolite AMPA in soils and their transfer to surface waters: A multi-scale approach in the Lavaux vineyards, western Switzerland

Thèse de doctorat

Présentée à la
Faculté des géosciences et de l'environnement de l'Université de Lausanne
par

Silwan Daouk

Maîtrise ès Sciences en Géosciences et Environnement
de l'Université de Lausanne

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FATE OF THE HERBICIDE GLYPHOSATE AND ITS METABOLITE AMPA IN SOILS AND THEIR TRANSFER TO SURFACE WATERS : A MULTI-SCALE APPROACH IN THE LAVAUX VINEYARDS, WESTERN SWITZERLAND

Lausanne, le 21 mars 2013

Pour le Doyen de la Faculté des géosciences et de l'environnement

Professeur Karl Föllmi

Preface

This work is the achievement of four years of research at the University of Lausanne, dedicated to study environmental implications of the agricultural use of pesticides. More specifically, it aims at understanding the fate of the most famous herbicide in the world, glyphosate, in western Switzerland, where the consequences of its use are poorly known. Indeed, when the idea to study this molecule first came into my mind, and after a little research, I realized that no public services or laboratories were monitoring it in Switzerland, despite its intense use for many types of crops. Meanwhile, people in EAWAG started to publish the results of their research, on which this one is partly based. Furthermore, in ACW Waedenswil, this herbicide is also analyzed for a short while. Indeed, as always in science, this work could not have been realized without the previous scientific knowledge gathered during previous years and centuries. Thus, I hope that this contribution will be continued somehow, somewhere by somebody, but, most of all, I hope it will be helpful to the society.

"As for me, all I know is that I know nothing"
Socrates, -470-399, Republic, 354c

« Ou l'humanité détruira les armements, ou les armements détruiront l'humanité. »
Albert Einstein, physicien, 1879-1955

« La perte de rigueur des enseignements scientifiques, signalée par nos plus grands mathématiciens, a rendu poreuse la frontière du savoir et du croire. Cette rupture peut faire craindre la naissance de générations en qui l'esprit critique aura été remplacé par une adhésion vague à la "tolérance". »
Danièle Sallenave, écrivain, 1940-, dieu.com, 2004

A mes parents qui m'ont toujours encouragé durant mes études

A Charlotte, avec amour

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Summary

The use of herbicides in agriculture may lead to environmental problems, such as surface water pollution, with a potential risk for aquatic organisms. The herbicide glyphosate is the most widely used active ingredient in the world and in Switzerland. In the Lavaux vineyards it is nearly the only herbicide molecule applied. This work aimed at studying its fate in soils and its transfer to surface waters, using a multi-scale approach: from molecular (10^{-9} m) and microscopic scales (10^{-6} m), to macroscopic (m) and landscape scales (10^{3} m).

First of all, an analytical method was developed for the trace level quantification of this widely used herbicide and its main by-product, aminomethylphosphonic acid (AMPA). Due to their polar nature, their derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) was done prior to their concentration and purification by solid phase extraction. They were then analyzed by ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). The method was tested in different aqueous matrices with spiking tests and validated for the matrix effect correction in relevant environmental samples. Calibration curves established between 10 and 1000 ng/l showed r^2 values above 0.989, mean recoveries varied between 86 and 133% and limits of detection and quantification of the method were as low as 5 and 10 ng/l respectively.

At the parcel scale, two parcels of the Lavaux vineyard area, located near the Lutrive River at 6 km to the east of Lausanne, were monitored to assess to which extent glyphosate and AMPA were retained in the soil or exported to surface waters. They were equipped at their bottom with porous ceramic cups and runoff collectors, which allowed retrieving water samples for the growing seasons 2010 and 2011. Results revealed that the mobility of glyphosate and AMPA in the unsaturated zone was likely driven by the precipitation regime and the soil characteristics, such as slope, porosity structure and layer permeability discrepancy. Elevated glyphosate and AMPA concentrations were measured at 60 and 80 cm depth at the bottom of the parcels, suggesting their infiltration in the upper parts of the parcels and the presence of preferential flow in the studied parcels. Indeed, a succession of rainy days induced the gradual saturation of the soil porosity, leading to rapid infiltration through macropores, as well as surface runoff formation. Furthermore, the presence of more impervious weathered marls at 100 cm depth induced throughflows, the importance of which for the lateral transport of the herbicide molecules was determined by the slope steepness. Important rainfall events (>10 mm/day) were clearly exporting molecules from the top layer of the soil, as indicated by high concentrations (1-100 µg/l) in runoff samples. A mass balance showed that total loss (10-20%) mainly occurred through surface runoff (96%) and, to a minor extent, by throughflows in soils (4%), with subsequent exfiltration to surface waters.

Observations made in the Lutrive River revealed interesting details of glyphosate and AMPA dynamics in urbanized landscapes, such as the Lavaux vineyards. Indeed, besides their physical and chemical properties, herbicide dynamics at the catchment level strongly depend on application rates, precipitation regime, land use and also on the presence of drains or constructed channels. Elevated concentrations, up to 4970 ng/l, observed just after the application, confirmed the diffuse export of these compounds from the vineyard area by surface runoff during main rain events. From April to September 2011, a total load of 7.1 kg was calculated, with 85% coming from vineyards and minor urban sources and 15% from arable crops. Small vineyard surfaces could generate high concentrations of herbicides and contribute considerably to the total load calculated at the outlet, due to their steep slopes (~10%). The extrapolated total amount transferred yearly from the Lavaux vineyards to the Lake of Geneva is 190 kg.

At the molecular scale, the possible involvement of dissolved organic matter (DOM) in glyphosate and copper transport was studied using UV/Vis fluorescence spectroscopy. Combined with parallel factor (PARAFAC) analysis, this technique allowed characterizing DOM of soil and surface water samples from the studied vineyard area. Glyphosate concentrations were linked to the fulvic-like spectroscopic signature of DOM in soil water samples, as well as to copper, suggesting the formation of ternary complexes. In surface water samples, its concentrations were also correlated to those of copper, but not in a significant way to the fulvic acids signature. Quenching experiments with standards confirmed field tendencies in the laboratory, with a stronger decrease in fluorescence intensity for fulvic-like fluorophore than for more aromatic ones.

Lastly, based on maximum concentrations measured in the river, an environmental risk for these compounds was assessed, using laboratory tests and ecotoxicity data from the literature. In our case and with the methodology applied, the risk towards aquatic species was found to be negligible (RF<1).

Résumé

L’utilisation d’herbicides en agriculture pose des problèmes environnementaux comme la pollution des eaux de surface, avec un risque potentiel pour les organismes aquatiques. L’herbicide glyphosate est la substance active la plus utilisée au monde et en Suisse. Dans le vignoble du Lavaux c’est quasiment la seule molécule appliquée. Ce travail étudie le devenir de cet herbicide dans les sols et son transfert aux eaux de surface, en adoptant un approche multi-échelle: du niveau moléculaire (10^{-9} m) et microscopique (10^{-6} m), à l’échelle macroscopique (m) et kilométrique (10^{3} m).

Tout d’abord, une méthode analytique a été développée pour la quantification de cet herbicide et de son principal métabolite, l’acide aminométhylphosphonique (AMPA). De par leur nature très polaire, une étape de dérivation de ces molécules est nécessaire à l’aide du 9-fluorenylmethyl chloroformate (FMOC-Cl), avant de les concentrer et les purifier par extraction sur phase solide. Elles sont ensuite analysées par chromatographie liquide ultra performante, couplée à un triple quadripôle (UPLC-MS/MS). La méthode a été testée dans différentes matrices aqueuses à l’aide de tests de fortifications. Avec des droites de calibrations montrant un coefficient de régression (r^2) supérieur à 0.989, des taux de récupération oscillants entre 86 et 133%, et une limite de détection et de quantification de 5 et 10 ng/l respectivement, la méthode a été validée pour la correction d’effet matrice dans les échantillons environnementaux.

A l’échelle macroscopique, deux parcelles du vignoble du Lavaux, situées près de la rivière Lutrive à 6 km à l’est de Lausanne, ont été choisies pour évaluer dans quelle mesure le glyphosate et l’AMPA sont retenus dans les sols ou exportés vers les eaux de surface. Elles ont été équipées avec des bougies poreuses en céramique et des capteurs de ruissellement, qui permirent de récolter des échantillons d’eau pour les saisons 2010 et 2011. Les résultats ont montré que la mobilité du glyphosate et de l’AMPA dans la zone non saturée est déterminée par le régime de précipitations et les caractéristiques du sol, comme la pente, la structure de la porosité et la différence de perméabilité entre les horizons. Des concentrations élevées de glyphosate et d’AMPA ont été observées à 60 et 80 cm de profondeur au bas des parcelles, suggérant leur infiltration en amont et la présence de flux préférentiels dans les parcelles étudiées. En effet, la succession d’événements pluvieux induit la saturation graduelle de la porosité du sol, et l’infiltration rapide à travers les macropores, ainsi que la formation de ruissellement de surface. De plus, la présence de marnes altérées plus imperméable à 100 cm de profondeur génère des flux de sub-surface, dont l’importance dans le transport latéral des molécules d’herbicide dépend de la pente. Les pluies importantes (>10 mm/j) exportaient clairement les molécules des couches superficielles, comme indiqué par des concentrations importantes dans les échantillons d’eau de ruissellement. Un bilan de masse a montré que la perte totale (10-20%) est réalisée principalement par

ruissellement de surface (96%), et dans une moindre mesure par flux latéraux de sub-surface (4%) et exfiltration vers les eaux de surface.

Les observations faites dans la Lutrive montrèrent des détails intéressants de la dynamique du glyphosate et de l'AMPA dans un paysage urbanisé comme le vignoble du Lavaux. En effet, à côté de leurs propriétés physico-chimiques, la dynamique de ces molécules à l'échelle du bassin versant dépend fortement des taux d'applications, du régime des précipitations, de l'occupation du sol et la présence de drains et de canaux construits. Des concentrations élevée, jusqu'à 4970 ng/l, mesurées peu après l'application ont confirmé l'exportation diffuse de ces composés des zone de vigne par ruissellement de surface lors d'événements pluvieux importants. D'avril à septembre 2011, une charge totale de 7.1 kg a été calculée à l'exutoire, avec 85% venant des surfaces viticoles et d'autres sources urbaines mineures, et pour 15% d'autres cultures. Les surfaces de vignes, pourtant relativement petites, ont généré de grandes concentrations d'herbicides et contribué considérablement à la charge totale calculé à l'exutoire, à cause des pentes importantes dans ce vignoble (~10%). La masse annuelle totale qui arrive dans le lac Léman, extrapolé pour l'ensemble du Lavaux, s'élève à 190kg.

A l'échelle moléculaire, la possible implication de la matière organique dissoute (MOD) dans le transport du glyphosate et du cuivre a été étudiée par spectroscopie de fluorescence UV/Vis. Cette technique, combinée à l'analyse factorielle parallèle (PARAFAC), a permis de caractériser la MOD des échantillons d'eau du sol et de rivière de la zone viticole étudiée. Les concentrations de glyphosate étaient liées aux signatures spectroscopiques de composés de type fulvique dans la solution du sol, ainsi qu'au cuivre, suggérant la formation de complexes ternaires. Dans les eaux de surface, ses concentrations étaient aussi corrélées à celles du cuivre mais pas aux composés fulviques.

Finalement, basée sur les concentrations maximales mesurées en rivière, une évaluation globale du risque environnemental pour ces composés a été réalisée, en combinant des tests en laboratoire avec des données d'écotoxicité de la littérature. Dans notre cas, ainsi que pour d'autres rivières en Suisse, le risque envers les organismes aquatiques a été estimé négligeable (RF<1).

Résumé grand public

L’utilisation d’herbicides en agriculture sert à limiter la compétition pour l’eau et les nutriments entre les mauvaises herbes et la culture. Ceci pose des problèmes environnementaux comme la pollution des eaux de surface, ce qui peut représenter un risque pour les organismes aquatiques. Le glyphosate est la substance herbicide la plus utilisée au monde et en Suisse. Ce travail s’intéresse au devenir de cet herbicide dans les sols et à son transfert aux eaux de surface dans le vignoble du Lavaux

Dans un premier temps, une méthode analytique, basée sur la chromatographie liquide et la spectrométrie de masse, a été développée afin de mesurer la quantité de cet herbicide et de son principal produit de dégradation, l’acide aminométhylphosphonique (AMPA). La méthode a été validée pour l’analyse d’échantillons environnementaux.

A l’échelle macroscopique, deux parcelles du vignoble du Lavaux, situées près de la rivière Lutrive à 6 km à l’est de Lausanne, ont été choisies pour évaluer dans quelle mesure le glyphosate et l’AMPA sont retenus dans les sols ou exportés vers les eaux de surface. Elles ont été équipées avec des capteurs d’eau du sol, des bougies poreuses en céramique, et des capteurs de ruissellement, qui permirent de récolter des échantillons d’eau pour les saisons 2010 et 2011. Les résultats ont montré que la mobilité du glyphosate et de l’AMPA dans le sol est ainsi déterminée par le régime de précipitations et les caractéristiques du sol, comme la pente, la structure des vides et la différence de perméabilité entre les couches. Des concentrations élevées de glyphosate et d’AMPA ont été observées à 60 et 80 cm de profondeur au bas des parcelles, suggérant leur infiltration en amont et la présence de flux d’eau rapides dans les parcelles étudiées. Les pluies importantes (>10 mm/j) exportent clairement les molécules des couches superficielles, comme indiqué par des concentrations importantes dans les échantillons d’eau de ruissellement. Un bilan de masse a montré que la perte totale (10-20%) vers les eaux de surface est réalisée principalement par ruissellement de surface (96%) et, dans une moindre mesure, par flux latéraux de sub-surface (4%).

Les observations faites dans la rivière à côté des vignobles montrèrent des détails intéressants de la dynamique du glyphosate et de l’AMPA dans un paysage urbanisé comme le vignoble du Lavaux. En effet, à côté de leurs propriétés physico-chimiques, la dynamique de ces molécules à l’échelle du bassin versant dépend fortement des doses appliquées, du régime des précipitations, de l’occupation du sol et de la présence de drains et de canaux construits. D’avril à septembre 2011, une charge totale de 7.1 kg a été retrouvée à l’exutoire, avec 85% venant des surfaces viticoles et d’autres sources urbaines mineures, et pour 15% d’autres cultures. Les surfaces de vignes, pourtant relativement petites, ont pu générer de grandes concentrations d’herbicides et contribuer considérablement à la charge totale calculé à l'exutoire, à cause des pentes importantes dans ce vignoble (~10%). La masse annuelle totale qui arrive dans le lac Léman, extrapolée pour l'ensemble du Lavaux, s'élève à 190kg.

Afin de comprendre plus précisément le fonctionnement du transport du glyphosate, les possibles interactions qu'il peut avoir avec la matière organique naturelle et le cuivre à l'échelle moléculaire ont été étudiées par spectroscopie de fluorescence. Cette technique a permis de prouver que les concentrations de glyphosate des échantillons d'eau du sol et de rivière étaient liées et à la matière organique et au cuivre, suggérant ainsi une triple association.

Finalement, basée sur les concentrations maximales mesurées en rivière, une évaluation globale du risque environnemental pour ces composés a été réalisée, en combinant des tests en laboratoire avec des données d'écotoxicité de la littérature. Dans notre cas, ainsi que pour d'autres rivières en Suisse, le risque envers les organismes aquatiques a été estimé négligeable.

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List of Abbreviations

ACW: Agroscope Changin-Waedenswil
AMPA: AminoMethylPhosphonic Acid
CEC: Cation Exchange Capacity
CFT: Colloid Filtration Theory
CHC: Chlorinated HydroCarbons
CIPEL: Commission Internationale pour la Protection des Eaux du Léman
DDT: DichloroDiphenylTrichloroethane
DIC: Dissolved Inorganic Carbon
DOC: Dissolved Organic Carbon
DOM: Dissolved Organic Matter
DT50: 50% Degradation Time
EC50: 50% Effect Concentration
ED: Endocrine's Disruptors
EDS: Energy Dispersive Spectroscopy
EDTA: EthyleneDiamineTetraAcetic acid
EEM: Excitation Emission Matrix
Eh: Redox potential
EPFL: Ecole Polytechnique Fédérale de Lausanne
EPSPS: 5-EnolPyruvylShikimate-3-Phosphate Synthase
ESI: Electrospray Ionization Source
FA: Fulvic Acids
FMOC-Cl: 9-fluorenylmethyl chloroformate
FOEN: Federal Office for the Environment
GR: Growth Rate
GSM: Global System Message
GUS: Groundwater Ubiquity Score
H: Humin
HA: Humic Acids
HC$_5$: 5% Hazardous Concentration
HCl: Chlorydric acid
HDPE: High Density PolyEthylene
HIX: Humification Index
HOC: Hydrophobic Organic Contaminants/Chemicals
HS: Humic Substances
ICP-MS: Inductively Coupled Plasma Mass Spectrometry
IPA: IsoPropylAmine
IS: Internal Standard
ISC: Inner Sphere Complex
KOH: Potassium hydroxyde
LOD: Limit Of Detection
LOQ: Limit Of Quantification
MEC: Measured Environmental Concentration
MeOH: Methanol
MRM: Multiple Reaction Monitoring
MW: Molecular Weight
NMR: Nuclear Magnetic Resonance
NOEC: Non Observed Effect Concentration
NOM: Natural Organic Matter
OEaux: Federal Ordinance on Water Protection
OECD: Organisation for Economic Co-operation and Development
OM: Organic Matter
OP: Organ phosphorous or organophosphate
ORRChem: Federal Ordinance on Chemical Risk Reduction
OSC: Outer Spher Complex
PAHs: Polycyclic Aromatic Hydrocarbons
PARAFAC: Parallel Factor analysis
PCA: Principal Component Analysis
PCB: Polychlorinated Biphenyls
PCP: Personal Care Products
PF: Preferential Flow
pH: H_3O^+ concentration
POEA: PolyOxyEthylene tallowAmine
POM: Particulate Organic Matter
POP: Persistent Organic Pollutants
PPP: Plant Protection Products
PPCP: Pharmaceuticals and Personal Care Products
PVC: PolyVinyl Chloride
RF: Risk Factor
SEM: Scanning Electron Microscope
S/N: Signal to Noise ratio
SOM: Soil Organic Matter
SPE: Solid Phase Extraction
SS: Suspended Solids
SSD: Species Sensitivity Distribution
SVWC: Soil Volumetric Water Content
T°: Temperature
TQD: Triple Quadrupole
TR: Toxic Ratio
UNESCO: United Nations Educational, Scientific and Cultural Organization
UPLC-MS/MS: Ultra Performance Liquid Chromatography tandem Mass Spectrometry
US EPA: United-States Environmental Protection Agency
WWII: World War II
WWTP: Waste Water Treatment Plant
XRD: X-Ray Diffraction
XRF: X-Ray Fluorescence

1 General introduction

1.1 Micropollutants in Switzerland

Recently, the urban population of the world has become more important than the rural one (52.1% in 2011)[1], implying increased needs in drinking water as well as wastewater treatments in cities. Freshwater resource management has thus become a priority in an urbanized world and the research project 'Léman21' (see §1.1), in the frame of which this study took place, aims at providing the scientific basis that will broadly redefine strategies for risk identification and sustainable management of lakes with a medium size, such as Lake Geneva, especially regarding their pollution with chemicals found at trace levels ('micropollutants') and their ecological impact.

Indeed, production of organic chemicals has increased considerably: at the end of the last century, 60 million metric tons were manufactured yearly in the US (Pierzynski et al., 1994). Most of them are of great concern dealing with environmental safety and human health. Furthermore, around 100'000 active substances are available in Europe (Hernando et al., 2008), and research on their potential threats to human and environmental health still lag behind their industrial production. Indeed, there are only 10'000 substances with known effects on human health and 1'000 which were analysed with regard of their environmental impacts (Chèvre, 2007). Thus, 'emerging pollutants' or 'micropollutants', such as personal care products (PCP), during the last decade have become a subject for environmental chemists and toxicologists (Daughton, 2001). They refer to several chemical groups *"used internally or externally with the bodies of humans and domestic animals and plants"*, including pharmaceuticals, contrast agent (used for X-ray radiography), fragrances (used in perfumes), sun-screen agents, excipients (manufacturing and formulating ingredients), etc. They all occur at trace concentration in the environment (µg/l or less) and most of them are polar and bioactive, which means that they are probably able to induce perturbations within organisms (Daughton, 2001). Indeed, many chemicals, including pesticides and pharmaceuticals, were confirmed as endocrine disruptors (ED), i.e. inducing hormonal perturbations. These chemicals were found to be released by waste water treatment plants (WWTP) and to have some harmful effects on aquatic organisms. In Switzerland, although the national research program on ED (PNR50) concluded that, in general, concentrations of ED in surface waters present no deleterious effects on fish populations, fish present some physiological problems due to oestrogens, downstream of some WWTPs at some key points in Swiss streams (NSF, 2008).

In this context, the "Strategy MicroPoll" was launched by the Federal Office for the Environment (FOEN) in 2006 and aims at developing decision tools and a strategy to reduce micropollutants inputs

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[1] Source: United Nations World Urbanization Prospects (http://esa.un.org/unpd/wup/index.htm), consulted Dec 8, 2012

in water bodies from urban effluents[2]. Its specific objectives are to evaluate micropollutant load in streams, its reduction and the means to reach this reduction, which have to be accompanied by financial and legislative supports. In the first stage, a prioritization of substances was performed: among the 30’000 substances used daily in Switzerland, 260 substances were pre-selected and finally 50 substances with substantial ecological impacts were chosen. In order to reduce the micropollutant load in Swiss rivers and lakes, four different measures are foreseen (Gälli et al., 2009):

1) Source reduction
2) Centralized technical measures
3) De-centralized technical measures
4) Organization

The first measure can be set through legislative tools, incentive taxes or labels and/or responsible individual behaviours, which will then lead to a different conception from the producer side. The second includes mainly the elimination of micropollutants in WWTPs with new technologies, and pilot systems were studied, such as in Lausanne-Vidy (Margot et al., 2011). The third measure recommends setting up a WWT unit in every house and applying technical solutions to reduce emissions from the principal polluters. The fourth one foresees to connect small WWTPs to big ones equipped with modern equipment. All these different measures cannot be implemented at the same time and their combination will induce different effects on pollutant loads, according to their use and chemical behaviour. For instance, the decentralized separation of urine and faeces in hospitals may be installed relatively rapidly, whereas the equipment of all households with decentralized WWT units may take a much longer time (Gälli et al., 2009).

1.2 Objectives of the research program ‘Léman21’

This Swiss National Science Foundation project deals with the environmental fate of micropollutants from the catchment to the deep waters of Lake Geneva. But as the whole watershed would have been difficult to assess, it focuses principally on the Lausanne agglomeration and the Vidy Bay on its lakeside. The project is divided in four different research modules which are briefly described thereafter:

1) Water and sedimentation dynamics
2) Catchment micropollutant inputs
3) Micropollutant behaviour
4) Microbial and ecological impacts

Research module 1 is divided into four sub-projects which aim at measuring different parameters (temperature, conductivity, wind directions, etc.), and using water current tracers and sediment traps,

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[2] Source: www.bafu.admin.ch, consulted July 20, 2012.

to obtain *“detailed 3-dimensional spatial and temporal modelling of the flows of water, energy, and sediments throughout the joint region linking the coastal zone and central lake”*, a physical-based support to assess the environmental fate of chemical pollutants. Therefore, two sub-projects deal with dynamic modeling of water currents in the Vidy Bay and in the entire lake, according to wind directions and strengths (PhDs of Le T. Ahn-Dao and Razmi Amir M.). Indeed, wind is considered the most important driving force for current dynamics. Another sub-project aims at understanding the sedimentation dynamics in the Vidy Bay and, with the help of different tracers and techniques (radionuclides, sediment traps), provides a basis for the interpretation of contaminants, transported preferentially bound to particles/colloids (PhD of Graham Neil). The last project deals more specifically with mercury (Hg) pollution of the Vidy Bay and its transport dynamics (PhD of Gascón Diez Elena).

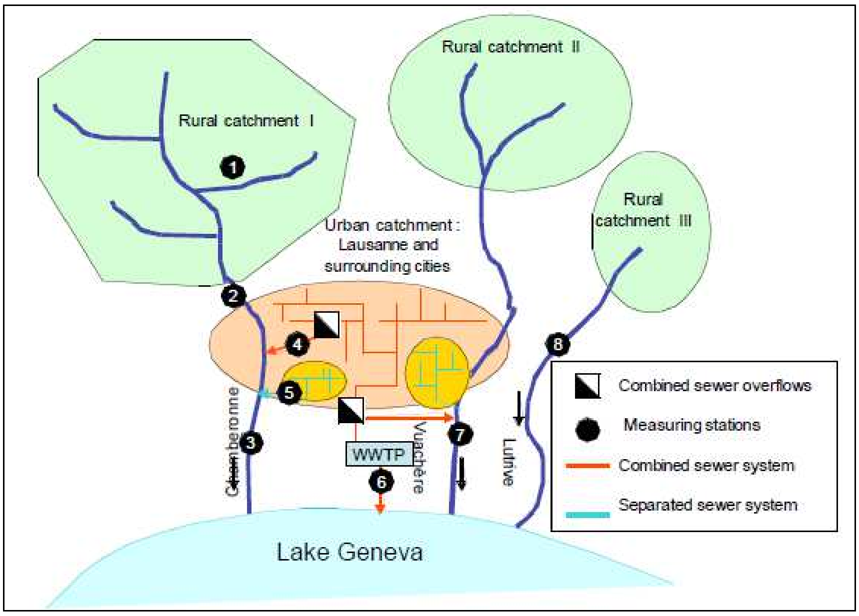

Figure 1.1: Proposed measuring sites for estimating micropollutants inputs (Pfeifer et al., 2008)

Research module 2 is divided into two parts, a rural-suburban and an urban area (Fig. 1.1), and aims at identifying micropollutant sources and possible vectors, estimating loads entering surface waters, a better understanding their dynamics and at developing modeling tools. One sub-project (this PhD) deals with agricultural inputs, mainly pesticides, from rural catchments that contribute to the lake pollution through rivers. Two other sub-projects deal with urban inputs that end up in the WWTP and are characterized by a mixture of pharmaceuticals, biocides used on building facades, residues of contraception pills (ethinylestradiol) or detergent derived products. One thesis (PhD of Coutu Sylvain) develops hydrological modeling tools at the city scale (Coutu et al., 2012a) and study the fate of pharmaceuticals, as well as biocides applied on building facades (Coutu et al., 2012c), where the other (PhD of Margot Jonas) assesses the different removal technologies available for WWTPs. Both physical and chemical techniques such as ozonation and adsorption on activated carbon (Margot et al.,

2011) and biological techniques, such as engineered constructed wetlands for small WWTPs, are studied.

Research module 3 deals with the fate of micropollutants in the Vidy Bay and farther out into Lake Geneva. The aim is to identify the main removal processes of micropollutants in lake waters and to discriminate dilution phenomena from degradation and sorption ones. To do so, lake water measurement campaigns of micropollutants and naturally occurring stable isotopes, compositions of water, laboratory simulations as well as molecular modeling of reactions that micropollutants are susceptible to experience in Lake Geneva have been set up. Thus, one sub-project (PhD of Bonvin Florence) deals with the occurrence of micropollutants in the Lake and processes involved in their removal (hydrolysis, direct and indirect photolysis, sorption, volatilization and biodegradation), combining field and laboratory approaches. Another sub-project (PhD of Tentscher Peter) deals with molecular modeling of the reactions of organic pollutants in lakes, mainly their oxidation potentials with quantum chemistry. A third sub-project (PhD of Halder Janine) investigates the stable isotopic composition of lake water samples, mainly H-, O- and C-isotopes, in order to trace water masses, identify their origin and dynamics and to understand their role in the fate of micropollutants in lake waters.

Research module 4 deals with the microbial and ecological impacts of micropollutants in the Vidy Bay and further in the Lake Geneva. Thus, the objective is to make a risk assessment of compounds, both as single substances and as a mixture, as well as bacteria entering the lake, for aquatic fauna and flora using new methods of risk assessment. More precisely, one sub-project (PhD of Czekalski Nadine) deals with microbial antibiotic resistance risk arising from exposures to high levels of antibiotics in the urban watershed of the city of Lausanne. A second sub-project (PhD of Gregorio Vincent) will assess ecotoxicological risks to aquatic organisms implied by mixtures of micropollutants, mainly herbicides, and evaluate new approaches for ecotoxicological risk assessment. Finally, a third sub-project (PhD of Borgatta Myriam) will investigate the effects of anticancer drugs on aquatic fauna.

Several results related to the project have been published already, mainly on the Vidy Bay, such as prioritization (Perazzolo et al., 2010), monitoring (Bonvin et al., 2011; Morasch et al., 2010) or methodological (Coutu et al., 2012b) studies. These papers deal mainly with pharmaceuticals in water, where others deal with bacteria (Czekalski et al., 2012; Haller et al., 2011; Poté et al., 2009) or heavy metal pollution (Poté et al., 2008; Thevenon et al., 2011), in both the water column and sediments of the Vidy Bay. Less was done with pesticides as more knowledge is already gathered, however some were monitored (Bonvin et al., 2011) and the effect of herbicide mixture on phytoplankton communities fluctuation was assessed (Gregorio et al., 2012). Nevertheless, some plant protection products may pose some environmental and health problems, and this study focusses on pesticide molecules and their diffuse transport from terrestrial to aquatic ecosystems (see below).

1.3 Choice of target substances

Though there are thousands of industrial compounds that can be found in soils and waters, this project does not deal specifically with mineral pollutants like metallic trace elements or macronutrients, such as N- or P-fertilizers, used in agriculture. Nevertheless, non-focused pollutants such as metallic trace elements and/or nutrients are often applied together with pesticides, or within the same time range, and may thus influence their behaviour and fate in the environment. Therefore, in specific cases it is necessary to assess their influence on micropollutant transfer dynamics. This project focuses on organic micropollutants such as pharmaceuticals, agricultural pesticides, veterinary products and/or personal care products (PCP). Veterinary products that are administrated to livestock that pastures on one field near a river can be transferred to rivers via excreta and thus may represent a diffuse pollution of surface water. However, since there are more crops than pastures in the study area, the main targeted micropollutants of our part remain plant protection products (PPP). Indeed, this study focuses only on agricultural pesticides and will not consider building treatments, veterinary products, pharmaceuticals and/or PCP.

In Switzerland, 400 PPP and 1150 veterinary products are homologated (Stamm, 2005). Among these 400 molecules, fungicides and herbicides are the most sold agrochemicals in Switzerland. Glyphosate [*N*-(phosphonomethyl)glycine], a broad spectrum, systemic, post-emergence herbicide widely used for weed and vegetation control (Delabays and Bohren, 2007), is the most sold active ingredient in the world and in Switzerland. In 2005, glyphosate sales were estimated at 191 t, representing 13% of all plant protection products and 30% of herbicides sold (SGCI, 2006). As it shows a strong sorption ability (log Koc = 4.34) and is rapidly transformed into aminomethylphosphonic acid (AMPA) in soils (DT50moy = 46d; Gimsing and dos Santos, 2005), it is often assumed that glyphosate would not reach water bodies. Indeed, it was not detected in the middle of the lake by the CIPEL[3] (Corvi et al., 2005). However, glyphosate and its metabolite AMPA are also soluble in water (12 and 5.8 g/l respectively) and were found to be leached or washed from soil surface layers and to reach ground and surface waters (Landry et al., 2005; Borggaard and Gimsing, 2008; Botta et al., 2009). Moreover, and despite its large use in agricultural areas as well as urban ones, there is only little knowledge available on its environmental fate in Switzerland yet (FOEN, 2004; Delabays and Bohren, 2007; Hanke et al., 2010). Indeed, there are still a lot of analytical problems for the quantification of this herbicide due to its zwitterionic structure and its complexation with metal ions (Hanke et al., 2008). Nevertheless, Hanke et al. (2008) recently analysed several water samples from Switzerland and found concentrations between 25 and 65 ng/l in surface water (Rhine River) but did not detect them in groundwater

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[3] CIPEL: International commission for the protection of Léman Lake's waters

samples. They concluded that the relative importance of the different type of sources and export dynamics of these compounds to surface waters needs further, more detailed investigation. Moreover, urban sewer systems and Waste Water Treatment Plants (WWTP) are known to release glyphosate and AMPA into surface waters (Kolpin et al., 2006; Botta et al., 2009; Hanke et al., 2010). However, due to their chemical properties, they are also fixed in sewage sludge during the wastewater treatment as shown by Ghanem et al. (2007). These authors compared concentrations in sludge of different French WWTPs and were able to discriminate the domestic origins of these aminophosphonates, with higher concentrations in separate sewer systems than in combined ones. The WWTP of the city of Lausanne has been found to reject more than 350 ng/l of glyphosate and AMPA after biological treatment (Margot et al., 2011). Thus, their fate in Lake Geneva's waters and sediments needs to be investigated. Furthermore, environmental implications of its agricultural use still need to be assessed in Switzerland. According to Klein et al. (2007), among the presupposed 20 most used active substances in Lake Geneva's Swiss watershed, glyphosate is among the 10 most applied substances for the main four types of crops. Furthermore, the expansion of minimum and no-till practices to avoid soil erosion and organic matter mineralisation leads to an increased application of non-selective herbicides such as glyphosate (Dill et al., 2010; Charles, oral comm.).

The use of herbicide molecules may lead to several environmental problems: not only to rapid water pollution, but also, in a more long-term perspective, to the development of resistance mechanisms in weeds. In viticulture, glyphosate is nearly the only herbicide used (Egger, 2009), leading to a delicate situation in terms of weed management (e.g. Dill et al., 2010, Shaner et al., 2012). The federal agricultural research institute (ACW) reported in 2009 the presence of an exotic species (*Conyza bonariensis*) in a vineyard parcel of the Lavaux vineyard[4]: the first one in Europe to develop a resistance to glyphosate (Delabays, 2007). To our knowledge, there are no research laboratories analysing this compound in western Switzerland. Thus, an analytical method and a better understanding of the diffuse sources of this "monopolistic" molecule are needed in western Switzerland, as well as its mobility among environmental compartments.

1.4 Purpose of the study, scientific questions and thesis outline

The specific objective of this work is to contribute to a better understanding of glyphosate transfer processes from agricultural fields to surface waters, with the suburban areas of the city of Lausanne and Lake Geneva as a case study. To do so, a multi-scale approach including microscopic (molecular), macroscopic and landscape levels has been proposed (Fig. 1.2), especially in order to assess the role of soil organic matter (SOM) in these transfer processes (Simpson, 2006). Indeed, hydrologic and/or chemical transfer processes are likely to take place at different scales, both temporally and spatially.

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[4] http://www.agroscope.admin.ch; News-Changins, Sep 3, 2009 (consulted, Sep 2010)

Scales of pesticide-related processes are obviously independent of scientific observations, whereas scales of these latter can be chosen but they are highly dependent on technical, logistic and economic limitations. Monitoring at the parcel scale (macroscopic-level) of soil and water samples will be the practical way to assess all inputs and outputs of pollutants in a limited agro-ecosystem. Furthermore, some measuring stations will be put into adjacent rivers (Fig. 1.1) in order to differentiate pesticide sources according to catchment land use, as well as to assess spatial dynamics in surface waters (landscape-level). Both approaches are complementary as they allow understanding the fate of pesticides at two different scales: the parcel scale (macroscopic level) and the catchment scale (landscape level). The challenge remains to establish a link between molecular observations in the laboratory and field results at the parcel and catchment scales.

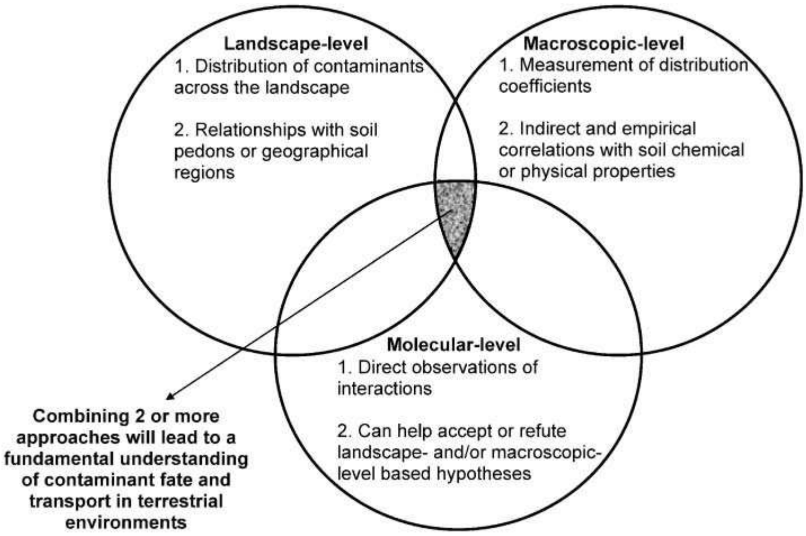

Figure 1.2: The multi-scale approach for the study of organic contaminant interactions with soils proposed by Simpson (2006)

Beside a multiple spatial resolution, the *temporal* sampling frequency has its importance in the study of diffuse water pollution by pesticides. Therefore, the sampling frequency determines a double strategy: sampling at a regular frequency and after ‘events’. Events in our case are:

1) Storms, during which a large amounts of runoff waters may enter the river leading to floods with a high load of micropollutants, either in a dissolved state or adsorbed on particles, and
2) Pesticide(s) applications.

This double strategy should be applied simultaneously in order to study seasonal variations of pesticide concentrations and loads during and between events. Then, the challenge is to understand the significance of measured concentrations in terms of transfer processes because of the multiple types of interactions between processes that govern pesticide environmental fate (see Chapter 2).

Nevertheless, it is meaningless to try to assess the transport of pesticides in agricultural parcels without considering water pathways. Therefore, soil hydraulic properties have to be assessed in parallel to pesticide quantifications. Furthermore, these hydraulic properties represent an essential prerequisite for the modeling of the fate of pesticides, whose outputs have to be confronted to field results, in order to confirm if a given model is valuable to predict concentrations, and to allow extrapolating observations at a larger scale or for other situations. All this may give more detailed information on water dynamics in investigated soils and parcels. Consequently, this could allow the potential role of preferential flows in the vertical and horizontal transport of pesticides to be assessed and thus, in the potential pollution of ground and surface waters.

The main scientific questions of this study were:

- Which pesticide molecules to study and with which analytical methods? (After having considered other molecules, such as isoproturon, the study was limited to glyphosate as described above.)
- How to practically assess the importance of runoff phenomena versus infiltration regarding glyphosate losses at the parcel scale?
- How to take into account preferential flow and colloid-associated transport in the transfer of glyphosate at different scales: soil column, parcel, and catchment scale?
- How efficient is an herbal or a forest fringe in between fields and rivers for the retention of glyphosate?
- How to assess multiple interactions between glyphosate and soil components (soil organic matter, clay minerals and oxides)?
- How to combine field measurements, laboratory experiments and modeling in order to minimize errors and obtain reliable results at the three proposed different scales, extrapolative to other situations?
- What are the overall diffuse inputs of glyphosate in Lake Geneva from a specific agricultural activity and area (here wine-production in the Lavaux vineyards)?

In **Chapter one**, the global project **‘**Scientific concepts for the sustainable management of mid-sized lakes in the 21st century’, into which this study was integrated, is first shortly presented. Second, the choice of pesticide molecules and the study specific objectives are presented and the study area is described. A literature review of the main processes involved in the environmental fate of agrochemicals follows, with focus on the importance of soil constituents in the transfer of pesticides from the agricultural field to surface waters (**Chapter two**). More specifically, these processes are reviewed for the environmental fate of the widely used herbicide glyphosate. Then, the developed analytical tools to quantify this latter molecule and its main degradation product (AMPA), as well as a similar herbicide (glufosinate) in environmental samples are described in parallel to the first proofs of their occurrence in surface water (**Chapter three**). Their fate in two vineyard parcels and their subsequent losses in the direction of the adjacent river, as a function of a specific soil configuration and the precipitation regime is presented (**Chapter four**). Then, their dynamics in the Lutrive River and the evaluation of the different sources participating in this pollution is evaluated, as well as an associated risk assessment (**Chapter five**). Finally, the role of dissolved organic matter (DOM) in these transfers is evaluated using UV/Vis fluorescence spectroscopy (**Chapter six)**. Both analyses of natural samples and quenching experiments in laboratory are performed. Finally, **Chapter seven** synthesizes the most important results with some perspectives for further research.

1.5 Study area, field equipment and soil hydraulic properties

1.5.1 *Study area*

As the three catchments initially planned (see Fig. 1.1) would have been impossible to cover with the proposed multi-scale approach within the normal period of a PhD thesis, it was decided to focus only on catchment III, i.e. on vineyards. The study area is located in the Lutrive watershed, north of Lutry, a village on the east side of the city of Lausanne, which is crossed by the river (Fig. 1.3). Its watershed has a total surface of 6.4 km^2 with different land uses, mainly agricultural fields (45%), with 4.1% of vineyards, urban and impervious surfaces (31%) and forests (24%). It can be divided into five sub-watersheds with vineyards located only in the southern sub-watershed. The Lutrive River has its source located on the Swiss plateau at an altitude of 842 m and its mouth on the level of Lake Geneva at 372 m, with a mean slope of 7.3% (Pfeifer et al., 2008). It has a pluvial recharge and a torrential regime that preoccupies local authorities; that is why the river stream has been corrected twice (De Cerenville 2006 *in* Egger, 2009). First it has been channelled in the Lutry village in the 1930s and, more recently (1998-2000), in the steepest parts the banks have been reinforced, and steps have been created to lower the velocity and thus bank erosion. The Lutrive represents the western limit of the Lavaux region, officially registered as one of UNESCO’s World Heritage Sites on 28th June 2007[5].

The Lavaux, originally “La Vaux de Lustrie” meaning “The Valley of Lutry”, is a vineyard area located in western Switzerland of 830 ha, comprising about 10’000 terraces on 40 km between Montreux and Lutry to the east of Lausanne (Fig. 1.3). This landscape is composed of moraine deposits and, with its steep slopes from 13 to 43% and the light reflected by the lake, it represents a very suitable environment for the growth of grapevines (Letessier and Fermond, 2004). The bedrocks are composed of Tertiary molasse deposits, which include conglomerates, sandstones and marls from the upper Oligocene epoch (23-28 Ma; Weidmann, 1988). These formations constitute the substrate of the Lavaux landscape, upon which, during the Quaternary, moraine materials were deposited by the Rhône glacier (see Appendix 1.1). More specifically, the area of Lutry is characterised by several

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[5] Source: http://www.lavaux-unesco.ch

faults separating the ‘coal molasse’ from the ‘red molasse’, respectively composed of sandy dark marls and silty red ones (Weidmann, 1988; Berger, 1998). All these geological formations, along with more recent landscape modifications, are at the origin of the large heterogeneity observed in soil profiles of the studied area.

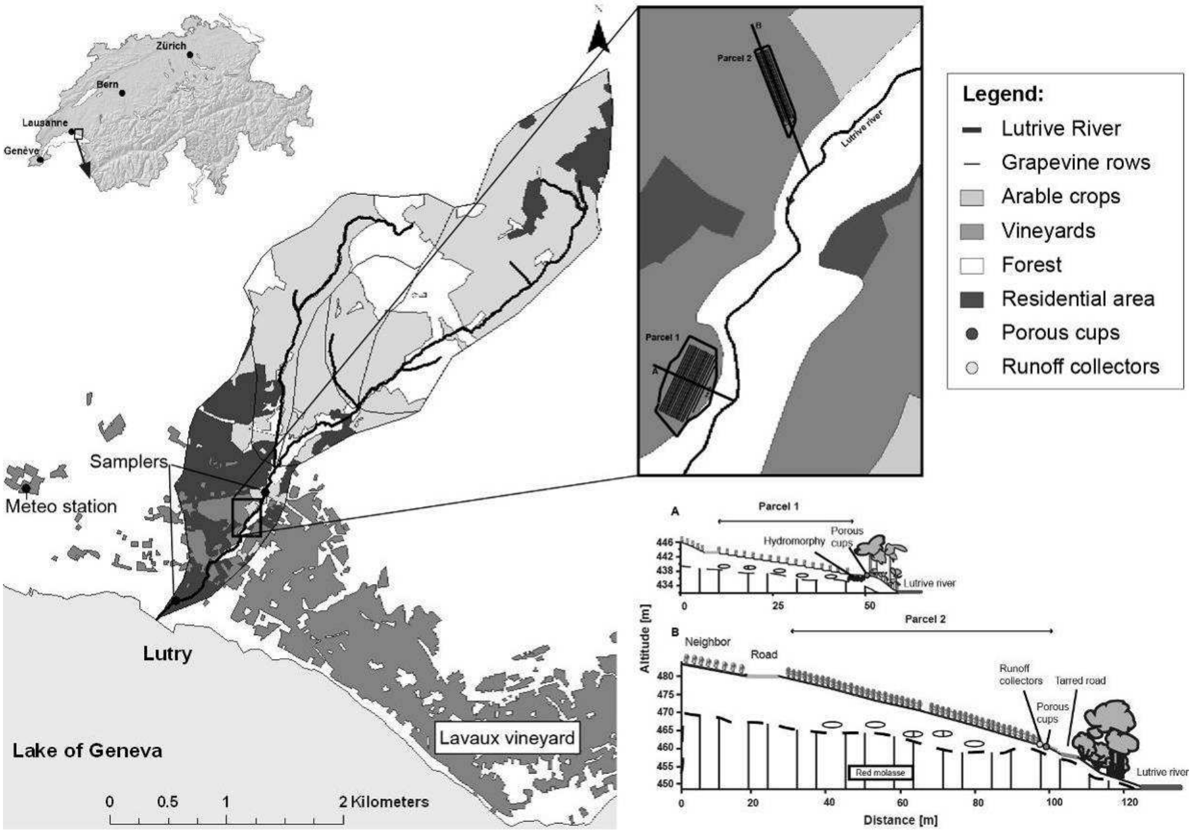

Figure 1.3: Lutrive watershed in western Switzerland with its different land use, the location of the Pully meteorological station and of the automatic samplers, as well as the one of the two investigated vineyard parcels and their transects with equipment at their bottom

Based on previous work dealing with risk assessment of pesticide transfer from vineyards to surface waters (Egger, 2009), two risky parcels were chosen in an area called ‘Flon-de-Vaux’ close to the Lutrive River (Fig. 1.3). The general character of the soils was obtained through drilling with an auger and digging two pits of a depth up to two meters (Fig. 1.5). Textures, colours and the presence of carbonates were noted in the field, whereas soil pH was measured in the laboratory on water extractions (Soil:H_2O=1:2.5). More precise grain size analyses by laser diffractometry (Malvern©, Mastersizer 2000) were obtained for samples of various depths at the bottom of both parcels, treated with HCl beforehand to remove carbonates (Emch and Pitteloud, 2010). Hourly precipitations of the Pully station (Fig. 1.3), located at 1.8 km from the parcel were obtained from Swissmeteo©.

Soils of both parcels are colluvial calcosols, according to the French classification (Baize and Girard, 1995), or calcaric cambisol, according to the FAO-WRB classification (IUSS, 2006), with anthropogenic influences (Fig. 1.4). The dominant colour, referring to the Munsell colour system

(Oyama and Takehara, 1992), was medium to light brown (10YR4/3) with a grey tendency (2.5Y5/3) in the deepest horizons. Both soils showed a *silt loam* texture and small differences were observed between plots and depths. HCl reactivity at all depths revealed the carbonate nature of soils, which was confirmed by pH-values between 8 and 9. Organic carbon content varied from the surface layer to deeper horizons between 1.7 and 0.7% in parcel 1 and 2.2 and 0.8% in parcel 2. Copper concentrations in top soils (0-30 cm) varied between 300 and 500 mg/kg in parcel 1 and 100 and 170 mg/kg in parcel 2 (Emch and Pitteloud, 2010).

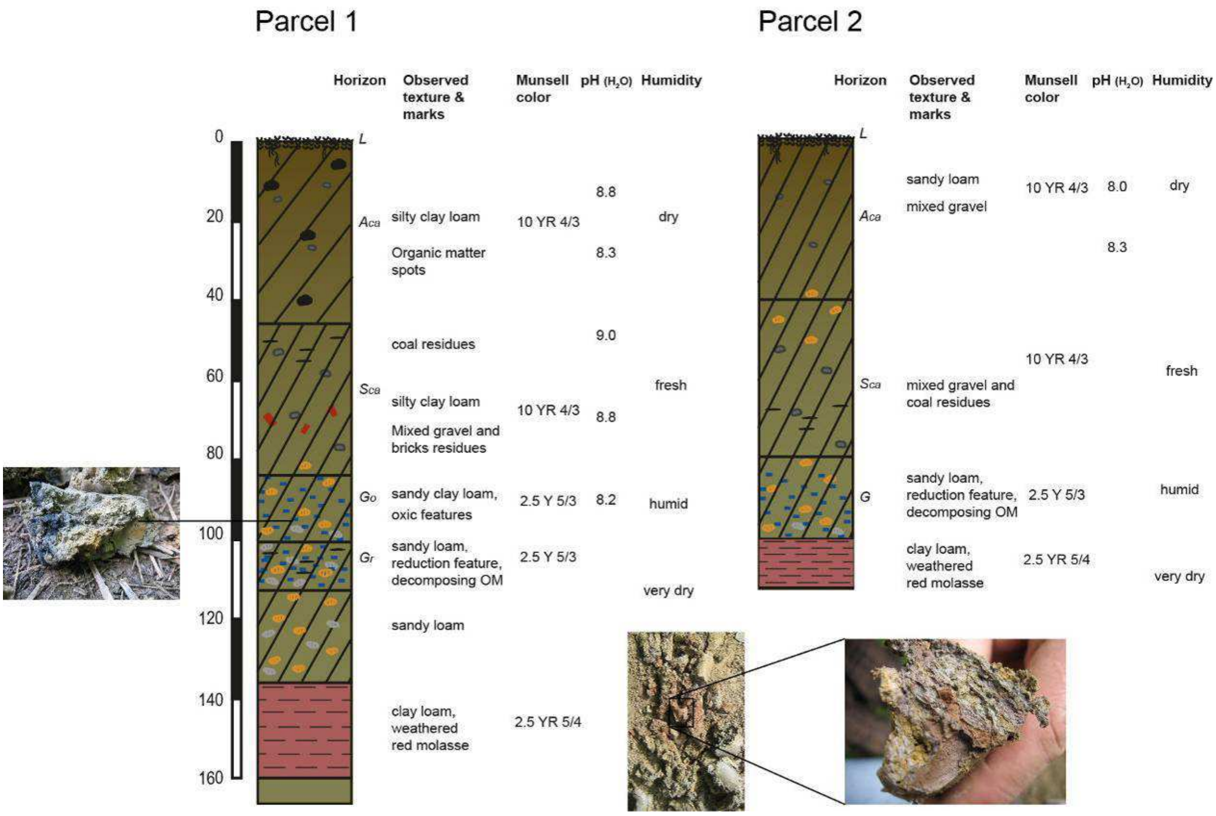

Figure 1.4: Description of soil profiles dug at the parcel bottom and observations.

Parcel 1 has a gentle slope of about 15% and the convergence of drainage waters induces temporary hydromorphism at its bottom, as already observed by Letessier and Fermond (2004). From 80 to 110 cm a more humid horizon with redoximorphic features was identified, more greyish in colour and the presence of iron oxides, and some decomposing organic matter (Fig. 1.4). A knife hole made in this latter horizon gave rise to pouring water with a constant flow of max. 1 dl/min (see Appendix 1.2). These observations, along with surface water stagnation during and after rainfall events, confirmed the hydromorphic nature of this parcel, i.e. the presence of a temporary perched water table. From 110 cm on, a more dry and clayey horizon was found and at 130 cm a reddish layer (2.5Y5/4) was observed, interpreted as the marls of the 'red molasse' beds cited before. This less permeable marly layer may explain the water saturation between 80 and 110 cm depth and the observed constant flow indicates the presence of subsurface lateral flows, known also as ‘throughflows’ or ‘interflows’, whether they reach directly or not a river or lake (Whipkey, 1978; Pidwirny, 2006). Velocities of these kinds of flows are usually slow: maximum flow rates occur on steep slopes and in permeable sediments with the lowest ones in flat soils, containing heavy clays.

Parcel 2 has a steeper slope (~25%) and drainage waters converge in the direction of the Lutrive River. As in parcel 1, a layer with a reducing hydromorphic regime was situated downwards from 80 cm depth, and at 115 cm a reddish layer was also encountered. In the middle of the plot, drillings revealed a more or less identical profile down to 85 cm depth, but the hydromorphic layer between 80 and 115 cm and the reddish horizon beneath were not always observed, suggesting a C-horizon consisting of dm- to m-sized discontinuous lenses of weathered marls (Fig. 1.3). All these observations are in agreement with the regional characterization of vineyard soils made by Letessier and Fermond (2004; see Appendix 1.3).

1.5.2 *Field equipment*

Both vineyard parcels were equipped at their bottom with four porous cups (SDEC SPS200, Ø63mm) at different depths: 20, 40, 60, 80 cm to get soil solution. Their implementation, in fall 2009, and utilization were done according to Bès and Sévenier (1999). They propose two different practical approaches: one is to induce an additional 0.3 bars tension to the hydraulic potential $\psi(\theta)$ of the concerned depth and the other is to apply always the same depression between 0.3 and 0.8 bars according to the soil nature. A 0.6 bars tension was always applied for each depth as soil is fine textured (*silt loam*).

As parcel 2 is more representative of the Lavaux region in terms of slope, it was fully equipped with complementary devices (Fig. 1.5). Three "hand-made" runoff collectors were built inspired from past studies (Schulz et al., 1998; Trevisan and Dorioz, 2001) and placed at the vineyard end in parcel 2, according to observed erosion paths. They were made up of a bottle buried in the soil, a funnel with a one mm sieve mesh on it to avoid macro fauna or big particles, a water conducting ramp made of PVC placed just under the root zone and ending above the funnel, and finally a roof to avoid direct rain inputs (see Appendix 1.4). Moisture capacitive sensors (SDEC HMS 9000) and tensiometers[6] (SDEC SMS 2500S) were installed at 20, 40, 60 and 80 cm depth, in duplicate for tensiometers (Fig. 1.5, Appendix 1.4). Data were recorded by an OTT Logosens-2 station-manager and retrieved from the laboratory via GSM-Data. All these instruments were powered by solar energy via a huge battery, located in a garden house with the station-manager and the GSM modem (Appendix 1.4).

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[6] Tensiometers are instruments made of a porous ceramic cup and a sealed plastic cylindrical tube. The ceramic cup represents a membrane through which water will move from the tensiometers to the surrounding soil matrix when they are subjected to negative soil water potentials. These latter induce a negative suction, known also as the matrix potential $\psi(\theta)$, in the tensiometers cylinder which is recorded in an *ad hoc* device at the top of it.

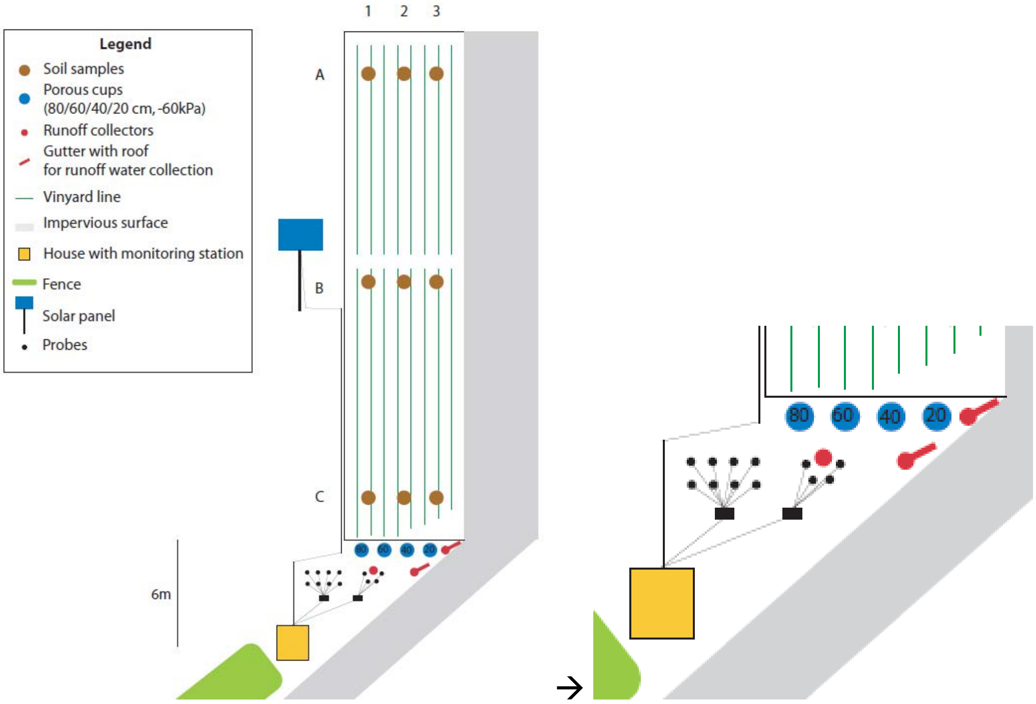

Figure 1.5: Sampling and monitoring devices in parcel 2: soil sampling points, the 4 porous cups at different depths, the 3 runoff collectors, as well as the 8 tension (black points; left down and right) and the 4 humidity (black points; left up) probes connected to a data logger in the house and powered by a solar panel

1.5.3 *Soil hydraulic properties*

Soil hydraulic properties were assessed in parcel 2 with different techniques thanks to the collaboration with the PROTEE laboratory from the University of Toulon-Var (USTV). Indeed, beside the assessment of soil texture, bulk density or other soil parameters that influence water fate in soils, infiltration and evaporation experiments should be performed *in situ* or in laboratory on undisturbed samples. This would allow the construction of the soil-water retention curve (Fig.1.6), which links soil water content (θ) with water potential (ψ), known also as soil suction or pressure head (h). This relation can be obtained for different soil horizons experimentally in the laboratory on undisturbed samples. It allows estimating the unsaturated hydraulic conductivity (K), which is difficult to measure because it is extremely variable in the field and its measure is time-consuming and expensive (Van Genuchten, 1980). This relation can be obtained in the laboratory, for instance with the Wind method (Wind, 1968), but also *in situ* with the implementation of humidity and tension[7] probes in the field. The determination of the hydraulic conductivity at saturation (K_{sat}) is easier to assess and allows the limit of infiltration rate of a soil to be determined (Musy and Soutter, 1991).

The silty texture of the soil was confirmed by laser granulometry and the horizon 80-90 cm of parcel 1 had a slightly higher clay content than the upper ones (Appendix 1.5), due to the presence of weathered marls at this depth. Bulk density of surface horizons was of 1.37 for parcel 2 and 1.17 for parcel 1, with a porosity of 44 and 50% (Appendix 1.6).

K_{sat} was approximated with a double ring infiltration experiment (Emch and Pitteloud, 2010; Appendix 1.7): with a mean infiltration rate of 3.6 mm/h, the saturated hydraulic conductivity (K_{sat}) was determined to be about 10^{-4} m/s, which is considered as representative of semi-pervious soils (Musy and Soutter, 1991). This was confirmed with the Wind method with a K_{sat} value of 1.8×10^{-4} m/s for the surface horizon, whereas the 60 cm horizon had a value of 4.4×10^{-5} m/s (de Carvalho, 2010).

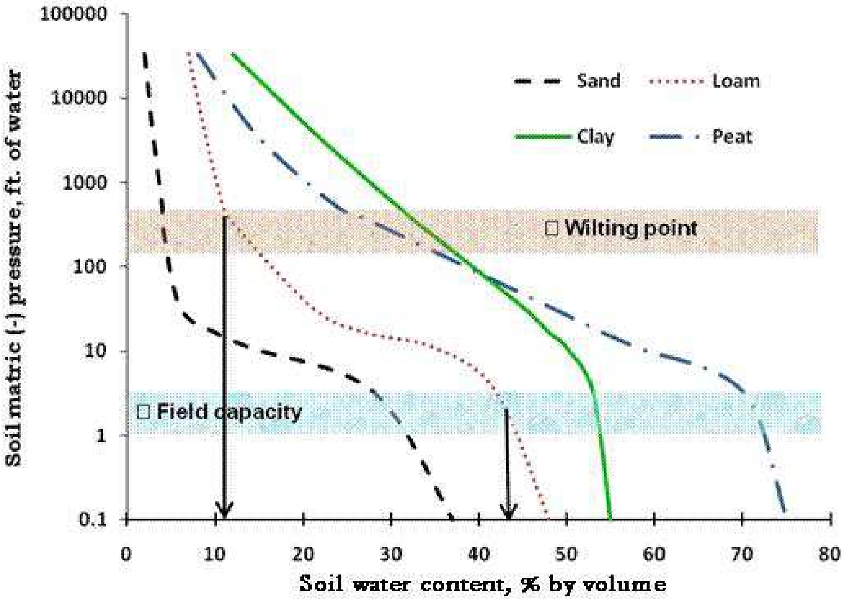

Figure 1.6: Soil-water retention curve for different soil types, with wilting point and field capacity regions (Source: NRCCA Soil and Water Management Study Guide; http://nrcca.cals.cornell.edu)

I*n situ* soil moisture content (θ; Fig. 1.7), temperature (Appendix 1.8) and water potential (ψ; Appendix 1.9) were monitored from April 2011 to April 2012. Monitored soil moisture content showed different patterns at the different depths during the growing season (Fig. 1.7). In general, a more reactive trend was observed in surface layers than in deeper ones, with an increase in moisture content after rain events and a decrease during dry periods. However, this general tendency showed some exceptions, for instance a more important increase at 60 cm has been noticed on June 9, 2011 from 25 to 40%. Moreover, some 'hydrologic events', with a rapid increase and decrease in moisture content after hourly precipitations of 10-15 mm, was detected at 80 cm several times: 06/18; 07/17; 09/05; 09/19. These events are proof of the presence of lateral flows in this soil horizon. As no significant increase was seen in the above layers, it can be concluded that the infiltration capacity of the bulk porosity of the soil was exceeded and the borrowing of macropore channels was assumed to take place further up in the parcel. Temperatures were similar at 20 and 80 cm, with naturally less

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[7] Soil tension or suction refers to the attractive forces exerted on water in soil voids.

variability at 80 cm, whereas they were five degrees less at 40 cm (Appendix 1.8). This corroborates the hypothesis of the influence of surface waters in the deep layer.

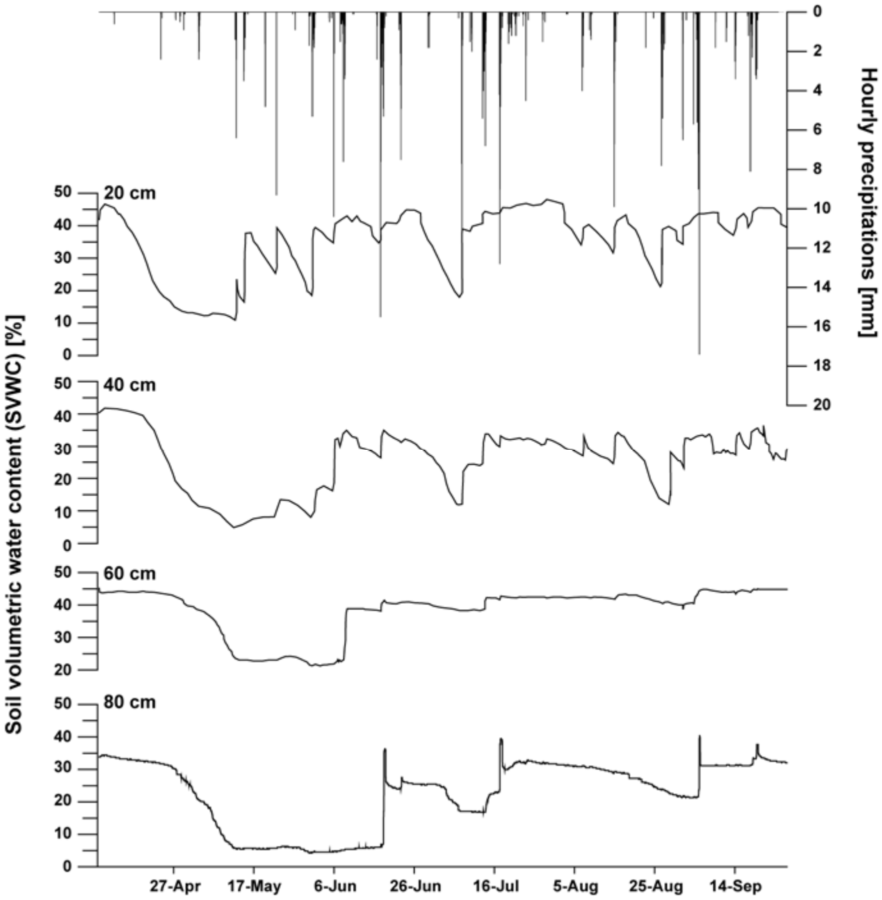

Figure 1.7: Soil volumetric water content (SVWC) at 20, 40, 60 and 80 cm from April to September 2011, with hourly precipitations obtained from the Pully meteorological station (Swissmeteo©).

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2 Environmental fate of pesticides with focus on the herbicide glyphosate

2.1 Introduction

The environmental fate of pesticides is reviewed in this chapter with first some definition and classification aspects. As complete knowledge of the huge amount of active substances used in agriculture is impossible, it consists solely of an overview of the main pesticide families and some of their properties. Second, their environmental fate is detailed, with the different parameters to consider in the processes they underwent after their application on the soil-plant system. In a third part, these processes are reviewed specifically for the widely used herbicide glyphosate, as well as a few eco-toxicological aspects.

2.1.1 *Definition*

Pesticides are defined as '*any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest*'[8]. They are mostly organic compounds produced to help farmers to control pests like weeds, insects, rodents or pathogens (Pierzynski et al., 1994). They are of great concern because of their high toxicity and potential threat to human and ecosystem health given their non-desired properties (Bliefert and Perraud, 2009).

2.1.2 *Classification*

The large diversity of pesticide molecules can be classified in two main different ways: a biological classification separating them by their targets (herbicides, insecticides, fungicides, etc.) or a chemical classification separating the different chemical families of substances showing distinct molecular nature. According to Calvet et al. (2005), the chemical classification makes a distinction between inorganic, organo-metallic and organic molecules. The two first classes of pesticides are mainly fungicides: inorganic fungicides are made of copper and sulphur like the famous Bordeaux mixture ($[Cu(OH)_2]_x$, $CaSO_4$) and organo-metallic fungicides are made of a complex between an organic anion (dithiocarbamate) and a metal (Zn and/or Mn). Organic pesticides are numerous and classified in different chemical families with specific properties dependent on functional groups and/or molecular structure. Other classifications of pesticides exist like the one proposed by Gevao et al. (2000), which separates the different chemical families of substances into ionic forms and non-ionic forms (Fig. 2.1). Though fate and persistence of pesticides molecules within the environment will depend not only on their ionic charge, but also on other physical and chemical properties like molecular structure, volatility, solubility and their reactivity towards other molecules especially enzymatic complexes (Yaron et al., 1996; Calvet et al., 2005), this latter classification was retained to present the main different types of pesticides below.

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[8] EPA : http://www.epa.gov/pesticides/about/index.htm, consulted December 15, 2008.

2.1.2.a Ionic pesticides

Ionic pesticides include two different kinds of pesticides: cationic pesticides and 'ionisable' or polar pesticides. These latter are characterized by functional groups that are either basic or acidic, and their environmental fate is strongly dependent on soil pH as they can be partially ionized within the natural range (4-9) (Kah, 2007). Their adsorption onto components of the soil depends indeed a lot on surface charges, where neutral pesticides are mainly adsorbed onto soil organic matter by hydrophobic interactions. Ionisable pesticides dissociate in solution and hence produce either H^+ or OH^- depending on whether they possess acidic or basic functional groups respectively. Dissociation, governed by the dissociation constant (pK_a), leads to the occurrence of anions for weak acidic molecules and partially protonate products for weak bases in the most common agricultural soil pH range (5-8) (Regnault-Roger et al., 2005).

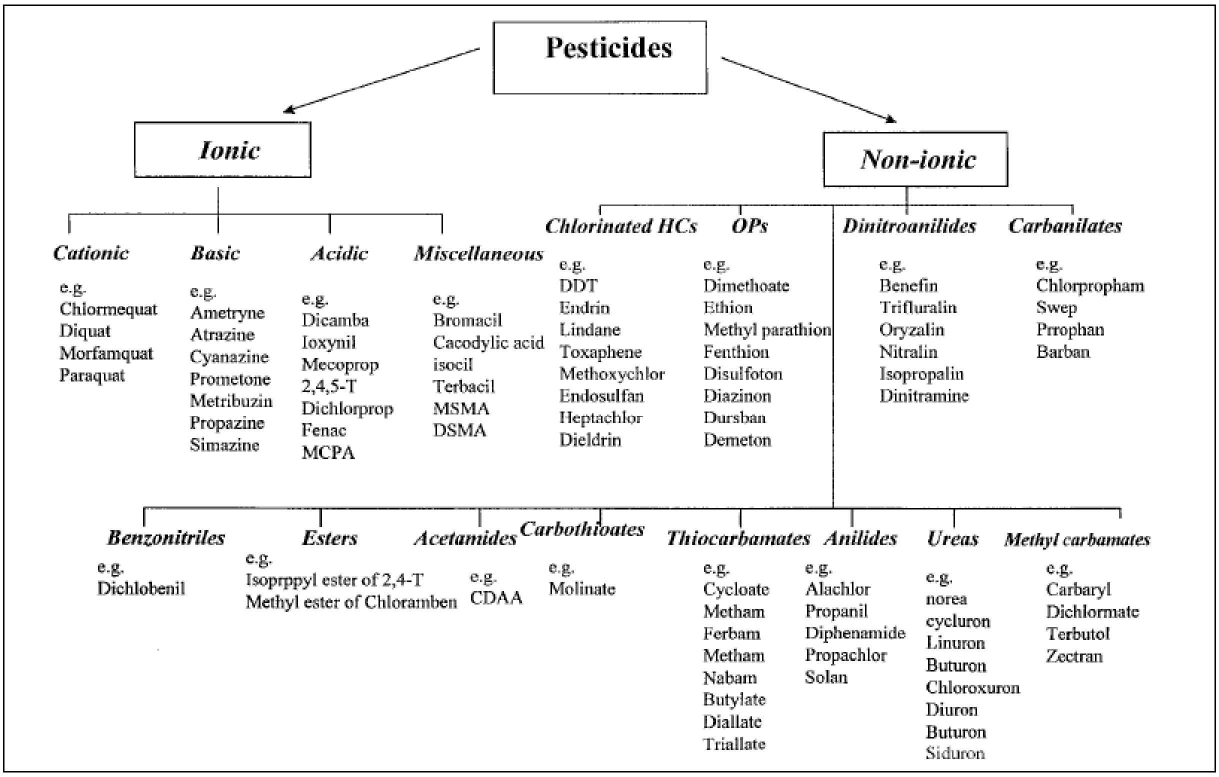

Figure 2.1: Classification of pesticides (Source: Gevao et al., 2000)

Cationic pesticides or highly basic pesticides, like the two main bipyridylium herbicides diquat and paraquat, are heterocyclic compounds characterized by a positive charge (Béland, 2006). These latter two contact herbicides are characterized by a high solubility in water and a low volatility and are subject to a rapid photolysis except when sorbed onto Particulate Organic Matter (POM) (Yaron et al., 1996). These kind of ionic pesticides are not sensitive to pH changes within natural soil pH range and thus will not dissociate because of their very low or high pK_a (Kah, 2007).

Basic pesticides, like several triazine type compounds, are composed of a ring structure containing nitrogen and carbon. Names are ending in a different way when the substituted group on the ring differs: *-azine* corresponds to a chlorine atom (R-Cl), *-tryn* to methylthio groups ($R\text{-}SCH_3$) and *–ton* to methoxy groups ($R\text{-}OCH_3$) (Yaron et al., 1996). Most of them are relatively volatile but their solubility is a function of the substituted functional group, with the methoxy substituted compounds having the highest solubility. Basic pesticides are also known to be electron donor and to form complexes with electron acceptor molecules, like OM, clays and Fe-/Al-hydroxides, when pH is lower than their pK_a (Béland, 2006). Functional groups involved in complexation possess N or S atoms like R-NH, $R\text{-}NH_2$, R-S or R-SH.

Acidic pesticides refer to several types of molecules: substituted phenols, chlorinated aliphatic acids, substituted benzoic acids, etc. (Yaron et al., 1996). They are characterized by the presence of carboxyl (R-COOH), thiols (R-SH) or phenolic (R-OH) functional groups able to be ionized in aqueous solutions leading to anionic moieties (Béland, 2006; Calvet et al., 2005). Their dissociation occurs when the pH of the solution goes above their pK_a and their adsorption is limited on OM and clays, because of repelling negative charges, and is more likely to occur on Fe/Al oxyhydroxydes which possess variable surface charges. Among acid pesticides, 2,4-D and 2,4,5-T are the two most used molecules and their structures include a phenyl ring bind to an aliphatic acid by an oxygen atom.

2.1.2.b Non-ionic pesticides

Non-ionic pesticides include a lot of different types of molecules, as shown in Figure 2.1, which do not ionize in aqueous media. All of these groups (chlorinated hydrocarbons (CHC), organophosphates (OP), carbamates, ureas, etc.) have different properties that will determine their fate in soils especially their adsorption behaviour (Yaron et al., 1996). This latter is more governed by the soil's constituents, especially SOM, than by the pH (Calvet et al., 2005; Béland, 2006). Indeed, it is proven that organic colloids play an important role in the adsorption processes of all non-ionic pesticides (Yaron et al., 1996; Fang et al., 1998; Wauchope et al., 2002), which are mainly adsorbed by Van der Waals forces and hydrophobic links (Béland, 2006; Kah, 2007).

Chlorinated HC are among the most known pesticides with the famous DDT[9] used during WWII against pests like typhus (Regnault-Roger et al., 2005), but include also lindane, chlordane, aldrin, dieldrin, etc. They exhibit a long persistence in soils and also relatively low water solubility (especially DDT) that is why their use decreased during the end of the last century (Yaron et al., 1996). The vapour pressure of CHC varies among the group from low (DDT, endrin) to very high (heptachlor) and thus DDT is relatively inert in soils with its low solubility and volatilization ability leading to a persistent pollution.

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[9] DDT : DichloroDiphenylTrichloroethane

Organ Phosphorus or organophosphate pesticides are less persistent in soils than CHC but more toxic to mammals and so they have greater solubility and vapour pressure. Thus, they replaced CHC in agriculture from the 50's on and are still the most used insecticides today (Regnault-Roger et al., 2005), maybe because of the large diversity of action mechanisms among the 150 OP numbered by A. Wood[10].

Carbamates are herbicides derived from the carbamic acid (R1-NH-COO-R2); they are characterized by a high specific selectivity (Yaron et al., 1996), which means that they have several activity types and can be used for some specific targets (Regnault-Roger et al., 2005). They can be divided into three categories but in two different ways depending on authors. Gevao et al. (2000) separate thiocarbamates like butylate, which contain a sulphur atom instead of oxygen, from methyl carbamates like carbaryl, which structure is characterized by the occurrence of a methyl group and carbanilate. Regnault-Roger et al. (2005) separate carbamates in three groups: C-cycle carbamates (like carbaryl), heterocyclic carbamates (like pyrimicarb) and aliphatic carbamates (like aldicarb).

Ureas are characterized by their no selectivity and when applied to soils, they reach the upper part of the plant via the roots. Substituted ureas are ureas with three H-substitution with some different groups: phenyl, methyl and/or methoxy (Yaron et al., 1996).

2.2 Environmental fate of pesticides

Fate of pesticides in the environment is extremely complex and depends on a large amount of parameters. They are generally applied on the soil-plant system where chemical, physical, and biological processes take place and influence their retention and their degradation in this environmental compartment as well as their transport to surface and ground waters (Gavrilescu, 2005; Holvoet et al., 2007). These three different types of processes controlling pesticide behaviour are summarized in Table 2.1.

Furthermore, mobility of pesticides in soils is mainly dependent on three factors: their physicochemical properties like water solubility, sorption ability, persistence, etc.; the soil characteristics like texture, permeability, depth, pH, SOM, topography, etc.; and the weather conditions like precipitations, temperature, etc. (Calvet et al., 2005; Holvoet et al., 2007; Gavrilescu, 2005).

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[10] http://www.alanwood.net/pesticides/class_insecticides.html

Table 2.1: Three types of processes influencing pesticides fate, adapted from Calvet et al. (2005)

Transport	Retention	Degradation
Runoff	Partitioning	Physical
Leaching	Volatilization	Chemical
Colloid-associated transport	Sorption	Biological
Capillary flows	Uptake	

After their transport within the soil system, the pollutants can be transferred into surface or ground waters leading to a potential threat for organisms or a pollution of drinkable waters. Within the surface water, they interact with aquatic organisms but also with river sediments, influencing also their retention and degradation as summarized in Figure 2.2.

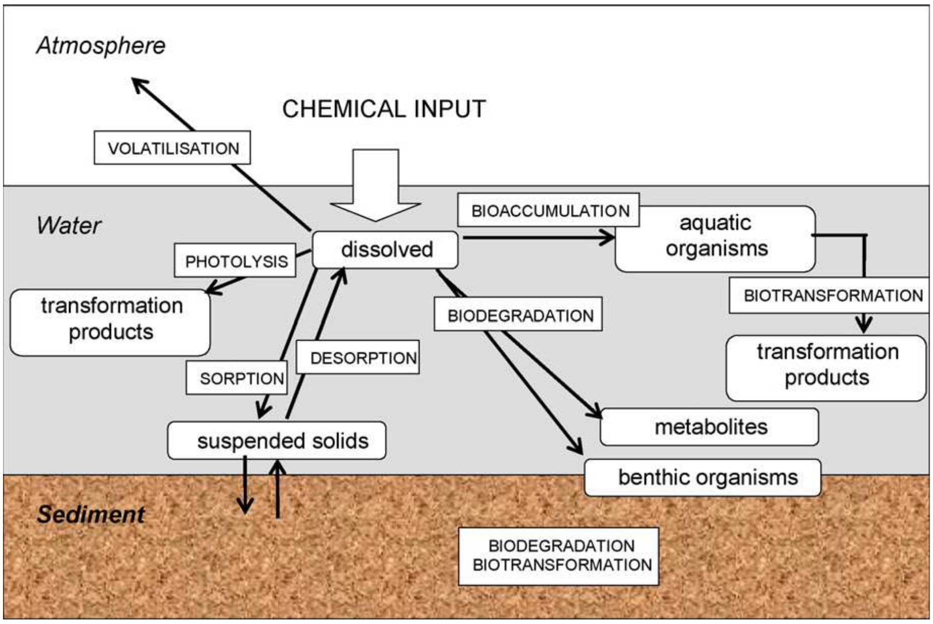

Figure 2.2: Processes governing pesticides fate in surface waters (Source: Holvoet et al., 2007)

Fate of pesticide within the soil system is largely governed by transport phenomena in the aqueous phase as well as the processes of binding and immobilization on the solid phase, *i.e.* particle surfaces. Soil solid constituents are controlling not only transport of pollutants, but also to a large extent their retention, release and surface-induced chemical degradation, especially those with a high surface area like clays and organo-mineral complexes (Yaron et al., 1996). Thus, retention processes are maybe the most important processes when assessing the fate of one pesticide within soils because it influences all other processes (transport and degradation) (Stevenson, 1994 *in* Béland, 2006). Theoretical background commonly used to assess these three types of processes is given thereafter, with first on the retention of pesticides in soils, then on the degradation they underwent after their application, and finally on the different transport processes they can be subject to in the environment.

2.2.1 *Partitioning and retention of pesticides*

Environmental partitioning is a term referring to the transfer dynamic of one substance of interest from one medium to another within the environment. More precisely, it deals with partitioning of chemicals among a soil-water-atmosphere interactive system. Transfers of organic compounds like pesticides between these three different phases will depend mainly on three factors:

1. Properties of the molecule
2. Nature of the environmental compartment
3. Temperature

In addition to their own properties, the composition and the temperature of the medium in which pesticide molecules evolve will determine their reactivity in this medium, known also as fugacity or chemical potential. Fugacity can indeed be defined as "*the measure of the tendency of a component to escape from a phase*"[11]. Boiling points, vapour pressure, octanol-water partition coefficient (K_{ow}), are some of the main properties to take into account when assessing the fugacity of one pesticide in environmental compartments. For example, hydrophobic compounds with a high K_{ow} coefficient will preferentially accumulate in soils or sediments with a high content in organic carbon, where more volatile compounds will reside more in soil gases and in the atmosphere than other compartments. Thermodynamics "*deals with energy levels and transfers of energy between systems and between different states of matter*"[12]. It is important to consider energy levels of molecules like pesticides because they will determine their ability to move from one phase to another in environmental systems, as well as their transformation in other molecules *i.e.* metabolites (Schwarzenbach et al., 1993).

Retention of pesticides is characterized by their ability to resist to a phase transfer within the soil system, which will depend largely on their properties, such as molecular structure, electrical charge and solubility. Though pesticide properties are important in their retention, they cannot be considered alone. Thus, for adsorption phenomenon, known as a transfer from fluids (water or gas) to a solid phase, soil parameters - organic matter and clay contents, moisture, texture, structure, pH and T° - have to be considered as well. For instance, an increase in soil temperature (T°) and moisture content (θ) leads generally to a decrease in adsorption rates or coefficient (K_d) (Kah, 2007). These relations can be explained by the exothermic nature of adsorption for the influence of temperature and by the increased competition with water molecules for sorption sites and/or a decrease in pesticide solubility because of its higher concentration. However, this trend is not always found and the influence of T°

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[11] Mackay and Peterson, 1981 *in* Binelli and Provini 2003 chemosphere 53 143-151.
[12] Anderson, G.M. (1996) Thermodynamics of Natural Systems, John Wiley & Sons, Inc. ed.

and θ on sorption seems to be complex and variable. Ionic strength is also a chemical parameter that influences ionic pesticide sorption but seems to have not much effect on uncharged molecules (Kah, 2007).

Pesticide retention in soils is not irreversible and they can be released into the soil liquid or gaseous phase by desorption from solid surfaces. A change in physicochemical properties of the soil solution in the close neighborhood of the soil aggregate that retains the pesticide molecules represents the reason of their release (Yaron et al., 1996). Theoretically, the release would have a similar isotherm than for the retention, but it is often not the case and a part of the retained molecules are not transferred back into solution: this phenomenon is known as 'hysteresis'. Hysteresis is thought to be partly the consequence of a slow diffusion of the adsorbed phase into organic matter complexes (Bewick, 1994), leading to an immobilization within the soil matrix (see §2.2.1.f).

The volatilization phenomenon is presented thereafter (§2.2.1.a), followed by the presentation of the classical tools to assess pesticide retention, adsorption isotherms (§2.2.1.b), then the influence of molecule properties (§2.2.1.c) and those of the soil constituents (§2.2.1.d) on the adsorption mechanisms (§2.2.1.e) will be discussed in details. Finally, the formation of pesticide bound residues and their uptake in organisms is discussed (§2.2.1.f).

2.2.1.a Volatilization

Volatilization is the transfer from solid or liquid phase into a gaseous or a vapour phase (Yaron et al., 1996). The volatilization potential for a pesticide can be appreciated by its Henry's law constant: H = vapour pressure/solubility. Although the vapour pressure is important for the ability to change the state, several other parameters are needed to assess volatilization phenomena: soil adsorption ability (and thus soil composition), humidity, temperature, and air fluxes (Regnault-Roger et al., 2005). Volatilization can be assessed by two distinct processes: vaporization and diffusion (Yaron et al., 1996). Vaporization is characterized by a phase change that can be thermodynamically described by a relation relating molar heat of sublimation (Hs) to these of fusion (Hf) and vaporization (Hv): $\Delta Hs = \Delta Hf + \Delta Hv$. On the other hand, diffusion is the dispersion of the vapours into the overlying atmosphere and can be expressed as a flux: $IF = kz\ (dp/dz)\ z$. The method of application is of great concern because of wind strength and potential drifts that can lead to pollution of adjacent water.

2.2.1.b Adsorption isotherms and coefficients

Numerous studies have been done on the sorption processes using the common batch or slurry method (Boivin et al., 2005; Lennartz and Louchart, 2007), which consist of an equilibrium technique measuring the amount of pesticide adsorbed from an initial concentration within the liquid phase by a determined mass of soil (Weber et al., 2004). The ability of a pesticide to be adsorbed by soil's particles is expressed by its adsorption coefficient which is $K_d = q/c$, where *q* is the concentration of pesticide adsorbed in the solid phase (g Kg^{-1}/g g^{-1}) and *c* is the concentration within the liquid phase at equilibrium (Wauchope et al., 2002; Frankki, 2006).

Sorption dynamics can be modelled in different ways using isotherms[13] (Fig. 2.3): Linear isotherm (Eqn. 1) describes a hydrophobic partitioning mechanism as "*a constant relationship between the concentrations of free analyte and sorbed compound*"[14]. Freundlich isotherm (Eqn. 3) is a non-linear empirical model including a *n* factor that deviate it from a linear model, except when $n = 1$. In these two cases, sorption ability, i.e. sorption sites, is thought to be infinite. However, as environmental surfaces are known to possess finite sorption sites, Langmuir isotherm (Eqn. 2) has been developed including the maximum adsorption capacity (q_{max}). It also includes a K_L constant that expresses the binding affinity. It describes a high affinity at the beginning of adsorption and a decrease in adsorption intensity as more binding sites are occupied followed by a final saturation.

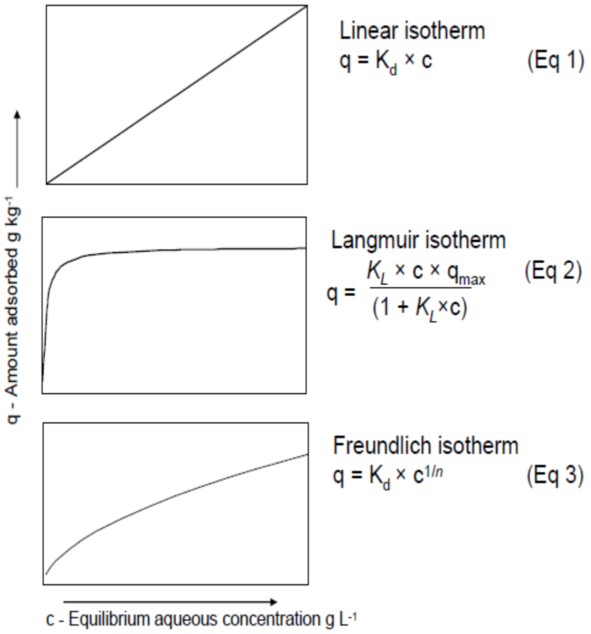

Figure 2.3: Linear, Langmuir and Freundlich sorption isotherms (Frankki, 2006)

As the soil organic matter (SOM) is assumed to be the main sorbent in soils because of its non-polar nature (Wauchope et al., 2002; Wu et al., 2004; Lennartz and Louchart, 2007), the adsorption coefficient is often normalized by the soil organic carbon fraction (F_{oc}) and becomes $K_{oc} = K_d/F_{oc}$. High K_{oc} values (>500) indicate a strong sorption ability, whereas low values (<500) show a clear tendency to leach or runoff (Wauchope et al., 2002). Beside these experimental predictions of how a pesticide will adsorbed on the soil matrix, there are several theoretical views and related parameters that have to be considered. For instance, the moisture content which implies a competition for binding sites on clays or SOM (Gavrilescu, 2005). Furthermore, expendable clays like smectite can become, in some cases, a more dominant sorptive phase than SOM, what implies to reconsider the K_{oc} use as universal adsorption coefficient. Indeed, some authors pointed out that K_{oc} is limited to non-ionisable pesticides and more polar compounds will adsorbed on other surfaces than SOM such as clay minerals or metal oxides (Gerstl, 2000; Wauchope et al., 2002). Furthermore, considerable variations in K_{oc} values for the same compound are still obtained (Gerstl, 2000; Warren et al., 2003). There are two

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[13] An isotherm can be described as "*a relation between the amount of an environmental chemical adsorbed per unit weight of adsorbent and the concentration of the environmental chemical (solute) in the solution at equilibrium*" Choudhry, G.G. (1984) Humic Substances.

[14] Frankki, S., (2006) Association of Organic Compounds to Dissolved and Particular Natural Organic Matter in Soils, Department of Forest Ecology, Faculty of Forest Sciences. Swedish University of Agricultural Sciences, Umea, p. 52.

major explanations: 1) the K_{oc} calculation is generally based on an average value of the OM content of a soil, 2) the nature of SOM can directly influences the partitioning of the compound. Indeed, due to a better hydrophobic environment, less polar OM gives higher K_{oc} values than more polar OM (Warren et al., 2003).

2.2.1.c Sorption and pesticides properties

Most of organic pesticides are compounds with low molecular weights and exhibit low water solubility (Khan, 1978). Logically, a pesticide with lower water solubility is more adsorbed than one with higher water solubility. One of the most important pesticide property governing its adsorption is its charge characteristics as the molecule can be polar or ionic (Khan, 1978). As far as hydrophobic pesticides are concerned, sorption can be related to a partitioning between a polar aqueous phase (soil solution) and a non-polar solid phase (mainly OM) and thus significant correlations have been found between sorption coefficient (K_d) and the octanol-water partition coefficient (K_{ow}) (Calvet et al., 2005; Kah, 2007). This latter coefficient determines the uptake and translocation of one molecule in plants, as well as its potential bioaccumulation in organisms, and, for ionisable compounds, it varies with pH (Chamberlain et al., 1996). While sorption of hydrophobic ionisable pesticides is pH dependent, as far as the degree of dissociation is "*a function of the dissociation constant and the pH of the soil solution*"[15], sorption of neutral compounds depends on OM content as their polarization is influenced by the availability of π electrons, which means that the presence of aromatic groups plays a key role in their adsorption on charged surfaces (Khan, 1978). Thus, pesticides molecular structure influences their sorption: depending on the nature of functional groups and substituting groups, the position of substituting groups as well as the presence and amount of unsaturated bonding, their chemical behaviour towards soil constituents will be different.

Cationic pesticides, like paraquat or diquat, generally have Langmuir isotherms when sorbed to SOM. However, surface charge density and cation charge spacing, as well as steric hindrance may be factors influencing the competition of the two insecticides for sorption on HS (Khan, 1978). *Basic* pesticides like s-triazine herbicides, generally protonate and are then adsorbed on negatively charged surface like SOM. Their adsorption on SOM is pH-dependent with a decrease of protonation at high pH and therefore the maximum adsorption is reached near the pK_a of the molecules (Khan, 1978). *Acidic* pesticides sorption on SOM involving the ionization of carboxyl or phenolic groups is lower than for cationic or basic molecules. *Nonionic* pesticides include a wide range of different chemical families with specific properties (CHC, OP, phenylureas, etc.), which largely govern their adsorption (Khan, 1978). Thus, pesticides properties influence largely their retention behaviour; however it will also depend on soil constituents (see below).

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[15] Kah, M., (2007) Behaviour of Ionisable Pesticides in Soils, Environment Department. University of York, York, p. 229.

2.2.1.d Sorption and soil constituents

Organic pollutant partitioning and sorption mechanisms are thought to be largely governed by the nature of soil's constituents and their surface type and activity (Yaron et al., 1996; Kah, 2007). Soil constituents involved in sorption of organic contaminants are mainly Soil Organic Matter (SOM) and clay minerals with their negatively charged surfaces. Soil Organic Matter (SOM) is indeed though to be one of the largest tributary to pesticide sorption in soils (Wauchope et al., 2002; Wu et al., 2004), especially for the hydrophobic partitioning of non-polar pesticides (Kah, 2007). SOM can be defined as a complex mixture of materials that can be classified in two categories (Senesi and Loffredo, 1999):

- A non-humic fraction composed of carbohydrates (polysaccharides), N-compounds (amino acids, proteins, etc.), P-compounds, S-compounds and lipids (waxes and resins).
- A humic fraction composed of Humic Substances (HS): Humic Acids (HA), Fulvic Acids (FA) and Humin (H).

Humic substances (HS) are not structurally identified but consist "*of polyelectrolytic organic acids having a wide range of molecular sizes, and being macromolecular*"[16]. HS are indeed thought to be constituted of relative low-molecular mass moieties bound together mainly by H-bonding and hydrophobic links (Sutton and Sposito, 2005). In aqueous solutions those interactions lead to the formation of micelles, with interior hydrophobic regions isolated from solutions by external hydrophilic parts, which have been proved to enhance the solubility of some organic pollutants. Because of their unknown structural complexity and the large diversity of their functional groups, HS are indeed able to create complexes with biocides in several ways (see §2.2.1.e), such as: ionic interactions, H-bonding, Van der Waals forces, Ligand exchange, hydrophobic partitioning, covalent bonding or sequestration (Jones and Bryan, 1998; Gevao et al., 2000). These interactions depend on both pesticide and HS properties and can occur simultaneously depending on the type of functional groups and the soil acidity (Gevao et al., 2000). Indeed, pH, as well as ionic strength and hydrophobic interactions, are known to change the conformational state of HS and thus influence sorption mechanisms (Martin-Neto et al., 2001). Furthermore, pH governs "*the ionization of the acidic functional groups on organic matter which may be available for cation exchange*"[17] expressed by their dissociation constant (pK_a). For instance, carboxyl groups, which are among the main functional groups involved in pesticides sorption, are known to be completely ionized at pH 7 (Thurman, 1985).

Dissolved Organic Matter (DOM) is defined as the part of the NOM smaller than 0.45µm composed of molecules of relatively low Molecular Weight (MW) (Zsolnay, 2003). DOM is often divided in two different fractions (Leenheer, 1981 *in* Cilenti et al., 2005):

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[16] McDonald, S., Bishop, A.G., Prenzler, P.D., Robards, K. (2004) Analytical chemistry of freshwater humic substances. Analytica Chimica Acta 527, 105-124.

- A hydrophilic fraction composed of organic acids, polyhydroxy phenols, carbohydrates, amino acids and amino sugars
- A hydrophobic fraction with hydrocarbons, fatty acids, nucleic acids and quinones

It is proved that DOM can increase the solubility of extremely insoluble organic compounds, such as pesticides like DDT, and by the way their mobility (Chiou, 2002), though it is not always the case (Pennington et al., 1991; Franco, 2001). Therefore, the potential enhanced leaching of contaminants (see §2.2.3), which depend on retention/mobilisation dynamic, can be influenced, beside soil and their properties, by the nature of DOM, *i.e.* its humification degree (Franco, 2001; Haberhauer et al., 2002). However, retention of DOM can also occur on solid surfaces like clay minerals and/or sesquioxides, thus reducing the availability of active site for pollutants (Kaiser and Zech, 2000; Zsolnay, 2003).

SOM-pesticides-soil minerals: a triple interaction

Sheng et al. (2001) reported the conclusions of several studies which were that sorption of neutral compounds on clay mineral surfaces are of little occurrence compared to SOM as the latter ones are polar and preferentially adsorbed water molecules. However, SOM is not the only sorbent phase in soils especially when more polar solute is concerned and/or soil OM content is low. Indeed, ionic pesticides, despite NOM, may also bind to clay and Fe/Al (hydr)oxides (Kah, 2007). Furthermore, clay minerals can become the preferred sorbent when soil moisture content is low (Wauchope et al., 2002) and/or as 2:1 clay types are concerned, because of their high surface areas and Cation Exchange Capacity (CEC) (Sheng et al., 2001). Clay mineral surfaces are generally negatively charged and the adsorption of charged solutes is governed by an electrochemical system where possible interactions are of both chemical and electrical nature (Yaron et al., 1996). The phyllosilicate nature and/or the type of surface's functional groups are also influencing the surrounding water's configuration by their charge distribution and the complexes formed with cations (Calvet et al., 2005). For 2:1 clay type, the nature of the interlayer cation plays certainly an important role in adsorption of pesticides. Coquet et al. (2004) showed that the clay content explained appropriately the adsorption coefficient (K_d) variations of some molecules on different subsoil materials, in this case atrazine, isoproturon and metamitron, and an adsorption coefficient normalized by the clay content (K_{clay}) has been proposed.

As soil matrix is composed of an heterogeneous mixture of Humic Substances (HS), clay minerals, metal oxides, $CaCO_3$, etc., some surface's coating can occur reducing or enhancing the ability of soil constituents to interact with contaminants (Khan, 1978; Franco, 2001; Ahangar et al., 2008). Indeed, because of their heterogeneous chemical nature, HS may bind to mineral surface in several different ways (ligand exchange, cation bridging, H-bonding and hydrophobic interaction) and act as a catalyser or an inhibitor of hydrophobic organic contaminant (HOC) sorption (Murphy and Zachara, 1995).

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[17] Khan, S.U., (1978) The interaction of organic matter with pesticides, in: Schnitzer, M., Khan, S.U. (Eds.), Soil Organic

Furthermore, these different reactions may take place simultaneously and geochemical conditions, like pH or ionic strength, may influence both sorption of HS to mineral surfaces and sorption of HOC to organo-mineral complexes. Sorption of HS increases when lowering pH because of the protonation of mineral surfaces which can lead to stable inner-sphere complexes (see §2.2.1.e), by a ligand exchange mechanism involving HS carboxylate (HS-COOH) and mineral surface hydroxyls (MS-OH) (Murphy and Zachara, 1995). Though the large diversity of possible interactions between humic substances and soil mineral constituents is of great importance in soils and sorption sites may be already occupied for exogenous organic compounds such as pesticides, organo-mineral complexes may remain sorbents for them (Khan, 1978). Considering both organic and inorganic soil constituents, their nature and reactivity, as well as the physical and chemical properties of pesticides, it is really difficult to experimentally assess the triple interaction between SOM, soil minerals and pesticides. However, summarizing several studies, Murphy and Zachara (1995) lead to the conclusion that HOC binding to mineral-associated HS involves a hydrophobic adsorption and that pH, ionic strength and cation valence are important variables influencing mineral-bound HS configuration and reactivity. In general, bound HS act as "*a hydrophobic "film", enhancing the apparent* [adsorption] *of HOC by the mineral phase*"[18]. However, the presence of divalent cations like calcium can reduce this apparent sorption enhancement by inducing a more compact structural conformation of HS "*through carboxylate site saturation and intersite bridging*"[18].

2.2.1.e Sorption mechanisms

Table 2.2 summarizes the different possible interactions involved in adsorption of organic compounds and the involved functional groups.

Cation exchange is a mechanism involved for cationic pesticides sorption on negatively charged surfaces of clay minerals and OM. Triazines, often in a protonate form, as well as positively charged bipyridylium like diquat or paraquat can interact with negatively charged sites of HS mainly carboxylate groups (Senesi, 1995 *in* Kah, 2007). *Anion exchange* is the opposite and involves anionic species and a positively charged site on soil surface. While anion exchange occurs mainly in tropical soils containing positively charged Al or Fe (hydr)oxides, cation exchange is typically of temperate soils with negatively charged constituents (Kah, 2007).

Charge transfer is an important mechanism which needs to be taken into account. It refers to an electron donor-acceptor mechanism known also as π-π reactions, *i.e.* electron exchanges with a relative overlapping of molecular orbitals (Kah, 2007). HS are here of concern because of the presence of chemical groups within their structure with different electron density leading to preferential

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Matter. Elsevier science Publishers B.V., Amsterdam, pp. 137-171.

[18] Murphy, E.M., Zachara, J.M. (1995) The role of sorbed humic substances on the distribution of organic and inorganic contaminants in groundwater. Geoderma 67, 103-124.

exchange: "*the formation of charge transfer complexes has been postulated as the possible mechanism involved in the adsorption of s-triazines onto soil organic matter*"[19].

Table 2.2: Mechanisms of adsorption for organic compounds in soil solutions (Source: Yaron et al., 1996)

Mechanism	Principal organic functional groups involved
Cation exchange	Amines, ring NH, heterocyclic N
Protonation	Amines, heterocyclic N, carbonyl, carboxylate
Anion exchange	Carboxylate
Water bridging	Amino, carboxylate, carbonyl, alcoholic OH
Cation bridging	Carboxylate, amines, carbonyl, alcoholic OH
Ligand exchange	Carboxylate
Hydrogen bridging	Amines, carbonyl, carboxyl, phenylhydroxyl
Van der Waals interactions	Uncharged, nonpolar organic functional groups

Protonation can be related to a charge transfer from a base to an acid, leading to a ionic moiety which can than be involved in an ion exchange adsorption (Khan, 1978). Thus, basic pesticides may protonate and than be adsorbed on OM dependent on the pH as this latter parameter governs the ionization of the acidic functional groups of OM, *i.e.* their availability for cation exchange. Indeed, basic compounds having a pK_a greater than soil pH may accept a proton and become cationic (Kah, 2007). Furthermore, soil organic matter may exhibit a pH as much as two units lower on its surface than within the near soil solution and hence basic pesticides protonate even if their pK_a is smaller than the pH of the aqueous solution (Khan, 1978).

Cation bridging is characterized by "*the formation of an inner-sphere complex between an exchangeable cation at a clay or OM surface and an anionic or polar functional group on a pesticide*"[20] (see Fig. 2.4). It means that the cationic moiety has been able to create a covalent binding with an organic functional group of the pesticide avoiding or displacing the surrounded water (Kah, 2007). *Water bridging* is referring to the situation where the surrounded water is not avoidable and the covalent link unfeasible, thus an ionic interaction occurs between the proton of the exchangeable cation and the organic functional group of the pesticide leading to an outer-sphere complex (Kah, 2007).

Ligand exchange is a replacement mechanism of weak ligands, like water, that hold polyvalent cations associated to soil components by adsorbent molecules like pesticides (Khan, 1978; Kah, 2007). The initial condition is therefore that the adsorbent must be a stronger chelating agent than that of the replaced ligands (Khan, 1978). This mechanism is thought to happen in two steps: the rapid formation of an outer-sphere complex and the slower rearrangement of bonds leading to an inner-sphere complex (Kah, 2007).

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[19] Khan, S.U., (1978) The interaction of organic matter with pesticides, in: Schnitzer, M., Khan, S.U. (Eds.), Soil Organic Matter. Elsevier science Publishers B.V., Amsterdam, pp. 137-171.

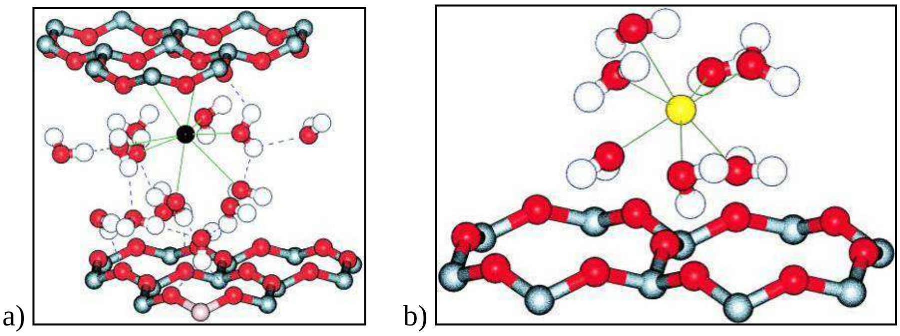

Figure 2.4: a) Inner-sphere complex (ISC) of K^+ and b) outer-sphere complex (OSC) of NA^+ in the interlayer region of a montmorillonite clay mineral (Source: Sposito et al., 1999)

Hydrogen bridging is a dipole-dipole interaction between the electropositive nature of the H nucleus of some functional groups (like –OH and –NH) and the electronegative one of atoms like N, F, O or oxygen containing functional groups of the sorbent surface (Kah, 2007). Hydrogen bonding can be considered as "*a partial charge transfer between two electronegative atoms, one being held by covalent bond and the other by electrostatic forces*"[21]. For instance, H-bonding greatly contributes to adsorption of several non-ionic polar pesticides on SOM, such as substituted ureas and phenylcarbamates, or ionic polar ones at pH values below their pK_a, like 2,4-D or atrazine (Gevao et al., 2000).

Van der Waal interactions are weak interactions "*comprising short-range dipolar or induced-dipolar attractions*"[20] and are thought to be involved in the adsorption of non-ionic and non polar pesticides (Khan, 1978). Though experimental evidence are scarce and they are thought to be weaker than H-bonding, they were suggested to be involved in the adsorption of many compounds, like 2,4-D or picloram (Gevao et al., 2000; Kah, 2007).

To all these different interactions the hydrophobic sorption or partitioning must be considered as well because it is thought to be "*the main mechanism for the retention of non-polar pesticides by hydrophobic active sites of HS or clay*"[22]. Indeed, HS possess within their structure some hydrophobic sites, mainly lipids which derived from waxes and/or resins: 50% of the aliphatic structures of HS are fatty acids esterified to phenolic groups (Khan, 1978). It has been shown to be an important mechanism in the adsorption of DDT and other organochlorine insecticides, as well as of the herbicide metolachlor on SOM (Gevao et al., 2000). Another phenomenon is the formation of hydrogen bonds within humic polymers leading to the creation of internal voids that can trap hydrophobic molecules, which can become preferential sorbents for neutral pesticides. Hydrophobic sorption is known as a

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[20] Kah, M., (2007) Behaviour of Ionisable Pesticides in Soils, Environment Department. University of York, York, p. 229.
[21] Khan, S.U., (1978) The interaction of organic matter with pesticides, in: Schnitzer, M., Khan, S.U. (Eds.), Soil Organic Matter. Elsevier science Publishers B.V., Amsterdam, pp. 137-171.

pH-independent mechanism: pH changes can induce conformational modifications of HS and thus affect the amount of hydrophobic sites. This can be referred to ‘*steric effects*’, which are related to the space needed by each moiety of one molecule, leading sometimes to a change in the molecule conformation and thus in its reactivity[23].

2.2.1.f Bound residues, ageing phenomenon and uptake by organisms

Bound residues are assumed to be “*compounds* […] *which persist in the* [soil] *matrix in the form of the parent substance or its metabolite(s) after extraction* [by] *method* [which do] *not significantly change the compounds themselves or the structure of the matrix”*[24]. Therefore, the extractability is operationally defined by the nature of the extractant and the experimental conditions. Sorption phenomenon can indeed lead to a stabilization/sequestration process with an immobilization of the chemical within the soil matrix, which can be reversible with changing conditions depending on the nature of the binding (Gevao et al., 2000). Somehow, the formation of bound residues can be associated with the ‘ageing’ process, which is in concern when contact time between a pollutant and the soil is increasing. Thus, strong links can be established whether they are of physical or chemical nature, involving van der Waals forces or electrostatical interactions respectively, reducing the fraction extractable by ‘mild’ extractant theoretically more bioavailable (Gevao et al., 2000). In the end, the bioavailability of pesticides residues, i.e. their ability to be taken up by plants and/or soil organisms, determines indeed their ecological significance. “*Uptake is the movement of pesticides into plants and animal*s”[25] and is influenced by environmental conditions, soil properties and pesticide properties. Indeed, plant species or organisms, pH, T°, OM content, nature and mode of action of pesticide are all influencing more or less pesticide uptake. Furthermore, the presence of surfactants in pesticide formulations influences sorption and uptake behaviour, like the enhancement of the apparent water solubility for hydrophobic molecules.

2.2.2 *Degradation and transformation of pesticides*

According to Kah (2007), degradation is the second most important parameter after sorption to predict the fate of pesticide in soils but as it is the result of complex interactions between several processes, it is difficult to assess. Indeed, after their application, pesticides are susceptible to breakdown in different ways within environmental compartments, such as physical, chemical and biological degradation. Although a large part of applied pesticides is degraded *in situ* or transformed into non-toxic compounds, numerous pesticides and their by-products (metabolites) are known to resist to degradation processes. Indeed, degradation processes lead not always to a complete mineralization and metabolites can also have a great environmental pollution potential and sometimes even a higher

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[22] Kah, M., (2007) Behaviour of Ionisable Pesticides in Soils, Environment Department. University of York, York, p. 229.
[23] http://en.wikipedia.org/wiki/Steric_effects, consulted 01.29.2009.
[24] Gevao, B., Semple, K.T., Jones, K.C. (2000) Bound pesticide residues in soils: a review. Environmental Pollution 108, 3-14.
[25] Ibid.

toxicity (Tixier et al., 2002). They can thus accumulate in the environment and organisms, leading to a potential threat to consumers, especially for highly hydrophobic molecules subject to the bio-amplification/magnification process along the food chain like the classical example of DDT.

2.2.2.a Photolysis

Photodegradation occurs on foliage, on the soil surface or in the air, when sunlight breaks the chemical bonds of the pesticide molecule (Gavrilescu, 2005), but is of minor importance within the soil system (Bewick, 1994). Gavrilescu (2005) makes a difference between direct and indirect photolysis: direct photodegradation is when the molecule absorbed photons where indirect photolysis concerns the absorption of light energy from another molecule which has been excited by photons. In this latter case, some specific moieties are concerned in regards of recent studies: °OH, $CO_3^{-\circ}$, O* and DOM* (Halladja et al., 2007; Chiron et al., 2008; Arsac et al., 2008). According to Gavrilescu (2005), several factors influence photocatalytic reactions including pesticides: method of application, intensity of light, time of exposure as well as the properties of the site and of pesticides.

2.2.2.b Chemical degradation

Chemical degradation includes three largely pH-dependent reactions: hydrolysis, redox reactions and ionization (Gavrilescu, 2005). Hydrolysis is commonly characterized by the replacement of some functional group by a hydroxyl ion in an aqueous phase. Therefore, hydrolytic reactions are strongly enhanced by the presence of hydroxide ions within the system and thus by its pH.

Oxidation-reduction reactions are important as they control oxidation numbers of metals as well as oxidation state and structures of organics compounds. Redox conditions, assessed by the redox potential (Eh), are important as far as it gives information about the electron-donors/acceptors availability for the soil microbial community, which is the most implied community in the biodegradation processes. Indeed, it is difficult to separate accurately the biotic and the abiotic nature of oxidation and reduction processes and of the origin of electron-donors/acceptors (Bewick, 1994).

Ionization refers to the strong influence of pH on acids or bases which determines if they are in a neutral or ionic form.

2.2.2.c Biological degradation

Biological degradation, or biodegradation, refers to the degradation of contaminants by different soil microorganisms, such as bacteria, actynomycetes and fungi. Indeed, it is largely accepted that microbial degradation is the main source of pesticide degradation in soils (Bewick, 1994; Gavrilescu, 2005). Populations of microorganisms can exceed 10^{10} in one gram of soil and the species diversity is not yet totally apprehended (Brady and Weil, 2002). Although fungi are able to degrade some chemicals, bacteria are the most efficient for pesticide degradation largely because of their ability to transfer genes from one stub to another. The pesticide’s degradation mechanisms involve most of the time some complementary enzymatic complexes belonging to several microbial stubs, which individually are unable to degrade contaminants (Regnault-Roger et al., 2005). According to Warren et al. (2003), there are five different ways involved in the degradation process:

- Metabolism, where the pollutant represent a carbon and energy supply for bacterial metabolism
- Co-metabolism, where microbes use another energy source for their metabolism which is transforming/mineralizing pollutants
- Polymerisation of the compound with NOM resulting from microbial reactions
- Absorption of the pollutant by organisms (§ 2.2.1.f)
- Secondary transformation as a result of microbial induced changes in Eh or pH (§2.2.2.b)

All this metabolic induced degradation pathways are not governed only by the nature of microbial communities and/or by their enzymatic complexes, but also by functional groups of the pesticide in concern (Calvet et al., 2005). In a kinetic point of view, degradation can be divided in three categories as shown in Figure 2.5. Most of the time, biodegradation of pesticides occurs not immediately because of several phenomena (Warren et al., 2003):

- Growth of the degrading population
- Induction, which refers to the increase of the appropriate enzyme's production.
- Adaptation, which is characterized by a mutation within the population

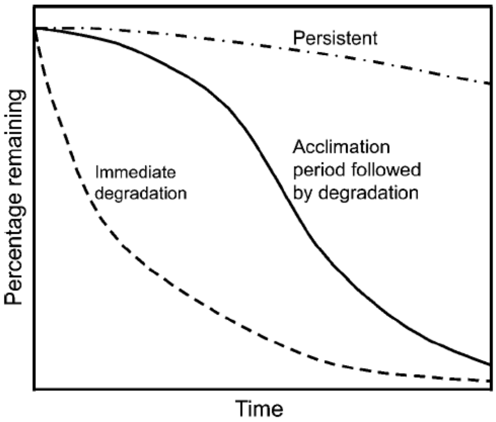

Figure 2.5: Degradation of organic pollutants (Gavrilescu, 2005)

However, if the microbial population is already adapted and numerous, an immediate degradation can occur but we cannot exclude that some chemicals are persistent when there is no specific biota to degrade them (Gavrilescu, 2005). The rate of pesticide degradation is governed by several factors such as soil parameters (T°, pH, water and OM content) and frequency of application. Indeed, the diversity of pesticide degradation's pathways is enhanced when alternating between different types or formulations of pesticide. Most of the time, degradation rates decrease with soil depth as related with the decline of microbial biomass related to a decrease in OM content (Soulas and Lagacherie, 2001;

Sonia Rodríguez-Cruz et al., 2006). However, in some cases, the reverse trend is observed (Karpouzas et al., 2001), and can be related to an increase in bioavailability or to a decrease in microbial competition (Sonia Rodríguez-Cruz et al., 2006).

2.2.3 *Transport of pesticides*

Transport mechanisms and pathways of pesticides differ among the parts of the soil: surface, unsaturated zone (vadose) and saturated one. Surface transport, known as ‘runoff’, depends on several surface properties like heterogeneity, structure as well as infiltration and adsorption ability (Lecomte et al., 2001). The vadose zone is characterized by the presence of oxygen, a high microbial activity linked to high concentration of OM and a large diversity of geochemical conditions (Kanti Sen and Khilar, 2006): the vertical transport of pollutants in the vadose zone is known as ‘leaching’. The anoxic saturated zone contains much less OM and bacterial activity and the geochemical conditions are much more homogeneous. Between the vadose zone and the water table, there is a ‘capillary fringe’ where capillary rises occur. In the unsaturated zone, transport of pollutants is mainly vertical whereas in the saturated zone and when runoff events are considered it is largely horizontal (Kanti Sen and Khilar, 2006).

Generally, a two phase approach is used to assess pollutant transport partitioning them between the immobile solid phase and the aqueous mobile one (McGechan and Lewis, 2002), without considering the colloidal phase. However, a part of the solid phase, colloidal in nature, can be mobile under specific conditions and may enhance or retard pollutants transport (Kanti Sen and Khilar, 2006). Thus, it is important to include this ‘third mobile solid phase’ in pollution studies, because it can sorb pesticides like the solid immobile phase but also be transported as fast as the aqueous phase. Indeed, sorption mechanism on colloids, like clayey or organic particles (mainly NOM), can play a determining role in accelerating the transfer of pollutants (Chiou et al., 1986; Jacobsen et al., 1998) and is of *“far greater importance than any movement of precipitated insoluble […] pesticides”*[26]. However, this phenomenon, often called ‘colloid-facilitated transport’ (Guyot, 1994), lead not always to an enhancement of solubility and thus it is better to call it ‘colloid-associated transport’ (Kanti Sen and Khilar, 2006). We will focus largely on this colloid-associated transport in chapter 2.2.3.c with a special interest in Dissolved Organic Matter (DOM), as it is known to be a carrier for Hydrophobic Organic Contaminants (HOCs) and often accelerate their transport within the soil column (Williams et al., 2000; Kanti Sen and Khilar, 2006). However, clay colloids, Particular Organic Matter (POM) and metal oxides can also be involved.

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[26] McGechan, M.B., Lewis, D.R. (2002) Transport of particulate and colloid-sorbed contaminants through soil, part 1: General principles. Biosystems Engineering 83, 255-273.

2.2.3.a Runoff

Transport of pesticides by runoff is one of the most important way pesticides from agricultural areas get directly into surface water bodies (Yaron et al., 1996; Gavrilescu, 2005). Indeed, the runoff-induced pesticides concentrations in surface waters may be much higher than those in groundwater after leaching (Bewick, 1994). However, sorption and dilution phenomena can reduce pesticides concentrations entering a water body by a runoff event. Total runoff includes water transfers on the soil surface (2) and in the subsoil (4-8) as shown in Figure 2.6.

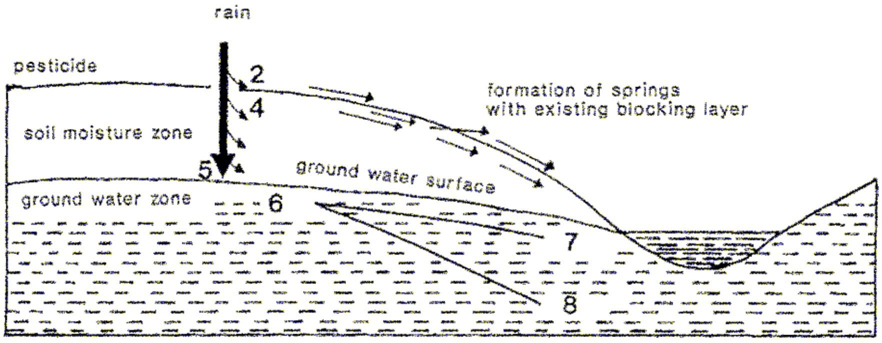

Figure 2.6: Runoff events including surface and subsurface (Source: Häfner, 1994)

Surface runoff takes place when precipitation or irrigation waters enter the soil faster than the infiltration rate. This phenomenon is primarily driven by water flow, for dissolved pesticides and those associated with DOM, but depends also on the movement of the particle to which pesticides are associated (§2.2.3.c). Indeed, pesticides may also bind to suspended materials, like Particular Organic Matter (POM) or colloidal clay minerals. Transport of pesticides bound on these latter materials is influenced by their release amount from soil matrix, the strength of the binding links and their mobility in water flow (Guyot, 1994; Wu et al., 2004). Their further deposition after a certain transport distance is off course a function of the carrier size, but is also dependent on their stability in water and on the flow velocity (Wu et al., 2004). It is well proved that the smallest the particles involved, the longer the transport distance of bound-pollutants and the slower the sedimentation rate (Yaron et al., 1996). Moreover small particles like clay minerals are known to be preferential sorbents for some pollutants. However, the nature of pesticides is also influencing sorption-desorption dynamic: a pesticide with a high K_d value is thought to run off only if the great intensity of rainfall leads to a soil erosion (Bewick, 1994). Pesticide hydrophobicity as well as the organic matter content of the suspended material, influence pesticide affinity to this latter one (Wu et al., 2004). As OM is thought to be the main sorbent for pesticides, there is a direct relationship between concentrations of POM and particle-bound pesticides (Wu et al., 2004). Subsurface runoff (interflows and/or throughflows, see Chapter 4), takes place when lateral convection is more important than vertical one (Regnault-Roger et al., 2005). This type of runoff is generated when the field is not flat and the B horizon has a low porosity. Thus, pesticides runoff is governed by several factors such as: climate (precipitations), topography, soil characteristics (texture, moisture, etc.), water income's rates, and vegetation and pesticide properties.

2.2.3.b Leaching

Leaching is the transport of pesticides by water within a soil profile through the vadose zone to ground water: it is a complex process governed by several soil and environmental factors (Köhne et al., 2009). The possible risk of a groundwater pollution will depend not only on the water movement but also on several degradation processes (§2.2.2) as well as sorption processes (§2.2.1). Pesticides mobility within fluids is governed by two types of movements, convection and diffusion, and the total solute flux (Js) can be expressed by a simple equation (Bewick, 1994):

$$Js = J_{DL} + J_{CL} + J_{DG},$$

Where J_{DL} and J_{CL} represent respectively the diffusive and the convective fluxes in the liquid phase and J_{DG} is the diffusive flux in the gas phase. Convective flux in the gas phase J_{DG} can be neglected except when dealing with extremely volatile compounds. This simple convective-diffusive equation cannot assess all the realities of pollutant transport in soils where two major leaching pathways are of concern: preferential flows (PF) and matrix flows.

"*Preferential flow is the process in which water and solute rapidly move through soil macropores, bypassing much of the soil matrix*"[27], leading to non-equilibrium conditions between macropores domain and soil matrix in terms of pressure heads and solute concentrations (Gerke, 2006). Preferential flows are the result of worm holes, root channels or shrinkage cracks (Kamra and Lennartz, 2005), but they can also be due to wetting front instabilities (Guyot, 1994). Gerke (2006) reports several differences in PF occurrence with several terms: *macropore* flow which refers to flow using biopores or fissures; *unstable* flow which refers to instabilities of the wetting front by water repellency or air entrapment; *'finger'-like* flow occurs mainly in top soils and *funnel* flow refers to lateral flow following textural boundaries. All different flow types referring to PF are known to play a greater role in solutes leaching when water incomes intensity arises as irrigation water or precipitations (Mdaghri-Alaoui, 1998; Gerke, 2006; Jarvis, 2007). However, despite the use of dye tracers by a few authors (Alaoui and Goetz, 2008) and as recommended by the Swiss federal office for the environment (FOEN; Alaoui 2007), there are few methods available to characterize PF in field studies. Nevertheless, their probabilities to occur are important to consider when assessing the potential risk of groundwater pollutions (Blackwell, 2000; Gärdenäs et al., 2006). Indeed, pesticide leaching to groundwater can occur *via* PF before degradation or adsorption can take place (Köhne et al., 2009).

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[27] Gavrilescu, M. (2005) Fate of Pesticides in the Environment and its Bioremediation. Engineering in Life Sciences 5, 497-526.

Matrix flow is characterized by a much slower migration of pollutants within the soil solution through the micropores of the soil matrix, leading to greater retention's probabilities because of longer contacts between soil water and particles. Also, leaching of pesticides through the soil profile is governed by their properties (solubility/hydrophobicity and degradability) as well as two soil parameters: permeability, with the importance of PF, and sorption ability. For example, a hydrophobic chemical will be strongly linked to the greater OM content in top-soil and thus won't leach easily, except using PF when associated with colloids (§2.2.3.c). All these parameters influence leaching rate once pesticides are applied and the way they are applied is also an important factor which determines their fate within the soil system.

Capillary flows are upstream flows in the limit zone between the saturated and the vadose zones just above the water table, called capillary fringe, and may be responsible for the upward movement of pesticides soils.

2.2.3.c Colloid-associated pollutant transport

Colloids are defined as solid particles with very low water solubility remaining suspended in solution due to their small size ranging from some µm to some nm as shown in Figure 2.7 (Atteia et al., 1998; McGechan and Lewis, 2002). Depending on authors size range limits vary: 1nm to 1µm (McGechan and Lewis, 2002), 10nm to 0.1µm (Kanti Sen and Khilar, 2006), 1nm to 0.2µm (Irace-Guigand and Aaron, 2003) or 10nm to 10µm (Sposito, 1989).

Contaminating colloids can be divided in two groups (Ibaraki, 1995 *in* McGechan and Lewis, 2002): true colloids, created when concentration of contaminants exceeds their solubility, and pseudo-colloids, which are colloids of a non-contaminating nature with sorbed pollutants on their surfaces. True colloids can be radionuclide or pesticide molecules in suspension when applied as wettable powders (Yaron et al., 1996). Pseudo-colloids can be inorganic like clays, Fe or Al oxides/hydroxides or organic like natural OM as HA or FA, root exudates or even bacteria and viruses (Yaron et al., 1996; Kanti Sen and Khilar, 2006). This latter group includes important transporters of pollutants, especially when soils and agriculture applications are discussed. As this work will focus on pseudo-colloids we chose to use the term 'colloids' referring to the classification of Brady and Weil (2002) which excludes biological colloids, like bacteria, viruses and exudates, but includes four different groups:

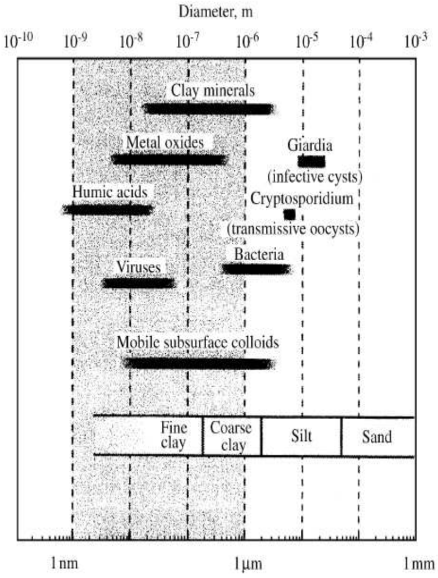

Figure 2.7: Size ranges of colloids (McGechan and Lewis, 2002)

1. Silicate clays (e.g. kaolinite or smectite)
2. Non-crystalline clays (e.g. allophane or imogolite)
3. Iron and aluminium oxides (e.g. gibbsite or goethite)
4. Humic colloids (e.g. HA or FA)

Colloids are of great concern when dealing with pollutants having a high ability to be adsorbed, characterized by a high K_d value and thought to be immobile in soils (Yaron et al., 1996). Indeed, because of their small size, their high specific area, their large amount of functional groups and their suspended nature soil's colloids are effective sorbents for hydrophobic organic contaminants (HOC) like pesticides (Ryan and Elimelech, 1996; Laegdsmand et al., 1999). Another property that influences their sorption ability is the common presence of negative electrical charges on their surfaces (Kanti Sen and Khilar, 2006). Particle size distribution and nature of colloids are two important parameters to take in account for a better understanding of colloid-associated pollutant transport (Guyot, 1994; Atteia et al., 1998). However, colloid-associated transport of pollutants takes place in soils after the three following steps (Ryan and Elimelech, 1996):

1. mobilization of colloid,
2. pollutant sorption on colloid
3. transport of the complex through the profile

Off course, sorption can occur before or after mobilization of colloidal particles. Kanti Sen and Khilar (2006) remind us that the first important criterion is the sufficient amount of colloids able to adsorb a sufficient amount of pollutant. However, these three steps are dependent on a large amount of parameters: size of colloids, properties of pollutants, texture and structure of soils, soil hydrodynamic and geochemical conditions (Laegdsmand et al., 1999). Soil type is an important parameter as far as texture and structure influence colloid-associated transport of pesticides: fine texture with small pores enhances it less than coarse texture and large pores (Yaron et al., 1996). Soil structure, especially the amount of preferential flows (PF), largely influences the extent of colloid-associated transport, enhancing it when contaminant-colloid complexes are located close to macropores (de Jonge et al., 2004).

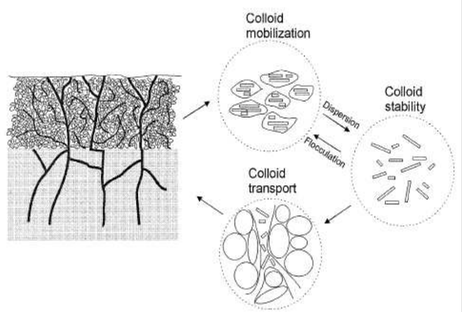

Figure 2.8: Main processes related to *in situ* colloid mobilization and transport (Source: de Jonge et al., 2004)

The origin of colloids in the vadose zone is thought to be *"the in situ release of water-dispersible colloids"*[28] (Fig. 2.8), but they can also result from raindrop impact and surface erosive flow

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[28] de Jonge, L.W., Kjaergaard, C., Moldrup, P. (2004) Colloids and colloid-facilitated transport of contaminants in soils: An introduction. Vadose Zone Journal 3, 321-325.

(Laegdsmand et al., 2004). Indeed, the mobilization or release of colloids corresponds to their detachment from the soil matrix when water passes through the pores (McGechan and Lewis, 2002). Release and dispersion of clay colloid is enhanced when leaching with ‘low–electrolyte-concentration water’, *e.g.* rainwater, occurs and thus pesticides adsorbed on the soil matrix in surface can migrate down the profile (Yaron et al., 1996). This is due to the lowering of the ionic strength (Laegdsmand et al., 2004). Another phenomenon to stress is when heavy rain comes just after pesticides application, impacts of rain drops onto the soil surface project pesticides-colloids complexes in the air which can than move through the soil profile using nearby macropores. This phenomenon and surface erosion are both related to a ‘mechanical stress’ and in a lower extent to a ‘moderate chemical dispersion’ from the lowering of the ionic strength (Laegdsmand et al., 2004).

Properties influencing the release of colloids are: pH, ionic strength, clay mineralogy, moisture content and preferential flow (PF) degree (de Jonge et al., 2004). The influence of physical and chemical conditions on aggregation of colloids can modify their sorption ability and transport: varying ionic strengths, for instance, may change flocculation-dispersion of clays (Atteia et al., 1998) and the conformation of humic molecules (Laegdsmand et al., 2004). The greater the ion concentration, e.g. $[Ca^{2+}]$, the more flocculated are the clay colloids and thus their immobilization occurs faster. As far as humic substances are concerned, an increase in ionic strength leads to a decrease in double layer interactions and thus humic materials are more aggregated (Baalousha et al., 2006). The authors stress the important influence of pH as well as the cation type on HS aggregation due to an increase in ionic strength: on one hand high pH leads to a greater dissociation of functional groups, on the other hand the divalent cation Ca^{2+} exhibit a higher capacity to neutralize negative charges than the monovalent cation Na^{+}. Moreover, a more condensed shape supposes less molecular voids and hence less sorption sites available for HOC (see §2.2.1.e).

Sorption, transport and fate of contaminant-colloid complexes

The amount of clay or OM released as well as the K_d value and the desorption kinetic of pesticide from the mobile colloids are important parameters to assess the extent of colloid-pesticides complexes transport. Despite a good comprehension of sorption mechanism of pollutants in soils (§2.2.1.e), the understanding of pollutant’s sorption on colloids is still poor (McGechan and Lewis, 2002; Irace-Guigand and Aaron, 2003) and there is few publications on this topic (Laegdsmand et al., 2004). However, sorption of HOC to colloidal fractions has to be considered in pollution studies and a partition coefficient between the colloidal phase and the ‘truly dissolved’ one (<5nm) can be used (Burgess et al., 1996; Irace-Guigand and Aaron, 2003). Colloid-associated transport will depend on regional climate and soil properties, mainly SOM characteristics and binding strength between pesticides and soil constituents (Guyot, 1994).

Immobilization of colloids can occur in three different ways: sedimentation, adsorption or clogging. This latter phenomenon is also called 'straining' or 'physical filtration' (Yaron et al., 1996; McGechan and Lewis, 2002; McGechan, 2002) and led to the 'colloid filtration theory' (CFT) which says that colloid transport "*will be limited to distances of meters to tens of meters*" in most aquifers (Ryan and Elimelech, 1996).

2.2.4 *Interactions between processes*

All the processes responsible of the fate of xenobiotics in the environment described before are of course not occurring in an independent way. Some interactions between different types of processes are more or less known, such as the influence of retention processes on microbial metabolism. Sorption phenomena are indeed governing in some way or another bioavailability of pesticides and thus their degradation rates. Biotic **degradation** is either restrained by sorption, because it occurs mainly in soil solution with enzymes, or favoured if adsorbents represent an energy source for co-metabolism of microorganisms. Abiotic transformations may also be enhanced by sorption, such as hydrolysis of molecules attached to soil aggregates in contact with soil water or photolysis of those fixed at the soil surface. Thus, K_d values are important factors which can correlate microbiological metabolism or photolytic degradation (Calvet et al., 2005). Moreover, desorption of pesticides from the inside of soil aggregates and their transport to the macro porosity, where soil solution with enzymatic complexes lies, determines also the rapidity of their degradation. **Retention** of pesticides is in its turn affected by their degradation, which limit quantities that could be retained or transformed on soil aggregates. It also depends on transport dynamics as the access to active sites is controlled by molecular diffusion in the liquid phase. **Transport** processes are also influenced by retention and degradation ones as these latter decrease the amount of molecules transported. Therefore, it is really complex to get a realistic overview of what is happening to pesticides once they are applied on crops within the field. Furthermore, there are few field studies compared to experimental ones (Schulz, 2008).

2.3 Environmental fate of the herbicide glyphosate

Fate of pesticides in the environment depends on several processes described before (§2.2) and is governed by different factors. Aqueous solubility is considered to be the major physicochemical parameter for the assessment of transport and fate of agrochemicals in soils, but their mobility in this media is also highly dependent on their sorption abilities (Stangroom et al., 2000; Ruggiero et al., 2002). Glyphosate [*N*-(phosphonomethyl)glycine] is the most used herbicide in the world for weed and vegetation control, leading to problematic situations in terms of weed resistance (Dill et al., 2010). Glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA), possess a ubiquitous character: they show relatively high water solubilities (10.5 and 5.8 g/l respectively), and also high sorption abilities (log K_{oc} = 4.34 and 3-4.4 respectively) and low leaching potentials (GUS index = -0.49 and 0.21 respectively)[29]. They are therefore assumed to have a limited mobility in soils and not to reach water bodies (Sprankle et al., 1975; Beltran et al., 1998). Nevertheless, binding of glyphosate is a complex phenomenon influenced by several soil parameters and constituents (Albers et al., 2009), and recent studies have demonstrated that glyphosate can be leached or washed from soil surface layers and reach ground and surface waters (Borggaard and Gimmsing, 2008).

Structures and important properties of glyphosate and AMPA are shown in Figure 2.9 and Table 2.3 respectively.

[redacted]

Figure 2.9: Molecular structures of a) glyphosate and b) aminomethylphosphonic acid (AMPA)

Table 2.3: Properties of glyphosate and aminomethylphosphonic acid (AMPA);
Source: Footprint poject[29] or noticed

Properties	Glyphosate	AMPA
Formula	$C_3H_8NO_5P$	CH_6NO_3P
MW [g/mol]	169.07	111.04
Water Solubility [mg/l]	10’500	5’800
Melting pt [°C]	189.5	-
Degradation pt [°C]	200	-
log K_{ow}	-3.2	-1.63
Density [g/ml]	1.71	1.64
pK_a	0.79, 2.29, 5.96 and 10.98*	0.9, 5.6 and 10.2**
vapP [mPa, 25°C]	0.0131	-
Henry cste [Pa m^3/mol]	2.1×10^{-7}	0.16
Soil degradation:	-	-
DT50 lab [20°C]	4-180	-
DT50 field	3-174**	76-240
GUS Index	-0.49	0.21
Sorption coefficient K_d / K_{oc} [l/kg]	222 / 1’435	- / 1’200-25’000***
Freundlich sorption coefficient K_f [l/kg]	0.6-303**	15.7-1570**

* Chamberlain et al., 1996, ** Laitinen, 2009, *** Reding, 2005

During the last decade, many studies have been conducted in soil columns in the laboratory to evaluate the factors governing the mobility of glyphosate and AMPA (de Jonge et al., 2000; Candela et al., 2007; Dousset et al., 2007; Zhao et al., 2009; Zhou et al., 2010; Bergstrom et al., 2011). Field studies

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[29] Pesticides properties data base, Footprint project. www.eu-footprint.org, consulted July 2012.

are more scarce and have been done principally in high latitude contexts (Kjaer et al., 2005; Siimes et al., 2006; Simonsen et al., 2008; Laitinen et al., 2009; Aronsson et al., 2010; Candela et al., 2010). As a reminder the three different types of processes governing the fate of pesticides in soils presented before are, according to Calvet et al. (2005): retention, degradation and transport. Studies dealing with environmental fate of glyphosate are reviewed hereafter according to these three types of processes, as well as those dealing with its mode of action and ecological toxicity.

2.3.1 *Retention and sorption behaviour of glyphosate*

The mechanisms of sorption involved with different constituents of the soil are important to consider when trying to understand the environmental fate of glyphosate after its application on agricultural soils. Glyphosate is indeed known to be rapidly and strongly adsorbed to the soil matrix (Beltran et al., 1998), but its binding is a complex phenomenon influenced by several soil parameters and constituents (Albers et al., 2009). For instance, adsorption of glyphosate appears to be weaker in sandy or calcareous soils than in clayey or granitic ones (Mamy, 2004). Glyphosate mobility in soils is pH-dependent as its charges vary from +1 to -3, depending on its four different dissociation constants (Sprankle et al., 1975; Sheals et al., 2002). Indeed, complexation phenomena with dissolved ions and/or soil constituents' surfaces involved its three functional groups: amine, carboxylate and phosphonic groups (Gimsing and dos Santos, 2005). In general, it is less adsorbed with an increase in pH (Zhao et al., 2009). However, in natural soil pH ranges (4-9) glyphosate possesses generally one or two negative charge(s) and thus is more likely to complex with positively charged moieties, mainly soil minerals with an important contribution of Fe and Al oxides (Borgaard and Gimmsing, 2008). It has been indeed proven to be sorbed onto iron and aluminium hydroxides such as goethite (FeOOH) and gibbsite ($Al(OH)_3$) (Gimsing et al., 2004c), onto clay minerals such as montmorillonite (Morillo et al., 1997) but also, to a lower extent, onto humic substances (Piccolo et al., 1996; Albers et al., 2009). Nevertheless, some ternary complexes with negatively charged surfaces can be formed with the intermediate of a positively charged moiety (Sprankle et al., 1975; Morillo et al., 2000). Thus, as reported by Gimsing and dos Santos (2005), several studies have shown that glyphosate sorption in soils is influenced by multivalent cations in the order $Fe^{3+} > Al^{3+} > Cu^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+} > Ca^{2+} \sim Mg^{2+}$.

2.3.1.a Sorption to soil minerals

Glyphosate adsorption in soils has been positively related to the iron and aluminium hydroxides (crystallised and amorphous) content (Morillo et al., 2000). These latter are formed from the direct weathering of primary silicates or from the hydrolysis and desilication of clay minerals like smectite and kaolinite (Sposito, 1989). Thus, they are often associated to silicate clays, forming coatings on external surfaces or ‘islands’ in 2:1 type’s interlayer, altering their adsorption behaviour (Brady and Weil, 2002). The specific area determines the sorption strength, which is thus higher for goethite than for hematite, this latter possessing lower specific area (McConnell and Hossner, 1985). Adsorption occurs through ionic interactions between positive charges of hydroxides and negative charges of glyphosate, decreasing with increasing pH and consequent negative charges on hydroxides (McConnell and Hossner, 1985).

Adsorption of glyphosate on clay minerals depends on the clay nature in the order: bentonite < kaolinite – illite < montmorillonite < nontronite (Sprankle et al., 1975; Mamy, 2004). Moreover, the exchangeable cation is influencing the sorption strength in the order: $Na^+ < Mg^{2+} < Ca^{2+} < Zn^{2+} < Mn^{2+} < Fe^{3+} < Al^{3+}$ (Sprankle et al., 1975; Mamy, 2004). Sorption mechanisms involved are ionic interactions and surface complexes with the intermediate of exchangeable cations (Morillo et al., 1997; Jonsson et al., 2008).

2.3.1.b Sorption to Soil Organic Matter (SOM)

Despite its affinity for soil mineral phases (Borgaard and Gimmsing, 2008), glyphosate has been proven to bind also to soil organic constituents, such as humic substances (Roy et al., 1989; Dousset et al., 2004). Humic substances (HS) are thought to be made of relatively low-molecular mass moieties bound together mainly by H-bonding and hydrophobic links (Sutton and Sposito, 2005). Their structure is sensitive to ambient conditions such as pH, ionic strength and also to their own concentration (Aoustin et al., 2001). At low pH, HS molecules exhibit a greater aggregation and condensation state (Baalousha et al., 2006), due to the protonation of acidic functional groups leading to more intramolecular H-bonding and a decrease in negative charges that repulse each other (McDonald et al., 2004). At high concentration of salts and/or HS themselves, those latter exhibit more compact sphero-colloidal structures also linked with a decrease in intramolecular repulsive forces. In these conditions, a decrease in the surface area and the disappearance of certain structural micropores are observed (Alvarez-Puebla and Garrido, 2005), diminishing their potential binding for pollutants.

According to Piccolo et al. (1996), glyphosate gets linked to humic substances (HS) mainly by H-bonding, the extent of which depends on their molecular size and degree of aromaticity. The larger the molecular size and the lower the aromatic content of humic molecules, the stronger was the adsorption of glyphosate. A large molecular size induces a higher potential for sorption because of a greater number of hydrogen bonding. Moreover, a lower abundance of aromatic rings in humic macromolecules enhances their stereochemical flexibility, leading to an easier access for the small herbicide molecule to their inner reactive sites. However a bias in this study has been pointed out with the use of $CaCl_2$ as a background electrolyte, leading to the creation of complexes between Ca^{2+} and glyphosate, and also the non-purified nature of used humic acids with the risk of metal ions interferences (Borggaard and Gimmsing, 2008; Albers et al., 2009). Ternary complexes between glyphosate and humus are indeed likely to be formed with intermediate di- and trivalent “bridging” metal cations, such as Fe or Al (Barrett and McBride, 2006; Borggaard and Gimmsing, 2008) but also Cu (Morillo et al., 1997). Therefore, no general sorption mechanism could have been pointed out and the role of soil organic matter (SOM) in glyphosate sorption remains controversial: SOM may either decrease glyphosate sorption by blocking sorption sites or increase it, as higher SOM content seems to enhance sorption ability of poorly ordered Al- and Fe- oxides (Borgaard and Gimmsing, 2008).

Dissolved organic matter (DOM) is defined as the part of the SOM smaller than 0.45µm composed of molecules of relatively low molecular weight (Zsolnay, 2003). Organic compounds, such as pesticides, can bind to DOM, which can increase their solubility and their availability for microorganisms (Pennington et al., 1991; Franco, 2001). In aqueous solutions and especially under acidic conditions, intramolecular interactions in HS lead to the formation of micelle-like aggregates, with interior hydrophobic regions isolated from solutions by external hydrophilic parts, which have been proven to enhance the solubility of some organic pollutants (Sutton and Sposito, 2005). However, the nature of ionic species in solution influence colloidal stability of aqueous dispersion, which is mainly controlled by the pH but also by the presence of multivalent cations like calcium (Baalousha et al., 2006). Therefore, the potential enhanced leaching of pesticides in soils, dependant on retention-mobilisation dynamic, can be influenced by the nature of DOM, *e.g.* its humification degree, as well as the soil composition that determine the nature of ionic species present in solution, and also by pesticide physico-chemical properties (Franco, 2001; Haberhauer et al., 2002). However, retention of DOM can also occur on surfaces of clay fraction and sesquioxides and thus reducing the availability of active site for pollutants (Kaiser and Zech, 2000; Zsolnay, 2003).

To our knowledge, DOM has been little investigated as a possible carrier for glyphosate. Only recently, Mazzei and Piccolo (2012) studied the interactions of glyphosate with dissolved humic acids (HA) and fulvic acids (FA) at pH 5.2 and 7 by NMR spectroscopy. With this technique, they confirmed the formation of H-bonds between glyphosate carboxyl and phophonate moities and protonated oxygen functions in dissolved humic substances, with a larger binding for FA and at pH 5.2 than for HA and at pH 7.

2.3.1.c Influence of ions on glyphosate sorption

As already mentioned, glyphosate, due to its structure, is known to form stable complexes with metallic ions through coordination linkages of its different functional groups. AMPA is also subject to complexation with metals but to a lower extent (Barrett and McBride, 2006). The stability of the complexes depends on the valence as trivalent cations formed more stable complexes than divalent cations: bi-dentate complexes (2:1) are more stables than mono-dentate ones (1:1) (Subramaniam and Hoggard, 1988). Indeed, Fe^{3+} and Al^{3+} and also Cu^{2+} complex strongly with glyphosate.

As vineyard soils are highly loaded with copper, Cu concentration is an important parameter to consider when assessing glyphosate transfer in this kind of agro-ecosystem (Dousset et al., 2007). These latter authors studied the effect of copper on leaching of glyphosate through one granitic and one calcareous soil column. They observed decreased leaching in the calcareous soil and an increase in leaching in the granitic soil as copper levels increased from the geochemical background (17 and 34 mg/kg) up to 500 mg/kg. They explained this result by the formation of soil-Cu-glyphosate ternary complexes at pH 9 and the formation of Cu-glyphosate complexes that then leached in acidic conditions referring to Morillo et al. (2002) and Sheals et al. (2002) respectively. Morillo et al. (1997) found out that the presence of copper leads indeed to desorption of glyphosate from the external sites (Al-OH groups) of montmorillonite and the formation of Cu-glyphosate complexes, which show a high stability and a lower sorption affinity than glyphosate alone. Inversely, glyphosate may enhance copper mobility by chelating with Cu^{2+} previously adsorbed on both mineral and organic surfaces (Barrett and McBride, 2006). Copper could thus have a significant influence on glyphosate mobility and potential transfer to ground and surface waters and vice versa.

The early study of Sprankle et al. (1975) demonstrated that the main glyphosate binding mechanism is an inner-sphere complex (ISC) through the phosphonic acid moiety and that the soil phosphate level is one of the main influencing factors. Indeed, the binding of glyphosate to soil is clearly influenced by phosphate sorption site availability (Day et al., 1997), and a sorption competition occurs with phosphate ions (PO_4^{3-}). As demonstrated by Gimsing et al. (2004a), fertilizing with P-fertilizers can lead to a glyphosate release and an increase in water pollution (Fig. 2.10). However, there are a lot of discrepancies between studies and it is hypothesized that two different kinds of sorption sites coexist: sites available for both phosphate and glyphosate and sites available for either one or the other (Borgaard and Gimmsing, 2008). From a different perspective, Zhao et al. (2009) conclude that addition of phosphate induce pH change, which subsequently contribute to glyphosate mobility.

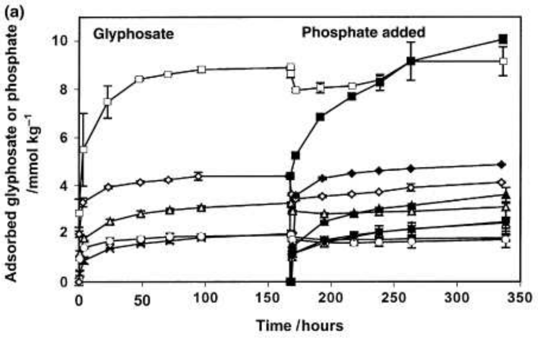

Figure 2.10: Adsorption of glyphosate followed by phosphate on 5 Danish soils (Gimsing et al., 2004a)

Glyphosate adsorption is thus a complex phenomenon influenced by pH, dissolved ions and soil components, especially hydroxides (Fe, Al), clay minerals and humic substances. From a general point of view, glyphosate is preferentially sorbed onto clay minerals and their associated iron oxides

(Borggaard and Gimmsing, 2008; Bergström et al., 2011). However, in two different studies, the highest sorption appeared in soils rich in soil organic matter (SOM), with low pH-value and high Fe/Al-oxide content (Autio et al., 2004; Albers et al., 2009). Therefore, pH conditions, the presence of cationic moieties and the multiple interactions between Al/Fe oxides, organic matter and clay minerals influence largely the retention of glyphosate and thus its mobility in soils (Sprankle, 1975; de Jonge and de Jonge, 1999).

2.3.2 *Degradation of glyphosate*

The main degradation pathway of glyphosate in the environment is known to occur mainly through microbial degradation in soils, as it has been proven that it is neither submitted to volatilization process nor hydrolytic and photolytic decomposition (Schuette, 1998). Degradation takes place both in aerobic and anaerobic conditions and can lead to the formation of either aminomethylphosphonic acid (AMPA) and glyoxylate or sarcosine (Fig. 2.11), before being mineralized in carbon dioxide and phosphate or ammonium. Nevertheless, it has been proved to be maximal at neutral pH and in aerobic conditions (Forlani et al., 1999).

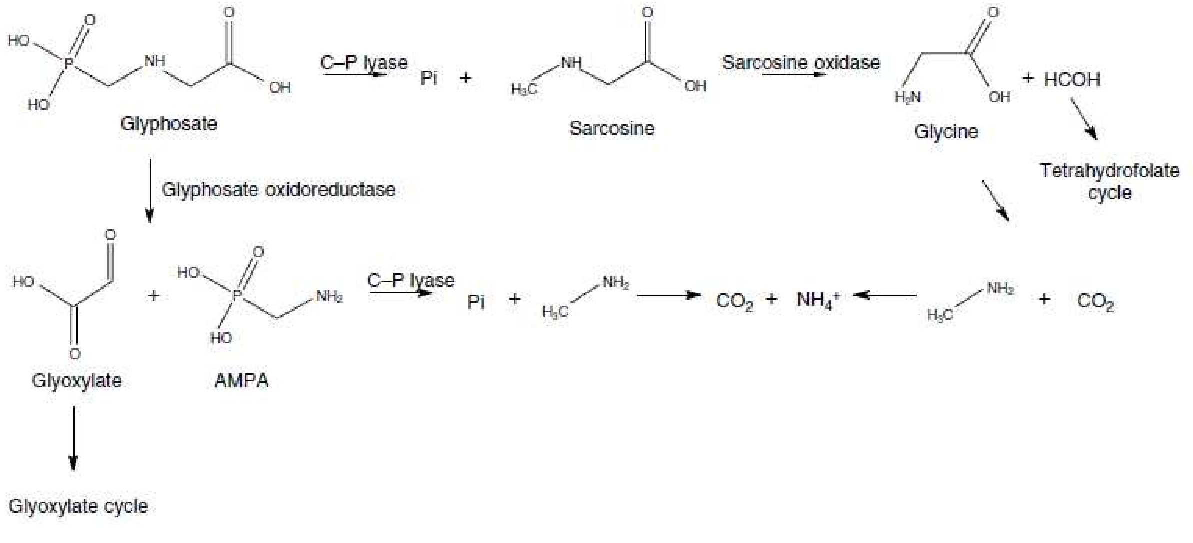

Figure 2.11: Degradation pathways of glyphosate in soils (Borgaard and Gimmsing, 2008)

AMPA is more persistent in soils (DT50 = 150d, 76-240d) than glyphosate maybe due to its stronger sorption through the phosphonate group and consequent protection against microbial degradation or its lower penetrability of microorganism cellular walls (Schuette, 1998; Borgaard and Gimmsing, 2008). Indeed, it has been proven that sorption/desorption glyphosate onto soil components surfaces greatly influences its availability for microbial degradation, leading to a large range of DT50 values (Schnurer et al., 2006; Borgaard and Gimmsing, 2008). As the non-extractible fraction, i.e. bound residues, increases with the contact time between one chemical and the sorbent phase, it becomes indeed less available for biodegradation (Barriuso et al., 2008). Recently, Gimsing et al. (2004b) correlated glyphosate mineralization rate with *Pseudomonas* species population size, but several microorganisms (fungi, actynomycetes, bacteria) are known to be able to degrade glyphosate though bacteria seem to be the most efficient (Borgaard and Gimmsing, 2008). Some uncultivable and thus unknown species may also be involved in the first steps of degradation (Forlani et al., 1999). Degradation of glyphosate in soils depends on several parameters such as soil type, pH and temperature (Simonsen et al., 2008).

2.3.3 *Transport of glyphosate and AMPA in soils and pollution of water bodies*

Although glyphosate is thought to be less mobile than other herbicides due to its important affinity for soil components (see above), it has been frequently found in surface waters of United States and France (Kolpin et al., 2006; Botta et al., 2012; Coupe et al., 2012). Indeed, according to a large national survey realised in 2003-2004 in France, more than 50% of about a thousand of samples contained AMPA, the most frequent pesticide molecule detected, and more than 30% contained glyphosate (IFEN, 2006). This occurrence is surely due to their relatively important water solubility (see Table 2.3), and the difference between them can be explained by the longer half life of the metabolite. Indeed, according to Stangroom et al. (2000) aqueous solubility is considered as the major physicochemical parameter for assessment of transport and fate of organic micropollutants.

The survey realised in groundwater samples pointed out a very small occurrence (2-3%) leading some authors, such as Al-Rajabs et al. (2008), to affirm that *"the contamination of groundwater does not seem to be a concern, regardless of the soil type, if the herbicide is used in accordance with good agricultural practice"*[30]. However, a recent study showed that more than 40% of the 139 groundwater samples taken in 11 different sites in Catalonia were polluted by glyphosate with an average concentration of about 200 ng/L (Sanchís et al., 2012). This occurrence discrepancy can be explained by two main reasons: the number of samples and the sampling time period, which are respectively much lower and more specific to the application in the Spanish study. Their results *"corroborate the hypothesis of previous studies pointing that glyphosate may exhibit a certain grade of mobility in soils"*[31].

The use of ^{14}C labelled glyphosate can reveal the influence of soil texture and macropore flow on the potential leaching of glyphosate (de Jonge et al., 2000; Bergström et al., 2011). Indeed, leaching of glyphosate was higher in a clay soil than in a sandy one, due to a slower degradation rate explained by

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[30] Al-Rajab A, Amellal S, Schiavon M. 2008. Sorption and leaching of 14C-glyphosate in agricultural soils. Agronomy for Sustainable Development 28(3):419-428.

[31] Sanchís, J., L. Kantiani, et al. (2012). "Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry." Analytical and Bioanalytical Chemistry: 1-11.

a strongest adsorption in the clay soil (Bergström et al., 2011). For its metabolite AMPA, they observed no leaching in the sandy soil but some in the clay soil and suggested, despite its more persistent nature, a faster degradation rate than for glyphosate. The same observation was done by de Jonge et al. (2000) with a larger leaching amount of glyphosate in a sandy loam soil than in a sandy soil. A reduced leaching with a 96h contact time before irrigation was also observed in the sandy loam soil, revealing the role of sorption kinetics for leaching, but no differences were observed in the sandy soil. This result, corroborated on a similarly textured soil by Candela et al. (2007), means that sorption kinetics allowing glyphosate to access sorption sites influence the leaching potential in fine textured soils. Moreover, it has been concluded that the observed difference is more related to the pore structure and the occurrence of macropore flow shortly after application, than to sorption properties (de Jonge et al., 2000). Thus, glyphosate is more susceptible to leach in well-structured fine-textured soils than in structureless sandy soils, as well as with a shorter contact time before water inputs.

More recently, some studies examined the difference between dissolved, colloidal and particle-bound transport of glyphosate. Particle-bound transport (>0.24 µm) accounted for only a small proportion (1-17%) in leaching experiments on soil columns (de Jonge et al., 2000; Gjettermann et al., 2009; Kjær et al., 2011). Colloid-associated transport (>0.02 µm) represents 68 ± 10% of the total glyphosate leaching in undisturbed soil columns from a ploughed system (Gjettermann et al., 2009). Discrepancies between studies have been suggested to depend on the time used to collect the leachate, because desorption of glyphosate from soil particles is kinetically driven (Gjettermann et al., 2011). Thus, the question whether they are preferentially transported adsorbed to particles (>1µm), in the colloidal (5nm-1µm) or in the “truly dissolved” phases (<5 nm) still remain unclear.

All the processes responsible of the fate of glyphosate in the environment described before are undoubtedly not occurring in an independent way. Moreover, factors controlling transport of glyphosate and AMPA to surface waters and their importance in space and time are still under investigation (Coupe et al., 2012). Glyphosate and AMPA transfer from fields to water bodies may indeed use several pathways depending on the field configuration (soil type, presence of tile-drains and/or discontinuities such as macropores, soil colloids, etc.), agricultural practices (application, tillage) and meteorological conditions (rainfall intensity and magnitude) (Coupe et al., 2012; Sanchís et al., 2012). Therefore it is really complex to get a realistic overview of what is happening to this herbicide once it is applied on crops within the field. Nevertheless, in a recent review Borggaard (2011) affirms that the presence of glyphosate and AMPA in ground- and surface-waters “ *indicates a land-to-water transport either in dissolved form and/or bound to soil particles, so-called colloid-*

facilitated transport, where the subsurface transport mainly takes place through macropores by preferential flow."[32]

2.3.4 *Mode of action and effects on non-target organisms*

2.3.4.a Mode of action

Glyphosate inhibits the EPSPS enzyme (5-enolpyruvylshikimate-3-phosphate synthase), which is involved in the elaboration of three essential aromatic amino acids: phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Try) (Sikorski and Gruys, 1997). A competitive inhibition takes place during the transformation of the shikimate into chorismate, which is the precursor of these three essential amino acids (Gauvrit, 2005). These latter are really primordial to the protein synthesis but also to lignin synthesis in which Phe is involved (Regnault-Roger et al., 2005). Most herbicides, as they are small sized, lipophilic and neutral, will penetrate in the target-plant in a passive way because of their affinity for polar lipids that constitute cell membranes (Tissut et al., 2006). They will accumulate in the tissues and be redistributed in the aqueous parts slowly. Glyphosate in contrary is highly hydrophilic and charged and can therefore not easily penetrate in the cell membrane. It has been shown that glyphosate is able to penetrate in an active way, i.e. physiologically controlled, by taking place on a phosphate transporter instead of $PO_4H_2^-$ ions (Tissut et al., 2006). Moreover, in humid conditions, the cuticle inflates and waxes are separating allowing water and solutes exchanges, such as hydrophilic xenobiotics like glyphosate. That explains the presence of surfactants in herbicides formulations, which change properties of molecules to enhance their efficiency. Glyphosate can be found in more than 400 commercial formulations but it is mainly sold as a water-dispersible isopropylamine salt suppressing its charge and conferring a more lipophilic characteristic to the active ingredient (Tissut et al., 2006).

After being absorbed into the plant cells through the leaf surface, glyphosate is readily translocated to merismatic tissues by the phloem and fixed on the active site of phospho-enolpyruvate instead of the EPSPS (Schuette, 1998). This latter is a key enzyme in the shikimic acid pathway, only found in plants and certain microorganisms (Sikorski and Gruys, 1997), which catalyzes the condensation of the shikimate-3-phosphate and of the phospho-enolpyruvate into 5-enolpyruvylshikimate-3-phosphate (chorismate). As this latter is not produced any more, the three essential aromatic amino acids (Phe, Tyr and Try) are no longer synthesized leading to the rapid death of organisms. Indeed, these latter are primordial to the whole protein synthesis but also, after desamination, to lignin synthesis in which Phe is involved, as 35% of the ultimate dry mass derived from this pathway (Sikorski and Gruys, 1997; Regnault-Roger et al., 2005). Plants are gradually wilting with a visible yellowing and than browning

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[32] Borggaard, O.K. (2011) Does Phosphate Affect Soil Sorption and Degradation of Glyphosate? – A Review. Trends in Soil & Plant Sciences Journal 2, 16-27.

of the above-ground parts with a concomitant degradation of the underground parts in a few days (Schuette, 1998). Glyphosate shows this effect on both mono- and dicotyledons.

2.3.4.b Effects on non-target organisms

Glyphosate has theoretically few toxic effects on animals which do not posses the shikimic acid pathway, such as mammals, birds, fish and invertebrates and affects only plants, bacteria and fungi (Dill et al., 2010). In general, glyphosate possesses low toxicity to terrestrial organisms with a median lethal dose ranged from 800 to 1,340 mg/kg in mammals, and 1,170 to >2,000 mg/kg in amphibians (McComb et al., 2008). Nevertheless, some studies revealed accumulation of glyphosate in an earthworm species with enzymatic perturbations (Contardo-Jara et al., 2009) and in another laboratory experiment also growth reduction (Springett et al., 1992). A recent lab study has also proven long-term effects of glyphosate on the development and fertility of a predator insect *Chrysoperla externa* (Schneider et al., 2009). Moreover, several studies have revealed more important ecotoxicological effects (Contardo-Jara et al., 2009; Mann et al., 2009) as well as toxicological effects (Richard et al., 2005) of glyphosate formulations than of its acid and/or isopropylamine salt forms. Indeed, glyphosate in its acid form is soluble at 1% (pH 1), in its salt form at 50% (pH 4-5), and with added surfactants glyphosate it is able to penetrate the waxy cuticle of plant foliage (Dill et al., 2010). According to a recent review (Mann et al., 2009), the most added surfactant in glyphosate-based products is a polyoxyethylene tallowamine (POEA) and it has been found in several cases to be responsible for the toxicity of glyphosate formulations to amphibians (see also Dinehart et al., 2009 and Giesy et al., 2000).

In freshwater ecosystems glyphosate shows, in general, a low toxicity, but seem to be more toxic in alkaline than in acidic waters (USDA, 1997). An acute exposure of the phytoplankton community to glyphosate formulations perturbs its composition and its growth, but also stimulates in some cases the primary production (Pesce et al., 2011). This latter effect is attributed either to the adding of nitrogen and phosphorus with glyphosate degradation, what stimulates the growth of periphyton organisms like cyanobacteria, or to the pressure caused by the herbicide on herbivorous organisms. Despite the few data available, chronic exposure at environmental relevant concentrations are not significantly affecting freshwater organisms (Pesce et al., 2011).

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3 The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part I: Method validation in different water matrices

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Abstract

An analytical method for the quantification of the widely used herbicide, glyphosate, its main by-product, aminomethylphosphonic acid (AMPA) and the herbicide glufosinate at trace level was developed and tested in different aqueous matrices. Their derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) was done prior to their concentration and purification by solid phase extraction. The concentrated derivates were then analyzed by ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Spiking tests at three different concentrations were realized in several water matrices: ultrapure water, Evian© mineral water, river water, soil solution and runoff water of a vineyard. Except for AMPA in runoff water, obtained regression curves for all matrices of interest showed no statistical differences of their slopes and intercepts, validating the method for the matrix effect correction in relevant environmental samples. The limits of detection and quantification of the method were as low as 5 and 10 ng/l respectively for the three compounds. Spiked Evian© and river water samples at two different concentrations (30 and 130 ng/l) showed mean recoveries between 86 and 109%, and between 90 and 133% respectively. Calibration curves established in spiked Evian© water samples between 10 and 1000 ng/l showed r^2 values above 0.989. Monitoring of a typical vineyard river showed peaks of pollution by glyphosate and AMPA during main rain events, suggesting the diffuse export of these compounds by surface runoff. The depth profile sampled in the adjacent lake near a waste water treatment plant outlet showed a concentration peak of AMPA at 25m depth, indicating its release with treated urban wastewater.

Key words: AMPA, glufosinate, glyphosate, liquid chromatography-tandem mass spectrometry, surface water.

3.1 Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is a non-selective, post-emergence herbicide world-wide used for weed and vegetation control (Delabays and Bohren, 2007). In Switzerland, it represented around 13% of all plant protection product sales in 2005, whereas in United States it was 26% in 2007 (SGCI, 2006; Grube et al., 2011). When applied in the field, it shows a strong tendency to sorption and is rapidly transformed into AMPA [aminomethylphosphonic acid] in soils. Glyphosate is therefore often assumed to not reach water bodies (Gimmsing and dos Santos, 2005). However, due to their high water solubility, glyphosate and AMPA were found to be leached or washed out from soil surface layers and to reach ground and surface waters (Landry et al., 2005; Booregaard and Gimmsing, 2008; Botta et al., 2009). A large survey recently carried out in France showed that more than 50% of the analyzed surface water samples contained AMPA and that more than 30% contained glyphosate (IFEN, 2006). The maximum concentrations reached 48 and 165 µg/l for AMPA and glyphosate respectively. In ground water samples, only 2-3% contained these molecules with a maximum concentration of 6.8 µg/l for glyphosate.

But due to its zwitterionic nature and its tendency to form stable complexes with metal ions, trace quantification of glyphosate remains an analytical challenge (Freuze et al., 2007; Ghanem et al., 2007; Hanke et al., 2010). Indeed, glyphosate is an amino acid with several acid dissociation constant values (pKa = 2, 2.6, 5.6 and 10.6), due to the ionization of its functional groups: phosphonate, carboxyl and amine (Sprankle et al., 1975), which renders the analytical method not trivial. Its main metabolite AMPA and the herbicide glufosinate [2-amino-4-(hydroxy-methylphosphinyl)butyric acid] are also aminophosphonates, but with primary amine groups, whereas glyphosate has a secondary one (Fig. 3.1). In order to analyze these compounds, gas chromatography was used by several authors (Kataoka, 1996; Börjesson and Torstensson, 2000; Kjaer et al., 2011), but this is time-consuming and needs a derivatization procedure, in order to confer volatility to these compounds (Stalikas and Konidari, 2001; Ibáñez et al., 2005). According to Stalikas and Konidari (2001), liquid chromatography is considered a more adequate technique to analyze chemicals with this kind of properties. Moreover, the recent use of tandem mass spectroscopy techniques, following liquid chromatography (LC-MS/MS), allows determining low concentrations of ionizable pesticides, possessing, like glyphosate, either acidic and/or basic functional group(s) (Ibáñez et al., 2005).

Glyphosate detection at trace levels, i.e. below 100 ng/l, needs a concentration step of the water samples. Among available methods, solid phase extraction (SPE) was proposed by several authors (Ibáñez et al., 2005; Vreeken et al., 1998; Tran Thi et al., 2009), but a derivatization step, in order to lower its polarity, is nonetheless required (Petit et al., 2004). To do so, the pre-column derivatization with FMOC-Cl [9-fluorenymethylchloroformate] is considered the most simple and showed better results than other derivatization agents (Le Fur et al., 2000; Colin et al., 2003). Moreover, the use of isotope labeled standards as internal standards (IS) can minimize the so-called 'matrix-effect'

(IUPAC, 1997), as well as derivatization variations (Ibáñez et al., 2005). Glyphosate analysis was also improved with a pre-acidification step at pH 1, in order to break complexes that are formed with some metals or with natural organic matter, especially in groundwater samples (Ibáñez et al., 2006). Furthermore, Freuze et al. (2007) proposed to add the complexing agent EDTA to the sample, because some complexes may be reformed at the required pH of 9.2 for the derivatization with FMOC-Cl. These latter improvements are included in the 'state of the art' analytical procedure proposed by Hanke et al. (2008) and developed for surface- and ground-water samples. These authors reached limits of quantification at the ng/l level and obtained very good recoveries (91-107%).

Figure 3.1: Chemical structures of studied molecules: A) Glyphosate; B) Aminomethylphosphonic acid (AMPA); C) Glufosinate.

Considering the analytical challenges posed by glyphosate, AMPA and glufosinate, the objectives of this study were twofold. First, we aimed at testing and validating an analytical method that allows quantifying glyphosate, its main by-product AMPA and the less used herbicide glufosinate, by UPLC-MS/MS in different typical environmental water samples (from surface water samples to soil solutions). The purpose was to test the reliability of the method and validate the matrix effect correction with internal standards for further analysis in relevant natural samples. Indeed and as mentioned earlier, matrix effects can alter the obtained signal. To do so, the significance of slope and intercept discrepancies between different matrices, spiked at several concentrations, was tested. Second, we intended at evaluating the occurrence of the three compounds in different kinds of aqueous media with different anthropogenic influence. To do so, we chose a small river of western Switzerland situated in a vineyard catchment and the Vidy Bay, part of Lake Geneva, which receives the effluents of the city of Lausanne.

3.2 Materials and methods

3.2.1 *Chemicals*

Glyphosate (PESTANAL®, 99.7%), glufosinate-ammonium (PESTANAL®, 99.2%) and AMPA (99%) were obtained from Sigma–Aldrich (Buchs, Switzerland). Glyphosate-FMOC (98.5%), AMPA-FMOC (97%), glufosinate-FMOC (94%) and the internal standards (IS) labeled with stable isotopes 1,2-$^{13}C_2$,^{15}N glyphosate (98%) and ^{13}C,^{15}N AMPA (99%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock solutions for each compound with concentrations of about 1 g/l were prepared in ultrapure water (18.2 MΩ*cm) and acetonitrile (V/V=1:1), except for IS, which were prepared in ultrapure water only with a concentration of 1 mg/l. The stock solutions were stored at -18°C, except for IS, which were stored at 4°C. A mixture solution with concentrations of 4 mg/l of the analytes was prepared in water and acetonitrile (V/V=1:1). This latter was used for sample fortification and internal calibration, whereas individual stock solutions of the derivatized products were used for the tuning of the triple quadrupole. The derivatizing agent 9-fluorenylmethylchloroformate (FMOC-Cl) and the complexing one, ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2, purity >99%), were both obtained from Sigma–Aldrich (Buchs, Switzerland). For the derivatization step, aqueous solutions of 50 g/l FMOC-Cl and of 50mM borate buffer ($Na_2B_4O_7$ $10H_2O$) were prepared weekly. These three solutions were stored at 4°C.

3.2.2 *Analytical procedure*

The analytical method was adapted from Hanke et al. (2008) (Fig. 3.2, for more details see Appendix 3.1). Briefly, a sample aliquot of 80 ml was transferred to a high density polyethylene bottle. In order to eliminate possible interactions of glyphosate with matrix components, the sample solution was acidified with 6M HCl to pH 1 for one hour according to Ibáñez et al. (2006). Subsequently, the sample was spiked with 50 µl of the internal standard (IS) mixture solution and neutralized with 6M KOH. Five ml of borate buffer and ten ml of acetonitrile were added, resulting in the best conditions for the derivatization with FMOC-Cl: pH 9.2 and 10% of acetonitrile (Ghanem et al., 2007; Petit et al., 2004; Hanke et al., 2008). The bottle was shaken vigorously and the derivatization was performed in the dark at room temperature. After two hours, acidifying the solution to approximately pH 3 by adding one ml of formic acid stopped the reaction. The solution was then filtered with a 250 ml bottle-top filtration unit, using a 0.45 µm cellulose acetate membrane filter (Schleicher & Schuell, n°OE67), allowing the removal of FMOC-Cl excess.

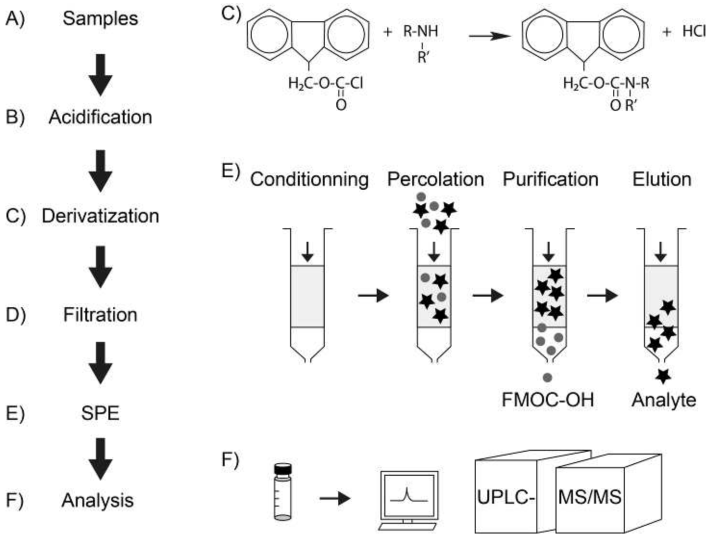

Figure 3.2: Main phases of the analytical procedure: A) Samples (80 ml); B) Acidification (1h); C) Derivatization with FMOC-Cl (2h); D) Filtration (0.45 µm); E) Solid-phase extraction (SPE); F) Analysis by UPLC-MS/MS.

In order to decrease the organic solvent content for the enrichment on reversed-phase, the sample was diluted with 100 ml of ultrapure water (Hanke et al., 2008). Then, eight ml of 0.5M EDTA were added to avoid complexation of the derivatized apolar compounds. The solid phase extraction (SPE) was applied as proposed by Hanke et al. (2008): conditioning of the 200 mg Strata-X© cartridges with 5 ml of methanol, followed by 5 ml of formic acid 0.1%, as well as the sample concentration and the removal of the hydrolysis product (FMOC-OH) with 3.5 ml of dichloromethane. The analytes were then eluted from the cartridges in glass tubes with 9 ml of MeOH without using vacuum, and reduced by evaporation to approximately 250 µl with a gentle flow of nitrogen gas at 40°C. The extracts were transferred to 1.5 ml amber glass vials and the volume was reconstituted with solvent A (see below) to approximately 0.5 ml in order to obtain the initial mobile phase conditions for the injection into the UPLC-MS/MS.

Table 3.1: UPLC-MS/MS parameters for glyphosate, AMPA and glufosinate and isotope labeled standard derivatives: retention times (R_t), MRM quantification (Q) and confirmation (C) transitions, cone voltage (CV) and collision energy (CE).

Analyte	R_t [min]	Transitions [m/z]	CV [V]	CE [eV]
Glyphosate-FMOC (Q)	5.3	390.17 → 168	16	24
Glyphosate-FMOC (C)	5.3	390.17 → 150	16	16
AMPA-FMOC (Q)	5.7	332.1 → 110	13	12
AMPA-FMOC (C)	5.7	332.1 → 135.6	13	20
Glufosinate-FMOC (Q)	5.7	402.17 → 134.2	19	50
Glufosinate-FMOC (C)	5.7	402.17 → 180.1	19	10
1,2-^{13}C^{15}N Glyphosate-FMOC (Q)	5.3	393.17 → 171	16	24
1,2-^{13}C^{15}N Glyphosate-FMOC (C)	5.3	393.17 → 153	16	16
^{13}C^{15}N-AMPA-FMOC (Q)	5.7	334.1 → 112	13	12
^{13}C^{15}N-AMPA-FMOC (C)	5.7	334.1 → 137.6	13	20

The target substances were then separated by liquid chromatography on an ACQUITY® Ultra Performance LC® system (Waters, MA, USA). For the best analyte separation, an alkaline mobile phase (pH 9-9.5) and a stationary hybrid reverse phase column (1.7 µm ACQUITY BEH C18, 2.1×50 mm; Waters, MA, USA) were used as recommended by N. Mazzella (pers.comm.). The mobile phase comprises a solvent A composed of 80% of water with 0.1% of triethylamine buffered at pH 9-9.5 with acetic acid and of 20% of acetonitrile, and a solvent B composed of 80% of acetonitrile and 20% of water with 0.1% of triethylamine buffered at pH 9-9.5 with acetic acid. The LC gradient for the separation was: from 0 to 3.5 min 95% A: 5% B, from 3.5 to 10 min 5% A: 95% B and from 10 to 15 min 95% A: 5% B. Initial conditions were re-established in 1 min resulting in a total run time of 16 min. The injection volume for UPLC-MS/MS analysis was 7.5 µl. The flow rate was 0.3 ml/min and the column temperature 30°C. The target substances were then detected by tandem mass spectrometry (MS/MS) using a TQD mass spectrometer (Waters MS Technologies, Manchester, UK). The instrument was equipped with an electrospray ionization source operated in the negative mode (ESI$^-$). Nitrogen was used as desolvatation gas, with a flow rate of 650 l/h at 350°C, and was extracted from room air by a N_2 generator (N_2-Mistral-4, Schmidlin Labor). The capillary voltage and source temperature were 3 kV and 150°C respectively. The cone was cleaned before each analysis. Optimization of the TQD for each target analyte was obtained by direct injection of the FMOC-standards in the multiple reaction monitoring (MRM) mode. Obtained retention time and optimum ionization and fragmentation conditions for every FMOC-analytes are given in Table 3.1.

3.2.3 *Method validation in different water matrices*

Spiking tests were performed in different water matrices in order to validate the method for further monitoring campaigns. The chosen matrices were: ultrapure water, Evian© water, river water, soil solution and runoff water. Natural water samples were collected close to the Lutrive River in a vineyard area located above the village of Lutry, Switzerland (Fig. 3.3).

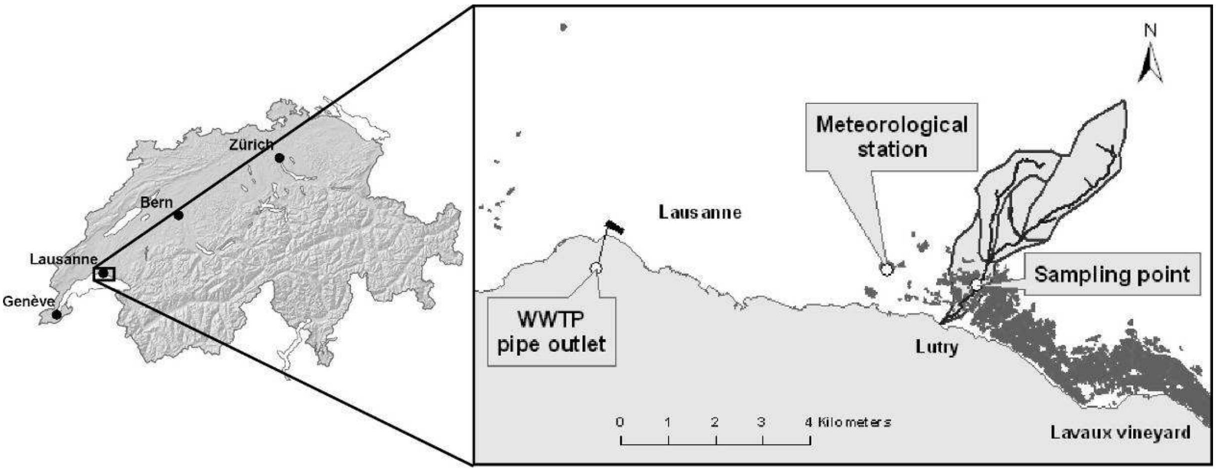

Figure 3.3: Study area in western Switzerland showing the location of the WWTP outfall in the Vidy Bay and the sampling point in the Lutrive River watershed.

Spiking tests were performed at three different concentrations (40, 80 and 120 ng/l) in all matrices; in natural waters blank subtraction was performed. In each case, samples were spiked and analyzed in triplicate. The main parameters of the different water samples are presented in Table 3.2: dissolved organic carbon (DOC) measurements were realized with a Liquitoc (Elementar©) and water hardness was calculated after Ca^{2+} and Mg^{2+} measurements with an ICS-1100 (Dionex-Thermo Fischer©) as following: $[CaCO_3] = 2.5[Ca^{2+}] + 4.1[Mg^{2+}]$. Linear curves were obtained by plotting the ratio of the analyte area to the IS area against the ratio of the theoretical concentration of the analyte to the IS one. The corresponding internal standards were used for glyphosate and AMPA, whereas AMPA IS was used for glufosinate as they are both primary amines (Hanke et al., 2008). The difference of slopes and intercepts for the curves were tested with the Prism® program according to Zar (1999). The p-values were fixed to 0.05.

The accuracy of the method was assessed by calculating mean recoveries between the measured and the spiked concentrations in Evian© and River water, at 30 and 130 ng/l in triplicates. The limits of detection (LOD) and quantification (LOQ) of the method were determined in ultrapure, Evian© and surface water samples as the lowest concentrations with a signal/noise ratio equal or above three and ten respectively (Shabir, 2003; Gros et al., 2006). The three different samples were spiked at four different concentrations (7, 14, 21, 28 ng/l), with blank subtraction for surface water. For quantification in environmental samples a larger calibration curve was built by spiking Evian© water at seven concentrations: 10, 25, 50, 100, 250, 500 and 1000 ng/l. The method reproducibility was assessed by calculating the standard deviations of responses from two standards (50 and 1000 ng/l) of three different calibration curves, performed at three different moments within two months. Standard deviations of the calibration curves slopes were checked as well.

Table 3.2: Main properties of analyzed water samples: pH, electrical conductivity (EC), dissolved organic carbon (DOC) and hardness, expressed in French degrees [°F].

	pH	**EC [µS/cm]**	**DOC [mg/l]**	**Hardness [°F]**
Evian© water	7.2	590	< 0.5	29.8
River water	8.2	331	4.5	14.6
Soil solution	8.5	450	1.5	41
Runoff water	8.4	110	15	11

3.2.4 *Environmental sampling*

The Lutrive is a local river in the east of the city of Lausanne, at the western limit of the Lavaux vineyard area (Fig. 3.3). Its small watershed (6.4 km^2) is characterized by different land uses: agricultural fields (45%), of which 4.1% are vineyards, urban and impervious surfaces (31%) and forests (24%). Grab samples were collected in the vineyard area during the growing season of 2010 and during both dry- and wet-weather conditions. Daily precipitation data of the meteorological station of Pully, located at 2 km west of the Lutrive River, were obtained from Swissmeteo© (Fig. 3.3). Lake Geneva was sampled during dry weather on the 1st of July 2010, in the Vidy Bay near the waste water treatment plant (WWTP) outlet at nine different depths: -2, -5, -10, -15, -18.5, -21, -23, -25 and -29 m. Corresponding real-time temperature and electrical conductivity data were obtained from Bonvin et al. (2011).

3.3 Results and discussion

3.3.1 *Linearity and matrix effect*

The response factors, *i.e.* the ratio area/IS area, for the different concentrations, normalized by IS concentration, showed a good linearity for the three compounds (Fig. 3.4). Coefficients of determination (r^2) were all above 0.916 except for AMPA in runoff water, which was only 0.324. The slopes were varying between 2.4 and 3.1 for glyphosate, 5.1 and 10.7 for AMPA and between 9.3 and 10.7 for glufosinate; intercepts were varying between -0.072 to 0.069. Both values, slopes and intercepts, were not significantly different between the different matrices samples for glyphosate and glufosinate. For AMPA however, a difference with the others was observed for the runoff sample with a slope of 5.1. The same was observed for the intercept that is higher than the others (0.32). These poorer results for AMPA in runoff samples can be explained either by substantial AMPA content in the spiked sample or by the high DOC concentration in this kind of sample (see Table 3.2).

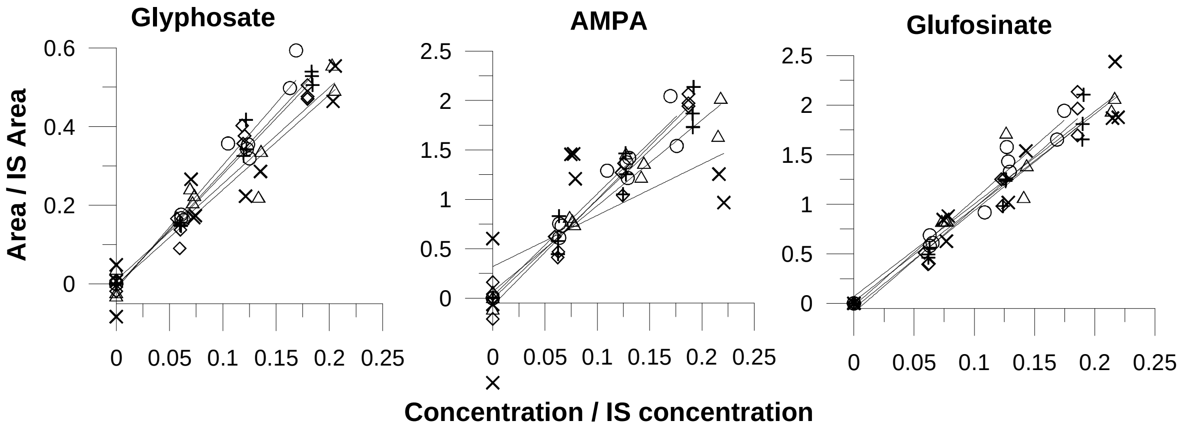

Figure 3.4: Performance of the developed method for the five water types tested: triplicates of spiked water samples of three concentrations (40, 80 and 120 ng/l) normalized by internal standards (IS) labeled with stable isotopes, with the different matrices: Ultrapure water (○), Evian© water (+), River water (◊), Soil solution (Δ) and Runoff water (×); blank subtraction were performed for soil solution and runoff water samples.

Nevertheless, in general the results confirm the ability of internal standards to compensate signal losses due to the matrix-effect, which was stronger for runoff samples and soil solution. Indeed, both showed considerable discrepancies in slopes when compared with ultrapure, Evian© or river water samples before normalization with IS (see Appendix 3.2). Thus, with the exception for AMPA in runoff water, the results show the applicability of the method for the monitoring of several types of environmental samples: surface water, soil solution and runoff samples. Moreover, they confirm the suitability of Evian© water as calibration matrix. Indeed and surely due to its mineral content, Evian© water showed more similar slopes to environmental matrices than ultrapure water, making it more suitable for building calibration curves.

3.3.2 *Precision and accuracy*

Calibration curves in spiked Evian© water samples were generated from 10 up to 1000 ng/l. They showed a linear behavior with the following equations and coefficients of determination (r^2): glyphosate = 1.222x + 5.204, r^2 = 0.991; AMPA = 1.325x + 1.707, r^2 = 0.989; glufosinate = 1.249x + 0.372, r^2 = 0.995. The inter-day variation of standards responses showed a good reproducibility with relative standard deviations of 9, 17 and 9% for glyphosate, AMPA and glufosinate respectively at 50 ng/l and of 8, 4 and 9% at 1000 ng/l; standard deviations of calibration curve slopes were varying of 3, 1.6 and 6.5% respectively. Despite elevated response variations for river water spiked at low concentrations (30 ng/l), the method showed a good accuracy with mean yields of spiked Evian© samples varying from 86 to 109%, whereas for spiked river water samples they were varying from 90 to 133% (Table 3.3). This variability is substantially reduced at higher concentration (130 ng/l) and can thus be related to blank subtraction. However, it could be due in part to several other factors: the presence of dissolved salts, the ionization efficiency and/or the influence of the property of the ionization cone.

Table 3.3: Mean recoveries of spiked water samples (n=3) [%, (SD%)].

Sample	Concentration [ng/l]	Glyphosate	AMPA	Glufosinate
Evian© water	30	109.1 (26.9)	86.3 (28.4)	88.0 (17.9)
Evian© water	130	101.1 (12.1)	105.1 (9.2)	104.0 (20.0)
River water	30	102.9 (133.8)	115.6 (13)	90.3 (47.8)
River water	130	115.9 (9.0)	133.1 (19.6)	109.8 (30.8)

3.3.3 *Limits of detection (LOD) and of quantification (LOQ)*

The limit of quantification (LOQ) in ultrapure and Evian© water samples was 7 ng/l, with a signal/noise ratio (S/N) equal or above 10 for the three compounds, whereas for river water samples S/N was lower. However, at 14 ng/l the S/N ratio was higher than 10. As the concentration of the first standard used to build the calibration curves is 10 ng/l, the LOQ can thus be fixed at this level. Spiked Evian© water at lower concentrations showed S/N ratios above 3 at 5 ng/l. In surface water sample S/N ratios above three were observed at 7 ng/l. Thus, the method LOD and LOQ were fixed at 5 and 10 ng/l respectively.

3.3.4 *Environmental samples*

Samples taken in the Lutrive River exhibited concentrations between the detection limit and maximum values of 800 ng/l and 300 ng/l for glyphosate and AMPA respectively (Fig. 3.4). These concentration peaks are well above the threshold value defined for pesticides in Switzerland (100 ng/l). This implicates that glyphosate and AMPA may be hazardous for surface waters. These values are in the range of previous results obtained with occasional sampling in different other Swiss rivers (Hanke et al., 2008; Corvi et al., 2005).

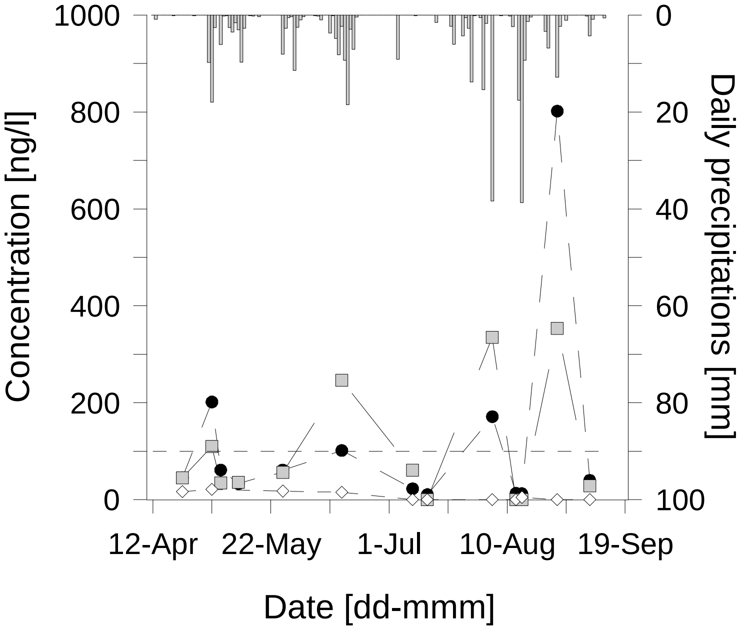

Figure 3.4: Results for the Lutrive River from April to September 2010: Concentrations of glyphosate (●), AMPA (■) and glufosinate (◊); daily precipitations from the Pully meteorological station (Swissmeteo©, histograms); threshold of the federal ordinance on water protection (Oeaux) for pesticides (100ng/l; --).

Glyphosate shows a typical pattern for chemicals applied in agriculture, with elevated concentrations during rain events, suggesting the transfer of these compounds from fields to surface water as already shown for other herbicides (Leu et al., 2004; Freitas et al., 2008). The peaks are well linked to main rain events (precipitations > 10-20 mm). April applications gave rise, with 27.8 mm of rain in two days, to the contamination peak of May 2: 201 ng/l of glyphosate. This transfer can occur by surface runoff (Huang et al., 2004), via preferential pathways through soils (de Jonge et al., 2000) and/or facilitated by drains (Brown and Van Beinum 2009; Kjaer et al., 2011).

The concentration pattern of AMPA also exhibits peaks, suggesting a similar transport pathway than for glyphosate. During summer rain events, AMPA concentrations became higher than glyphosate concentrations, revealing a possible transformation of glyphosate into AMPA in soils, for example catalyzed by microorganisms (Gimmsing et al., 2004). However, in late August glyphosate concentrations increased again, the highest concentration having been reached on August 27 (glyphosate: 801 ng/l; AMPA: 353 ng/l). Two hypotheses may be formulated: one being a late application in vineyards, glyphosate can be applied from April to August, and the other seeding bed preparation for crop in the upper part of the catchment (Hanke et al., 2010).

The herbicide glufosinate was not often detected and at concentrations just above LOQ, even during rain events, although it was applied in the catchment. This is possibly due either to its minor use or its low mobility and/or rapid degradation in soils ($DT50_{field}$=7d)[33]. Nevertheless, the first hypothesis is more realistic as glyphosate shows only a slightly slower degradation in soils ($DT50_{field}$=12d), in contrary to its metabolite AMPA which shows a much more persistent character ($DT50_{field}$=151d).[32]

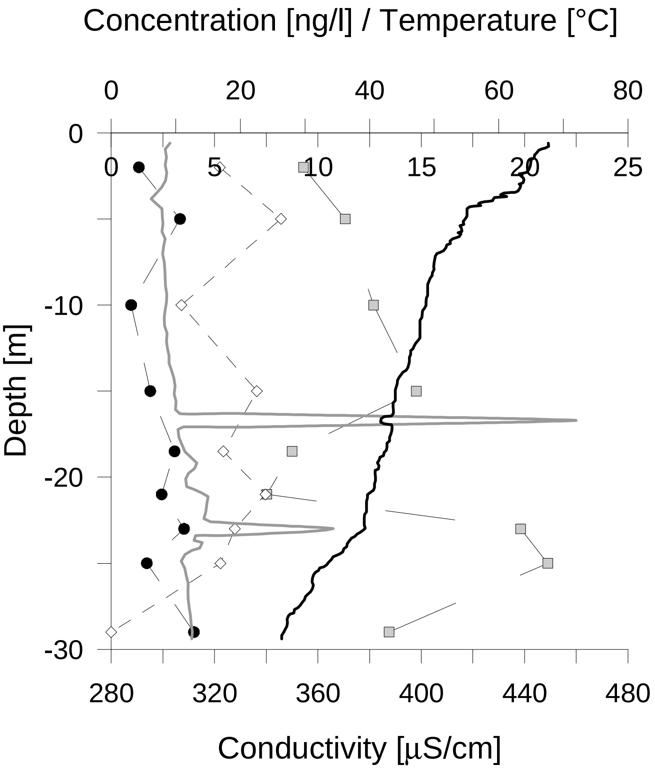

Figure 3.5: Results for the lake depth profile sampled above the WWTP outlet in Vidy Bay, Lake Geneva, the 1st of July 2010; glyphosate (●), AMPA (■) and glufosinate (◊) concentrations; temperature (-) and conductivity (-) profiles.

Results of the depth profile from the Vidy bay of Lake Geneva in July 2010 (Fig. 3.5) showed glyphosate concentrations in general below the LOQ. Glufosinate and AMPA were detected in higher concentrations reaching a maximum of 26 and 67 ng/l, respectively, suggesting possible other sources than for glyphosate. For AMPA, the highest concentrations were found at 25 m depth, at which depth dissolved organic carbon (DOC) and major ions measurements (Na^+, Ca^{2+}, K^+, Mg^{2+}, Cl^-, NO_3^-) show also a concentration peak (Appendix 3.3). In a recent publication, Bonvin et al. (2011) highlighted the influence of the WWTP outlet and the release of treated wastewater at this specific depth, as confirmed by temperature and conductivity anomalies. This may explain the increase in concentrations of the metabolite AMPA and major ions at this depth as shown for other micropollutants such as

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[33] Pesticide properties data base, Footprint project, www.eu-footprint.org, consulted May 30, 2012.

pharmaceuticals (Bonvin et al., 2011). It has been suggested that the degradation of phosphonic acids in detergents was also an important source of AMPA in wastewater, especially during dry periods (Botta et al., 2009). No substantial concentration peaks are observed for the herbicides glyphosate and glufosinate and thus, diffuse losses by terrestrial runoff are more probable as shown for other pesticides by Bonvin et al. (2011).

3.4 Conclusions

The validation of the method to quantify the herbicide glyphosate, its metabolite AMPA and the herbicide glufosinate at trace level in several types of natural waters was successful and allows these potentially hazardous molecules to be followed in the environment. Further investigations to better understand their behavior in soils after their application and their transport to surface water will be possible. Preliminary results of field studies show that river water samples exhibit a frequent pollution by the studied herbicides, which finally end up in Lake Geneva. Several samples showed concentrations above the legal threshold of 100 ng/l. This highlights the importance of monitoring these substances in the aquatic system.

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4 The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part II: The role of infiltration and surface runoff

In press in *Journal of Environmental Science and Health, part B: Pesticides, Food Contaminants, and Agricultural Wastes*

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Abstract

Two parcels of the Lavaux vineyard area, western Switzerland, were studied to assess to which extent the widely used herbicide, glyphosate, and its metabolite AMPA were retained in the soil or exported to surface waters. They were equipped at their bottom with porous ceramic cups and runoff collectors, which allowed retrieving water samples for the growing seasons 2010 and 2011. The role of slope, soil properties and rainfall regime in their export was examined and the surface runoff/throughflows ratio was determined with a mass balance. Our results revealed elevated glyphosate and AMPA concentrations at 60 and 80 cm depth at parcel bottoms, suggesting their infiltration in the upper parts of the parcels and the presence of preferential flows in the studied parcels. Indeed, the succession of rainy days induced the gradual saturation of the soil porosity, leading to rapid infiltration through macropores, as well as surface runoff formation. Furthermore, the presence of more impervious weathered marls at 100 cm depth induced throughflows, the importance of which in the lateral transport of the herbicide molecules was determined by the slope steepness. Mobility of glyphosate and AMPA into the unsaturated zone was thus likely driven by precipitation regime and soil characteristics, such as slope, porosity structure and layer permeability discrepancy. Important rainfall events (>10 mm/day) were clearly exporting molecules from the soil top layer, as indicated by important concentrations in runoff samples. The mass balance showed that total loss (10-20%) mainly occurred through surface runoff (96%) and, to a minor extent, by throughflows in soils (4%), with subsequent exfiltration to surface waters.

Keywords

Glyphosate, AMPA, infiltration, runoff, throughflows, vineyards, Switzerland.

4.1 Introduction

Aqueous solubility is considered to be the major physicochemical parameter for the assessment of transport and fate of agrochemicals in soils, but their mobility in this media is also highly dependent on their sorption abilities (Stangroom et al., 2000; Ruggerio et al., 2002). Herbicide loss from agricultural fields depends on their physical and chemical properties, but also on meteorological and site-specific factors, as well as anthropogenic ones, such as agricultural practices (Salmon-Monviola et al., 2011). The use of herbicides represents several environmental threats such as rapid water contamination and, in a more long term perspective, the development of resistance mechanisms in weeds (Delabays et al., 2004). Glyphosate [*N*-(phosphonomethyl)glycine] is the most used herbicide in the world for weed and vegetation control, and, with the recent banning of other molecules, such as atrazine, it became almost the only applied molecule in viticulture in Switzerland, leading to a delicate situation in term of weed resistance (Delabays and Bohren, 2007; Egger, 2009; Dill et al., 2010).

Glyphosate, and its main metabolite aminomethylphosphonic acid (AMPA), possess a ubiquitous character: they show relatively high water solubilities (10.5 and 5.8 g/l respectively), and also high sorption abilities (log Koc = 4.34 and 3-4.4 respectively) and low leaching potentials (GUS index = -0.49 and 0.21 respectively; www.eu-footprint.org). They are therefore assumed to show a limited mobility in soils and to not reach water bodies (Sprankle et al., 1975; Beltran et al., 1998). Nevertheless, binding of glyphosate is a complex phenomenon influenced by several soil parameters and constituents (Albers et al., 2009), and recent studies have demonstrated that glyphosate can be leached or washed from soil surface layers and reach ground and surface waters (Landry et al., 2005; Boorgaard and Gimmsing, 2008).

Glyphosate mobility in soils is pH-dependent as its charges vary from +1 to -3 depending on its four different dissociation constants (Sprankle et al., 1975; Sheals et al., 2002). In general, it is less adsorbed with increasing pH (Zhao et al., 2009). In natural soil pH ranges (4-9) glyphosate has generally one or two negative charge(s) and thus is more likely to complex with positively charged moieties, mainly soil minerals with an important contribution of Fe and Al oxides (Boorgaard and Gimmsing, 2008). It has been indeed proved to sorb on iron and aluminium hydroxides such as goethite ($FeOOH$) and gibbsite ($Al(OH)_3$) (Gimmsing et al., 2004), but also on clay minerals, such as montmorillonite (Morrillo et al., 1997) and, generally to a lower extent, on humic substances (Piccolo et al., 1996; Albers et al., 2009). Some ternary complexes with negatively charged surfaces can be formed with the intermediate of a positively charged moiety (Sprankle et al., 1975; Morrillo et al., 2002; Barrett and McBride, 2006). Glyphosate sorption in soils is influenced by multivalent cations in the following order: $Fe^{3+} > Al^{3+} > Cu^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+} > Ca^{2+} \sim Mg^{2+}$ (Gimmsing and dos Santos, 2005). Therefore, pH conditions, the presence of cationic moieties and the multiple interactions between Al/Fe oxides, organic matter and clay minerals influence largely the retention of glyphosate and thus its mobility in soils (Sprankle et al., 1975; de Jonge and de Jonge, 1999).

Soil texture and pore structure have also been proved to influence the potential infiltration processes of glyphosate (de Jonge et al., 2000; Bergstrom et al., 2011). It was higher in a clayey soil than in a sandy one, due to a slower degradation rate explained by a strongest adsorption in the clayey soil (Bergstrom et al., 2011). For its metabolite AMPA, no infiltration was observed in the sandy soil, but some in the clay soil, which suggests, despite its more persistent nature, a faster degradation rate than for glyphosate. A similar observation was made by de Jonge et al. (2000) with a larger amount of leached glyphosate in a sandy loam soil than in a sandy one. However, the observed difference is thought to be more related to the pore structure and the occurrence of macropore flows shortly after application, as well as non-equilibrium sorption, than texture discrepancies (de Jonge et al., 2000; Candela et al., 2007). Thus, glyphosate is more susceptible to be leached in well-structured fine-textured soils than in structure-less sandy soils, as well as with a shorter contact time prior a rain event. Furthermore, preferential flow and associated colloidal transport have been proved to represent important mechanisms in the transfer of this strongly sorbed herbicide to water bodies (Gjettermann et al., 2009; Boorgaard, 2011; Kjaer et al., 2011).

All these processes responsible of the fate of glyphosate and AMPA in the environment are obviously interdependent in a complex manner, and factors controlling their transport to water bodies, and their importance in space and time, are still under investigation (Coupe et al., 2012). Many studies have already been conducted in soil columns in the laboratory to evaluate the factors governing the mobility of glyphosate and AMPA (de Jonge et al., 2000; Candela et al., 2007; Dousset et al., 2007; Zhou et al., 2010). Field studies are much more scarce and have been done, either in high latitudes contexts (Kjaer et al., 2005, 2011; Simonsen et al., 2008; Laitinen et al., 2009; Aronsson et al., 2010) or with different management practices (Landry et al., 2005; Candela et al., 2010). The work of Landry et al. (2005) is, to our knowledge, the only one in vineyard soils, which are highly loaded with copper, due to the classical use of Cu-based fungicides. It is interesting to assess glyphosate transfer in this kind of agro-ecosystem as copper has been proved to have a significant influence on glyphosate mobility and potential transfer to ground- and surface waters and vice versa (Barrett and McBride, 2006).

In this study, the fate of glyphosate and its metabolite AMPA was assessed in two parcels of the Lavaux vineyard area in western Switzerland. The transport dynamics of these molecules was examined with monitoring equipments during the 2010 and 2011 growth cycles of grapevines. The role of different parameters, such as slope, soil properties and rainfall regime, was studied regarding their potential infiltration and/or surface transport by runoff, with subsequent potential threats to ground- and surface waters. The expected implication of soil macropores and preferential water pathways in their transport was examined, as well as the one of soil particles and colloids. This study is, to our knowledge, the first dealing with glyphosate fate in agricultural soils in Switzerland.

4.2 Material and methods

4.2.1 *Study area and soil features*

The Lavaux is a vineyard area located in western Switzerland of 830 ha including about 10’000 terraces on 40 km between Montreux and Lutry to the east of Lausanne (Fig. 4.1a). This landscape is composed of moraine deposits and, with its steep slopes from 13 to 43% and the light reflexion on the Lake of Geneva, it represents a very suitable environment for the growth of grapevines (Letessier and Fermond, 2004). The bedrocks are composed of Tertiary molasse deposits, which include conglomerates, sandstones and marls from the upper Oligocene epoch (23-28 Ma) (Weidmann, 1988). These formations constitute the substrate of the Lavaux landscape, upon which, during the Quaternary, moraine materials were deposited by the Rhône glacier. More specifically, the area of Lutry is characterised by several faults separating the ‘coal molasse’ from the ‘red molasse’, respectively composed of sandy dark marls and silty red ones (Weidmann, 1988; Berger, 1998). All these geological formations, along with more recent landscape modifications, are at the origin of the large heterogeneity observed in soil profiles of the studied area.

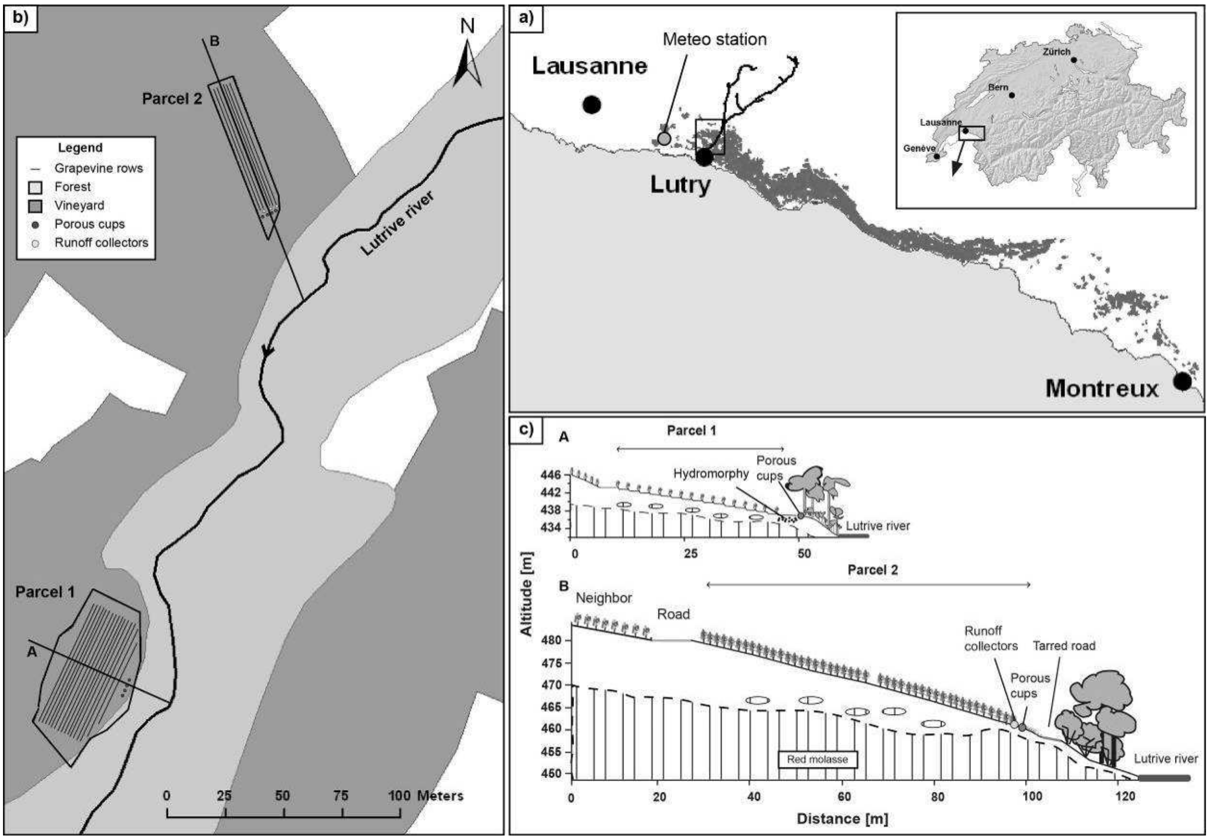

Figure 4.1: Study area: a) Lavaux vineyard area in western Switzerland, located at Lake Geneva near Lausanne, the location of the meteorological station of Pully (MeteoSwiss©) is also shown. b) The two vineyards parcels close to the Lutrive River and the location of studied transects from the top of the parcels down to the river. On parcel 1 grapevines form horizontal rows, planted on small terraces, whereas on parcel 2, they are arranged vertically, following the slope. c) Cross sections, showing the unsaturated zone and inferred molasse substrate as well as the sampling devices to collect soil and runoff water (porous cups and runoff collectors for parcel 2).

Based on a previous work dealing with risk assessment of pesticides transfer from vineyards to surface waters (Egger, 2009), two risky parcels were chosen close to the Lutrive River (Fig. 4.1b). The general character of the soils was obtained trough drilling with an auger and digging two pits of a depth up to two meters at their bottom. Textures, colours and the presence of carbonates were determined in the field, whereas soil pH was measured in the laboratory on water extractions (Soil:H_2O=1:2.5). More precise grain size analyses by laser diffractometry (Malvern©, Mastersizer 2000) were obtained for samples of various depths at the bottom of both parcels, treated with HCl beforehand to remove carbonates.

Soils of both parcels are colluvial calcosols, according to the French classification (Baize and Girard, 1995), with anthropogenic influences. The dominant colour, referring to the Munsell colour system (Oyama and Takehara, 1992), was medium to light brown (10YR4/3) with a grey tendency (2.5Y5/3) in the deepest horizons. Both soils showed a *silt loam* texture and light differences were observed between plots and depths. HCl reactivity at all depths revealed the carbonate nature of soils, which was confirmed by pH-values between 8 and 9. Organic carbon contents varied from the surface layer to deeper horizons between 1.7 and 0.7% in parcel 1 and 2.2 and 0.8% in parcel 2 (Appendix 4.1). Copper concentrations in top soils (0-30 cm) varied between 300 and 500 mg/kg in parcel 1 and 100 and 170 mg/kg in parcel 2 (Appendix 4.2).

Parcel 1 has a gentle slope of about 15% and the convergence of drainage waters induces temporary hydromorphism at its bottom (Fig. 4.1c), as already observed by Letessier and Fermond (2004). From 80 to 110 cm a more humid horizon with redoximorphic features was indeed identified, more greyish in colour and the presence of iron oxides, and some decomposing organic matter. A knife hole made in this latter horizon gave rise to pouring water with a constant flow of max. 1 dl/min. These observations, along with surface water stagnation during and after rainfall events, confirm the hydromorphic nature of this parcel, i.e. the presence of a temporary perched water table. From 110 cm on, a more dry and clayey horizon was found and at 130 cm a reddish layer (2.5Y5/4) was observed, interpreted as the marls of the 'red molasse' beds cited before. This less permeable marly layer may explain the water saturation between 80 and 110 cm depth and the observed constant flow indicates the presence of subsurface lateral flows, known also as ‘throughflows’ or ‘interflows’, whether they reach directly or not a river or lake (Whipkey, 1978; Pidwirny, 2006). Velocities of these kinds of flows are usually slow: maximum flow rates occur on steep slopes and in pervious sediments with the lowest ones in flat soils containing heavy clays.

Parcel 2 has a steeper slope (~25%) and drainage waters converge in the direction of the Lutrive River. As in parcel 1, a layer with a reducing hydromorphic regime was situated downwards from 80 cm depth, and at 115 cm a reddish layer was also encountered. In the middle of the plot, drillings revealed a more or less identical profile down to 85 cm depth, but the hydromorphic layer between 80 and 115 cm and the reddish horizon beneath were not always observed, suggesting a C-horizon consisting of dm- to m-sized discontinuous lenses of weathered marls (Fig. 4.1c).

4.2.2 *Sampling and analytical methods*

In both parcels, the herbicide glyphosate was applied the same day and only under the rows, leaving a grass band in between them. It is mainly applied in spring time to avoid a nutrient and water competition between grapevines and weeds during the growing season. Application data were obtained from winegrowers and are summarized in Table 4.1. Precipitation data were obtained from the closest meteorological station of Swissmeteo©, located in Pully at 1.8 km from the two parcels (Fig. 4.1a).

Table 4.1: Quantities of applied products and their active ingredients in studied parcels and dates of herbicide application

Parcel	Product	Quantity [l/ha]	Active Ingredient (A.I.)	A.I. applied [g/ha]	Application date
1, 2	Glyfos®[1]	3	Glyphosate, isopropyl amine salt	1080	20.04.2010 15.04.2011

[1] ®Cheminova Inc.

In order to sample the soil solution, both parcels were equipped at their bottom with porous ceramic suction cups (SDEC SPS200, Ø63 mm, porosity=1 µm) at four different depths: 20, 40, 60 and 80 cm (Fig. 4.1c). Their implementation, in fall 2009, and use were done according to Bès and Sévenier (1999). The applied tension was 0.6 bars and the recovery of samples was done every week or more frequently during intense rainfall periods. As the slope of parcel 2 is more representative of the Lavaux region, it was equipped with three runoff collectors. They were built inspired from previous studies (Schulz et al., 1998; Trevisan and Dorioz, 2001), and placed at the end of grapevine rows according to observed erosion paths. They comprised a bottle buried in the soil, a funnel with a sieve of 1 mm size to avoid macro fauna or large particles, a PVC conducting ramp placed just under the root zone and ending above the funnel, and a roof to avoid direct rain inputs.

Samples were collected in 250 ml high density polyethylene bottles and transported to the laboratory in a cool box. They were placed in a freezer until their analysis, for which they were then gently de-frozen. Electrical conductivity and pH were determined in the field.

The herbicide glyphosate and its metabolite AMPA were quantified by LC-MS/MS with a previously developed method (Daouk et al., submitted), based on their pre-column derivatization with FMOC-Cl and their enrichment by solid phase extraction. The limit of quantification was 10 ng/l and it was tested successfully for the matrix effect that could occur by analyzing soil solution and runoff samples. Dissolved organic and inorganic carbon (DOC/DIC) concentrations were measured with a C-analyzer (Liquitoc Elementar©). Ion concentrations were obtained with an ion chromatography system (ICS-1100/2100, Dionex-Thermo Fischer©).

A principal component analysis (PCA) was performed on the soil water samples using the R software to help interpreting all the analyses and discriminating the observations made in the two different parcels (R Development Core Team, 2009). Prior to it, each of the parameters was normalized to zero mean and unit variance, by subtracting the mean value of the variable and by dividing by the standard variation, allowing them to have the same influence in the PCA.

In order to determine the surface runoff/throughflows ratio, a mass balance was done for both surface runoff and soil solution samples of parcel 2 (surface = 845 m^2). As glyphosate was applied only under the grapevine rows, the initial quantities correspond to half of the surface. The mass (M) of glyphosate and AMPA were obtained by multiplying the concentrations (C) with cumulated precipitations that fell on the parcel surface between two sampling events (mmINT): M [g] = C [g/l] × mmINT [l].

4.3 Results and discussion

4.3.1 *Soil water samples*

Soil water samples had in general pH values between 7.3 and 8.4 and electrical conductivities (EC) between 300 and 550 µS/cm (Fig. 4.2). Higher range of values was observed at 20 cm in parcel 1 and at 60 and 80 cm in parcel 2, with pH and EC values of 8.4-9 and 450-700 µS/cm respectively. This certainly reflects the presence of throughflows deep in the profile as previously observed. Dissolved organic carbon (DOC) contents varied in general between 2 and 10 mg/l, except in parcel 2 at 20 cm, where they were between 7 and 24 mg/l. Inorganic carbon (DIC) concentrations were found between 35 and 60 mg/l, with slightly higher values at 80 cm in parcel 2 (between 60 and 75 mg/l). Ion analysis revealed a calcium-dominated composition, with variable magnesium, sodium, nitrate and sulphate contents (Appendix 4. 3). The variability for the latter was certainly due to the application of sulphur in the two parcels to prevent fungal diseases. A surprising difference in HCO_3 discriminated samples from the two parcels, with high content for half of the samples from parcel 1 and very low ones for the others. This, together with an increase in Mg, may in part be related to the temporary reducing conditions in parcel 1, and the reversible processes of reductive-dissolution of Fe oxides, and Fe^{2+} fixation on clay minerals, as shown by Boivin et al. (2002).

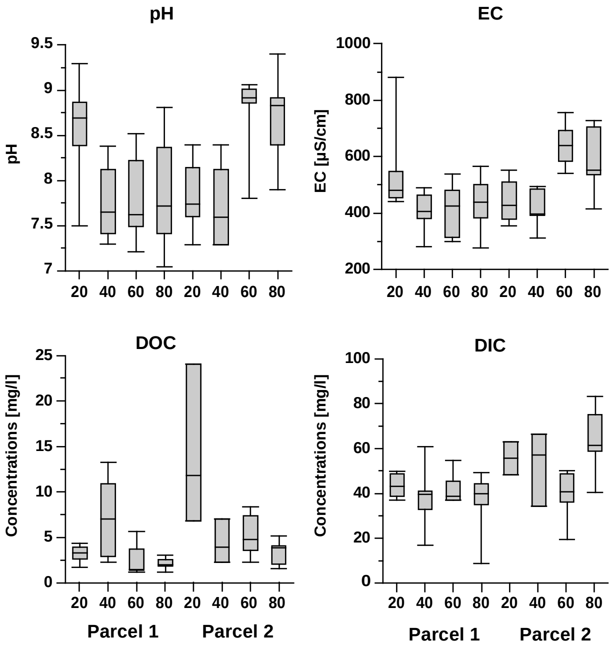

Figure 4.2: pH, electrical conductivity (EC), dissolved organic and inorganic carbon (DOC/DIC) contents in soil water samples at 20, 40, 60 and 80 cm of both parcels.

For both parcels, in 2010, glyphosate and AMPA in soil solution were generally found at concentrations higher than 300 ng/l only at 20 and 80 cm (Fig. 4.3). In 2011, much higher concentrations were observed, especially in the surface layers: up to 4.7 and 1.75 µg/l at 20 cm in parcel 1, respectively 12 and 6.5 µg/l at 40 cm in parcel 2 (Fig. 4.4). This discrepancy can be attributed to several factors: higher application rates or repeated treatments in the neighbourhood parcels, a different rainfall regime, with subsequent different water pathways, or maybe also the ageing of the ceramic porous cups. The obtained soil solution at the different depths is indeed likely to depend on the local pore size distribution, and the degree of equilibration between water in matrix pores and in macropores (Williams and Lord, 1997). In certain cases, the sampling area of porous cups has been shown to decrease with increasing infiltration rates (Weihermuller et al., 2005), and aged ceramic cups showed better passage rates for pesticides than new ones (Domange et al., 2004).

In parcel 1, in 2010 (Fig. 4.3a), concentrations above 500 ng/l were found at 20 cm on June 15 for glyphosate (785 ng/l) and on June 15 and 17 for AMPA (1696 and 876 ng/l respectively). Some smaller increases in concentrations were also observed for glyphosate at 20 cm on May 27 (247 ng/l), and for AMPA at 60 cm on May 18 (344 ng/l) and at all depths on August 27 (120-240 ng/l). Concentration peaks were always related to cumulated rainfall during the previous days, leading to the observed punctual water logging at the surface of this parcel.

The succession of important rainfall events in August (>10 mm/day) in the end, induced the presence of AMPA at all depths, revealing a higher mobility than for glyphosate, despite its lower water solubility. Its constant production by soil microorganisms, as well as its slightly different sorption properties may also in part explain this observation.

In 2011 (Fig. 4.4a), glyphosate concentrations were in general higher at 20 and 60 cm than at 40 and 80 cm, but only the 20 cm-samples exhibit concentrations at the µg/l level in April-May. Glyphosate concentrations in the 60 cm samples decreased with time, but showed important variations, from 50 to 700 ng/l, linked to important rain events. They first decreased from 700 to 100 ng/l and then re-increased in mid-May to up to 500 ng/l and also in late June up to 400 ng/l.

AMPA concentrations in parcel 1 at the same depth showed similar variations, but in contrast, often increased with time (Fig. 4.4a). Important rain events of more than 20 mm in one day, such as the one of early June, induced also a rise in concentrations at 40 and 80 cm. Furthermore, a much more important increase in concentrations was noticed at 80 cm than with more than 40 mm precipitation in two days, such as on June 17 to 18.

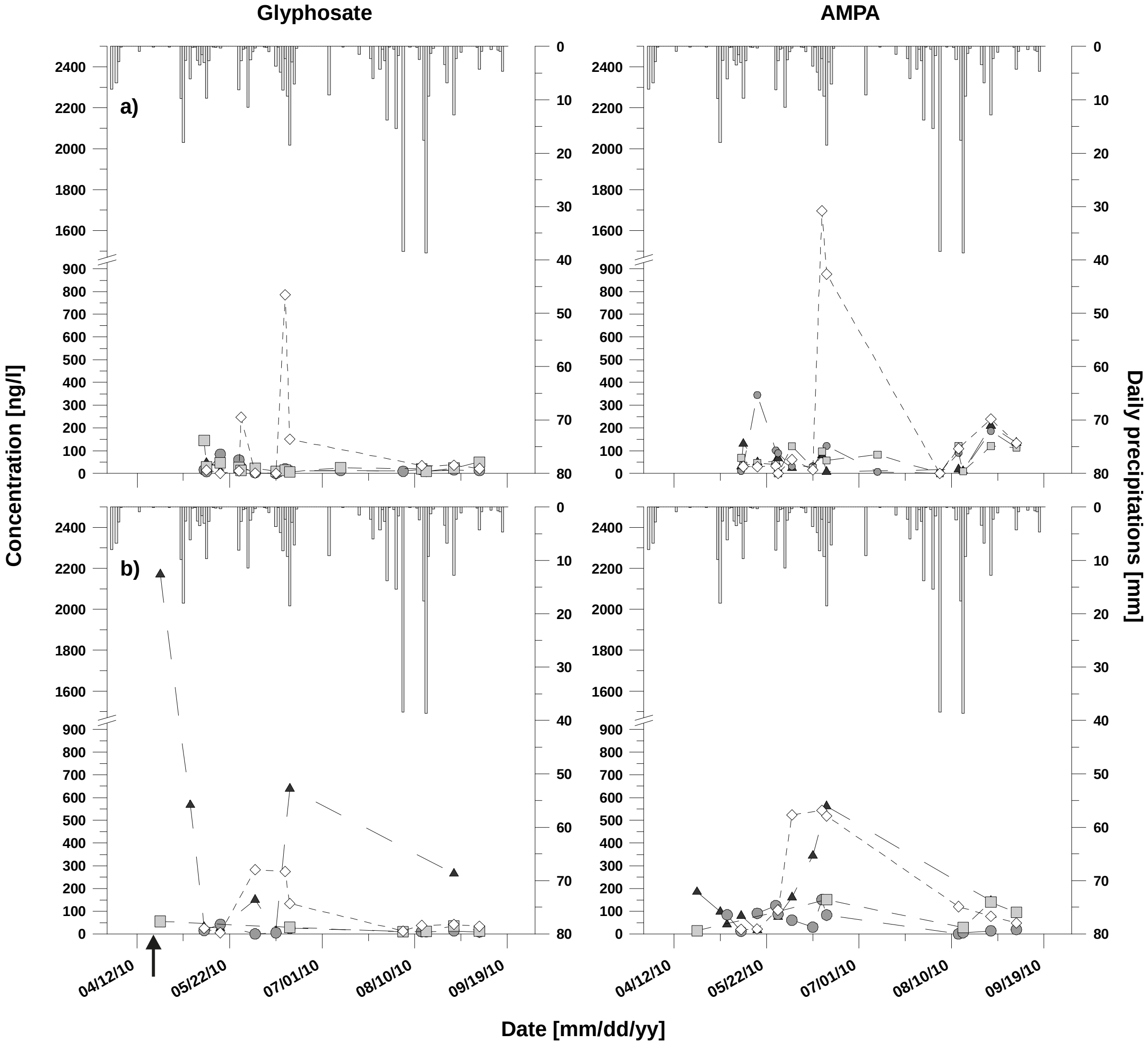

Figure 4.3: Growing season 2010. Concentrations of glyphosate and AMPA in soil solution at the bottom of parcel 1 (a) and 2 (b), at 20(◇), 40(■), 60(●) and 80 cm (▲) for the period April-September 2010. The black arrow indicates the date of glyphosate application and the daily precipitations are shown as bars (scale at the right side of the graph).

Concerning the infiltration processes in parcel 1, the important rainfall events of June 2010 seem to have significantly leached the soil surface layer, desorbing in part glyphosate and AMPA molecules. Mainly located in the first layer due to their sorption properties, they were then mobilized and, surely due to the presence of cracks or root channels at the surface, they infiltrated and were retrieved in the 20 cm depth porous cup. Nevertheless, part of the herbicides can be transported downhill by surface runoff and either be retained in the forested buffer zone (see Fig. 4.1b), or participate to the contamination of the Lutrive River, as previously observed (see Chapter 3).

In parcel 2, in 2010 (Fig. 4.3b), the highest concentration of glyphosate in soil solution (2171 ng/l) was found at 80 cm depth, two days after its application (April 22, 2010). This elevated concentration can be explained by both the small amount of rain (0.1 mm in 5 days) and the short time between application and sampling, resulting in a highly concentrated soil water sample. Indeed, the second sample, taken at 80 cm on May 5 after 36.5 mm of rain in five days, showed a lower concentration of 567 ng/l. Then concentration peaks of up to 640 ng/l for glyphosate and 562 ng/l for AMPA were found at 80 cm depth on June 17. These peaks were also observed at 20 cm, but not at 40 and 60 cm.

The gradual rise observed in AMPA concentrations in parcel 2 in June 2010 at 80 cm depth (Fig. 4.3b) suggests an increasing water saturation of the soil pore space, and certainly the further use of preferential pathways by the soil solution in this parcel. In contrast to parcel 1, the occurrence of these peaks at 80 cm is certainly explained by the steeper hillslope, which represents, with the presence of more impervious reddish marls remnants in the subsoil (Fig. 4.1c), favourable conditions for the formation of lateral water circulation within the unsaturated zone.

However, infiltration apparently took place in the upper part of the parcel, and, due to their high affinity for soil colloids, preferential flows and/or colloid-associated transport are likely to occur, as demonstrated in previous laboratory studies (de Jonge et al., 2000; Gjettermann et al., 2009).

In 2011, still in parcel 2 (Fig. 4.4b), a surprisingly high concentration peak of glyphosate in late July and a corresponding one of AMPA in early August was noticed at 40 cm. This could reflect either a second application in the neighbourhood parcels or a change in the pore system at this depth, with different inferred water pathways.

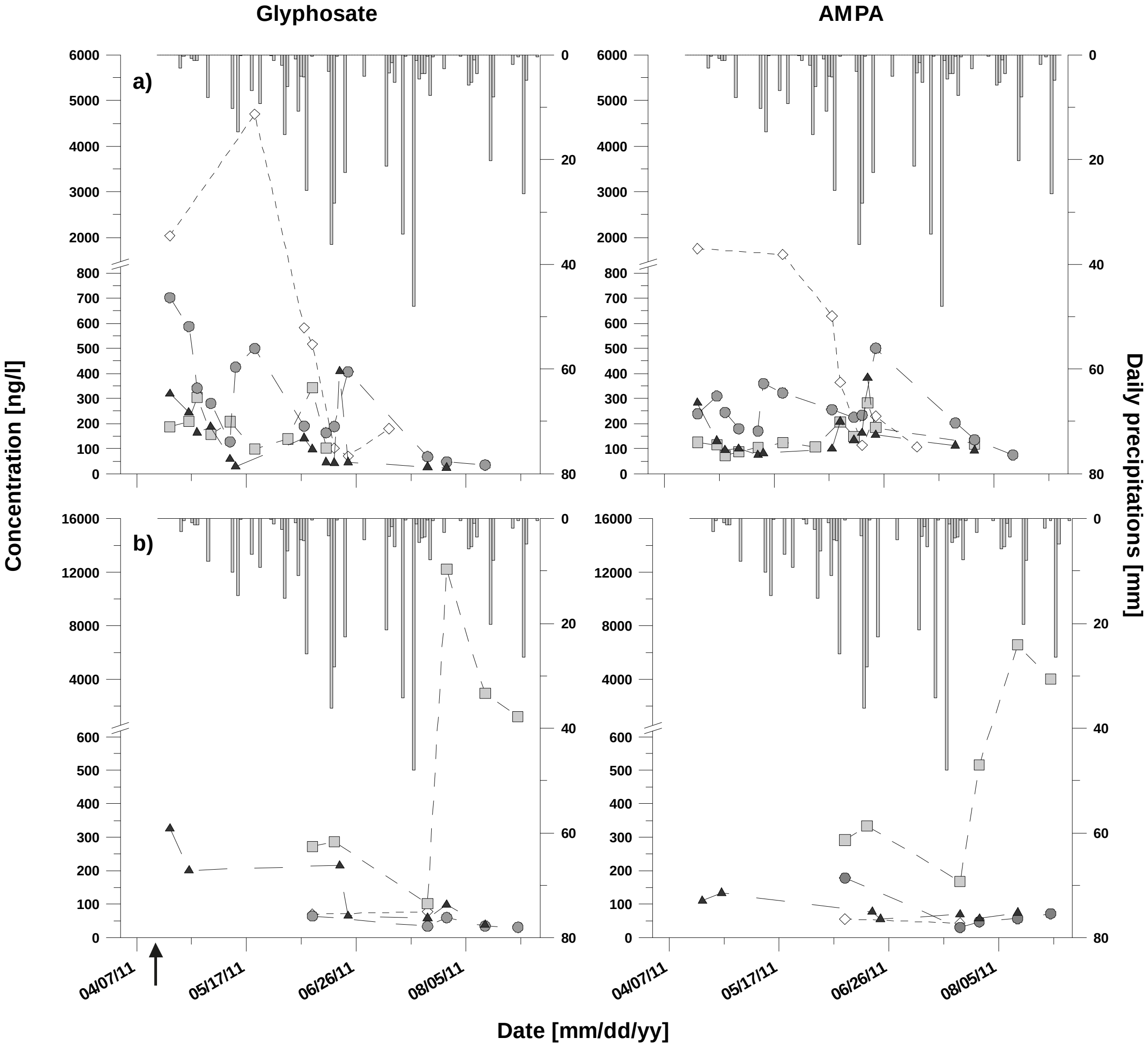

Figure 4.4: Growing season 2011. Concentrations of glyphosate (left) and AMPA (right) in soil solution at the bottom of parcel 1 (a) and 2 (b), at 20(◇), 40(■), 60(●) and 80 cm (▲) for the period April-September 2011. The black arrow indicates the date of glyphosate application and the daily precipitations are shown as bars (scale at the right side of the graph).

Thus, concerning the infiltration behaviour in both parcels, the variable intensity of rain events and the presence of the more impervious reddish layer or lenses around 100 cm are certainly the two most important parameters explaining the occurrence of glyphosate and AMPA at 60 and 80 cm at the bottom of both parcels. Leaching and infiltration of glyphosate and AMPA further up in the parcel and their subsequent transport via throughflows are conditioned by the specific soil characteristic, as well as by to the succession of significant rain inputs.

Figure 4.5 shows glyphosate and AMPA concentrations as a function of cumulated rainfall between two sampling periods. In general, with the exception of highly concentrated samples (>2.5 µg/l) and the effect of degradation with time, cumulated rain fall seem to govern glyphosate and AMPA concentration dynamics in the vadose zone in the following way: 1) cumulated rainfalls up to 10 mm decrease herbicide concentrations due to a dilution effect, 2) quantities between 10 and 30 mm lead to a concentration rise, certainly due to an increase in the kinetic energy of the soil solution, with the consequent formation of preferential flow in the parcels with colloid-associated transport, and 3) from 30 mm of cumulated rainfall, the increased surface runoff and dilution are responsible for the decrease in concentration.

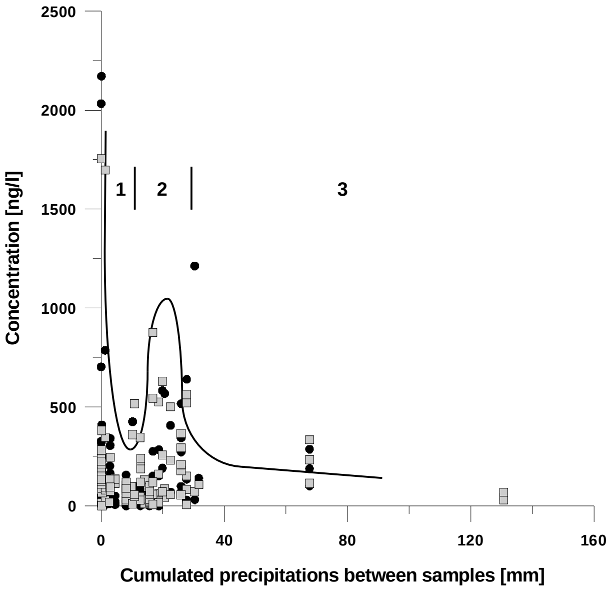

Figure 4.5: Influence of cumulated rainfall between two sampling periods on glyphosate (●) and AMPA (■) concentrations in soil waters of both vineyard parcels. Three phases can be distinguished. 1: An important decrease due to a dilution effect, 2: At medium rainfall, an important increase due to preferential flows and colloid-associated transport and 3: At cumulated rain amounts greater than 30 mm, a levelling-off decrease, due to the combined effect of increased surface runoff and dilution.

In addition, chemical parameters of the soil solution, such as the alkaline pH conditions and the presence of copper in considerable amounts, are known to diminish glyphosate and AMPA sorption, participating thus in their release (Morillo et al., 1997, Barrett and McBride, 2006; Zhao et al., 2009). Furthermore, the often slightly reducing conditions encountered at 80 cm depth may also have led to a lower sorption capacity, with Fe^{3+} reducing to Fe^{2+} (Gimmsing and dos Santos, 2005).

4.3.1.a Principal component analysis (PCA)

The PCA performed on the normalized matrix of different parameters for all samples retrieved in the porous cups of both parcels showed five components with Eigen values above 1, which accounted for 67% of the dataset variance. As shown in Fig. 4.6a, the two first components are able to discriminate soil water samples between the two parcels by their differences in chemical composition, along an axis pH, Mg, DIC on one hand, and Cl, SO_4, Na on the other. The reducing conditions, more present in parcel 1 and related to temporary hydromorphic conditions, certainly explain in part this difference.

However, glyphosate and AMPA concentrations were poorly linked with these chemical parameters. They were more related to cumulated precipitations between samples (mmINT) along the Y axis formed by the 5th component (Fig. 4.6b), which account for 8.1% of the variance. Observations linked to this Y axis are mainly from parcel 2 and showed indeed a concentration gradient, with highly loaded samples linked with small cumulated precipitations and inversely. This confirms the higher variability observed in parcel 2 and the dilution by the rainfall events, and thus the important role of rainfall regime on the transport dynamics, as already shown in Fig. 4.5. Though laboratory results are difficult to extrapolate to field situation, the rain fall intensity and the soil pore structure were previously found to play a prevalent role on the sorption properties for glyphosate leaching in soil columns (de Jonge et al., 2000).

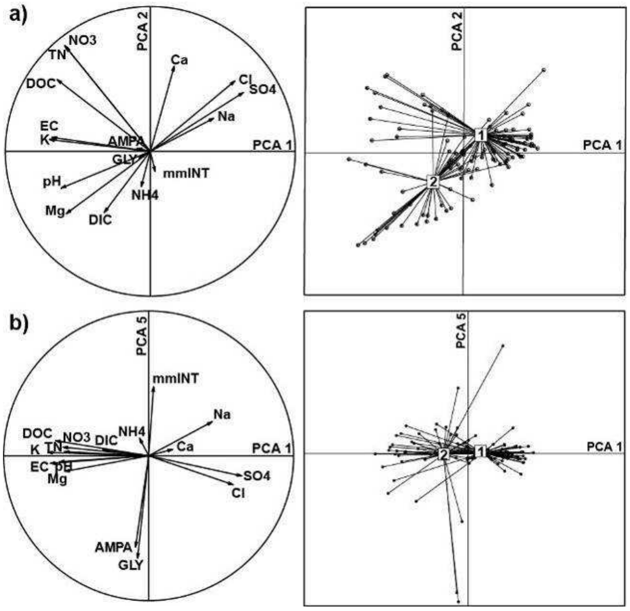

Figure 4.6: Principal Component analysis (PCA) done with R-software on different normalized parameters of the soil water samples: a) correlation circle and projection of the different parameters on the 1st (X) and 2nd (Y) component axis diagram, accounting for 40.8% of the variance, and relative positions of the observations with parcels as gravity centres; b) Idem on the 1st (X) and 5th (Y) component axis diagram, accounting for 32.4% of the variance.

4.3.2 *Surface runoff water*

Runoff water samples collected in parcel 2 showed pH between 8.3 and 8.7 and electrical conductivity between 50 and 105 µS/cm. In 2010, high concentrations of glyphosate and AMPA were found in the two first unfiltered (but decanted) runoff samples after the application (Fig. 4.7): 73 and 110 µg/l, respectively 9 and 14 µg/l. This result is in agreement with the relatively high concentration found (567 ng/l) in the soil solution at 80 cm for the same period (05/05/10). Glyphosate concentrations dropped down to 7 µg/l in May and then to 4 µg/l in early June, before decreasing to 1 µg/l after the succession of rainy days in mid-June.

For AMPA, the decrease in concentrations was less drastic, what can be explained by the fact that it is assumed to be constantly produced by glyphosate degradation. The high concentration (~9 µg/l) observed in early July occurred after only one rainy day after a dry period that probably allowed soil microorganisms to decay glyphosate into AMPA more actively.

In 2011, concentrations were in the same range of values and their decrease was also observed, but to a lower extent. In contrast to 2010, AMPA concentrations were never higher than those of glyphosate. At the end of June, high concentrations were observed again with 95 µg/l of glyphosate and 9 µg/l of AMPA. These values are in same range than right after the application in late April, revealing an application on neighbourhood parcels. Indeed, the important rainfall of more than 40 mm in two days induced certainly a huge runoff, possibly passing across the road situated above the parcel, and penetrating it.

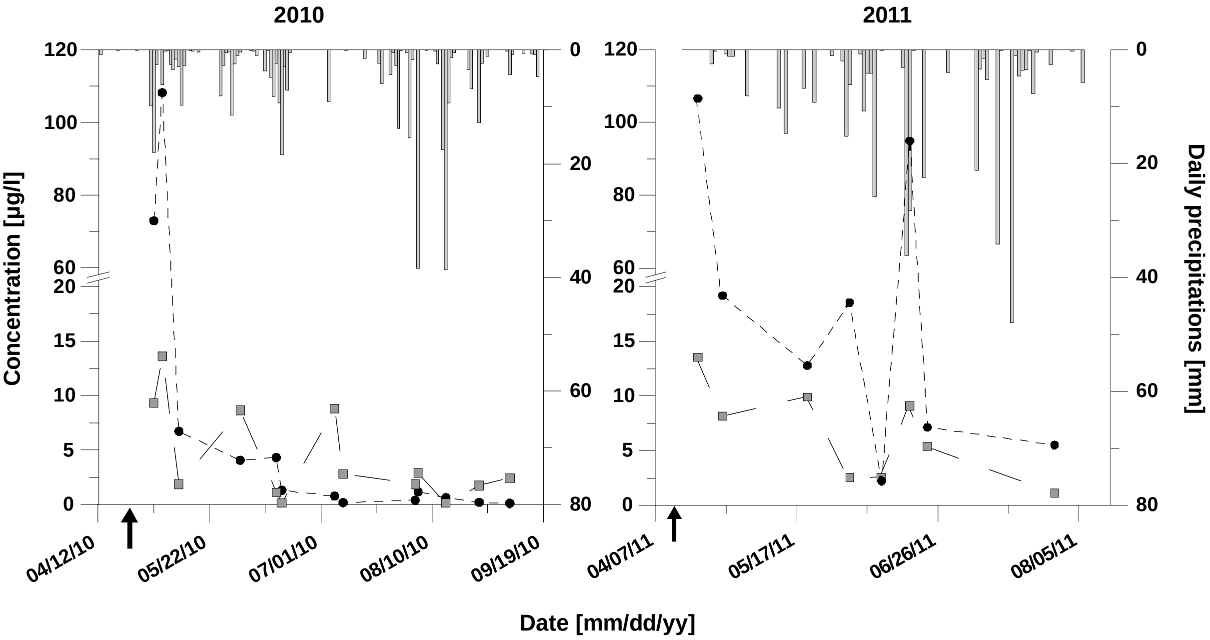

Figure 4.7: Runoff of parcel 2. Glyphosate (●) and AMPA (■) concentrations in unfiltered, but decanted runoff water samples in 2010 (left) and 2011 (right); the black arrow again indicates the date of glyphosate application and the daily precipitations are shown as bars (scale at the right side of the graph).

In order to determine whether glyphosate and AMPA were transported in the dissolved state or bound to soil particles, a syringe filtration (Nylon filters) of runoff samples was made: the fraction <0.45 µm still carried between 70 and 90% of the total concentration, with medians of 78% and 73% for glyphosate and AMPA respectively (n=10, Appendix 4.5). Thus, transport of glyphosate and AMPA associated to coarse particle (>0.45 µm) accounted for 20-30%, which is more than in previous studies despite a smaller cut-off (0.24 µm) (de Jonge et al., 2000; Kjaer et al, 2011).

The presence of considerable amounts of copper in the surface soil, may in part explain this discrepancy. The soil particles collected after sample decantation showed indeed a mean copper concentration of 150 mg/kg, what is in the same range than in the top soil of this parcel (see above). The presence of copper may lead to the desorption of glyphosate from solid surfaces and the formation of aqueous Cu-glyphosate complexes, which show a high stability and a lower sorption affinity than glyphosate alone, as demonstrated by Morillo et al. (1997, 2002). Inversely, glyphosate may also enhance copper mobility by chelating with Cu^{2+} previously adsorbed on both mineral and organic surfaces (Barrett and McBride, 2006). However, the alkaline conditions (pH 9) were previously shown to favour the binding of Cu-glyphosate complexes to soil constituents, certainly with the formation of ternary surface complexes as suggested for goethite with spectroscopic investigations (Sheals et al., 2002; Dousset et al., 2007).

4.3.3 *Mass balance*

The total amount of glyphosate and AMPA retrieved in both type of samples from parcel 2 (surface = 845 m^2), and likely to be exported from it, was 4.3 g in 2010 and 9.1 g in 2011 (Table 4.2). This represents respectively 10 and 20% of the initial amount, which, despite the uncertainty of such kind of calculations, is in agreement with previous studies (Gavrilescu, 2005; Simonsen et al., 2008). The 80-90% remaining were either retained, and possibly as bound residues after some time, or degraded in the soil (Sprankle et al., 1975; Boorgaard and Gimmsing, 2008; Simonsen et al., 2008), as volatilization is not likely to happen due to their properties[28]. The relative contribution of throughflows in the unsaturated zone versus surface runoff in our case was 3-5% versus 95-97%.

Table 4.2: Mass balance for glyphosate in parcel 2 for both growing seasons, with amounts retrieved in both types of samples, soil solution and surface runoff, according to cumulated precipitations, as well as percentages of the applied amount and of the relative contribution of throughflows and surface runoff. 1 g of AMPA was considered arising from 1 g of glyphosate.

Year	Applied amount [g]	Soil water [g]	[%]	Runoff [g]	[%]	Throughflows [%]	Runoff [%]
2010	45.6	0.145	0.32	4.179	9.16	3.36	96.64
2011	45.6	0.466	1.02	8.659	18.98	5.10	94.90

4.3.4 *Synthesis*

The presence of variable amounts of glyphosate and AMPA previously observed in the Lutrive River (see Chapter 3), indicates a significant export of these molecules from the vineyards. Both infiltration processes in the unsaturated zone with subsequent exfiltration to the river and surface runoff are at the origin of this export. Figure 4.8 summarizes the processes which can be inferred from our observations.

The occurrence of glyphosate and AMPA at 60 and 80 cm depth in significant amounts confirms their infiltration, whose extent depends on the precipitation regime and physical soil characteristics. As suggested with rainfall events of more than 10 mm/day (see Fig. 4.5), the increase in rain intensity leads obviously to the saturation of soil matrix pore space with water, and thus macropore flows with certainly associated colloid-transport are likely to happen, transporting herbicide molecules relatively rapidly from the soil top layer down the profile. With more important rainfall (>30 mm/day) both micro- and macropores are getting saturated and surface runoff becomes the dominant process.

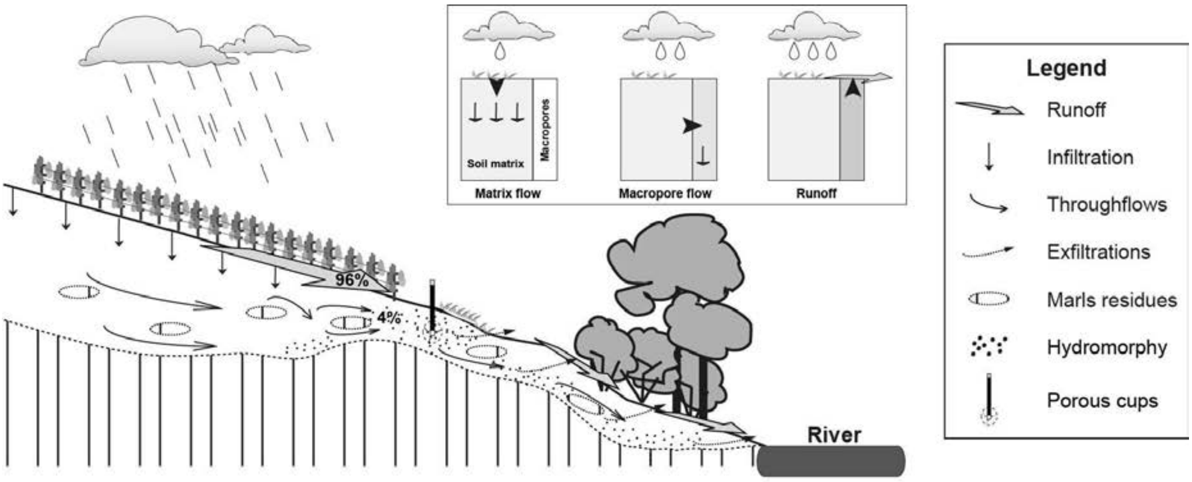

Figure 4.8: Synthesis illustration of water pathways in vineyard parcels with infiltration and throughflows, leading to temporary hydromorphy and exfiltration, as well as surface runoff responsible for the export of glyphosate and AMPA molecules in the river direction. Gradual saturation of the soil porosity is shown as insert as rain intensity increases, with matrix flow and then the formation of macropore flows and surface runoff.

Then, the presence of a more impervious layer or lenses around 100 cm depth in the investigated parcels induces throughflows, undoubtedly leading to temporary saturated zones, leaking to the river. When comparing the two parcels, the steepness of the hillslope determines the importance of these throughflows in herbicide transport. Furthermore, the precipitation regime seems to govern more strongly glyphosate and AMPA transport in soils than chemical interactions as suggested by the PCA. However, the observed peaks of glyphosate and AMPA in the Lutrive River during rain events remain however the result of surface runoff processes (96%). In these latter, particle-associated transport (>0.45 µm) plays a minor role, whereas around 75% of the surface runoff happen in the dissolved fraction and/or associated with small colloids. In contrast, exfiltration of soil water that underwent throughflows participate in a minor extent to the contamination of the river (4%).

In our case, the absence of an aquifer possibly receiving these molecules and the presence of a more impervious horizon at 100 cm limited the risk of groundwater pollution, but it can be important in other contexts. The national survey on groundwater quality, realized by the Federal Office for the Environment (FOEN) in 2004-2006, reports indeed a frequent contamination (>0.1 µg/l) with AMPA (10% of the monitoring stations) and, to a lower extent, with glyphosate (3%) (OFEV, 2009).

4.4 Conclusions

This study presents clear evidence for the mobility of the herbicide glyphosate and its metabolite AMPA in the vadose zone despite their high sorption abilities. Though chemistry of soil solution does not play an important role in their transport, mainly governed by rainfall regime and soil permeability, the presence of copper and the alkaline pH conditions in the studied vineyard soils certainly participate in their mobility by influencing their sorption. Thus, in fine-textured layered soils with significant slope, the increase in moisture content leads to the formation of throughflows just above the more impervious layer, which actively participate in the transport of glyphosate and AMPA downhill. Nevertheless, their transfer from fields to adjacent surface water happens mainly by surface runoff, in a dissolved state or bound to small colloids, representing potential threats for aquatic organisms.

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5 Dynamics and environmental risk assessment of the herbicide glyphosate and its metabolite AMPA in a small vineyard river of the Lake Geneva catchment.

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Abstract

The use of pesticides may lead to environmental problems, such as surface water pollution, with a risk for aquatic organisms. In this work, a typical vineyard river of western Switzerland was first monitored in order to measure discharged loads, identify sources and assess the dynamic of the herbicide glyphosate and its metabolite AMPA. Second, based on river concentrations, an associated environmental risk was calculated, using laboratory tests and ecotoxicity data from the literature. Measured concentrations confirmed the mobility of these molecules with elevated peaks during flood events, up to 4970 ng/L. From April to September 2011, a total load of 7.1 kg was calculated, with 85% coming from vineyards and minor urban sources and 15% from arable crops. Compared with existing literature, this load represents an important fraction (6-12%) of the estimated amount applied, due to the steep vineyard slopes (~10%). The associated risk of these compounds towards aquatic species was found negligible in our case, as well as for other rivers in Switzerland. Growth stimulation was nevertheless observed for the algae *Scenedesmus vacuolatus* with low concentrations of glyphosate, which could indicate a risk of perturbation in aquatic ecosystems, such as eutrophication. Combining field and ecotoxicity data allowed to perform a realistic risk assessment for glyphosate and AMPA and should be applied to other pesticide molecules.

Keywords

Glyphosate, AMPA, Vineyard river, Species sensitivity distribution, Risk assessment

5.1 Introduction

Contamination of surface water by pesticide molecules represents an unwanted side effect of their use and a potential risk for aquatic organisms, especially in agricultural areas. Their transfer from fields to surface water happens mainly by rainfall-induced surface runoff, but may also occur in part by sub-surface runoff and via drainage pipes according to landscape configuration (Freitas et al., 2008; Brown and van Beinum, 2009). This transfer depends largely on the intrinsic properties of molecules, but weather conditions, application practices, as well as soil types and land use are also important parameters to consider (Rabiet et al., 2010; Salmon-Monviola et al., 2011).

The widely used herbicide glyphosate [*N*-(phosphonomethyl)glycine] and its metabolite AMPA [aminomethylphosphonic acid] are among the main pesticide molecules found in surface waters worldwide (Battaglin et al., 2009). Main properties of these two molecules are given in Table 1. In 2010 in France, 46% of more than a thousand surface water samples contained AMPA and 25% contained glyphosate, the two most frequent pesticide molecules detected (www.statistiques.developpement-durable.gouv.fr). In Switzerland, there are only scarce data of their occurrence in surface waters yet, despite the extensive use of glyphosate in both urban and agricultural areas. Hanke et al. (2008) analyzed several water samples and found low concentrations of glyphosate and AMPA in the Rhine River and two lakes of eastern Switzerland (up to 75 ng/L). However, tributaries of the two lakes showed higher concentrations, up to 390 ng/L.

Table 5.1: Main properties of glyphosate and aminomethylphosphonic acid (AMPA); Source: www.eu-footprint.org or noticed.

Properties	***Glyphosate***	***AMPA***
Formula	$C_3H_8NO_5P$	CH_6NO_3P
Molecular weight [g/mol]	169.07	111.04
Water solubility [g/L]	10.5	5.8
Log Kow	-3.2	-1.63
DT50 field [d]	3-174[a]	76-240[a]
Sorption coefficient Kd [L/kg]	222	-

[a] Source: Laitinen, 2009

In agriculture, the expansion of minimum and no-till practices to avoid soil erosion and organic matter mineralisation, as well as the recent banning of other molecules such as atrazine, led to an increased application of non-selective herbicides, such as glyphosate (Delabays and Bohren, 2007; Dill et al., 2010). In the Swiss watershed of Lake Geneva, glyphosate is among the 10 most used pesticides and is applied in many different types of culture (Klein et al., 2007). For grapevine for instance, a crop mainly located in western Switzerland, glyphosate is nearly the only herbicide used (Dubuis et al., 2011), which may result in a delicate situation in terms of weed management, with an elevated risk to develop resistance (Delabays and Bohren, 2007; Shaner et al., 2012). The few grab samples taken in Swiss rivers showed elevated concentrations, often above the legal threshold of 100 ng/L for each molecule of pesticide, defined in the Swiss federal ordinance on water protection (Oeaux) (Corvi et al., 2005; Hanke et al., 2008). It is therefore important to further monitor glyphosate and AMPA in Swiss streams and assess the related risk for aquatic ecosystems.

Glyphosate has theoretically a low toxicity towards animals, such as mammals, birds, fish and invertebrates, which do not possess the targeted metabolic pathway, and would affect mainly plants, bacteria and fungi (Dill et al., 2010). Indeed, it shows a median lethal dose that ranges from 800 to 1,340 mg/kg in mammals, and from 1,170 to >2,000 mg/kg in amphibians (McComb et al., 2008). Nevertheless, some studies revealed that glyphosate accumulated and reduced the growth of earthworm species (Springett and Gray, 1992; Contardo-Jara et al., 2009), or showed long-term effects on the development and fertility of the predator insect *Chrysoperla externa* (Schneider et al., 2009). Several studies showed that the glyphosate formulation, i.e. the commercial form of glyphosate, is more toxic (Richard et al., 2005) and ecotoxic (Contardo-Jara et al., 2009; Mann et al., 2009) than the acid and/or isopropylamine salt forms. The polyoxyethylene tallowamine (POEA) surfactant has been indeed found to be largely responsible for the toxicity of glyphosate formulations (Tsui and Chu, 2003; Cedergreen and Streibig, 2005).

In freshwater ecosystems glyphosate shows, in general, a low toxicity, but seem to be more toxic in alkaline than in acidic waters (USDA, 1997; Solomon and Thompson, 2003). Exposures to short, but high concentrations of glyphosate formulation perturb the composition and the growth of phytoplankton communities, but also stimulate in some cases the primary production (Pesce et al., 2011). This latter effect is attributed either to the increase of nitrogen and phosphorus by glyphosate degradation, which stimulates the growth of periphyton organisms, such as cyanobacteria (Vera et al., 2012), or to the pressure caused by the herbicide on herbivorous organisms. Growth stimulation due to glyphosate exposure was also observed in single algae tests (Cedergreen et al., 2007) and with ameba (Perruchoud et al., 2011). For algae, this stimulation, called hormesis effect, also concerned photosynthesis and chlorophyll-a synthesis.

The objectives of this work were double: first to get a more precise understanding of the sources and dynamics of the herbicide glyphosate and its metabolite AMPA in a typical vineyard catchment. Second, to assess the environmental risk of the measured concentrations of glyphosate and AMPA in the river draining this catchment. To our knowledge, this is the first study that combines transport dynamics and global risk assessment of this widely used herbicide in a real situation.

5.2 Material and methods

5.2.1 *Study Area And Sampling*

The Lutrive is a small river in the east of the city of Lausanne with its spring located on the Swiss plateau at an altitude of 842 m, and its mouth on Lake Geneva at 372 m, with a mean slope of 7.3% (Fig. 5.1). Its small watershed (6.4 km^2) is characterised by different land uses: agricultural fields (45%), urban and impervious surfaces (31%) and forests (24%). Vineyards account for 4.1% of the agricultural surface and are mainly located in its lower southern sub-catchment (1.2 km^2). This latter shows a steeper slope (~10%) and comprises also some urban and impervious surfaces (Lutry village), whereas land use in the upper part of the catchment (5.2 km^2) is mainly characterized by crop fields and forests. The Lutrive River shows a rain-dominated regime with only little contribution of snow melting in winter (December-February). Hourly precipitations data of the meteorological station of Pully, located at 2 km of the Lutrive River, were obtained from MeteoSwiss, the Swiss official meteorological network (Fig. 5.1).

In vineyards, glyphosate is generally applied from April to August (Dubuis et al., 2011). In the studied catchment, glyphosate application data were extrapolated from interviews of a few wine-growers (n=6; 3.5% of the vineyard area). Typical application rates ranged from 3 to 5 L/ha of formulated products containing 360 g/L of active ingredient. The application is made manually, due to the important slope of the plots, and generally only under vine rows, letting a grass cover in between them. Glyphosate was applied once in spring (mid-April) with possible other sporadic and local application(s) later in the season, if weed development is important. For arable crops (e.g. for seed bed preparation for new crops), glyphosate is mainly applied in August and September (Hanke et al., 2010).

The Lutrive River was sampled during the year 2011 upstream and downstream of the vineyard area (Fig. 5.1). Two automatic samplers (ISCO 6712, Teledyne Inc.) equipped with polyethylene bottles and ultrasonic flow modules to measure the water level were implemented. These two locations were chosen in order to discriminate the sources of glyphosate (vineyard + urban versus agricultural) and to study the concentration dynamics at different time scales. The water level (h) was monitored every five minutes and the rating curve for water discharge (Q) was estimated on the basis of NaCl dilution gauging experiments.

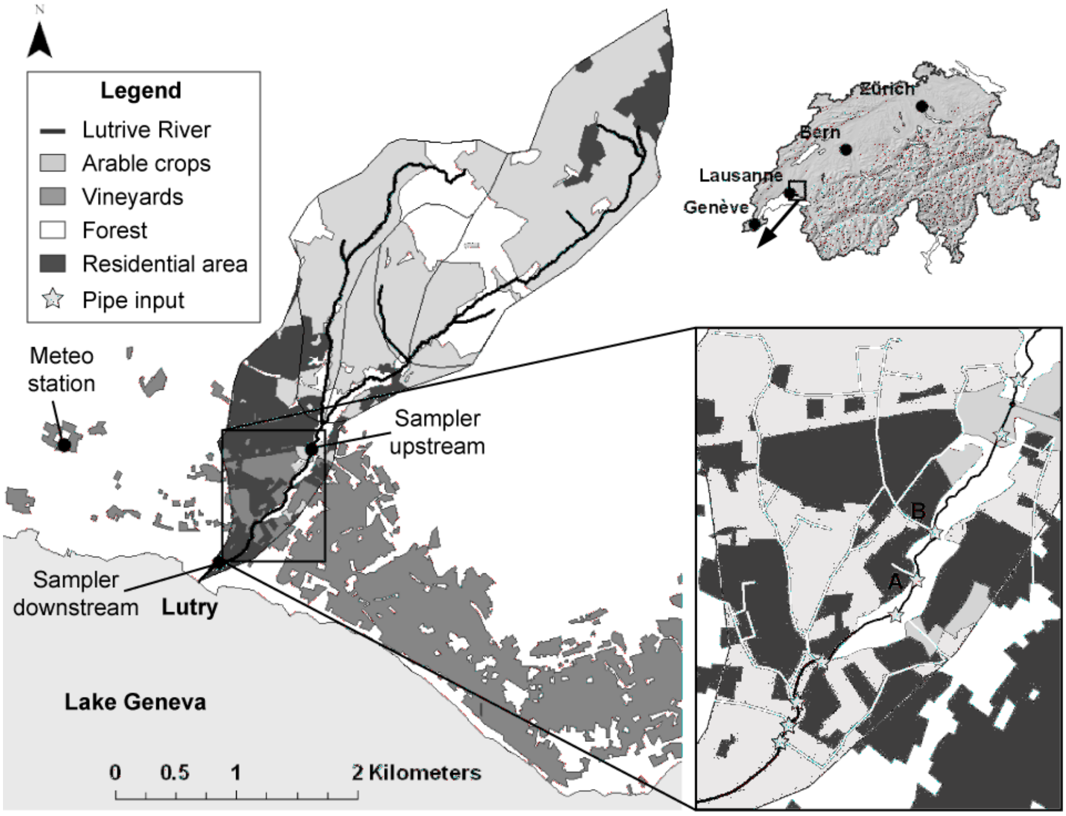

Figure 5.1: Map of the Lutrive River watershed in western Switzerland with locations of the two automatic samplers, upstream and downstream the vineyard area above the Lutry village, and of the meteorological station of Pully (MeteoSwiss); the zoomed area shows the clear water network in the vineyard area with the drainage pipe (A) and the stormwater channel (B) sampled in May and June 2011.

The automatic samplers were programmed for two types of sampling: a regular and an event-based sampling; a third of the bottles were dedicated to the regular sampling and the other two thirds to the event-based sampling. The first was done every 4 days and the second when the water level reached a predefined level, indicating the start of a rain event, and then every two hours. 50 samples for the downstream site and 20 for the upstream site were selected from April to October 2011 and analyzed. Twenty of them, 15 downstream and five upstream, were regularly collected during dry periods to estimate background levels of glyphosate and AMPA.

Many incoming pipes were observed along the river (Fig. 5.1, ‘star’ symbols), discharging potentially polluted waters, coming from either agricultural fields or residential areas: 13 grab samples were collected from one agricultural drainage pipe (Fig. 1, A), situated approximately at an average depth of 60cm, and seven from one stormwater channel (Fig. 1, B) in the vineyard area in May and June 2011 for pesticide analysis.

5.2.2 *Analytical Methods*

Electrical conductivity and pH were measured in the field; samples were then stored in high density polyethylene (HDPE) bottles at -20°C until analysis. Dissolved organic and inorganic carbon (DOC/DIC) concentrations were measured with a C-analyzer (Liquitoc Elementar©). Major ion concentrations (Na^+, Ca^{2+}, K^+, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}) were obtained with an ion chromatography system (ICS-1100/2100, Dionex-Thermo Fischer©). The herbicide glyphosate and its metabolite AMPA were quantified by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), after their derivatization with FMOC-Cl followed by solid-phase extraction (SPE). Previously, the method was adapted from Hanke et al. (2008) and validated with a LOQ of 10 ng/L and a good reproducibility (see Chapter 3). In order to evaluate the relative proportion of AMPA compared to glyphosate, its molar ratio was calculated according to Coupe et al. (2012):

$$\% \text{ AMPA} = [\text{AMPA}] / ([\text{glyphosate}] + [\text{AMPA}]) \times 100, \quad (1)$$

with [AMPA] and [glyphosate] their respective molar concentrations in water.

Glyphosate and AMPA loads were estimated with a basic numeric integration (US EPA, 2003):

$$\text{Load} = k\, \Sigma\, c_i\, q_i\, t_i, \qquad (2)$$

where k is a unit conversion factor, c_i the concentration of the sample i, q_i the discharge at its sampling time (t_i) and $t_i = 0.5(t_{i+1} - t_{i-1})$. Then, AMPA loads were transformed proportional to the glyphosate ones, by a multiplication with their molecular weight ratio (1.52) (Coupe et al., 2012).

5.2.3 *Risk assessment*

The aquatic risk factor (RF) for a given pesticide can be evaluated using Equation 3 (EC, 1996):

$$\text{RF} = \text{MEC}/\text{HC}_5, \qquad (3)$$

where MEC is the measured environmental concentration of the pesticide and the HC_5 is the hazardous concentration for 5% of the species, derived from the species sensitivity distribution (SSD), which is based on NOEC data (Posthuma et al., 2002). The HC_5 may be divided by a safety factor between 1 and 5 depending on the available ecotoxicological data (EC, 1996). In our case, a factor of 5 was used, as only 11 NOEC data were used covering just three taxonomic groups.

The NOEC data for glyphosate were obtained from the US EPA ECOTOX Database (www.epa.gov/ecotox) and from the literature. Classically, only the most sensitive species are considered to construct the SSD curve for pesticides. But for glyphosate, they were difficult to determine. According to Solomon and Thompson (2003), diatoms seem to be the most sensitive species, whereas Relyea (2005) considered amphibians as the most sensitive species. For this reason, all available data were therefore considered to build the SSD curve. However, only data expressed in concentration of active ingredient (µg a.i./l) were considered to build the SSD. Thus, when the formulation was tested and expressed in µg/l but not according to the active substance (µg a.i./l), data were not considered. Furthermore, a difference was made between the tests with the active ingredient (more than 95% of purity, glyphosate acid and IPA salt of glyphosate) and the formulation, expressed in active ingredient (Table 5.2 and Fig. 5.6). Indeed, the formulation is assumed to have a greater effect than the active ingredient, which could influence the risk analysis (Solomon and Thompson, 2003).

Table 5.2: NOEC data for the active ingredients glyphosate acid and IPA salt. The geometric mean was calculated for species with 2 or more NOEC values found in the literature. The data in grey corresponds to NOEC obtained from tests using only the active ingredient (more than 95% of purity) as tested substance.

Species Scientific Name	***Species Group***	***Exposure Duration [Days]***	***NOEC [µg/L]***	***Source***
Chlorella pyrenoidosa	Algae, Moss, Fungi	4	108000	Anton et al., 1993
Scenedesmus acutus	Algae, Moss, Fungi	4	2000	Saenz et al., 1997
Scenedesmus acutus	Algae, Moss, Fungi	4	3200	Saenz et al., 1997
Scenedesmus quadricauda	Algae, Moss, Fungi	4	770	Saenz et al., 1997
Scenedesmus quadricauda	Algae, Moss, Fungi	4	1250	Saenz et al., 1997
Bufo americanus	Amphibians	16	1000	Relyea, 2005
Hyla versicolor	Amphibians	16	1000	Relyea, 2005
Lithobates clamitans clamitans	Amphibians	16	1000	Relyea, 2005
Lithobates pipiens	Amphibians	16	1000	Relyea, 2005
Lithobates sylvaticus	Amphibians	16	100	Relyea, 2005
Rana catesbeiana	Amphibians	16	1000	Relyea, 2005
Carassius auratus	Fish	4	2880000	Anton et al., 1994
Carassius auratus	Fish	4	3431000	Anton et al., 1994
Oncorhynchus mykiss	Fish	4	823500	Anton et al., 1994
Cyprinus carpio	Fish	4	1700	Liong et al., 1988
Oreochromis niloticus	Fish	4	310	Liong et al., 1988
Utterbackia imbecillis	Molluscs	1	10040	Conners and Black, 2004

No NOEC data were found for AMPA. Therefore, a SSD for AMPA was predicted, based on the SSD of glyphosate, following a methodology proposed by Chèvre et al. (2006). This requires determining the toxic ratio (TR) between glyphosate and AMPA, which was done by conducting an acute toxicity test on the alga *Scenedesmus vacuolatus*. The toxic ratio (TR) was determined using Equation 4 (Chèvre et al., 2006):

$$TR = EC50_{glyphosate}/EC50_{AMPA} \qquad (4)$$

EC50 were obtained from the dose-response curve fitted with a log-logistic model. Using this method assumes that glyphosate and AMPA have a similar mode of toxic action, which was not proven yet. This approach allows therefore a first risk evaluation, but more ecotoxicity data will be needed to assess a reliable risk for AMPA.

5.2.4 *Acute toxicity test with algae*

The toxic ratio (Eqn. 4) was determined through laboratory bioassays testing glyphosate and AMPA toxicity with the algae *Scenedesmus vacuolatus* (Chlorophyceae; strain 211-8b, Shihira and Krauss, Philadelphia, PA, USA). Glyphosate (Pestanal®, 99.2%) and AMPA (aminomethylphosphonic acid, 99%) were purchased from Sigma-Aldrich (Buchs, Switzerland), whereas the algae *Scenedesmus vacuolatus* were transmitted by the Department of Bioanalytical Ecotoxicology at Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany. Algae were cultured in 50 mL of a sterile inorganic medium (OECD, 2011), in 250 mL sterile Erlenmeyer flasks. They were maintained in a HT Infors shaker at 25°C and shaken at 90 rpm (Vallotton et al., 2009), with a continuous illumination of 70 µmol/m^2/s provided by cool-white fluorescent lamps in order to have optimal conditions of exponential growth.

Standard acute toxicity tests for glyphosate and AMPA were made according to the OECD procedure (OECD, 2011). The exposure concentrations varied from 2 to 80 mg/L for glyphosate and from 60 to 160 mg/L for AMPA. Each concentration and control was done in triplicate. No adsorption or degradation was assumed during the test, and thus tested concentrations were the nominal concentrations. The algae growth was determined by measuring the absorbance at a wavelength of 690nm with a microplate reader (ELx800™, BioTek® Instruments, Winooski, Vermont) at the beginning, and at the end of the test. The effect, i.e. the growth inhibition, was then assessed with a linear regression of the natural logarithm of optical density over time. Growth rates for the different concentrations (GR_E) and the control (GR_C) were measured simultaneously (Vallotton et al., 2009). The growth inhibition (Inh_{growth}) for each tested concentration was then deduced from these growth rates:

$$Inh_{growth} = 100 \times (GR_C - GR_E) / GR_C \qquad (5)$$

The NOEC was determined using a t-test, comparing the growth of a given concentration to the growth of the control.

5.3 Results and discussion

5.3.1 *Precipitations and discharge*

During 2011, the total annual precipitation in the study area was 916 mm, with a daily maximum of 48 mm (July 17). This is less than the mean annual rainfall of 1101 mm/y calculated for 30 years (1961-1991). This is certainly due to the occurrence of two significant dry periods in January-February and November (Fig. 5.2). Mean discharges were of 0.163 m^3/s and 0.198 m^3/s at the upstream and downstream station respectively. Discharge was in general lower than 1 m^3/s, except during several flood events, which are mainly occurring during intense summer rainfall (May-July). Discharge of 1.59 m^3/s was reached May 22 after 9.3mm of rain fallen in one hour, whereas discharges of more than 3 m^3/s were reached in June-July with rainfall of more than 20 mm/day. The maximum of 3.47 m^3/s upstream and 5 m^3/s downstream was reached on September 5 at 5am due to 17 mm fallen in one hour.

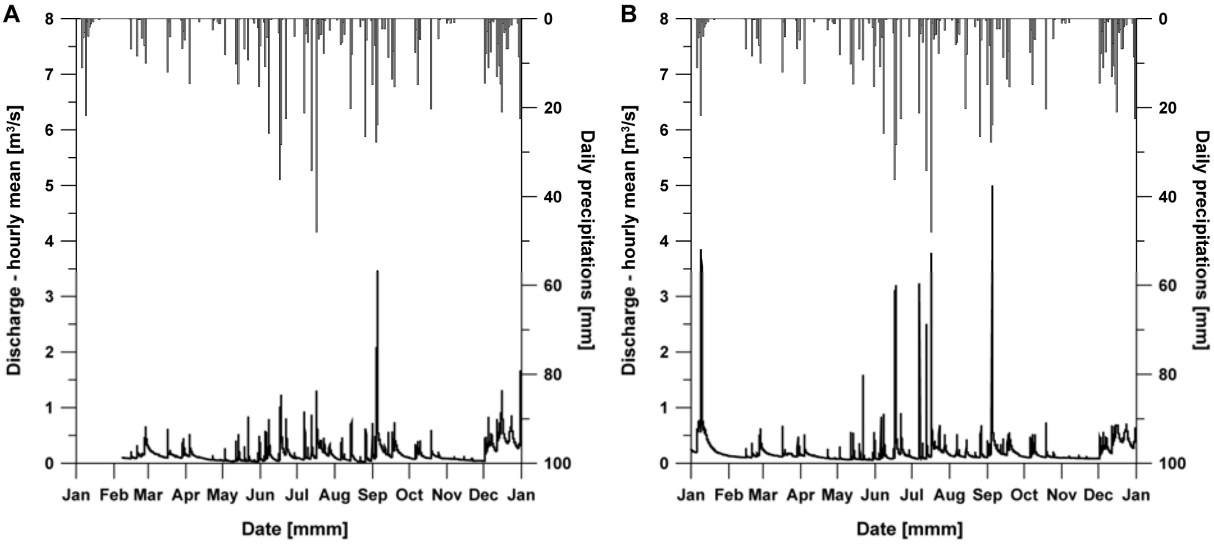

Figure 5.2: Hourly discharge of the Lutrive River measured at the upstream (A) and the downstream (B) station for 2011. Daily precipitations from the meteorological station of Pully are shown on the right axis (Source: MeteoSwiss).

5.3.2 *River samples*

The chemical composition of the river water was dominated by calcium (Ca^{2+}) and bicarbonate (HCO_3^-) ions (Appendix 5.1), and showed a mean pH of 8.2 and a mean electrical conductivity of 300 µS/cm, with minimum and maximum values of 90 and 430 µS/cm respectively. These alkaline conditions are known to enhance the potential toxicity of glyphosate as already mentioned (USDA, 1997; Solomon and Thompson, 2003).

For glyphosate and AMPA, several concentration peaks were found largely above the 100 ng/L threshold for each molecule of pesticide (Fig. 5.3). These peaks were well related to the main rain events (>10-20 mm/day), revealing the transfer of these compounds from fields to surface water mainly by surface runoff. Concentration ranges of glyphosate and AMPA are in agreement with previous results of other rivers in Switzerland (25-3940 ng/L) (Corvi et al., 2005; Hanke et al., 2008), but lower than in other countries (2-700 µg/L) (IFEN, 2006; Peruzzo et al., 2008). Concentrations were much higher downstream than upstream, as the main vineyard area is located in the southern sub-catchment (Fig. 5.1). This may also be explained by urban sources occurring in the same sub-catchment, such as private gardening and weed management along railways, which are known to represent sources for surface water pollution by glyphosate (Botta et al., 2009; Hanke et al., 2010).

The highest glyphosate concentration (4970 ng/L) was observed at the outlet during the first rain after the application, April 15, despite its low intensity (2.5 mm) and a period of eight days of dry weather between (Fig. 5.3b). Then, glyphosate concentrations decreased down to 3000-3500 ng/L in mid-May and then to 1000-2000 ng/L in early June, though rainfall events became more important (>10 mm/day). This decrease certainly reflects the degradation of glyphosate into AMPA in vineyard soils. However, the important rainfall of June 17 (36 mm) gave rise to a higher concentration (3420 ng/L), suggesting either a higher transfer rate or other applications in the vineyard. This peak was equivalent to the one observed in early August with much lower rain (11 mm in 2 days), which could reveal a late application in the vineyards, but also the beginning of the contribution of glyphosate applied on arable crops in the upper part of the catchment (Fig. 5.3a; 1000 ng/L). Indeed, the highest contamination peak upstream resulting mainly of application on crops was observed in late August (2620 ng/L).

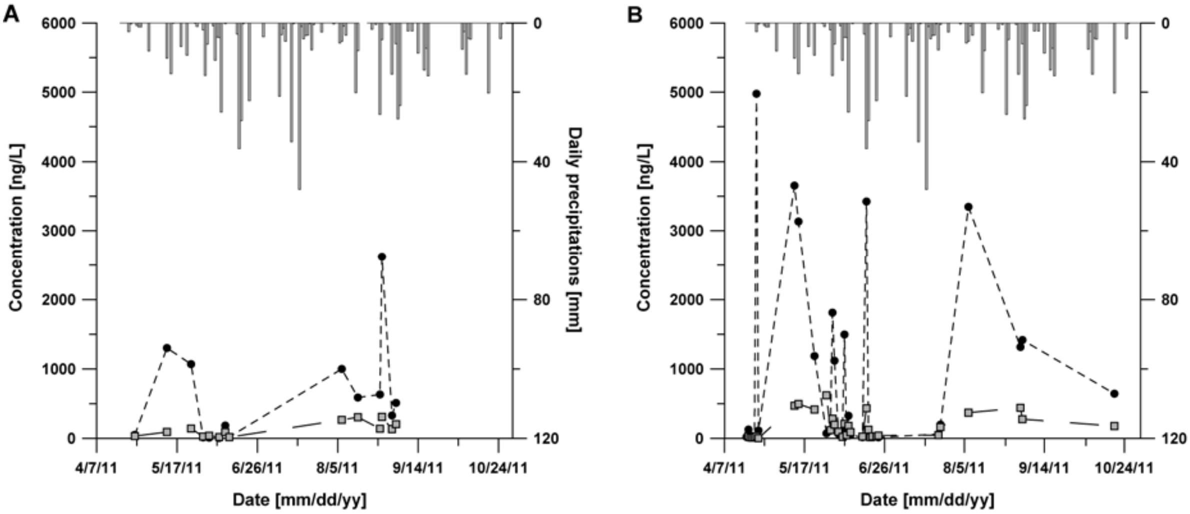

Figure 5.3: Concentrations of glyphosate (●) and AMPA (■) upstream (A) and downstream (B) of the vineyard sub-catchment for the period April-October 2011. Daily precipitations from the meteorological station of Pully are shown on the right axis (Source: MeteoSwiss). Dashed lines between samples were added for a better dynamic visibility, though they are totally hypothetic.

AMPA concentrations did not vary much, with a mean concentration of 170 ng/L and a median concentration of 160 ng/L at the outlet and a maximum of 620 ng/L observed on May 28. Its downstream molar ratio (Eqn. 1), increased from 10-30% one month after glyphosate application up to 60-90% after two months, revealing glyphosate biodegradation in soils. The ratio decreased to 20% after every important rain event, revealing new inputs of glyphosate in the river by surface runoff. Nevertheless, its median value was 55%, suggesting a slightly higher proportion relative to glyphosate in most samples. This is in contrast to previous results for a French vineyard river (Coupe et al., 2012), where a median value of 31% was observed. This discrepancy may be explained by the bigger size of the catchment and the larger proportion of urban area in our case, with the possible associated release of AMPA resulting from the degradation of phosphonate detergent molecules (Botta et al., 2009), and/or from glyphosate applications in private gardens, paved forecourts and streets (Hanke et al., 2010). Although these two latter types of application are forbidden in Switzerland by the chemical risk reduction ordinance (ORRChem), they were proved to be not negligible (Hanke et al., 2010). Thus, the urban environment can also be an important contributor to glyphosate and AMPA in surface waters in some cases (Botta et al., 2009; Hanke et al., 2010), as already mentioned. Nevertheless, in our case, urban sources are likely to be minor compared to the vineyard contribution.

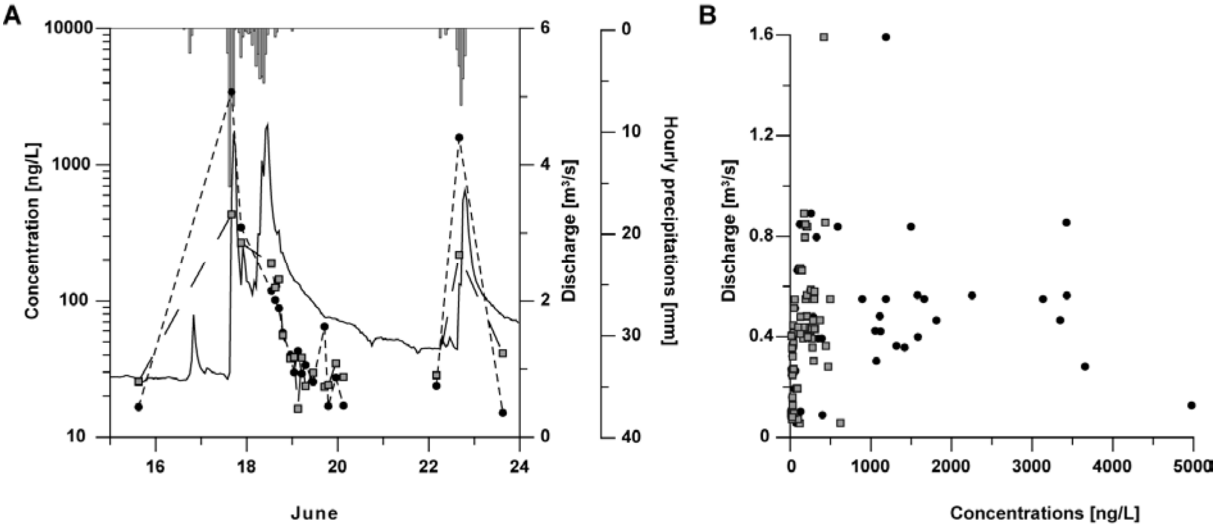

Figure 5.4: A) Concentration dynamics of glyphosate (●) and AMPA (■) at the downstream station during the rain events occurring between June 15 and 23, with discharge and hourly precipitations (Source: MeteoSwiss). Note the logarithmic scale; dashed lines between samples were added for a better dynamic visibility, though they are totally hypothetic. B) Discharge of the Lutrive River (m^3/s) versus glyphosate (●) and AMPA (■) concentrations at the downstream station for the period April-October 2011.

During rain events, glyphosate and AMPA concentrations increased rapidly with river discharge, reaching a maximum almost at the same time, and decreased also rapidly after (Fig. 5.4a). A lag time of only 1h is observed between the rainfall and the discharge peak, reflecting the small size of the catchment. This suggests a fast transfer of glyphosate and AMPA to the river at the catchment scale, such as runoff on impervious surfaces, as previously suggested by Hanke et al. (2010), and also the concentration of this runoff water in channels of different types (see section 5.3.4 and Fig. 5.1). In general, glyphosate and AMPA concentrations increased with river discharge, with an exception for concentrations above 3000 ng/L with relatively small discharge ($<0.6m^3/s$), which are representative of sampling just after the application (Fig. 5.4b). Though sources cannot be differentiated clearly between vineyard and residential areas, due to the mixed land use in the considered sub-catchment, the steepness of the vineyards (~10%) likely gives rise to a fast transport of the herbicide by surface runoff to the river, after spring applications, due to the presence of surrounding impervious surfaces.

5.3.3 *Total loads*

When cumulating glyphosate and AMPA loads (see Eqn. 2), a total amount of 7.1 kg was found downstream from mid-April to early September, which is three times higher than previously found in other areas of Switzerland (Hanke et al., 2010). When assuming an application of glyphosate on 50 to 100% of the total agricultural area and at the recommended rate of 1080 g/ha, this total load represents 12 to 6% respectively, which is much higher than the 2% or less found in previous studies (Siimes et al., 2006; Coupe et al., 2012). This discrepancy can be attributed to the steepness of the vineyard area and also, in part, to the uncertainties of the extrapolated applied amount. During the same period, a total amount of 1.04 kg was found upstream of the vineyard area, which represents 15% of the load found downstream. Note that this upper part corresponds to 80% of the total watershed area. Thus, the relative contribution of vineyards (85%), and for a minor part applications in residential areas, is more important than for arable crops, where other herbicide molecules may be in use at the same time. These results highlight the importance of vineyards, even with relatively small surfaces (20%), to the potential pollution of receiving waters.

5.3.4 *Drainage and stormwater samples*

Concentrations of glyphosate and AMPA observed in grab samples, taken in the drainage pipe and the stormwater channel, were highly variable (Fig. 5.5). Glyphosate and AMPA contamination peaks of 2050 and 350 ng/L respectively, were observed in the drainage pipe (Fig. 5.5a) in early May, after 8 mm of rain. The second peaks of 940 and 590 ng/L of glyphosate and AMPA respectively, were observed on June 20, after 68 mm of rain in 2 days. These latter peaks apparently did not contribute to the ones observed in the Lutrive River outlet (3420 ng/L of glyphosate), which occurred at the beginning of the rain event (June 17). Indeed, percolation water, carrying glyphosate and AMPA molecules, took obviously 2 days to reach the depth of the drainage pipe and was then carried away towards the river. In fact the drainage waters of this pipe are not directly discharged into the river, but in the forest strip along it, where retention of glyphosate and AMPA could occur. In any events, these results confirm the leaching of the strongly sorbed herbicide glyphosate (mean K_d = 222; www.eu-footprint.org/ppdb) and the importance of drainage pipes in its transfer to surface water in agricultural areas, as previously observed (Kjaer et al., 2011).

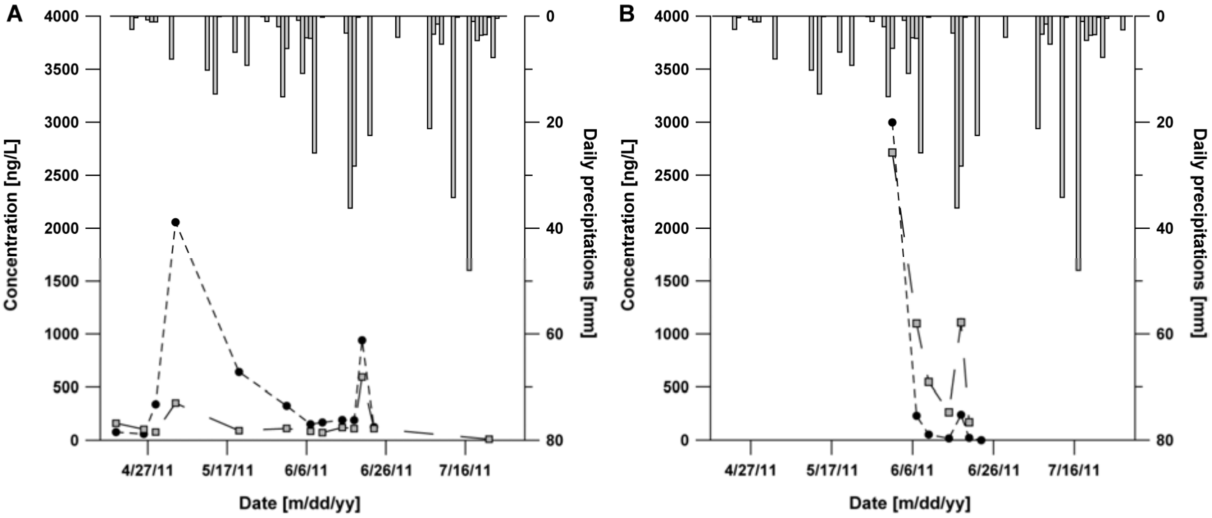

Figure 5.5: Concentrations of glyphosate (●) and AMPA (■) observed in sporadic grab samples of a drainage pipe in a vineyard parcel (A) and of a stormwater channel in the vicinity of the vineyards (B), from April to June 2011. Daily precipitations are shown on the right (Source: MeteoSwiss). Dashed lines between samples were added for a better dynamic visibility, though they are totally hypothetic.

The stormwater samples taken in the vicinity of vineyard parcels (Fig. 5.5b) showed higher concentrations than those taken in the drainage pipe (Fig. 5.5a). This channel carried runoff waters coming from many vineyard parcels, located above a road, as well as a residential area, whereas the drainage pipe carries percolation waters of only a few vineyard parcels (see Fig. 5.1). Except for the first sample, AMPA concentrations were up to four times higher than for glyphosate, suggesting either another urban source or its slower degradation in soils ($DT50_{field}$ = 151d, glyphosate = 12d; ; www.eu-footprint.org/ppdb).

5.3.5 *Risk assessment*

The results of the risk assessment approach are based on the SSD curves for glyphosate and AMPA (Figure 5.6). The SSD of glyphosate was obtained from the available NOEC data (Table 5.2), whereas the SSD of AMPA was extrapolated from the SSD of glyphosate, using the toxic ratio. This latter is based on EC50 values, resulting from the standard acute toxicity test, for the algae *Scenedesmus vacuolatus*. These EC50 are presented in Table 5.3, as well as values found in the literature for several algae in freshwater, and for the active ingredient with a purity higher than 95%. Thus, according to Equation 5, $TR \leq 43361/160000 \leq 0.27$. Interestingly, the literature NOEC data obtained for tests conducted with glyphosate and with tests conducted with the formulation (re-calculated in glyphosate) do not differ. This is surprising, as the glyphosate formulation is known to be more eco-toxic than the active ingredient, due to the important toxicity of the POEA surfactant (Solomon and Thompson, 2003; Tsui and Chu, 2003; Cedergreen and Streibig, 2005). But this may be due to the few available ecotoxicity data (11 NOEC values used in our calculation, from table 5.2)

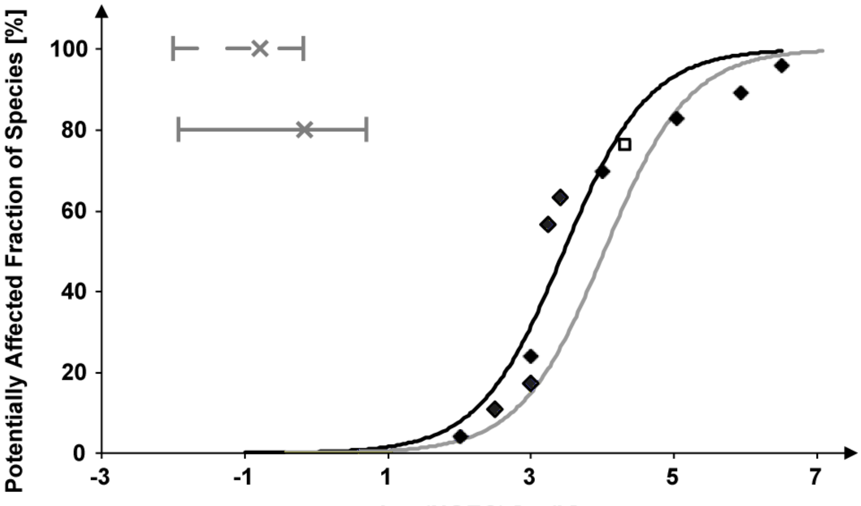

Figure 5.6: SSD curve for glyphosate (black line) obtained from literature data (black and grey diamonds) and predicted SSD for AMPA (grey line). The grey diamonds correspond to literature NOEC-values obtained with the active ingredient (more than 95% of purity, glyphosate acid and IPA salt of glyphosate), and the black ones with the formulated products, expressed in active ingredient. The white square corresponds to the EC50 of glyphosate measured in the laboratory. The minimum, maximum and mean concentrations of glyphosate (grey solid line) and AMPA (grey dotted line) measured in the Lutrive River are represented on the top left of the figure.

The minimum, maximum and mean concentrations of glyphosate and AMPA measured in the Lutrive River were lower than those of the two SSD-NOEC curves of glyphosate and AMPA and thus are expected to have a very low effect on aquatic species. Indeed, for the glyphosate maximum concentration of 4970 ng/L measured in the Lutrive River, the fraction of affected species is 0.92%, whereas for a minimum concentration of 10 ng/L it goes down to 0.01%. For AMPA, the maximum concentration of 620 ng/L leads to 0.07% of affected species, and less than 0.05% for the minimum concentration of 10 ng/L.

Table 5.3: EC50 values [μg/L] for several algae found in the literature with their confidence interval when this latter is available. The values in grey correspond to values obtained in the laboratory. For AMPA, no published data was found.

Species Scientific Name	EC50 [μg/L]	Source
Scenedesmus vacuolatus (Glyphosate)	43361 [40190 ; 46783]	This study
Scenedesmus vacuolatus (AMPA)	>160000	This study
Pseudokirchneriella subcapitata	5555	Ma et al., 2006
Scenedesmus quadricauda	7200 [4400 ; 8900]	Saenz et al., 1997
Scenedesmus acutus	10200 [10400 ; 11 200]	Saenz et al., 1997
Pseudokirchneriella subcapitata	24700 [22800 ; 26700]	Tsui and Chu, 2003
Scenedesmus quadricauda	70500	Ma et al, 2003
Pseudokirchneriella subcapitata	129000 [108000 ; 158000]	Pereira et al., 2009
Pseudokirchneriella subcapitata	270000 [224400 ; 315600]	Cedergreen and Streibig, 2005

For glyphosate, the HC_5 was found to be 52 μg/L and approximately 193 μg/L for AMPA. The risk factor is therefore equivalent to 0.48 and 0.016 for glyphosate and AMPA respectively. As these values are much less than one, the risk can be considered small for the Lutrive River. However, for AMPA, the results should be considered as a preliminary evaluation. Indeed, the SSD was predicted from the SSD of glyphosate (Fig. 5.6). Accordingly, the risk caused by glyphosate and AMPA maximum concentrations measured in other rivers or streams of the Lake Geneva catchment on aquatic species was also considered negligible (Table 5.4). Indeed, the risk factor calculated for glyphosate and AMPA concentrations found in the Venoge River and in the small Charmilles stream in the Geneva area was also very low (Table 5.4). However, the risk factor depends likely on the river size, as it is attenuated with highly diluted concentrations in larger rivers, such as the Venoge or the Rhine River, as compared to smaller ones, such as the Charmilles or the Lutrive River in our case. It may be different worldwide, as the risk of glyphosate and AMPA is high in some parts of France or in Argentina, as illustrated in Table 5.4. The much higher concentrations found in Argentina can be linked to the more intensive nature of current agriculture practices, such as direct sowing in glyphosate-tolerant transgenic soybean cultivation, combined with a soil, highly susceptible to hydric erosion (Peruzzo et al., 2008). This leads to a higher amount applied than in Swiss vineyards, and a large transfer of glyphosate from the near fields to adjacent streams with precipitations, attached to particles for a part (see Chapter 4), due to the low retention of bared soils associated with this practice.

Table 5.4: Minimum and maximum concentrations in µg/L and maximum risk factors in several rivers and streams of the Lake Geneva catchment and of other countries in the world, for glyphosate and AMPA. For Venoge and Charmilles only one measure was available. Maximum risk factors exceeding the norm of 1 were indicated by an asterisk.

	Glyphosate		***AMPA***	
HC_5/5 [µg/L]	10.4		38.6	
Rivers and streams (Source)	**Concentrations (min-max) [µg/L]**	**Maximum risk factors [-]**	**Concentrations (min-max) [µg/L]**	**Maximum risk factors [-]**
Lutrive (This study)	(0.015-4.97)	0.48	(0.01-0.62)	0.016
Venoge (Corvi et al., 2005)	0.12	0.012	0.12	0.003
Charmilles (Corvi et al., 2005)	2.8	0.27	3.94	0.102
Rhine river (CH) (Hanke et al., 2008)	(0.025-0.055)	0.005	(0.055-0.065)	0.002
Streams in Buenos Aires Province (Argentina) (Peruzzo et al., 2008)	(100-700)	67*	-	-
Rivers in France (IFEN, 2006)	(2-165)	16*	(2.1-48.1)	1.25*

Although glyphosate seems to not present a great risk for the aquatic ecosystem, the stimulation effects at low concentrations represent an interesting result. In the conducted algae test growth stimulation was observed with the three first concentrations, 2000, 10000 and 20000 µg/L (see Appendix 5.4). Corresponding inhibition values, with 95% confidence interval, were of -0.015 (-0.043; 0.013), 0.006 (-0.008; 0.019) and -0.015 (-0.055; 0.025). This refers to an hormesis effect, which was also observed with other algae or species (Cedergreen et al., 2007; Perruchoud et al., 2011; Vera et al., 2012). A possible explanation for this could be the N- or P-content of glyphosate (Pesce et al., 2011). If such an effect does not constitute a risk (no inhibition is observed), one could argue that a stimulation is also an effect. It can then be supposed that low concentrations of glyphosate, in rivers or lakes, could promote the development of algae. This may be considered as a risk, as such changes can affect the ecosystem, as already demonstrated with phosphorus pollution and eutrophication (Smith, 2009).

5.4 Conclusions

Our results confirm the mobility of the herbicide glyphosate and its metabolite AMPA, which are leached and/or washed from vineyard and urban areas and enter surface waters with different pathways. Small vineyard surfaces could generate high concentrations of herbicides and contribute considerably to the total load calculated at the outlet. They also reveal interesting details of herbicide dynamics in urbanized landscapes, such as the Lavaux vineyard. Indeed, besides their physico-chemical properties, they strongly depend on application rates, precipitation regime, land use and also on the presence of drains or constructed channels in the catchment.

In our case, and with the methodology applied, the ecotoxicological risk, linked to the maximum concentrations of glyphosate and AMPA observed in the Lutrive River, was found negligible (RF<1). However, RF was assessed for the active ingredient, and surfactants are known to participate actively to glyphosate-formulated product toxicity towards aquatic organisms. In addition, growth stimulation on the algae *Scenedesmus vacuolatus* in the laboratory was observed illustrating a potential hormesis effect for this active ingredient. Furthermore, in order to come up with a realistic risk assessment, other pesticide molecules applied in vineyards should be taken into account to perform mixture toxicity tests. Another improvement for the risk assessment could be the use of pulse exposure tests in the laboratory. Indeed, input of herbicides into the aquatic environments often occurs in pulses of a couple of hours, rather than as continuous flows, resulting in fluctuating exposure of aquatic organisms to these pollutants. Future integration of the dynamic of concentrations in risk assessment will help to better characterize a potential risk of glyphosate.

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6 Multiple interactions between the herbicide glyphosate, dissolved organic matter (DOM) and copper in a vineyard environment assessed with UV/Vis fluorescence spectroscopy

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Abstract

UV/Vis fluorescence spectroscopy was used to study the possible interactions of dissolved organic matter (DOM) with the herbicide glyphosate and copper-based fungicide used in vineyards. The study focussed on the role of DOM in the transport of these micropollutants from parcels to surface waters (river, lake). Soil solution and river water samples were collected in the Lavaux vineyard area, western Switzerland. Their fluorescence excitation emission matrices (EEM) were decomposed using parallel factor (PARAFAC) analysis, and compared to their content in glyphosate and copper. Quenching experiments with DOM standards were also performed in the laboratory to confirm field observations. PARAFAC analysis of EEM of both samples showed the contribution of protein-like and humic-like fluorophores. In soil water samples, complexes between fulvic-like and humic-like fluorophores of DOM, copper and glyphosate were likely formed. In surface water, DOM-copper and glyphosate-copper interactions were observed, but not between glyphosate and DOM. Quenching experiments proved the higher affinity of glyphosate and copper for the fulvic-like fluorescent part of the dissolved humic acid standard.

Keywords

Dissolved organic matter, Glyphosate, Copper, Fluorescence spectroscopy, Parallel Factor Analysis, Lavaux vineyards.

6.1 Introduction

The diffuse pollution of water bodies by herbicide molecules is determined by their mobility and their ability to be retained and degraded in agricultural soils. Glyphosate [*N*-(phosphonomethyl)glycine] is the most used herbicide active ingredient in the world. The extent of glyphosate sorption in soils depends on different parameters, such as soil constituents and pedoclimatic conditions, and their combination. Despite its affinity for soil mineral phases (Piccolo et al., 1994), glyphosate has been proved to bind also to soil organic matter (SOM), such as humic substances (Dousset et al., 2004; Roy et al., 1989). The strongest adsorption in soils rich in organic matter, occurred with low pH-values and high Fe/Al-oxide contents (Albers et al., 2009; Autio et al., 2004).

Humic substances (HS) are constituted of relative low-molecular mass moieties bound together mainly by H-bonding and hydrophobic links (Sutton and Sposito, 2005). Their structure is sensitive to ambient conditions such as pH, ionic strength and also to their own concentration (Aoustin et al., 2001). At low pH, HS molecules exhibit a higher aggregation and condensation state (Baalousha et al., 2006), due to the protonation of acidic functional groups leading to more intramolecular H-bonding and a decrease in negative charges that repulse each other (McDonald et al., 2004). At high concentration of salts and/or HS themselves, they exhibit more compact sphero-colloidal structures, also linked with a decrease in intramolecular repulsive forces. Under these conditions, a decrease in the surface area and the disappearance of certain structural micropores are observed (Alvarez-Puebla and Garrido, 2005), diminishing their binding potential for pollutants.

Dissolved organic matter (DOM) is defined as the part of the SOM smaller than 0.45µm composed of molecules of relatively low molecular weight (Zsolnay, 2003). Organic compounds, such as pesticides, can bind to DOM, which can increase their solubility and their availability for microorganisms (Franco, 2001; Pennington et al., 1991). In aqueous solutions and especially under acidic conditions, intramolecular interactions in HS lead to the formation of micelle-like aggregates, with interior hydrophobic regions isolated from solutions by external hydrophilic parts, which have been proved to enhance the solubility of some organic pollutants (Sutton and Sposito, 2005). However, the nature of ionic species in solution influence colloidal stability of aqueous dispersion, which is mainly controlled by the pH, but also by the presence of multivalent cations such as calcium (Baalousha et al., 2006). Therefore, the potential enhanced leaching of pesticides in soils, dependant on retention-mobilisation dynamic, can be influenced by the nature of DOM, e.g. its humification degree, as well as the soil properties that determines the nature of ionic species present in solution, and also by pesticide physico-chemical properties (Franco, 2001; Haberhauer et al., 2002). However, retention of DOM can also occur on surfaces of the clay fraction (clay minerals and sesquioxides) and thus reducing the availability of active site for contaminants (Kaiser and Zech, 2000; Zsolnay, 2003).

According to Piccolo et al. (1996), glyphosate gets linked to humic substances (HS) mainly by H-bonding, which extent depends on their molecular size and degree of aromaticity. The larger the molecular size and the lower the aromatic content of humic molecules, the stronger was the adsorption of glyphosate. A large molecular size induces a higher potential for sorption because of a more important number of possible hydrogen bonding. Moreover, a lower abundance of aromatic rings in humic macromolecules enhances their stereochemical flexibility, leading for the small herbicide molecule to an easier access to their inner reactive sites. Ternary complexes between glyphosate and humus are also likely to be formed with the intermediate of di- and trivalent “bridging” metal cations, such as Fe^{3+} or Al^{3+} (Barrett and McBride, 2006; Borggaard and Gimsing, 2008) but also Cu^{2+} (Morillo et al., 1997). Indeed, these authors showed that the presence of copper leads to glyphosate desorption from the external sites (Al-OH groups) of montmorillonite and the formation of Cu-glyphosate complexes, which show a high stability and a lower sorption affinity than glyphosate alone. Inversely, glyphosate may enhance copper mobility by chelating with Cu^{2+} previously sorbed on both mineral and organic surfaces (Barrett and McBride, 2006). Thus, copper could have a significant influence on glyphosate mobility and potential transfer to ground and surface waters and vice versa. Thus, the role of SOM in glyphosate sorption remains controversial: SOM may either decrease glyphosate sorption by blocking sorption sites or increase it, as higher SOM content seems to enhance sorption ability of poorly ordered Al- and Fe-oxides (Borggaard and Gimsing, 2008).

To our knowledge, DOM has been little investigated as a possible carrier for glyphosate. Only recently, Mazzei and Piccolo (2012) studied the interactions of glyphosate with dissolved humic acids (HA) and fulvic acids (FA) at pH 5.2 and 7 by NMR spectroscopy. With this complex technique, they confirmed the formation of H-bonds between glyphosate carboxyl and phophonate moities and protonated oxygen functions in dissolved humic substances, with a larger binding for FA and pH 5.2 than for HA and pH 7. Other spectroscopic techniques can be used to study the interactions of pollutants with DOM, such as UV/Vis fluorescence spectroscopy (Larrivee et al., 2003; Fu et al., 2007). This latter is largely used to characterize DOM (Chen et al., 2003; Hudson et al., 2007; Nebbioso and Piccolo, 2012). Furthermore, when combined with multivariate analysis, such as parallel factor analysis (PARAFAC), a qualitative and quantitative discrimination of fluorophores present in complex samples of different origin and nature is possible (Nebbioso and Piccolo, 2012, Christensen et al., 2006). Furthermore, this technique is also valuable to study interactions of DOM with pollutants by performing quenching experiments (Danielsen et al., 1995). Indeed, their presence will change the spectroscopic properties of DOM by occupying some binding sites on the fluorescent parts of the humic molecules.

The objective of this work was to study the role of dissolved/colloidal organic matter in the transport of glyphosate and copper from vineyard parcels to surface waters. The means were double: first the characterization of DOM by fluorescence spectroscopy of natural samples, soil water and river samples, and second, to study in the laboratory the interactions between DOM, glyphosate and copper using quenching experiments.

6.2 Material and methods

6.2.1 *Natural samples*

Soil solution samples from two vineyard parcels were collected in 2011 with porous ceramic suction cups (SDEC SPS200, Ø63 mm, porosity = 1 µm) at four different depths at the bottom of the parcels: 20, 40, 60 and 80 cm (Fig. 6.1). Their implementation, in fall 2009, and use were done according to Bès and Sévenier (1999). The applied tension was 0.6 bars and the recovery of samples was done every week or more frequently during intense rainfall periods. The Lutrive River was sampled during the year 2011 upstream and downstream the vineyard area, with two automatic samplers (ISCO 6712, Teledyne Inc.), equipped with polyethylene bottles and ultrasonic flow modules (Fig. 6.1). They were filtrated at 0.45µm with Nylon syringe filters.

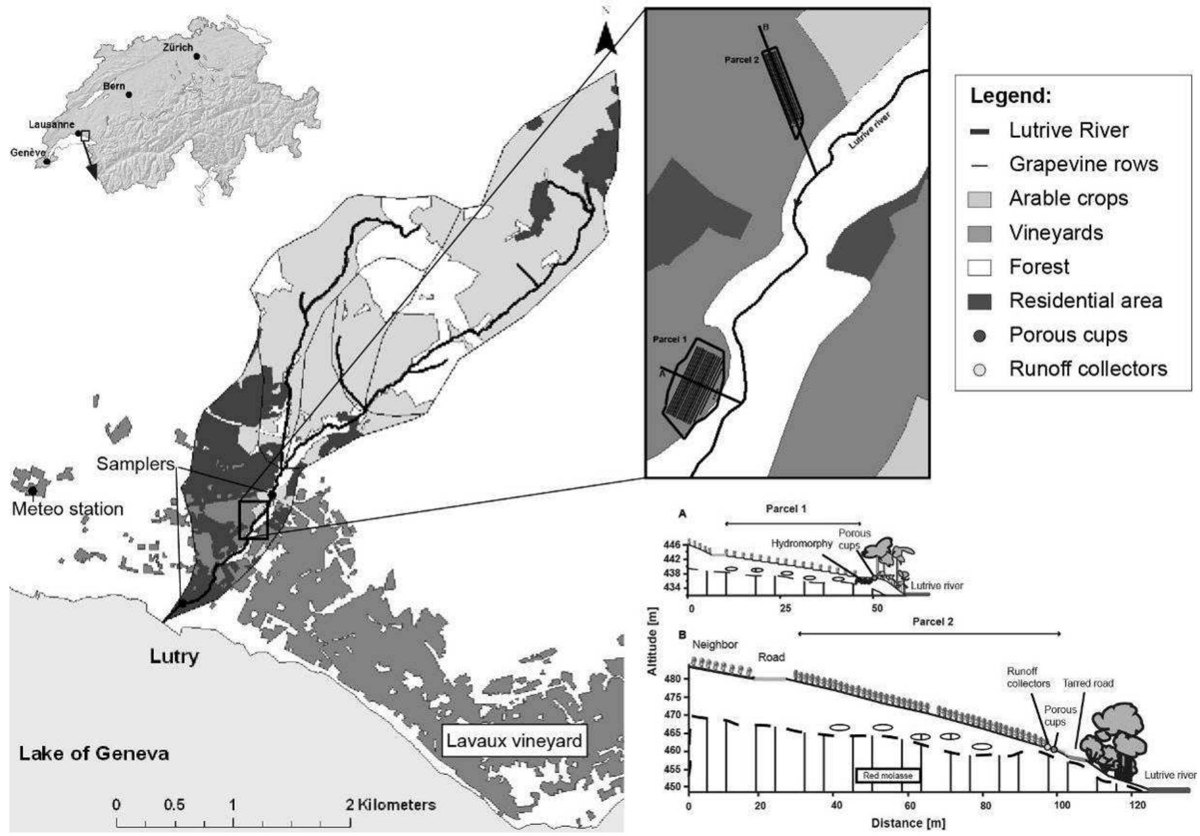

Figure 6.1: Lutrive watershed in western Switzerland with its different land use, the location of the Pully meteorological station and of the automatic samplers, as well as the one of the two investigated vineyard parcels and their transects with equipments at their bottom: porous cups and runoff collectors.

Both types of samples were then analyzed by UV/Vis fluorescence spectroscopy and 3D excitation-emission matrixes (EEM) were obtained with a spectrofluorometer (FluoroLog®-3, Horiba). Excitation wavelengths varied between 240 and 450 nm with a 5 nm increment and emission ones between 290 and 550 nm with a 2.5 nm increment. Parallel factor analysis (PARAFAC) of fluorescence EEM was done using a MATLAB (MathWorks©) routine developed at the University of Copenhagen (Bro, 1997; Bro and Vidal, 2010), but it was adapted for GNU Octave (GNU General

Public License). In order to apply PARAFAC analysis, attention should be pay to some factors affecting the decomposition, such as quenching and scattering (Christensen et al., 2006), and some preprocessing steps have been done. First, sample absorption were checked to be less than 0.1 at 254nm for the inner filter effect (Mounier, pers. comm.). Second, the Rayleigh and Raman first and second order bands were corrected using a simple algorithm. Lastly, EEF matrices were cut using a matrix-cutting function, because they have to constitute a three-way data array X in which they have the same dimensions (Christensen et al., 2006). For soil water samples, due to the presence of an artifact at 240-245 nm of excitation, their EEM were cut at 260 nm for PARAFAC decomposition. The appropriate number of contributing factors for the model is defined by the quality of the spectral contribution separation, which can be expressed as a percentage called "corcondia", for core consistency diagnostic (Bro and Kiers, 2003). A value of 50% would be problematic, whereas value above 90% can be interpreted as "very trilinear". Zhao (2011) suggests that a corcondia value above 60% is acceptable.

The herbicide glyphosate and its metabolite AMPA were quantified by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), after their derivatization with FMOC-Cl followed by solid-phase extraction (SPE). Previously, the method was adapted from Hanke et al. (2008) and validated with a LOQ of 10 ng/L and a good reproducibility (see Chapter 3). Dissolved organic carbon (DOC) concentrations were measured with a C-analyzer (Liquitoc Elementar©). Trace metallic ions (Fe^{3+} and Cu^{2+}) were quantified by ICP-MS (ELAN® 6100 DRC, Perkin Elmer©) on selected samples, previously acidified with HNO_3 and treated with H_2O_2 to avoid possible interference with organic matter. Pearson correlation matrices were obtained using the R software (R Development Core Team, 2009).

6.2.2 *Quenching experiments*

Glyphosate (PESTANAL®, 99.7%) and a humic acid (HA) standard were obtained from Sigma–Aldrich (Buchs, Switzerland). Glyphosate solutions with concentration varying between 10 and 150 mg/l were prepared in ultrapure water (18.2 MΩ×cm). HA solution with concentration of 100 mg/l was prepared in NaOH 0.1M and pH was adjusted to 8 in order to be representative of the natural pH conditions of the studied vineyard environment. The copper standard was obtained from Merck (Switzerland) and solutions with concentrations of 0.1 to 0.5 mM were prepared. The Stern-Volmer equation was then applied (Esteves da Silva et al., 1998):

$$I_0/I = 1 + K\,[M],$$

with I_0 the initial fluorescence intensity, I the quenched fluorescence intensity, K the stability or static quenching constant and [M] the maximum added concentration.

6.3 Results and discussion

6.3.1 *Soil water samples*

Fluorescence excitation and emission matrices (EEM) of soil water samples (see Appendix 6.1) showed mainly the presence of fulvic-like fluorophores, characterized by a peak A (λex: λem = 250:440) and humic-like fluorophores, characterized by a peak C (λex: λem = 315:440). A correlation between intensities of these two fluorophores was observed (Fig. 6.2). Peak A showed however always a higher intensity than peak C (Fig. 6.2). In a few samples, a peak B (λex: λem = 260:310) was also observed, indicating a protein-like fluorophore.

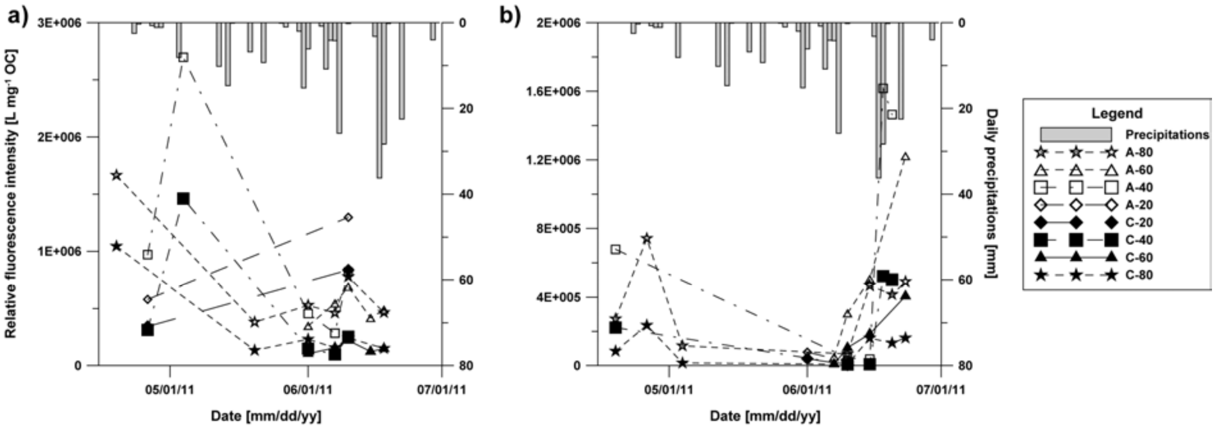

Figure 6.2: Relative fluorescence intensities of peak A (white symbols) and C (black symbols) of soil water samples of a) parcel 1 and b) parcel 2 at different depths (20, 40, 60, 80 cm) from April to July 2011, with daily precipitations.

Parallel factor analysis (PARAFAC) applied to these samples showed the contribution of three components (Fig. 6.3). The two fluorophores representing humic substances (A and C) in EEM could not be discriminated with PARAFAC analysis, confirming the “new views” on humic substances (Sutton and Sposito 2005; Kelleher and Simpson 2006), i.e. low-molecular mass moieties bind together by interactions of relatively low energy, such as H-bonding. However, PARAFAC decomposition discriminated two different peak couples: A1/C1 (λex: λem = 250:400/315:400) and A2/C2 (λex: λem = 250:475/340:475). This suggests that dissolved humic substances present in soil solution samples can be divided in low- (A1/C1) and high-MW (A2/C2) compounds, as the fluorescence signal of these latter is localised at longer wavelengths, for both excitation and emission. Indeed, compounds with high MW were shown to fluoresce at longer wavelengths, in both excitation and emission, due to more important energy losses in their more complex structures (Kalbitz et al., 2000; Ohno et al., 2007). Furthermore, comparing fluorescence signals from the two different parcels, fluorophores C1 and C2 were of lower intensity in parcel 1 (Appendix 6.3) than in parcel 2 (Appendix 6.4). This could be explained by the steeper slope of this latter, inducing a more important role of throughflows in soil solution transport as previously shown (see Chapter 4). The fluorophore B was present in some of the samples in both parcels (Corresponding names of samples in Fig. 6.3d are listed in Appendix 6.5).

6.3.2 *Lutrive River samples*

PARAFAC analysis applied to EEM of samples taken in the Lutrive River (Appendix 6.6) showed also the presence of three fluorophores linked to the presence of protein-like compounds (Fig. 6.6a) and humic-like ones (Fig. 6.6b,c). The first one is representative of the release coming from the urban area, whereas the two others are naturally occurring in freshwaters and linked to the breakdown of organic material (Hudson et al., 2007).

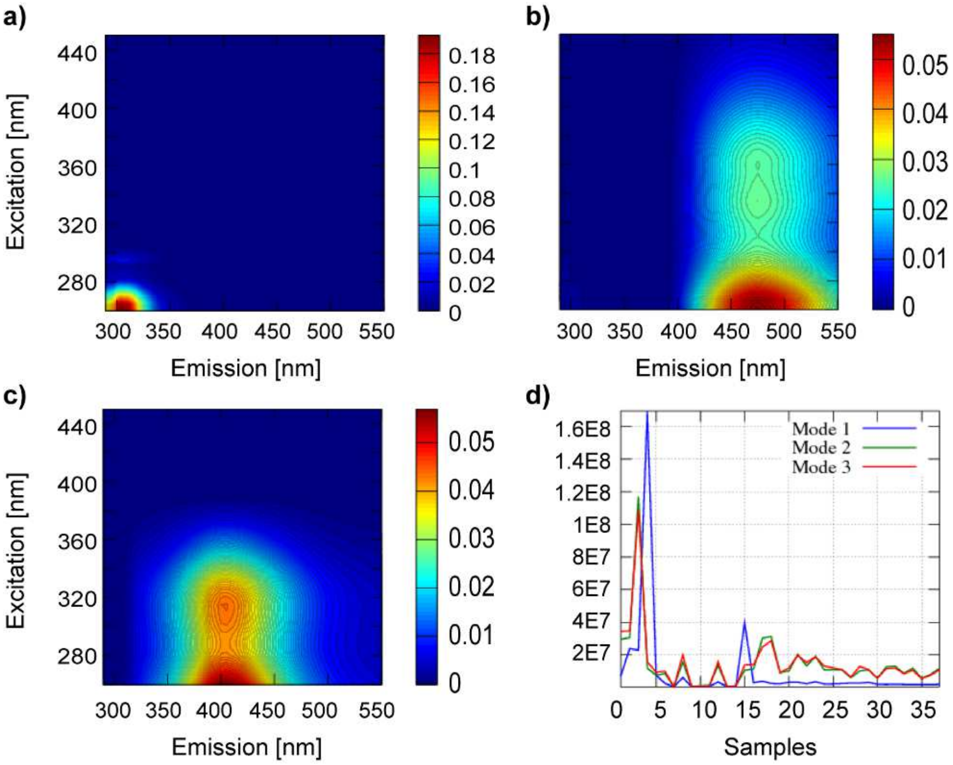

Figure 6.3: Parallel factor analysis (PARAFAC) of soil water samples with three components: a) protein-like (mode 1), b) (mode 2) and c) (mode 3) humic-like fluorophores, and d) their relative weight in the different samples with fluorophore a) in blue, b) in green and c) in red. Core consistency was of 80.2% (Appendix 6.2)

The two peaks representing humic substances could not be discriminated with the parallel factor analysis (peak A and C, Fig 6.4c), confirming again the "new views" on humic substances as for soil water samples (Sutton and Sposito 2005; Kelleher and Simpson 2006). Indeed, this was confirmed by a correlation observed between their intensities for both upstream and downstream sites (Fig. 6.5 and Appendix 6.7). Nevertheless, peak A showed always a higher intensity than peak C (Fig. 6.5). Though peak C2 was present, the peak C1 was absent. This could mean that low-MW humic-acid like compounds did not reach the freshwater system, but high-MW ones did, suggesting the transfer of these from soil surface layers to the Lutrive river by surface runoff during rainfall events.

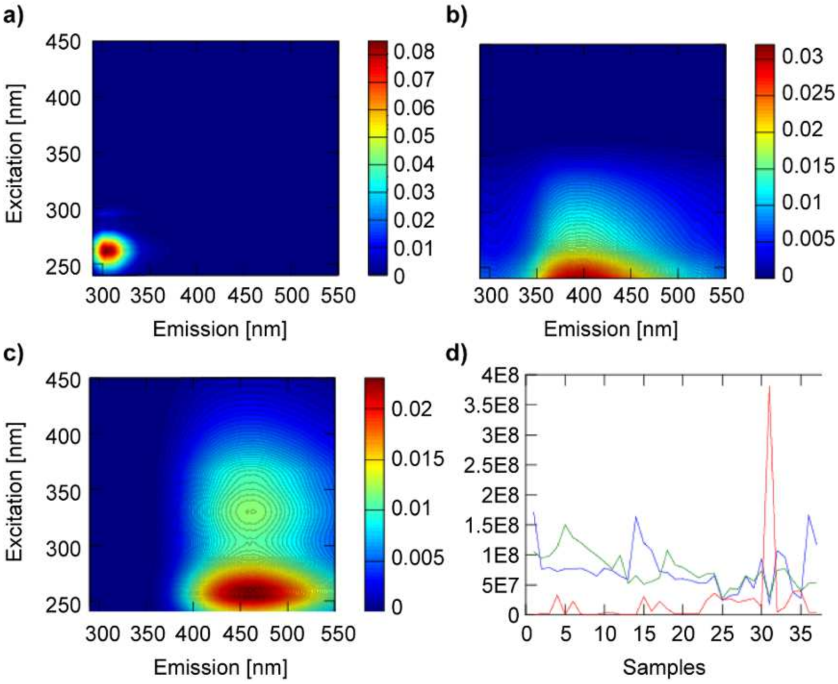

Figure 6.4: Parallel factor analysis (PARAFAC) of 37 river water samples with the three contributing fluorophores: a) protein-like, b) fulvic-like and c) humic-like fluorophores, and d) their relative weight in samples with fluorophore a) in red, b) in blue and c) in green. Core consistency was of 90.7% (Appendix 6.8)

Protein-like fluorophore (peak B) seemed to show an inverse behaviour than humic-like ones (A and C), and decreased when those increased, but this was not confirmed by any significant correlation (Table 6.1).

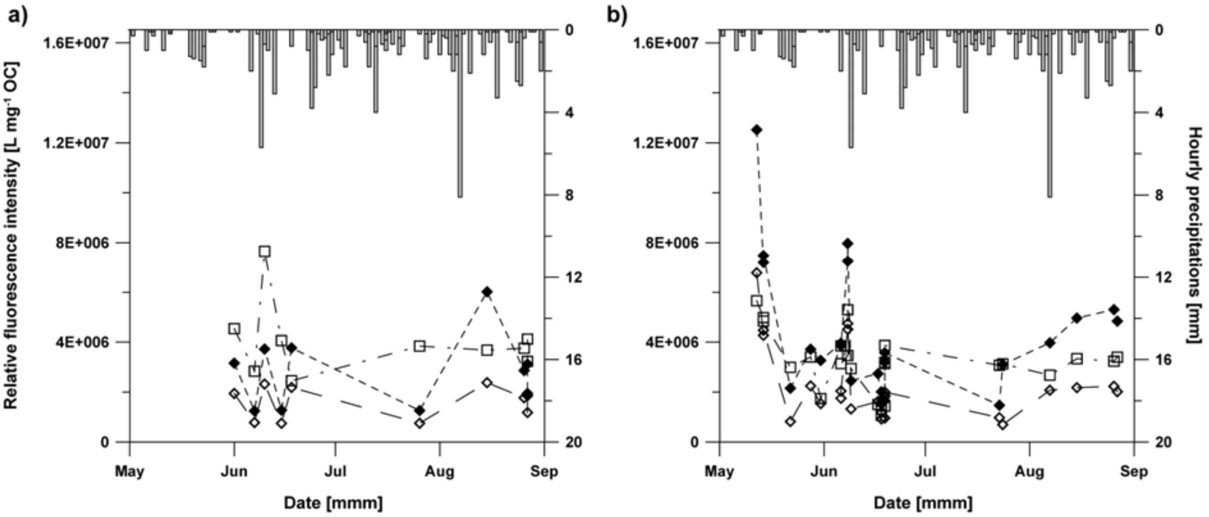

Figure 6.5: Variations of the relative fluorescence intensities of peak A (♦), B (□) and C (◊) in Lutrive river samples at a) the upstream and b) the downstream site for the period May-August 2011.

6.3.3 *Humification index*

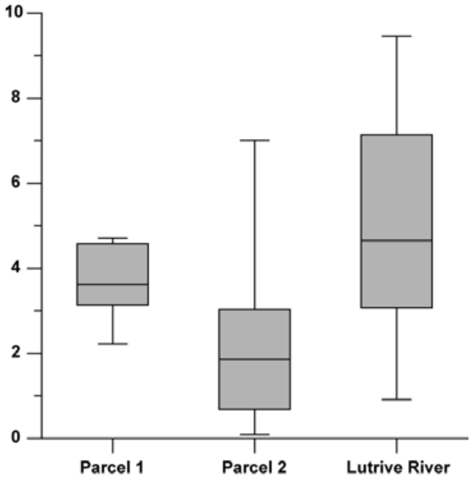

Figure 6.6: Box plots of the humification index (HIX; Zsolnay et al., 1999) for samples of parcel 1 and 2, and of Lutrive River.

For soil water samples, the average humification index was higher in parcel 1 than parcel 2 (Fig. 6.6), but variation of the humification index at different depths with time and precipitations was more important in parcel 2 than in parcel 1 (Fig. 6.6 and 6.7a,b). The higher mean value in parcel 1 may be explained by the proximity of the forest, and thus the continuous input of fresh organic matter in the top soil, whereas the higher variability of HIX values in parcel 2 is certainly due to the steepness of the slope. In general, HIX values were higher in superior layers (20 and 40 cm) than in deeper layers (60 and 80 cm), which could suggest the higher solubility and mobility of less humified compounds (fulvic-like compounds) reaching deep layers (Kalbitz et al., 2000). However, the presence of throughflows at 80 cm depth may also explain the mobility of colloidal more humified compounds in parcels and therefore the slightly higher HIX values at 80 cm than at 60 cm in parcel 1. Though no general relation between precipitations and HIX values could be pointed out, in parcel 1 these latter seem to increase with rainfall and decrease during dry periods, which is clear at 60 cm depth. In parcel 2 however, this tendency is not very clear. This absence of tendency is certainly due to the more important influence of preferential pathways taken by soil solution in this parcel, as previously observed (see Chapter 4). The significant increase of HIX values in the subsoil (80 cm) during the intense rainfall of mid-June following a dry period reveals this phenomenon. Indeed, the presence of preferential flowpaths, combined with the accumulation of water-soluble organic compounds in the soil caused, for example, by elevated temperature or dry periods without soil leaching, lead to elevated concentrations and fluxes of DOM in subsoil horizons at the beginning of intense rainfall (Kalbitz et al., 2000). Thus, precipitations and soil structure are likely to be driving forces for the solubilisation and the mobilization of humic compounds, principally localized in the top soil layer, and their leaching and horizontal transport throughout the parcels. This could be of major importance for copper and glyphosate transport. Indeed, DOM export from the top soil during intense rainfall events via rapid water flow, e.g. through macropores, is likely more reactive towards metals and organic xenobiotics, than during rainfall of low intensity via matrix flow (Kaiser and Guggenberger, 2005).

HIX values of river samples varied between 1 and 9, with high values found during the high rainfall events of early and mid-June (Fig. 6.6 and 6.7c). This confirms the transfer of more humified organic compounds from the top soil layer to the river by surface runoff during these events. Furthermore, no correlation could have been pointed out between humification index values and glyphosate or AMPA concentrations (Table 6.1b). However, HIX values were inversely correlated to fluorescence intensity of peak B, which is logical because this latter is representative of less-humified compounds than peaks A and C.

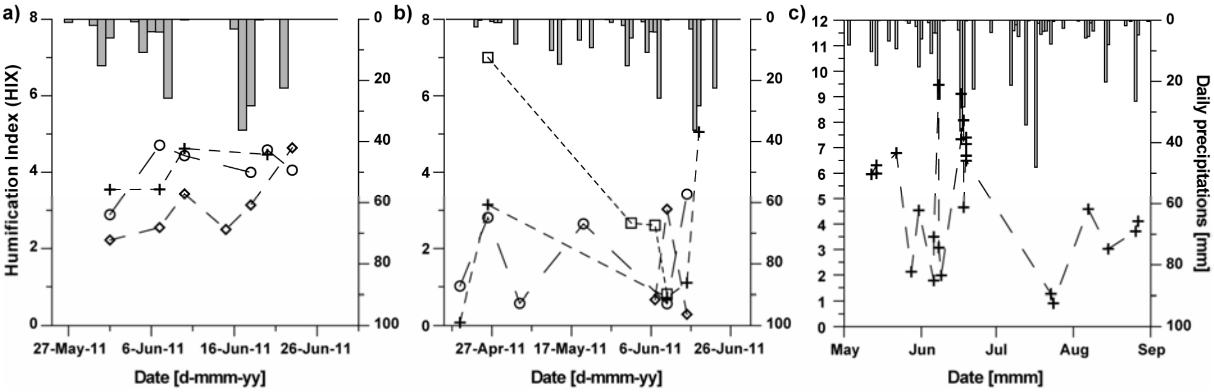

Figure 6.7: Variations of the humification index (HIX; Zsolnay et al., 1999) with daily precipitations in soil water samples of a) parcel 1 and b) parcel 2 at different depths (□ 20 cm, + 40 cm, ◊ 60 cm and ○ 80 cm) for the period April-June 2011, as well as c) in Lutrive River samples for the period May-September 2011.

6.3.4 *Correlations*

Looking at the potential correlations between spectroscopic signatures of DOM and pollutants of interest in soil water samples, fulvic-like fluorophores (peak A) was significantly correlated with both glyphosate and copper (Table 6.1a), beside to the ratio A/C. This was also the case for peak C, which confirms the hypothesis of the formation of ternary complexes between dissolved humic substances, glyphosate and copper as previously observed (Piccolo et al., 1992; Maqueda, 1998). Glyphosate was also correlated to both copper and iron concentrations, suggesting their possible implication in the transport of the herbicide. Chelation of glyphosate with metals has been indeed proved to happen and with a relatively high stability in solution (Subramaniam and Hoggard, 1988; Barja and dos Santos Afonso, 1998). However, no significant correlation was observed between HIX values and glyphosate or copper. This means that DOM humification degree was not a determining factor in the transport of these pollutants. Although all these statistical considerations should be taken with precaution, due to the low number of samples (12), the use of UV/Vis fluorescence spectroscopy was an appropriate choice to study the role of DOM in the fate of glyphosate and copper in vineyard soils.

Looking at the potential correlations between fluorescence signatures of DOM and pollutants of interest in Lutrive River samples, fulvic-like fluorophores (peak A) was significantly correlated with both iron and copper (Table 6.1b). This confirms the formation of fulvic-metals complexes in freshwater environment as previously observed with fluorescence spetroscopy (Larrivee et al., 2003; Hudson et al., 2007). Concentrations of glyphosate were also linked to both metals, suggesting that in the Lutrive River glyphosate-metal complexes are likely to be present as previously shown (Barja and dos Santos Afonso, 1998). However, no significant correlation was observed between glyphosate and intensities of peak A. Nevertheless, p-value was slightly higher than 0.01 (0.015), and when considering p-values < 0.05 glyphosate was correlated to peak A, as well as AMPA, and peak C to peak B and both metals. This only suggests a potential implication of DOM, both humic and fulvic acids, in the transport of glyphosate and copper in freshwater ecosystems.

Table 6.1: Spearman correlation coefficients between fluorescence intensities of peak A, C, the ratio A/C and the humification index (HIX), as well as the concentrations of glyphosate, AMPA, iron and copper for a) 12 soil water samples and b) 21 Lutrive downstream samples, with significant correlations in bold (p-values < 0.01).

a)

Correlation	**A**	**C**	**A/C**	**HIX**	**Glyphosate**	**AMPA**	**Fe**	**Cu**
A	1	0.995	-0.755	0.142	0.707	0.539	0.213	0.909
C	**0.995**	1	-0.788	0.106	0.717	0.574	0.201	0.889
A/C	**-0.755**	**-0.788**	1	-0.031	-0.489	-0.310	0.055	-0.599
HIX	0.142	0.106	-0.031	1	-0.052	0.259	-0.094	0.019
Glyphosate	**0.707**	**0.717**	-0.489	-0.052	1	0.640	0.753	0.819
AMPA	0.539	0.574	-0.310	0.259	0.640	1	0.333	0.457
Fe	0.213	0.201	0.055	-0.094	**0.753**	0.333	1	0.498
Cu	**0.909**	**0.889**	-0.599	0.019	**0.819**	0.457	0.498	1

b)

Correlation	**A**	**B**	**C**	**A/C**	**HIX**	**Glyphosate**	**AMPA**	**Fe**	**Cu**
A	1	-0.207	0.897	0.214	0.064	0.525	0.382	0.565	0.561
B	-0.207	1	-0.457	0.542	-0.631	-0.088	0.107	-0.093	0.098
C	**0.897**	-0.457	1	-0.220	0.156	0.404	0.343	0.537	0.447
A/C	0.214	0.542	-0.220	1	-0.084	0.243	0.209	0.072	0.242
HIX	0.064	**-0.631**	0.156	-0.084	1	0.041	-0.143	0.073	-0.055
Glyphosate	0.525	-0.088	0.404	0.243	0.041	1	0.468	0.669	0.786
AMPA	0.382	0.107	0.343	0.209	-0.143	0.468	1	0.226	0.270
Fe	**0.565**	-0.093	0.537	0.072	0.073	**0.669**	0.226	1	0.910
Cu	**0.561**	0.098	0.447	0.242	-0.055	**0.786**	0.270	**0.910**	1

6.3.5 *Quenching experiments*

Quenching of the dissolved humic acid standard with the addition of increasing concentrations of copper, as well as of glyphosate, gave rise to a decrease in fluorescence intensity of fulvic-like fluorophore (peak A, λex: λem = 275:515) much more important than for humic-like (peak C, λex: λem =335:515) and lignin-like (peak H, λex: λem = 450:535) fluorophores (Fig. 6.8a and b). This confirmed the great affinity of copper and glyphosate for the fulvic-like fluorescent part of DOM observed in field samples. However, when added together, no significant decrease in fluorescence intensity of peak A was observed (Fig. 6.8c), suggesting a stronger chelation capacity for glyphosate towards copper than for DOM. This confirms the potential formation of copper-glyphosate complexes in freshwater ecosystems, certainly more stable than glyphosate- and copper-DOM ones.

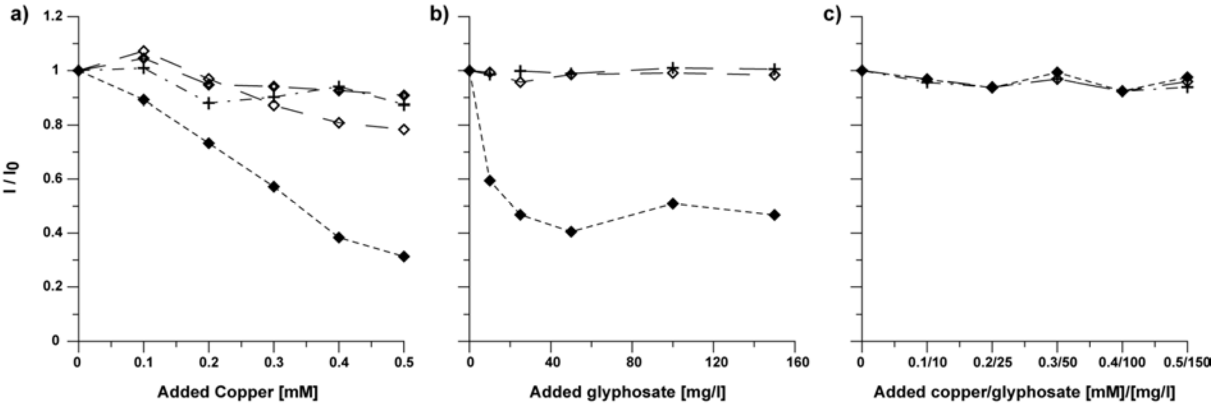

Figure 6.8: Results of the quenching experiments made at pH 8 using Sigma-Aldrich© dissolved humic acid standard: Addition of increasing concentrations of a) copper, b) glyphosate and c) both, with the relative decrease in fluorescence intensities of peaks A (♦), C (◊) and H (+).

The Stern-Volmer equation fitted well to the fluorescence quenching of peak A with added copper (Fig. 6.9), with a regression coefficient of 0.879 and a stability quenching constant of 3.731. The quenching of peak A with glyphosate was not linear and thus the model could not be applied. Although these results should be taken with caution to the low number of points, they showed a different DOM quenching behaviour between copper and glyphosate, with a linear decrease in the case of copper and a saturation plateau from 25 mg/l of glyphosate on. This suggests that, at this concentration, all the possible H-bonding between glyphosate and DOM standard were occurring.

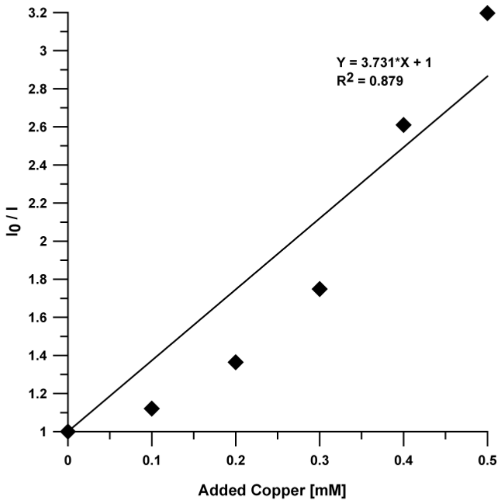

Figure 6.9 : Stern-Volmer model applied to the fluorescence quenching of the peak A with the addition of increasing concentration of copper

6.4 Conclusions

UV/Vis fluorescence spectroscopy, combined with parallel factor (PARAFAC) analysis, allowed to characterize in a proper way DOM of soil and surface water samples from the studied vineyard area. Furthermore, with this technique, the multiple interactions between the herbicide glyphosate, its metabolite AMPA, copper and iron could be assessed in the collected natural samples. Results, showed the affinity of both metals for fulvic-like DOM in soil solution, but only copper in the river. Glyphosate was also linked to the fulvic-like spectroscopic signature in soil water samples, as well as to both metals. In surface water samples, its concentrations were also correlated to iron and copper ones, but not in a significant way to fulvic-like signature. The higher affinity for fulvic-like compounds of these agrochemicals largely used in vineyards were confirmed by quenching experiments in laboratory.

Ackowledgments

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7 Synthesis and outlook

The aim of this synthesis is to summarize the main results of this work and to provide an overall picture of the fate of the ubiquitous herbicide glyphosate after its application in vineyards and of the subsequent pollution of surface waters. First of all, results of the development of analytical tools to study this herbicide and its metabolite are briefly summarized (§7.1). The adopted multiple scale approach (Fig. 7.1) allowed to help follow the fate of the herbicide glyphosate and its metabolite AMPA in an urbanized vineyard landscape (Lavaux, western Switzerland). More precisely, it permitted to better understand:

1. The possible physical, chemical and biological reactions that these molecules can undergo at the molecular and microscopic level (§7.2; Fig. 7.1a),
2. The physico-chemical processes that they are subject to at the soil column and parcel level (macroscopic level) (§7.3; Fig. 7.1b), and
3. Their overall fate at the river catchment level (landscape level) and the associated risk assessment (§7.4; Fig. 7.1c).

[redacted]

Figure 7.1: The multiscale approach to study the fate of the herbicide glyphosate and its metabolite AMPA with a) microscopic, b) macroscopic and c) landscape levels

7.1 Analytical development

The validation of the method to quantify the herbicide glyphosate, its metabolite AMPA and the herbicide glufosinate at trace level in several types of natural waters was successful. Indeed, with a low limit of quantification (10 ng/l) and a good reproducibility (86-133%), it is possible to track these xenobiotics in the environment. Preliminary results of grab samples taken in 2010 showed that river water samples exhibit a frequent pollution by the studied herbicides, often above the 100 ng/l threshold for each pesticide defined in the federal ordinance on water protection (Oeaux, 1998), which finally end up in Lake Geneva. Samples taken in the Vidy Bay had higher concentrations for AMPA than for glyphosate, which was also the case for the sediments analyzed (see Appendix 7.1). Although solid analysis was not validated, extractions with potassium hydroxide (KOH) gave relatively coherent results despite a lower accuracy of the method (glyphosate and AMPA yields = 58 and 126%; see Appendix 7.2). Indeed, the evolution of glyphosate and AMPA concentrations in the surface soil layer (0-3 cm) at the bottom of parcel 2 in 2010 was logical (see Appendix 7.3), with AMPA being more persistent than glyphosate. Thus, some more analytical development should be done in order to analyse these compounds correctly in soils and sediments.

7.2 Molecules, colloids and particles

Glyphosate in the studied vineyard environment shows, in theory, a global double negative charge, due to the alkaline pH conditions (8-9). Once applied, it is known to sorb readily onto soil components, especially mineral phases such as iron and aluminium hydroxides (Fig. 7.1a), but also 2:1 clay minerals with intermediate divalent cations (Fig. 7.1b; Piccolo et al., 1994). It has been also proven to interact with organic consituents such as humic substances mainly through H-bonding (Roy et al., 1989; Piccolo et al., 1996; Dousset et al., 2004). Indeed, both glyphosate and humic molecules contain various electronegative atoms, which can act either as H-donor or H-acceptor groups (Piccolo et al., 1996). However, these authors showed that "*the occurrence of H-bondings between glyphosate and HS is controlled not only by the content of hydrogen donor and acceptor groups but also by the molecular size and stereochemical flexibility of the humic material*"[34].

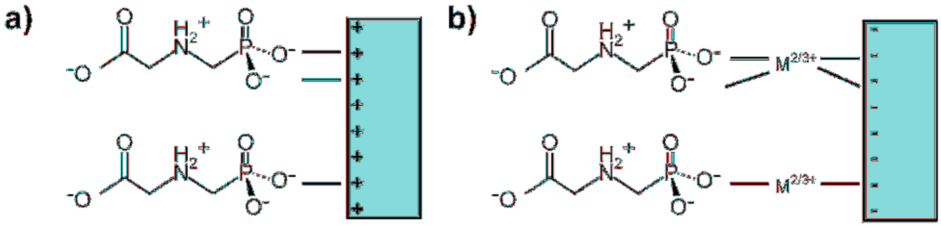

Figure 7.1: glyphosate adsorption on both a) positively and b) negatively charged surfaces, with in both cases the possible formation of monodentate mononuclear (down) and bidentate binuclear (up) complexes

In our case, due to the alkaline conditions, the glyphosate amine group is theoretically the only possible H-donor functionality as both carboxylate and phosphonate groups are deprotonated, and humic molecules have a larger size, resulting in a more flexible structure for the formation of H-bonds. Furthermore, ternary complexes between glyphosate and negatively charged surfaces, such as organic matter or clay minerals, are also likely to be formed with intermediate di- and trivalent "bridging" metal cations, such as Cu^{2+}, Fe^{3+} or Al^{3+} (Fig. 7.1b; Morillo et al., 1997; Barrett and McBride, 2006; Borggaard and Gimsing, 2008). Morillo et al. (1997) found out that the presence of copper can also lead to glyphosate desorption from the external sites (Al-OH groups) of montmorillonite and the formation of Cu-glyphosate complexes, which have a high stability and a lower sorption affinity than

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[34] Piccolo, A.; Celano, G.; Conte, P. Adsorption of Glyphosate by Humic Substances. J. Agric. Food Chem. 1996, 44(8), 2442-2446.

glyphosate alone. Inversely, glyphosate may enhance copper mobility by chelating with Cu^{2+} previously sorbed on both mineral and organic surfaces (Barrett and McBride, 2006). However, the alkaline conditions were previously shown to favour the binding of Cu-glyphosate complexes to soil constituents, certainly with the formation of ternary surface complexes (Fig. 7.1b; Sheals et al., 2002; Dousset et al., 2007). These inner-sphere complexes are predominantly monodentate complexes, but for a minor part bidentate ones can also be formed (Sheals et al., 2002).

It has been also shown that there is a competition for binding sites as both positive and negative surfaces can be already occupied by organic matter and hydroxides respectively (Tipping and Cooke, 1982; Day et al., 1997). This can induce a change in surface charge and, according to conditions, glyphosate may either remain in solution and be transported further through the soil porosity, or being adsorbed onto the mineral-organic surface complexes. Furthermore, other compounds, such as phosphate, also have a competitive adsorption with glyphosate on Fe- and Al-oxides (Gimmsing et al., 2004; Borggaard et al., 2005).

Thus, in the collected soil or river water samples, natural colloids (<1 µm), such as organic matter, clay minerals or hydroxides, and/or their possible associated form, could have acted as carriers for glyphosate. Indeed, colloid-associated transport is known to participate in glyphosate transfer from the top soil layer to deeper horizons (de Jonge et al., 2000; Gjettermann et al., 2009; Kjaer et al, 2011), which could also be the case in rivers. With the help of UV/Vis fluorescence spectroscopy, we studied the possible involvement of organic colloids in glyphosate and copper transport in both soil water and river samples. Combined with parallel factor (PARAFAC) analysis, this technique allowed characterizing DOM of soil and surface water samples from the studied vineyard area. Furthermore, the multiple interactions between the herbicide glyphosate, its metabolite AMPA, copper and iron could be assessed in the collected natural samples. Results showed the affinity of both metals for fulvic-like DOM in soil solution, but only for copper in the river. Glyphosate was also linked to the fulvic-like spectroscopic signature in soil water samples, as well as to both metals. This confirms the formation of ternary complexes DOM-copper-glyphosate as previously shown (Piccolo et al., 1992). In surface water samples, the concentration was also correlated to those of iron and copper, but not in a significant way to the DOM fulvic-like signature. The higher affinity for fulvic-like compounds of these agrochemicals largely used in vineyards was confirmed by quenching experiments in the laboratory. These showed indeed a stronger decrease in fluorescence intensity for fulvic-like fluorophores than for more aromatic ones (humic-like and lignin-derived ones) in the tested humic acid standard solution.

7.3 Soil pore system, throughflows and transport at the parcel scale

Transport of glyphosate and AMPA at the soil column level, i.e. their infiltration and/or leaching, likely depends on the soil pore system structure (connectivity, tortuosity, macropores, etc.). Indeed, the obtained soil solution at the different depths is likely to depend on the local pore size distribution, and the degree of equilibration between water in matrix pores and in macropores (Williams and Lord, 1997). At the parcel scale, herbicide transport depends likely on the formation of lateral water preferential pathways, i.e. the presence or absence of discontinuities in the arrangement of soil horizons, as well as on the formation of surface runoff, which depends on the soil infiltration capacity. Thus, both vertical and lateral transport of glyphosate and AMPA in soils are highly linked to the precipitation regime, as well as to soil texture and structure. Both infiltration processes in the unsaturated zone, with subsequent exfiltration to the river, and surface runoff relative to our observations are summarized in Figure 7.2.

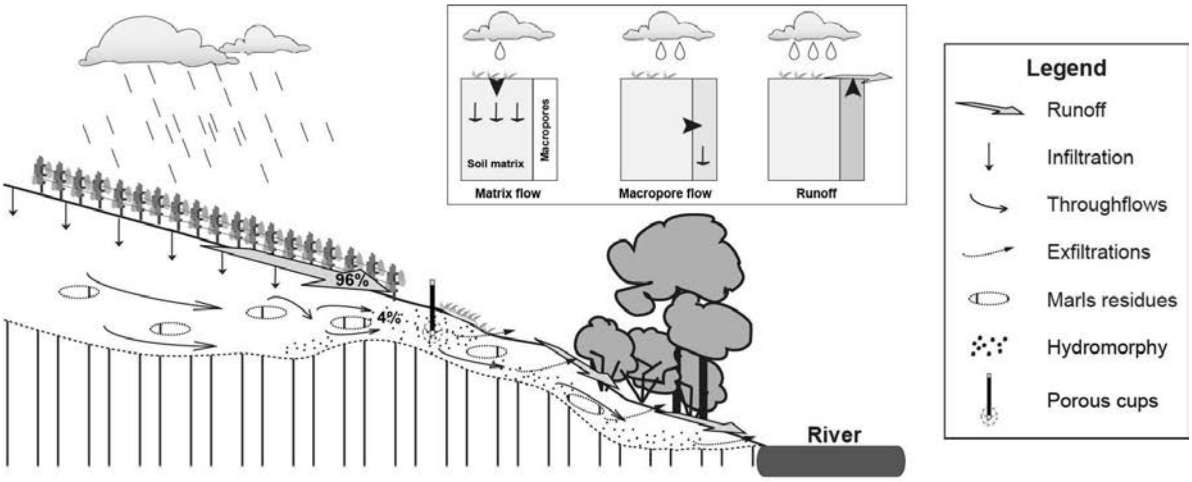

Figure 7.2: Synthesis of water pathways in vineyard parcels with infiltration and throughflows, leading to temporary hydromorphy and exfiltration, as well as surface runoff responsible for the export of glyphosate and AMPA molecules in the river direction. Gradual saturation of the soil porosity as rain intensity increases is shown as an insert, with matrix flow and the formation of macropore flows and surface runoff.

Concerning the infiltration behaviour in both parcels, the variable intensity of rain events and the presence of the more impervious reddish marl layer or lenses around 100 cm depth are certainly the two most important parameters explaining the occurrence of glyphosate and AMPA at 60 and 80 cm at the bottom of both parcels. Leaching and infiltration of glyphosate and AMPA further up in the parcel and their subsequent transport via throughflows are conditioned by the specific soil characteristic, as well as by to the succession of significant rain inputs. Indeed, concentration peaks were always related to cumulated rainfall during the previous days. Thus, with rainfall events of more than 10 mm/day, the increase in rain intensity leads to the saturation of soil matrix pore space with water, and thus macropore flow with associated colloid-transport is likely to happen, transporting herbicide molecules relatively rapidly from the top layer of the soil down the profile. The presence of a more impermeable layer or lenses around 100 cm depth in the investigated parcels induces throughflows, undoubtedly leading to a temporarily saturated zone, like in parcel 1, that may leak to the river. When comparing the two parcels, the steepness of the hillslope determines the importance of these throughflows in herbicide transport.

Furthermore, the precipitation regime seems to govern more strongly glyphosate and AMPA transport in soils than chemical interactions described above. Although laboratory results are difficult to extrapolate to field situations, the rain fall intensity and the soil pore structure were previously found to play a prevalent role on the sorption properties for glyphosate leaching in soil columns (de Jonge et al., 2000). Indeed, cumulated rain fall seems to govern glyphosate and AMPA concentration dynamics in the vadose zone in the following way:

1. Cumulated rainfall up to 10 mm decreases herbicide concentrations due to a dilution effect,
2. Quantities between 10 and 30 mm lead to a rise in concentration, certainly due to an increase in the kinetic energy of the soil solution, with the consequent formation of preferential flow in the parcels with colloid-associated transport, and
3. From 30 mm of cumulated rainfall on, the increased surface runoff and dilution are responsible for the decrease in concentration.

Nevertheless, although the chemical composition of soil solution does not play an important role in their transport, mainly governed by the rainfall regime and soil permeability, the presence of copper and the alkaline pH conditions in the studied vineyard soils certainly participate in their mobility, by diminishing their sorption (Morillo et al., 1997, Barrett and McBride, 2006; Zhao et al., 2009). Furthermore, the often slightly reducing conditions encountered in deeper horizons may also have led to a lower sorption capacity, with Fe^{3+} reducing to Fe^{2+} (Gimmsing and dos Santos, 2005), inducing a higher availability of glyphosate for biodegradation in the soil solution. This could, in part, explain the observed decrease in glyphosate concentrations with time in parcel 1 at 60 cm, and the simultaneous increase in AMPA.

The export of glyphosate and AMPA from parcels to the Lutrive River during rain events remains, however, the result of surface runoff processes (96%). With more important rainfall (>30 mm/day) both micro- and macropores get saturated and surface runoff becomes the dominant process. Concentrations of glyphosate in runoff samples were very high just after the application and decreased drastically with time and the rain events afterwards. For AMPA, the decrease in concentrations was less drastic, as it is assumed to be constantly produced by glyphosate degradation. In surface runoff processes, particle-associated transport (>0.45 µm) plays a minor role, whereas 78% of glyphosate and 73% of AMPA in surface runoff happen in the dissolved fraction and/or associated with small colloids, which is less than in previous studies despite a smaller cut-off (0.24 µm) (de Jonge et al., 2000; Kjaer et al, 2011). The presence of considerable amounts of copper in the surface soil, may in part explain this discrepancy. In contrast, exfiltration of soil water that underwent throughflows participate in a minor extent to the contamination of the river (4%). The total amount of glyphosate and AMPA retrieved in both type of samples from parcel 2, and likely to be exported from it, was 4.3 g in 2010 and 9.1 g in 2011. This represents respectively 10 and 20% of the initial applied amount, which, despite the uncertainty of such kind of calculations, is in agreement with previous studies (Gavrilescu, 2005; Simonsen et al., 2008). The 80-90% remaining was either retained, or degraded in the soil (Sprankle et al., 1975; Boorgaard and Gimmsing, 2008; Simonsen et al., 2008).

7.4 River dynamics, Lavaux landscape and global risk assessment

In the samples retrieved from the Lutrive River, several concentration peaks of glyphosate and AMPA were found largely above the 100 ng/L threshold for each molecule of pesticide defined in the federal ordinance on water protection (Oeaux). These peaks, up to 4970 ng/l, were related to the main rain events (>10-20 mm/day), revealing the transfer of these compounds from fields to surface water, mainly by surface runoff, confirming results at the parcel scale. Concentrations were much higher downstream than upstream, as the main vineyard area is located in the southern sub-catchment. This may also be explained, for a minor part, by urban sources occurring in the same sub-catchment, such as private gardening and weed management along railways, which are known to represent sources for surface water pollution by glyphosate and AMPA (Botta et al., 2009; Hanke et al., 2010). A few drainage and stormwater samples of the vineyard area contained elevated concentrations of glyphosate and AMPA. Herbicide molecules transported downhill by these pathways are either retained in the forested buffer zone, or contribute to the pollution of the Lutrive River.

During main rainfall events, colloids and particles, potentially carrying copper, glyphosate and/or AMPA, as described in section 7.2, are transferred to aquatic ecosystems. Once in rivers, they are transported as suspended solids (SS), with a more important proportion of coarse particles (50-2000 µm) found at the beginning of a flood event, which decreases afterwards to the advantage of smaller ones (<50 µm; Appendix 7.4). The minerals in SS were first identified by scanning electron microscopy and energy dispersive spectroscopy (SEM-EDS), and then confirmed by X-ray diffraction (XRD): phyllosilicates (clay minerals, biotite), calcite, quartz, K-feldspar, Na-plagioclase and sometime goethite (Appendix 7.5 and 7.6). Organic debris, such as fibres and diatoms, were also identified by SEM (Appendix 7.6).

The mass balance revealed a total load of 7.1 kg at the river outlet from mid-April to early September, which represents 6 to 12% of the total amount applied in the catchment, assuming a glyphosate application of 1080 g/ha on 100 to 50% of the total agricultural area, respectively. This high proportion was attributed to the steepness of the vineyard area and also, in part, to the uncertainties of the extrapolated applied amount. During the same period, a total amount of 1.04 kg was found upstream of the vineyard area, which represents 15% of the load found downstream. Note that this upper part corresponds to 80% of the total watershed area. Thus, the relative contribution of vineyards (85%), and, for a minor part, applications in residential areas, is more important than for arable crops, where other herbicide molecules may be in use at the same time. These results highlight the importance of vineyards, even with relatively small surfaces (20%), to the potential pollution of receiving waters. When extrapolating the 6 kg/year from our small vineyard sub-watershed (26 ha) to the whole Lavaux vineyard surface (830 ha), a total yearly glyphosate input to Lake Geneva of 190 kg can be estimated. Nevertheless, this extrapolation should be considered with caution, due to the large uncertainties of its calculation and the large diversity of weed management practices, as well as in the landscape configuration, which can be found in the Lavaux vineyard.

In our case, and with the methodology applied, the ecotoxicological risk, linked to the maximum concentrations of glyphosate and AMPA observed in the Lutrive River, was found to be negligible (RF<1). Future integration of the dynamic of concentrations in risk assessment will help to better characterize a potential risk of glyphosate. Indeed, input of herbicides into the aquatic environments often occurs in pulses of a couple of hours rather than continuous flows, resulting in fluctuating exposure of aquatic organisms to these pollutants. Thus, an improvement for the risk assessment could be the use of pulse exposure test in the laboratory. Furthermore, these latter could be done not only with the active ingredient, but also with surfactants found in glyphosate-formulated products, which are known to participate actively to its toxicity towards aquatic organisms. Lastly, and in order to come up with a realistic risk assessment, other pesticide molecules applied in vineyards, such as organic fungicides, should be taken into account to perform mixture toxicity tests.

As a perspective for future research, all the gathered field data should be compared with the outputs of different transport models which are currently in use in both research and political/administrative institutions. Indeed, many transport models are available for the transport of pesticides at the soil column level, and some are currently in use for the authorization of new pesticide molecules, while others also take into account the macropore domain and the presence of preferential flows, such as MACRO, HYDRUS, RZWQM (Köhne et al., 2009). Modeling the transport of glyphosate in rivers according to the precipitation regime and land use would also be interesting, especially as colleagues at EPFL developed such tools (e.g. Coutu et al., 2012).

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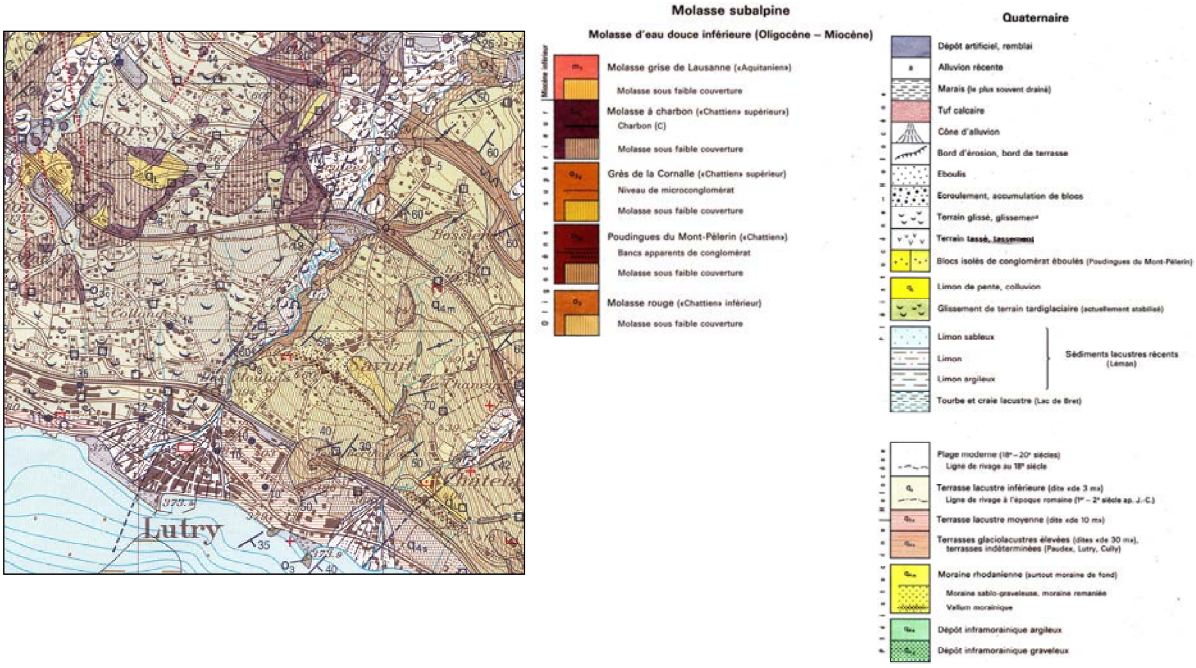
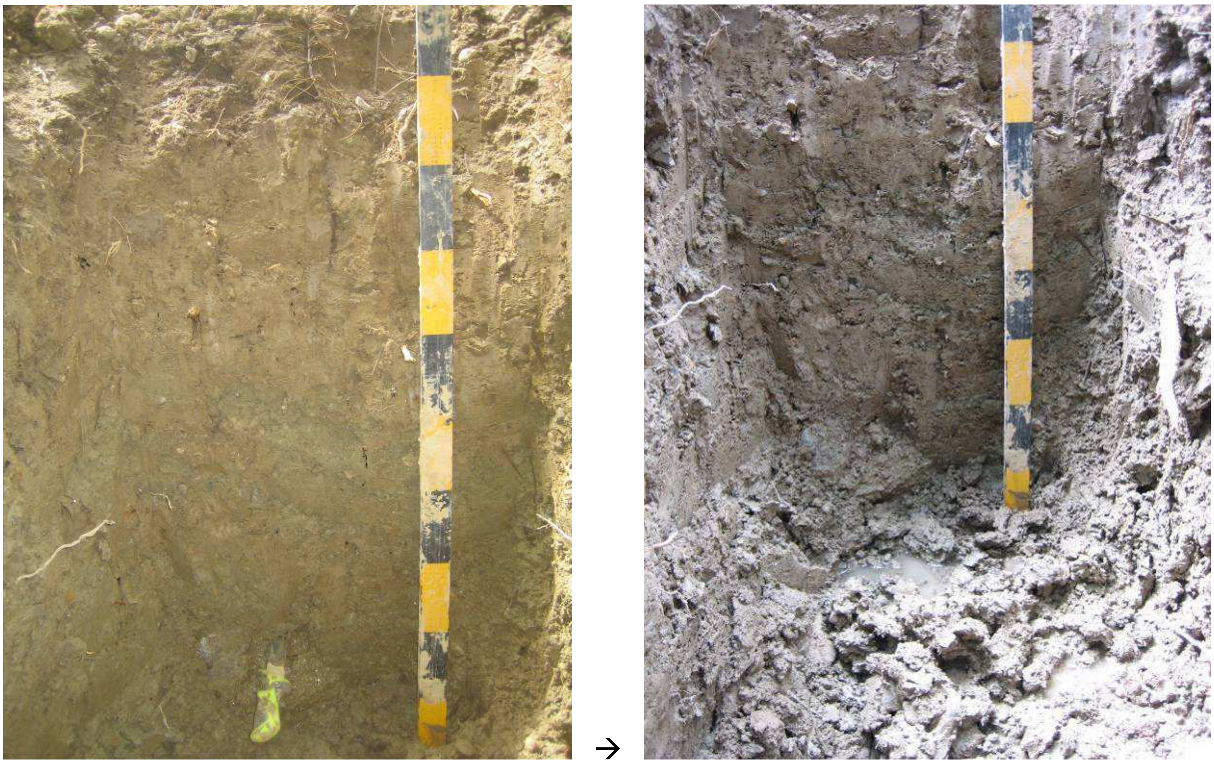
8 Appendices

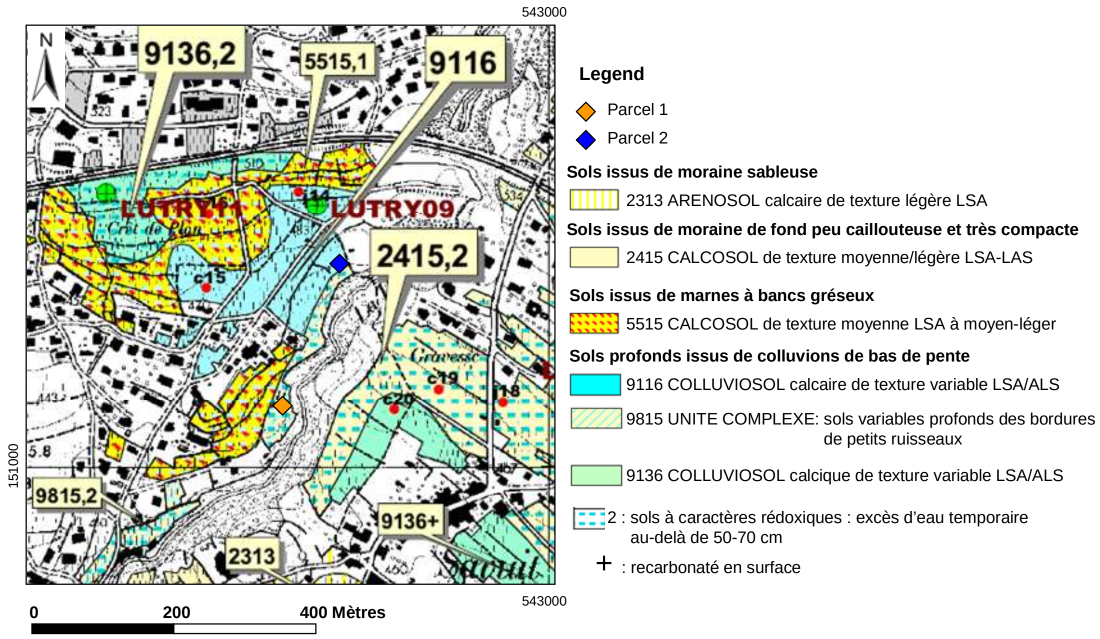
Appendix 1.1: Geology in the Lutry region (Geological Atlas 1: 25 000, ©Swisstopo Data)



Appendix 1.2: Pictures of the soil profile in parcel 1 with pouring water with a constant flow of max. 1 dl/min after a knife's hole made at 85 cm.



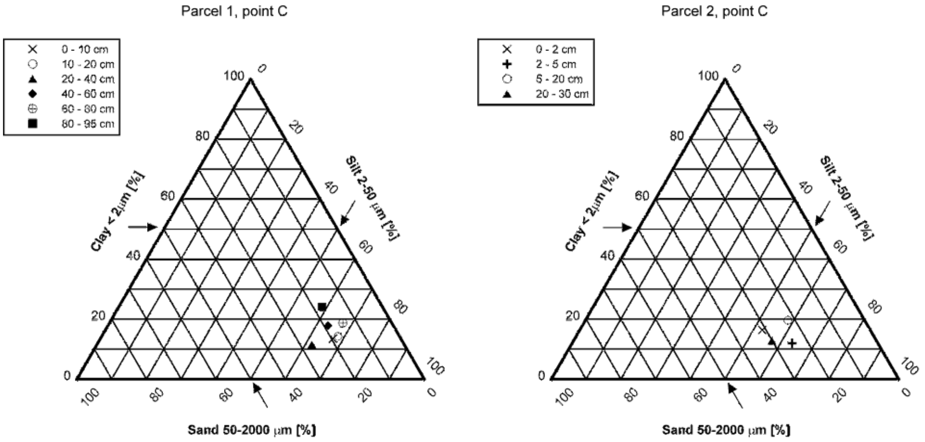
Appendix 1.3: Map of soil units in the area of the the two studied vineyard parcels (adapted from Letessier and Fermond, 2004)



Appendix 1.4: Monitoring station in parcel 2, with a) the house in which are b) a data logger (green device), a power regulator (black device, up left), a GSM modem (grey device, up left in the box) and a battery powered by c) a solar panel; d) runoff collector and e) overall picture with the implementation of probes in April 2011 with C. Martino.


a) b) c)
d) e)

Appendix 1.5: Soil texture for different horizons of profiles dug at the bottom of parcel 1 and 2, and analyzed by laser granulometry after HCl and H_2O_2 treatments (Emch and Pitteloud, 2010).



Appendix 1.6: Soil bulk density for surface horizons sampled at the bottom of parcel 1 and 2 with the cylinder technique (Emch and Pitteloud, 2010).

Parcel	Parcel 2 (0-10 cm)					Parcel 1 (0-10cm)					Parcel 1		
N° cylinder	62	102	53	58	45	96	90	57	86	44	99		
Name	**B1**	**B2**	**B3**	**B4**	**B5**	**D1**	**D2**	**D3**	**D4**	**D5**	**D 20 cm**		
Weight cylinder - empty [g]	138.5	138.1	144.2	142.8	142.8	138.2	132.6	132.9	145.6	142.9	143.9		
Weight field [g]	304	298	305	294	291	279.6	274.4	279.6	294.2	298	332		
Weight dry [g]	282	277	285	275	272	247	245	250	268.5	268.5	307		
Weight sat. [g]	325	321	328.5	320	317	300	299	300	316	316	342		
Weight soil - field [g]	165.5	159.9	160.8	151.2	148.2	141.4	141.8	146.7	148.6	155.1	188.1		
Weight water - field [g]	22	21	20	19	19	32.6	29.4	29.6	25.7	29.5	25		
Weight soil - dry [g]	143.5	138.9	140.8	132.2	129.2	108.8	112.4	117.1	122.9	125.6	163.1		
Weight water - sat. [g]	43	44	43.5	45	45	53	54	50	47.5	47.5	35	Mean P2 0 10cm	Mean P1 0 10cm
Bulk density [g/cm3]	1.44	1.39	1.41	1.32	1.29	1.09	1.12	1.17	1.23	1.26	1.63	**1.37**	**1.17**
Particule density [g/cm3]	2.52	2.48	2.49	2.40	2.35	2.31	2.44	2.34	2.34	2.39	2.51	**2.45**	**2.37**
Porosity [%]	43.0	44.0	43.5	45.0	45.0	53.0	54.0	50.0	47.5	47.5	35.0	**44.10**	**50.40**
V [cm3]	100												

Weight field = **Weight field - weight empty** **V = Volume cylinder**

Weight water - field = **Weight field - weight empty - weight soil dry**

Weight soil - dry = **Weight dry - weight empty**

Weight water - sat. = **Weight sat. - weight empty - weight soil dry**

ρ_a **= Ws / Vtot** Source: ALAOUI, 2009

ρ_p **= Ws / Vp** $\rho_p = \mathrm{Wsoil} / (W_{\mathrm{water\ tot}} - M_{\mathrm{water\ only}}) 1 / \rho_w$ $\rho_p = \mathrm{W\ soil}/[((V*\rho_w) - \mathrm{W\ water\ sat})\ 1 / \rho_w]$

$\Phi = 1-(\rho_a / \rho_p)$

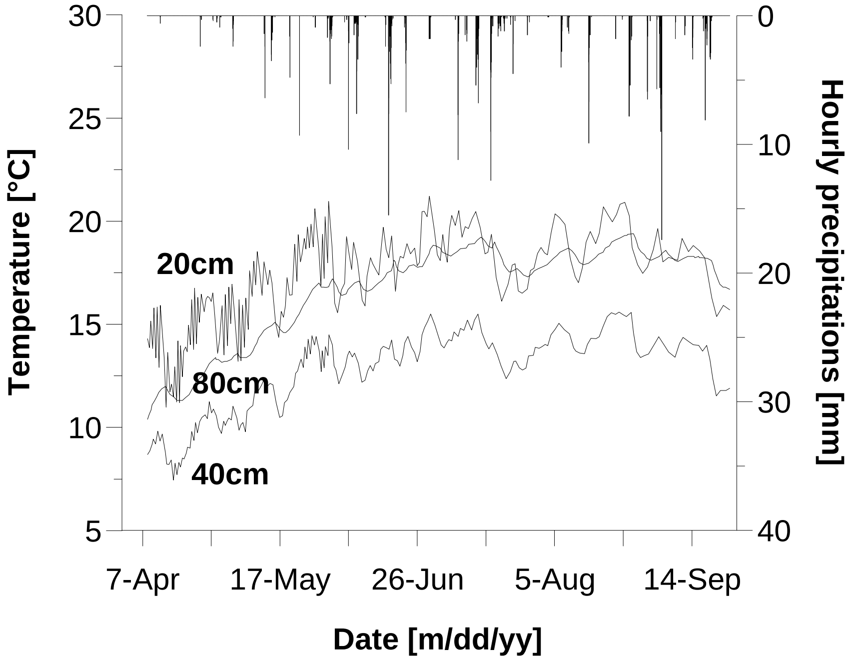
with **Ws = solid weight** ρ_a **= bulk density** ρ_w **= water density = 1 g/cm**^{3}

Vtot = total volume ρ_p **= particle density**

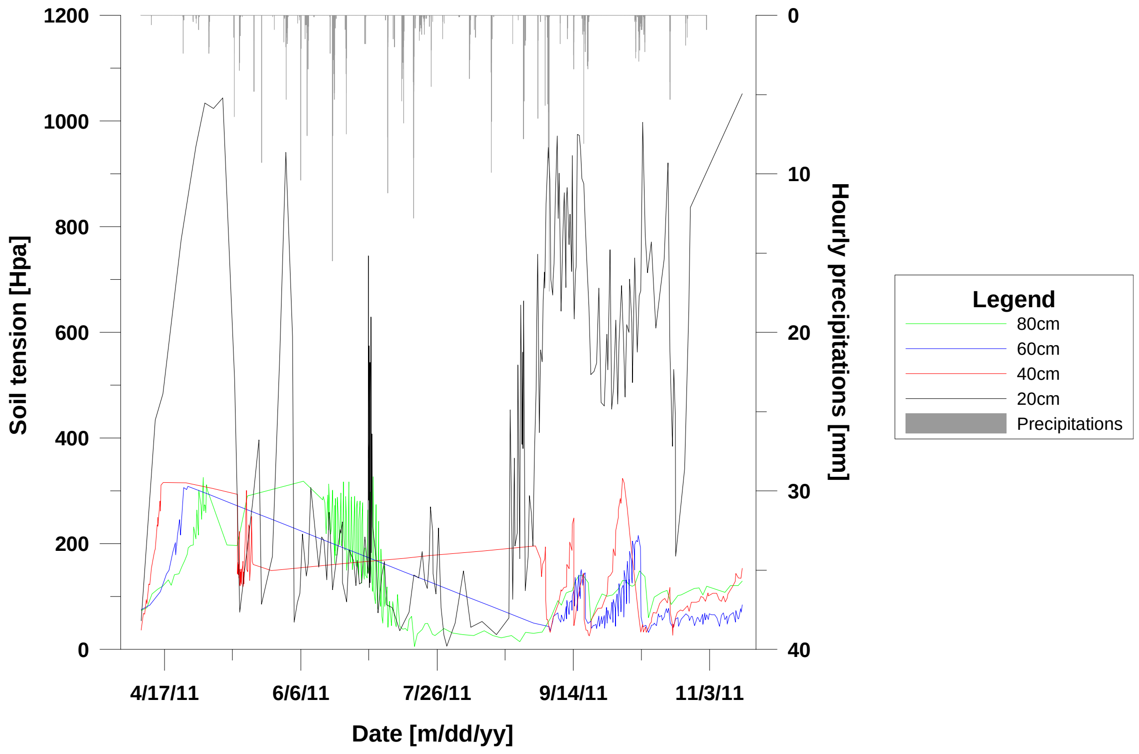
Vp = particles volume Φ **= porosity**

Appendix 1.7: Double ring infiltration experiment

Appendix 1.8: Soil temperature for the period April 2011-September 2011 with hourly precipitions



Appendix 1.9: Soil tension for the period April 2011-September 2011 with hourly precipitions



Appendix 1.10: Equation for the transformation of permittivity signals to soil volumetric water content (SVWC).

$$\varepsilon = A' \times SVWC + B' \leftrightarrow \varepsilon = 33.5 \times SVWC + 15.07 \rightarrow SVWC = \varepsilon / 33.5 - 15.07$$

Appendix 3.1: Detailed protocole for the analysis of glyphosate, AMPA and glufosinate by UPLC-MS/MS (in French)

DOSAGE DU GLYPHOSATE, DE L'ACIDE AMINOMETHYLPHOSPHONIQUE (AMPA) ET DU GLUFOSINATE PAR UPLC-MS/MS

Introduction :

Le glyphosate est un herbicide systémique de la famille des acides aminés de type phosphonométhyle, l'AMPA est son produit de dégradation principal et le glufosinate est un herbicide de contact de la même famille à la structure similaire. Afin de quantifier ces composés dans des échantillons naturels (eaux + sols), une méthode par chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS) a été développée basé sur le travail d'autrui (Ibanez et al. 2005, Hanke et al., 2008 et Tran-Thi et al., 2009). Avant tout, une étape de dérivation (substitution nucléophile avec du FMOC-Cl) de ces molécules polaires est nécessaire afin de les rendre apolaires. Suite à cette dérivation et étant mis en excès, du FMOC-Cl résiduel ainsi que son produit hydrolysé (FMOC-OH) pouvant péjorer la qualité de l'analyse sont à éliminer respectivement par filtration et purification avec un solvant polaire aprotique (dichlorométhane). Enfin, une étape de concentration par extraction sur phase solide (SPE) est souhaitable afin de pouvoir quantifier de faibles teneurs par LC-MS/MS. Du fait de leur caractère polaire, des phases inverses sont nécessaires que ce soit pour la SPE ou la LC.

Réactifs

- Standards :
    - Glyphosate (N° 45521 chez Sigma-Aldrich)
    - AMPA (N° 324817 chez Sigma-Aldrich)
    - Glufosinate-ammonium (N° 45520 chez Sigma-Aldrich)
    - Glyphosate 13C15N (Dr. Ehrenstorfer)
    - AMPA 13C15N (Dr. Ehrenstorfer)
- Acétonitrile, Méthanol, Dichlorométhane, H_2O milli-Q, HCl 6M, NaOH 6M, KOH 0.6M
- Acide formique 99% et 0.1%
- Pastille de KOH
- EDTA (CAS N° 60-00-4, N° ED chez S.-A.)
- Sodium tetraborate decahydrate (CAS N° 1303-96-4, N° 71999 chez S.-A.)
- FMOC-Cl (CAS N° 28920-43-6, N° 23186 chez S.-A.)
- Triethylamine (CAS N° 121-44-8, N° 17924 chez S.-A.)

Matériel :

- Une bouteille en HDPE et une en verre de 250ml par échantillon
- Micropipettes avec embouts
- Vaisselle de laboratoire habituel, (béchers, tubes de 10-15ml, pipettes pasteurs,…)
- pH-mètre
- Unité de filtration (Nalgène©) avec filtres 0.45um acétate de cellulose (ou cellulose régénérée)
- Cartouches SPE Strata-X (200mg, 6ml, Phenomenex©, N° 8B-S100-FCH chez Brechbuhler) avec capillaires (N° 57059 chez S.-A.)
- Système SPE 'off-line' (Supelco©)
- Bloc chauffant avec évaporateur N_2
- UPLC-MS/MS
- Vials avec bouchons pré-percés

Préparation des solutions stocks:

Solutions uniques non dérivées (~ 1 mg/ml) :

Peser 5.0mg dans un tube à centrifuger en PP de15ml et noter la masse exacte et ajouter 5ml d'un mélange 50/50 H2O/ACN et noter les masses. Le volume est ensuite recalculé avec la densité de 0.8915 pour H2O/ACN

Solutions mix non dérivées (~ 4 ug/ml) :
Une fois les trois solutions prête, peser 0.2ml de chaque solution dans un nouveau tube à centrifuger en PP de 50ml, noter les masses. Ajouter 50ml du mélange 50/50 H2O/ACN noter la masse.

Standard Interne (13C/15N) :
Solution aqueuse mix des deux analytes (glyphosate + AMPA) labellisés aux isotopes stables (^{13}C et ^{15}N) d'une concentration de 1000 ng/ml réalisée à partir des standards uniques de 100 ng/µl (dilution 100 !)

Tampon Borate-Na : 50mM dans H_2O → 1.91 g / 100ml

EDTA 0.5M : Peser 73g pour 500ml d'eau avec ajout de pastilles de KOH pour le dissoudre

9-enylmethylchloroformate (FMOC-Cl) : 50mg/ml dans acétonitrile → 1g /20ml

Préparation des éluants et des solutions de rinçage (le jour d'analyse):

A : Dans un cylindre gradué mesurer 400/600/800 ml d'eau milli-Q (selon le nombre d'échantillons, il faut compter 32min/ech ou 16min/injection) et transvaser dans une bouteille d'éluant pour UPLC. Puis ajouter 0.1% de TEA (soit 0.4/0.6/0.8ml), bien mélanger et ajouter de l'acide acétique glacial jusqu'à atteindre un pH d'environ 9.5.
B : Dans un cylindre gradué mesurer 400ml d'acétonitrile et transvaser dans une bouteille d'éluant pour UPLC.
A et B : Il faut mélanger 20ml de A dans le B et vice-versa.
Eluant A : 95% = H_2O + 0.1% triethylamine + acide acétique -> pH 9.5 ; 5% = Acétonitrile
Eluant B : 95% = Acétonitrile ; 5% = H_2O + 0.1% triethylamine + acide acétique -> pH 9.5

Préparer une solution de 80/20 eau milli-Q/Acétonitrile pour le weak needle wash / seal pump wash (au moins 400ml car il faut compter 3ml/injection!!) et une solution de 50/50 eau milli-Q/Acétonitrile pour le strong needle wash (il faut compter 0.5 ml/injection)

Mode opératoire :

Préparation des échantillons de sol	- Sur une balance analytique, peser 5g de l'échantillon (préalablement séché à 50°C durant 1h et tamisé à 2mm) dans un tube à centrifuger de 15 ml - Ajouter 10 ml de KOH 0.6M - Agiter pendant 30 min - Centrifuger à 4000 RPM pendant 30 min - Transvaser dans une bouteille en HDPE, peser et compléter à 80 g avec de l'eau MQ en notant bien les masses et continuer comme décrit ci-après
Préparation des échantillons d'eau	- Sur une balance analytique, peser 80g de l'échantillon dans une bouteille en HDPE (si l'échantillon risque d'être très concentré, une dilution à l'eau MQ est recommandée !)* - Ajouter 50µl de la solution labellisée aux isotopes stables (^{13}C et ^{15}N ; 1000ng/ml)* - Acidifier avec HCl 6M (jusqu'à pH1) puis attendre 1h. - Neutraliser avec NaOH/KOH 6M. * Attention à relever les masses !

Dérivation	- Ajouter 5ml de tampon Borate afin d'obtenir un pH de 9.2 et agiter - Ajouter 10ml d'acétonitrile (10% du volume) et bien agiter - Ajouter 600 µl de FMOC-Cl, bien agiter et laisser réagir 2h dans l'obscurité et à température ambiante (20-25°C) - Acidifier la solution à pH 3 en ajoutant 1ml d'acide formique 99% - Filtrer à 0.45um avec des filtres en acétate de cellulose ou en cellulose régénérée dans des bouteilles en verre (250ml) - Rincer la bouteille en HDPE avec 30ml d'eau milli-Q - Ajouter 70ml d'eau milli-Q - Ajouter 8ml d'EDTA, bien agiter et laisser reposer 5min
SPE :	Préparer le montage (cartouches avec nom + capillaires)
Conditionnement	- Injecter 5ml de méthanol (MEOH) puis 5ml d'acide formique 0.1%
Percolation	- Injecter les 200ml d'échantillon à l'aide du système SPE 'off-line' de type gouttes à gouttes - Sécher ensuite les cartouches durant ~ 30mn
Purification	- Injecter 3.5ml de dichlorométhane afin d'enlever les produits hydrolysés de la dérivation (FMOC-OH) - Sécher à nouveau les cartouches durant ~ 30mn Si l'analyse par LC-MS/MS est effectuée un autre jour, les cartouches SPE peuvent être congelées emballées dans du papier d'aluminium !
Elution	- Eluer avec 9ml de méthanol (MEOH) dans un tube en verre (10-15ml) adapté au bloc chauffant avec évaporateur N_2. - Réduire sous flux d'azote jusqu'à obtenir un volume de 0.25 ml et mettre en Vial avec au moins 50% d'éluant A1 (Vtot = 0.5ml)* * Peut varier en fonction de la concentration attendue !!!

Calibration

La calibration est une calibration interne réalisée avec 6 standards allant de 20 à 1000 ng/l, préparés exactement comme des échantillons naturels en dopant 80ml d'eau d'Evian© à l'aide de la solution mère « std mix » et de sa solution *fille** diluée 10x (ACN : eau = 1 :1) comme dans l'exemple si dessous :

Standard	**1**	**2**	**3**	**4**	**5**	**6**
Concentration théorique [ng/l]	**20**	**40**	**80**	**200**	**400**	**800**
Masse pipetée [g]	*0.150**	*0.300**	*0.600**	0.015	0.030	0.060
Volume de SI [µl]	50	50	50	50	50	50

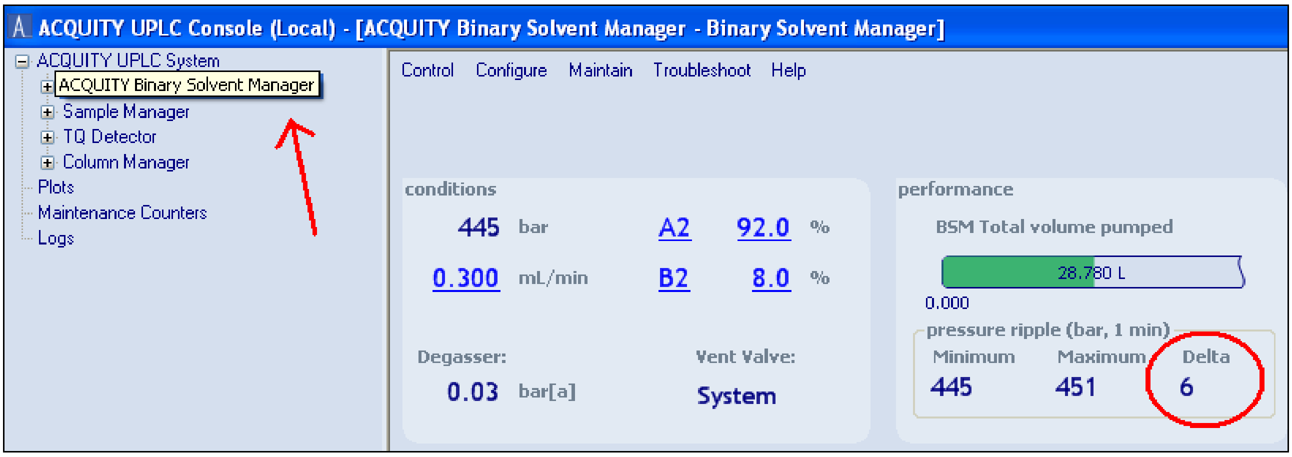
N.B. : Il ne sert à rien de calibrer plus élevé que 800 ng/l au risque de polluer le cône, il est préférable de diluer les échantillons que l'on imagine être très chargé !!

Analyse par LC - MS/MS :

Allumer les trois modules de l'UPLC, le triple quadripôle (MS/MS) n'est jamais éteint. Allumer l'ordinateur et ouvrir le programme « *MassLynx* », Appuyer sur « open projet » puis « Yes » puis « c:/MassLynx_Projet_GlyphosatePro », normalement il ouvre la dernière séquence d'analyse réalisée. Initialiser le système en cliquant sur « Intellistart » en bas à gauche de l'écran, ce qui ouvre l'« Acquity UPLC console » avec les paramètres des différents modules.

Revenir sous Masslynx et dans la colonne « Inlet File » de la séquence d'analyse → clic droit sur la methode « glyphosate_7 » puis edit. Dans la nouvelle fenêtre de l'« Inlet method » (c'est ici que l'on peut jouer avec les gradients, l'autosampler ou les conditions des colonnes), vérifier que les affichages d'état « ready » et « OK » soient au vert, puis cliquer sur« load method », le bouton en haut à droite de l'écran avec la flèche bleue. L'affichage d'état « pump on » doit se mettre au vert.

Sous « Acquity UPLC system », aller sous « Acquity Binary Solvent Manager », vérifier que la bonne colonne (n°2) ait été choisie, puis aller sous « Control » et « Prime A/B solvent » : sélectionner A1/B1 et mettre un cycle de 3 minutes. Une fois que la purge est terminée, le bouton « Load method » de « l'Inlet method » est à nouveau disponible, recliquer dessus (les éluants A1/B1 se remettent à 95/5 avec un débit de 0.3ml/min sous Acquity BSM) puis attendre que la stabilisation se fasse : le delta 1 min dans l'encadré « pressure ripple » de l'Acquity BSM devrait être de 0 ou 1 avant de lancer l'analyse !



445 bar
A2 92.0 %
0.300 mL/min
B2 8.0 %
Degasser: 0.03 bar[a]
Vent Valve: System
BSM Total volume pumped: 28.780 L
0.000
Minimum 445
Maximum 451
Delta 6

Finalement, sous Masslynx enregistrer la « sample list » sous un autre nom, rentrer le nom des échantillons, vérifier l'emplacement des vials et s'il n'y a pas deux fois le même nom. Avant de lancer, vérifier si le message « Only batch shutdown enabled » apparaît en bas à droite de la fenêtre si vous voulez que le système s'arrête après l'analyse!!

Pour lancer la séquence, cliquer sur le bouton « Start Run ».

Interprétations :

Dans un classeur Excel rentrer toutes les pesées des standards : m(Evian), m(Std), m(SI), m(Vial), m (Vial + Ech + Eluant A), et calculer les concentrations théoriques. Rentrer ces dernières, au préalable dédoublées, dans la « sample list » sous les colonnes Conc A, Conc B et Conc C correspondant au glyphosate, AMPA et glufosinate respectivement. Indiquer « standard » dans la colonne sample type pour les standards (…) et lancer l'interprétation (Process samples). Intégrer chaque pic de la même façon et exporter ensuite le tout (« all groups summary »). Coller sous excel et faire ensuite la moyenne des deux injections pour chaque échantillon.

Validation (limite de détection, taux de récupération ; Daouk et al., in press) :

LOQ = 10 ng/l (ratio S/B > 10) et LOD = 5 ng/l (ratio S/B > 3)

Taux de recuperation [%]:

Sample	Concentration [ng/l]	Glyphosate	AMPA	Glufosinate
Evian© water	30	109.1 (26.9)	86.3 (28.4)	88.0 (17.9)
Evian© water	130	101.1 (12.1)	105.1 (9.2)	104.0 (20.0)
River water	30	102.9 (133.8)	115.6 (13)	90.3 (47.8)
River water	130	115.9 (9.0)	133.1 (19.6)	109.8 (30.8)

Références :

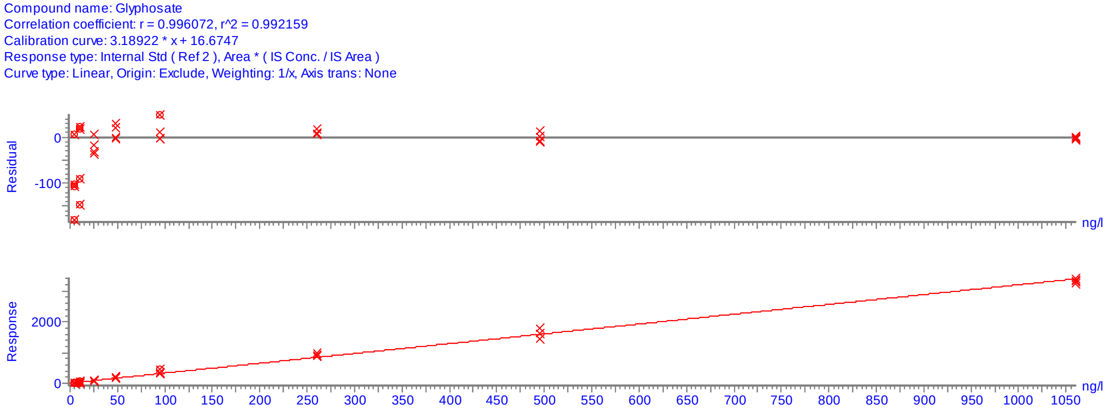
Daouk, S.; Grandjean, D.; Chèvre, N.; De Alencastro, L.F.; Pfeifer, H.-R. The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part I: Method validation in different water matrices. J. Environ. Sci. Health, B. **In press**.

Hanke, I., H. Singer, and J. Hollender. **2008**. Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. Analytical and Bioanalytical Chemistry 391:2265-2276.

Ibáñez, M., Ó.J. Pozo, J.V. Sancho, F.J. López, and F. Hernández. **2005**. Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry. Journal of Chromatography A 1081:145-155.

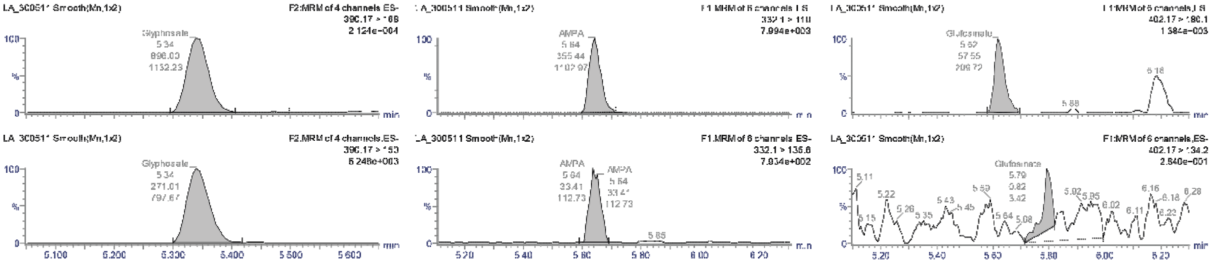
Tran-Thi, N.-T., N. Mazzella, B. Delest, and F. Delmas. **2009**. Développement et validation d'une méthode permettant le dosage du glyphosate et de l'AMPA dans les eaux de surface par HPLC-ESI-MS/MS. XXXIXème congrès. Groupe Français des Pesticides, Toulouse.

Exemple de calibration :

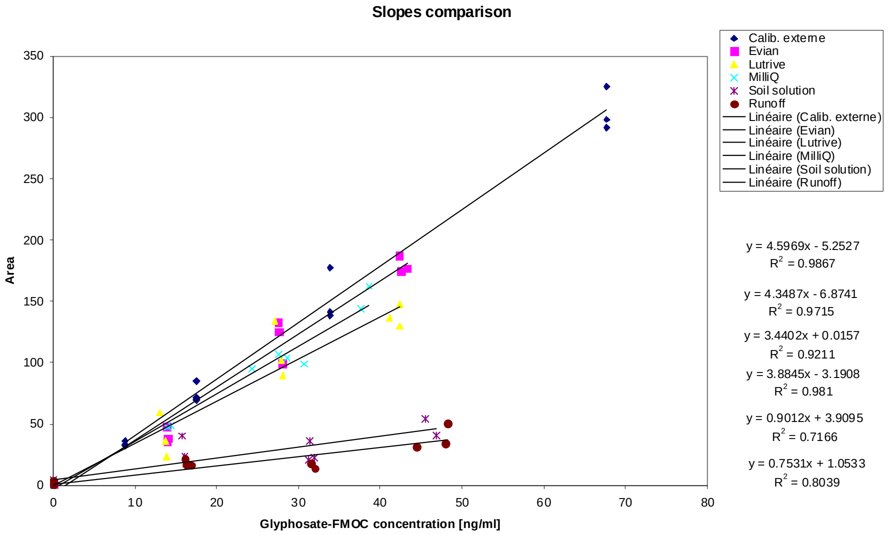


Compound name: Glyphosate
Correlation coefficient: r = 0.996072, r^2 = 0.992159
Calibration curve: 3.18922 * x + 16.6747
Response type: Internal Std (Ref 2), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

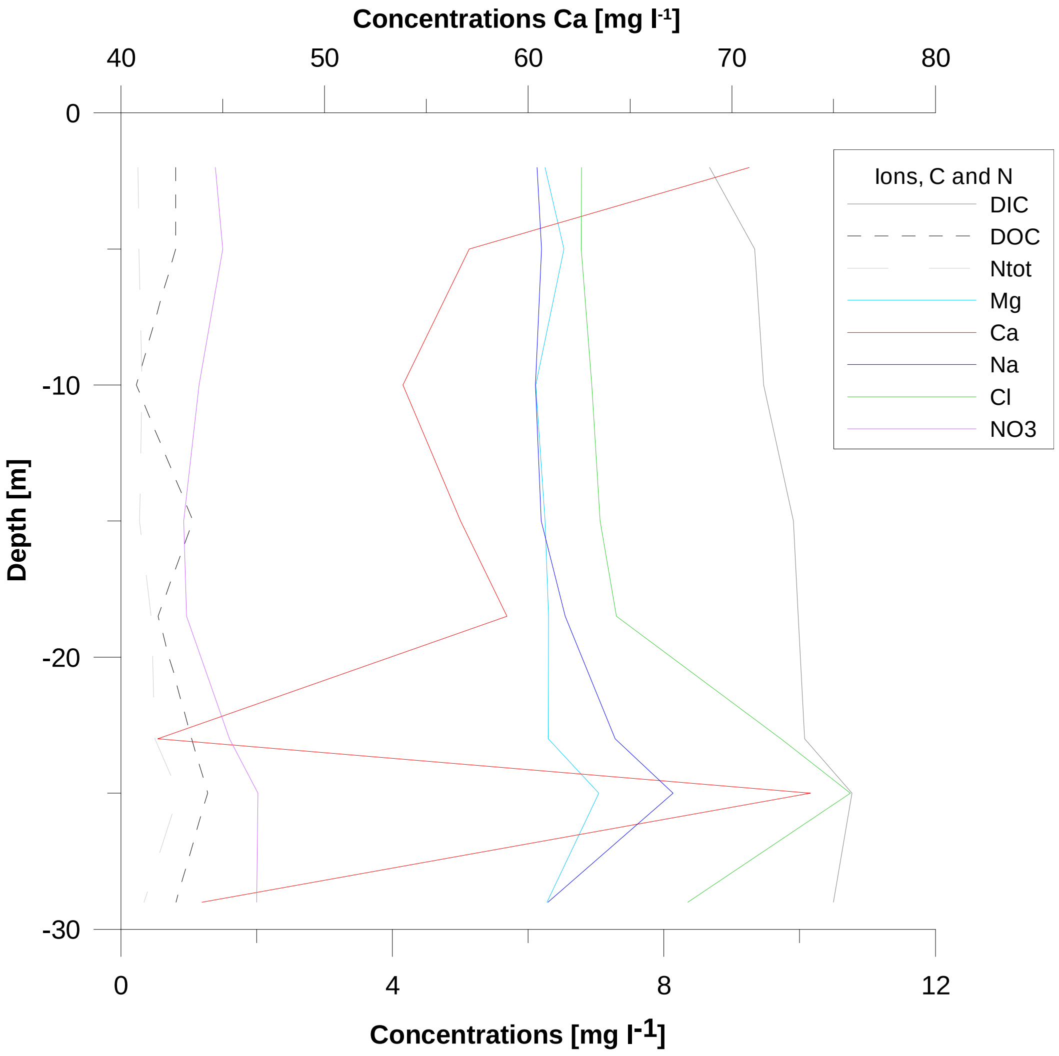
Exemple de chromatogrammes :



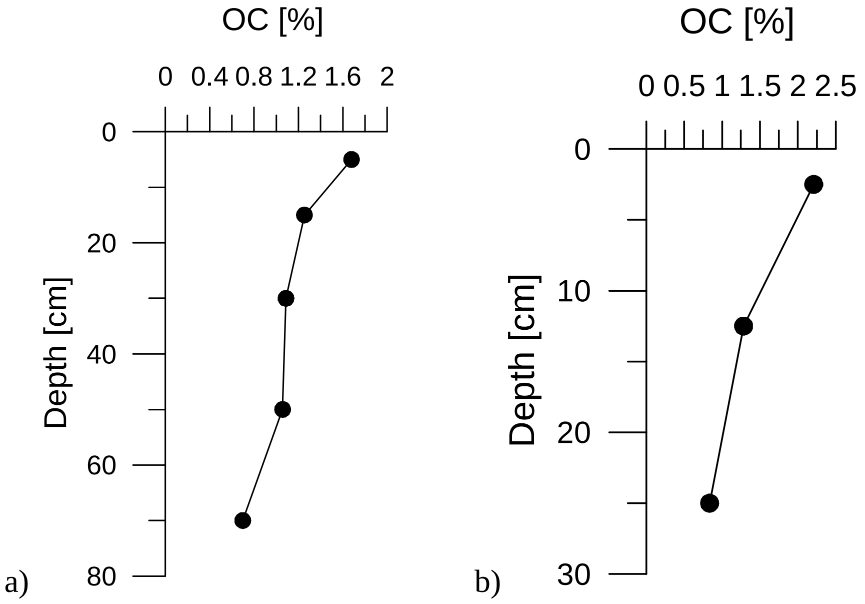
Appendix 3.2: Matrix effect for glyphosate in soil solution and runoff samples before normalization with internal standards



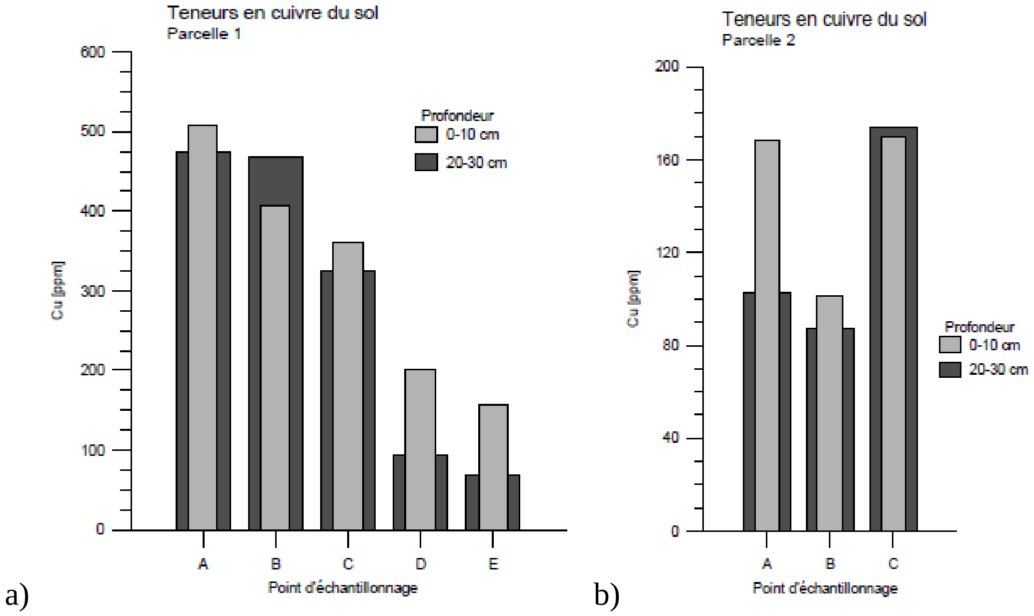
Appendix 3.3: Major ions in the Vidy bay profile, June 30, 2010



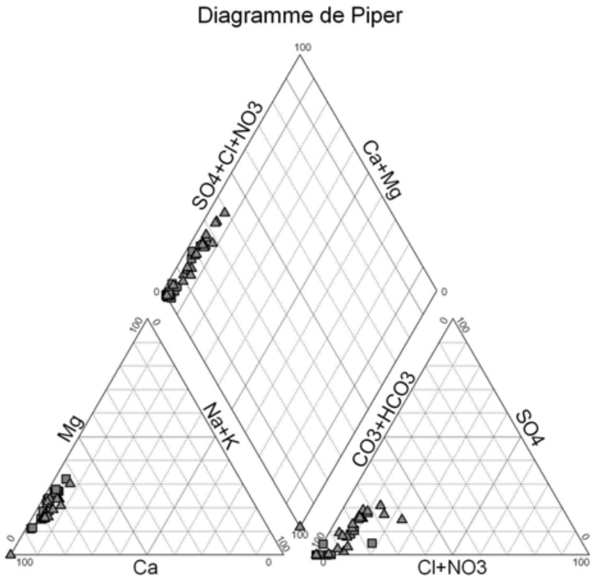
Appendix 4.1: Percentage of organic carbon (OC) in soils of a) parcel 1 and b) parcel two



Appendix 4.2: Copper concentrations in transects of a) parcel 1 and b) parcel 2



Appendix 4.3: Piper diagram representing major ions in soil water samples of parcel 1 (▲) and parcel 2 (■).



Appendix 4.4: Glyphosate and AMPA concentrations in 1) runoff samples and 2) soil water samples for the year a) 2010 and b) 2011

1)

a)

Sample	GLY [ug/l]	AMPA [ug/l]
C3_02_05	72.94	9.33
C3_0505	108.24	13.62
C3_1105	6.72	1.86
C3_02_06	4.07	8.63
C1_1506	4.31	1.12
C1_1706	1.31	0.14
C3_1706	1.44	7.03
C1_0607	0.77	8.79
C3_0907	0.17	2.82
C1_0408	0.40	1.87
C3_05_08	1.18	2.90
C3_1508	0.62	0.13
C3_2708	0.20	1.74
C3_0709	0.11	2.42

b)

Date	PP [mm]	GLY [µg/l]	AMPA [µg/l]
04.19.11	0	106.6	13.6
04.26.11	0	19.2	8.2
05.20.11	0	12.8	9.9
06.01.11	6.1	18.6	2.6
06.10.11	0.2	2.2	2.6
06.18.11	28.3	94.9	9.1
06.23.11	0	7.1	5.4
07.29.11	0	5.5	1.1

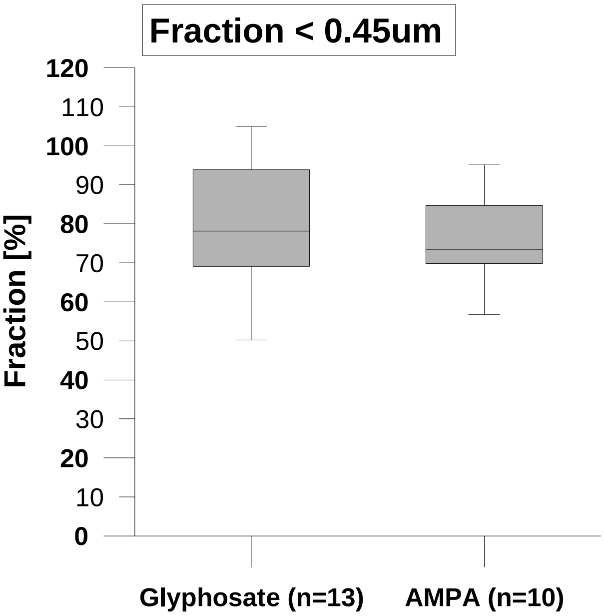
2) a)

Date	Parcel	Depth	GLY [ng/l]	AMPA [ng/l]
05.11.10	1	40	144	68
05.11.10	1	60	16	11
05.11.10	1	80	19	31
05.12.10	1	20	14	30
05.12.10	1	40	29	34
05.12.10	1	60	8	30
05.12.10	1	80	54	130
05.18.10	1	20	<LOD	29
05.18.10	1	40	47	46
05.18.10	1	60	84	345
05.18.10	1	80	18	49
05.26.10	1	20	10	33
05.26.10	1	40	26	39
05.26.10	1	60	59	101
05.26.10	1	80	<LOD	52
05.27.10	1	20	247	0
05.27.10	1	40	12	0
05.27.10	1	60	25	89
05.27.10	1	80	<LOD	66
06.02.10	1	20	<LOD	61
06.02.10	1	40	22	120
06.02.10	1	60	<LOD	30
06.02.10	1	80	<LOD	24
06.11.10	1	20	<LOD	15
06.11.10	1	40	9	24
06.11.10	1	60	<LOD	30
06.11.10	1	80	<LOD	27
06.15.10	1	20	786	1697
06.15.10	1	40	13	96
06.15.10	1	60	20	n.d.
06.15.10	1	80	8	80
06.17.10	1	20	151	877
06.17.10	1	40	6	58
06.17.10	1	60	11	121
06.17.10	1	80	10	7
07.09.10	1	40	25	83
07.09.10	1	60	12	7
08.05.10	1	60	9	0
08.13.10	1	20	34	109
08.13.10	1	40	20	120
08.13.10	1	60	16	90
08.13.10	1	80	10	18
08.15.10	1	40	9	9
08.15.10	1	60	10	10
08.15.10	1	80	8	11
08.27.10	1	20	35	239
08.27.10	1	40	22	120
08.27.10	1	60	15	186
08.27.10	1	80	14	209
09.07.10	1	20	21	133
09.07.10	1	40	50	113
09.07.10	1	60	12	114
09.07.10	1	80	6	135
04.22.10	2	40	55	14
04.22.10	2	80	2171	185
05.05.10	2	80	567	98
05.11.10	2	20	24	19
05.11.10	2	60	15	12
05.11.10	2	80	30	80
05.18.10	2	20	<LOD	21
05.18.10	2	60	42	91
05.18.10	2	80	26	17
05.26.10	2	60	n.d.	125
05.27.10	2	20	n.d.	105
05.27.10	2	60	n.d.	87
05.27.10	2	80	n.d.	76
06.02.10	2	20	283	524
06.02.10	2	60	<LOD	60
06.02.10	2	80	151	161
06.11.10	2	60	<LOD	30
06.11.10	2	80	13	345
06.15.10	2	20	275	543
06.15.10	2	60	n.d.	151
06.17.10	2	20	134	519
06.17.10	2	40	29	150
06.17.10	2	60	25	83
06.17.10	2	80	639	562
08.05.10	2	20	13	n.d.
08.05.10	2	40	9	n.d.
08.13.10	2	20	38	120
08.13.10	2	60	10	<LOD
08.15.10	2	40	11	27
08.15.10	2	60	10	<LOD
08.27.10	2	20	42	78
08.27.10	2	40	36	140
08.27.10	2	60	14	14
08.27.10	2	80	266	146
09.07.10	2	20	34	48
09.07.10	2	40	13	94
09.07.10	2	60	9	19

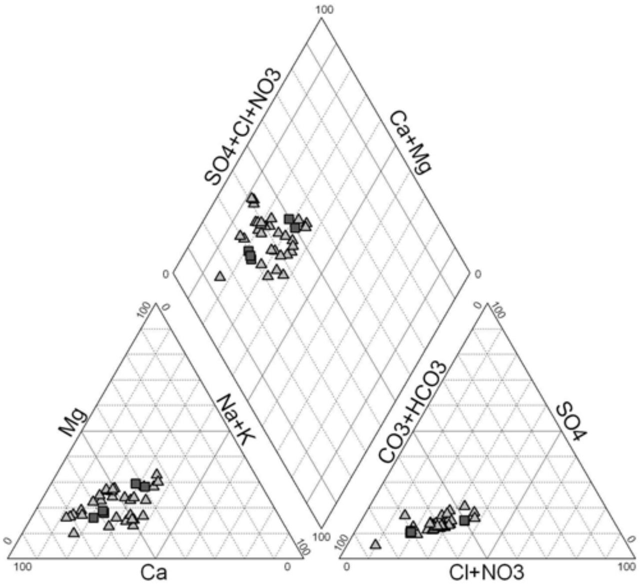
b)

Date	Parcel	Depth	GLY [ng/l]	AMPA [ng/l]
04.19.11	1	20	2032	1754
04.19.11	1	40	187	125
04.19.11	1	60	702	239
04.19.11	1	80	319	282
04.29.11	1	40	305	73
04.29.11	1	60	341	244
04.29.11	1	80	164	95
05.04.11	1	40	156	87
05.11.11	1	60	128	170
05.13.11	1	60	425	359
06.01.11	1	40	139	107
06.07.11	1	20	582	629
06.07.11	1	60	190	256
06.07.11	1	80	48	71
06.10.11	1	20	516	365
06.10.11	1	40	343	206
06.10.11	1	80	98	208
06.15.11	1	40	102	149
06.15.11	1	60	163	225
06.15.11	1	80	46	134
06.18.11	1	20	101	113
06.18.11	1	60	188	233
06.20.11	1	80	409	381
06.23.11	1	20	69	229
06.23.11	1	60	406	500
04.19.11	2	80	326	110
04.26.11	2	80	201	133
06.10.11	2	20	70	55
06.10.11	2	40	272	292
06.10.11	2	60	64	178
06.18.11	2	40	286	334
06.20.11	2	80	215	76
06.23.11	2	80	65	56
07.22.11	2	60	33	30
07.22.11	2	80	57	69
07.29.11	2	40	12230	516
07.29.11	2	60	59	47
07.29.11	2	80	98	57
08.12.11	2	40	2949	6583
08.12.11	2	60	34	57
08.12.11	2	80	38	74
08.24.11	2	40	1212	4024
08.24.11	2	60	31	71

Appendix 4.5: Proportion of glyphosate and AMPA in the dissolved (<0.45µm) fraction of runoff samples



Appendix 5.1: Piper diagram of water samples from the Lutrive River sampled at the downstream (▲) and upstream sites (■).



Appendix 5.2: Glyphosate and AMPA concentrations in water samples from Lutrive River at the a) downstream and b) upstream sites, with daily precipitations.

a)

Date	Daily P [mm]	Gly	AMPA
04.18.11	0	20	n.d.
04.19.11	0	127	33
04.20.11	0	12	14
04.21.11	0	20	10
04.22.11	0	14	15
04.23.11	2.5	4976	31
04.24.11	0.3	113	<LOD
05.12.11	10.2	3655	471
05.14.11	14.7	3134	496
05.22.11	9.3	1187	416
05.28.11	0	72	622
05.30.11	2	113	118
05.31.11	15.2	1812	282
06.01.11	6.1	1119	197
06.03.11	0	64	96
06.05.11	10.8	91	22
06.06.11	4.1	1497	210
06.07.11	4.2	42	46
06.08.11	25.8	325	181
06.09.11	0	48	90
06.15.11	0	17	26
06.17.11	36.2	3425	434
06.18.11	28.3	101	125
06.19.11	0.2	43	16
06.20.11	0	17	28
06.22.11	22.5	24	28
06.23.11	0	15	41
07.23.11	7.8	61	47
07.24.11	0.4	198	163
08.07.11	5.3	3347	370
09.02.11	0	1317	441
09.03.11	6	1418	277

b)

Date	Daily P [mm]	GLY	AMPA
26.04.2011	0	50	33
12.05.2011	10.2	1302	92
24.05.2011	0	1070	142
30.05.2011	2	15	26
02.06.2011	0	10	38
07.06.2011	4.2	6	20
10.06.2011	0.2	185	91
12.06.2011	0	20	18
07.08.2011	5.3	1000	266
15.08.2011	0	589	305
26.08.2011	26.5	633	140
27.08.2011	4.8	2621	310
01.09.2011	14.8	330	134
03.09.2011	6	511	204

Appendix 5.3: Glyphosate and AMPA concentrations in a) drainage and b) stormwater sanples

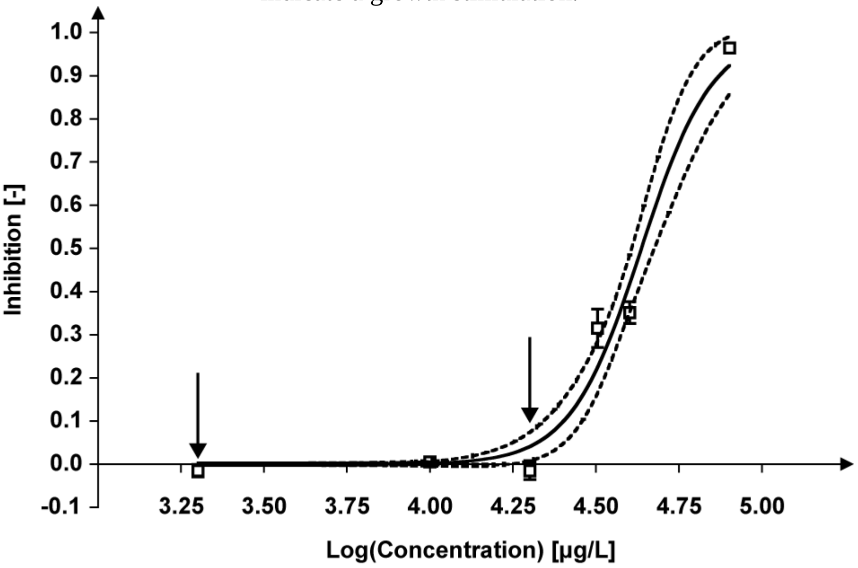
Date	Daily P [mm]	GLY [ng/l]	AMPA [ng/l]
04.19.11	0	78	161
04.26.11	0	60	104
04.29.11	1.1	339	78
05.04.11	0	2057	351
05.20.11	0	640	91
06.01.11	6.1	325	112
06.07.11	4.2	152	85
06.10.11	0.2	169	73
06.15.11	0	192	122
06.18.11	28.3	190	110
06.20.11	0	940	593
06.23.11	0	126	112

a)

Date	Daily P [mm]	GLY [ng/l]	AMPA [ng/l]
06.01.11	6.1	2997	2713
06.07.11	4.2	231	1099
06.10.11	0.2	53	546
06.15.11	0	17	263
06.18.11	28.3	240	1108
06.20.11	0	22	169

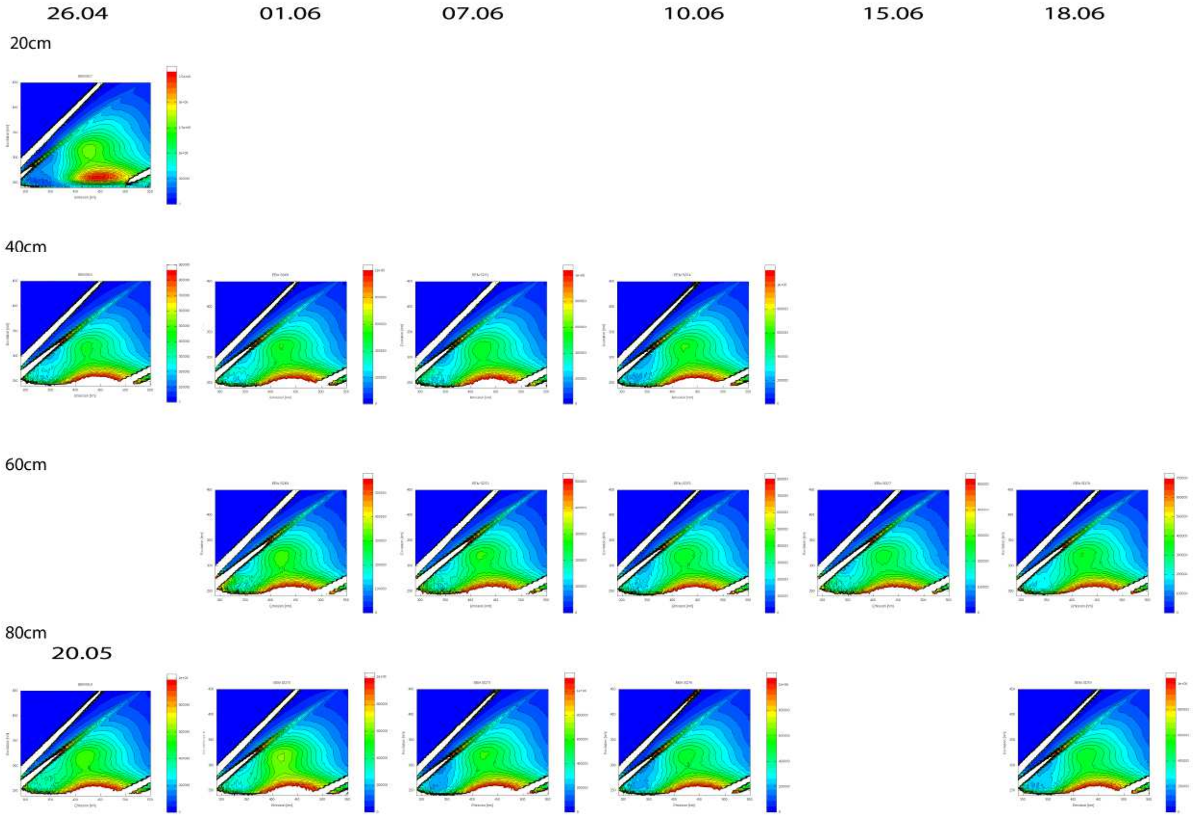
b)

Appendix 5.4: Dose response curve for glyphosate (black curve) with its confidence interval (dotted curves). Tested concentrations in the laboratory were illustrated by white squares with their standard deviation. Arrows indicate a growth stimulation.

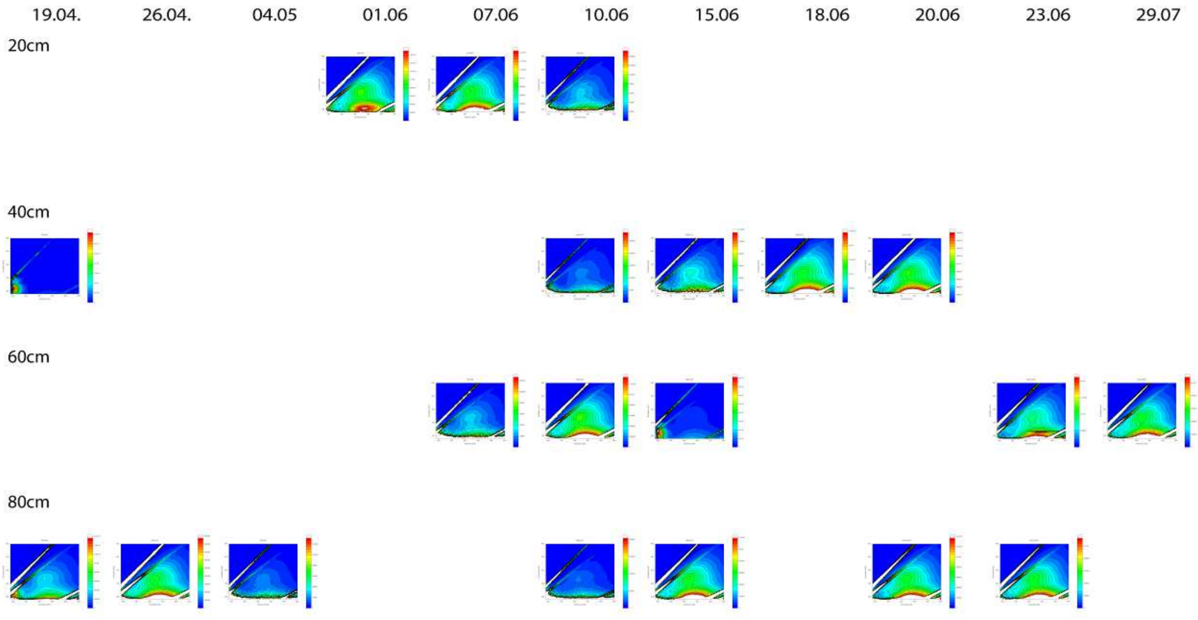


Appendix 6.1: Excitation-Emission Matrices (EEM) obtained by UV/Vis fluorescence spectroscopy from soil water samples from parcels 1 and 2.

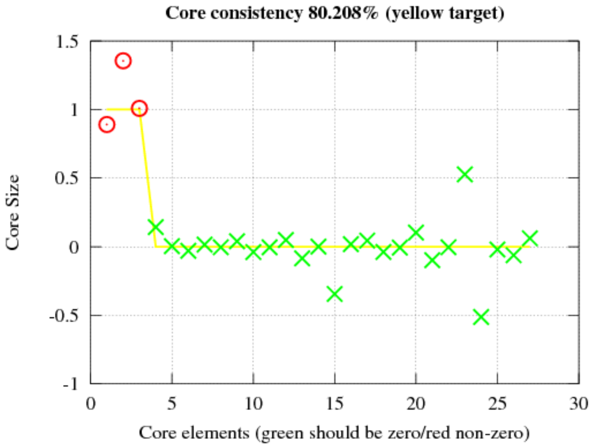
Parcel 1



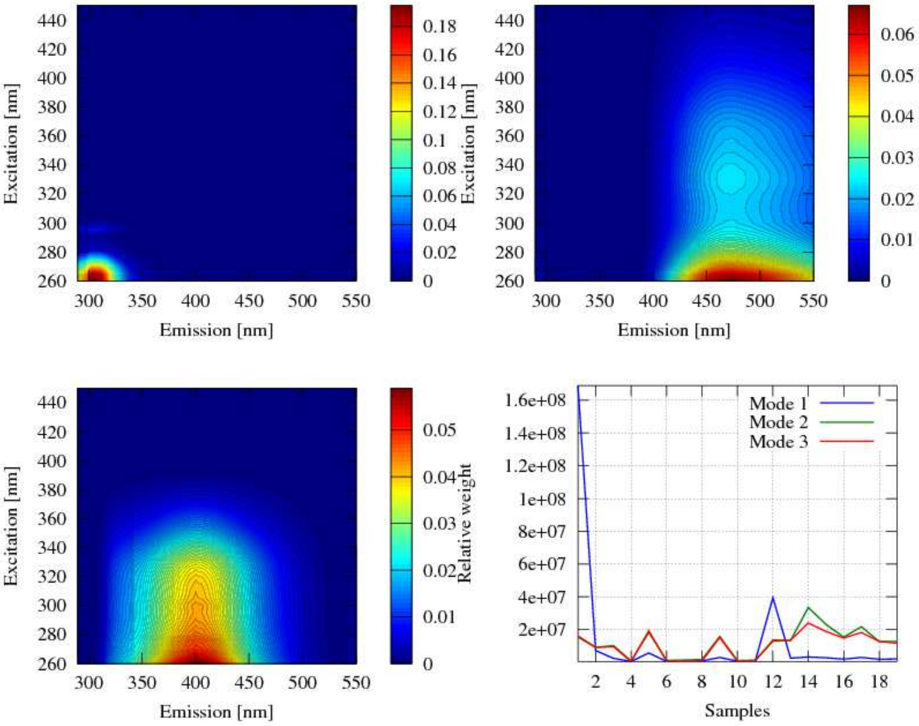
Parcel 2

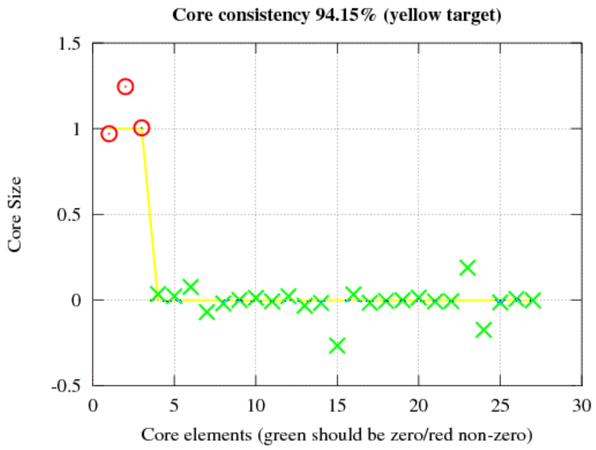


Appendix 6.2: Corcondia for the PARAFAC of all soil water samples with three fluorophores

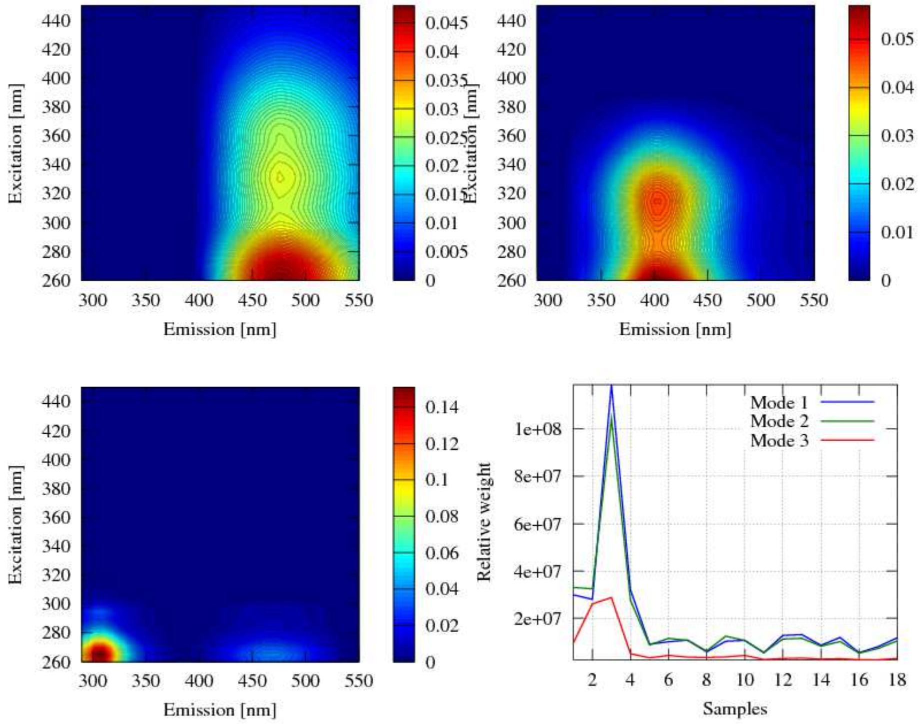


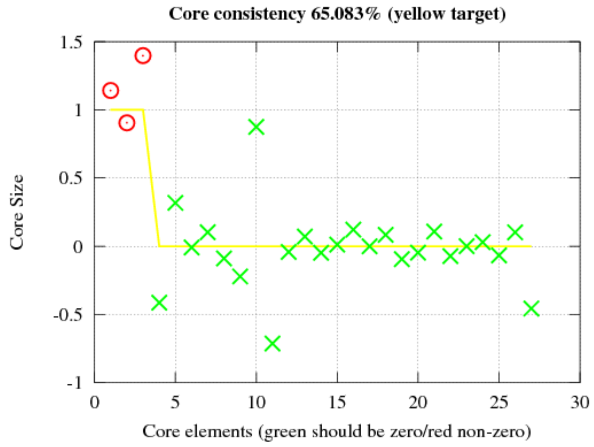
Appendix 6.3: PARAFAC of soil water samples of parcel 1 with three components with corcondia





Appendix 6.4: PARAFAC of soil water samples of parcel 2 with three components with corcondia



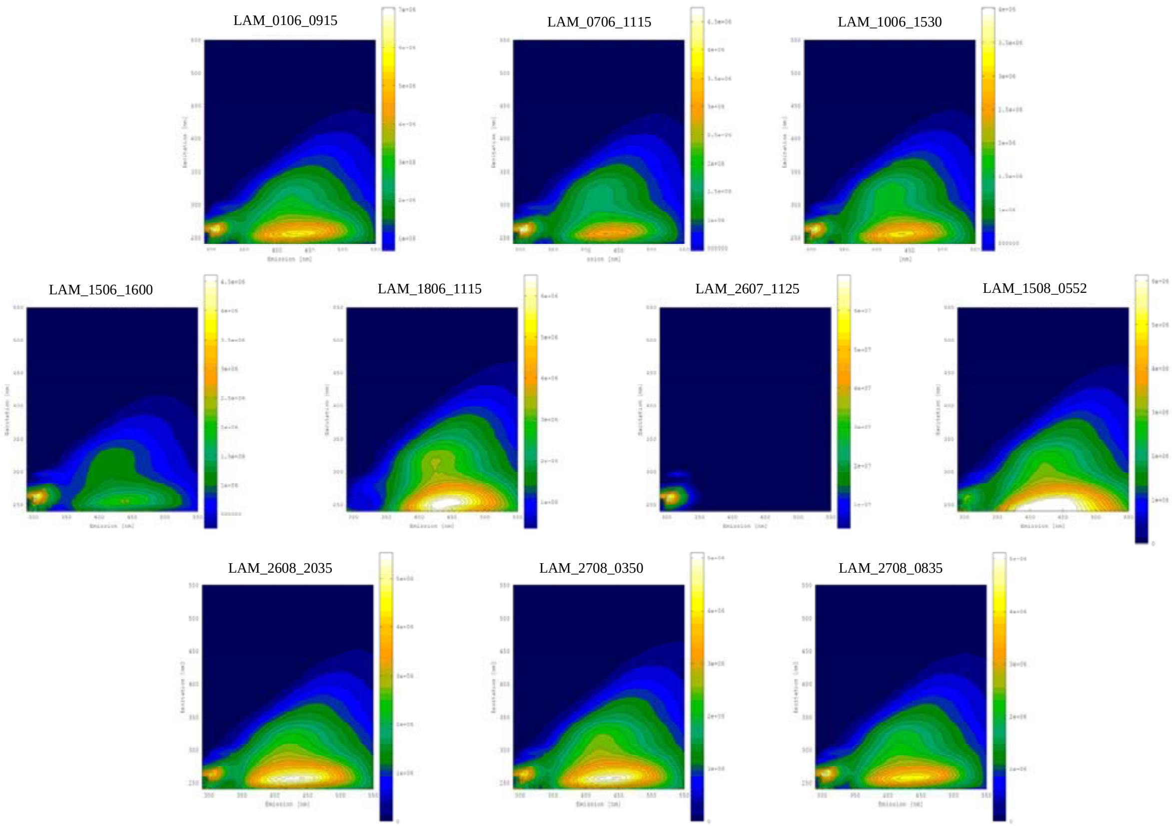


Appendix 6.5: Corresponding names of samples in Fig. 6.3d

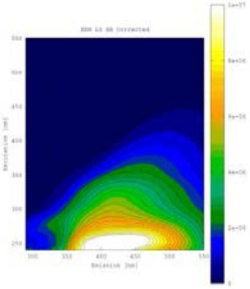
No	**Sample**	**Parcel**	**Depth**	**Date**
1	SD124	1	80	19.04.2011
2	SD127	1	40	04.05.2011
3	SD82	1	20	10.06.2011
4	B2	2	40	19.04.2011
5	B3	2	80	19.04.2011
6	B4	2	80	26.04.2011
7	B5	2	80	04.05.2011
8	B6	2	20	01.06.2011
9	B8	2	60	07.06.2011
10	B9	2	20	10.06.2011
11	B10	2	40	10.06.2011
12	B11	2	60	10.06.2011
13	B12	2	80	10.06.2011
14	B13	2	40	15.06.2011
15	B14	2	60	15.06.2011
16	B15	2	80	15.06.2011
17	B16	2	40	18.06.2011
18	B17	1	20	26.04.2011
19	B18	1	40	26.04.2011
20	B19	1	80	20.05.2011
21	SD48	2	40	20.06.2011
22	SD49	2	80	20.06.2011
23	SD50	2	60	23.06.2011
24	SD51	2	80	23.06.2011
25	SD52	2	60	29.07.2011
26	SD68	1	40	01.06.2011
27	SD69	1	60	01.06.2011
28	SD70	1	80	01.06.2011
29	SD71	1	40	07.06.2011
30	SD72	1	60	07.06.2011
31	SD73	1	80	07.06.2011
32	SD74	1	40	10.06.2011
33	SD75	1	60	10.06.2011
34	SD76	1	80	10.06.2011
35	SD77	1	60	15.06.2011
36	SD78	1	60	18.06.2011
37	SD79	1	80	18.06.2011

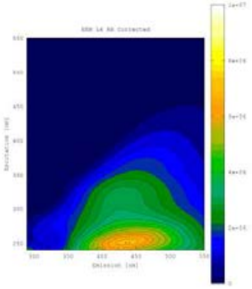
Appendix 6.6: Excitation-Emission Matrices (EEM) obtained by UV/Vis fluorescence spectroscopy from Lutrive River samples.

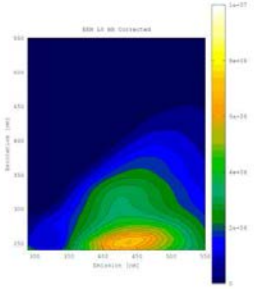
<u>Upstream samples (Note the different scales)</u>

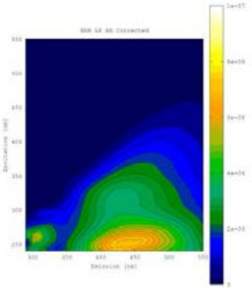


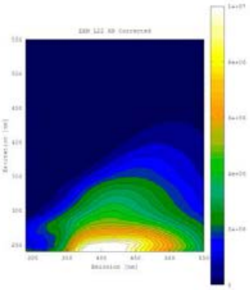
Downstream samples (Note the different scales)

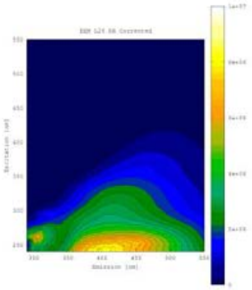


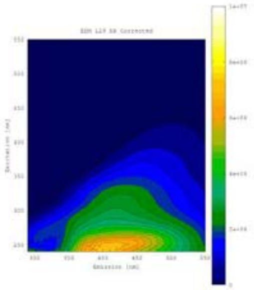


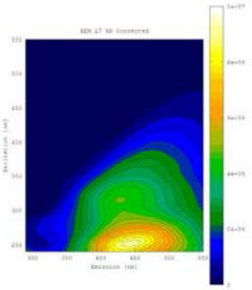


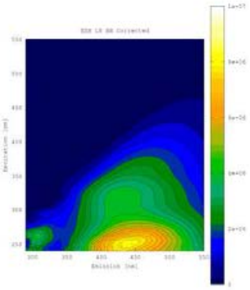


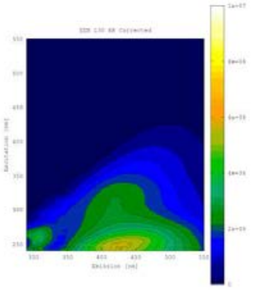


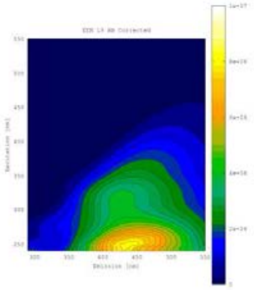


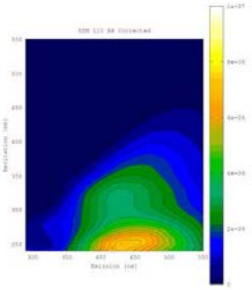


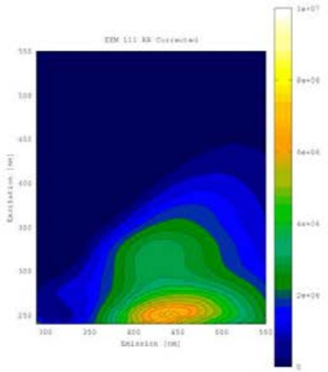


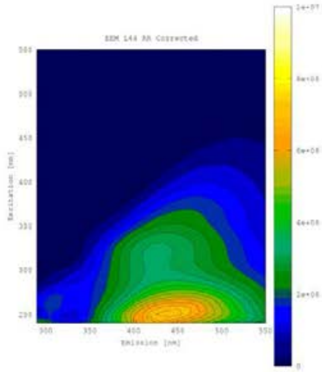


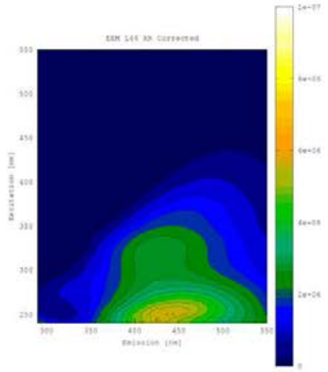


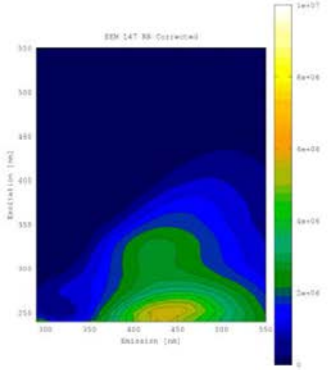


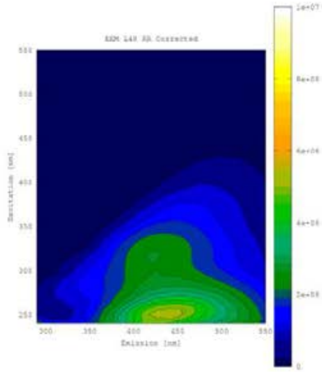


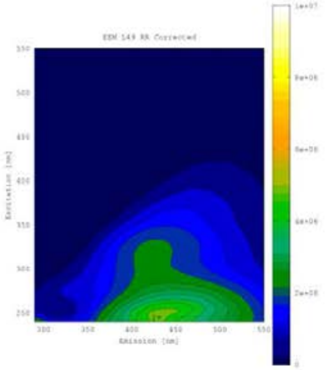


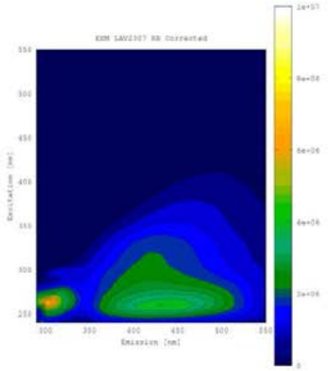


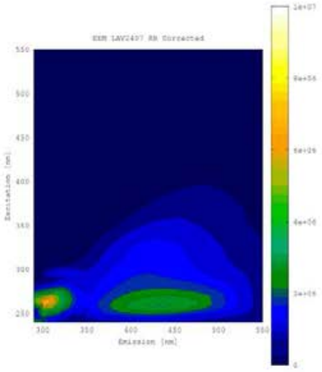


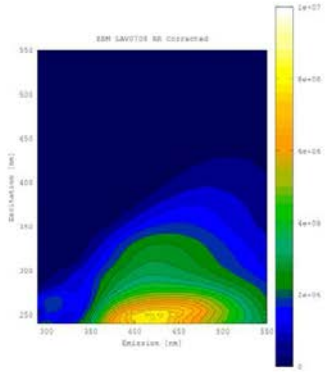




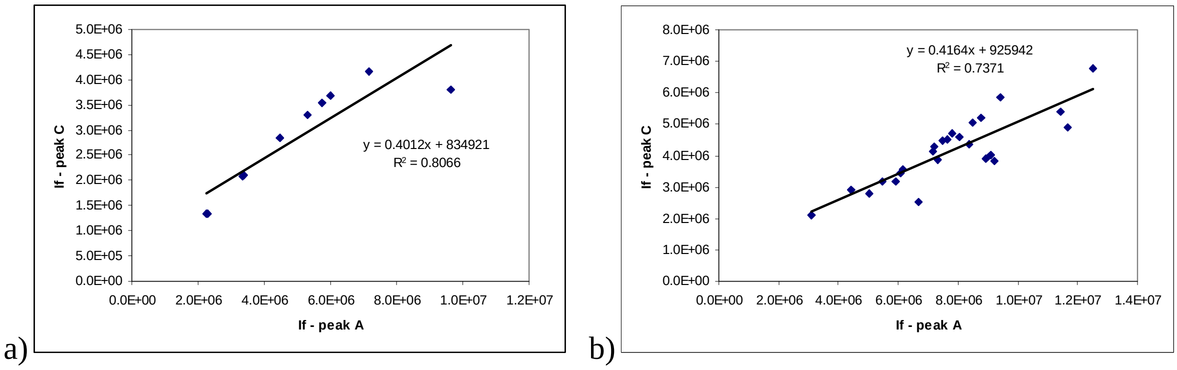




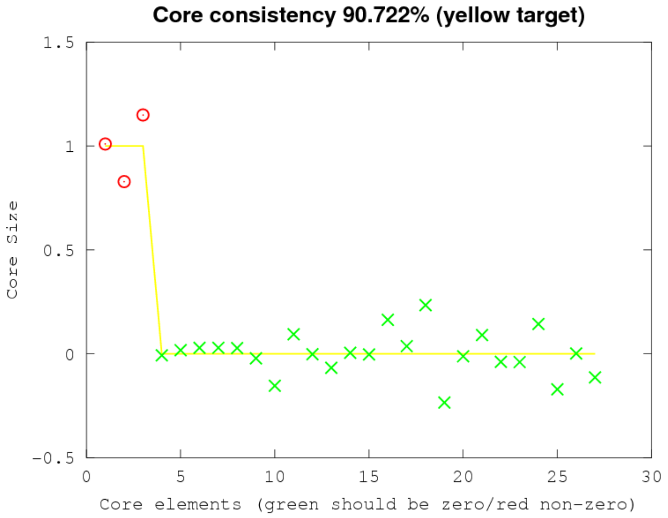




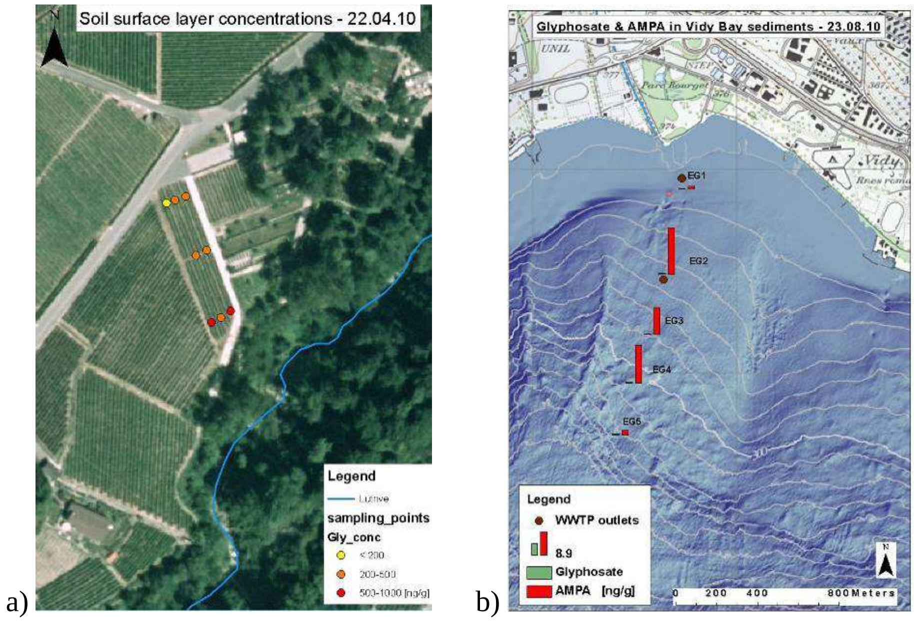
Appendix 6.7: Correlation between fluorescence intensities of peak A and C in Lutrive samples for a) upstream and b) downstream site, with equations and regression coefficients (R^2)



Appendix 6.8: Corcondia for the PARAFAC decomposition of all river water samples with three components



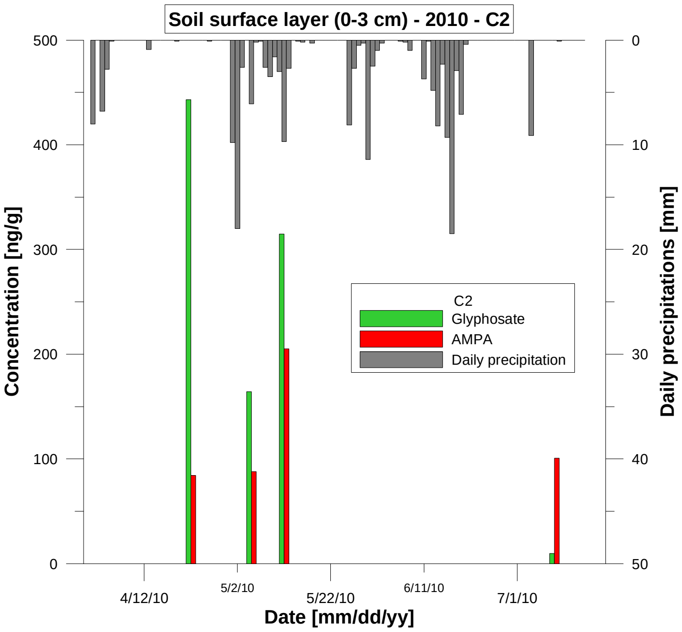
Appendix 7.1: First results of glyphosate and AMPA analysis in solid samples: a) soil and b) sediments



Appendix 7.2: Mean recovery rates [%] and (standard deviation) of spiked soil samples with 10ng/g of glyphosate, AMPA and glufosinate and extracted with 0.6M KOH and analyzed according to Appendix 3.1.

Soils (10 ng/g; n=5)	Glyphosate	AMPA	Glufosinate
Non corrected	0.58 (0.15)	1.26 (0.2)	0.98 (0.22)
Corrected with IS	1.21 (0.09)	2.47 (0.46)	1.9 (0.46)

Appendix 7.3: Concentrations in a few soil samples in 2010



Appendix 7.4: Total suspended solids (TSS) and size distribution during flood events in the Lutrive River

Date	Houre	Station	TSS [mg/l]	< 2µm	2- 20µm	20- 50µm	50- 200µm	200- 2000µm
08.07.11	00:05	Upstream	92.4	4.8	51.5	29.1	12.7	1.9
08.07.11	01:05	Upstream	52.0	3.8	48.1	33.4	13.3	1.4
08.07.11	01:25	Upstream	46.4	3.6	45.8	29.9	18.4	2.4
08.07.11	11:25	Upstream	10.0	0.0	41.3	30.1	20.2	8.4
08.15.11	04:40	Upstream	102.4	3.2	42.6	32.1	19.7	2.4
08.15.11	05:50	Upstream	36.4	3.2	45.4	34.1	15.7	1.7
08.15.11	06:00	Upstream	34.0	3.4	46.7	33.2	15.3	1.4
08.15.11	06:10	Upstream	29.6	3.5	48.8	33.3	13.6	0.8
08.15.11	06:15	Upstream	30.8	3.6	49.3	33.0	13.4	0.7
07.23.11	23:10	Downstream	52.4	3.3	39.4	34.9	19.1	3.4
07.24.11	01:10	Downstream	87.2	5.9	55.0	25.3	12.2	1.5
07.24.11	01:25	Downstream	71.2	6.5	58.2	23.6	10.7	1.0
07.24.11	01:35	Downstream	71.6	7.3	60.2	22.0	9.8	0.7
07.24.11	02:10	Downstream	49.6	5.3	56.5	26.2	11.0	1.0
08.07.11	01:05	Downstream	397.0	6.2	55.4	23.8	12.4	2.2
08.07.11	01:10	Downstream	162.0	5.0	47.2	27.8	16.8	3.2
08.07.11	02:05	Downstream	134.0	6.8	58.1	23.5	10.9	0.6
08.07.11	03:05	Downstream	98.0	4.1	43.6	30.3	18.9	3.1
08.07.11	07:40	Downstream	45.0	3.7	49.5	33.1	13.5	0.2
08.24.11	17:25	Downstream	n.a.	1.3	18.1	32.5	41.8	6.2
08.26.11	21:05	Downstream	72.0	5.4	49.3	26.6	16.4	2.3
08.26.11	21:15	Downstream	82.0	5.9	52.6	25.2	14.0	2.3
08.26.11	21:20	Downstream	31.3	5.6	53.8	25.5	13.7	1.4
08.27.11	03:40	Downstream	49.3	5.2	54.8	25.4	13.0	1.6
08.27.11	17:25	Downstream	n.a.	3.6	41.9	27.8	24.6	2.1

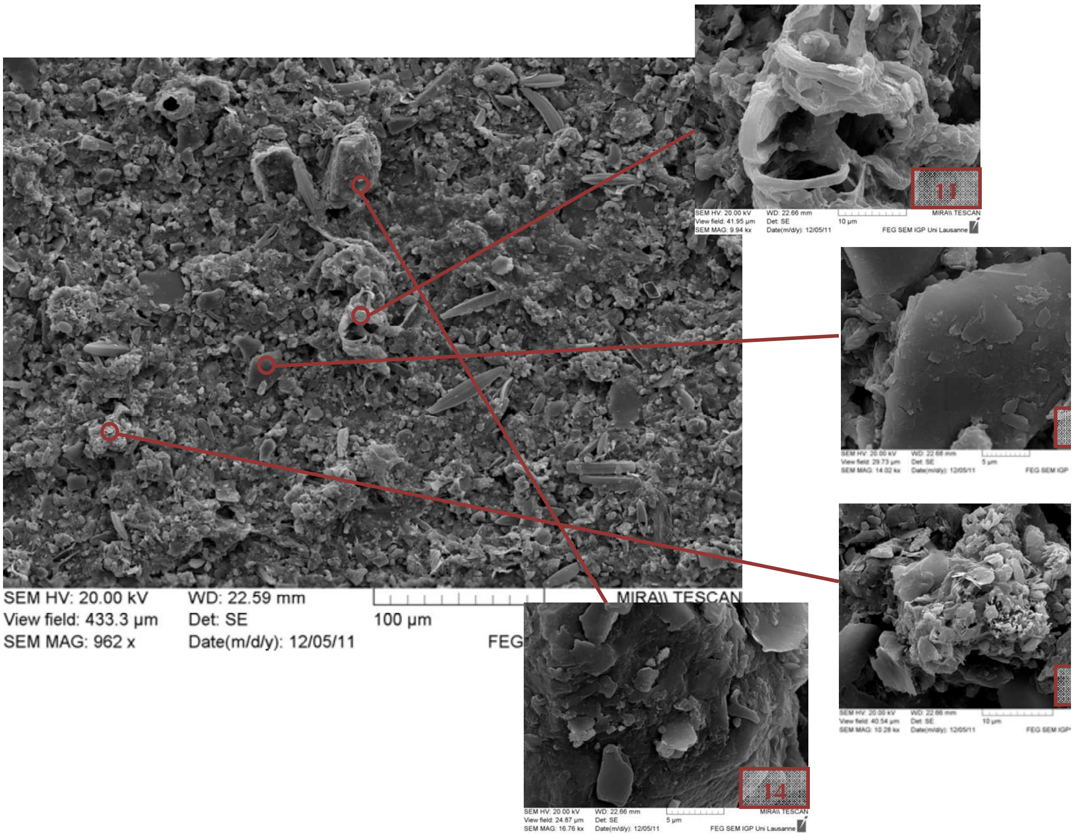
Appendix 7.5: X-ray diffraction analysis of suspended solids on 1.2um cellulose filters

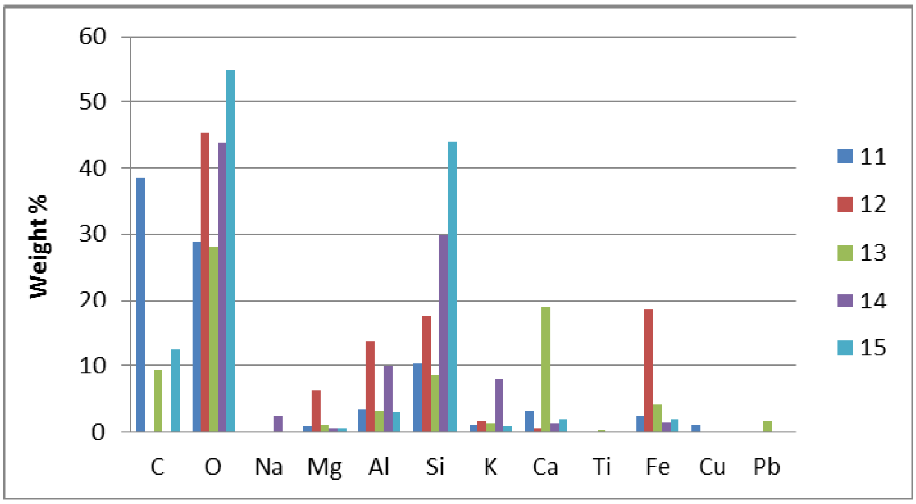
Samples	Phyllosilicates	Quartz	K-Feldspar	Na-Plagioclase	Calcite	Dolomite	Goethite	Unquant.
SD8 FT	35.9	11.2	2.6	3.8	10.8	2.0	3.2	30.5
SD6 FT	33.7	14.4	2.3	3.4	16.7	2.1	0.0	27.4
LAV FT	29.5	11.4	2.2	2.9	6.9	1.4	3.2	42.5
LAM FT	23.7	8.1	2.5	1.8	10.5	1.2	0.0	52.3
CL FT	29.9	4.9	1.9	2.1	4.4	1.2	4.1	51.6
36 FT	28.0	4.9	2.5	1.5	7.9	1.6	4.4	49.4

Appendix 7.6: Scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) analysis of suspended solids on cellulose filters of different size

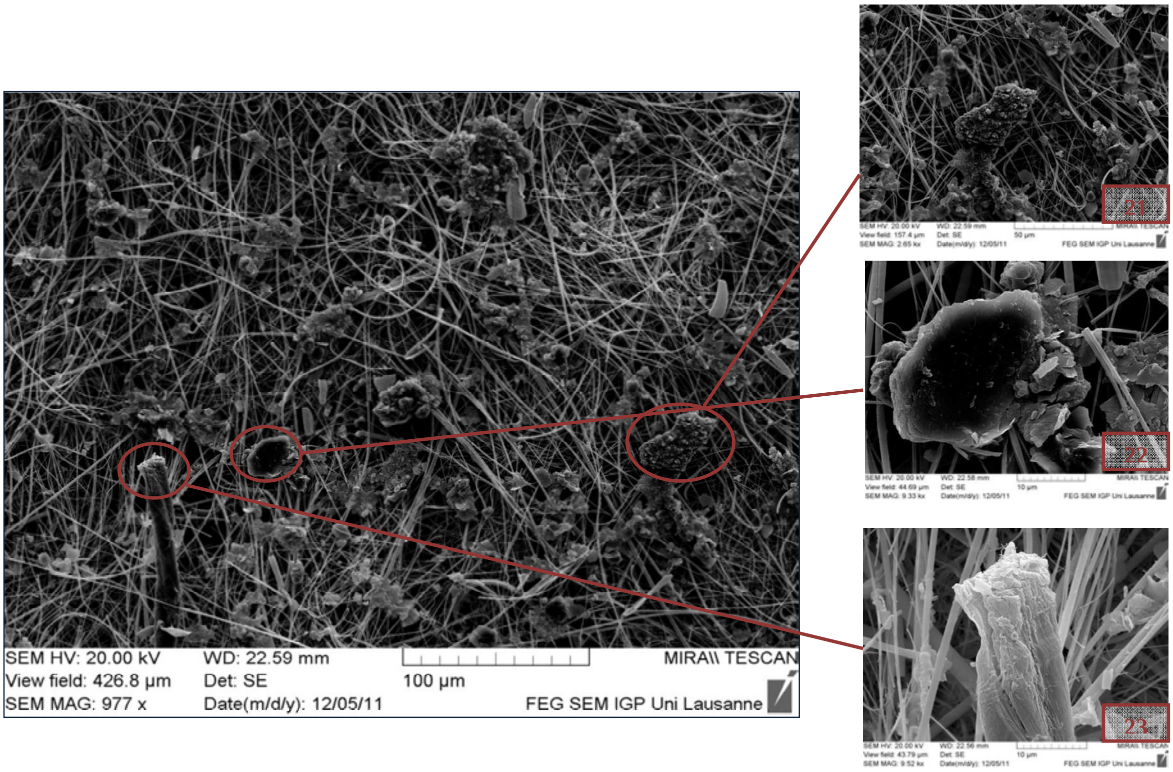
Sample	Filter (µm)	Section	Characterization
1	0.45	11	Calcium carbonate (calcite)
		12	phyllosilicate/Biotite (black mica)
		13	Compound with calcium carbonate
		14	Potassium Feldspar
		15	Quartz
2	1.2	21	Calcium carbonate (calcite)
		22	Sodium Feldspar
		23	Organic
		24	Alkali feldspar
3	1.7	31	Biotite (black mica)
		32	Organic
		33	Alkali feldspar and Organic (diatom)
		34	Biotite (black mica)
		35	Calcium carbonate (calcite)
		36	Diatom
4 (Downstream)	1.2	41	Biotite (black mica)
		42	Biotite (black mica) and Calcium carbonate (calcite)
		43	Mixture
5(upstream)	1.2	51	Biotite (black mica) and Calcium carbonate (calcite)
		52	Biotite (black mica) and Calcium carbonate (calcite)
		53	Quartz and Calcium carbonate (calcite)
		54	Quartz
		55	Calcite and Biotite (black mica)
		56	Calcium carbonate (calcite) and quartz

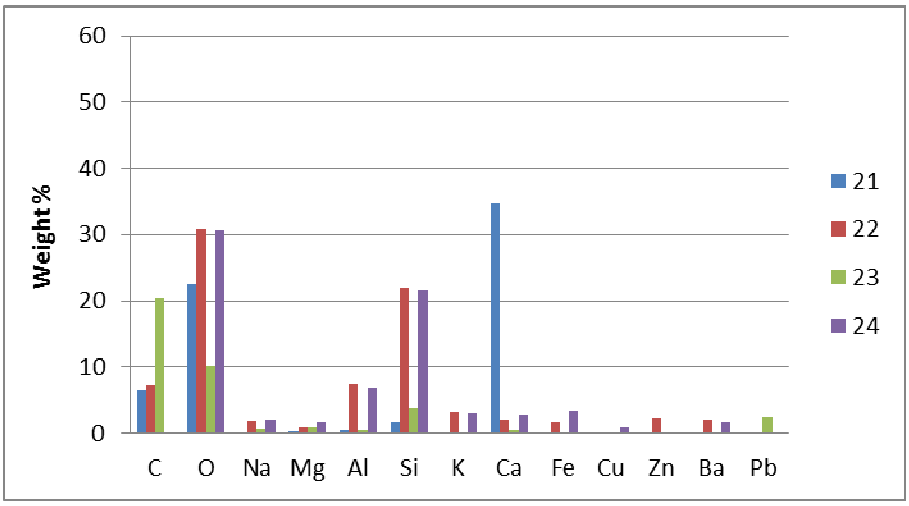
SEM-EDS: sample 1



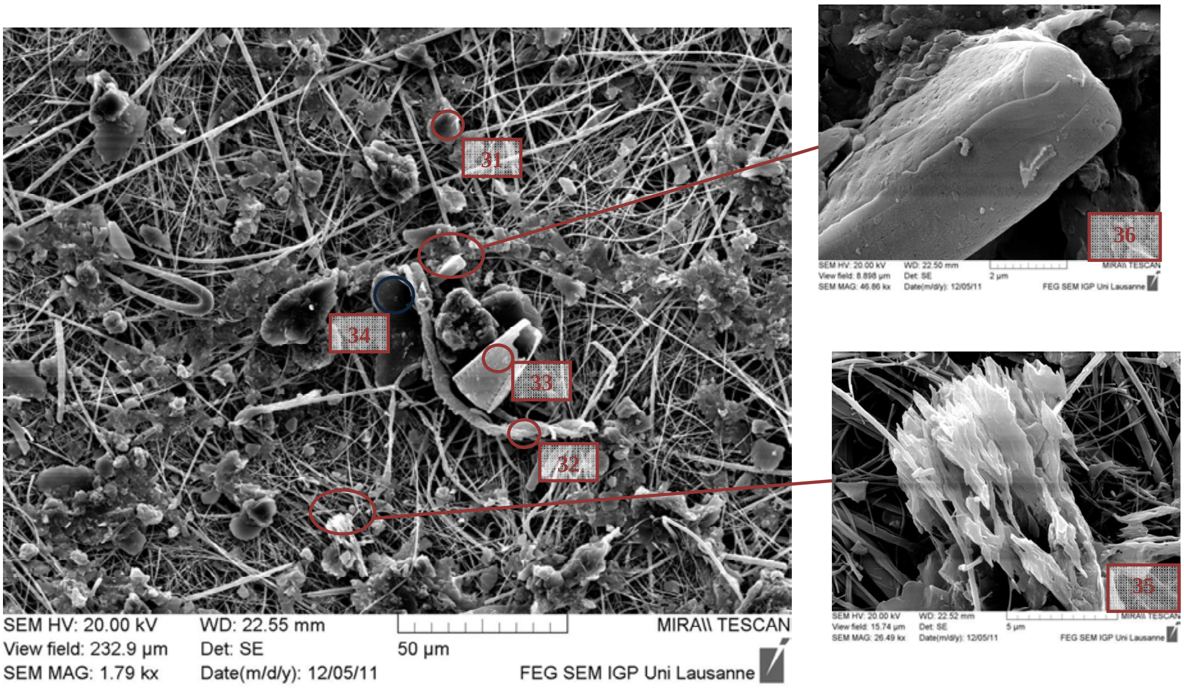


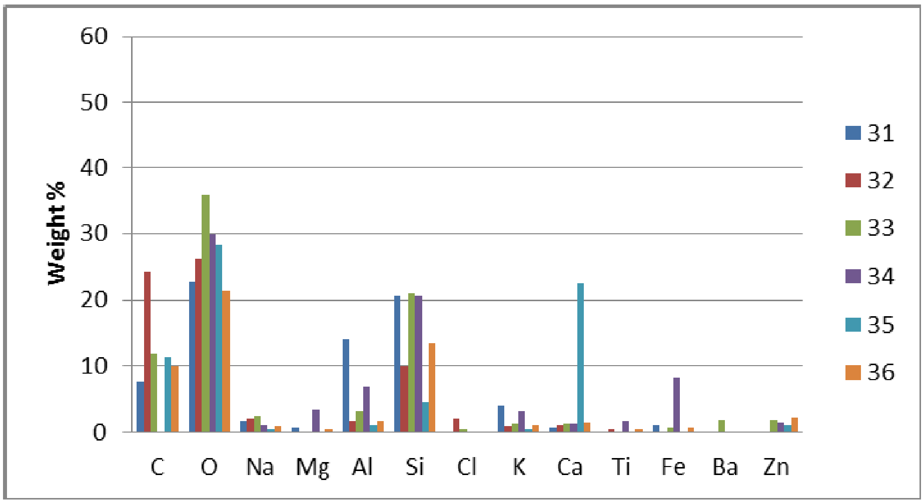
SEM-EDS: sample 2



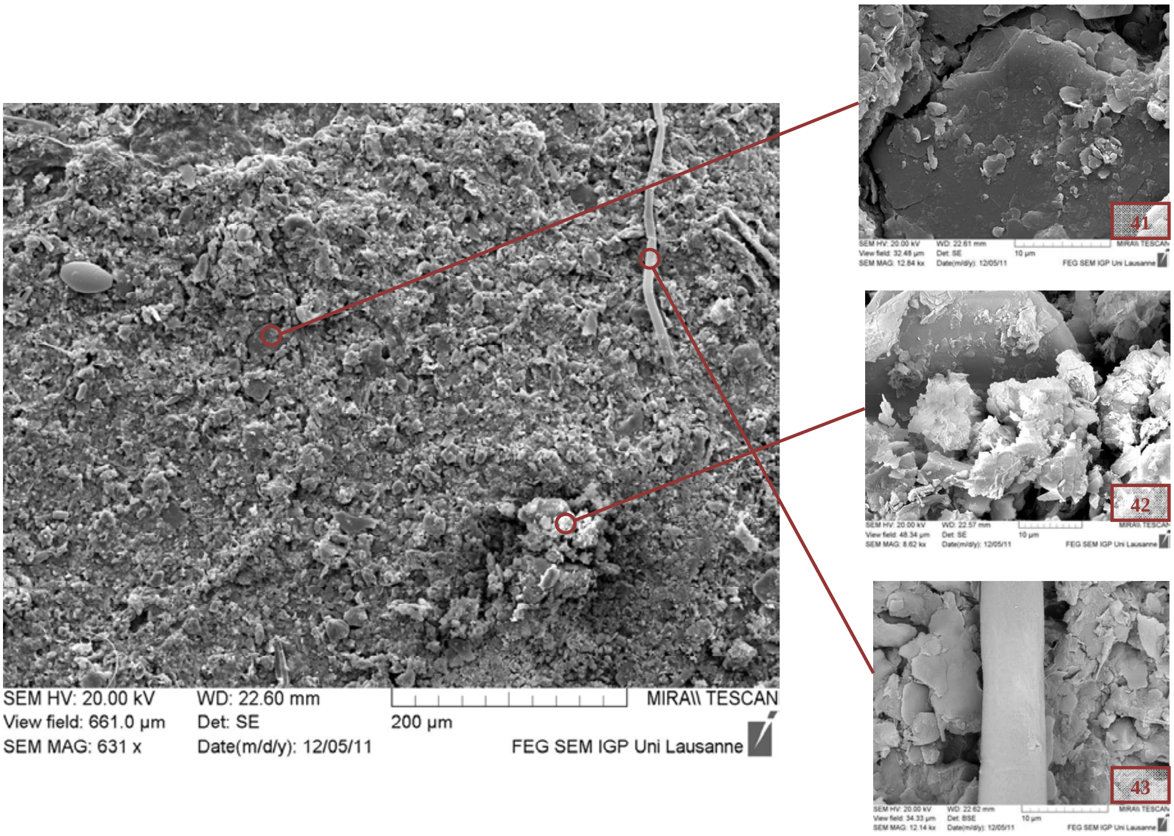


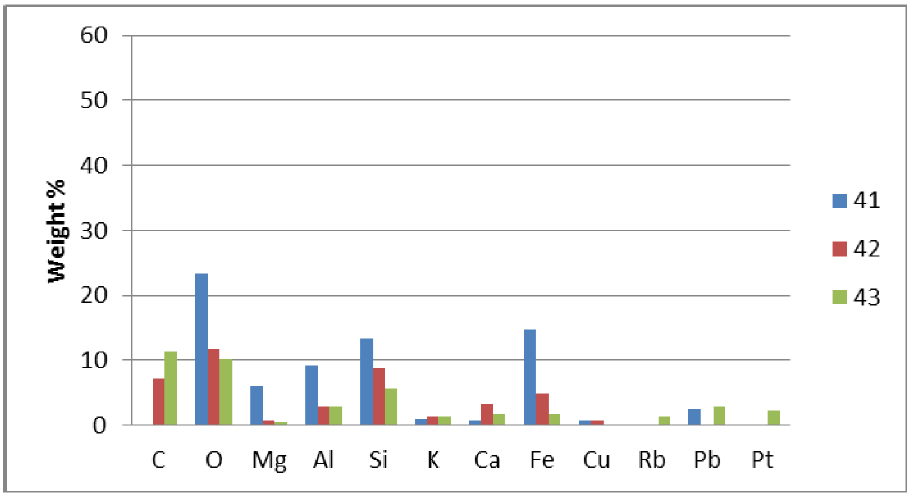
SEM-EDS: sample 3





SEM-EDS: sample 4





SEM-EDS: sample 5